

Snake Mortality Associated with Late Season Radio-transmitter Implantation

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Radio-telemetry is an increasingly used procedure to obtain data on the biology of free-living snakes (Reinert 1992, 1994). In Texas and Louisiana we have been using the surgical technique of Weatherhead and Anderka (1984) to implant transmitters in timber rattlesnakes (*Crotalus horridus*) and Louisiana pine snakes (*Pituophis melanoleucus ruthveni*) to obtain information on biology and habitat preferences. In New Jersey transmitters were surgically implanted following the procedure described by Reinert and Cundall (1982), with recent improvements and modifications (Reinert 1992), to investigate the biology and habitat preference of timber rattlesnakes (Reinert and Zappalorti 1988a, 1988b) and northern pine snakes (*P. m. melanoleucus*) (Burger and Zappalorti 1988, 1989, 1991). During the course of this research we have implanted SI-2T transmitters (Holohil Systems Ltd.) in 30 *C. horridus* and 28 *P. m. ruthveni*; and SM-1 transmitters (AVM Instrument Co.) and LF-1 transmitters (L. L. Electronics, Inc.) in 37 *P. m. melanoleucus*, 28 *C. horridus*, and several in other snake species.

Snakes were captured, returned to the laboratory, and surgically implanted with transmitters using the two techniques cited above. In Texas and Louisiana all transmitters were implanted subcutaneously. In New Jersey, all transmitters were implanted in the coelomic cavity (Reinert and Cundall 1982). Snakes were maintained in the laboratory for 2–15 days, with access to a heat source to facilitate healing (Smith et al. 1988), prior to release. Snakes received replacement transmitters at approximately 6–18 month intervals and were entered in the data set repeatedly for each surgery.

In New Jersey, to avoid poor healing or infection during hibernation (Reinert, pers. comm.), snakes rarely were implanted with transmitters after September 1, unless there was a specific need or important reason to do so, e.g., discover an unknown hibernation site, monitor winter body-temperature, or to prevent the loss of a long-term study specimen. In Texas and Louisiana, as research progressed, we suspected that elevated mortality and behavioral anomalies might be occurring in snakes that received implants 1–2 months prior to hibernation in the fall. Observations after release suggested that snakes receiving transmitters in the fall suffered higher mortality during subsequent hibernation than snakes receiving transmitters during spring and summer. In addition, snakes undergoing fall surgeries appeared to exhibit a greater tendency to be present on the surface during the subsequent winter than snakes operated on in the preceding spring and summer.

We tested these hypotheses using Texas data accumulated over a four-year period between 1993 and 1997. A total of 39 rattle-snake surgeries were performed. Three snakes were deleted from the data set because of causes of death unrelated to surgery (two

killed on roads and one died of a pre-existing fungal disease). Five individuals were lost after 1–6 months because of unknown causes and also were deleted. Of the remaining 31 surgeries, 21 were performed between 1 February and 31 August (early surgeries) and 10 were performed between 1 September and 31 October (late surgeries). Six snakes died following surgery and release, 2 of 21 following early surgeries, and 4 of 10 following late surgeries. This difference is significant ($\chi^2 = 4.03$, $P = 0.045$).

Also, we examined the influence of surgery date on timber rattle-snake behavior between a sample of snakes subjected to early surgery, and those subjected to late surgery, specifically the occurrence of the snakes above or below ground during the primary hibernation period (Dec.–Feb.). For this three-month period, snakes subjected to early surgery (N = 19) were on the surface near their hibernacula during only 8 of 74 relocations (10.8%). Of the six snakes subjected to late surgery, 10 of 24 relocations (41.7%), including observations of all six individuals, were of snakes on the surface. This difference is highly significant ($\chi^2 = 11.51$, $P = 0.001$). Of the four snakes that ultimately died during the hibernation period following late surgery, an even higher percentage of relocations were of snakes on the surface (71.4%).

Similar results were obtained for Louisiana pine snakes. A total of 37 surgeries were performed. Eight were deleted from the data set for reasons similar to those for rattlesnakes (three killed on roads, five lost). Of the remaining 29, 25 were early surgeries and 4 were late surgeries. A total of 8 snakes died following surgery and release, 4 of 25 after early surgeries and 4 of 4 after late surgeries. This difference is highly significant ($\chi^2 = 12.18$, $P = 0.001$).

Limited data for pine snakes also are available in relation to surface activity during Dec.–Feb. For the three-month period, snakes subjected to early surgeries were on the surface near their hibernacula during 2 of 37 relocations (5.4%). Snakes subjected to late surgeries were on the surface during 4 of 5 relocations (80%) prior to their deaths. This difference is highly significant ($\chi^2 = 20.02$, $P = 0.001$).

Although the sample sizes are small due to our discontinuation of late surgeries, the differences are unequivocal. Snakes receiving surgically implanted transmitters in the fall experience a significantly higher mortality rate than snakes implanted earlier in the season. They also spend significantly more time on the surface during the subsequent winter. We suggest that individuals subjected to late surgeries that spend more time on the surface during the subsequent winter may be attempting to thermoregulate in an effort to hasten healing of their surgical wounds. Individuals operated on earlier in the season have had more time to heal prior to hibernation and do not exhibit thermoregulatory behavior to the same extent.

How the above scenario translates to higher mortality rates is open to speculation. Several additional observations are perhaps relevant. Of the eight snakes of both species that died following late surgeries, all died within 1–4 months after surgery, in all cases prior to the end of the winter hibernation period for these populations. Of the eight snakes in question, five were observed 7–16 days prior to being found dead. All five were in apparent good health and showed no obvious signs of infection. When verified as dead, two were isolated transmitters, four were skeletal remains and transmitters, one a badly decayed carcass, and one an extremely fresh carcass with no indication of cause of death on necropsy. This last individual was found mid-morning following a night of sub-freezing temperatures. All eight transmitters or remains were on the surface within 5 m of the snake's hibernaculum. We suspect that all eight snakes died on the surface. The

limited information available suggests that predation, and perhaps hypothermia, contributed to cause of death.

If the snakes were more vulnerable to predation while on the surface at low temperature, and this was a factor in the above results, the differences between species may be relevant. Following late surgeries, rattlesnakes survived at a higher rate (6 of 10) than pine snakes (0 of 4). It is possible that even when exposed at low temperatures, the larger and venomous rattlesnakes are better able to deter avian and mammalian predators.

Similar results were observed in New Jersey. A total of 75 early surgeries (April–August) and 9 late surgeries (September–October) were performed on six species of snakes, primarily timber rattlesnakes and northern pine snakes. A total of eight snakes died, four following early surgery (5%) and four following late surgery (44%). This difference is highly significant, $\chi^2 = 14.26$, $P < 0.001$). As observed in Texas, snakes that received late implants (September–October) spent more time basking and remained on the surface longer than those that did not (Zappalorti et al. 1983; Zappalorti and Burger 1985). Additionally, in New Jersey late implant timber rattlesnakes ($N = 3$) and northern pine snakes ($N = 3$) emerged sooner in the spring and spent more time basking than snakes receiving early implants.

These results strongly suggest that researchers should refrain from surgical procedures and release of snakes immediately prior to hibernation if possible. These data also suggest that effects of surgical procedures can alter snake behavior, apparently for several months in some situations, following relatively minor surgery. The implications for conclusions based on resulting data are obvious.

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A Simple Stomach Flushing Method for Small Frogs

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Amphibian dietary studies conventionally employ dissection of fixed animals. This kind of study requires large samples, sometimes difficult to justify in light of amphibian population declines (Wake 1991). Another drawback is the destruction of specimens that could otherwise be useful for other purposes. Moreover, destructive sampling may interfere with population studies by affecting density-dependent processes.

Non-destructive techniques have been used in a variety of dietary studies on large anurans including direct observation (Murphy 1976), stomach eversion, and stomach flushing (Gittins 1987; Legler and Sullivan 1979; McAlpine and Dilworth 1989). These methods, when carried out properly, cause low or no mortality, do not disrupt population processes, and allow individuals to be sampled repeatedly over time. However, dietary studies of small frogs still rely on the collection and dissection of animals (Lima and Moreira 1993; Vitt and Caldwell 1994).

In this report I describe an adaptation of the stomach flushing method for use in small frogs. The method was used to study the diet of the small leptodactylid frog *Hylodes asper* (Müller), an endemic species of the Atlantic forest of southeast Brazil. The range of snout-urostyle length (SUL) of postmetamorphic individuals of this species varies from 20 to 47 mm.

After capture, frogs that were too strong to immobilize with one hand (usually animals >35 mm SUL), were anesthetized for a few seconds in a plastic bag containing cotton soaked with chloroform. In preliminary tests of the procedure, three frogs died after being exposed to chloroform for more than 20 seconds; 10 seconds of exposure seems to be a safe limit. Small individuals were not anesthetized to avoid unnecessary trauma. I used chloroform instead of liquid (Leclerc and Courtois 1993) or injected anesthetic (Letcher 1992) due to the faster recovery rate and because no anesthetic remains on the frog's body.

Anesthetization prevented frogs from using their hands to remove the flushing tubes from their mouths. After anesthetization I introduced a thin flexible silicone tube (Sigma Equipment: 1