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Will Saltwater Slow Sperm and Stop Frog Fertilization?

Increased salinity may lower sperm activity and fertilization rates in Squirrel Treefrogs (*Hyla squirella*)

As global warming causes sea levels to rise and storm activity to intensify, animals living in freshwater systems along the coast are in danger of increasing salinity in their environment. A new study on squirrel treefrogs in Charleston, SC hopes to answer important questions about how saltwater intrusion could affect frogs at their earliest life history stages: gametes and fertilization.

Cecilia Bueno, an REU intern at Grice Marine Laboratory, College of Charleston, looked at how low levels of salinity affect the reproduction of a small treefrog (*Hyla* squirella) native to the freshwater Coastal Plains habitat. The Coastal Plains, like all freshwater systems, has a salinity of around 0 parts per thousand (ppt), while ocean water is 35ppt. When salinity increases, the change can pose a threat to the treefrogs that need freshwater to live and breed.

"Amphibians cannot tolerate much salinity, especially in their early life stages," says Dr. Allison Welch, an assistant professor at the College of Charleston who specializes in amphibians, "This makes them vulnerable to habitat salinization, which can happen when salts are introduced to freshwater environments, for example from storm surge or road salt runoff." Previous research in Dr. Welch's lab on southern toads (*Anaxyrus terrestris*), squirrel treefrogs (*Hyla squirella*), and green treefrogs (*Hyla cinerea*) has found detrimental effects of even low levels of salinity on tadpole growth, fertilization rates, and sperm activity.

Building off of previous research on the squirrel treefrog and its close relative the green treefrog, Bueno hoped to answer the question "Do differences in sperm function at increased levels of salinity correlate with fertilization success?"

In order to answer this question, she conducted an experiment to look at how 6ppt salinity affected fertilization rates and then how salinities ranging from 4ppt to 8ppt affected sperm activity. By comparing fertilization rate with sperm tolerance, Bueno hoped to see whether an increased sperm tolerance could predict greater fertilization success.

Bueno found a great amount of variation in fertilization success at 6ppt- from nearly 100% success to near 0% success- between the 32 males and females studied. Interestingly, a significant amount of this variation can be attributed to both male and female components within the different crossed pairs.

Bueno also found that salinity had a significant negative effect on sperm activity, though this effect was nonlinear. Between the 30 males analyzed for sperm activity, there was a lot of variation in response to increasing salinity. This variation could be attributed to males, in which different males had significant effects on how its sperm would react to increased salinity. This is important as it suggests variation in sperm tolerance to salinity, which could potentially allow for adaptation if increased salinity continues.

Despite our predictions, there was no correlation between sperm activity at 6ppt and male fertilization success at 6ppt, suggesting the variation for both could be attributed to different mechanisms.

The frogs for this study were taken from Dixie Plantation in Hollywood, SC, close to the city of Charleston. As ocean levels rise, coastal areas such as Charleston are predicted to become inundated as tidelines rise well above their normal levels. The salinization of coastal freshwater habitats is likely to continue as global climate change causes such environmental changes, making its effects on all life-stages of freshwater organisms increasingly relevant.

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Male Squirrel Treefrog at Dixie Plantation in Hollywood, SC

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Full paper will be available in Dr. Robert Podolsky's inbox on August 10, 2017. Further information about this and other REU projects can be found at https://blogreu.wordpress.com/.

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