2008 Grant Follow-up Report

Trailing of maternal chemical cues by neonate Timber Rattlesnakes, *Crotalus horridus*

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A life history can be thought of as a set of genetically based rules that, through interaction with the environment, determine the allocation of time to competing behaviors, energy to competing physiological functions, and the packaging of reproductive effort into individual offspring in order to maximize lifetime reproductive success (Dunham et al., 1989). The packaging of reproductive effort is based on “trade-offs” between energy expended on current reproduction and future reproductive success (Shine, 1980). Trade offs in the allocation of reproduction energy affect such parameters as clutch size, reproductive effort, reproductive mode, reproductive frequency, and lifetime clutch production in a species (Shine, 1980). Parental care is one way to maximize reproductive value for the present reproductive event, but it comes at a cost. Parental care is associated with increased adult mortality and a consequent reduction in future offspring production (Winkler, 1987).

In many reptile species, parental care is limited to locating a suitable nest for oviposition, the laying of eggs, because the implications of choosing a good nest site can be the difference between successful hatching and mortality (Blouin-Demers et al., 2004). Maternal nest site selection can also affect the phenotype of the offspring (Shine and Harlow, 1996). In Australian skinks (*Bassiana duperreyi*) the mother tends to select nest sites with temperatures that are better suited to develop more fit offspring (Shine and Harlow, 1996). The evolution of viviparity, the retention of offspring to full-term, can be interpreted as a form of parental care. Even a short retention of the eggs in the female body cavity increases the rate of survival (Shine, 1985). Some lizards, such as *Anolis aeneus*, have been found to retain shelled oviducal eggs during periods of drought (Stamps, 1976). Unfortunately, viviparity can be costly for the mother. Lower clutch frequency, increased mortality risks to the mother, higher metabolic costs, and decreased food intake (Tinkle and Gibbons, 1977, Shine 1985) are all costs associated with viviparity. Some of these associated risks can be lessened by the usage of rookeries and communal dens (Graves and Duvall, 1995)**.**

Chemical cues are important aspects in the natural history of many animals. Animals use chemical cues to find food and mates, among other things (Cooper, 1995). Lizards (Cooper, 1995) and snakes are known to use scent trails to locate prey and to find foraging sites with a high frequency of prey activity. Furthermore, viperids such as *Agkistrodon piscivorus,* utilize chemical cues to locate incapacitated prey items after envenomation (Chiszar et al., 1986).

Conspecific, or intraspecific, chemical cues are also important in many aspects of the natural history of snakes. Female garter snakes, *Thamnophis marcianus,* use pheromones to attract males during the breeding season (Ford and O'Bleness, 1986). Brown and Maclean, (1983) found that in communally denning species such as *Crotalus horridus,* chemical trailing may be a important factor in the location of a den by neonates, a necessary factor for offspring survival. Chemical trailing by neonates may indicate that females may "lead" their offspring to the den, which would be a form of parental care that increases offspring survival at a cost to the mother.

The Timber Rattlesnake (*C. horridus*) is a large bodied member of the family Viperidae that ranges from New Hampshire west to Minnesota, south to Northern Florida and Southwest Texas. The total range encompasses 30 U.S. states (Brown, 1993). Timber Rattlesnakes show male larger sexual dimorphism with males reaching a total length of approximately 111 cm and a weight of 900g, while females reach 97cm and 600g (Brown, 1993). *C. horridus* is a communally denning species that is viviparous and utilizes rookeries (communal birthing sites). The dens utilized are typically south facing rocky hillsides, though den site variation does occur depending mostly on latitude (Ernst, 1992). The rookeries are used by pregnant females to regulate the temperature of the developing offspring (Ernst, 1992).

The goal of this research project is to determine if neonate Timber Rattlesnakes use chemosensory searching to follow their mothers from the birthing site to the den site for overwintering. By utilizing radio telemetry I will be able to repeatedly locate females and neonates for determining location using GPS. Once a large enough data set is obtained, I will be able to use GIS software to determine if neonates are following the same path as their mother to reach den sites.

Material and Methods

A mark-recapture study of Timber Rattlesnakes was conducted from 27 April 2008 until 1 November 2008. Snakes of all sizes and sexes were located by actively searching suitable habitat within the field site. At the site of capture we recorded date, time, and location (UTM coordinates using a Garmin

GPS II Plus, Garmin Intl., Olathe, KS). Captured snakes were either processed in the field, or transported to MG 3035 on Truman State University campus for proccessing. We measured snake body mass (± 0.1 g), snout-vent length (SVL), head length (HL), head width (HW), and tail length (TL). All lengths and widths (± 0.1 cm) were measured using a squeezebox (error rate ± 1.0% of total length; Quinn and Jones, 1974). In addition, we recorded sex (by probing for the presence/absence of hemipenes), reproductive condition (by palpation), and stage class. All snakes were individually marked using PIT-tags (passive integrated transponder; Avid, Inc.). PIT tags are 11mm x 2.1 mm polymer encased microchips that allow for easy identification of each individual by scanning the area where the chip is injected. All snakes were released at the site of capture as soon as possible, usually within 2 days.

1 adult female, that was suspected to be gravid, and one post-parturitive female were surgically implanted with temperature sensitive radio transmitters (model SB-2T; Holohil Systems Ltd.) following a modified version of the protocol of Reinert and Cundall (1982). Once a female was implanted with the transmitter, she was tracked approximately every other day.

Upon parturition, neonates were collected for initial measurement, including sex, mass (g), and snout- vent length (SVL), and housed communally with their littermates and mother at MG 3035 until the first ecdysis (approx. 1 week after birth). Radio transmitters (Holohil BD-2HX, 1.65g) were then glued to 6 neonates (3 from each litter), using cyanoacrylic glue (superglue), externally on the right dorsolateral surface, approximately 70% of the SVL to the tail (Cobb et al. 2005). The neonates and mother were tracked approximately every other day until they entered the hibernacula (den).

Results

Demographics- In total, 26 timber rattlesnakes were captured between 27 April 2008 and 30 August 2008. 2 litters were located with an adult female present (presumably the mother) totaling 15 neonates (litter 1 [21-Aug-08] n = 7 [4:3]; litter 2 [30-Aug-08] n = 8 [7:1]). The other 9 captured individuals consisted of: 4 one year-olds (0:4), 3 juveniles of unspecified age (2:1), and 2 mature adults (1:1).

Radio-tracking- The presumed mother of litter 1 was captured, with litter, on 21 August 2008 and implanted with a radio transmitter. Transmitters were glued onto 3 of the neonates in litter 1 and were released with the adult female and littermates at the site of capture (and birth). One of the neonates in this litter lost its transmitter in < 5 days of release, but the other two were able to be tracked until ingress into the hibernaculum. The 2 tracked neonates entered the same hole as the female at ingress and were found within 1m of the adult female on several tracking days prior to ingress.

The presumed mother of litter 2 was captured on 8 July 2008 and, due to the presence of 8+ follicles (located by palpation), implanted with a radio transmitter, and released at the site of capture. This individual was tracked until it was found with a litter on 30 August 2008 and transported, with the neonates, to Truman State University for holding. Transmitters were glued onto 3 of the neonates, who were then released at the site of capture with the female. All 3 of the snakes lost transmitters in < 5 days of release and were therefore unable to be tracked. The female was tracked until ingress at a different location than the female and 2 neonates from litter 1.

Discussion

Demographics- Of the 26 marked individuals, only two of them were relocated without the aid of radio telemetry, 1 juvenile male and the female from litter 1. Unfortunately, this is too small of a data set to determine population size at the site. An assessment of population size would also be confounded by the relatively short time of search pressure on the neonate rattlesnakes, which make up 58% of the recorded population, as compared to the snakes found at the beginning of the season (both recaptured snakes were first captured within the first 2 weeks of field work at the site).

Radio tracking- Although the use of external radio transmitters is a relatively new technique, it has been successfully utilized in several studies on juvenile snake movement and behavior (Cobb et al., 2005; Reinert, 2005; Jellen and Kowalski, 2007; Figueroa et al., 2008). The main problem that we encountered was that 4 out of 6 of the radio transmitters fell off of the neonates. Due to the short window of time between parturition and ingress, we essentially lost over half of our tracking data set due to this. To minimize potential transmitter loss in future seasons of this research, more attention will be devoted to ensure that the transmitters are more securely attached to the snakes before release.

The 2 neonates that we did follow provided some interesting preliminary data. Studies by Cobb et al. (2005) and Jellen and Kowalski (2007) indicated that neonate rattlesnakes may use pheromone trailing to follow the mother to the den site as well as make coordinated movements with siblings. The selection of the 2 neonates to den at the same hole as their mother, as opposed to denning elsewhere, could indicate similar behavior.

Future directions- Due to the relatively small amount of information that is known about the movement and behavior of juvenile rattlesnakes, and juvenile snakes in general, as well as the positive preliminary data collected, it is apparent that more work needs to be done on this topic and that this project should be continued at Truman State University.

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