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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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Inaba et al. (2003) already found that motility arrest of fish sperm by CO_2 is only found in flatfish species but not in other teleosts including freshwater and seawater species. What is then new information we can learn from this study? I would recommend the authors to compare their results with those published data (probably used CO2 levels higher than projected future atmospheric CO2), and discuss the importance of their own findings.

The study by Inaba *et al*. 2003 did not aim to test the effect of ocean acidification (increase in CO_2 coupled with a decrease in pH) on fish sperm. The methods used in their study therefore differ quite substantially from ours:

1. Their method of applying "a gentle stream of [pure?] CO_2" cannot control the pH and CO_2concentrations in the water. They did not run their experiments at different pH levels, but rather used well-buffered solutions that only permitted investigation of the effects of mild hypercapnia, not hypercapnia and pH change simultaneously (as we did). We used defined CO_2 concentrations coupled with changing pH to test the effect of a certain predicted level of ocean acidification, and therefore the studies are not at all comparable.

2. Inaba *et al.* do not discuss what effect "a gentle stream of CO_2" might have on the swimming behaviour of the sperm. We measured swimming speed in closed (and known) pCO_2 conditions. Not only would it not have been possible to apply a stream of CO_2 to the observation chamber, but for the reasons outlined above this would have been uninformative for the hypothesis we were testing.

3. Inaba *et al.* state that they measured sperm velocity and beat frequency "according to Cosson *et al.* (1985)". However, Cosson *et al*. do not mention measurement of sperm speed in their manuscript. Consequently it's not clear, how Inaba *et al*. measured speed, and hence even if it were relevant (see above comment regarding difference in treatments) it would not be possible to compare Inaba *et al*'s measurements with those from our study.

4. Temperature is a key determinant of sperm physiology. We held the sperm under constant, environmentally-relevant, cold conditions. Inaba *et al*. worked at "room temperature (18–20˚C)". Moreover, Inaba *et al*. make no mention of how they accounted for heating by the microscope, and it is therefore possible that the sperm experienced substantially higher temperatures under the intense light focussed by the microscope's condenser (pers. obs.). Again, this reduces comparability between our studies.

5. Inaba *et al*. provide no data for the claimed lack of response of (non flatfish) teleosts to increased CO_2 (or decreased pH) other than that they lack the same

(presumed) CA enzyme that the flatfish have. While this may provide support for an inference regarding likely responses of sperm to CO_2, the absence of this enzyme cannot be taken as evidence of absence of an effect.

It is somewhat curious to see that the authors did not discuss their earlier data on a sea urchin and an oyster, in which low seawater pH had significant effects on sperm only in the urchin but not in the oyster. Question here is why sperm of some animals are CO2-sensitive but those from other animals are not? Do the authors have some information about this point?

The effects of ocean acidification have already been found to vary substantially between populations/species/genus/families etc (see e.g. Kroeker et al., 2010, *Ecology Letters*). Organisms that can regulate their ion exchange are more robust to ocean acidification than organisms that cannot (Melzner *et al*., 2009, *Biogeosciences*). Several researchers (Havenhand, Renborg, Williamson, Mifsud, unpublished) have found the effects of ocean acidification on fertilization success, to vary markedly even between closely related species. This may well be due to adaptation to different pH environments at the time of spawning. For example, two sympatric species of sea urchins can have very different responses to low pH levels if one spawns early in the year when pH levels are stable and high, while the other spawns later in the year when pH levels fluctuate widely (Stumpp *et al*, in prep). Furthermore, there seems to be substantial intraspecific variation in the responses to ocean acidification, probably due to local adaptation in different populations. The effect of high CO_2 levels on fertilization success has been found to differ between females of a sea urchin species, reflecting genetic variation in tolerance to CO_2 (Kurihara, 2008). Furthermore, the literature reveals wide intra- and inter-species variability in the ionic composition of seminal plasma and sperm even between fish species (Billard et al., 1995) that could account for different responses to ocean acidification, as the mechanisms of motility initiation differ.

For reasons of brevity we have chosen to not include this discussion in our manuscript here.

The manuscript contains several errors which should be corrected.

Specific comments

Page 5863 line 2-4: How many sperm were observed for each replicate?

An average of 200 paths per slide were tracked for each of the 5 replicate slides for each male.

Page 5863 line 3: What is the "motile sperm direction"?

This is a mistake, the word "direction" should not be there. It has been removed.

Page 5863 line 13-14: It is stated that mean control SW pH was 8.056 and acidified SW pH was 7.554. These values are different from those given in Table 1 (8.080 and 7.558 calculated) and in Table 2 (7.929 and 7.504 measured). Which is correct? Why calculated and measured values differ this much?

Again, this was a mistakeand has been corrected. The actual measured values are those given in table 2, while the calculated values are those in table 1. The difference between measured and calculated pH values most likely arise from the use of NBS buffers in seawater, which was necessary for practical reasons. Other sources of change can occur due to the storage of the water and in measurements of DIC and TA, but we assume the calculated pH values to be closest to the actual values we had in the water.

Page 5863 line 14: "36-50 cm" is standard or total length?

Total length. This has been added in the text.

Page 5864 line 24: Alavi and Cosson 2005 is not a review paper and does not say "the

pH of the swimming medium has little influence on sperm motility". This is probably another Alavi and Cosson 2005 paper in Cell Biol. Int. 29, 101 "Sperm motility in fishes. I. Effects of temperature and pH: a review"? It is confusing here. In line 19, it is stated that the external pH is of crucial importance... (Alavi and Cosson 2005, 2006), which is opposite to the statement in line 24 but referring to the same Alavi and Cosson 2005 paper. Which is correct?

Apologies for the confusion. The Alavi and Cosson 2005 paper cited was the review paper in Cell Biol. Int. 29, 101 "Sperm motility in fishes. I. Effects of temperature and pH: a review", in which the authors state in the abstract that "the pH of the swimming medium, and thus the intracellular pH of spermatozoa, has less influence on sperm motility parameters in cyprinids, salmonids and sturgeons" than other factors. Their quote was preceded by an "although" in our manuscript meaning that the literature as yet does not seem to agree on whether pH has much of an influence or not. That is why we go on to argue that the effect of pH on semen depends on the mode of pH manipulation, and that when pH is lowered by increasing CO_2 concentrations the effect is much more pronounced compared to acid addition. SImilar results have been reported elsewhere (e.g. Kurihara & Shirayama 2004 *Mar.Ecol.–Prog.Ser.*).

Page 5865 line 2-6 According to Inaba et al. 2003, fishes other than flatfishes did not show inhibition of sperm motility in elevated CO2 conditions. Then, what is the new information we can learn from this paper.

See above for discussion of the Inaba *et al.* 2003 paper and the comparison to our manuscript.

Table 1

This table is not commented upon in text.

This was due to mis-referencing of tables in the text, which has now been corrected.

There are two fish with #7.

Again this was an error and the relevant individual has been relabelled (to 8).

pHC is quite different between fish #1-#7 (7.858) and fish #8-#18 (7.985). Why?

These are the measured values of the pH in the activation water used. For fish numbers 8-18, a new batch of water was taken from the storage barrels of acidified and control water and these had a slightly different pH values.

How was the sperm swimming speed determined? These data are means of 5 replicates for each fish?

The data in table 2 are the means of 5 slides per fish, and an average of 200 paths per slide, from which swimming speeds were determined.

Table 3

Are the numbers of df correct? Why not integers?

These are the corrected df calculated by the statistical package and hence are non-integers.

Technical corrections

Page 5863 lines 14 and 16 "Table 1" should read "Table 2".

Page 5863 line 22 and Page 5864 line 3 "Table 2" should read "Table 3".

Mislabelling of tables has been corrected.

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