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Enzymatic Activity in the Chorion for Hatching in the California Grunion

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Abstract
Leuresthes tenuis is a small, silverside fish that spawns on the beaches during some of the highest tides of the summer months. Of the many unique traits to the species including that the eggs develop fully out of water to the point of hatching competence but will not hatch until presented with an environmental cue, which causes them to hatch in less than a minute. The purpose of this study is to better understand the role of enzymes called chorionases, which act to break down the chorion (egg membrane). I hypothesize that the chorion begins to weaken in this species when it is hatching competent but before it receives the stimulus to hatch. Unlike most organisms, the grunion embryo reaches hatching competence when it is fully developed, once it reaches competence, it stalls development and waits for an environmental cue. Before this, the egg is not hatching competent and will not hatch even if the trigger is there. The fact that it hatches so quickly could be evidence that an enzyme is acting to break down the chorion and this is what I hypothesize causes hatching competence. Some related fish have two chorionases while some only have one. To see whether one or two enzymes act in hatching in the grunion, I made a solution of the hatching enzyme, concentrated it, ran both SDS-PAGE and native-PAGE gels to separate out the protein by size, and cut out and sent those bands away for sequencing.

Introduction
The California Grunion lays its eggs on beaches [Thompson and Thompson, 1919]. There are many unique characteristics of this species, including that the eggs, which develop terrestrially, reach hatching competence but do not hatch until an environmental cue [Martin et al., 2013]. This environmental cue happens when the tide rises preceding the new and full moon, and the eggs are washed out of sand into the waves. Another unique trait is that these fish hatch in less than a minute, whereas related fish take 30-60 minutes to hatch [Martin et al., 2011]. In medaka, there are two enzymes which break down the inner layer of the chorion, high chorionic enzyme and low chorionic enzyme [Yasumasu et al., 2010]. The inner layer is thicker, HCE acts first to weaken it, and LCE breaks it down completely, while the outer layer is broken by the fish mechanically. In zebrafish, only one enzyme acts to break down the chorion, HCE, so the process is altered [Sano et al., 2016]. The purpose of this experiment is to see if both chorionic enzymes act in the hatching of L. tenuis, and if so, why the eggs hatch so quickly. I hypothesize that HCE is released once the grunion reaches hatching competence, and LCE is released once the environmental stimulus occurs. This way, the chorion is already weakened once it comes time for the fish to hatch, and it can do so rapidly.

Methods

Hypothesis one:
We are still waiting on the results of the protein identification. However, in regards to the second part of the hypothesis, a preliminary study suggested that there was a significant difference in egg rigidity between pre-hatching competent and hatching competent eggs. However, due to variation in the population and the fact that the eggs may have been weakened being in the refrigerator and the embryos dying, this data could not be trusted. By comparing a single sample egg from the same clutch when they were pre-hatching competent, then a few days later when they were hatching competent, we minimized this variability and found that there was no significant difference in rigidity. We still believe that further testing is required. The chorion is supposed to protect eggs from infection. Eggs in the wild have increased rate of infection the longer they stay in the sand, so this could be a sign of weakening. Furthermore, we did not allow the eggs to remain at hatching competence for long, they had just reached hatching competence when these data were recorded. Most of the egg did hatch, but in the preliminary study, eggs had been hatching competent for at least three days. If enzymes are released once the egg becomes hatching competent, it may take slightly longer for enzymatic activity to begin. We plan on re-measuring these values in a few days. The eggs we tested were on only seven days old. At temperatures near the coast it can take 8-30 days for eggs to become hatching competent [Moravek and Martin, 2011]. Lastly, 0.245 N has may not been enough force to pull a significant difference. Though these results were not significant, allowing the eggs longer to develop or testing chorion strength with a more sensitive test could show a significance that we cannot with the current method.

Results

Discussion

References

Conclusion

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Figure 1: A grunion male and female at a grunion run. Photo by Doug Martin.