Effects of Ocean Acidification on Sperm Motility in the Red Abalone, *Haliotis rufescens*

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ABSTRACT

Fossil fuel emissions are changing global temperature and ocean water chemistry. These changes are already altering the seasonal upwelling events that bring deeper ocean water with lower temperature, dissolved oxygen, and pH to shallower areas of the California Current Large Marine Ecosystem. For example, increase absorption of CO_2 by the ocean is expected to lower the pH of current upwelling events (observed to be ~7.5) by a further 0.4 pH units. These changes in seawater chemistry are expected to affect reproduction, growth, and survival for many coastal marine invertebrates. To gain insights into this process, this study seeks to evaluate the effects of ocean acidification on the swimming behavior of sperm from the red abalone (*Haliotis rufescens*), an economically and ecologically important species along the California coast. Abalone and many other marine organisms reproduce by releasing gametes into the ocean and fertilization success may be influenced by ocean conditions during this process. The effects of pH on sperm swimming behavior, which likely play an important role during fertilization, are unknown for red abalone. Thus, evaluating sperm motility under different pH conditions may reveal how fertilization could be affected by seawater chemistry. In this experiment, sperm were exposed to a pH of 8.0 and pH of 7.2 for one minute. Samples of sperm were recorded by video and analyzed using ImageJ

ManualTracking software to measure speed. No significant difference in sperm speed was found between the high and low pH for this experiment. These findings will help gain insights into the effects of ocean acidification on the reproductive success of red abalone and other coastal marine invertebrates with similar reproductive modes.

INTRODUCTION

Burning of fossil fuels results in an increase in atmospheric carbon dioxide (CO_2). This CO_2 is absorbed by the ocean where it undergoes several reactions to achieve chemical equilibrium. Hydrogen ions are released into the ocean, thus lowering ocean pH. The 2007 IPCC report predicts the global ocean surface pH to drop a further 0.4 pH units. This change has effects on early life stages of coastal marine invertebrates, specifically reproduction, growth, and development (Pechenik, 1999; Cowen et al., 2000; Raven et al., 2005). Ocean acidification is already known to impede fertilization success in coastal marine invertebrates (). This study seeks to assess the effect of pH on sperm swimming behavior, which may have an effect on fertilization success. The organism chosen to study these effects was the red abalone (Haliotis refescens), an economically relevant species in California. The red abalone reproduce by broadcast spawning, making them a model organism to study pH effects on sperm motility for the red abalone and for species with similar reproductive modes. The pH treatments chosen to study their effects on sperm motility were a High and a Low pH. The High (control) treatment was ~8.0 pH, from incoming filtered seawater in the Monterey Bay. The Low treatment was ~7.2 pH, the future projected upwelling pH conditions brought to coastal ecosystems.

MATERIALS AND METHODS

SPAWNING

Gravid male red abalone were purchased at American Abalone Farms in Davenport, CA for this experiment. Once purchased they were immediately brought to the lab where they were measured for length and then placed in 40 L in-flow brooding

tanks, kept in 24 hour darkness, with incoming seawater at 11° C. They were fed *Macrocystis perifera* ad libitum for three weeks while acclimating to these conditions.

After three weeks, Tris buffer and Hydrogen Peroxide were used to spawn the abalone. Once spawning began, a glass pipette was used to collect sperm from the respiratory holes. Sperm were collected into a beaker to highest concentration. Concentration was checked with a hemacytometer and diluted to create the sperm stock solution in which samples were taken from.

EXPERIMENT

Sperm were exposed to two pH treatments; High and Low. The High (control) treatment was ~8.0 pH, from incoming filtered seawater. The Low treatment was ~7.2 pH, the future projected upwelling pH conditions seen in the Monterey Bay. There were 6 replicates (n=6) for each High/Low treatment and each treatment was administered over three time points. These time points were 0, 4, and 8 hours post-spawning. The treatment vessel used was a 2 ml Eppendorf tube. For one sample, 1.5 ml of treated sea water (TSW) went into the Eppendorf tube followed by 75 uL of sperm from the stock sperm solution. Once the tube was filled, it was rested in a 13° C water bath and the exposure time, set at 1 minute, began. Immediately after exposure, 150 uL from the Eppendorf tube was micropipetted onto a glass slide with a 0.9 mm height Viton rubber O-ring and coverslip on top. The O-ring was used to mitigate wall effects on sperm swimming speed (Havenhand et al, 2008). Sperm were examined under a Zeiss Axioplan 2 Compound Scope at 40x magnification. A 10 s video, (12 frames/second) was recorded as soon as possible after exposure (within one minute) to reduce effects of seawater chemistry and temperature change.

DATA EXTRACTION FROM VIDEOS

To assess sperm swimming speed, videos were analyzed by ImageJ ManualTracking software. Ten random sperm per sample were tracked over 1 s (12 frames). Speed in μm/s was measured using ImageJ ManualTracking. The average speed of the 10 sperm per video sample was taken for each replicate.

RESULTS

Results from One Way ANOVA ($\alpha = 0.05$) suggest there is no significant difference in mean sperm speed between the High/Low pH treatments. One Way ANOVA ($\alpha = 0.05$) did show a significant difference in mean sperm speed among time the three time points. A Tukey Post-Hoc test revealed a difference between 0 and 4 HPS and 0 and 8 HPS, but no significant difference in means between 4 and 8 HPS.

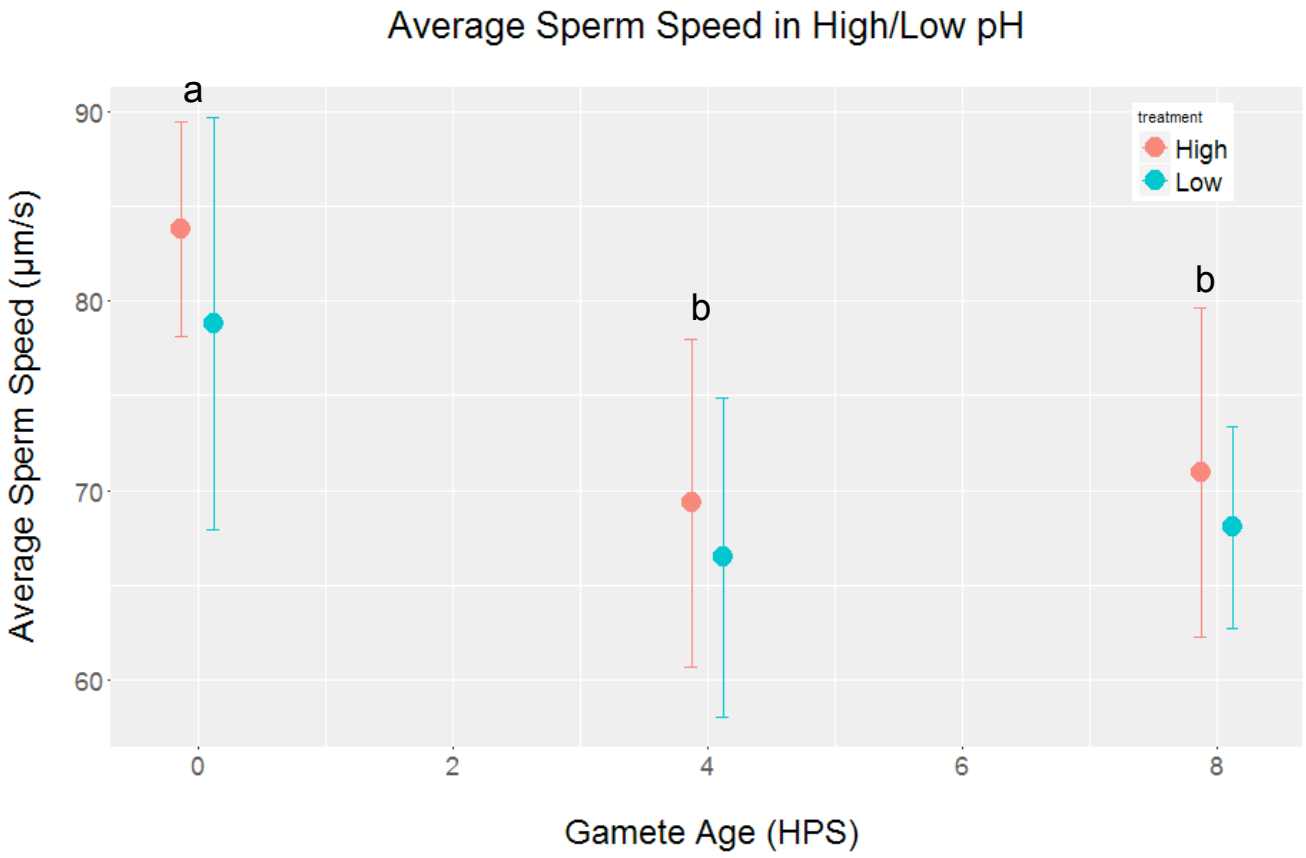

Figure #: Data are expressed as mean ± standard deviation (n = 6).

DISCUSSION

While there was no significant difference in mean sperm speed between the High/Low treatments, the mean for High was still higher than the Low for each time point. This is an interesting signal that should be investigated. Possible reasons for this

small signal are short exposure time, small experimental volume of TSW, and too few replicates. The exposure time, which was set at 1 minute, may not have been long enough to see a significant effect of pH on sperm speed. The volume of TSW with sperm that was recorded was 150 µl. Volumes at this small of a scale can undergo rapid changes in temperature and seawater chemistry. While the videos were recorded within one minute of the exposure time, these factors were not measured after the recording so it's unknown how these factors may have affected sperm speed. Replicates for the High/Low at (n=6), gave a small dataset to work with. An increased number of replicates may have shown by statistical analysis a larger difference in treatments.

CONCLUSIONS/RECOMMENDATIONS

No significant difference in mean sperm speed between High/Low was found. A significant difference among time points was found, suggesting gamete age effects on sperm speed. A small signal of mean sperm speed from the High being consistently higher than the Low sperm speed was found. In order to increase this signal seen between the treatments, it's recommended to repeat this experiment with longer exposure time and increased replicates. The knowledge gained from this work will serve as a basis towards future NSF-funded ocean acidification research.

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