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RH: Telemetry effects on snakes • Weatherhead and Blouin-Demers

### **Long-term effects of radiotelemetry on black ratsnakes**

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**Abstract:** We assessed the effect of radiotelemetry on growth, reproduction, and survival of black ratsnakes (*Elaphe obsoleta*) using data from a 6-year study.

Transmitters were surgically implanted for more than 1 year and were less than 2.5% of the snakes' mass and 3.8% of the snakes' length. Compared to snakes

without transmitters, snakes with transmitters exhibited lower annual growth in mass but not length, and females produced lighter clutches of eggs relative to their body size. Although results were equivocal, radiotelemetry also may have adversely affected survival. Potential explanations for the negative effect of radiotelemetry include direct effects of transmitters on snakes (impaired behavior, cost of transportation, infection) and indirect effects (increased disturbance by researchers). Because we conducted the study at the northern limit of the species' distribution, snakes in this population may be particularly sensitive to the negative effects of radiotelemetry. Despite the apparent costs, we believe that use of radiotelemetry is still warranted because of the benefits realized.

**Key words:** black ratsnakes, *Elaphe obsoleta*, growth, radiotelemetry, radiotransmitter, reproduction, survival

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Radiotelemetry has allowed important advances in our understanding of the behavior and ecology of wild animals. Whereas radiotelemetry has enhanced the study of relatively easily observed animals such as birds, it has arguably revolutionized the study of more secretive animals such as snakes. As with any technology employed in wildlife research, it is important that the data obtained from radiotelemetry accurately reflect the natural state of the study species, rather

than being an artifact of that technology. Furthermore, when radiotelemetry is used to study species of conservation concern, it is particularly important that any negative effects on the study animals arising from use of the technology be minimal. Despite current widespread use of radiotelemetry in field studies of snakes, we are unaware of any systematic analysis of possible long-term effects of transmitters on snakes. Even the few attempts to assess short-term effects of radiotelemetry have been narrow in scope and limited in sample size (Lutterschmidt and Reinert 1990, Charland 1991, Graves and Duvall 1993, Lutterschmidt and Rayburn 1993). In this paper we assess whether radiotelemetry affects growth, reproduction, or survival of black ratsnakes (*Elaphe obsoleta*).

A formal test of the hypothesis that telemetry does not alter study animals is impossible for some aspects of behavior (e.g., movements) because it would require collecting data from a control sample of animals studied without the benefit of telemetry. Other variables of ecological interest, however, can be compared for animals with and without transmitters. Birds have been studied most in this regard and evidence for transmitter effects is mixed. For example, Gessaman and Nagy (1988) found that carrying a transmitter and harness increased flight times by as much as 31% and metabolic costs of flight by as much as 100% for homing pigeons (*Columbia livia*). In contrast, other studies have found no short-term effect of transmitters (e.g., Neudorf and Pitcher 1997). Studies investigating long-term effects (e.g., reproduction, survival) of transmitters on

birds have also produced mixed results, with some finding no effect (e.g., Sodhi et al. 1991, Powell et al. 1998) and others clear negative effects (e.g., Foster et al. 1992). Unlike birds, snakes do not have to contend with the aerodynamic consequences of carrying a transmitter, and the cost of transporting the additional mass of the transmitter should also be much lower for snakes. Thus, the prediction we test here is that transmitters should not have a detectable negative effect on growth, reproduction, or survival of black ratsnakes.

Black ratsnakes are oviparous colubrids. Males reach maximum snout-vent lengths (SVL) of approximately 1500 mm and females 1400 mm. Demographic analysis of the same population studied here indicates that individuals can live as long as 30 years (Blouin-Demers et al. 2002). Black ratsnakes are excellent climbers that prey on a wide variety of birds' eggs and nestlings (e.g., Fitch 1963, Stickel et al. 1980). As the species' name suggests, however, black ratsnakes also prey extensively on small mammals (Fitch 1963; Stickel et al. 1980, Weatherhead et al. 2003), which they kill by constriction.

## **Methods**

### **Field and laboratory methods**

From 1996 through 2001, we used radiotelemetry to study various aspects of the thermal ecology of black ratsnakes at the Queen's University Biological Station in eastern Ontario, Canada (e.g., Blouin-Demers and Weatherhead 2001).

Our transmitters (model SI-2T, Holohil Systems, Inc.) were originally developed for research on black ratsnakes (Weatherhead and Anderka 1984), but are now widely used by researchers studying a variety of snake species (F. W. Anderka, Holohil Systems, Inc., personal communication). The cylindrical transmitters measured 40 x 11 mm (excluding the antenna), weighed 8.6 g and provided an expected battery life of 2 years. Transmitters never exceeded 2.5% of the mass of any snake at implantation or 3.8% of its SVL.

We implanted transmitters using a modification of the surgical technique of Reinert and Cundall (1982), a method commonly used in radio-telemetry studies of snakes. Using sterile techniques, we implanted a radio-transmitter in the body cavity of the snake, suturing the transmitter to a rib or inter-costal muscle to prevent its migration inside the coelom. We left the flexible antennae extended, lying between the epidermis and outer body wall. We anesthetized snakes using isoflurane gas delivered via a precision vaporizer (Blouin-Demers et al. 2000). Following surgery we injected sterile fluids (0.9% lactated Ringer's solution at a dosage of 50 ml/kg) into the snake's coelom to promote healing and avoid damage caused by the antibiotic if the snake was dehydrated. We then injected snakes subcutaneously with gentamicin sulfate (reptile dose = 2.5 mg/kg) or enrofloxacin (reptile dose = 10 mg/kg). We gave a second injection before release, 24 hours post-surgery. Snakes were kept in captivity and provided warmth for 3 days following surgery and then released at their point of capture.

We located snakes with transmitters approximately every 48 hours throughout the active season (late April to mid October). When a snake was visible, we marked its location with flagging tape (usually within approximately 1.5 m of the snake) to allow later quantification of habitat variables after the snake had moved. We also marked locations of snakes that we could not see (e.g., under ground or cover, in a tree) as close as possible to their presumed position. We excluded from this study snakes implanted with transmitters that we fed as part of a thermoregulation study. However, we included snakes with transmitters that we captured occasionally during the active season. For example, some individuals were used in thermal preference trials requiring 1 week in captivity (Blouin-Demers and Weatherhead 2001). Also, in mid-June we captured each female that had a transmitter and palpated the females to determine their reproductive status. Non-gravid individuals were not captured again that season. Females determined to be gravid were subsequently captured and palpated (to determine whether they had laid their eggs) each time we located them and they were visible. This resulted in gravid females being captured 5-8 times within the active season.

When we located gravid females at nests we brought them into the laboratory. Females were housed individually and provided with moist peat moss in which to lay, which usually occurred within a few days. Fresh clutches were weighed using an electronic scale and then incubated as part of another study. Females were released at their point of capture. Because ratsnakes nest

communally, we regularly captured gravid females without transmitters at nests used by females we were tracking. By also bringing those individuals into the laboratory to lay, we obtained clutch mass data for comparison with values from radio-tracked females.

We conducted a mark-recapture study concurrently with the radio-telemetry study. We captured snakes at hibernacula during spring emergence and opportunistically during the active season. We marked all snakes individually with passive integrated transponder (PIT) tags and determined sex by probing their cloacae for hemipenes. At each capture we measured SVL by running a flexible measuring tape along the snake's venter and weighed snakes with spring scales (transmitter mass was deducted for implanted snakes). All field and laboratory methods were approved by the Carleton University Animal Care Committee (#B97-01) and the University of Illinois Institutional Animal Care and Use Committee (#01240).

### **Data analysis**

*Growth.* We generated annual growth increments for both mass (in g) and SVL (in mm) using only spring captures at hibernacula. This ensured that snakes had empty guts when weighed and that mass increments represented body mass. We used annual growth increments because growth varies among years and we wanted to use year of growth as a control variable in our analyses. For snakes with transmitters this meant that growth was determined for a year in which the

snake carried the transmitter for the entire year, having had the transmitter implanted the previous year. For snakes without transmitters, we included only mature individuals ( $\geq 1050$  mm SVL; Blouin-Demers et al. 2002) in the analyses because these individuals were the focus of our radio-telemetry studies. Each individual was included only once in our analyses, producing annual growth increments for 90 individuals without transmitters and 19 individuals with transmitters.

We analyzed growth data using multivariate analysis of covariance (MANCOVA). Dependent variables were annual SVL increment and annual mass increment and the independent variable was whether or not the snake had a transmitter. We included sex, mean SVL (mean of the 2 values used to calculate an individual's annual growth increment) and year as control variables because they affect growth. Because we have detailed those effects elsewhere (Blouin-Demers et al. 2002), we only report results here for the main effect of carrying a transmitter. We did not include interaction terms because a fully factorial design would have produced empty cells because of the number of variables involved. We used MANCOVA instead of 2 separate ANCOVAs because this kept the analysis-wide  $\alpha$  at 0.05 and because multivariate approaches are preferable when independent variables are suspected to influence more than 1 dependent variable (Stevens 1996). We confirmed with box plots and residual plots that dependent variables followed a multivariate normal distribution in each group, that

population covariance matrices for dependent variables were equal, and that the relationship between the dependent variables and the covariate was linear.

*Reproduction.* We calculated relative clutch mass (RCM) using clutch mass expressed as a percentage of the post-oviposition mass of the mother. With individuals represented only once in our analyses, we had RCM data for 36 females without transmitters and 9 females with transmitters. We analyzed RCM data using analysis of covariance (ANCOVA). Our dependent variable was RCM and the independent variable was whether or not the snake had a transmitter. We included maternal SVL as a covariate because RCM varies with size. We confirmed with box plots and residual plots that the dependent variable followed a normal distribution and that the relationship between the dependent variable and covariate was linear.

Because we were testing the hypothesis that carrying a transmitter does not have a detectable negative effect on growth or reproduction of black ratsnakes, we used 1-tailed tests significant at  $\alpha = 0.05$ . All analyses were conducted with JMP version 5 (SAS 2002).

*Survival.* Formal analysis of survival differences between snakes with and without transmitters was not possible because we lacked comparable data for the two groups (tracking snakes allowed us to know when an individual died, a level of precision unavailable for snakes without transmitters). Informally, we were able to compare annual survival estimates of snakes without transmitters from

mark-recapture analyses (Weatherhead et al. 2002) with mortality estimates for snakes with transmitters based on known times of death. We could not use Pollock et al.'s (1989) modified Kaplan-Meier procedure to estimate survivorship from radio-telemetry data because this method was not designed to estimate overall annual survival (or mortality) probability, but to estimate how survival probability varies over time. Moreover, to get good precision with this method, a minimum of 40-50 animals would have to be tagged at all times, far more than were available in our study.

## Results

### Growth

The whole model MANCOVA was significant (Wilk's  $\Lambda = 0.59$ ,  $F_{(14,200)} = 4.30$ ,  $P < 0.001$ ). The multivariate main effect of whether or not the snake had a transmitter was significant in a 1-tailed test ( $F_{(2,100)} = 2.98$ ,  $P = 0.028$ ). Thus, carrying a transmitter negatively affected growth of black ratsnakes. To identify the relative importance of growth in mass versus SVL to the whole model, we conducted univariate  $F$ -tests, adjusting  $\alpha$  to 0.025 because we conducted 2 tests (i.e.,  $0.05/2$ ). In 1-tailed tests, mass increment contributed to the multivariate significance ( $F_{(1,101)} = 4.78$ ,  $P = 0.016$ ) while SVL increment did not ( $F_{(1,101)} = 0.27$ ,  $P = 0.302$ ). Therefore, transmitters negatively affected mass gain, but not SVL gain (Figure 1). The mean annual mass gain of snakes with transmitters (4.6 g) was only 9.6% of the mean annual mass gain of snakes without transmitters (47.9 g).

The power of the test (Cohen 1977) of whether carrying a transmitter affected mass increase was good ( $1 - \beta$  at  $\alpha = 0.05 = 0.58$ ) owing to a medium standardized effect size ( $D = 0.21$ ). Conversely, the power of the test of whether carrying a transmitter affected SVL increase was poor ( $1 - \beta$  at  $\alpha = 0.05 = 0.08$ ), but this was due to a smaller standardized effect size ( $D = 0.05$ ) and not to higher unexplained variance. Therefore, the conclusion that carrying a transmitter affected mass increase, but not SVL increase, appears reasonable.

### **Reproduction**

The whole model ANCOVA for RCM was significant at  $\alpha = 0.05$  ( $R^2 = 0.189$ ,  $F_{(2,42)} = 4.88$ ,  $P = 0.012$ ). The main effect of whether or not the snake had a transmitter was significant in a 1-tailed test ( $F_{(1,42)} = 6.32$ ,  $P = 0.008$ ). Female black ratsnakes with transmitters had RCM 7.2% lower than females without transmitters (31.9% vs. 39.1%, Figure 1). The power of the test (Cohen 1977) was good ( $1 - \beta$  at  $\alpha = 0.05 = 0.69$ ) despite a medium standardized effect size ( $D = 0.34$ ).

### **Survival**

We implanted transmitters in 86 mature black ratsnakes between 1996 and 2001. Because many snakes were followed over multiple years (up to 5), we had data for 150 snake-years. In total, 39 individuals died while being tracked (21 from predation, 12 during hibernation, and 6 roadkills), yielding a crude estimated annual mortality probability of  $39/150 = 0.26$  for radio-implanted snakes. Weatherhead et al. (2002) studied annual variation in survival for black

ratsnakes not implanted with radio-transmitters in the same population. From 1981 to 1998, mean annual mortality probability for all snakes was 0.29, but was 0.22 for mature snakes (i.e., the group most comparable to snakes with transmitters). The upper bound of the 95% confidence interval for the mortality estimate for mature snakes was 0.264. Thus, by this (imperfect) approach, the higher mortality of snakes with transmitters was just within the error estimate of the mortality probability of snakes without transmitters.

### **Discussion**

Biologists employing radiotelemetry in their research on wild animals will always be concerned that telemetry yields reliable data while not causing serious harm to the animals. Most often the only evidence available in this regard is whether the animals appear to behave normally. In our research on black ratsnakes we have observed both male and female snakes with transmitters mating, and as we have demonstrated here, females with transmitters reproduce successfully. Also, both males and females with transmitters grow and appear to survive well. By these subjective criteria, therefore, we did not anticipate finding a negative effect of transmitters on black ratsnakes. Contrary to that expectation, our results indicated that snakes with transmitters gained mass more slowly than snakes without transmitters, despite maintaining comparable rates of growth in SVL. Furthermore, females with transmitters produced lighter clutches relative to their body mass compared to females without transmitters. These negative effects

of transmitters on growth and reproduction may also have reduced survival, although results were equivocal. Collectively our results indicate that radiotelemetry does not prevent black ratsnakes from engaging in all the activities normal for this species, but that transmitters do have some adverse effects on the snakes.

There are several possible proximate explanations for our results, none of them mutually exclusive. First, transmitters could impair feeding, accounting for slower mass growth and reduced RCM of reproductive females. A rigid transmitter in the body cavity and a flexible antenna running under the skin could affect a snake's constricting ability, although the transmitters were always less than 3.8% of a snake's SVL. Second, the cost of transporting transmitters could require energy that would otherwise be allocated to mass gain. Again, the fact that transmitters never exceeded 2.5% of a snake's mass, and the fact that these snakes are relatively inactive (Blouin-Demers and Weatherhead 2002), makes this explanation seem implausible. Third, despite our efforts to maintain sterile conditions during surgery and our application of antibiotics following surgery, it is possible that infections associated with surgery or with transmitters required snakes to allocate resources toward fighting infection rather than toward growth and reproduction. We did not systematically record information on the snakes' health when we removed transmitters. In all cases, however, transmitters had been encapsulated in some connective tissue. In approximately 20% of cases, there

was some evidence of infection, indicated by caseous exudate in the area of the transmitter. Infections appeared pronounced in only 2 cases, both of which appeared to involve some reopening of the incision from the original surgery.

A fourth possible explanation is that the detrimental effect of radiotelemetry was a consequence of disturbance experienced by snakes with transmitters rather than a direct effect of the transmitters. Although snakes without transmitters were captured opportunistically during the active season, on average individual snakes were disturbed less than once each year. By contrast, snakes with transmitters were approached every 48 hours, and just the presence of human activity can depress snake activity (Parent and Weatherhead 2000). Some snakes with transmitters were also brought into captivity for short periods, further disrupting their normal activity. All these explanations could be tested experimentally, although the expense of such an extensive experiment may be prohibitive.

Two general issues arise from our study. How applicable are our results likely to be to other species and locations, and how should these results affect the use of radiotelemetry in future research on snakes? One unique feature of our study that might reduce its general relevance is that our study population of black ratsnakes is at the northern extreme of the species' distribution. Snakes in this population spend more than half the year hibernating, so with such a brief active period, any cost associated with radiotelemetry might be magnified.

Nevertheless, because many threatened animal populations are at distribution extremes, our results should be relevant to such snake species.

Given that the negative effects of radiotelemetry are not unique to our study population, it might be possible to use a life-history approach to predict how those effects will be manifested in other populations and species. In an experiment with juvenile adders (*Vipera berus*), Forsman and Erik (1996) found that somatic growth (SVL) was less sensitive than condition (mass adjusted for SVL) to food limitation, presumably because body size is more important to fitness. The greater effect of telemetry on growth in mass than length that we found is consistent with Forsman and Erik's (1996) results. How different snake species respond to telemetry may also vary with longevity. For example, in the same way that life history should affect whether an organism that is parasitized directs resources into survival versus reproduction (e.g., Forbes 1993), short-lived snakes might respond to transmitters by increasing reproductive effort, while long-lived species may maximize survival. Future tests of telemetry effects on snakes should take into account life history attributes of the study species.

Finally, assessing the consequences of our results for future radiotelemetry studies of snakes involves evaluating both the resulting costs and benefits. Although detectable, the costs appeared not to be substantial. Growth and reproduction were lower, and if there was a survival cost it was slight. In addition, it should be possible to reduce these costs further through measures such

as disturbing snakes with transmitters less, and perhaps increasing the period of post-operative recovery of snakes prior to releasing them. The efficacy of these measures can be explored experimentally. With regard to the benefits derived from using telemetry, in the case of our own research our subjective view is that the benefits have justified the costs. In addition to any basic ecological contributions, our research has substantially informed efforts to conserve black ratsnakes in Canada (Prior and Weatherhead 1997). Knowing that radiotelemetry can negatively affect snakes will require that costs and benefits of any proposed study be evaluated. Meanwhile, research designed to identify both the costs of radiotelemetry and ways to minimize those costs will be valuable.

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**Figure heading**

Figure 1. Annual growth in snout-vent length (SVL) and mass of male and female black ratsnakes, and relative clutch mass (RCM) of female black ratsnakes with and without radio-transmitters. Plotted values and corresponding scale for the y-axis are least square mean increments ( $\pm 1$  SE) of SVL (mm) and mass (g), and least square mean clutch mass expressed as a % of maternal mass.