Is Sperm Stored over Winter in Juveniles? A Study of *Crotaphytus collaris* Reproduction

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Abstract: Females of many reptiles, including several species of lizards, are known to be able to store sperm over long periods of time for later use in fertilization of their eggs. This is especially true for species found in areas of low population density where encounters with members of the opposite sex may be few and far between. While Collared lizards (*Crotaphytus collaris*) are not found in areas of low population density, both adults and juveniles participate in pair bonding where Juvenile females bond with a juvenile male in the fall and are then part of that male's harem in the spring mating season. In addition to this, some larger juvenile males have been found to produce sperm near the end of their first fall. This raises the question of whether or not it is possible that juvenile males and females mate during the fall and if so whether or not females are able to store this sperm over winter to fertilize their eggs in the spring. To test this question, we captured juvenile females in the fall and induced them into a simulated brumation in the laboratory over the winter. In the spring, when outside temperatures began to warm up, we began bringing them out of brumation. Now, we will place males with half of the captured females as a control for laboratory conditions and leave the other half of the captured females separated from any males. The production of viable or non-viable eggs from the females without access to males in the spring will determine the results of our study.

Keywords: Collared Lizards, Brumation, Over-Winter Sperm Storage

Introduction

Collared lizards (*Crotaphytus collaris*) are mid-sized lizards that show sexual dimorphism in both coloration and body size (Fitch 1956). Males are typically green or orange in coloration with orange or yellow heads and orange dorsal spots, bars, or speckled patches across much of their dorsum; females are not as colorful but develop orange spots or bars during the mating season (Linsted 2000). Both males and females have black bands around their necks (Linsted 2000). Collared lizards occur throughout Oklahoma along rocky slopes and outcroppings often near rivers, lakes, or streams (Webb 1970). Young are hatched in late July and early August and enter brumation, winter dormancy in reptiles where metabolic rates are reduced, in mid to late October (Sexton 1992; Mayhew 1965). All ages and sexes emerge from brumation around late March and early April (Sexton 1992). Mating then occurs (Trauth 1979), and females lay their eggs in late June and early to mid-July with 2-21 eggs per clutch (Sexton 1992, Porter 1972). As adults, female collared lizards bond with males and become part of their harem during the mating season (Landers et al. 2016). A similar behavior is seen in juvenile lizards where females tend to bond with males before brumation; this behavior is thought to be a form of precocial sexual selection that gives an added mating advantage during the next breeding season (Landers et al. 2016). While no one has observed mating between juvenile males and females prior to the spring mating season, some large juvenile males have been found to produce sperm in

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late October before entering brumation (Landers et al. 2016).

In our study, we want to discover if mating occurs between juveniles before brumation, and if mating occurs whether or not females are able to store the sperm over winter for fertilization of their eggs in the spring. Females of many reptilian species have the ability to store sperm for large periods of time between mating and fertilization (Sever and Hamlett 2002). In fact, several species of sea turtles are well-known for this ability and can store sperm for years before fertilizing and laying their eggs (Sever and Hamlett 2002). Some species of lizards–including *Anolis carolinensis, Microsauria pumila, Chameleo ellioti, Holbrookia propinqua,* and *Conolophus subcristatus* –exhibit this trait as well (Birkhead 1993). Seminal receptacles, otherwise known as sperm storage tubules, are small tubes that connect to the oviducts of many reptiles and are thought to be places that sperm can be stored for later use in fertilization (Sever and Hamlett 2002, Cuellar 1966a). Cuellar (1966a) found that females of the species *Uta stansburiana* have seven seminal receptacles. Cuellar (1966b) also found that females of this species were able to store sperm for use in fertilization of their eggs for a period of 81 days or longer. Female collared lizards have at least six of these seminal receptacles (Cuellar 1966a), so it may be possible that adult females are capable of storing sperm over the summer for fertilization of multiple clutches, and possibly even over the winter for use the following spring. We believe, however, that it is unlikely that juvenile collared lizards mate before brumation and store sperm over the winter because collared lizards do not live in areas of low population density, fall copulation between juvenile lizards has never been observed, and males have been found to have the greatest levels of sperm production during the same time in the spring and early summer that ovulation occurs in the reproductive cycle of females. (Trauth 1979).

Methods

To begin our study, we went to the field in late October and captured both male and female juvenile collared lizards from several locations (Glass Mountains and shore of Sooner lake on OG&E property) in Oklahoma using a cane pole and noose. We then took the measurements of snout vent length (mm), total body length (mm), and mass (grams) of each lizard. In addition to these measurements, we took cloacal smear samples from every male using the process described by Landers et al. (2016). The cloacal smears allowed us to further tell if some of the juvenile males in the areas that we captured females had fully developed sperm at this time. Once we had finished our measurements, we released the males back where we found them and took the 12 females back to our laboratory to keep over winter. Keeping the females over winter insures that only our control group will mate with males in the spring. We placed the 12 females that we brought back to the laboratory in glass aquariums with gravel substrate and larger stacked rocks that the lizards could hide under to feel more protected. The lizards were fed and their water was refreshed daily until we stopped feeding a few days before we induced brumation. We stopped feeding them prior to inducing brumation to simulate natural behavior and insure that the lizards' digestive systems were emptied once brumation was induced because decaying

food in the digestive system can cause illness (De Vosjoli 2012). We then induced brumation by shortening the amount of light the lizards received and lowering the room temperature a few degrees every few days. Once we could not lower the temperature of the room farther, we transferred the lizards into a cold temperature incubator. We continued to drop the temperature a few degrees and the light cycle a few minutes every couple of days until we reached 10ºC and total darkness. Simulating brumation helps to most closely mimic the behavior of collared lizards in the wild. When our target temperature of 10ºC and total darkness were reached, we stopped providing water or food and checked on the lizards once a week over the winter months. This allowed us to make sure all the lizards were still alive and remove any lizards that may have died. Then in the spring, we reversed our previous steps to bring the females back out of brumation. One lizard expired over the winter months and another died shortly after bringing the lizards out of brumation, cutting our number of total females down to 10. Once the lizards were returned to room temperature they were returned to the glass tanks and we resumed daily feeding and care. Then, for unknown reasons, we began to experience much more mortality, subjects that were eating and appeared healthy suddenly grew weak and died within 24 hours. Some had eyes sealed shut. One subject was examined by an OSU veterinarian and she recommended euthanasia since the subject had become very weak and moribund. We are awaiting necropsy results. Our sample size fell to three healthy females.

The following describes the methods we intended to employ with a larger sample size. Following that description is an explanation of what we will now do with just a sample size of three.

Everything to this point has taken place from October of 2016 to April of 2017. The rest of our study will take place from May of 2017 to July of 2017. The females will be split into two groups, one to serve as the control and one to serve as the experimental group. We will place males in with our control group and no males in with our experimental group. Putting males in with the control group allows the females to mate like they normally would in their natural setting and controls for the artificial conditions of laboratory captivity. Having no males in with our control group ensures that the last time they had the possibility to mate was in the fall. Then, once the females lay their eggs in June, we will collect them and record which group and lizard each egg came from before placing them in an incubator. After collecting the eggs, we will release both the males and females back at the sites where we originally captured them. We will incubate any eggs produced by both groups until it is apparent that all the eggs that were going to hatch have done so. This should allow us to determine if juvenile female collared lizards are indeed able to store sperm over the winter and use them to fertilize eggs that remain viable and hatch.

If any of the eggs laid by our experimental group, the one without access to a male in the mating season, yield hatchlings, then that would strongly indicate that juvenile female collared lizards can store sperm over winter since the last opportunity for these females to mate would have been in October. This outcome would not support our hypothesis, but would further increase our understanding of collared lizard mating and reproductive behavior. The other possible outcome of our

study would be that the eggs of our experimental group produce no hatchlings. While not as interesting as the first possible outcome, this would support our hypothesis that juvenile female collared lizards do not store sperm over the winter.

However, because our sample size was reduced to only three lizards due to heavy post-brumation mortality, we will not have a control group and instead simply determine if the three females without exposure to males in the spring lay eggs, and if so, if the eggs are fertile and viable and if hatchlings actually appear.

Expected Results

We induced the females we captured into brumation from November of 2016 to April of 2017 to simulate their natural behavior as closely as possible. Because we kept them in brumation for the same amount of time that they would normally brumate in the wild, our project is still ongoing. We expect that the females without access to males in the spring will not produce viable eggs and therefore not produce offspring, while those given access to males in the spring will produce viable eggs and offspring. We believe this because males are common enough that females should have no trouble finding a mate in the spring, and males and females both reach their peak reproductive ability during the same period in the spring.

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Literature Cited

Birkhead, T.R., and A.P. Møller. 1993. Sexual selection and the temporal separation ofreproductive events: sperm storage data from reptiles, birds and mammals. Biological Journal of the Linnean Society 50: 295-311.

Cuellar, O. 1966b. Oviducal anatomy and sperm storage structures in lizards. Journal of Morphology 119: 7-19.

Cuellar, O. 1966a. Delayed fertilization in the lizard *Uta stansburiana*. Copeia: 549-552.

De Vosjoli, P. 2012. The lizard keeper's handbook. i5 Publishing.

Fitch, H.S. 1956. An ecological study of the collared lizard (*Crotaphytus collaris*). University of Kansas.

Landers J., J. Wiggins, J. Agan, S. Fox. 2016. Determining sexual maturity of *Crotaphytus collaris*, Research poster presented at Oklahoma State University 2016 Research Week.

Linsted, M. 2000. "*Crotaphytus collaris*" (On-line), Animal Diversity Web. Accessed November 15, 2016 at http://animaldiversity.org/accounts/*Crotaphytus collaris*

Mayhew, W.W. 1965. Hibernation in the horned lizard, *Phrynosoma m'calli*. Comparative Biochemistry and Physiology 16: 103-119.

Porter, K.R. 1972. Herpetology. W. B. Saunders Company, Philadelphia, Pennsylvania

Sever, D.M., and W.C. Hamlett. 2002. Female sperm storage in reptiles. Journal of Experimental Zoology 292: 187-199

Sexton, O.J., R.M. Andrews, and J.E. Bramble. 1992. Size and growth rate characteristics of a peripheral population of *Crotaphytus collaris* (Sauria: Crotaphytidae). Copeia: 968-980.

Trauth, S.E. 1979. Testicular cycle and timing of reproduction in the collared lizard (*Crotaphytus collaris*) in Arkansas. Herpetologica:184-192.

Webb, R.G. 1970. Reptiles of Oklahoma. University of Oklahoma Press, Norman, Oklahoma.