

Lizards need their coffee, too: Could caffeine help bring threatened species back from the brink?

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In an article just published in *Conservation Physiology*, Macquarie University's Simon Clulow and colleagues reveal a new sperm freezing



and revival technique that shows considerable promise—and involves a surprising ingredient.

The group took <u>sperm</u> from a <u>number</u> of Yellow Spotted Monitors—a <u>giant lizard</u> or "goanna" species that has suffered severe population declines in recent years as cane toads moved into their habitat. For a large lizard, a toad makes a tasty—and lethal—treat. With populations crashing by as much as 97 percent, with huge knock-on effects throughout its ecosystem, the Yellow Spotted Monitor is in urgent need of help.

The researchers set out to discover which freezing technique works best, and which was the best protocol for getting frozen sperm moving again after thawing it out. They found that one common cryoprotectant, dimethyl sulfoxide or DMSO for short, worked well. "We were excited to find that we could use a relatively simple cryoprotectant to prevent the sperm from being ripped apart by ice crystals during the freezing process" said Clulow. "It was a promising start. But as with a small number of previous attempts to freeze <u>reptile</u> sperm, we weren't able to retrieve large numbers of mobile, swimming sperm post-thaw."

The team decided they needed to try a technique not used with lizards before if they were to make a breakthrough in retrieving greater numbers of motile lizard sperm. This method had increased movement in fresh sperm samples of mammals and birds, but had never been applied to reptile sperm after freezing. "We added caffeine to stimulate the sperm when we thawed them out" explains Lachlan Campbell, Ph.D. student and first author of the study. "To our delight, we saw a huge increase in the number of moving sperm after freezing and thawing. It turns out, frozen lizard sperm need their morning coffee to get started just like us!"

After a series of experiments to perfect the protocol, the team found



they were able to retrieve nearly half the sperm cells they froze, producing the greatest recovery of motile sperm reported for any reptile species.

This technique offers a promising new path for reptile conservation worldwide, and particularly for Australian <u>lizards</u>. It provides new opportunities to build up the Kimberley Ark gene bank—an ambitious conservation insurance project established by Clulow and colleague Dr. Sean Doody at the University of South Florida. The project aims to restore genetic diversity after the sadly inevitable wildlife devastation caused by the westward march of cane toads across Northern Australia. "We were ecstatic with the outcomes of this study!" said Clulow. Doody agrees. "It's a large step in the right direction to protecting the <u>genetic</u> <u>diversity</u> of northern Australia's unique reptile predators."

More information: Lachlan Campbell et al. A model protocol for the cryopreservation and recovery of motile lizard sperm using the phosphodiesterase inhibitor caffeine, *Conservation Physiology* (2020). DOI: 10.1093/conphys/coaa044

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