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Biogeosciences Discussions

Interactive comment on "Effect of ocean acidification on marine fish sperm (Baltic cod: *Gadus morhua*)" *by* A. Y. Frommel et al.

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Authors studied the effects of acidification on sperm motility in a marine fish cod. Their findings showed no significant effects of pH at 7.55 on sperm motility. Abstract should contain results and major findings of the study and a conclusion. The first six lines are an introduction, could be omitted.

In keeping with modern practice we include introductory information in the abstract so that our findings can be interpreted in context. The remainder of the abstract presents our findings and a conclusion as recommended by this Reviewer.

Please describe what are effective acid-base regulatory systems in adult fish?

Acid-base regulatory systems in adult fish are not relevant to this study, as the cod we

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Interactive Discussion

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studied are free-spawners and the sperm is activated in the ocean. Therefore it is the regulatory system of the sperm cells, not that of the adult fish, that is interesting to its behaviour. We have not included information on adult acid-base regulation in the manuscript.

To study the role of pH, at least 3 different values of pH are needed to test!

More than two levels are required to estimate the form of the response of sperm motility with pH, but are not necessary to determine whether a given pH change will have an impact (see e.g. recent review by Kroeker *et al.* 2010, *Ecology Letters*). Unfortunately more treatment levels were not realizable in the experimental setting on board our research vessel.

Most of the study published on fish sperm, showing effects of pH at <7.0. I will recommend to decrease pH to 7 and even 6.5.

Our aim was not to determine at what pH sperm motility is affected, but rather to assess the likely impact of near-future changes in ocean pH on cod sperm motility. The Reviewer's suggestion would yield data of interest to sperm physiologists, but have no ecological relevance in the context of ocean acidification, which was the aim of this study.

Which results become achieved if you remove effects of males in statistical analysis? I not sure that it is a good idea to look for the effects of males when you are talking about the effects of acidification? However, if the effect of males is included, authors should discuss it.

The effect of males was included because "male" is the relevant biological replicate, i.e. the level at which any effects of ocean acidification on sperm motility are likely to vary.A statistically significant interaction between "male" and "treatment" (pH) would indicate variability in the sensitivity of different males to the same pH change, indicating potential for adaptation. Excluding this factor by leaving out the effect of "male" (as the

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Reviewer suggests) would reduce the statistical power of our test, and (potentially) confound the results. Our results showed that the effect of males was significant while the effect of CO2 was not.

This sentence used in discussion is absolutely incorrect; "The pH of cod seminal fluid lies between 7.9 to 8.4 (Suquet et al., 2005) and therefore, lowering the pH of the surrounding activating fluid to 7.55 should prevent the triggering of sperm motility (Cosson et al., 2008; although the review by Alavi and Cosson, 2005, states that the pH of the swimming medium has little influence on sperm motility)." It is wrong to say that pH lower than that of the seminal plasma should prevent initiation of sperm motility. Alavi and Cosson (2006) did not say this hypothesis. In this article, the authors clearly talk about a pH-independent mechanism that trigger the of sperm motility in most of the fish. The most important parameters are osmotic or ionic depend signals. Another point is the effects of ions in the activation medium, which probably remove the effects of pH. There are a lot of different cations (especially Na+, Ca2+ and K+) in seawater used for activation of sperm in this study. I will recommend using different types of activation medium to follow up the effects of pH, for example sucrose based activation medium, sweater based and NaCl based activation medium. Then change the pH by Phosphate buffer and look for the effects of pH. So far, the effect of CO2 has been shown in flat fish sperm motility. Authors discussed a nice research published by Inaba et al., 2003. I did not see such a effects on cod sperm. In addition, I believe the initiation of sperm motility in cod is an osmotic-signal dependent following the activities of ion channels that lead to axonemal beating.

The sentence was re-written to clarify the argument that an activation medium with a pH lower than the seminal plasma might lead to inhibition of activity. See Alavi and Cosson (2005) section on pH for a more detailed description of the effects of lowering the external pH on the sperm motility in other fish species. The aim of this study was to determine whether ocean acidification would have an effect on cod sperm swimming behaviour and therefore influence reproductive success in this species under predicted

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future scenarios of global change. The study was not aimed at investigating singular effects of pH at different activation media since cod spawn in seawater. Previous literature discusses the initiation of sperm motility by osmotic changes at entering seawater. This is completely correct but has no consequence for our study in that a decrease in pH arising from an increase in CO_2 in a future ocean acidification scenario might still have an effect on the activity, as has been reported for several invertebrate species. Sperm motility has further been shown to be influenced by the ionic composition of the surrounding medium and ocean acidification would change that.

A technical note about the evaluation of sperm motility in marine fish is their stickiness into the slides if you do not avoid it using for example bovine serum albumin (BSA).

We are aware of this and included this in our methodology: "To prevent sperm from adhering to the glass surfaces, slides and cover slips were pre-coated with bovine serum albumin (see Bolton & Havenhand, 1994)." Methods, lines 22-24

Interactive comment on Biogeosciences Discuss., 7, 5859, 2010.