

Simulated hibernation aids MSU toad work

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Mississippi State University researchers successfully promoted egg laying in threatened Boreal toads when they moved the amphibians out of the refrigerator and into the wine chiller.

Mississippi Agricultural and Forestry Experiment Station researchers at MSU are working with a group of 52 threatened Boreal [toads](#) native to the Colorado Rockies. The toads are housed in a special lab in the MSU Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology.

Natalie Calatayud, a postdoctoral fellow, and Cecilia Langhorne, a graduate student pursuing her doctorate in animal physiology, manage the lab and care for the toads. Their work is part of a long-standing partnership with the Memphis Zoo.

"Our goal is to increase the numbers of the wild populations," Calatayud said. "We are establishing reproduction protocols to promote breeding in captivity and produce animals for reintroduction to their natural habitat."

In their [native habitat](#), Boreal toads hibernate for six months before mating. When the MSU project began, research focused on using hormones to get the toads to breed without [hibernation](#), but that approach was unsuccessful.

"When that didn't work, we decided to hibernate the females," Calatayud said.

To encourage hibernation, they use a refrigerator, a wine chiller and a water cooler.

Four females are housed in plastic, lidded boxes and placed in the refrigerator. The boxes have a layer of moist soil for the toads to sit on, and temperatures are kept at a constant 39.2 degrees.

This lower temperature is used to simulate conditions found in the wild, where Boreal toads spend the cold winters underground or in beaver dams.

"The females use their fat reserves to develop eggs while in hibernation," Langhorne said. "They actually gain weight in the refrigerator as their bodies hold moisture from the damp environment, and eggs develop."

As a test, four females were kept in hibernation for one month in the refrigerator, four others for three months and a last set of four for six months. Toads in the six-month treatment came out of hibernation July 14.

"We have had success with females laying eggs a week later," Calatayud said.

These studies determined the need for hibernation to get the animals to reproduce successfully.

"We had a 50 percent success rate for both the one-month and three-month hibernations," Calatayud said. "That is a great success because no female had ever laid eggs in the two years before this test."

Taking a toad from hibernation in a refrigerator is a two-step process. Researchers first placed the female toads in a wine chiller with slightly higher temperatures of 46.4-50 degrees. After a week in the wine chiller,

the toads were moved back into their regular laboratory habitat with 46- to 50-degree water supplied to their ponds from a water cooler.

"Temperature, humidity and altitude may be very important factors hindering our efforts," Calatayud said, but the researchers are learning how to work around these challenges.

After collecting data on the initial hibernation study, the researchers will adjust hormone treatment, hibernation length and other factors to help optimize egg production. They are also working on timing the production of eggs and sperm to create ideal conditions for in vitro fertilization.

Scott Willard, head of the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, said the MSU study is funded through the Memphis Zoo as part of a larger Institute for Museum and Library Services grant.

"This work is important because amphibians are sentinel species," Willard said. "They are indicators of ecosystem health, which is important to all of us. While this project is centered on the captive propagation of endangered amphibians and working out the kinks of how to do this, there are larger implications to the work in an ecological context regionally and nationally."

Provided by Mississippi State University

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