

## Project information

### Project title

Influence of ocean acidification and temperature on sperm motility, fertilization and embryonic development in Atlantic cod

### Year

2011/2012

### Project leader

Helge Tveiten, Nofima

### Participants

- Helge Tveiten (project leader), Nofima, P.B. 6122, N-9291 Tromsø
- Marianne Frantzen, Akvaplan-niva, Fram Centre, N-9296 Tromsø
- Geir Rudolfson, Norwegian Radiation Protection Authority, Fram Centre, N-9296 Tromsø

### Flagship

Ocean acidification, Theme: Effect of ocean acidification on species and ecosystems

## Summary of Results

In order to investigate the effect of temperature and pH on Atlantic cod sperm characteristics and fertilization ability, two experiments (exp. I and exp. II) were conducted during the natural spawning period in April 2011. In both experiments sperm from five males and eggs from one female were utilized. All gametes were stripped from broodfish of the National Cod Breeding Programme at the same day as the experiments were conducted.

### Sperm characteristics:

Sperm characteristics were investigated using Computer Assisted Sperm Analysis (CASA) according to the established cod protocol (Rudolfson et al., 2005). Sperm was activated in seawater at different temperatures (4 °C and 9 °C) and pH (8.1 [control], 7.7 and 7.4) and recorded at different time intervals (0, 10 and 20 minutes post activation). The relationships between sperm quality over time (0, 10 and 20 min post activation) and stressors (temp and pH) were analyzed in mixed effects models with sperm velocity and motility as the dependent variable, individual as within subject and random factors, and pH, temperature and time since activation as the fixed factor. We ran three different models where in the full model all factors could interact, and compared this with two reduced models, one with all two way interaction or additive effects only. We followed Baayen et al. 2008 and used the 'anova' function in the lme4 package to compare the quality of the fit between models. Model fit and the significance of including the interactions were tested using AIC (Akaike's Information Criterion) and log-likelihood ratio statistics (LLR  $\lambda^2$ ) (Bates 2005). Model fitting and estimates were obtained with linear mixed-effects (lmer) package lme4 in R (version 2.11.0, R Development Core Team, 2007) using maximum likelihood estimates (ML). Significance of coefficients are gauged by checking the summary for whether the absolute value of the t-statistic exceeds 2. Finally, the model fit was verified using visual examination of normal probability plots and residual plots.

### Fertilization ability:

Eggs (1000 eggs per replicates, three replicates per treatment) were fertilized in seawater at different temperatures (4°C and c. 9°C) and pH (8.1 [control], 7.7 and 7.4) using a protocol under conditions where fertilization is sperm-density dependent (sperm to egg ratio = 5 x 10<sup>5</sup>: 1, gamete contact time < 30 min.; own unpublished results; Butts et al., 2009). In exp. I fertilization was undertaken immediately after sperm activation and fertilized eggs were incubated under pH and temp conditions identical to the fertilization conditions. In exp. II the number of incubators was doubled and eggs were fertilized with sperm either activated at fertilization or 10 minutes pre-fertilization. In this experiment fertilized eggs were incubated at temp conditions identical to fertilization conditions but at ambient pH (pH 8.1). Fertilization rate was monitored, and fertilized eggs were investigated with respect to early cell cleavage according to Tveiten et al. (2001).

## Results

### Sperm characteristics:

Parameters included in this report are mean curvilinear velocity (VCL,  $\mu\text{m s}^{-1}$ ) and percentage of motile cells (MOT) of exp. I and II combined (n=10 males per treatment) (see table I and fig.1 in attachment).

The results revealed no effect of pH on sperm VCL or MOT. However, increased temp (9°C) induces a significantly stronger temporal decrease in both VCL and MOT compared to lower temp (4°C).

### Fertilization ability:

Unexpected and surprising results were obtained regarding the fertilization ability in both exp. I and exp. II where the eggs in general, independent of pH, fail to fertilize at 4 °C (ambient temperature; control). Image analyses of the eggs fertilized at 9 °C

did not revealed any effect of pH on percentage fertilized eggs in any of the experiment.

However, in exp. I there was a tendency of higher prevalence of activated eggs that had failed to initiate any cell cleavage, and eggs with abnormal cell cleavage at pH 7.7 and 7.4 compared

to pH 8.1 (see fig. 2 and 3 in the attachment). The fertilization success were in general lower in exp. I than exp. II indicating a higher egg quality in the latter exp. The tendency of increased abnormal egg development at reduced pH in exp I may indicate that future ocean acidification scenario can influence the recruitment potential of populations suffering a suboptimal physiological condition caused by unidentified stressors. This hypothesis needs further investigation. Also, the failure of eggs to fertilize at 4 °C needs to be further investigated.

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## Published Results/Planned Publications

The results from the project will be submitted as a "short communication" to a relevant peer review journal (e.g. Marine Biology)

## Communicated Results

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## Interdisciplinary Cooperation

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### Budget in accordance to results

The funding from the Fram Centre have made it possible to start up some activities which are part of a bigger research project that has not yet succeeded to receive the necessary funding. The results from the Fram Centre project are important for emphasizing the objectives and strengthen the feasibility for getting the big project funded. Further, throughout the Fram Centre project we have been able to identify needs for optimization of the experimental exposure systems and contacts have been established to ensure appropriate water chemistry analysis in future experiments.

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### If Yes

Yes, the results from the project are highly relevant for optimization of fertilization protocols utilized by the cod aquaculture industry (juvenile producers).

### Conclusions

The result of no fertilization at 4 °C is highly interesting and need further investigation. The experiments conducted in this project are only snapshots of the parameters that may be affected regarding reproduction by temp and pH and further experiments need to also consider effect on the reproductive development prior to spawning (effects on the broodfish) as well as effects on egg quality, larvae and juveniles. Also, effects of temp and pH in combination with other stressors (i.e. environmental pollutants) are of highly relevance.

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