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Effects of pH on Sea Urchin's Fertilization Success Rate

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1. Introduction

A lot of marine organisms are sensitive to changes in pH level, though not all(Johnson, 2022). For example, a previous study looking at the fertilization rate of the short-spined sea urchin discovers that low pH does not pose any impact under even the lowest sperm concentration on the species' fertilization rate(Byrne et al., 2010). In some cases, a positive correlation between fertilization and low pH is suggested. For example, the blue mussel is recorded to have a higher fertilization rate when the sperm swims in pH of 7.6 instead of the ambient sea with a pH of 8.0(Lymbery et al., 2019). In sea urchin *Strongylocentrotus nudus*, however, a slightly lower pH range of 7.94 to 7.96 can impair sperm and drastically decrease fertilization rate(Sung et al., 2014). Those previous studies indicate that species can demonstrate very distinct pH tolerance.

In this experiment, *Strongylocentrotus purpuratus*, the purple sea urchin, are studied. They are herbivorous echinoderms abundant in the Pacific coasts of North America(Clemente et al., 2012). They usually reside in rocky intertidal zones or among the kelp forest(Pearse, 2006). Predators of *S. purpuratus* like spiny lobsters, sheep-head fish, and sea otters maintain their abundance for the health of kelp forests(Pearse, 2006). *S. purpuratus* also competes with algal grazers like abalones and red sea urchins(Pearse, 2006). A previous study on *S. purpuratus* has revealed a decreased larval size relating to low pH capacity(Kelly et al., 2013). Though abundant genetic variation of body size was also observed in the same study, suggesting that it is an evolvable adaptation toward future ocean acidification.

This study attempts to elucidate the relationship between low pH and fertilization rate of the *S. purpuratus,* investigating possible adaptation for them to survive in ocean acidification. The study can be used to predict abundance of *S. purpuratus* under ocean acidification and how they will alter the ecosystem, especially the kelp forests. The hypotheses are that 1) *S. purpuratus* is sensitive to pH variation, which can be shown through changes in its fertilization rates; 2) Decrease in pH level will decrease the fertilization rate of *S. purpuratus.*

2. Material and Method

This study was conducted at Meiyen Lab, a business lab in CA, in 2022. *S. purpuratus* were collected from Marinus Scientific, LLC. Before the lab, 0.53M KCl was injected into 10 females and 10 males to induce spawning. The eggs were collected in artificial filter seawater with 35 ppt. The sperm was collected dry. HCL or NaOH was added to filtered sea water to obtain a desired pH, tested with a pH meter. 1mL of washed eggs were prepared in tubes with pH 7.6, 7.9, 8.2, and 8.5. Sperms at 10^{-2} concentrations (50 uL concentration sperm in 4.95 uL sea water) were put in tubes with the same pH scale. The rest of the procedure is divided into sperm dilution, fertilization reaction, and egg scoring.

2.1. Sperm dilution

4 new tubes were labeled with 10^{-3} and the corresponding pH values. Each tube was filled with 4.95 mL seawater with the correct pH. 500 uL of the 10^{-2} sperm concentration was pipetted to the corresponding 10^{-3} tubes.

2.2. Fertilization reaction

1 mL of 10^{-3} sperm were added to the matching pH tubes containing eggs. Fertilization was allowed to continue for 2 minutes before 2 mL of 0.5M KCl was pipetted for stopping. The process was repeated for each tube.

2.3. Scoring eggs

Successful fertilization was identified under the microscope by the presence of fertilization envelopes of cortical reactions, which forms after the fusion of the plasma membranes of the sperm and the oocyte. The percentage of fertilization was determined out of the first 100 egg samples. 4 trials were run and the final values were the average percentage. Standard errors were calculated for the amount of variation around the mean and a t-test was performed for the difference in rates of fertilization(a=0.05).

3. Result

The changes in percent fertilizations at various pH levels are all insignificant(Fig.1). In pH 7.6, the fertilization rate is 77.75% +/- 7.14% (1 S.D.). In pH 7.9, the fertilization rate is 83.25% +/- 5.38% (1 S.D.), P-value=0.175 from pH 7.6 to 7.9 and therefore the value is insignificant. In pH 8.2, the fertilization rate is 81.00% +/- 5.29% (1 S.D.), P-values=0.335 from pH 7.9 to 8.2 and the value is insignificant. In pH 8.5, the fertilization rate is 82.75% +/- 3.95% (1 S.D.), P-values=0.722 from pH 8.2 to 8.5 and the value is insignificant.

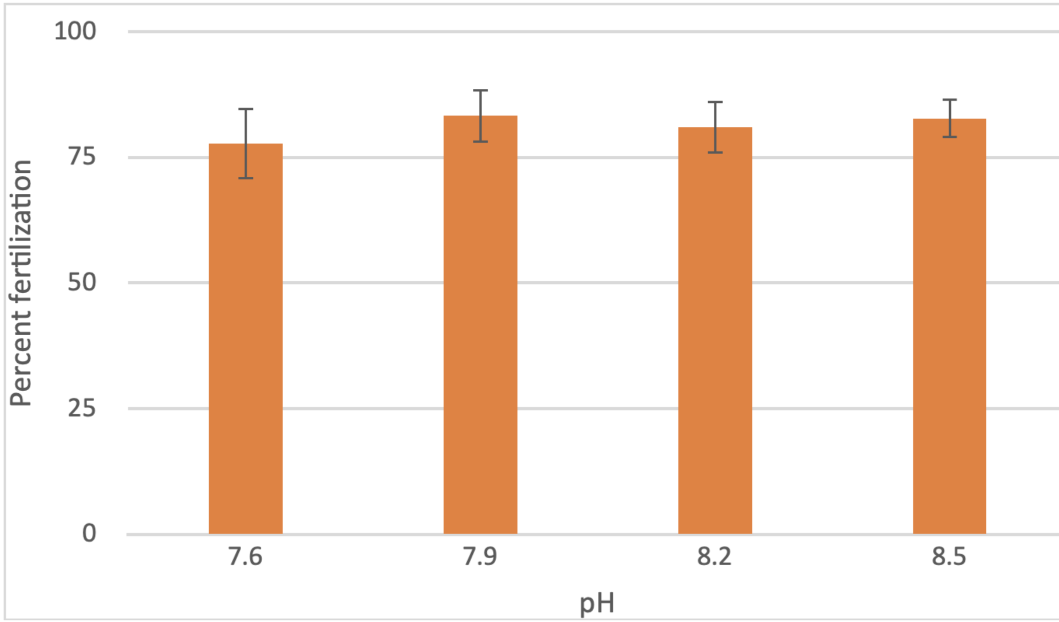

Figure 1. Percentage of fertilization rate in *Strongylocentrotus purpuratus* under various pH levels. The graph presents the average percentage of 4 trials. 1mL of 10^{-3} sperm were fertilized with 1mL of washed eggs in respective pH for 2 minutes. The error bars indicate standard deviations.

4. Discussion

The result shows that pH level 7.6, 7.9, 8.2, and 8.5 has no significant effect on the fertilization rate of *S. purpuratus.* Although there is no direct research on the pH regulatory mechanism of sea urchins, some studies provide insight into the pH regulatory mechanism of fish, which also use external fertilization. It is pointed out that an altered pH affects an organism's endocrinological mechanisms, which are related to sperm motility activation(Mishra et al., 2018). The main axis under influence is the hypothalamic-pituitary-testes axis, in which the somatic cells are stimulated to release

17α-hydroxyprogesterone, causing the production of 17α, 2β-dihydroxy-4-pregnen-3-one in spermatozoa to regulate sperm motility(Mishra et al., 2018). A further experiment is needed to determine if this mechanism applies to sea urchins.

There are several reasons to explain the unsupported hypothesis about decreased fertilization and pH sensitivity of *S. purpuratus*. It is found that the effect of pH differed by site for species(Kapsenberg et al., 2017). The information below is from Kapsenberg et al. Sea urchins originating from sites with the narrowest pH fluctuation demonstrate the greatest pH sensitivity, meaning that the adaptive capacities of the species are determined by their distribution. Therefore, species in regions with large pH variations develop better resistance to low pH. In the example region, upwelling in the area lower pH periodically and *S. purpuratus* living in the area exhibit a wider range of pH tolerance. Given the information, it is plausible that the pH range used in this experiment is within the tolerance of *S. purpuratus*, which can be why no significant change in fertilization is observed.

Another possible danger of low pH not shown in this experiment is the risk of polyspermy. In a study from Reuter et al. of the red sea urchin, it is suggested that a decline in pH increases sperm limitation and reduces the efficiency of fast blocks(egg membrane depolarizes after the entry of the first sperm). The conclusion is that low pH can aggravate low fertilization rate in low-density populations or areas with turbulence(Reuter et al., 2011). Although the study of Reuter et al. focuses on a different species of sea urchin, it is feasible that *S. purpuratus* can exhibit a similar pattern of sperm limitation and risk of polyspermy below its minimum pH tolerance.

For the understanding of the result from this study, *S. purpuratus* exhibits a wide pH tolerance. It is an advantage to their survival in comparison with other species under future ocean acidification, but a potential threat of regime shift to the kelp communities and the ecosystems dependent on them(Johnson, 2022). Works have been put into estimating species' sensitivity to ocean acidification to predict the impact of ocean acidification on the entire ecosystem(Busch & McElhany, 2016).

The stand deviation and caveat in the experiment mainly derive from the scoring of fertilized eggs. Since the fertilization rate remained fairly high even with standard deviation, it might be difficult to distinguish between individual eggs and their fertilization envelopes. Other possible errors include imprecision in the amount of sperm pipetted to proceed to fertilization and imprecise input of KCl. Inadequate mixing in samples extracted for microscope examination can also cause errors. More trials should be run to avoid flaws.

Future work on the pH regulatory mechanism of sea urchins and how low pH reduces sperm motility can be conducted. Such study can help to develop a better understanding of possible adaptation toward lowered pH. It is also crucial to find the minimum tolerance pH of *S. purpuratus* and if altered pH poses significant changes in their fertilization rates below that tolerance. The future study can adapt the same procedure, except for a modified lower pH level to find a significant difference between fertilization rates. If a low pH indeed impacts fertilization rates of *S. purpuratus*, tracking the embryo development can be the next step in examining possible polyspermy.

5. References

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