# Conservation Biology of Black Rat Snakes: Ecological, Demographic, and Genetic Approaches

by

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# Abstract

My primary goal in this thesis was to investigate ecological, demographic, and genetic aspects of the conservation biology of black rat snakes (*Elaphe obsoleta obsoleta*) in Ontario (Canada). In the first chapter, I quantify the surface features of hibernacula to determine whether new hibernacula could be found by searching for characteristic habitat. Rocky, south-facing slopes typify hibernacula habitat. However, the ubiquity of such features in the landscape precludes their use as keys for locating new sites. Thus, I conclude that subterranean structure and micro-climatic characteristics probably determine the suitability of sites for hibernation and that radio-telemetry remains the best method of finding new hibernacula.

In the second chapter, I detail the demography (e.g., growth rate, size-composition) of rat snakes in Ontario using multi-year, capture-recapture data. The study population exhibits slow growth and delayed maturation, traits that would stall recovery of populations following decline. Geographically separate populations within the study area display temporally-correlated patterns of abundance and survivorship, but contrasting trends in the proportion of adults. Methodological implications of these data are that unbiased estimates of population composition are best obtained by sampling hibernacula populations, although juvenile snakes are underrepresented in hibernacula. Collectively, these results suggest that populations should be monitored at multiple hibernacula and on relatively fine temporal schedules.

In the third chapter, I employ randomly amplified polymorphic DNA (RAPD) markers to investigate the distribution of genetic variation in populations of rat snakes at three spatial scales. Highly remote (> 1000km apart) populations are strongly divergent, whereas sub-populations (≤ 50km apart) composing a regional metapopulation exhibit moderate divergence. There is little evidence of genetic structure between neighboring hibernacula, except in one case where habitat alteration may have interrupted gene flow. These findings indicate that rat snake populations are highly structured genetically and that local populations should be managed to maintain this diversity.

drafting the species' national status report for the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). This review suggests that the black rat snake should be regarded as a Threatened Species in Canada, since population extinctions are likely to occur if human-induced disturbances are not countered.

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"When I was young in the mountains,
we listened to frogs sing at dusk and awoke
to cowbells outside our windows. Sometimes
a black snake came in the yard, and my
Grandmother would threaten it with a hoe.
If it did not leave, she used the hoe
to kill it. Four of us once draped a very
long snake, dead of course, across our necks
for a photograph."

- excerpted from the award-winning children's book,
*When I was young in the mountains* (Rylant 1982).

"I live in the land that's like the Garden of Eden," he said.
"But it can't be Eden without a snake.
We're terrified it may become a true Eden.
How we long to remain unharmed!
And how carefully we identify the harm
in the obvious places,
without too much inner searching."

- excerpted from *Daughter Buffalo* (Frame 1973)

# Table of Contents

Page

Chapter 1. Habitat Features of Black Rat Snake Hibernacula in Ontario

Chapter 2. Demography of Black Rat Snakes in Ontario

# Table of Contents

(continued)

Chapter 3. Population Genetic Structure in Black Rat Snakes

Chapter 4. Conservation Status of the Black Rat Snake in Canada

# List of Tables

Table Page

# List of Figures

# List of Figures

(continued)

# General Introduction

The relative success of management programs designed to promote the recovery of endangered species is often contingent upon the degree to which a species' ecology is both known and conceptually/empirically linked to the problem at hand (Clark *et al.* 1994; Caughley & Gunn 1996). More specifically, a comprehensive understanding of the ecological characteristics of a species' enables conservation biologists and natural resource managers to, (1) accurately assess and assign conservation status (before, during, and after recovery), (2) precisely describe the symptoms of population jeopardy (how the decline is manifested) and identify the probable cause(s) of decline, (3) propose, test, and implement appropriate recovery actions, (4) gauge the effectiveness of recovery actions (through population monitoring), and (5) provide early warning of subsequent changes to population health/viability. Unfortunately, there is at present a fundamental lack of such broad-based empirical data in many on-going recovery programs. Furthermore, various ecological components (e.g., behaviour, demography, genetics) are often inappropriately regarded as isolated processes (Mace & Ballou 1990; Sæther & Jonsson 1991; Curio 1996; Sugg *et al.* 1996). These deficiencies may be particularly true in efforts to conserve threatened snakes. Because snakes are a relatively poorly studied taxon, conservation plans for snakes are often developed on ill-informed foundations (Oliver 1982; Greene & Campbell 1992; Mittermeier & Carr 1994).

With these general principles in mind, my primary aim in developing this thesis was to provide distinct, yet interrelated, research perspectives on the conservation biology of black rat snakes (*Elaphe obsoleta obsoleta*) in Canada. Those aspects of black rat snake biology that I address include; the structure and availability of hibernation habitat, demographic composition and sampling methodology, population genetic structure, and national conservation status.

There are a number of reasons that this species is well suited to a study of conservation biology. First, the current distribution of black rat snakes provides the opportunity to study populations that (a) occur at different latitudes and are therefore subjected to dissimilar environmental conditions (e.g., harsh vs. benign climates), and (b) differ with respect to their conservation status (i.e., threatened and non-threatened). Black rat snakes occupy a broad latitudinal distribution in eastern North America, extending from Ontario (Canada) at ca. 44° N to Louisiana (U.S.) at ca. 33° N. As such, some populations are constrained by a brief 5 month active season (due to a 7 month inactive overwinter period), while others enjoy almost year-round activity. Furthermore, small, highly threatened, peripheral populations can be found in Ontario while relatively abundant, non-threatened, contiguous populations are located throughout many parts of the eastern U.S. (e.g., see Durner & Gates 1993). (Accordingly, concern regarding the conservation of black rat snakes in Canada addresses the status of isolated populations, rather than the global status of the species *per se*). Comparisons of populations found at different latitudes may enable one to assess the degree to which local environmental conditions influence life history traits (e.g., growth rate, age of maturity). An understanding of this relationship can be useful for predicting how a population might respond to ecological disturbance and in estimating the capacity of a population to recover from decline. Comparisons of populations that differ in their conservation status may permit one to assess how populations have reacted to geographic isolation and reductions in population size. Conversely, such comparisons may also provide objective benchmarks or goals for recovery by defining the traits of healthy (and recovered) populations of rat snakes.

A second, related reason why black rat snakes make good study subjects for conservation biology is that they are not so seriously threatened (even in Ontario) that sufficient data on jeopardized populations are unobtainable. In particular, black rat snakes are moderately abundant across the Frontenac Axis region of eastern Ontario. Furthermore, the species' habit of hibernating communally at traditional sites (hibernacula) means that population sampling can be conducted relatively efficiently. Therefore, field studies of this population are feasible because there are adequate numbers of snakes to study.

A third argument favoring a study of rat snakes in Ontario is that, thanks to the efforts of several research/management teams, there is a considerable amount of background data available. For example, the locations of numerous hibernacula (sample localities) are already known, long-term monitoring of two local populations on the Frontenac Axis provides a wealth of (previously unanalyzed) demographic data, and the basic behavioural ecology of the species (e.g., activity patterns, habitat use) has been investigated (e.g., Weatherhead & Charland 1985; Weatherhead & Hoysak 1989; Durner & Gates 1993), providing an empirical foundation for the development of conservation-related hypotheses (see below).

Overall, my selection of the black rat snake as a study subject fulfills a dual purpose. On the one hand, ecological research on black rat snakes contributes information that is of direct value to the conservation of threatened populations of this species in Ontario, serving as a step toward the development of an informed national recovery plan. On the other hand, to the extent possible, this study may also serve as a model for the conservation biology of other (more seriously threatened) Colubrids (e.g., *Drymarchon corais couperi, Elaphe vulpina gloydi, Pituophis m. melanoleucus*) - species for which the opportunity to generate detailed ecological datasets may be limited.

## Chapter 1 - Habitat Features of Black Rat Snake Hibernacula in Ontario

Communal hibernacula are critical habitats for black rat snakes in Ontario since they enable the snakes to survive the long cold winters typical of the region.

In addition, black rat snake populations are most vulnerable to catastrophe while they occupy hibernacula because many members of a population are concentrated in one location. Researchers have traditionally found hibernacula by means of radio-telemetry. Snakes are implanted with a radio-transmitters during the summer and followed to their hibernacula in the autumn. A simpler method of finding hibernacula would be one which used biophysical habitat features to identify sites from a combination of remotely-sensed (e.g., satellite imagery) and ground survey data. My goal in Chapter 1 was to characterize the surface habitat features of black rat snake hibernacula to determine if generalized searches for such attributes in the field could be employed to find new (previously unidentified) hibernacula.

There are at least four reasons why it may be advantageous to locate new hibernacula. First, identifying additional hibernacula might provide the opportunity to protect critical habitats and local populations from further disturbance. Second, distinguishing important hibernacula elements may offer guidance to habitat restoration efforts. Third, compared with opportunistic captures, hibernacula-based sampling is a more efficient method of censusing populations. Thus, locating hibernacula aids population monitoring for both conservation and non-applied purposes. Fourth, knowing whether or not the habitat features of hibernacula are uncommon in the landscape will help determine the degree to which hibernacula availability regulates the northern limit of the species' distribution.

Therefore, Chapter 1 focuses on a test of the following specific hypotheses; (1) Knowledge of the above-ground habitat of hibernacula will enable new hibernacula to be located, and (2) Hibernacula habitat features are limiting in the landscape such that the availability of suitable overwinter retreats is restricted.

## Chapter 2 - Demography of Black Rat Snakes in Ontario

Detailing the demographic traits of a species' is often a necessary step toward effective conservation planning and population management. For example, knowledge of the age of sexual maturity, frequency of reproduction, and fluctuation in rates of juvenile recruitment provides an indication of a population's potential to recover from low numbers and which segments of the population might be most critical to that process. At the same time, such information could help identify those management practices which might be most effective in stimulating recovery.

Therefore, this chapter details the demographic properties (e.g., growth rate, sex- and size-composition) of black rat snakes in Ontario. I use data obtained through multi-year, capture-recapture studies on the Frontenac Axis and data obtained from the literature (representing separate geographic localities) to address questions relating to the spatial and temporal variability of demographic traits. The general question I ask is, how similar to one another are rat snake populations that are separated in space and/or time? Answering this question provides some measure of how populations fluctuate - background data that can then be used as benchmarks for conservation purposes since they represent expectations for how "other" populations (e.g., stable, declining, or recovering) might/should be structured. Furthermore, because some of my longitudinal data were gathered using different sampling techniques I can compare field methods with respect to potential sampling biases and thereby recommend appropriate scales and techniques for population monitoring.

## Chapter 3 - Population Genetic Structure in Black Rat Snakes

Because a species' population genetic structure may reveal demographic patterns (e.g., restricted gene flow, genetic isolation) otherwise undetectable through traditional population studies (e.g., capture-recapture), I sought to document the genetic patterns exhibited by black rat snakes sampled at different spatial scales. Data presented in this chapter provide the opportunity to identify genetically-based "management units", enabling conservation practices to be focused appropriately. In addition, by contrasting the current genetic variability within populations I am able to make inferences about the potential response of populations to isolation/decline and to classify populations with respect to their relative, genetic conservation status.

The specific questions I address in Chapter 3 include; (1) Is there evidence of significant genetic structure among populations at any one of three distinct spatial scales (i.e., geographically isolated populations, local sub-populations within the Frontenac Axis, adjacent hibernacula)?, (2) At which spatial scale is the greatest amount of genetic variation found?, (3) Does the degree of population genetic structure and gene flow detected conform to expectations founded on behavioural and demographic data? and, (4) Do relatively small, isolated populations exhibit evidence of reduced genetic diversity?

### Chapter 4 - Status of the Black Rat Snake in Canada

While Chapters 1 through 3 present data that relate to the conservation of black rat snakes, Chapter 4 imparts my judgment of the conservation status of the species as informed by these results. (I appeal for tolerance by readers for the unavoidable overlap between material presented in Chapter 4 and those of previous chapters).

Chapter 4 is formatted as a draft species status report for the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). COSEWIC is a committee comprised of representatives from federal, provincial, and private agencies which assigns national status to species at risk in Canada. The primary goals of this chapter are to review the pertinent biological, historical, and cultural information and to make an assessment of the species' conservation status in Canada.

The primary question addressed in this chapter is the following; Is there a need for a conservation effort directed toward black rat snakes in Canada? In addressing this (potential) conservation problem I present the presumed historical range and current distribution of extant populations, a qualitative assessment of the probability of population extinction (relative jeopardy) including prevailing threats and limiting factors, and an estimation of what ecological/cultural significance extirpation of the species from Canada would have. Addressing these issues requires both a synthesis of the current state of ecological knowledge and identification of critical knowledge gaps. Collectively, this chapter is intended to act as the initial step toward focusing public/political attention on the conservation status and future recovery of the black rat snake in Canada.

# Chapter 1.

# Habitat Features of Black Rat Snake Hibernacula in Ontario

This chapter formed the basis of a paper of the same title
published in the *Journal of Herpetology*
and coauthored by Kent A. Prior and Patrick J. Weatherhead.

# Introduction

Different life history features of wildlife are often closely tied to precise habitat needs. Even relatively sedentary taxa like snakes may have specific and contrasting habitat requirements for over-wintering, breeding, nesting, and foraging (e.g., Burger & Zappalorti 1986; Burger *et al.* 1988; Scott *et al.* 1989; Barry *et al.* 1992). As such, the use of traditional sites for communal hibernation (hibernacula) by snakes (Gregory 1984; Sexton *et al.* 1992) is of particular interest to both conservation biologists and behavioral/evolutionary ecologists.

From a conservation perspective, communal hibernation necessarily places local snake populations (or snake communities in the case of multi-species hibernacula) in potential jeopardy. This is because traditional hibernacula may be used by many indivdiuals representing a significant proportion of a local population (e.g., 60-80%, see *Chapter* 2) which is collectively and annually susceptible to both natural and human-induced catastrophe. In fact, humans have exploited knowledge of the hibernation behavior and habitat requirements of rattlesnakes (Crotalidae) to such an extent (i.e., "rattlesnake round-ups") that populations have seriously declined or been extirpated altogether from parts of the U.S. (Galligan & Dunson 1979; Warwick 1990; Brown 1993). Thus, protection of hibernacula may be critical to the conservation of threatened species. If hibernacula are found to occur in very specific habitats, then one could protect hibernacula by protecting essential habitat.

Behavioral and evolutionary ecologists recognize that the quantification of the over-winter habitat needs of species may also be useful in understanding whether populations are limited by appropriate habitat for hibernation (Gregory 1984). The selection of suitable hibernacula by snakes is fundamental to the persistence of populations in cold climates and thus, may dictate the relative position of the northern (or southern) range limits for a number of species (see Rosen 1991). That communal hibernation may be a response to limited availability of suitable habitat does not preclude the possibility that the behavior serves some social/physiological function(s) as well (e.g., enhances mating success, minimizes probability of fatal over-winter dehydration).

While snake hibernation per se has received some attention (e.g., Gregory 1982, 1984; Costanzo 1989; Ultsch 1989; Weatherhead 1989), there have been very few quantitative descriptions of communal hibernacula (Drda 1968; Burger *et al.* 1988). As suggested above, contributions to this field may be particularly valuable when they focus on threatened species and/or peripheral populations. Black rat snakes (*Elaphe o. obsoleta*) occurring in Ontario meet both criteria. The most northerly population of this species (the "Frontenac Axis population") occurs in eastern Ontario and northern New York and has been provisionally designated as "threatened" in Canada (see *Chapter 4*). Active season habitat does not appear to be particularly limiting within the range of this population (Weatherhead & Charland 1985). Their preferred habitat, a mixture of deciduous forest, old field, and forest/field edge, is more abundant now than it was 100 years ago at the height of land-clearing for timber and agriculture (Kelly 1990; M$^c$Innis 1990). However, nothing is known about the availability of suitable communal hibernacula across this region.

Therefore, the objectives of this chapter were to (1) quantify variability in the above-ground habitat characteristics of communal hibernacula occupied by black rat snakes at the northern edge of the species' distribution, and (2) contrast hibernacula habitat with that available in the surrounding landscape in order to determine whether appropriate habitat might be limiting.

## Methods

### *Study Area*

At the extreme northeastern part of the species' range, the Frontenac Axis population of black rat snakes is confined to an area of approximately 5000 $km^2$ in eastern Ontario and northern New York (ca. 44°15' — 44°55' N, 75°20' — 76°45' E, see maps in Conant 1975; Cook 1984). Detailed surveys have shown this population to be geographically disjunct from all others, the nearest population being ≈ 120 km to the south, near Syracuse, New York (A. R. Briesch - unpubl. data; W. F. Weller and M. J. Oldham - unpubl. data). The Frontenac Axis itself is an exposed, south-easterly extension of the Precambrian (Canadian) Shield and represents the dominant geological feature of the study area. Topography throughout the region is irregular with a strongly rolling upland terrain (Rowe 1972), numerous granite outcrops, and many lake and river systems. The soils are composed of thin, very stony glacial till (Hoffman *et al.* 1967; Gillespie *et al.* 1966). Extensive second growth, mixed coniferous-deciduous forests (Great Lakes-St. Lawrence Forest

Region; after Rowe 1972) cover much of the area while the abandonment of marginal agricultural land has also resulted in considerable shrubland.

*Sampling*

I studied 10 communal hibernacula distributed across the Ontario portion of the Frontenac Axis population range. All hibernacula were originally identified during radio-telemetry studies (e.g., Weatherhead & Hoysak 1989) and are located at Murphy's Point Provincial Park (3 sites), Queen's University Biological Station (2 sites), Charleston Lake Provincial Park (2 sites), La Rue Mills (1 site), and on Hill Island within St. Lawrence Islands National Park (2 sites). For conservation reasons exact locations of hibernacula remain confidential.

At each hibernacula I quantified 5 structural habitat variables that I believed adequately characterized the sites and might also reflect the relative suitability of the sites for snakes. The habitat features I measured included, (1) slope in degrees (mean of 3 measurements with an inclinometer taken 5 m from the main opening of the hibernaculum), (2) aspect in degrees (orientation of slope at the main opening measured with a hand-held compass), (3) percent canopy closure (25 point samples [x4] within a 45° cone while standing at the main opening [modified after Reagan 1974]), (4) percent shrub cover (mean percent cover within 4, randomly positioned 1-m$^2$ quadrats located within 10 m of the main opening), and (5) percent rock cover (mean percent surface rock and bedrock cover within 4, randomly positioned 1-m$^2$ quadrats located within 10 m of the main opening). I was unable to quantify the underground structure of the hibernacula because the openings were too small to allow access and the threatened status of the population precluded excavation of the sites.

I also measured the same 5 variables at one "potential hibernacula," and two randomly positioned sites per hibernaculum, for a total of 40 sites collectively. Potential hibernacula were located during time-constrained searches (1 h), fell within 50-500 m of the hibernaculum, and represent sites that I *felt* appeared as if they might serve as suitable hibernation sites for rat snakes on the basis of visual inspection of habitat (i.e., prior to any empirical quantification). In essence, potential sites were found using my familiarity with the 10 actual hibernacula across the region as a guide. As a necessary criterion, all potential hibernacula had to include existing holes or cavities leading underground, though I had no way of determining the extent of these spaces. Comparisons among actual and potential hibernacula were used to assess whether any other hibernacula-type habitat was available in the surrounding landscape. Random sites were located through selection, by means of a random number generator, of a compass bearing and distance (between 50-500 m) from the hibernaculum. At these sites, I also searched the immediate area until I found an underground opening and began sampling from there. Comparisons among hibernacula and random sites provided an assessment of whether hibernacula habitat differed from the surrounding habitat generally. All potential hibernacula and random sites were checked a minimum of five times during spring emergence (1994) in order to document possible use of these sites by snakes.

Following emergence from hibernation and prior to dispersal to summer home ranges, black rat snakes in the Frontenac Axis population typically bask arboreally for several days within the immediate vicinity of the hibernaculum (Prior & Weatherhead, unpubl. data). During spring emergence (mid-April - late May) in both 1993 and 1994, I quantified the arboreal basking habitat used by rat snakes at 8 of the hibernacula. (Emergence at the two Charleston Lake hibernacula was not monitored, so I have no detailed information on the basking habits of snakes at these sites). During daily visits to hibernation sites throughout the emergence period I and an assistant carefully scanned the trees for snakes for an average of 30 min per site. Because snakes in coniferous trees were relatively more inconspicuous than those found in (bare) deciduous trees I was particularly thorough about searching coniferous trees for snakes in order to minimize any potential bias in my data. For the purpose of analysis I defined snakes as "basking arboreally" when I saw them in a stationary position, either draped or coiled on the branch or trunk of a tree, at a height of 1 m or more off the ground. When possible, basking snakes were captured by hand, measured, weighed, sexed, marked (with both a PIT-tag and non-toxic acrylic paint) for future identification, and then released back at the place of capture.

I recorded four features of the trees used by the snakes, including; tree species, diameter (cm) at breast height (DBH), the presence or absence of cavities and hollows in the branches and trunk, and a "relative condition rank." The condition rank of basking trees was assessed by visual inspection according to the following criteria; 1 = healthy tree with no visible sign of decay, 2 = live tree with as much as 50% of the crown dead, 3 = live tree with greater than 50% of the crown dead and numerous dead branches intact, and 4 = dead snag with few or no branches intact. I then matched each basking tree with a randomly selected, "unused tree" for which I quantified the same four features. Unused trees were chosen by selecting the first tree encountered along a transect (orientation randomly selected) radiating from individual basking trees. By definition, unused trees were never seen to be occupied by rat snakes during three years of study (1992-1994). By comparing the attributes of basking and the randomly selected unused trees at hibernacula I was able to determine whether the snakes might be actively selecting a subset of the trees available to them.

I also quantified five randomly selected trees at each potential hibernaculum and random point (see above). As such, randomly selected trees were representative of those available to rat snakes had there actually been hibernacula at those locations. Comparisons among randomly selected trees at both potential hibernacula and random locations and the pooled sample of basking and unused trees at hibernacula allowed us to assess whether the type of trees available to snakes for basking at hibernacula were unique and thus, potentially limiting.

### *Statistical Analyses*

I used correlation-based principal components analysis (PCA) to objectively reduce the dimensionality of the original habitat variable set to a smaller number of mutually uncorrelated composite variables or components. Slope aspect (°) was re-coded to binary form (i.e., 90-269° = south, 270-89° = north) prior to inclusion in the PCA (see Green, 1979, pp. 73-95 for a discussion of the use of binary data in PCA). Analysis of variance (ANOVA) was then used to test for differences among samples sites (i.e., hibernacula, potential hibernacula, random points) with respect to habitat-based PC scores. Scheffé F-tests were used for post-hoc comparisons of means when required. As above, I also used PCA and ANOVA to test for differences among (1) basking and unused trees at hibernacula, and (2) sample sites (i.e., hibernacula, potential hibernacula, random) with respect to the trees available for basking. Data on tree type (deciduous/coniferous) and the existence of cavities (presence/absence) were coded as binary variables prior to inclusion in the PCA. Where necessary, data were either arcsine- or log-transformed to achieve normality. All analyses were performed using STATVIEW II software (Abacus Concepts 1990).

## Results

Typical habitat descriptions for each of the three sample sites (actual hibernacula, potential hibernacula, random points) can be derived using information obtained from the mean habitat variables for each type (**Table 1-1**). In general, hibernacula tended to be situated on moderately sloping and relatively rocky hillsides that faced south-south-east. However, this data summary does not convey a sense of the great variability in hibernacula habitats used by rat snakes across the region. The following examples illustrate this point. Hibernacula occurred (1) on a talus slope in a steep-sided valley of broken rock and boulders, (2) on an open grassy slope, 30 m from a beaver (*Castor canadensis*) pond, (3) on relatively level ground amid a mature, closed-canopy forest, (4) on the steep, rocky shore of an island in a large lake, and (5) in a shallow depression, atop a forested ridge.

A PCA of habitat features reduced the original 5 structural variables to two orthogonal components that collectively explained 70% of the total variation among all sites (**Table 1-2**). Additional components had eigenvalues of less than 1.0 and were therefore considered uninformative

(Dunteman, 1989). The first component primarily reflected variation in slope and percent rock cover, and to a lesser degree the aspect of the slope. PC2 was interpreted as a contrast between percent shrub cover and percent canopy closure.

A plot of the two sets of PC scores for all sample types illustrates an apparent distinction among random sites and both actual hibernacula and potential hibernacula along the PC1 axis (**Fig. 1-1**). Unlike random sites, actual hibernacula and potential hibernacula tended to have relatively high PC1 scores (i.e., steeper and more rocky with southern aspect). This impression was supported statistically by an ANOVA of the PC1 scores ($F = 21.24$, df = 39, $P = 0.0001$) and post-hoc means tests (Scheffé F-test; actual hibernacula vs random sites, $F = 12.82$, $P \leq 0.05$ and potential hibernacula vs random sites, $F = 15.44$, $P \leq 0.05$). No differences were found among the sites with respect to PC2 scores ($F = 1.89$, df = 39, $P = 0.164$) suggesting that hibernacula were not unique with respect to degree of shrub cover and canopy closure.

The most common tree species found at the hibernacula covered a broad range including; oaks (*Quercus spp.*, 31%), white pine (*Pinus strobus*, 23%), white cedar (*Thuja occidentalis*, 15%), maples (*Acer spp.*, 11%), and ironwood (*Ostrya virginiana*, 8%). With the exception of white cedar, both potential hibernacula and random sites exhibited similar species composition, respectively; oaks (23%, 20%), white pine (13%, 24%), cedar (2.5%, 2.5%), maples (28%, 16%), and ironwood (10%, 10%). The predominance of cedar in the actual hibernacula samples was largely due to its' abundance at a single site at Murphy's Point.

Throughout the two spring emergence periods I observed a total of 163 individual black rat snakes basking in trees near (≤ 30 m from the main opening) all but one of the eight hibernacula investigated in this regard. Many trees were occupied by more than one snake at a time and some individuals were observed repeatedly in the same tree(s). However, in order to avoid pseudoreplication, any tree that was occupied is represented only once in the dataset and following analyses.

The number of basking trees used by snakes at each hibernaculum ranged from 0 to 11. In total, 39 basking trees were quantified, the majority of which (51%) were oaks. In general, basking trees tended to be older, partly dead or decaying deciduous trees with numerous cavities in the trunk and larger limbs (**Table 1-3**).

A PCA of the characteristics of trees found at actual hibernacula reduced the original 4 variables to two orthogonal components that collectively explained 74% (PC1 = 51.5%, PC2 = 22.9%) of the total variation among basking and unused, randomly selected trees (**Table 1-4**). The first component reflected variation in the presence of cavities, DBH or girth, and general health status or condition, while PC2 explained variation due to tree type (i.e., coniferous vs. deciduous). (Note that for the purposes of the PCA the existence of cavities in a tree was coded as follows; 0 = presence and 1 = absence. As such, a negative factor loading for this variable under PC1 is indicative of a high probability of the presence of cavities). Basking trees tended to have relatively high PC1 scores (**Fig. 1-2**) indicating that they were usually the largest and most unhealthy trees available in the vicinity of hibernacula. In contrast, there was no apparent difference between basking and unused trees with respect to PC2 scores suggesting that trees used for basking were no more likely to be deciduous than they were coniferous. An ANOVA of PC scores supported this interpretation. I found a significant difference between the attributes of basking and unused trees at hibernacula as described by PC1 ($F = 52.38$, df = 77, $P = 0.0001$), but not PC2 ($F = 0.76$, df = 77, $P = 0.385$). Thus, rat snakes appear to be selecting the older, decaying trees for basking among the wide assortment of those available to them around hibernacula. Whether such trees are deciduous or coniferous does not seem to be particularly important to the snakes.

Finally, a PCA of the characteristics of trees found at actual hibernacula, potential hibernacula, and random locations derived two orthogonal factors that collectively explained 69% (PC1 = 44.9%, PC2 = 24.5%) of the total variation among sites (**Table 1-4**). As in the previous analysis, PC1 reflected variation in the presence of cavities, DBH, and condition, while PC2 explained variation due to tree type (i.e., coniferous vs. deciduous). Ordination of the PC scores provides no clear distinction between site types along the PC1 axis (**Fig. 1-3**). ANOVA supported this view ($F = 0.51$, df = 197, $P = 0.598$), implying that the general structure of the trees around the three types of sites was similar and therefore that "potential basking trees" (large and decaying with cavities) were not absent from either potential hibernacula or random sites. However, potential hibernacula exhibited relatively low PC2 scores (i.e., predominantly deciduous, see **Fig. 1-3**). Indeed, ANOVA revealed a significant difference in the attributes of trees found across sites with respect to PC2 values ($F = 5.1$, df = 197, $P = 0.0065$). A post-hoc means test confirmed that potential hibernacula had disproportionately more deciduous trees than were found around actual hibernacula (Scheffé F-test, $F = 5.1$, $P \leq 0.05$). However, because my previous analyses suggested that rat snakes do not necessarily prefer to bask in coniferous over deciduous trees, or vice versa, I conclude that trees which are apparently suitable for basking are readily available at potential hibernacula.

**Table 1-1. Surface habitat characteristics (mean ± S.E.) of black rat snake hibernacula (N = 10), potential hibernacula (N= 10) and random sites (N= 20).**

| Variable | Hibernacula | Potential hibernacula | Random sites |
|---|---|---|---|
| Slope (°) | 17.56 ± 3.12 | 20.85 ± 1.84 | 7.53 ± 1.11 |
| Aspect (°) | 158.0 ± 17.0 | 191.0 ± 25.0 | 28.00 ± 80.0 |
| % Canopy closure | 74.0 ± 3.92 | 84.4 ± 2.10 | 84.90 ± 3.57 |
| % Shrub cover | 10.28 ± 2.71 | 3.88 ± 1.65 | 10.89 ± 2.34 |
| % Rock cover | 27.20 ± 10.61 | 31.50 ± 6.18 | 11.13 ± 2.42 |

**Table 1-2. Factor loading matrix for principal-components analysis of 5 habitat variables.**

| Variable | PC1 | PC2 |
|---|---|---|
| Slope (°) | + 0.844 | + 0.068 |
| Aspect (°) | + 0.694 | - 0.108 |
| % Canopy closure | - 0.431 | - 0.789 |
| % Shrub cover | - 0.339 | + 0.837 |
| % Rock cover | + 0.814 | - 0.048 |
| % Variance explained | 43.1 | 26.8 |

**Table 1-3. Characteristics (mean ± S.E.) of basking trees and randomly selected unused trees found at hibernacula, potential hibernacula, and random sites.**

| Sample site | Tree class | N | DBH (cm) | % Deciduous | Condition | % with cavities |
|---|---|---|---|---|---|---|
| Hibernacula | Basking | 39 | 31.15 ± 2.4 | 69.2 | 1.82 ± 0.18 | 87.2 |
| | Unused | 39 | 17.37 ± 1.3 | 50.0 | 1.25 ± 0.08 | 3.3 |
| | Pooled | 78 | 24.26 ± 1.6 | 57.7 | 1.54 ± 0.10 | 50.0 |
| Potential hibernacula | Unused | 40 | 25.08 ± 1.9 | 66.3 | 1.72 ± 0.15 | 32.5 |
| Random sites | Unused | 80 | 24.82 ±1.3 | 66.3 | 1.63 ± 0.11 | 27.5 |

**Table 1-4. Factor loading matrices for principal-components analyses of 4 tree characteristics within hibernacula (basking, unused trees) and among sites (hibernacula, potential hibernacula, random).**

| Treatment | Variable/<br>% *Variance explained* | PC1 | PC2 |
|---|---|---|---|
| Hibernacula | Tree DBH | + 0.798 | + 0.314 |
| | Deciduous/coniferous | - 0.467 | + 0.839 |
| | Tree condition | + 0.731 | - 0.142 |
| | Presence/absence of cavities | - 0.817 | - 0.300 |
| | % *Variance explained* | 51.5 | 22.9 |
| Among sites | Tree DBH | + 0.752 | + 0.253 |
| | Deciduous/coniferous | - 0.306 | + 0.917 |
| | Tree condition | + 0.688 | - 0.141 |
| | Presence/absence of cavities | - 0.812 | - 0.231 |
| | % *Variance explained* | 44.9 | 24.5 |

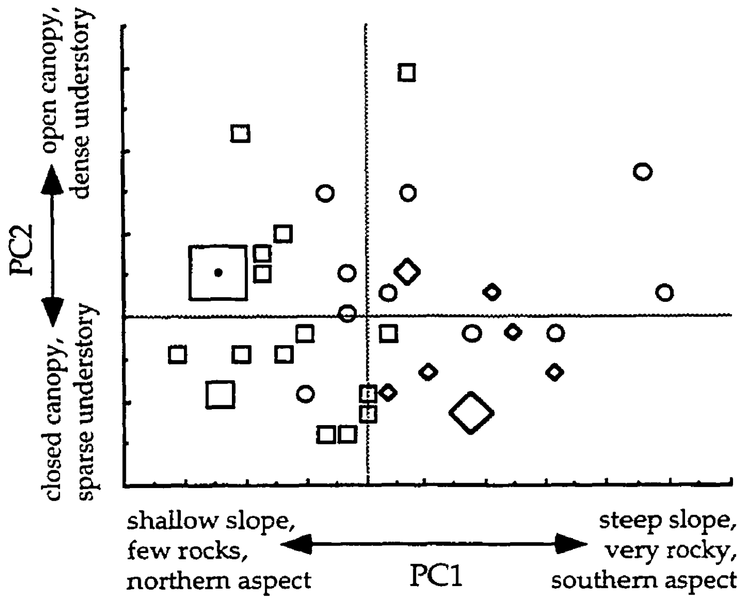

**Figure 1-1. Plot of principal components scores derived from 5 habitat variables. Sample sites include; actual hibernacula (circles), potential hibernacula (diamonds), and random locations (squares). Enlarged symbols represent coinciding data. Symbol size is proportional to the number of coinciding datapoints.**

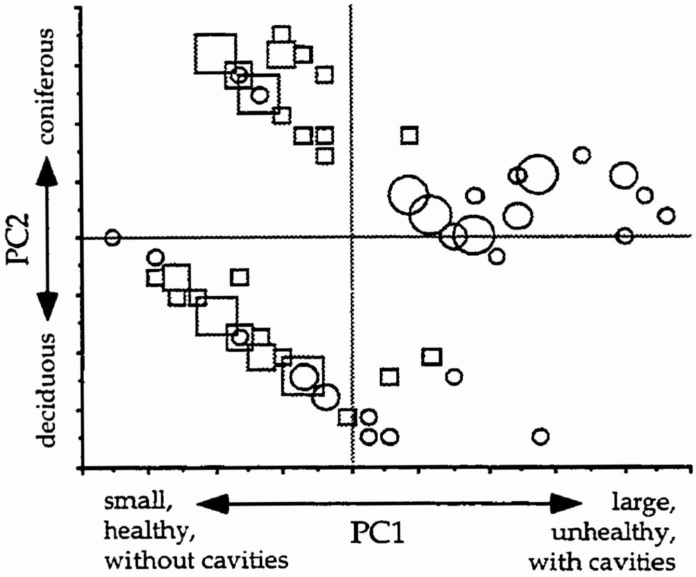

**Figure 1-2.** **Plot of principal components scores derived from 4 characteristics of trees measured at hibernacula. Trees sampled include; actual basking trees, (circles) and unused trees (squares). Enlarged symbols represent coinciding data. Symbol size is proportional to the number of coinciding datapoints.**

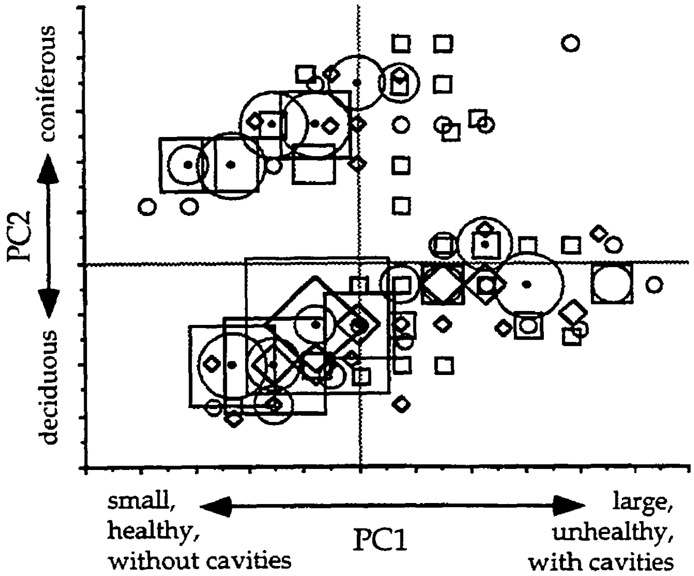

Figure 1-3. Plot of principal components scores derived from 4 characteristics of trees measured at different sites including; actual hibernacula (circles), potential hibernacula (diamonds), and random locations (squares). Enlarged symbols represent coinciding data. Symbol size is proportional to the number of coinciding datapoints.

## Discussion

Communal hibernacula occupied by black rat snakes of the Frontenac Axis population are typically situated on relatively rocky, south-facing slopes. These habitat features were sufficiently unique to distinguish hibernacula from a series of randomly selected locations in the surrounding landscape. However, for several reasons I regard these findings to be of limited value in the establishment of guidelines to identify and protect hibernacula throughout this region of eastern Ontario.

First, the broad variability in habitat features exhibited by the hibernacula I surveyed implies that many hibernacula would not be detected if one searched for them only on southern oriented rocky slopes. For example, at least four of the communal hibernacula I quantified during this study do not fit the image of an "average hibernacula" as conveyed by my data. As such, these four major sites clearly would not have been found had natural resource managers or biologists simply used "rocky, south-facing slopes" as a search image in their quest for hibernacula.

Second, I was able to locate many "potential hibernacula" which were, by my criteria, indistinguishable from real ones. Needless to say, none of the potential hibernacula proved to be (previously undiscovered) actual hibernacula. This strongly suggests that looking for hibernacula on the basis of surface habitat features alone would only rarely result in the discovery of actual hibernacula.

While surface habitat features may not be a good predictor of hibernacula locations, the trees used for arboreal basking at extant hibernacula seem to be distinct from those generally available. The snakes tended to prefer relatively large, partially dead and/or hollow trees as opposed to the younger, more healthy trees available to them. This suggests that rat snakes select specific attributes or a class of trees for basking around hibernacula. The presence of high quality, "potential" basking trees at both potential hibernacula and random sites suggests that basking-type trees are not limiting in the landscape generally.

From the perspective of species conservation, these findings indicate that both the identification and protection of black rat snake communal hibernacula in Ontario will have to proceed on a site by site basis. One cannot predictably find new hibernacula by simply looking for specific or critical habitat features within a landscape. Therefore, radio-telemetry remains the most effective and practical means of locating hibernacula in large, semi-remote areas like parks and ecological reserves. The preferential use of specific trees by rat snakes suggests that opportunities for arboreal basking should be considered in management plans designed to preserve the suitability of communal hibernacula. Specifically, mature and decaying trees and snags should not be cleared from the vicinity of actively used hibernacula. Similarly, where possible such features might be re-created at degraded sites prior to attempts at repatriating extirpated populations.

From a biological or evolutionary perspective my findings are equivocal as to whether or not appropriate hibernacula habitat is limiting in the Frontenac Axis region of eastern Ontario. At this point I am unable to determine if rat snakes at this (extreme) latitude are (1) geographically constrained by the limitations of over-winter habitat, and/or (2) hibernating communally simply in response to low habitat availability. However, it is unlikely that communal hibernation in black rat snakes is a consequence of some social function or advantage as has been proposed for other species (Gregory 1984; Duvall *et al.* 1993). For example, there is no evidence that black rat snakes realize enhanced mating success from hibernating communally since they mate during early summer after dispersal from hibernacula (pers. obs.).

Surface characteristics alone appear to be insufficient to ascertain the suitability of a site to serve as a communal hibernaculum for rat snakes. That is, surface features may not necessarily reflect essential subterranean structure and conditions, though features like percent rock, degree of slope, and aspect (as they relate to thermal and hydric conditions) are probably not irrelevant. That all hibernacula I studied faced "south" (i.e., mean aspect = 158.0 ± 17.0°) probably holds some biological significance. Southerly exposed hibernacula are likely to experience minimal frost penetration and early spring snow melt, both of which may ameliorate over-winter conditions (see Sexton *et al.* 1992).

Critical internal attributes of hibernacula may be both distinctive and not particularly common in the environment. Unfortunately, little is known about the internal composition and environment of natural hibernacula. The information available to date has come from either destructive excavations (Burger *et al.* 1988), investigations of hibernacula occurring in caves large enough to allow human access (Drda 1968; Sexton & Hunt 1980), and telemetry studies (e.g., Jacob & Painter 1980; Weatherhead 1989). A steady decline in the internal temperature through the over-winter period may be typical of communal hibernacula (Drda 1968; Sexton & Hunt 1980), including those used by black rat snakes at this latitude (Weatherhead 1989). Beyond this, traditional communal hibernacula used by rat snakes must generally be

(1) structurally stable, (2) deep enough that the snakes do not reach fatal, low temperatures (0-2° C), and (3) sufficiently humid to preclude over-winter dehydration. The use of remote sensing technology (e.g., ground-penetrating radar) in the future may allow detailed mapping of the internal characteristics of otherwise inaccessible hibernacula. Ultimately, we require additional information about the characteristics of good hibernating sites (e.g., subterranean structure, internal micro-climatic conditions) and need to assess these variables at potential sites more thoroughly before we can draw conclusions regarding the availability of hibernacula (Gregory 1984).

# Chapter 2.

# Demography of Black Rat Snakes in Ontario

# Introduction

Knowledge of the demography of wild populations is fundamental to the development of a comprehensive understanding of a species' ecology (Pielou 1974; Caughley 1977). Long-term studies are of particular value in this regard (especially for long-lived species), since they may reveal important patterns of temporal variation in demographic parameters (e.g., age-structure, sex ratio) and life-history traits (e.g., growth rate, age of maturity), otherwise unrecognized in short-duration studies. Similarly, studies that compare the attributes of ecologically and/or spatially isolated populations provide insights regarding among-population variation unattainable in investigations of single populations. Not only do such data contribute to a general advance in population ecology and life-history theory research (e.g., Gregory & Larsen 1993, 1996; Madsen & Shine 1994; Aldridge & Brown 1995) but increasingly, they may also serve conservation purposes by establishing a baseline against which the demographic properties of declining (and recovering) populations may be compared (Congdon *et al.* 1993; Garber & Burger 1995).

Snake ecologists have generally lagged behind those studying other vertebrates in detailing essential population attributes (see reviews in Parker & Plummer 1987; Dunham *et al.* 1988a). This state is due, at least in part, to the fact that many snake species are highly intractable to study, despite advances in telemetry and marking technologies. As such, ecologists seeking candidate models for research have often avoided snakes in favor of more easily monitored groups, including mammals, birds, and lizards (Seigel 1993).

Consequently, the paucity of long-term, comparative studies on snake populations has meant that; (1) general patterns of snake demography remain largely undefined, and (2) conservation programs for some of the most threatened snake species have had to proceed in the absence of adequate data on demographic traits (Greene & Campbell 1992; Mittermeier & Carr 1994).

My primary objective in this chapter is to summarize the demographic properties of an isolated (meta-)population of black rat snakes (*Elaphe o. obsoleta*). The data were principally obtained during the course of two independent, long-term (14 and 15y each) capture-recapture studies of eastern Ontario's Frontenac Axis population. My specific aims arise both from a general opportunity to conduct comparative analyses and contribute data of intrinsic value to the field of snake ecology and from specific concerns regarding the conservation status of the black rat snake in Canada.

The Frontenac Axis black rat snake population occurs at the northern periphery of the species' distribution (ca. 44° 55'N) and is the largest and least jeopardized of five isolated populations persisting in Ontario (see *Chapter 1*). Demographic data for the four highly threatened populations located in southwestern Ontario are non-existent and are unlikely to be obtained in the near future since there are no on-going field studies in this region. Thus, I regard data from the Frontenac Axis as essential to the development of our understanding of the demography of black rat snakes at northern latitudes. By comparing these data with those available in the literature I will assess the generality of the demographic attributes of black rat snakes in Ontario. Furthermore, these results may be directly applied to conservation of the species in Canada. Specifically, demographic patterns exhibited by the Frontenac Axis population may be useful for comparative purposes in as much as they represent empirically derived expectations for how other populations (threatened or otherwise) might be structured. For example, suppose that communally hibernating populations in southwestern Ontario have proportionately few reproductive adults relative to hibernacula populations on the Frontenac Axis. Recognition of such a difference in the age-structure or proportion of sexually mature individuals could alert recovery planners to potential problems associated with adult survival in southwestern Ontario populations.

As such, my primary objective is to assess temporal and ecological/spatial variation in the demographic structure of black rat snakes, in order to determine how similar populations are to one another. To meet this objective, I quantified a series of demographic attributes for the Frontenac Axis population (i.e., sex- and age-specific growth, survivorship, and recruitment rates; size-structure; sex ratio; population size, density) and tested for evidence of certain patterns which I outline below.

Due to its geographic position, the Frontenac Axis population experiences relatively brief active seasons (ca. 5 mo) followed by long, cold winters which require the snakes to hibernate at traditional underground hibernacula. Short active seasons severely limit the annual opportunity to accomplish basic physiological maintenance, foraging, growth, and reproductive activities. Extended periods of overwinter dormancy (ca. 7 mo) also exert costs since the snakes must cope with low temperatures, dehydration, a suppressed immune response, and only stored lipid reserves for nutriment; stresses which can result in overwinter mortality (e.g., Gregory 1982, 1984; Costanzo 1989; Blem & Blem 1996; Prior & Shilton 1996). It is reasonable to expect that the constraints imposed by these temporally variable environmental conditions might promote conservatism in growth and sexual maturation in the study population (Roff 1992). Indeed, strong negative relationships between the length of the active season and rates of growth and sexual maturation and reproductive frequency have been found in several snake populations (e.g., Brown 1991; Larsen & Gregory 1989; Martin 1991, 1992; May *et al.* 1996). Accordingly, I predicted that the brevity of the active season should result in relatively slow growth, delayed sexual maturity, and perhaps greater longevity compared with populations inhabiting less variable environments with longer active seasons (e.g., those found at southern latitudes).

Male-biased sexual size dimorphism is characteristic of adult black rat snakes (Ernst & Barbour 1989) and appears to be a sexually-selected trait thought to have evolved in response to male-male competition (especially combat) for females (Shine 1978, 1994). The discrepancy in size between the sexes of adult rat snakes results from the fact that males tend to grow more rapidly than females (Stickel *et al.* 1980), and not from any size advantage males might have at hatching (Fitch 1963; Clark & Pendleton 1995; S. Mullin - pers. comm.). The predicted demographic consequence of such a difference in the growth rates of males and females is that the faster-growing sex should predominate the adult age-class. Indeed, adult sex ratios have been found to be male-biased in a black rat snake population studied in Maryland (Stickel *et al.* 1980). Accordingly, I expected to find evidence of similar patterns of sex-specific growth rates and male-biased adult (or breeding) sex ratios in the Frontenac Axis population.

A decline in growth rate after sexual maturation is well-documented in snakes generally (Parker & Plummer 1987; Houston & Shine 1994; Madsen &

Shine 1994) and has been suggested qualitatively for black rat snakes in particular (Fitch 1963; Stickel *et al.* 1980). Sexually mature individuals may allocate energy resources to reproduction rather than growth for a variety of behavioural (e.g., mate-searching) and physiological (e.g., provisioning of embryos) reasons. Thus, given these energy costs associated with reproduction, I predicted that growth rates for rat snakes should decline following sexually maturity.

Certain demographic characteristics of the Frontenac Axis population might also reflect the relative costs of reproduction experienced by males and females. As with other taxa, male and female snakes are generally regarded as making unequal investments in reproduction, with females incurring relatively greater costs (e.g., Madsen & Shine 1993). The energetic costs of reproduction for male rat snakes result primarily from increased behavioural activities including mate-searching and male-male combat. Female rat snakes may incur relatively higher energy costs associated with vitellogenesis, the provisioning of embryos, and a general inability to actively forage while gravid. The short active season on the Frontenac Axis may limit a female's capacity to replenish energy reserves following a reproductive bout, potentially restricting her reproductive frequency (e.g., biennial vs. annual cycles?) and reducing her probability of surviving long overwinter periods (Madsen & Shine 1992, 1994; Farrell *et al.* 1995; Diller & Wallace 1996). If so, then I expected to find evidence of relatively higher mortality rates in females (i.e., male-biased survivorship) and a commensurate bias in the breeding sex-ratio of the population (i.e., male-biased sex-ratio).

I also quantified the size distribution of recruits to hibernacula (hibernacula participants that are new captures/members), recruitment rates , and the level of fidelity to hibernacula exhibited by individuals in the study population. Female black rat snakes deposit eggs at oviposition sites that may be remote from traditional communal hibernacula, and neonates generally do not attend these communal sites. Therefore, by characterizing the size distribution of hibernacula recruits I will be able to specify the average age at which young black rat snakes begin to participate in traditional hibernacula. My intent in quantifying recruitment rates was to provide an index of (annual) reproduction, a valuable measure from the perspective of population and species conservation. If viewed as a baseline, these data could provide the opportunity to assess the efficacy of conservation management of the Frontenac Axis population in the future. For instance, suppose that in 50 years recruitment is found to have declined well below the range documented during the period of the present study. Confronted with such an observation, resource managers might reasonably assume that programs they have implemented to protect oviposition sites, enhance hatching success, and increase neonate survival have failed and require adjustment. Only through the availability of baseline data could such informed comparisons be made, enabling progressive recovery planning and the refinement of management actions.

By estimating site fidelity I will be able to judge the degree to which communal hibernacula can be regarded as demographically "closed" sub-populations. Collectively, data on recruitment patterns and site fidelity will serve to improve our understanding of how representative hibernacula are of the surrounding local populations (see also methodological objectives below) and how demographically distinct hibernacula are from one another. In doing so I will judge the suitability of these social assemblies as sampling units for both population monitoring and conservation genetic management.

Temporal fluctuations in population size are also closely tracked by conservation biologists and resource managers charged with monitoring endangered species. A critical aim in the analysis of such data is to distinguish between the effects of human impacts and natural fluctuations (e.g., Pechmann *et al.* 1991, Seigel et al. 1995). I know of no major environmental or anthropogenic disturbances (e.g., landscape-scale habitat loss, elimination of major hibernacula) that have had a negative impact on rat snake populations on the Frontenac Axis during the 15y study period. Therefore, I predicted that local population size estimates would probably fluctuate randomly over the course of the study. Alternatively, I thought it prudent to regard any evidence of a longitudinal trend in population size as a possible signal of population decline or improvement, depending upon the direction of such a trend.

In meeting these primary biological objectives I was also able to address several secondary methodological objectives, the results of which may be of value to snake ecologists generally. My secondary aims are made possible by the availability of longitudinal demographic datasets resulting from two different sampling methods (i.e., opportunistic captures vs. hibernacula-based captures). Ecologists generally recognize that different field techniques designed to sample the same attribute potentially can yield contrasting datasets and ultimately confounding interpretations as a result of inherent sampling biases (e.g., Dufour & Weatherhead 1990; Dodd 1991; Kolb 1994). An obvious example in the field of snake ecology was noted during early applications of telemetry technology to assess movement patterns and habitat use. Prior to the advent of telemetry, data were obtained via opportunistic captures of individuals, a technique biased toward highly visible (i.e., relatively large and active) members of the population. By contrast, data obtained using telemetry are not contingent on individual visibility and therefore, are more representative of actual patterns of movement and habitat use. Documenting the discrepancies between telemetry and opportunistic capture data was a key step in allowing researchers to develop a more accurate understanding of the spatial ecology of snakes (Weatherhead & Charland 1985; Burger & Zappalorti 1988; Weatherhead & Prior 1992).

For many species, opportunistic captures remain the only method for quantifying snake population parameters such as size and age distributions, or for monitoring population size through time. While this probably results in biased samples (Fitch 1961; Mertens 1995; Seigel *et al.* 1995), there is no independent method for assessing whether that is the case. However, in species which form social groups (e.g., mating aggregations, communal hibernacula), alternative sampling techniques may be available which can yield more reliable population samples. Although tests for biased catchability are recommended whenever possible (Parker & Plummer 1987), few studies have attempted to quantify the degree to which data produced by different snake sampling techniques may be skewed (Shine 1986a& b; Iverson 1990).

My data for the Frontenac Axis population were obtained both by collecting and trapping directly at communal hibernacula during spring emergence and by opportunistic captures of snakes at large during the active season. As such, one methodological objective of this chapter is to provide a direct comparison of the demographic data resulting from each technique, thereby testing the validity of each approach for population sampling. As above, I expect that human observers are more likely to detect highly visible members of the population being sampled. Reproductive males tend to be more active than females, making them more prone to detection and capture through opportunistic encounters (Weatherhead & Hoysak 1989). Similarly, older (large) black rat snakes are generally more conspicuous than young (small) individuals because of their larger body size and less cryptic body colouration (uniform shiny black vs. grey overlaid with irregular-shaped, dark-brown dorsal and lateral blotches; Ernst & Barbour 1989). Thus, males and relatively large individuals should be disproportionately captured during opportunistic encounters, a sampling method that is reliant on the conspicuousness of those being sampled.

Aside from the potential biases outlined above, it is important to recognize that these two field techniques effectively sample different spatial/ecological scales of a population. Fine-scale, hibernacula-based samples are representative of non-arbitrarily defined social groups; traditional communal hibernacula populations. By contrast, coarse-scale, opportunistic captures sample "local populations"; collections of individuals which may be members of different neighboring hibernacula *and* young individuals not yet attending hibernacula. Data collected at these two distinct scales may differ qualitatively and/or quantitatively, thereby providing contrary impressions of the demographic composition of a study population(s). Because previous studies had quantified rat snake demography at the local population scale (Fitch 1960, 1963; Stickel *et al.* 1980) I was obliged to characterize the Frontenac Axis population at this scale as well in order to make direct comparisons among these regional populations.

Another of my methodological objectives arises both from the longitudinal (14-15y) nature of the sampling effort and from the wide variety of spatially separated populations sampled. Ideally, conservation-based population monitoring programs should be biologically informative, efficient, and cost-effective. Population surveys conducted too frequently will result in redundant data, wasted time and effort, and perhaps negative effects on the study subjects (e.g., disturbance resulting in increased movement). By quantifying temporal variation in the demographic characteristics of the study populations across 14-15y I was able to assess the potential value of conducting less frequent, cross-sectional surveys. For example, I considered whether population samples taken in alternate years would be capable of detecting demographic trends that are readily apparent in data resulting from annual surveys conducted over a 15y period. Similarly, the availability of demographic data representing seven hibernacula and two distinct local populations allowed me to quantify among-population variability across the Frontenac Axis. Such among-site comparisons permit me to ask whether samples obtained from a single local population, or two hibernacula for instance, represent adequately black rat snake populations across the entire region. In addressing these issues of (ecological) scale, I sought to recommend levels of temporal and spatial resolution appropriate for monitoring black rat snake populations.

## Methods

### *Study Sites and Field Methods*

My analyses are conducted on datasets resulting from both long- and short-term capture-recapture studies of four local populations of black rat snakes distributed across the Frontenac Axis region of eastern Ontario, including those found at Murphy's Point Provincial Park, Queen's University Biological Station, La Rue Mills, and Hill Island (**Fig. 2-1**). Within each study site, two general approaches were used to capture snakes; (1) opportunistic encounters with snakes found in the field away from communal hibernacula and (2) captures made at, or in the immediate vicinity of hibernacula. For hibernacula-based captures, I employed three different techniques; drift-fences (1.5 m high barriers of plastic sheeting fixed to a wood frame of posts and rails, bottom edge of plastic buried, funnel traps at both ends of fence) established in the vicinity of hibernacula, perimeter-traps (circular drift-fences, as above, that surrounded hibernacula openings), and careful searches of the ground and trees at hibernacula.

Long-term data were gathered between 1981 and 1995 at the Queen's Station and Hill Island (part of St. Lawrence Islands National Park) study sites (ca. 40km apart) and associated hibernacula (Curtis and Lower Hill, respectively). Where appropriate, these data were used to quantify growth, sexual maturation, survival, and recruitment rates, sex-ratio, size-structure, size, and density of rat snake populations at both local population and hibernacula sampling scales. These data were also used to investigate patterns of change through time in certain population parameters.

Short-term capture-recapture programs also provided demographic data for five additional hibernacula including; Cow Island (Queen's Station), Upper Hill (Hill Island), Hydro, Silver Queen (Murphy's Point), and La Rue Mills for the period between 1992 and 1995. These data, plus those obtained from Curtis and Lower Hill, were used to quantify among-hibernacula variation. Below I provide detailed descriptions of the study areas and sampling methods applied at each.

The Queen's Station study site is a ca. 1.80km$^2$ area composed of second-growth mixed deciduous-coniferous forest, old field, pasture, lawn, wetland, buildings and a network of gravel roads and driveways on the shores of Lake Opinicon. On the basis of both radio-telemetry and recapture data (Weatherhead & Charland 1985; Weatherhead & Hoysak 1989), the study site is thought to encompass the primary activity ranges for snakes occupying the Curtis and Cow Island hibernacula (ca. 1.2km apart). From 1981-95 snakes were captured during ground and tree searches at the two hibernacula at the time of spring emergence (ca. mid-April through May for the entire Frontenac Axis region) and while the snakes were at large during the remainder of the active season. In 1992-95, I also established a perimeter-trap to capture snakes at the Curtis hibernaculum and in 1992 only, I erected a drift-fence at the Cow Island hibernaculum (sloping, rocky terrain and the presence of many large trees prevented the use of a perimeter-trap at this location).

Hill Island is a 5.60km$^2$ island covered by second-growth mixed deciduous-coniferous forest and old field habitat in the St. Lawrence River. The study area (ca. 2km$^2$) is located to the east of a two-lane paved highway which divides the island. There are ca. 30 seasonal and permanent residences distributed primarily along the shores of the island and two (low volume traffic) gravel roads that service these homes. From 1982-95 snakes were captured during ground and tree searches in the vicinity of both the Upper and Lower Hill hibernacula (ca. 1km apart) at the time of spring emergence and only rarely away from these two hibernacula during the remainder of the active season. Hibernacula openings were located during a radio-telemetry study conducted in 1984. However, traps were not established at these sites until the spring of 1987. From 1987-94 a drift fence was established at the Upper Hill hibernaculum and from 1987-95 a perimeter-trap was used at the Lower Hill hibernaculum to capture snakes dispersing in the spring. Fieldworkers from 1981-91 and 1995 consisted primarily of Parks Canada employees (3-5) and occasionally contract biologists. During the 1992-1994 field seasons I was personally responsible for most captures, supplemented by those made by Parks Canada staff.

The Murphy's Point Provincial Park study area (ca. 5.0km$^2$) is located adjacent to the northwestern shore of Big Rideau Lake. Two hibernacula were sampled at this study site; Hydro and Silver Queen (ca. 2 km apart). The surrounding habitat includes second-growth mixed deciduous-coniferous forest, old field, wetlands, a network of gravel roads, and a few seasonal residences. A seasonal campground is located on the periphery of the study area. In 1992 I established a drift-fence near the Hydro hibernaculum and in 1993-94 I used perimeter-fences (one at each of two major openings) to capture dispersing snakes. Snakes at this site were also captured during ground and tree searches. Captures at the Silver Queen hibernaculum from 1992-94 largely resulted from ground searches (few snakes use trees at this site), although in 1992 I used a perimeter-trap during the early part of the emergence period.

The La Rue Mills study site is a single hibernaculum set in a mixed coniferous-deciduous forest adjacent to a major four-lane highway (Hwy 401), on properties managed by the St. Lawrence Parks Commission. From 1992-94 I used a drift-fence and ground and tree searches to capture snakes during emergence.

Snakes captured at all sites were processed in a similar manner, though different fieldworkers were responsible for data collection in different years. Upon capture, individual snakes were sexed by gently probing for the presence of hemipenes, measured for snout-vent length (SVL) to the nearest mm with a metric tape, weighed to the nearest g with a calibrated spring-scale (except at Hill Island from 1982-91 and 1995), marked by heat-branding the caudal scutes in a unique numeric pattern from 1981-91 or by PIT-tagging (Germano & Williams 1993) after 1991 (except at Murphy's Point where PIT-tagging was not begun until 1994), and released as soon as possible (usually minutes after, and always within 24 h of capture) at the point of capture. For purposes associated with other aspects of my research on black rat snakes I, also collected data on body temperatures with a cloacal thermometer and obtained a small volume of blood from caudal blood vessels from several individuals during 1991-94 only.

### *Data Analyses*

Long and short-term fieldwork across the Frontenac Axis study area between 1981 and 1995 collectively resulted in a total of 1128 captures (including recaptures) from Murphy's Point (174 captures, 1992-94), Queen's Station (551 captures, 1981-95), La Rue Mills (65 captures, 1992-94), and Hill Island (338 captures, 1982-95).

Before conducting formal analyses I considered the possibility that some of my data may contain systematic errors as a function of the field methods employed. In particular, I assessed the potential for error associated with the measurement of SVL. Lengths of live, large-bodied snakes (including members of the genus *Elaphe*) are notoriously awkward to measure, yet this is the most common form of size measurement in field studies (Fitch 1987). If field measurements of SVL exhibit low repeatability (i.e., if estimates of individual SVLs are highly imprecise) then their value as an index of the relative size of individuals is diminished. Thus, I quantified measurement error associated with my estimates of SVL for black rat snakes using data for 60 individuals (ranging in size from 450-1600mm SVL) for which I had two replicate measurements. Replicates were obtained as follows: with the aid of an assistant holding the snake, a flexible measuring tape was run along the snake's ventral surface from the tip of the rostrum to the posterior edge of the anal plate. The snake was then allowed to relax and the measurement was repeated within 5 min. Following Yezerinac *et al.* (1992), I estimated measurement error using the following formula:

$$\textbf{\% Measurement Error} = [S^2 \textbf{ within}/(S^2 \textbf{ within} + S^2 \textbf{ among})] \times 100$$

where $S^2$ within is the within-individual variance component and $S^2$ among is the among-individual variance component resulting from an analysis of variance (ANOVA, individuals as treatments). I calculated measurement error to be less than 1% (0.809) for my SVL values, suggesting that measurements of SVL for my study population are highly reliable estimates of the relative size of individuals.

I used recapture records from the Queen's Station study population to estimate growth rates as a linear function of body length separately for female and male black rat snakes (Van Devender 1978). Changes in length ($\Delta$SVL) and growth interval ($\Delta$T) for recaptured snakes were used to calculate growth rates (GR) as follows:

$$\mathbf{GR = \Delta SVL/\Delta T,}$$

where ΔSVL equals the SVL growth increment measured in mm and ΔT equals the number of days elapsed between captures less 215 days (ca. 7 mo) of hibernation per year (after Weatherhead 1989), under the assumption that snakes did not grow during this period. I then generated equations to describe male and female growth rates as a function of initial SVL using simple linear regression, i.e.,

$$\mathbf{GR = a - bSVL,}$$

where a and b represent the intercept and the slope of the regression line, respectively. Because I expected that snakes in my study population might exhibit relatively slow grow rates, and in order to maximize my ability to detect limited growth, I only included snakes for which the inter-capture interval exceeded 150 days (or 1 growing season). Estimated growth rates for males and females were compared using analysis of covariance (ANCOVA).

In order to provide some measure of the importance of environmental factors in shaping life-history strategies, I sought to quantify differences in growth rates (and other demographic traits) between populations inhabiting harsh vs. relatively benign climates. To do this I obtained raw recapture records from a study of black rat snakes conducted by Stickel *et al.* (1980) at the Patuxent Wildlife Research Center in Maryland (L. Garrett - pers. commun.) Using these data and the methods described above, I developed independent growth models for male and female rat snakes for this Maryland population. Stickel *et al.* (1980) regard the principal active period for rat snakes in

Maryland to encompass the 5 mo from 1 May to 30 September (i.e., equivalent to Ontario). However, Maryland is located ca. 600km south of the Frontenac Axis resulting in an 58-day difference in the annual frost-free period (mean frost-free period = 200d for Baltimore, MD vs. 142d for Ottawa, ON). As such, I reasoned that the Patuxent population would experience a relatively favorable environment. Therefore, I predicted that rat snakes in Ontario should exhibit slow growth rates relative to those in Maryland. As above, estimated growth rates of males and females and the two populations were compared using ANCOVA.

I had no data on individuals of known age (e.g., marked neonates followed through time). Therefore, in order to estimate age of maturity and longevity, and thus apply these values to my interpretation of demography and life history, it was necessary to generate an equation relating age to body size (SVL). Therefore, independent models of age relative to SVL were derived for females and males by integrating the growth rate equations above, i.e.,

$$\mathbf{Age\ (Years) = (1/b)\ LN(a + bSVL) + c}$$

where a and b are defined as above (both derived from the initial growth rate equation), and c is a constant of integration (Van Devender 1978). For both males and females this constant was derived by correcting the equation to ensure that neonates were aged "0 y". Neonate size was calculated to be ca. 255mm SVL based on the mean size of 30 (unsexed) hatchlings (representing 6 litters) from the study population. (There is no evidence that the sexes differ in size at hatching in *Elaphe obsoleta*; Fitch 1963; Clark & Pendleton

1995; S. Mullin - pers. comm.). Using the equation above, I estimated the age of males and females from the size of neonates through to maximum size at 10mm increments.

I assessed the accuracy with which the growth models predicted a snake's age based on its SVL by using the model-estimated ages to estimate the elapsed time between two successive captures of an individual and then comparing this estimate with the known elapsed time. In cases where a model is highly accurate one would expect to see close agreement between the estimated elapsed time (predicted age) and the known elapsed time (actual age).

To assess whether black rat snakes exhibit a decline in growth following maturation I determined differences in growth rates between females and males and between immature and mature reproductive-classes using analysis of variance (ANOVA). Both free-ranging and captive-raised male and female black rat snakes are considered to be sexually mature once they have reached ca. 900 and 1000mm SVL, respectively (Fitch 1963; G. Blouin-Demers - pers. comm.). In the absence of field data on minimal size at maturity in my study population I adopted the same size criteria and accordingly classified males ≥ 900 and females ≥ 1000mm SVL as being sexually mature. These values represent ca. 59 and 68% of maximum SVL recorded for males and females, respectively in the Frontenac Axis. Since most snakes mature at 60-75% of their maximum length (Parker & Plummer 1987), my minimum sizes of maturity are in general agreement with the patterns described for other species and populations.

In order to quantify demographic structure in the study populations it was useful to organize individual males and females into size-classes as follows; (1) < 900mm SVL, (2) ≥ 900 and < 1100mm SVL, (3) ≥ 1100 and < 1300mm SVL, and (4) ≥ 1300mm SVL. My use of these four size-classes represents a compromise between the number of categories (classes) and the need to retain adequate sample sizes for contingency table analyses. Furthermore, this classification allowed a direct comparison with data available from other studies (Fitch 1960, 1963; Stickel *et al.* 1980). Indices of the sex-composition were made by calculating breeding (adult) sex ratios, defined as the percent of females (i.e., ≥ 1000mm SVL) among the sample of sexually mature individuals.

I used hibernacula-based data from Queen's Station and Hill Island, for the period between 1988-95, to quantify the size distribution of recruits and recruitment rates. By restricting my analysis of recruitment patterns to this period (6-7y after the initial mark-recapture studies began) I ensured that the majority of new snakes captured during this interval were indeed new recruits and not simply members of the hibernacula that had yet to be sampled.

To test for inherent biases in my sampling methods I looked for evidence of sex- and/or size-biased catchability in opportunistic vs. hibernacula-based samples by comparing the latency to recapture (time between first and second capture minus days of hibernation) for females and males. If relative catchability was a function of sex, size, or some interaction between these two variables, then I expected that those snakes recaptured early would, on average, differ in one or more of these respects from those recaptured at a later date. In addition to identifying potential sampling biases, such comparisons also provide a direct contrast of demographic samples obtained from different spatial/ecological scales (i.e., local populations =

opportunistic captures vs. hibernacula populations = hibernaculum-based captures). By describing discrepancies between the two I can assess the relative efficacy of each technique for population surveys.

I used the Jolly-Seber capture-recapture model A to estimate local population sizes and survival rates from my annual capture data by employing the software JOLLY (Pollock *et al.* 1990). Among the several models available in this program I chose to use Model A because it is a conservative estimator, allowing for both death and immigration.

I also employed logistic regression to confirm results derived via the Jolly-Seber model regarding sources of variation in survival. In particular, I tested for evidence of differential survivorship based on sex and body size (SVL). For the logistic regression analyses, survival was represented as a binary response variable (survive = 1 vs. presumed dead = 0), with sex and body size serving as predictor variables. Individual snakes were presumed dead if they were not recaptured within 2y of initial capture. This time allowance provided a conservative estimate of actual survival since preliminary analyses demonstrated that only ca. 2% of known survivors eluded recapture for more than 2y following initial capture at hibernacula.

Statistical analyses were conducted using either JMP 3.0 (SAS Institute 1994) and STATVIEW II (Abacus Concepts 1990) software packages on a Macintosh computer. All proportional data were arcsine-transformed before analyses (Sokal & Rohlf 1981). All means are reported with standard error estimates.

## Results

### *Growth and Age Determination*

Growth rates (mm SVL/d) declined with increasing body size (SVL) for males and females in both the Frontenac Axis (Ontario) and Patuxent (Maryland) populations. In each case, the relationship between growth rate and SVL was best described by simple linear regression (Frontenac Axis males $y = -0.0006x + 0.9861$, $r^2 = 0.315$, $F = 28.08$, $P < 0.0001$, N = 63 and females $y = -0.001x + 1.2284$, $r^2 = 0.451$, $F = 37.86$, $P < 0.0001$, N = 48; Patuxent males $y = -0.001x + 1.641$, $r^2 = 0.665$, $F = 97.62$, $P < 0.0001$, N = 51 and females $y = -0.001x + 1.748$, $r^2 = 0.886$, $F = 203.61$ $P < 0.0001$, N = 28 ; **Fig. 2-2**). I expect that the "negative growth rates" exhibited by some individuals in both populations (see **Fig. 2-2**) actually represent instances of measurement error (assuming that they did not shrink between captures). Nevertheless, I elected not to remove these negative errors since I was unable to identify and eliminate the "positive errors" that should presumably be equally prevalent. Thus, deleting the negative errors alone would have biased my datasets.

To compare growth rates between males and females within each population I first conducted an analysis of covariance (ANCOVA) in which growth rate was the variable under consideration, sex was the treatment factor, and SVL was the covariate. For the Frontenac Axis, the growth rate versus SVL relationship did not differ between the sexes (ANCOVA - sex*SVL interaction, $F = 3.46$, df = 1, 107, $P = 0.065$; **Fig. 2-2**). When I removed the interaction term from the ANCOVA model and simply considered the main effects I found support for the expectation that males grow significantly faster than females (least squares mean = 0.348 ± 0.024 vs. 0.222 ± 0.028mm/d, respectively; ANCOVA - $F$ = 10.91, df = 1, 108, $P$ = 0.0013). Similarly, the relationship between growth rate and SVL did not differ between the sexes at Patuxent (ANCOVA - sex*SVL interaction, $F$ = 2.05, df = 1, 75, $P$ = 0.156). Growth in males and females declined at an equal rate with increasing body size. As with the Frontenac Axis population, the reduced ANCOVA model demonstrated that males grow significantly faster than females at Patuxent (least squares mean = 0.359 ± 0.017 vs. 0.203 ± 0.024mm/d, respectively; ANCOVA - $F$ = 24.13, df = 1, 76, $P$ < 0.0001, **Fig. 2-2**).

To determine whether growth rates of rat snakes at Patuxent exceeded those from the Frontenac Axis, I conducted separate ANCOVAs for males and females. In each case, growth rate was response variable, population was the treatment variable, and SVL served as the covariate. For males, I restricted the analysis to a sample of snakes ranging in size from 1000-1400mm SVL in order to ensure that the distribution of body sizes in each population was roughly equivalent. The initial ANCOVA for males indicated that the relationship between growth rate and SVL did not differ significantly between populations ( ANCOVA - population*SVL interaction, $F$ = 0.14, df = 1, 68, $P$ = 0.709). As a result, I constructed a reduced model by eliminating the interaction term and considered the main effects alone. As predicted, males from Patuxent were found to grow significantly faster than those from the Frontenac Axis (least squares mean = 0.354 ± 0.035 vs. 0.228 ± 0.0265mm/d, respectively; ANCOVA - $F$ = 16.54, df = 1, 69, $P$ = 0.0001, **Fig. 2-3**). As with males, I used a restricted dataset in my comparison of female growth rates (size range = 900-1300mm SVL). The association between growth rate and SVL did not differ significantly between Patuxent and the Frontenac Axis (ANCOVA -

population*SVL interaction, $F = 0.084$, df = 1, 46, $P = 0.773$). Therefore, I removed the interaction term and simply tested for the significance of the main effects. Consistent with my result for males, growth rates for females from Patuxent were significantly higher than those from the Frontenac Axis (least squares mean = 0.410 ± 0.041 vs. 0.142 ± 0.029mm/d, respectively; ANCOVA - $F = 40.04$, df = 1, 47, $P < 0.0001$, **Fig. 2-3**).

It is noteworthy that growth rates of rat snakes appear to be much more variable in Ontario than in Maryland (see **Fig. 2-2 & 2-3**). Though not one of my original predictions, I tested whether the variance in growth rates was higher in Ontario by comparing residuals derived from the growth rate versus size regressions for each of the two populations (analysis separate by sex). Among females from Ontario and Maryland, variance about the line of best fit was significantly higher for Ontario (Levene's Test; $F = 9.54$, $P = 0.003$, **Fig. 2-3**). For males, there was no detectable difference (Levene's Test; $F = 0.19$, $P = 0.664$, **Fig. 2-3**).

A decline in growth rate following sexual maturation is not uncommon in snakes (Parker & Plummer 1987; Houston & Shine 1994; Madsen & Shine 1994). However, Stickel *et al.* (1980) provide some evidence of continued rapid growth for several years after maturity in female black rat snakes from Maryland. I used ANCOVA to investigate the association between growth rate and reproductive status (i.e., immature vs. mature), while controlling for SVL, in the Frontenac Axis population. (Too few records of growth rates of immature individuals from Patuxent precluded a similar analysis of that population). My initial ANCOVA model detected a marginally significant interaction between sex and reproductive status (ANOVA - sex*reproductive status, $F = 3.54$, df = 1, 106, $P = 0.062$), implying that the relation between growth rate and reproductive status differs for males and females. Relative to males, females appear to experience a rather abrupt decline in growth rate following reproductive maturity (least squares means for females = 0.342 ± 0.050 immature vs. 0.146 ± 0.036mm/d mature and least squares means for males = 0.358 ± 0.082 immature vs. 0.335 ± 0.028mm/d mature). When I assessed variation in growth rate associated with reproductive status for each sex independently I found a significant effect in females (ANCOVA - $F = 4.63$, df = 1, 47, $P = 0.036$), whereas the effect in males was non-significant (ANCOVA - $F = 0.01$, df = 1, 62, $P = 0.900$). Between immature and mature reproductive stages, growth rates declined by 57.30% in females and only 2.3% in males.

Because male and female rat snakes grow at different rates, growth models were developed separately for each sex (**Fig. 2-4**). The resulting models suggest that males in the Frontenac Axis population reach maturity (900mm SVL) at an average age of 7.13y, whereas females mature (1000mm SVL) at ca. 9.44y. However, differential rates of growth among individuals within each sex mean that there is considerable variation in the age at which snakes in this population reach sexual maturity (see **Fig. 2-2**). In the Patuxent population, males and females mature at ca. 3.86 and 5.17y, respectively (growth model not shown). Growth rates calculated for the Frontenac Axis also suggest that some individuals (both males and females) in this population may be capable of reaching 30+ y (**Fig. 2-4**). By comparison, maximum longevity for rat snakes at Patuxent is estimated to be 20+ y.

To examine the accuracy with which my growth models predict a snake's age based on body size, I contrasted model-estimated ages (predicted ages) and "actual ages" of individuals at second capture. Actual ages were derived using the growth model to predict the age of an individual at the time of initial capture ($t_1$) and then adding to this value the number of days elapsed (less days in hibernation) to second capture ($t_2$) (i.e., actual age = predicted age at $t_1$ + no. days elapsed to $t_2$). Were my growth models highly accurate I expected to find little difference between the actual age of an individual (at $t_2$) and that predicted by the model based on SVL (at $t_2$). For males and females in both populations, I found a significant difference between the actual and predicted ages (Paired *t*-values = 7.69, 5.38 for males and 3.15, 3.98 for females at Frontenac Axis and Patuxent, respectively, all *P*'s $\leq 0.0016$). For males and females in each population, actual ages were greater than predicted ages, suggesting that my growth models underestimate age based on SVL. The mean difference between actual ages and predicted ages ranged from 1.52 (Frontenac Axis females) to 2.96y (Maryland males).

### *Composition of Hibernacula Populations*

Spring capture records provided the data used to quantify the structure of rat snake hibernacula populations across the Frontenac Axis. All hibernacula were occupied primarily by sexually mature adults (overall mean = 76.30 ± 2.53% mature, **Table 2-1**), though the range in the proportion of adults sampled in these aggregations was quite broad (43-100%).

The overall distribution of size-classes differed significantly among the seven hibernacula I studied ($\chi^2 = 78.27$, df = 18, $P < 0.0001$). Among these, Curtis and Lower Hill represent the most reliable samples, because most captures at these sites were obtained by perimeter-traps. These two sites had a similar overall distribution of size-classes ($\chi^2 = 4.37$, df = 3, $P = 0.224$, **Table 2-2**). The pattern of size-structure at each was one of relatively few individuals in both the smallest (< 900mm SVL) and largest (≥ 1300mm SVL) size-classes. Very few snakes under 600mm SVL (i.e., neonates to 3 year-olds) were ever captured at the Curtis or Lower Hill hibernacula (1.7-4.7%, respectively). Clearly, individuals in this size-class must be more common than these values suggest, otherwise there would be no source for the larger individuals that are regularly recruited into these hibernacula (see below & **Table 2-2**). The probable explanation for this finding is that most rat snakes under 3y of age do not attend the traditional communal hibernacula I studied. From this perspective, hibernacula populations must be regarded as non-random samples of the general (local) population, since they tend not to include the very youngest individuals.

The sex-composition of hibernacula populations, measured as breeding sex ratio, was highly variable, and both male- and female-biases were observed (range = 0-69% females, **Table 2-1**). I had sufficient data to test for differential sex ratios across size-classes within two rat snake hibernacula on the Frontenac Axis. My expectation was that males, the faster growing sex, should predominate the adult age-classes. Sex ratios did differ significantly among size-classes at Curtis ($\chi^2 = 14.04$, df = 3, $P = 0.0028$), with females being under-represented in the largest size-class (≥ 1300mm SVL). Males also predominated the large size-classes at Lower Hill as well, although overall sex ratios differences were not significant ($\chi^2 = 4.01$, df = 3, $P = 0.259$). While such unequal sex ratios could come about simply due to the disproportionate accumulation of the faster growing sex (i.e., males) in the larger size-classes, differential survival of males and females (i.e., higher mortality rates for females) could also contribute to this disparity.

The size-distribution of recruits (i.e., individuals participating in hibernacula for the first time) differed significantly from that of those already attending the Curtis and Lower Hill hibernacula ($\chi^2 = 44.16$, df = 3, $P < 0.0001$, $\chi^2 = 73.63$, df = 3, $P < 0.0001$, **Fig. 2-5**). Relative to recaptured individuals, recruits tended to be smaller and thus, younger. For example, individuals < 900mm SVL comprised 36 and 41% of recruits vs. 7 and 3% of recaptures at the Curtis and Lower Hill hibernacula, respectively. Recruits tended to be male (i.e., 52.46 - 58.62% males), although the sex-bias was non-significant at both Curtis and Lower Hill (Fisher's Exact Test, $\chi^2 = 0.149$, df = 1, $P = 0.401$ and $\chi^2 = 5.545$, df = 1, $P = 0.993$). Individuals captured for the first time at Curtis and Lower Hill averaged 926 and 964mm SVL, respectively. However, it is likely that some individuals may have been erroneously classified as recruits. That is, some "recruits" had undoubtedly been attending these hibernacula for one or more years but had simply managed to elude capture, in which case their inclusion in the calculation of the mean size of recruits would have resulted in an overestimate of mean body size. Individuals between 500 and 700mm SVL (2-4y), comprising ca. 10% of hibernacula populations, are probably more representative of the size of true recruits.

One way in which larger individuals could be true recruits would be if some individuals move between hibernacula. To examine this possibility, I estimated the degree to which the hibernacula I studied exhibited consistent (faithful) adult membership. In particular, I considered whether relatively large recruits might be predominated by highly active or transient males (Weatherhead & Hoysak 1989) that wander among hibernacula. While the majority of recruits were male at both Curtis and Lower Hill (52 and 59%, respectively), "large recruits" ($\geq$ 1100mm SVL) were no more likely to be male than were "small recruits" (< 1100mm SVL) at either site (Fisher's Exact Test,

$\chi^2 = 0.682$, df = 1, $P = 0.430$ and $\chi^2 = 1.13$, df = 1, $P = 0.385$). Estimates of site fidelity based upon multiple captures of individuals suggest that most individuals are quite faithful to particular hibernacula. Out of 473 recaptures made at adjacent pairs of hibernacula at Queen's (i.e., Curtis/Cow Island - 1.2 km apart) and Hill Island (i.e., Lower Hill/Upper Hill - 1 km apart), only 6 (1.26%) were cases in which individuals had switched hibernacula in successive years. Each of the six exchanges I documented occurred between Lower Hill and Upper Hill, all the snakes were > 900mm SVL (i.e., probably sexually mature), and five of them were males. Overall, these data indicate that rat snakes on the Frontenac Axis exhibit high hibernaculum site fidelity, but that males may be somewhat less site-faithful than females. Thus, it would appear that true recruits are predominantly younger individuals (2-4y old) entering hibernacula for the first time but that, assessments of the mean size of recruits may be inflated by the mis-classification and inclusion of relatively large and previously unmarked individuals.

In addition, I estimated annual recruitment rates at hibernacula as the number of unmarked snakes relative to the total number of snakes captured each year. Recruitment at Curtis and Lower Hill averaged less than 50% of the entire sample each study year (mean = 33.30 ± 9.19 and 42.43 ± 4.00%, respectively). Because the majority of recruits are between 2-4y, annual recruitment is largely representative of reproduction (and survival) within the local population from several years previous.

### *Composition of Local Populations*

I quantified the size- and sex-composition of local populations by pooling samples from captures made in the vicinity of neighboring hibernacula and opportunistic captures obtained from the surrounding area (i.e., active season range ca. 2km radius from hibernacula). I chose to characterize rat snake demography at this scale as well, because in the absence of knowledge of the exact location of hibernacula opening(s), or in cases where trapping is not practical (e.g., due to terrain), this is the spatial scale at which many snake ecology studies are conducted (e.g., Fitch 1963; Stickel *et al.* 1980). As such, I was able to compare my results directly with those obtained by others. Furthermore, the longitudinal nature of these data (14-15y) allowed me to assess both temporal variability in demographic characteristics and the potential value of cross-sectional surveys.

As with hibernacula samples, I used capture records to quantify local population structure at separate sites on the Frontenac Axis. Sexually mature adults (males and females combined) predominated local population samples at both Queen's Station and Hill Island across all study years (1981-95), averaging 71.83 ± 1.61 and 76.26 ± 3.01% of all snakes captured, respectively. In addition, both populations exhibited significant annual trends in the percent of adults sampled, though the trends were in opposite directions (**Fig. 2-6**). Queen's showed an increase in the percent of adults present between 1981 and 1995 ($r = 0.560$, $P = 0.029$), while Hill Island displayed a decrease over the same time period ($r = -0.591$, $P = 0.033$). Why should neighboring populations of rat snakes exhibit such contradictory patterns? I tested the possibility that opposing trends in juvenile recruitment at Queen's and Hill Island over the study period were responsible for this phenomenon. That is, a decline in recruitment at Queen's and an increase at Hill Island may have contributed to such patterns. Contrary to this expectation, correlation analysis was unable to detect any significant relationship between recruitment rate and sampling year at either site (r = -0.534, $P$ = 0.172 and r = 0.428, $P$ = 0.289 for Queen's and Hill Island, respectively). Although the mechanism remains uncertain my results highlight the fact that local rat snake populations are not static with respect to age-structure and that neighboring populations can exhibit very different demographic patterns.

Because among-year variation in age-structure seems to be the norm for these two local populations, relatively fine-scale temporal monitoring (i.e., annual) would appear to be necessary to detect subtle, though significant, longitudinal patterns. In order to test the sensitivity of these results to sampling frequency I eliminated alternate years from both datasets, thereby simulating semi-annual monitoring schedules. As expected, under these hypothetical schedules I was no longer able to detect significant relationships between the percent mature and study year for either population (e.g., r = 0.647, $P$ = 0.0823 and r = -0.514, $P$ = 0.237 for Queen's and Hill Island, respectively).

Expressed as a ratio of adults to sub-adults, the Queen's population averaged 2.7:1 (range = 1.92:1 - 4.17:1), whereas Hill Island averaged 3.5:1 (range = 1.33:1 - 8.50:1). Stickel *et al.* (1980) estimated this ratio to be 5.0:1 for a Maryland population of black rat snakes. I used data presented by Fitch (1961, 1963) to calculate an adult to sub-adult ratio of 1:1.33 for his Kansas study population.

Although there was a statistically significant difference in the overall size-class distribution between Queen's and Hill Island ($\chi^2$ = 12.09, df = 3, $P <$ 0.0071), these two local populations exhibited very similar patterns of size-structure (**Fig. 2-7**). Both populations were characterized by a steady increase in the percentage of individuals in size-classes 1 through 3 (up to 1300mm

SVL), followed by a sharp decline in the percentage in size-class 4 (≥ 1300mm SVL). In contrast, comparisons between populations at a more coarse geographic scale yielded major differences in size-structure. I found striking differences between the two Ontario populations on one hand, and those from Kansas and Maryland on the other ($\chi^2 = 379.27$, df = 9, $P < 0.0001$, **Fig. 2-7**). In particular, small snakes (≤ 900mm SVL) predominated in the Kansas population (Fitch 1961, 1963), while the Maryland population (Stickel *et al.* 1980) had an abundance of large (≥ 1000 - < 1300 mm SVL) and very large (≥ 1300 mm SVL) individuals (**Fig. 2-7**).

In order to characterize the sex composition of local populations and determine whether the relative proportion of females to males varied among years, I calculated breeding sex ratios for both Queen's and Hill Island. Sex ratios in both populations tended to be male-biased (mean = 38.84 ± 1.90 and 46.42 ± 2.37 % females, respectively), as expected if primary sex ratios are 50:50 and males mature at a younger age. Interestingly, Queen's exhibited an increase in the proportion of females present over the 15y study period (r = 0.829, $P = 0.0001$), whereas no significant relationship was detected between the proportion of females and sample year at Hill Island (r = 0.470, $P = 0.0895$; **Fig. 2-8**). What might be the cause for a positive trend in the percentage of females at Queen's? One proximate cause may be that the female recruitment rates increased (or conversely male recruitment decreased) over the study period. However, female recruitment appears to have declined significantly through time at Queen's (r = -0.785, $P = 0.036$), while the relationship between male recruitment and sampling year was found to be non-significant (r = 0.166, $P = 0.692$). Thus, sex-specific patterns of recruitment do not appear to explain the sex ratio trend I detected in the Queen's population.

At Queen's, the longitudinal increase in the percent of females was relatively strong, implying that a less frequent sampling schedule would illustrate the same trend. Indeed, when I eliminated alternate years from the Queen's dataset, to simulate semi-annual surveys, the result remained qualitatively unchanged ($r = 0.865, P = 0.005$). By contrast, sex ratios at Hill Island were far more variable among years and a simulated semi-annual sampling regime resulted in a non-significant relationship ($r = 0.459, P = 0.299$).

Within each of the Ontario, Maryland, and Kansas populations, sex ratios differed significantly across size-classes (Queen's - $\chi^2 = 57.79$, df = 3, $P < 0.0001$; Hill Island - $\chi^2 = 27.99$, df = 3, $P < 0.0001$; Maryland - $\chi^2 = 56.26$, df = 3, $P < 0.0001$; Kansas - $\chi^2 = 24.26$, df = 3, $P < 0.0001$). The pattern at Queen's, Hill Island, and Maryland was that of proportionately fewer large females while in the Kansas population females were under-represented in the smallest size-class (**Table 2-3**).

### *Sampling Bias: Opportunistic vs. Hibernacula-based Captures*

To compare the reliability of hibernacula vs. opportunistic capture samples, I first asked whether a snakes' sex and/or body size (mm SVL) might influence its susceptibility to capture by either of these two sampling methods. Accordingly, I contrasted the latency to recapture (measured as no. active days) for females and males across a range of sizes within both hibernacula and opportunistic samples. If catchability is a function of sex, size, or some interaction between these two variables, I expected that snakes recaptured sooner would, on average, differ in one or more of these respects from those recaptured at a later date.

ANCOVA of opportunistic samples made at Queen's indicated that the latency to recapture vs. SVL relation did not differ between the sexes (ANCOVA - sex*SVL interaction, *F* = 0.226, df = 1, 128, *P* = 0.634). Therefore, I removed this interaction from the final model and assessed the significance of the main effects. This analysis indicated that inter-capture intervals were significantly longer for smaller snakes (ANCOVA - *F* = 4.319, df = 1, 129, *P* = 0.039) and females (ANCOVA - *F* = 4.666, df = 1, 129, *P* = 0.032, least squares mean latency = 222.26 ± 16.39 and 174.14 ± 14.29 days for females and males, respectively), suggesting that estimates of local population structure based on opportunistic samples alone may be somewhat biased toward relatively large snakes and males. However, unlike Queen's, opportunistic captures on Hill Island did not exhibit a size- or sex-bias (reduced ANCOVA model; size effect - *F* = 0.007, df = 1, 22, *P* = 0.932 and sex effect - *F* = 0.474, df = 1, 22, *P* = 0.498, least squares mean latency = 301.29 ± 66.55 and 239.05 ± 58.72 days for females and males, respectively), though this may have been due principally to a limited sample size (N = 25). Relative to Queen's, typically fewer person-hours were spent in the field at Hill Island and less effort was made in capturing snakes after spring emergence. As such, opportunistic captures (and subsequent recaptures) of the Hill Island population were relatively infrequent.

In contrast to opportunistic captures, hibernacula-based samples were not sex- and/or size-biased. Latency to recapture did not differ significantly between the sexes (reduced ANCOVA model; *F* = 1.872, df = 1, 37 *P* = 0.179, least squares mean latency = 143.33 ± 17.86 vs. 110.62 ± 15.27 days for females and males, respectively) or vary with size (*F* = 0.848, df = 1, 37 *P* = 0.362) in samples collected at the Curtis hibernaculum. I obtained a similar result when testing for these effects using a dataset obtained from the Lower Hill hibernaculum (reduced ANCOVA model; $F = 0.149$, df = 1, 41 $P = 0.861$, least squares mean latency = 176.87 ± 13.23 vs. 118.94 ± 13.23 days for females and males, respectively). This analysis shows that sampling at hibernacula is more representative of who is in the hibernaculum, but not necessarily the local population (see below).

With these biases in mind, I compared the demographic characteristics described by the two sampling methods for the Queen's and Hill Island populations. Data from both Queen's and Hill Island supported the expectation that capture-method biases can lead to important discrepancies in the (inferred) demographic attributes of sampled populations. Contingency table analysis identified a significant difference in the size-structure resulting from opportunistic vs. hibernacula-based samples obtained at Queen's ($\chi^2 = 90.46$, df = 3, $P < 0.028$, **Fig. 2-9**). Analysis of the Hill Island datasets also identified a significant difference in size-structure between opportunistic and hibernacula samples ($\chi^2 = 16.24$, df = 3, $P < 0.001$, **Fig. 2-9**).

The absence of a size-bias in hibernacula-based samples implies that data obtained for both Curtis and Lower Hill accurately reflect the size-structure of these hibernacula. In short, hibernacula are in fact dominated by adults. Nevertheless, these samples (**Fig. 2-9**) must be viewed as imperfect representations of local population structure since the smallest snakes generally do not to attend hibernacula (see *Composition of Hibernacula Populations*). Likewise, opportunistic samples (at least at Queen's) show a marked discrimination against small snakes, suggesting that they too provide a biased illustration of the size-structure of local populations. Therefore, I expect that a more accurate image of the size-structure of local populations would be realized through an increase in the percentage of small snakes (size-class 1) and a corresponding decrease the percentage of large snakes (size-class 4). The result would be a size-class distribution that is positively skewed to the left (**Fig. 2-9**). Similarly, because opportunistic samples may be biased toward males, females are probably more prevalent in local populations than such data imply (i.e., > 39% - see *Composition of Local Populations*). By contrast, sex ratios of hibernacula samples appear to be accurate reflections of the sex-composition of hibernacula populations, because such samples are not sex-biased (see *Composition of Hibernacula Populations*).

### *Survival Rates and Population Size Estimates*

In order to assess the importance of both sex and body size (SVL) as a source of variation in survival, I used logistic regression analysis. For this analysis, survival was treated as a binary response variable (survive = 1 vs. presumed dead = 0), and sex and SVL were entered as predictor variables. I used perimeter-trap captures (Curtis and Hill Lower) gathered between 1992-95 since such samples did not exhibit sex- or size-biases in catchability (see *Sampling Bias* above). Individual snakes were presumed dead if they were not recaptured within 2y of initial capture. This time allowance provided a conservative estimate of actual survival since only ca. 2% of known survivors eluded recapture for more than 2y following initial capture. The full logistic model indicated that the relationship between survival and SVL did not differ between males and females (sex*SVL, G = 0.339, df = 1, $P$ = 0.560). As such, I removed this interaction from the final logistic model and assessed the significance of the main effects. The reduced logistic regression analysis failed to detect a significant difference in the survival probabilities between males and females overall (G = 0.176, df = 1, $P$ = 0.674). Nevertheless, body size did prove to be a significant predictor, indicating that individuals realize improved survival prospects with increasing SVL (G = 5.521, df = 1, *P* = 0.018). That is, small, young snakes suffer greater mortality than do larger, older individuals.

I used Jolly-Seber models to estimate annual survival rates (sexes pooled) based on capture records for local populations. Annual survival probabilities at Queen's Station and Hill Island averaged 0.714 ± 0.022 and 0.651 ± 0.077, respectively. Neither population exhibited a significant longitudinal trend over the 14-15y study period (Queen's, r = 0.173, *P* = 0.570 and Hill Island, r = -0.154, *P* = 0.669; **Fig. 2-10**). However, there was a notable correlation in annual survivorship estimates *between* the two populations (r = 0.654, *P* = 0.040).

I also used Jolly-Seber models to calculate annual population size indices for Queen's and Hill Island. Estimates for each population averaged 69.11 ± 4.86 and 46.31 ± 9.55, respectively. Neither population exhibited any significant long-term trend (Queen's, r = -0.276, *P* = 0.360 and Hill Island, r = -0.339, *P* = 0.279; **Fig. 2-11**). However, as with survival rates, I found a highly significant correlation in the annual population size estimates for these two demographically independent local populations (r = 0.784, *P* = 0.002). It should be emphasized that these estimates are relative indices of population size that have limited value for absolute comparisons. This is because the two study sites encompass tracts of differing area. Direct comparisons of estimated mean relative densities (Queen's Station = 3.83 snakes/ha and Hill Island = 2.31 snakes/ha) may be more appropriate.

**Table 2-1. Population structure of black rat snake hibernacula across the Frontenac Axis.**

| **Locality**<br>*Hibernaculum* | Year | Total individual captures | Percent mature | Breeding sex ratio (% females) |
|---|---|---|---|---|
| **Murphy's Point** | | | | |
| *Silver Queen* | 1992 | 15 | 86.67 | 53.84 |
| | 1993 | 11 | 81.82 | 33.33 |
| | 1994 | 16 | 62.50 | 40.00 |
| *Hydro* | 1992 | 30 | 83.33 | 56.00 |
| | 1993 | 42 | 90.48 | 55.26 |
| | 1994 | 60 | 83.33 | 52.83 |
| **Queen's Station** | | | | |
| *Cow Island* | 1992 | 12 | 75.00 | 55.55 |
| | 1993 | 7 | 100.00 | 57.14 |
| | 1994 | 11 | 90.91 | 30.00 |
| *Curtis* | 1992 | 27 | 62.91 | 47.05 |
| | 1993 | 23 | 69.57 | 56.25 |
| | 1994 | 34 | 82.35 | 46.42 |
| | 1995 | 21` | 80.95 | 35.29 |
| **La Rue Mills** | | | | |
| *La Rue Mills* | 1992 | 13 | 84.62 | 27.27 |
| | 1993 | 25 | 72.00 | 44.44 |
| | 1994 | 29 | 65.52 | 36.84 |
| **Hill Island** | | | | |
| *Upper Hill* | 1992 | 7 | 57.14 | 50.00 |
| | 1993 | 13 | 76.92 | 20.00 |
| | 1994 | 4 | 75.00 | 0.00 |
| | 1995 | 7 | 42.86 | 66.66 |
| *Lower Hill* | 1992 | 19 | 73.68 | 42.85 |
| | 1993 | 21 | 85.71 | 50.00 |
| | 1994 | 20 | 80.00 | 68.75 |
| | 1995 | 25 | 68.00 | 64.70 |

**Table 2-2. Percentage (sample size) size-class distribution of hibernacula samples from the Frontenac Axis.**

| | Hibernacula | |
|---|---|---|
| Size-class (mm SVL) | Curtis | Lower Hill |
| < 900 | 22.41 (26) | 16.47 (14) |
| ≥ 900 and < 1100 | 25.00 (29) | 38.82 (33) |
| ≥ 1100 and < 1300 | 38.79 (45) | 36.47 (31) |
| ≥ 1300 | 13.79 (16) | 8.24 (7) |

**Table 2-3. Comparison of sex ratios (% females) by size-class of black rat snake populations from Ontario (this study), Maryland (Stickel *et al.* 1980), and Kansas (Fitch 1961).**

| | Population | | | |
|---|---|---|---|---|
| Size-class (mm SVL) | Queen's Station - Ontario | Hill Island - Ontario | Maryland | Kansas |
| < 900 | 56.10 | 44.83 | 56.67 | 24.21 |
| ≥ 900 and < 1100 | 55.26 | 63.04 | 62.86 | 49.38 |
| ≥ 1100 and < 1300 | 45.55 | 57.14 | 67.62 | 50.00 |
| ≥ 1300 | 7.58 | 24.64 | 23.61 | 50.00 |

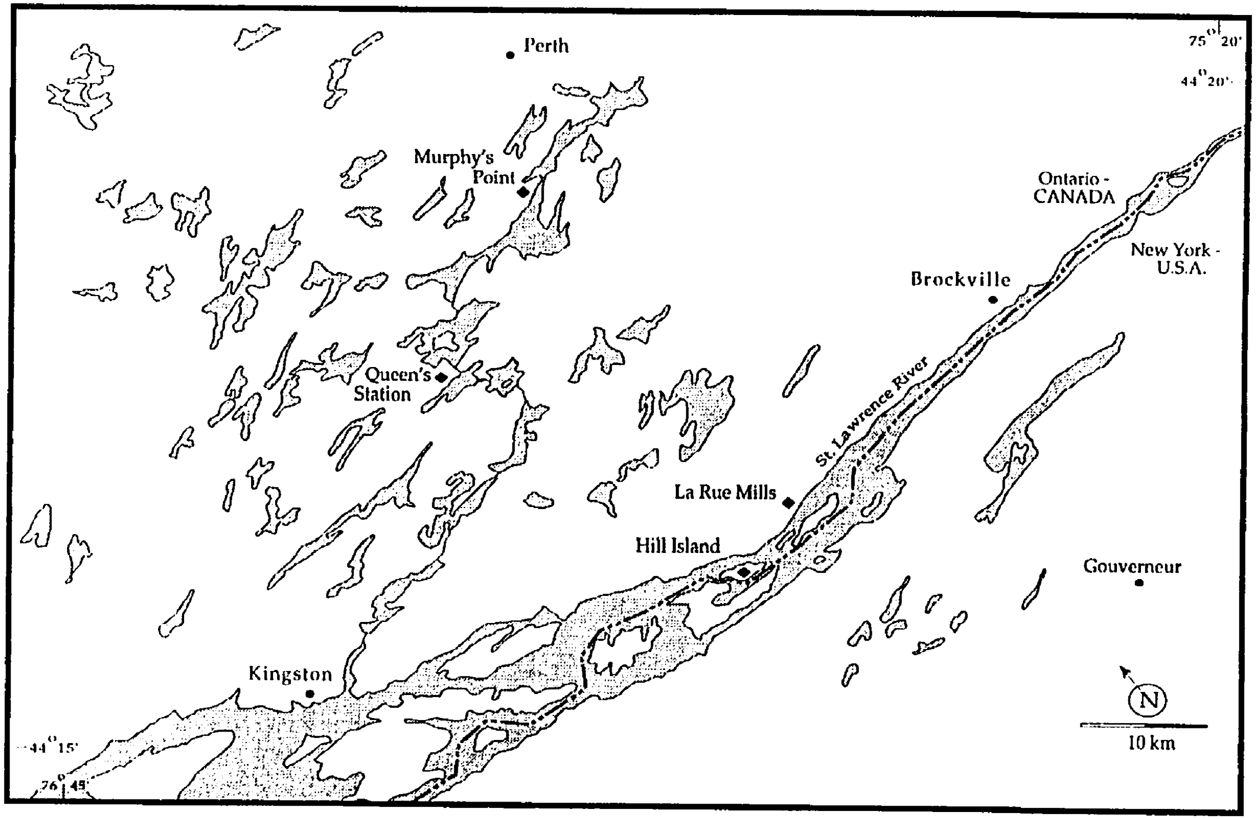

Figure 2-1. Range of the Frontenac Axis population in eastern Ontario and upper New York (shaded area). Primary study locations including; Murphy's Point, Queen's Station, La Rue Mills, and Hill Island are also indicated.

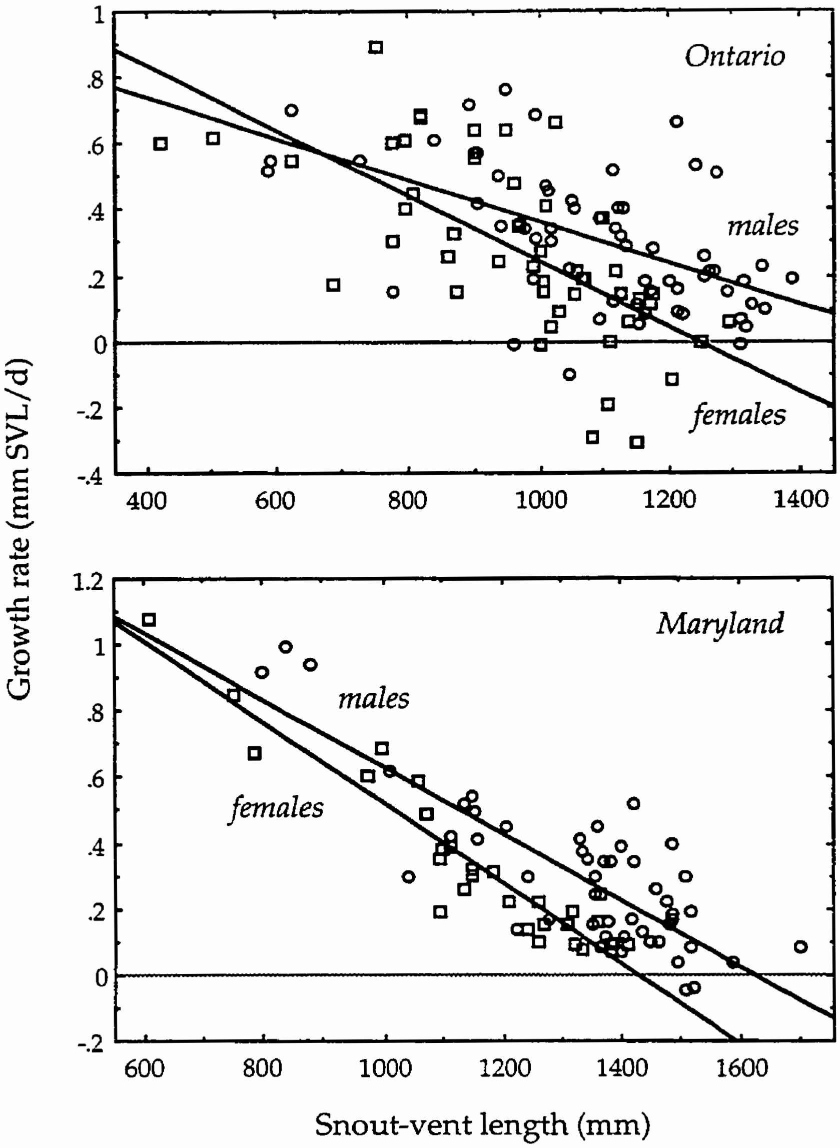

**Figure 2-2. Relationship between growth rate (mm SVL/d) and snout-vent length (mm) for male (circles) and female (squares) black rat snakes in Ontario (upper panel, this study) and Maryland (lower panel, Stickel *et al*. 1980). Linear equations of best fit are represented by lines.**

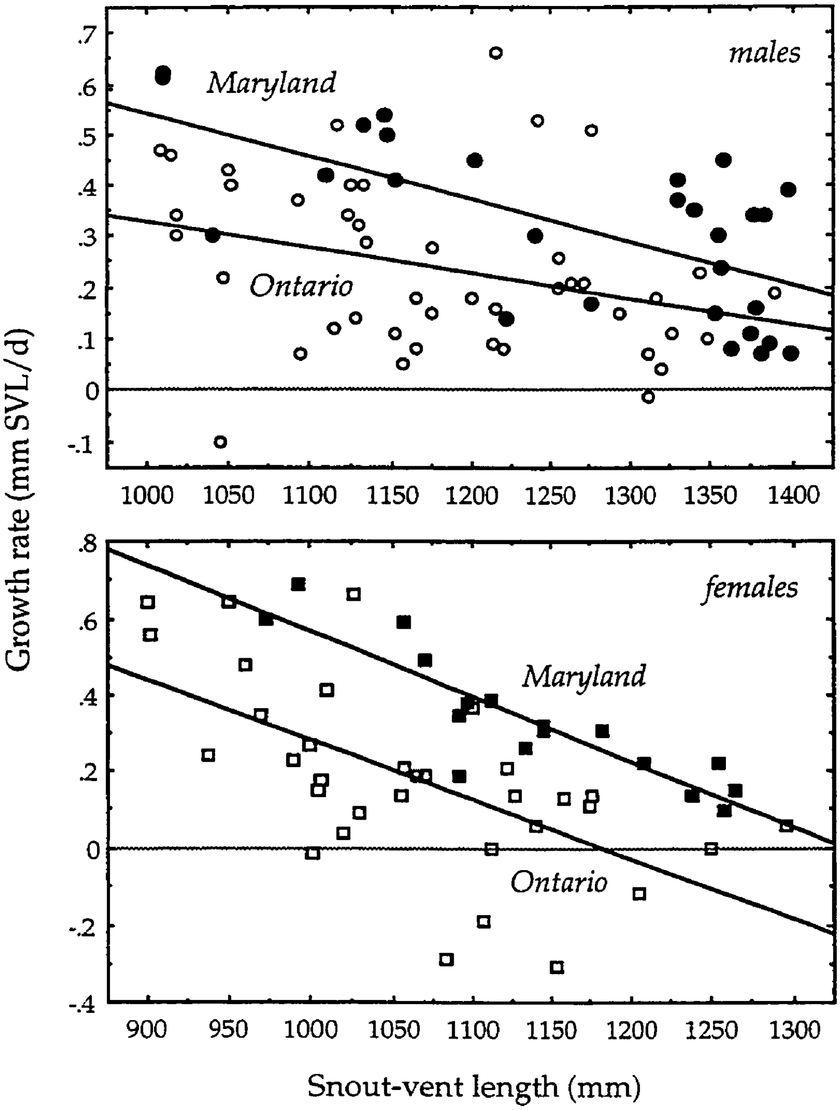

**Figure 2-3. Relationship between growth rate (mm SVL/d) and snout-vent length (mm) for male (upper panel) and female (lower panel) black rat snakes in Ontario (open symbols, this study) and Maryland (filled symbols, Stickel *et al.* 1980). Linear equations of best fit are represented by lines.**

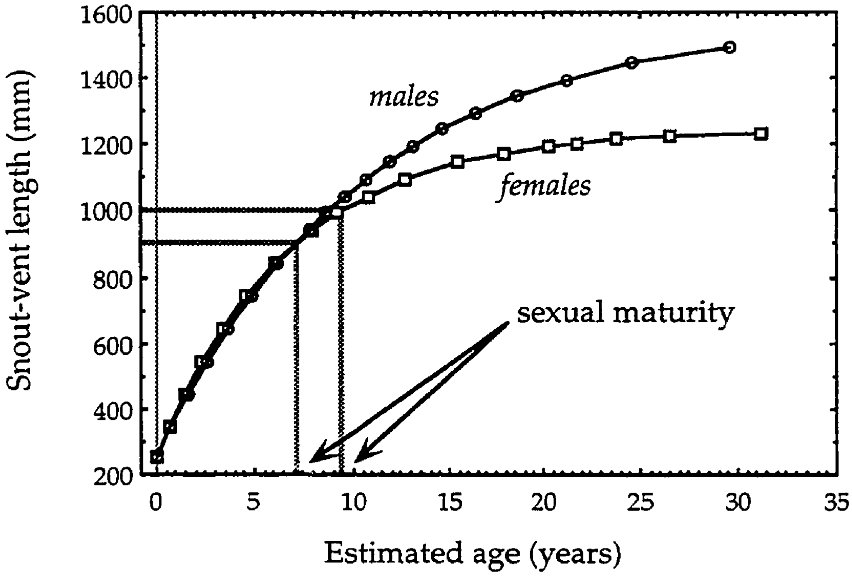

**Figure 2-4. Growth curves relating snout-vent length (mm) to estimated age in years for male and female black rat snakes from Ontario's Frontenac Axis population. Arrows indicate estimated age of sexual maturation for males (at ca. 900mm SVL) and females (at ca. 1000mm SVL).**

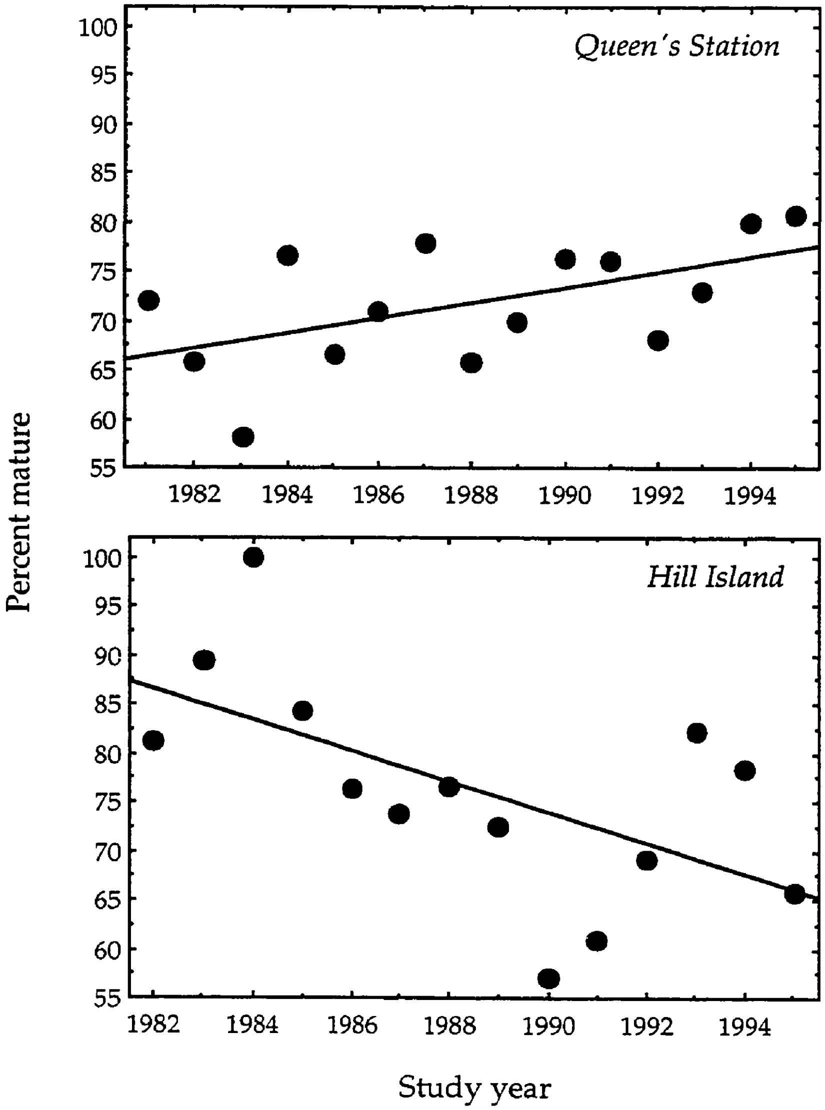

**Figure 2-6. Percentage of the population estimated to be sexually mature by study year at Queen's Station and Hill Island.**

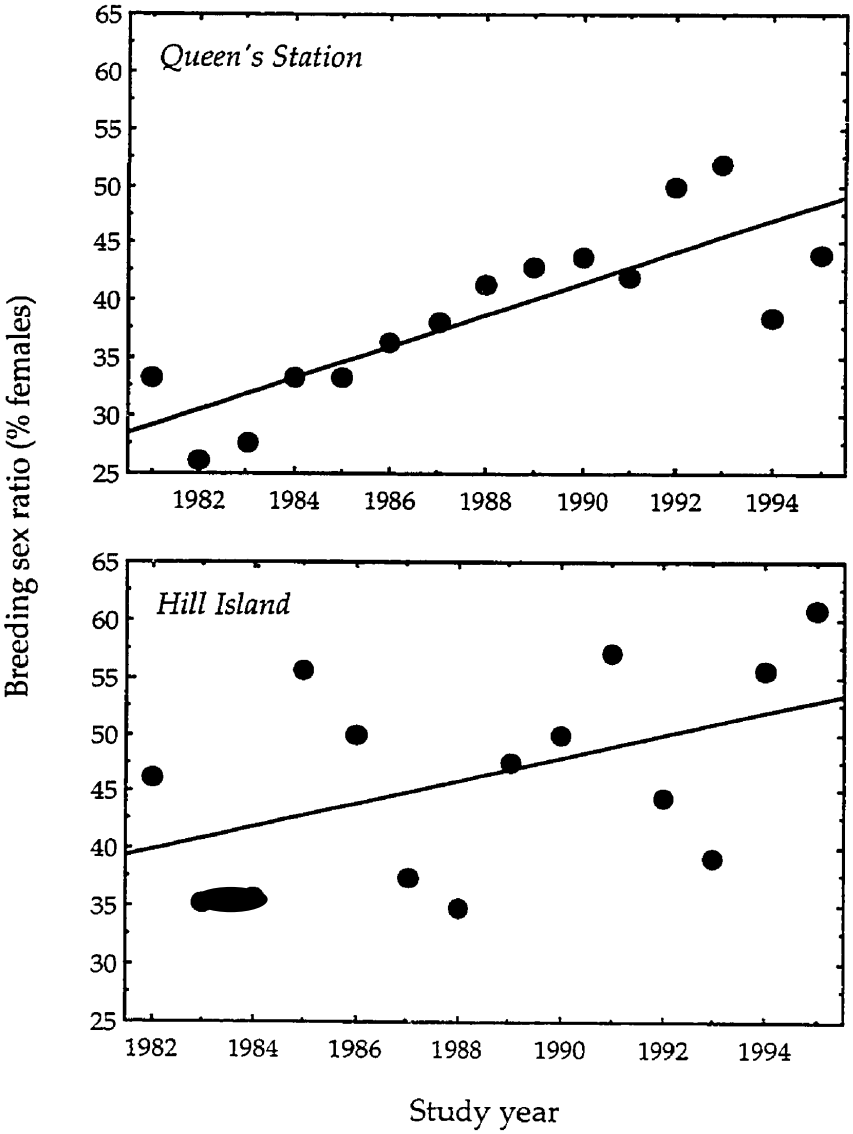

Figure 2-8. Estimated sex ratio (% females) by study year at Queen's Station and Hill Island.

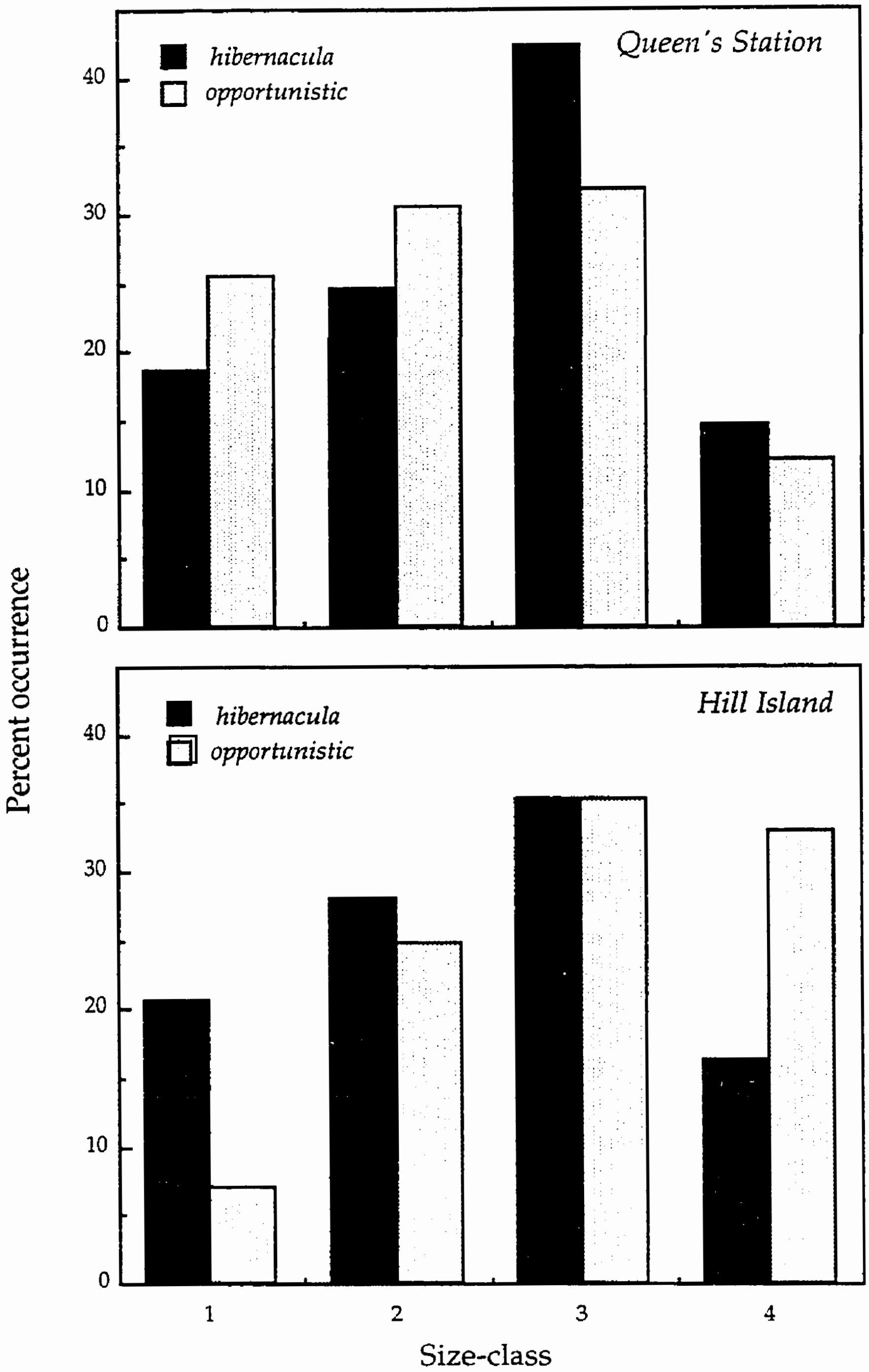

**Figure 2-9. Size-structure of hibernacula-based vs. opportunistic captures at Queen's Station and Hill Island. See text for size-class definitions.**

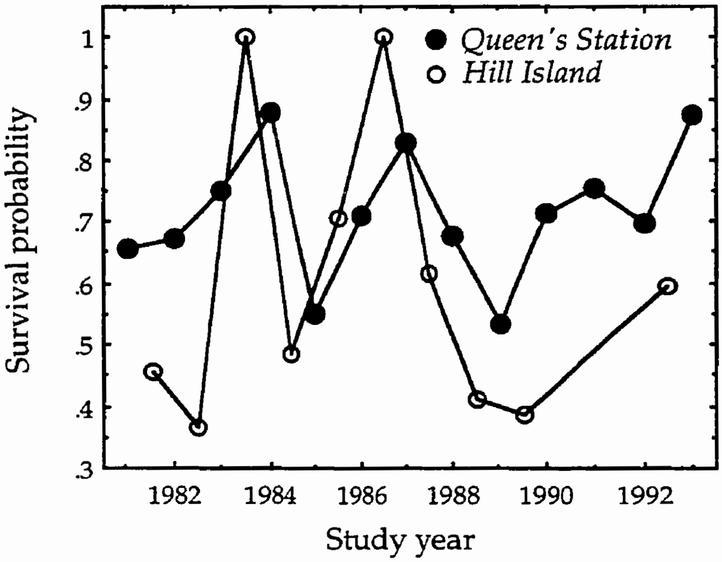

**Figure 2-10. Survival probability by study year at Queen's Station and Hill Island. Data for Hill Island have been offset slightly on the horizontal axis for illustrative purposes.**

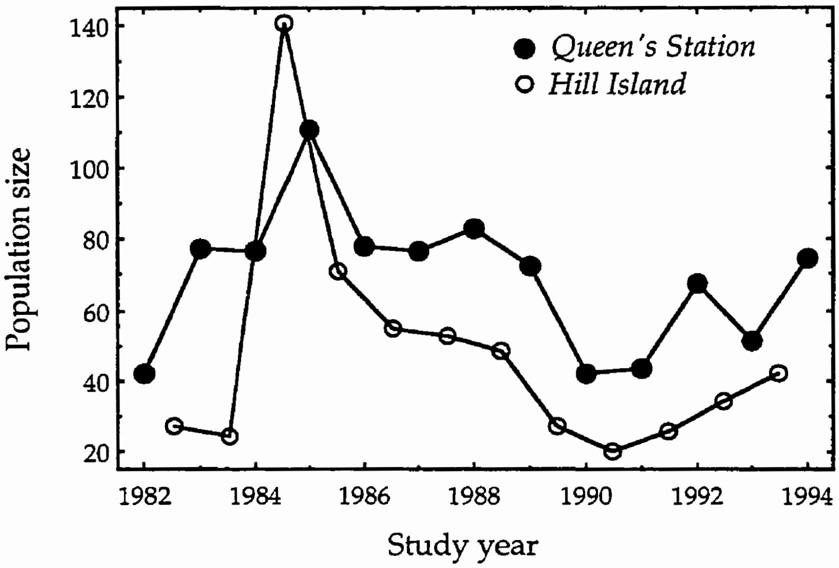

**Figure 2-11. Estimated population size by study year at Queen's Station and Hill Island. Data for Hill Island have been offset slightly on the horizontal axis for illustrative purposes.**

## Discussion

My analysis of the demographic structure of the Frontenac Axis black rat snake population serves multiple purposes. First, these results are of general value to snake ecologists in that they provide insights on patterns of spatial (regional and local) and temporal variation in the composition of snake populations. Second, my comparisons of the demographic characteristics resulting from sampling snake populations at different spatial/ecological scales (i.e., hibernacula vs. local populations) are of interest to those working on either theoretical or practical issues of snake ecology, since they identify biases inherent in certain field techniques and illustrate the need to consider methodology carefully when designing population monitoring programs. Third, these data are of value to the field of conservation ecology because they supply recovery planners and natural resource personnel with longitudinal baseline information on the demographic properties of a threatened population of black rat snakes.

### *Growth Rates and Demography*

Because growth rates play a major role in determining the demographic structure of snake populations (e.g., size-class distributions, sex composition, age at maturity), knowledge of how individuals and populations vary in this life history trait contributes to our understanding of population structure. Male rat snakes were found to grow relatively faster than females, as has been qualitatively demonstrated by Stickel *et al.* (1980). These sex-specific growth trajectories are responsible for three related demographic attributes. First, relatively fast, post-maturity growth by males results in the male-biased sexual size dimorphism, characteristic of the species. Second, differential growth rates may result in a disproportionate number of members of the faster growing sex in large size-classes, leading to male-biased adult sex ratios. Third, relatively rapid growth by males means that they take less time to reach sexual maturity (i.e., 7y for males vs. 9y for females). Rat snakes in this study also exhibited relatively rapid growth preceding sexual maturity and a subsequent slowing with increasing size in both sexes, a pattern they share with many species (Parker & Plummer 1987; Houston & Shine 1994; Madsen & Shine 1994).

My data indicate that growth rate may decline more precipitously with increasing size for females than it does for males in the Frontenac Axis (Ontario) population. By contrast, the relationship between growth rate and SVL did not differ between the sexes in the Patuxent (Maryland) population. This implies that the energy costs associated with maturation and reproductive activity are roughly equivalent for males and females in Maryland whereas, they may be relatively higher for females in Ontario. Why should reproduction be more costly for female rat snakes in Ontario? Perhaps the ultimate costs of reproduction are higher for Ontario females because, the short active season (May-September) limits their ability to recover body reserves lost through reproduction. Females in this population lay eggs in mid to late July, leaving them ca. 1-1.5 mo to acquire energy that must be allocated to growth, overwinter reserves, and possibly subsequent vitellogenesis. In comparison, the mating season ends in mid to late June, allowing males to focus on foraging for as much as 1 mo in advance of females. It may also be significant that Ontario males are able to concentrate on foraging during the peak of bird breeding activity, since eggs and nestlings represent major prey items for black rat snakes (Stickel *et al.* 1980; Weatherhead & Charland 1985).

Although Stickel *et al.* (1980) report the primary active period of the Patuxent population to be ca. 5 mo as well, rat snakes in Maryland can be found at large much later (and earlier) in the year (e.g., captures in January) than is the case in Ontario (pers. obs.). Furthermore, the average number of frost-free days differ by as much as 58 days between the two sites. As such, the Patuxent population seems likely to have a 1-2 mo longer growing season than the Frontenac Axis population. The greater opportunity to forage and recover reserves spent on reproduction before hibernation may be sufficient for reproductive females in Patuxent to maintain growth rates on par with males.

The influence of environmental conditions on growth rate was further illustrated by population level differences I found between Ontario and Maryland. Overall, my results are in general agreement with those of previous studies indicating that brief and variable active seasons (typical of high latitudes/altitudes) promote relatively slow growth and late sexual maturity in snake populations (Larsen & Gregory 1989; Brown 1991; Martin 1992, 1993). Rat snakes on the Frontenac Axis exhibited relatively slow growth, delayed maturity, and high longevity compared with those at Patuxent. Furthermore, the highly variable growth rates exhibited by females on the Frontenac Axis suggest that growth rate is influenced more by environmental conditions than by age *per se*. Differing energy expenditures (e.g., reproduction) and fluctuations in prey availability may have significant effects on inter-annual growth rates of rat snakes on the Frontenac Axis. A shorter growing season may mean that chance plays a much greater role in determining growth rates (e.g., number of meals per summer) for females in Ontario than it does in Maryland. These findings corroborate those of other studies that have documented strong correlations between growth rates of snakes and various environmental and ecological factors (Brown & Parker 1984; Platt 1984; Forsman 1993; Madsen & Shine 1993).

The estimated age of maturity for females on the Frontenac Axis (ca. 9y) is comparable to some populations/species regarded as noteworthy for exhibiting particularly late maturation. For example, females of temperate populations of timber rattlesnakes delay maturity until they reach 8-10y (Brown 1991; Martin 1992, 1993) and female Arafura filesnakes (*Acrochordus arafurae*), a low-energy specialist, do not mature before ca. 7y (Houston & Shine 1994). However, a caveat must accompany interpretation of my growth models (and thus, age estimates). Dunham (1978) recognized that modeled growth trajectories tend to systematically underestimate actual growth rates of reptiles, attributing this to the inability of stationary models to adequately describe within-season variation in growth rates (e.g., "growth spurts"). However, it seems equally likely that individuals in some populations might experience (otherwise undetectable) periods of stalled growth or quiescence, resulting in a tendency toward overestimation of growth rates by stationary models. That the models I developed for both the Frontenac Axis and Patuxent populations appear to overestimate growth rates (and underestimate ages) of black rat snakes supports this view. An implication of this variation is that my growth models are unable to provide highly accurate predictions of age based on body size. Nevertheless, comparisons of predicted ages for Frontenac Axis and Patuxent appear valid since models for both populations exhibited a similar degree of (im)precision (mean difference between actual and predicted ages = 1.5-3.0y).

### *Hibernacula Population Structure*

As is typical of the majority of species exhibiting communal overwintering behaviour (reviewed by Gregory 1984), black rat snake hibernacula populations on the Frontenac Axis tend to be composed largely of adults (ca. 76%). However, the variation in the percentage of adults associated with the seven hibernacula I studied was quite wide (43-100%). These hibernacula were dominated by mid-sized individuals (≥ 900 - < 1300mm SVL), with relatively few individuals in both the smallest (< 900mm SVL) and largest (≥ 1300mm SVL) size classes. While neonates of some Crotalidae rattlesnakes are known to attend traditional communal hibernacula (Galligan & Dunson 1979; Charland 1989), this age-class is usually absent from the hibernacula of most species, including those of rat snakes. However, this may be partly due to the difference in birth locations for the two groups. For at least some rattlesnakes species (or populations thereof), traditional gestation and overwintering sites may be relatively close together (0-100m apart, e.g., Gannon & Secoy 1985; Duvall *et al.* 1985). Thus, neonate rattlesnakes often do not have to travel long distances between birth sites and hibernacula. By contrast, rat snake oviposition sites, and thus neonate hatching locations, can be quite remote from communal hibernacula. As such, neonate rat snakes often may be unable to commute between birth locations and traditional hibernacula before the onset of their first inactive season.

The majority of new recruits to hibernacula I surveyed were probably 2-4y old, comparable to the age of recruitment in most other Colubrid species (e.g., Parker & Brown 1973; Parker 1976; Larsen & Gregory 1989). The most reasonable explanation for the occurrence of large "recruits" in my hibernacula-based samples is that such individuals were actually established members of hibernacula that had simply eluded capture for several years. Even the most carefully censused of the hibernacula I studied (Curtis, Lower Hill) probably did not provide entirely comprehensive samples since my perimeter-traps may not have enclosed all the potential hibernacula openings (exits) and some individuals may have been able to escape the traps.

Sex ratios of hibernacula populations across the Frontenac Axis tended to be highly variable, with roughly equivalent cases of male and female biases. Sex composition was generally even in all size-classes except for the largest (≥ 1300mm SVL), in which males predominated. Though male-biased sex ratios have been documented for other species, it is often the case that sex-biased catchability contributes to such disparities (e.g., Parker 1976; Brown & Parker 1984; Larsen & Gregory 1989). My samples at Curtis and Lower Hill were apparently not plagued by problems of differential catchability (either sex or size). As such, I am confident that males are truly more prevalent in the large size-classes of black rat snake hibernacula populations of my study area.

Recruitment to hibernacula tended to fluctuate annually, with an average of less than 50% of the population each season being new participants. Given that the majority of recruits may be 2-4y old, recruitment rates serve as crude indices of past reproduction. Because we currently know very little about either the spatial distribution of oviposition (nest) sites in relation to hibernacula or the dispersal patterns of young rat snakes, it is difficult to determine the degree to which local natality and immigration contribute recruits to specific hibernacula.

The choice of a suitable hibernaculum is crucial to overwinter survival for black rat snakes on the Frontenac Axis, since the use of an inappropriate site could prove fatal (e.g., due to freezing temperatures or dehydration). Such high risks suggest that selection should favor high site fidelity (Larsen & Gregory 1989). In keeping with this expectation, hibernacula site fidelity across the Frontenac Axis appears to be on the order to ≥ 95%, indicating that these social groups tend to have relatively consistent adult membership. Fidelity may be relaxed, promoting occasional switches between sites, when an individual's summer range encompasses more than one communal hibernacula (e.g., Hill Lower and Hill Upper at 1km apart). In such cases, changing sites (i.e., lower fidelity) would not be a particularly risky venture since the "new" hibernaculum has been proven suitable by virtue of its occupancy by conspecifics.

### *Local Population Structure*

My data imply that sharp contrasts occur in the overall size-class distribution of regional populations of black rat snakes. However, I believe that the patterns exhibited by the Kansas and Maryland samples are probably a function of the specific capture methods employed in these studies and do not reflect real biological differences among these rat snake populations. Small snakes were probably over-represented in the Kansas sample because the majority of captures in that study were obtained by "funnel traps primarily made and set for small snakes (which) were not highly effective for the capture of large adult rat snakes" (p. 650, Fitch 1963). Therefore, capture-success in the Kansas population was biased toward relatively small snakes. In contrast, most snakes caught in the Maryland study were visually located and captured by hand during incidental encounters in the field (Stickel *et al.* 1980), a method which apparently biased this population sample toward large (i.e., readily detectable) individuals. Therefore, I am unable to judge whether the size-structure of local rat snake populations differs among regions. Nevertheless, my data for the Frontenac Axis appear to be the least biased characterization of the demographic structure of rat snake populations documented so far.

Within the Frontenac Axis region I was unable to detect any clear differences in the general size-class distribution of local populations. Averaged over the entire study period, the local populations at Queen's Station and Hill Island exhibited very similar patterns of size-structure. Furthermore, the percentage of females sampled throughout the study period simultaneously increased in both populations (trends that were not accounted for by sex-biased recruitment). However, these similarities belie the fact that the two populations also exhibited *opposing* trends in the proportion of adults present. Queen's exhibited a positive trend, whereas Hill Island followed a negative trend in the percentage of adults sampled. While opposing patterns of general recruitment at each site (negative recruitment at Queen's and positive recruitment at Hill Island) offer a partial explanation for this particular result, it is perhaps more instructive generally to recognize that contrary trends in the demographic structure of snake populations can occur over relatively short geographic distances (40km). Differences in the size structure of snake populations are perhaps more commonly investigated and detected between populations occupying disjunct spatial distributions and/or those exposed to strongly differing environments (e.g., offshore island populations of *Notechis ater niger*, Schwaner 1985).

### *Sampling Bias: Opportunistic vs. Hibernacula-based Captures*

The results of my assessment of biases associated with specific population sampling methods indicate that opportunistic captures obtained at the scale of local populations may often be biased in favor of large snakes and males. By contrast, captures made at hibernacula appear to be more representative, because these samples did not preferentially select any particular size- or sex-class. Nevertheless, because individuals under 2y of age do not attend hibernacula, these samples provide no information on very young snakes in the population. The sampling biases associated with opportunistic captures and the restricted size-composition of hibernacula mean that these two types of samples yield different portraits of the demographic structure of rat snake populations. In particular, assessments of the composition of local populations based on opportunistic captures need to consider the fact that females and smaller snakes are more prevalent than such samples suggest. Similarly, small snakes are certainly more common in the general population than depicted by data from hibernacula samples. Applications of these sampling methods should take such discrepancies into account.

### *Survival Rates and Population Size Estimates*

My data on survivorship for black rat snakes indicate that small (younger) individuals suffer higher mortality rates than larger (older) individuals. I expect that overwinter freezing/dehydration and predation may be the major sources of mortality for juvenile black rat snakes on the Frontenac Axis. If juveniles hibernate solitarily, then their selection of a suitable overwintering site is likely to be somewhat fortuitous. This is because the "quality" of a solitary site remains undetermined until it has been used. In other words, such sites have not been "pre-tested". By contrast, traditional communal hibernacula are, by definition, high quality sites and this information may be conveyed to potential occupants by the presence of either scent trails or conspecifics themselves (see Reed & Dobson 1993). Predation is likely to be an important cause of mortality for small rat snakes due to the relatively broad suite of potential predators they may be subjected to. Nevertheless, there is considerable evidence that large adults in the study area are also taken by predators including, raptors (e.g., *Buteo lineatus*, pers. obs.) and carnivorous mammals (e.g., *Procyon lotor*, *Mustela vison*, G. Blouin-Demers - pers. commun.; S. Thompson - pers. commun.). Furthermore, adults are probably at greater risk of mortality resulting from encounters with humans and cars because their large size causes them to be both highly conspicuous and relatively big "targets".

Interestingly, I found no evidence of differential mortality between males and females, implying that the two sexes may incur roughly equal mortality costs due to reproduction. Though growth costs of reproduction appear to be greater for females in Ontario (see *Growth and Age Determination*), this does not seem to result in reduced survivorship. In fact, the costs of reproduction may be close to equivalent for males and females in many oviparous species. That is, the costs for females may be less, while the costs for males may be more than is usually thought to be the case. For example, females of some species appear not to need substantial body reserves for vitellogenesis (e.g., *Opheodrys aestivus*, Plummer 1983; *Pituophis melanoleucus*, Diller & Wallace 1996), though it is not known if this is the case for black rat snakes (especially at northern latitudes). Conversely, male dark green snakes (*Coluber viridiflavus*) have been demonstrated to require large reserves to participate in breeding and suffer high mortality rates resulting from mate-searching activity (Bonnet & Naulleau 1996). Male black rat snakes are known to exhibit high movement rates during the breeding season (Weatherhead & Hoysak 1989) and participate in strenuous bouts of combat with rival males (Rigley 1971). Clarification of the relative importance of the various potential sources of mortality for different size- and sex-classes of black rat snakes will require careful monitoring of the behaviour and actual fates of individuals and field experiments including manipulations of prey availability or food intake so as to control energy reserves (cf. Lindell & Forsman 1996; Brown & Weatherhead - in prep.).

Estimates of overall annual survival were relatively high for both the Queen's Station and Hill Island local populations, with no significant longitudinal trends apparent at either site. Neither did I detect any temporal trends in estimated population sizes. However, Queen's and Hill Island exhibited high correlations in both annual survival and population size over time. Because survival and population size estimates are both derived from formulae that relate the number of snakes captured in one year to the number captured subsequently, the two indices are necessarily auto-correlated. As such, it is not surprising to find that Queen's and Hill Island exhibit correlations for *both* estimates. What was unexpected was that demographic parameters of the two populations varied in concert.

There are at least two possible reasons why such a correlation might arise between geographically separated populations. First, the correlations might simply represent artifacts of sampling shared between the study sites.

While the sampling protocols were similar for the two sites (see *Study Sites and Field Methods*), most fieldwork was conducted by independent teams that did not coordinate their sampling efforts. That is, capture effort at one site was entirely independent of capture effort at the other. As such, I feel confident in ruling out the possibility that between-population correlations are merely sampling artifacts. A second possibility is that these correlations reflect common, population-level responses to ecological perturbation(s). Perhaps region-wide fluctuations in seasonal weather (e.g., wet springs, severe winters) impact the two populations similarly such that survival and capture probabilities vary equally (Parker & Plummer 1987), despite their geographic separation from one another. Correlated changes in population sizes through time by pairs of species (rather than different populations of a single species) at the same study site have been attributed to common, or at least, related responses to human predation (*Masticophis t. taeniatus, Crotalus viridis lutosus*; Parker & Brown 1973), fluctuations in annual precipitation/prey abundance (*Pituophis melanoleucus deserticola, Coluber constrictor mormon*; Parker & Brown 1973), and severe drought (e.g., *Nerodia fasciata, Seminatrix pygaea*; Seigel *et al.* 1995). To my knowledge, Klimstra's (1958) multi-year data on different snake communities in the US mid-west is the only study to have previously suggested that populations distributed over a relatively wide geographic region may fluctuate in synchrony. At the very least, my results point to our limited understanding of the dynamics of rat snake populations and the need to explore further the potential reasons why demographic parameters may be synchronous regionally. As suggested by Larsen and Gregory (1989), a detailed knowledge of local conditions is required to understand the dynamics of particular populations.

### *Implications for Conservation and Population Monitoring*

Many of the findings presented in this chapter have either direct or indirect implications for the monitoring and conservation of threatened snake populations. The life history traits and composition of black rat snake populations (e.g., slow growth, delayed maturity, low adult mortality rates, high longevity, large percentage of adults) are incompatible with the concept of rapid population turnover (Parker & Plummer 1987). With respect to conservation, such characteristics make it unlikely that populations of rat snakes are capable of recovering quickly from either large overall declines in numbers or even increased mortality rates of adults alone (see Brooks *et al.* 1991 and Congdon *et al.* 1993 for case studies of turtles). High road densities and traffic volume (e.g., as found throughout southwestern Ontario population ranges) probably elevate mortality rates for adult rat snakes above background levels, though this hypothesis should be tested. Furthermore, the wanton killing of even a few individual adult snakes by humans each season could have a severe cumulative impact, particularly in isolated populations where the number of reproductive individuals may already be critically low.

The slow growth rates exhibited by rat snakes also imply that even catastrophic reproductive failures, causing the absence of entire year-class cohorts, may go unrecognized by natural resource managers until several years after the event. This is because, neonates and young juveniles cannot be reliably sampled, since opportunistic captures are biased toward large individuals and young do not attend hibernacula. Therefore, the absence of a given year-class might not be detected until they fail to be recruited into a hibernaculum, 2-4y after they would have been born.

Because hibernacula are composed largely of (reproductive) adults, the extinction of a single hibernaculum population could have a major negative impact on the local population size and its capacity for recovery. Furthermore, even if the structural integrity and quality of the hibernaculum habitat were to remain high following such an extinction, it could be several years before the site is re-used, since occupation may require a threshold number of individuals in attendance to stimulate conspecific attraction (see Reed & Dobson 1993).

My data for the Frontenac Axis indicate that hibernacula population samples are unbiased representations of ecologically-delimited populations (see McArdle & Gaston 1993). By contrast, opportunistic captures of snakes at large tend to be both sex- and size-biased samples of vaguely defined local populations. Hibernacula populations also exhibit fairly consistent adult memberships. Furthermore, in most cases, hibernacula populations can be relatively easily sampled, requiring a limited field effort (daily site visits) during the brief spring emergence period (1.5 mo max. on the Frontenac Axis) each season. For these reasons, hibernacula-based samples would appear to best serve natural resource managers in their efforts to monitor the dynamics of rat snake populations (and perhaps other communally-hibernating species) over time. Radio-telemetry remains the most efficient way of locating hibernacula, although resource managers and researchers need to be aware of the possibility that several hibernacula can occur within a relatively small area (e.g., $1\text{km}^2$).

Despite their similarities, however, hibernacula populations across the Frontenac Axis are apparently not uniform, at least in terms of size- and sex-composition. This suggests that monitoring only a few of the several hundred hibernacula that may be distributed across the Frontenac Axis would not provide a reliable overview of the status of the larger regional population. Furthermore, if park and ecological reserve managers wish to obtain a realistic understanding of populations under their jurisdiction, they need to view local populations within a regional context. A widely distributed network of hibernacula sample sites is likely the best means of providing such complimentary data. Such a system of monitoring stations would allow both an assessment of the efficacy of local management actions, while at the same time permitting early detection of any problems associated with population health at a more coarse spatial scale.

Various short-term or cross-sectional sub-sets of my survival and population estimate data might easily have been interpreted as (human-induced) declines rather than as natural fluctuations in the context of the complete data set. For example, the ca. 3-fold decrease from 1985 to 1990 in the population size at Queen's portrays a pattern that is entirely different from the one illustrated by all 15y of data (see **Fig. 2-11**). Similarly, biennial sampling regimes would have been incapable of detecting certain trends in the sex ratio and size-structure of local populations. Furthermore, while I had no *a priori* reason to expect either population to exhibit trends in any demographic parameters, sampling *both* populations revealed contrasting trends - a reality that is unlikely to have been imagined had only one site been surveyed. For instance, it would have seemed reasonable to assume that the percentage of adults was increasing (and recruitment was decreasing) across the entire Frontenac Axis had only the Queen's Station population been sampled (see **Fig. 2-6**). Because a primary aim of population monitoring for conservation purposes is early disclosure of population distress (Caughley & Gunn 1996), my results suggest that annual monitoring at multiple sites across the Frontenac Axis would yield the highest quality information in this regard. While the benefits of such long-term, fine-scale monitoring of snake populations have been acknowledged elsewhere (Dodd 1992; Seigel *et al.* 1995), there remain few examples of this type of work in the literature.

# Chapter 3.

# Population Genetic Structure in the Black Rat Snake: Implications for Management

This chapter formed the basis of a paper of the same title
currently in press in *Conservation Biology*
and coauthored by Kent A. Prior, H. Lisle Gibbs, and Patrick J. Weatherhead.

# Introduction

Genetic data now serves an important role in guiding the management of endangered species (e.g., Hedrick & Miller 1992; Loeschcke *et al.* 1994). Conservation biologists commonly use both data that define the genetic structure of populations and data that provide comparative measures of within-population diversity. Data on the genetic structure of populations is of particular interest because it may reveal evidence of restricted gene flow or genetic isolation that is undetectable through traditional demographic studies (e.g., capture-recapture data). Such knowledge can be used to identify genetically-based "management units" (Moritz 1994), enabling conservation practices to be focused appropriately. Estimates of within-population diversity also are of value because they can reveal recent, or on-going, changes in population structure and dynamics. For instance, habitat destruction could fragment previously contiguous populations, thereby disrupting gene flow and resulting in a loss of within-population variation via genetic drift (Franklin 1980). Conservation of genetic diversity is prudent given evidence of a positive correlation between genetic variability and both individual fitness traits and population viability (e.g., McAlpine 1993; Jimenez *et al.* 1994; although see Milligan *et al.* 1994). Thus, estimates of current genetic variability within populations may allow one to rank populations with respect to their genetic conservation status (e.g., relative vulnerability).

The availability of both types of genetic data can contribute to the management of endangered species by providing an informed basis for allocating limited funds for conservation and for assessing the efficacy of conservation strategies (e.g., has genetic variability changed as a result of management?). While the benefits of genetic studies seem apparent, relatively few taxonomic groups (mostly mammals, birds, and fish) have been examined (reviewed by Avise *et al.* 1995). In particular, snakes are poorly represented in studies of conservation genetics (Dodd 1993). The general goal of this chapter is to provide one of the first studies of the population genetic structure of a threatened snake from a conservation perspective.

Five distinct sub-species of *Elaphe obsoleta* are currently recognized (Conant & Collins 1991). The black rat snake (*Elaphe o. obsoleta*) is the most widely distributed of the five, ranging across much of the eastern half of the U.S. (**Fig. 3-1**). Although abundant in some regions (Durner & Gates 1993), in Ontario this sub-species occurs in as few as five isolated populations in two separate parts of the province. The populations of "Skunk's Misery," "Big Creek," "Oriskany," and "Niagara" are all found in southwestern Ontario (north of Lake Erie), while the "Frontenac Axis" population is located in eastern Ontario (**Figs. 3-1 & 3-2**). The small size and high degree of isolation of these remnant populations have raised concerns regarding their conservation status, resulting in black rat snakes being provisionally designated as "threatened" in Canada (see *Chapter 4*).

The primary objective of this chapter was to quantify genetic population structure and estimate levels of gene flow in black rat snakes at three distinct spatial scales: (1) geographically separate regional populations (>100 km apart), (2) local sub-populations (15 - 50 km apart) within a regional population, and (3) communal hibernacula (1 - 5 km apart) within local populations (see **Fig. 3-3**). My reasons for choosing these scales for comparison were twofold. First, previous work by Paik and Yang (1987) on a congeneric species (*Elaphe dione*) found some evidence of genetic structure at the regional scale. Second, sampling at the scale of hibernacula reflects a non-arbitrary social/ecological assortment of individuals within local populations. That is, across much of the northern half of their range, black rat snakes hibernate communally at traditional sites to which individuals exhibit strong inter-annual fidelity (i.e., >95% - see *Chapter 2*). Gene flow among neighboring hibernacula is expected to be high for three reasons. First, mating takes place during the late spring and early summer after dispersal from the hibernacula (Weatherhead & Hoysak 1989). Second, the activity ranges of adults from adjacent hibernacula regularly overlap. Third, females may lay their eggs remote from the maternal hibernacula, so many juveniles may hibernate at sites different from those used by their mothers.

My secondary objective was to characterize the genetic diversity of the small, isolated rat snake populations found in Ontario relative to larger, non-isolated populations sampled in the central core of the species' range. I predicted that, in the absence of gene flow, small, isolated populations would exhibit reduced levels of genetic diversity (heterozygosity) as a function of genetic drift (e.g., Lesica & Allendorf 1995).

Collectively, these data will contribute to an understanding of the ecology and biogeography of black rat snakes. More specifically, my results are expected to help clarify the genetic status and composition of Ontario's threatened populations of black rat snakes, thus aiding those charged with preparing plans for the management of the species in Canada.

## Methods

### *Study Populations and Sample Collection*

I sampled 210 individual black rat snakes from regional populations, sub-populations within a regional population, and communal hibernacula within local sub-populations as described above. At the coarsest scale, the 210 individuals represent four distinct regional populations, distributed across the species' range, including two isolated populations in Ontario (Big Creek, Frontenac Axis) and two non-isolated populations from the U.S. (Maryland and Arkansas; see **Fig. 3-1**).

The Big Creek population occurs north of Lake Erie in southwestern Ontario (ca. 42°75'N, 80°50'E). Neighboring populations (Skunk's Misery, Oriskany, Niagara) are all a minimum of 50 km away with largely unsuitable habitat (intensive agriculture) in between.

The disjunct Frontenac Axis population occurs in eastern Ontario and northern New York state, bridging the St. Lawrence River at the east end of Lake Ontario (ca. 44°50'N, 76°50'E, see **Fig. 3-2**). The nearest population is found ca. 120 km to the south near Syracuse, New York (A. R. Briesch, pers. commun.). The fact that black rat snakes are absent from naturalists' surveys of upper New York around the turn of the century (e.g., De Kay 1842) suggests that the disjunction of the Frontenac Axis population from southwestern Ontario populations may have preceded European settlement and land-clearing.

The Maryland population was sampled in the vicinity of the City of Joppatowne (39°25'N, 76°22'E; **Fig. 3-1**). This is a non-isolated population occurring within the mid-eastern core of the species' range. Black rat snakes are abundant in some parts of the Joppatowne study site, an urban landscape bisected by (semi-)natural habitats (old field, riparian forest).

Finally, the Arkansas population sample was obtained from both the Ozark and Ouachita National Forests (ca. 35°50'N, 93°50'E) in western Arkansas (J. H. Withgott, pers. commun.). As with the Maryland population, the Arkansas population may be regarded as occuring within the core of the species' range.

Snakes were hand-captured and sampled opportunistically throughout both the Big Creek (1991-93) and Arkansas (1993) study areas. In contrast, snakes obtained from Frontenac Axis (1991-93) and Maryland (1992-93) were collected at only a few specific localities, including communal hibernacula. This feature of my sampling protocol provided the opportunity to sub-divide hierarchically the Frontenac Axis and Maryland regional population samples, allowing me to investigate within-population genetic structure. Specifically, the Frontenac Axis population sample is composed in part of individuals obtained from five (sub-population) localities ("Murphy's Point Provincial Park," "Queen's University Biological Station," "Charleston Lake Provincial Park," "La Rue Mills," and "Hill Island" - **Figs. 3-2 & 3-3**). The Queen's Station sample can be further sub-divided since it is composed in part of individuals associated with either the "Curtis" or "Cow Island" hibernaculum (inter-hibernacula distance = 1.2 km). La Rue Mills and Hill Island are also hibernacula samples (inter-hibernacula distance = 17 km - **Figs. 3-2 & 3-3**) and therefore, for the purpose of analyses, these samples were regarded at two hierarchical scales (i.e., representative of local sub-populations *and* communal hibernacula). The Maryland samples were also collected from two adjacent hibernacula (i.e., "Overpass" and "Riverside,"; inter-hibernacula distance = 1.6 km, see **Fig. 3-3**).

Once a snake was captured I drew ca. 100 ul of blood from a caudal vessel using an 0.5 cc insulin syringe fitted with a 28 gauge needle. Blood samples were immediately mixed with 800 ul of lysis buffer (Seutin *et al.* 1991) and refrigerated until DNA extraction. In all cases, snakes were permanently marked (i.e., clipping of caudal scutes or PIT-tagging) upon capture to eliminate the possibility of resampling the same individual on subsequent occasions.

### *DNA Extraction and RAPD Amplifications*

Genomic DNA was extracted from blood samples using standard techniques (Sambrook *et al.* 1989). Approximately 400 ul of the lysis buffer and blood solution was suspended in additional lysis buffer to a total volume of 4 ml, and rocked gently overnight at 37° C until no clots remained. Samples were then digested with proteinase K, extracted 2 - 3 times with a 70:30 mix of phenol:chloroform, and once with chloroform. The DNA was then precipitated by adding 1/10 volume of 3M sodium acetate (pH 5.5), and two volumes of 100% ethanol, spooled out using a Pasteur pipette, washed in 70% ethanol, and then allowed to dry for five minutes. It was then re-dissolved in 0.3 - 1.0 ml of TE depending on the yield of DNA, and gently rocked overnight at 37° C. I quantified the amount of DNA using a Hoefer TKO 100 flourometer. A test aliquot of DNA was digested and the concentration and quality of the sample DNA assessed by comparison with known standards of snake DNA. If the estimates of concentration were inaccurate, then the samples were allowed to re-dissolve at 37° C and the quantification procedure was repeated.

To generate RAPD profiles from the black rat snake DNA I used 20, ten base pair primers (A1 - A20) from the Operon Technologies Primer Kit A in PCR amplifications. Amplification reactions were performed in 25 ul volumes containing 10 mM Tris (pH 8.3), 50 mM KCl, 2 mM MgCl2, 0.001% gelatin, 10 mM dNTP (Pharmacia), 5 uM primer (Operon), 0.5 units of Taq DNA polymerase (Perkin Elmer Cetus), 25 ng of genomic DNA, and dH2O. Negative controls in which water was substituted for DNA were also run to check for the possibility of contamination. The reaction was overlaid with mineral oil and amplified in a Perkin Elmer Cetus Model 480 DNA Thermal Cycler programmed for 45 cycles of 1 min at 94° C to denature, 1 min at 36° C for annealing of primer, and 2 min at 72° C for extension. After the final cycle, samples were held at 4° C prior to analysis. Amplification products were separated according to size on 1.2% agarose gels electrophorised in 1 X TBE, stained with 35 ul ethidium bromide (10 mg/ml) in 700 ml 0.5 X TBE, de-stained for 30 minutes in water and visualized under ultraviolet light. A 1 Kb DNA ladder was run alongside RAPD products on each gel in order to confirm the sizes/identities of fragments. Black and white photographic negatives were taken of the gels and the profiles of individuals were scored for the presence/absence of fragments for each primer by projecting the photographic negative image on a white screen.

### *Primer Selection and Fragment Scoring*

Initially, I surveyed all 20 (A1 - A20) of the Operon primers for a sample of 20 black rat snakes. Based on clarity and resolution of the profiles produced, I chose five of these primers (A1, A5, A7, A10 and A20) which were then used to generate profiles for a larger sample of individuals. To quantify the consistency with which DNA bands of the five primers used could be reproduced, I ran two separate amplifications for the same 20 individuals. After blind scoring the presence (+) or absence (-) of specific bands in these 20 individuals I calculated a "repeatability" score for each locus based on the consistency with which a particular band was either present or absent in the two sets of amplifications. For example, if a particular band was consistently present or consistently absent between amplifications in all 20 individuals, then that locus was given a repeatability score of 1.0. In contrast, if 10 of 20 individuals were scored differently for a particular locus on the two amplifications, then the locus would be assigned a repeatability score of 0.50. Only those loci which exhibited complete reliability (i.e., repeatability score of 1.0) were subsequently used for analyses.

### *Statistical Analysis of RAPD Profiles*

Fragment patterns generated by the five RAPD primers were analyzed both directly as phenotypes and by estimating allele frequencies at corresponding loci, enabling more traditional genetic analyses. As a preliminary test of genetic structure I conducted an Analysis of Molecular Variance (AMOVA). AMOVA is equivalent to a classical analysis of variance (ANOVA) in that it computes (molecular) variance components from a matrix of squared (molecular) distances between pairs of observations (i.e., RAPD phenotypes). I calculated pairwise Euclidean distance measures between RAPD phenotypes using the software program RAPDistance (Armstrong *et al.* 1994) and then ran the AMOVAs with WINAMOVA (Excoffier *et al.* 1992). The significance of the variance component estimates were computed by nonparametric permutation procedures in WINAMOVA.

WINAMOVA allows for a three-level hierarchical partitioning of genetic variation. Therefore, in my first set of analyses the regional populations of Frontenac Axis, Big Creek, and Maryland were pooled to form an "Eastern" group and Arkansas served as a "Western" group (see **Figs. 3-1 & 3-3**). As such, I was able to partition phenotypic variation among individuals within populations, among regional populations within groups, and among groups (East vs. West). Population sample sizes for these analyses were as follows, Big Creek (N = 15), Frontenac Axis (N = 29), Maryland (N = 30), and Arkansas (N = 26). Though as many as 139 samples from the Frontenac Axis population were available to me, I elected to use only a randomly selected sub-sample of 29 at this level of analysis in order to avoid potentially confounding effects of widely disparate samples sizes (e.g., Lynch & Milligan 1994) and because WINAMOVA limits the number of samples for analysis to 100.

My second AMOVA analysis assessed genetic structure within the Frontenac Axis population. Here I pooled the sub-population samples of Murphy's Point and Queen's Station to form a "Northern" group and Charleston Lake, La Rue Mills, and Hill Island to form a "Southern" group (**Figs. 3-2 & 3-3**). As above, I was then able to partition phenotypic variation among individuals within sub-populations, among sub-populations within groups, and among groups (North vs. South). Because of WINAMOVAs limitations on sample size, my sub-population sample sizes were as follows; N = 23 for Murphy's Point (a random sample of the 33 available), N = 21 for Queen's Station (a random sample of the 48 available), N = 13 for Charleston Lake, N = 27 for La Rue Mills, and N = 16 for Hill Island. I assessed the possibility that the results of these AMOVAs (and subsequent analyses of allele frequencies) might be influenced by sampling effects by comparing results generated during five replicate analyses. For each replicate analysis I used unique random samples to represent Frontenac Axis (for population AMOVA) and Murphy's Point and Queen's Station (for sub-population AMOVA).

In addition, I estimated allele frequencies at the corresponding RAPD loci using the software RAPDFST 3.0 (Black 1995) in order to conduct standard genetic analyses of population structure. In all species studied to date, most RAPD polymorphisms are due to the presence of a dominant (+) and a recessive (-) allele at the same locus (e.g., Bowditch *et al.* 1993, Apostol *et al.* 1995). The dominant allele determines the presence of the band such that +/+ and +/- individuals have the (+, present) phenotype and -/- individuals have the (-, absent) phenotype. As in previous studies (e.g., Haig *et al.* 1994), I assumed that all the loci scored had this property and that all population samples were in Hardy-Weinberg equilibrium, so that my estimate of the frequency of $q$ of the recessive allele (-) equaled the square root of the frequency of the null phenotype (-). Calculation of Wright's (1978) $F_{ST}$ (among-population differentiation) was carried out in RAPDFST, using the weighted mean frequency of alleles formula. This method expresses $F_{ST}$ as the ratio of the observed variance in the frequency of an allele among sub-populations relative to its maximum variance in the total population. Following this, a $\chi^2$ value was calculated to determine if the estimate of $F_{ST}$ differed from zero (i.e., indicative of significant population differentiation). I also applied Wright's (1978) qualitative guidelines for interpretation of $F_{ST}$ values. That is, an $F_{ST}$ of 0-0.05 = little differentiation, an $F_{ST}$ of 0.05-0.15 = moderate differentiation, an $F_{ST}$ of 0.15-0.25 = great differentiation, and an $F_{ST}$

above 0.25 = very great differentiation. Estimates of effective gene flow were determined using Wright's (1931) formula, $Nm = (1 - F_{ST})/4F_{ST})$ where; $N$ = deme size and $m$ = migration rate among demes, assuming current equilibrium between migration and genetic drift. I then used the allele frequency data to generate measures of genetic distance (*D*, Nei, 1978) between populations and estimates of mean (expected) heterozygosity within populations with the software BIOSYS-1.7 (Swofford & Selander 1989).

Lynch and Milligan (1994) recently provided a set of guidelines for the analysis of population genetic structure using RAPD markers. In order to ensure unbiased estimates of population genetic parameters they advise researchers to restrict their analyses to the use of RAPD loci having alleles which occur at only moderate frequency across the populations under study. Specifically, only loci with alleles exhibiting an observed frequency of less than 1 - (3/N) should be used (where N = the population sample size). Consequently, I analyzed both "complete" (use of all loci) and "restricted" (use of loci with frequency less the 1 - (3/N)) RAPD datasets and considered both sets of results. However, I found few quantitative and no qualitative differences between the two. Therefore, because of their more conservative nature, I present only the results of my analysis of restricted datasets.

To put my results in context I summarized published data on snake population genetics. In addition to studies reviewed by Dessauer *et al.* (1987), I found nine other studies that reported measures of heterozygosity and genetic distance among snake populations based on protein variability.

# Results

## *RAPD Profiles*

A total of 40 bands was produced by the five RAPD primers, with 6 to 11 individual bands being generated by each primer (mean = 8.00 ± 2.09 S.E.). Band repeatability was relatively high overall, with the 40 bands exhibiting the same state (+,+ or -,-) between two independent RAPD-PCR amplifications pooled across 20 snakes on average 91.39 % (± 1.89 S.E.) of the time. However, only 16 (40%) of the 40 bands showed 100% reliability. Of those, 14 exhibited variability among individuals at the population level and these bands (or "loci") formed the "complete" datasets (see above). Applying the criteria of Lynch and Milligan (1994) for pruning RAPD loci (observed frequency less than 1-(3/N)) necessitated that I eliminate 7 (loci 1, 3, 5, 6, 10, 11, and 14; **Table 3-1**) of the original 14 loci. Therefore, data derived using the remaining 7 loci formed my "restricted" datasets on which I report here.

## *Regional Population Structure*

I identified two population-specific RAPD markers. First, 10 members of the Arkansas population were the only individuals to express the (+) allele at Locus 7. Second, a single individual from Arkansas was the only snake that exhibited the (-) allele at Locus 14 (**Table 3-1**).

AMOVA results based on RAPD phenotypes demonstrated significant genetic differences among regional groups (East vs. West), isolated populations (Frontenac Axis, Big Creek, Maryland, Arkansas), and individuals within each of the four populations (in all cases $p < 0.001$). Of the total genetic diversity, 12% was attributable to regional differences, ca. 8% to population differences within these regions, and 80% to individual differences among rat snakes within populations. Five replicate AMOVAS, using unique random samples of 29 individuals to represent Frontenac Axis, yielded little quantitative and no qualitative differences from these original results. Thus, sampling effects do not appear to have been significant at this spatial scale.

This general pattern of population genetic structure was clarified by my analysis of allele frequency data generated by RAPDFST (Black, 1995). Estimated allele frequencies at RAPD loci varied among regional populations (**Table 3-1**); mean among-population differentiation ($F_{ST}$) was 0.266 ± 0.062 S.E. (**Table 3-2**). In other words, an important component (26.6%) of the overall genetic variation was due to among-population divergence (Big Creek vs. Frontenac Axis vs. Maryland vs. Arkansas). Conversely, 73.4% of allelic variation was found within the isolated regional populations. Both the statistical ($\chi^2$ = 50.54, df = 14, $p < 0.001$) and qualitative significance of the total F value are indicative of considerable genetic differentiation among the four regional populations. That individual F values associated with the majority (57%) of the 7 loci comprising my restricted dataset were significant ($\alpha = 0.0036$ for multiple comparisons) suggests that the magnitude of the overall F was not due to differences in allelic frequency at only a few loci.

To identify exactly where genetic disjunctions occurred at the regional scale I calculated pairwise population estimates of $F_{ST}$ and Nei's (1978) genetic distance using RAPDFST and BIOSYS-1.7, respectively. These analyses identified considerable structure between Arkansas and each of the other three populations, indicating great to very great genetic divergence between

Arkansas and each of Big Creek, Frontenac Axis, and Maryland (**Table 3-3**). Though significant $F_{ST}$ values were also calculated between Maryland and both Big Creek and Frontenac Axis, such differences qualitatively represent only minor genetic divergence (i.e., $F_{ST} = 0.05\text{-}0.15$). My contrast between Frontenac Axis and Big Creek resulted in a non-significant $F_{ST}$ implying little genetic differentiation between the two populations. Between-population $F_{ST}$ averaged 0.164 ± 0.059 S.E. over all pairwise comparisons.

Estimates of Nei's (1978) genetic distance among regional populations averaged 0.190 ± 0.080 S.E. (ranging from 0.004 to 0.440). Though RAPD and protein variability data may not be directly comparable I felt it was important to consider my results in the context of other studies that have investigated genetic variation in snakes. My review of this literature indicates that few other studies have detected such strong genetic differentiation (average Nei's distance = 0.044 ± 0.011 S.E., N = 10 studies) between snake populations (**Table 3-4**). Most of these studies of protein variability were conducted on relatively smaller geographic scales (i.e., populations separated by 10-100s km rather than 100s-1000s km). The geographically closest pairs of isolated rat snake populations I surveyed (i.e., Big Creek, Frontenac Axis, Maryland - between 450-600 km apart) averaged as little as 1.5% difference, a value of at least the same magnitude (4.4%) as that found in most of the studies I reviewed. As above, replicate analyses using unique samples of individuals to represent Frontenac Axis, yielded little quantitative and no qualitative differences.

### *Sub-Population Structure*

I also found evidence of genetic structure among sub-populations within the isolated Frontenac Axis population. AMOVA results at this scale demonstrated significant genetic differences among sub-populations within the North/South groups (12.96% of total variance, $p < 0.001$), but not between the North/South groups themselves (1.00% of total variance, $p = 0.771$). Significant variation (86.04% of total variance, $p < 0.001$) was also detected among individuals within sub-populations. Thus, of the total genetic diversity accounted for at this spatial scale, ca. 13% was attributable to local sub-population differences, and ca. 86% to individual differences within sub-populations. Five replicate AMOVAs, using unique random samples to represent Murphy's Point and Queen's Station, yielded little quantitative and no qualitative differences from these original results.

RAPD allele frequency data also revealed moderate differentiation among sub-populations of rat snakes (overall $F_{ST} = 0.058 \pm 0.022$ S.E.). By this estimate, ca. 6% of allelic variation in the Frontenac Axis is found among sub-populations. Thus, gene flow ($Nm$, Wright 1931) among local sub-populations 24-34 km apart is relatively high (i.e., $Nm > 1$), or at least sufficient to preclude major genetic divergence. Pairwise comparisons supported this interpretation, with $F$ values and genetic distance measures generally indicative of moderate to little genetic differentiation or isolation among sub-populations (**Table 3-3**). Sub-population $F_{ST}$ averaged $0.038 \pm 0.007$ S.E. Based on protein variability, King and Lawson (1995) documented similar measures of divergence ($0.032 \pm 0.005$ S.E.) among water snake (*Nerodia sipedon*) sub-populations. That some of the most widely separated sub-population pairs (e.g., Queen's Station vs. La Rue Mills and Hill Island, 40 km apart) exhibited little differentiation further demonstrates the lack of distinct sub-division across much of the Frontenac Axis. Nevertheless, I also found evidence of moderate divergence between some sub-populations. For example, Charleston Lake vs. La Rue Mills yielded an $F_{ST} = 0.081$, despite being separated by a relatively short geographic distance (15 km). It is perhaps not insignificant that a major four-lane highway occurs between these two local sub-populations.

Genetic distances between rat snake sub-populations across the Frontenac Axis averaged 0.014 ± 0.002 S.E. As noted above, several studies of protein variability in snakes have derived similar estimates of genetic distance among "populations" (e.g., Merkle 1985, Paik & Yang 1987, Hedges 1989).

Finally, replicate analyses using unique samples of individuals to represent Murphy's Point and Queen's Station, yielded minor quantitative and no qualitative differences in my results.

### *Inter-Hibernacula Structure*

Genetic structure was also evident between some neighboring hibernacula (**Table 3-3**). Analysis of RAPD allele frequency data identified a significant, though minor, divergence between La Rue Mills and Hill Island. Given the geographic distance separating them, it is highly unlikely that members of these two hibernacula have direct contact with one another (see **Fig. 3-2**). Thus, current gene flow between La Rue Mills and Hill Island would involve snakes using hibernacula between these two sites. As a result, La Rue Mills and Hill Island exhibit genetic distance and gene flow estimates representative of sub-population structure and more in keeping with comparisons made at that spatial scale (see **Table 3-3**).

In agreement with our understanding of the movement patterns and reproductive ecology of black rat snakes in Ontario, I found no evidence of divergence between the hibernaculum populations of Curtis and Cow Island (**Table 3-3**), members of which have overlapping home ranges (Weatherhead & Hoysak 1989). Unexpectedly, the geographically proximate (1.6 km) Riverside and Overpass hibernacula sampled in Maryland were found to be considerably more divergent (**Table 3-3**).

### *Population Diversity*

As predicted, the isolated populations of Big Creek and Frontenac Axis were less heterozygous (0.104 ± 0.055 S. E. and 0.144 ± 0.051 S. E., respectively) than the core populations of Maryland and Arkansas (0.213 ± 0.069 S. E. and 0.162 ± 0.069 S. E., respectively), though the differences were not significant (ANOVA; $F = 0.547$, df = 27, $p = 0.655$). Estimates of mean expected heterozygosity per locus for the four regional populations averaged 0.155 ± 0.022 S.E., a value which is more than three times the average (0.046 ± 0.004 S.E.) derived from a review of 33 studies that have investigated protein variation in snakes. Estimates of heterozygosity for the three Frontenac Axis sub-populations averaged 0.188 ± 0.028 S.E. (Murphy's Point = 0.160 ± 0.067 S. E., Queen's Station = 0.160 ± 0.050 S. E., Charleston Lake = 0.244 ± 0.067 S. E.). Analysis of variance indicated no significant differences among the three ($F$ = 0.086, df = 41, $p = 0.917$). Heterozygosity estimates for the six communal hibernacula average 0.161 ± 0.017 S.E. (Curtis = 0.150 ± 0.046 S. E., Cow Island = 0.152 ± 0.056 S. E., La Rue Mills = 0.089 ± 0.043 S. E., Hill Island = 0.166 ± 0.059 S. E., Overpass = 0.190 ± 0.061 S. E., Riverside = 0.218 ± 0.079 S. E.), and no significant differences could be detected among them (ANOVA; $F = 0.558$, df = 41, $p = 0.730$). Bellemin *et al.* (1978 ) found communally hibernating populations of red-sided garter snakes (*Thamnophis parietalis*) exhibited heterozygosities ranging from 0.011 to 0.028, with a mean of 0.019.

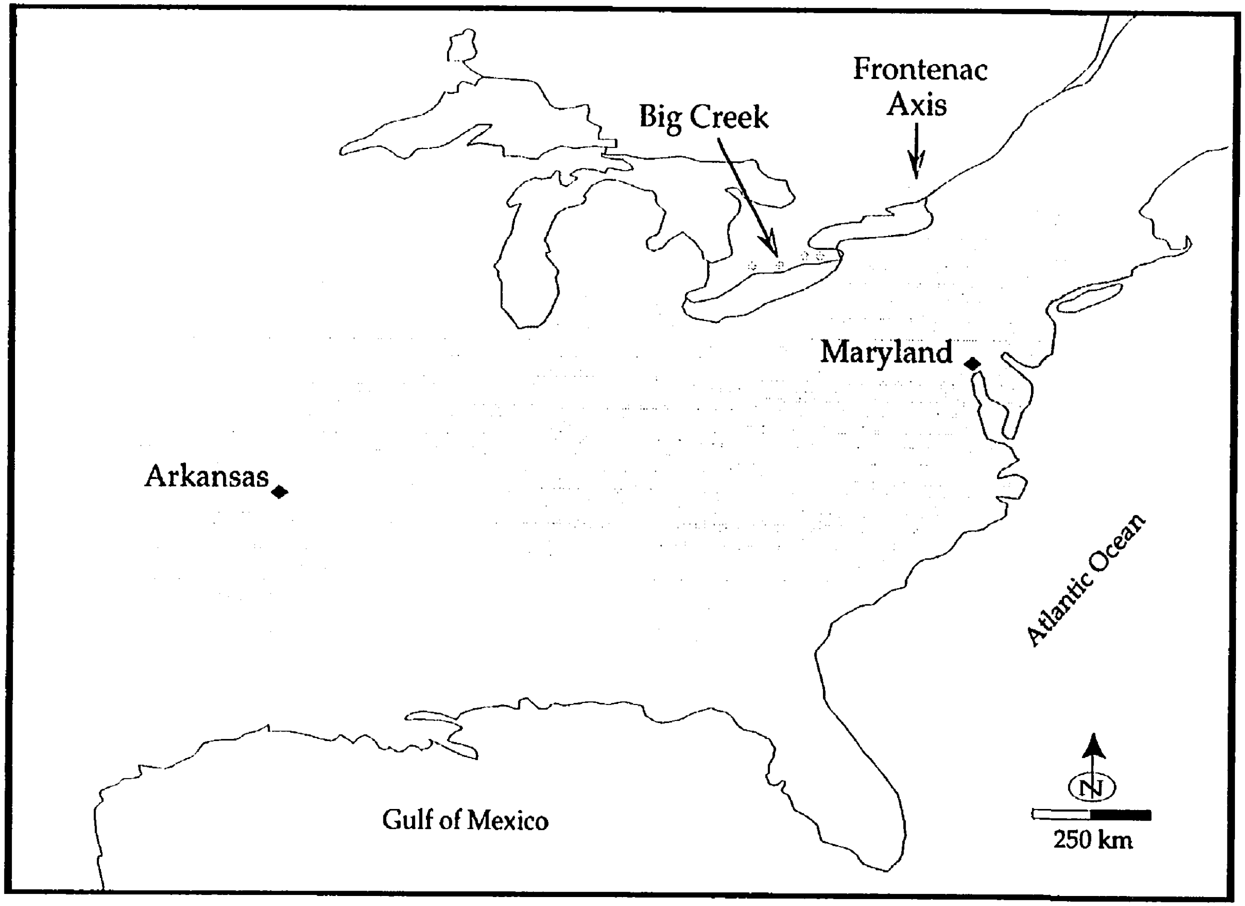

**Figure 3-1.** General distribution of the black rat snake (shaded area) and the relative positions of the four study populations in Canada and the U.S. including; Big Creek, Frontenac Axis, Maryland, and Arkansas.

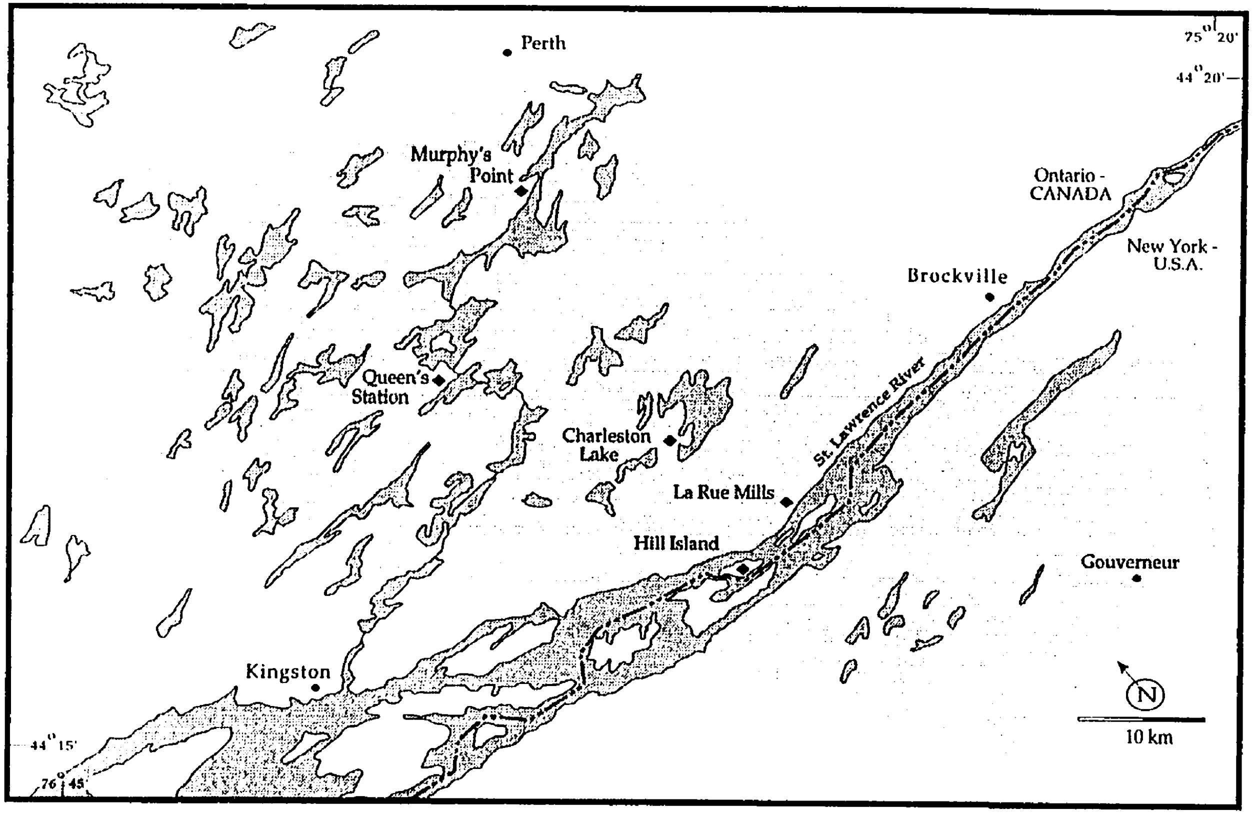

Figure 3-2. General range of the Frontenac Axis population (shaded area) located in eastern Ontario and northern New York and the relative positions of sub-populations (Murphy's Point, Queen's Station, Charleston Lake, La Rue Mills, and Hill Island) sampled across the region.

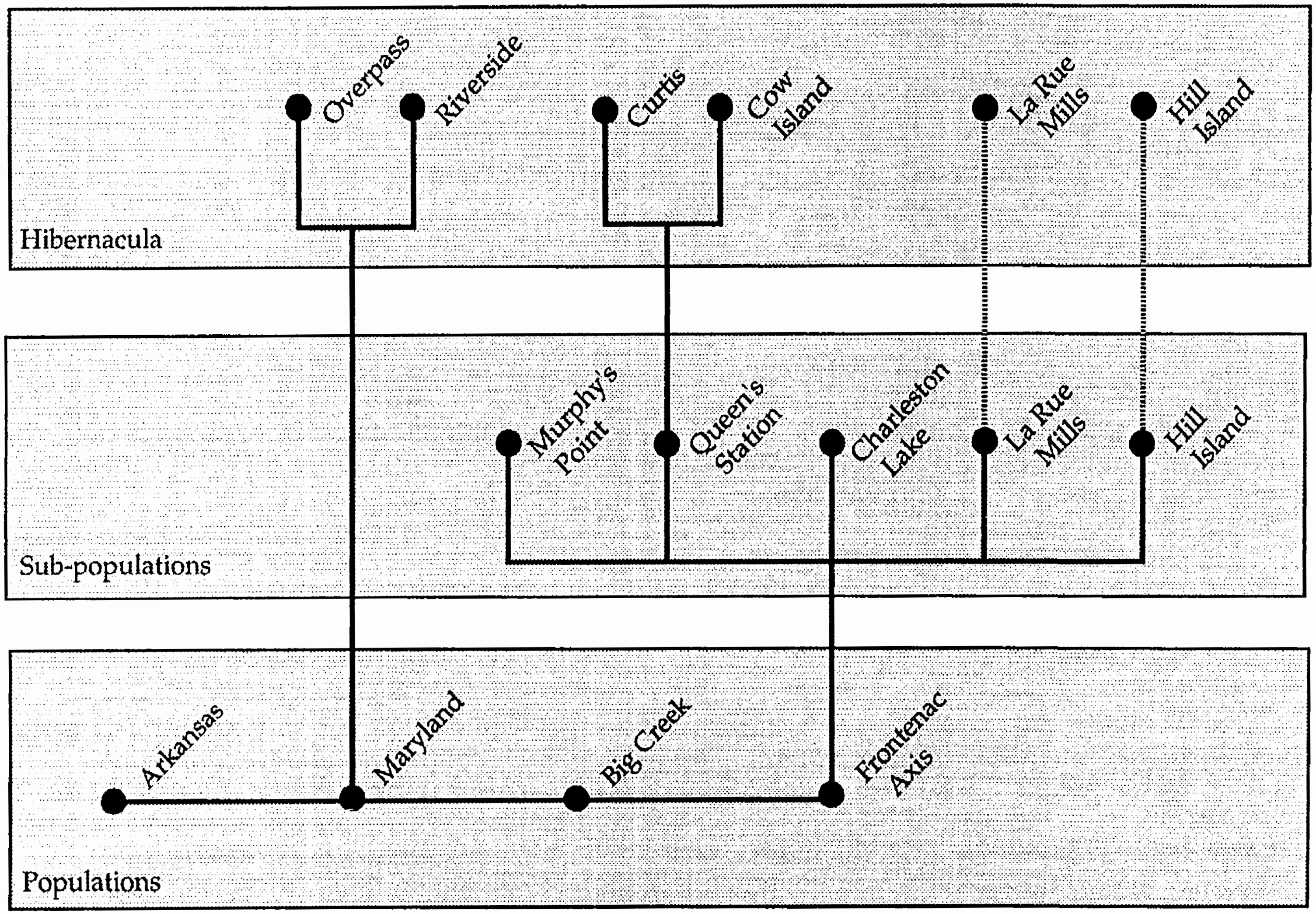

**Figure 3-3. Schematic of the hierarchical relationship among black rat snake samples used in genetic analyses. Note that samples collected from La Rue Mills and Hill Island hibernacula were used at two levels in the hierarchical analyses.**

Table 3-1. Phenotypic band (and allelic) frequencies among 14 RAPD loci for four populations of black rat snakes. (+/- indicates the presence/absence of a variable band). The 7 loci used to form "restricted datasets" are identified by asterisks.

| | Population and sample size | | | |
|---|---|---|---|---|
| Locus | Frontenac Axis<br>29 | Big Creek<br>15 | Maryland<br>30 | Arkansas<br>26 |
| Locus 1 | | | | |
| + | 0.333 (0.208) | 0.066 (0.033) | 0.800 (0.550) | 1.000 (1.000) |
| - | 0.666 (0.792) | 0.933 (0.967) | 0.200 (0.450) | 0.000 (0.000) |
| Locus 2* | | | | |
| + | 0.083 (0.083) | 0.133 (0.067) | 0.233 (0.117) | 0.961 (0.808) |
| - | 0.833 (0.917) | 0.866 (0.933) | 0.766 (0.883) | 0.038 (0.192) |
| Locus 3 | | | | |
| + | 0.958 (0.792) | 0.866 (0.633) | 0.866 (0.633) | 0.730 (0.481) |
| - | 0.041 (0.208) | 0.133 (0.367) | 0.133 (0.367) | 0.269 (0.519) |
| Locus 4* | | | | |
| + | 0.125 (0.063) | 0.133 (0.067) | 0.266 (0.150) | 0.000 (0.000) |
| - | 0.875 (0.938) | 0.866 (0.933) | 0.733 (0.850) | 1.000 (1.000) |
| Locus 5 | | | | |
| + | 0.958 (0.792) | 0.933 (0.733) | 1.000 (1.000) | 1.000 (1.000) |
| - | 0.041 (0.208) | 0.066 (0.267) | 0.000 (0.000) | 0.000 (0.000) |
| Locus 6 | | | | |
| + | 0.916 (0.708) | 1.000 (1.000) | 0.966 (0.817) | 1.000 (1.000) |
| - | 0.083 (0.292) | 0.000 (0.000) | 0.033 (0.183) | 0.000 (0.000) |
| Locus 7* | | | | |
| + | 0.000 (0.000) | 0.000 (0.000) | 0.000 (0.000) | 0.384 (0.212) |
| - | 1.000 (1.000) | 1.000 (1.000) | 1.000 (1.000) | 0.615 (0.788) |
| Locus 8* | | | | |
| + | 0.041 (0.021) | 0.000 (0.000) | 0.600 (0.367) | 0.923 (0.731) |
| - | 0.958 (0.979) | 1.000 (1.000) | 0.400 (0.633) | 0.076 (0.269) |
| Locus 9* | | | | |
| + | 0.333 (0.188) | 0.466 (0.267) | 0.466 (0.267) | 0.076 (0.038) |
| - | 0.666 (0.813) | 0.533 (0.733) | 0.533 (0.733) | 0.923 (0.962) |
| Locus 10 | | | | |
| + | 1.000 (1.000) | 1.000 (1.000) | 1.000 (1.000) | 0.500 (0.288) |
| - | 0.000 (0.000) | 0.000 (0.000) | 0.000 (0.000) | 0.500 (0.712) |
| Locus 11 | | | | |
| + | 1.000 (1.000) | 1.000 (1.000) | 1.000 (1.000) | 0.961 (0.808) |
| - | 0.000 (0.000) | 0.000 (0.000) | 0.000 (0.000) | 0.038 (0.192) |
| Locus 12* | | | | |
| + | 0.375 (0.208) | 0.000 (0.000) | 0.166 (0.083) | 1.000 (1.000) |
| - | 0.625 (0.792) | 1.000 (1.000) | 0.833 (0.917) | 0.000 (0.000) |
| Locus 13* | | | | |
| + | 0.041 (0.021) | 0.066 (0.033) | 0.000 (0.000) | 0.000 (0.000) |
| - | 0.958 (0.979) | 0.933 (0.967) | 1.000 (1.000) | 1.000 (1.000) |
| Locus 14 | | | | |
| + | 1.000 (1.000) | 1.000 (1.000) | 1.000 (1.000) | 0.961 (0.808) |
| - | 0.000 (0.000) | 0.000 (0.000) | 0.000 (0.000) | 0.038 (0.192) |

**Table 3-2. Black rat snake population differentiation (*F*ST) and migration rate (*Nm*) estimates based on allele frequency data derived using 7 variable RAPD loci. Geographically isolated populations contrasted include; Big Creek, Frontenac Axis, Maryland, and Arkansas.**

Restricted dataset (7 loci)

| Locus | $F_{ST}$ | $\chi^2$ | d.f. | *P* | *N m* |
|---|---|---|---|---|---|
| Locus 2 | 0.479 | 91.01 | 3 | <0.001 | 0.3 |
| Locus 4 | 0.045 | 8.55 | 3 | 0.034 | 5.4 |
| Locus 7 | 0.166 | 31.54 | 3 | <0.001 | 1.3 |
| Locus 8 | 0.386 | 73.34 | 3 | <0.001 | 0.4 |
| Locus 9 | 0.061 | 11.59 | 3 | 0.008 | 3.8 |
| Locus 12 | 0.707 | 134.33 | 3 | <0.001 | 0.1 |
| Locus 13 | 0.017 | 3.23 | 3 | 0.357 | 14.4 |
| **Means/ Totals** | **0.266** | **50.54** | **14** | **<0.001** | **0.70** |

**Table 3-3. Estimates of genetic differentiation ($F_{ST}$), genetic distance, and migration rate (*Nm*) between pairs of (a) regional populations, (b) sub-populations within the Frontenac Axis, and (c) neighbouring communal hibernacula of black rat snakes. Estimates are based upon 7 variable RAPD loci. Asterisks denote significant differentiation between sample pairs. Values for comparison between La Rue Mills vs. Hill Island are included in calculation of sub-populations means but not hibernacula means.**

| | | Restricted dataset (7 loci) | | |
|---|---|---|---|---|
| Pairwise comparison | Distance (km) | $F_{ST}$ | Genetic distance | *Nm* |
| Regional populations | | | | |
| Big Creek vs. Arkansas | ~1500 | 0.323* | 0.440 | 0.5 |
| Frontenac Axis vs. Arkansas | ~1900 | 0.273* | 0.339 | 0.7 |
| Maryland vs. Arkansas | ~1700 | 0.287* | 0.317 | 0.6 |
| Big Creek vs. Maryland | 590 | 0.039* | 0.020 | 6.1 |
| Frontenac Axis vs. Maryland | 560 | 0.041* | 0.022 | 5.8 |
| Frontenac Axis vs. Big Creek | 465 | 0.019 | 0.004 | 12.9 |
| Mean values | ~1119 | 0.163 | 0.190 | 4.4 |
| Sub-populations within Frontenac Axis | | | | |
| Murphy's Pt vs. Hill Island | 50 | 0.037* | 0.021 | 6.4 |
| Murphy's Pt vs. La Rue Mills | 45 | 0.035* | 0.017 | 6.9 |
| Queen's Station vs. La Rue Mills | 40 | 0.009 | 0.000 | 26.3 |
| Queen's Station vs. Hill Island | 40 | 0.017 | 0.008 | 14.2 |
| Murphy's Pt vs. Charleston Lk | 35 | 0.056* | 0.017 | 4.2 |
| Murphy's Pt vs. Queen's Station | 25 | 0.038* | 0.019 | 6.4 |
| Queen's Station vs. Charleston Lk | 25 | 0.038* | 0.016 | 6.3 |
| Charleston Lk vs. Hill Island | 20 | 0.031 | 0.008 | 7.9 |
| La Rue Mills vs. Hill Island | 18 | 0.034* | 0.011 | 7.2 |
| Charleston Lk vs. La Rue Mills | 15 | 0.081* | 0.023 | 2.8 |
| Mean values | 31 | 0.038 | 0.014 | 8.8 |
| Communal hibernacula | | | | |
| La Rue Mills vs. Hill Island | 18 | 0.034* | 0.011 | 7.2 |
| Overpass vs. Riverside | 1.6 | 0.039* | 0.026 | 6.1 |
| Curtis vs. Cow Island | 1.2 | 0.006 | 0.000 | 39.5 |
| Mean values | 1.4 | 0.022 | 0.013 | 22.8 |

Table 3-4. **Indices of genetic variability and structure (ranges in parentheses) for snake populations.**

| Species | Mean heterozygosity | Mean genetic distance (Nei 1978) | Mean distance (km) between populations | Reference |
|---|---|---|---|---|
| *Nerodia harteri* | 0.00 | 0.00 | < 100 | Rose & Selcer 1989 |
| *Thamnophis sirtalis* | 0.060 (0.041 - 0.083) | 0.081 (0.030 - 0.090) | 144 (1.5 - 255) | Sattler & Guttman 1976 |
| *Thamnophis parietalis* | 0.019 (0.011 - 0.028) | --- | 6.3 (2 - 150) | Bellemin *et al.* 1978 |
| *Thamnophis sauritus* | 0.077 | 0.072 | 800 | Gartside *et al.* 1977 |
| *Thamnophis proximus* | 0.092 | ? (0.020 - 0.061) | 450 (50 - 800) | Gartside *et al.* 1977 |
| *Elaphe dione* | 0.049 (0.037 - 0.087) | 0.034 (0.009 - 0.079) | 220 (25 - 485) | Paik & Yang 1987 |
| *Phyllorhynchus arenicolus* | 0.029 (0.017 - 0.041) | 0.026 (0.020 - 0.040) | 20 | Murphy & Ottley 1980 |
| *Typhlops jamaicensis* | 0.045 (0.000 - 0.081) | 0.026 (0.000 - 0.072) | 68 (8 - 175) | Hedges 1989 |
| *Typhlops platycephalus* | 0.068 (0.000 - 0.120) | 0.101 (0.020 - 0.220) | 75 (15 - 120) | Hedges & Thomas 1991 |
| *Typhlops hypomenthes* | 0.046 (0.000 - 0.080) | 0.042 (0.010 - 0.090) | 54 (35 - 90) | Hedges & Thomas 1991 |
| *Notechis scutatus-ater* | 0.037 (0.000 - 0.130) | --- | --- | Schwaner 1990 |
| *Agkistrodon piscivorus* | 0.016 (0.009 - 0.029) | 0.015 (0.001 - 0.036) | ?? | Merkle 1985 |
| *Crotalus scutulatus* | 0.042 (0.038 - 0.045) | 0.002 (0.001 - 0.003) | sympatric? | Wilkinson *et al.* 1991 |

## Discussion

The two general goals of this chapter were to make an initial assessment of the genetic structure and levels of gene flow in black rat snake populations at three spatial scales, and to characterize the genetic diversity of remnant populations found in Ontario relative to populations sampled in the central core of the species' range. Using 7 variable RAPD markers, I found evidence of genetic differences between geographically separated populations of black rat snakes. Regional populations appear to be strongly divergent from east to west across the species' range. Gene flow over this distance (1500 km) is insufficient ($Nm < 1.0$) to have kept these regional groups from diverging. Across shorter distances (500 km) and presumably more recent isolation, regional populations were less genetically divergent. Limited genetic differentiation between Big Creek and Frontenac Axis (1.9%) implies that historically these two populations may have been continuous. The Frontenac Axis population might represent the remnant of a range extension for the subspecies that expanded along the south and (or) north shore(s) of Lake Ontario from the southwestern part of the province (see **Fig. 3-1**) following the last glaciation. The rate of historic gene flow between southwestern and eastern Ontario appears to have been sufficient for Big Creek and Frontenac Axis to retain relatively high genetic similarity, despite their demographic and spatial isolation for at least the past 150 years.

I identified a moderate level of divergence among sub-populations across the Frontenac Axis, suggesting that there is genetic and demographic structure within this isolated population. Habitat heterogeneity and fragmentation, aspects of the species' behavioral ecology (e.g., dispersal patterns), patterns of human activity, and simple geographic distance might all contribute to an interference in the rate of gene flow among sub-populations 25-35 km apart.

Overall, the degree of genetic structure I found for black rat snakes using RAPDs was higher than most previous studies have found for other species using protein electrophoresis. This difference may reflect the methods used rather than biological differences among species. Given that RAPD loci appear to be more variable than protein loci, one should be better able to detect subtle patterns of genetic structure in populations using RAPDs. If so, then my results for black rat snakes suggest that other snake species may be more highly structured genetically than previous studies have indicated. Thus, other species need to be studied using highly variable genetic markers to determine whether genetic sub-structure is present in relatively small regional populations (i.e., at the scale of 100s rather than 1000s of km).

The range in estimates of genetic distance I found between adjacent hibernacula was unanticipated. Radiotelemetry studies (Weatherhead & Hoysak 1989; Blouin-Demers & Weatherhead, unpubl. data) demonstrate that residents of hibernacula that are 1-2 km apart (e.g., Cow Island and Curtis) probably interbreed, implying that effective population sizes may be quite large. The 17 km between La Rue Mills and Hill Island precludes contact between individuals associated with these hibernacula and thus, their relatively greater divergence in comparison to Curtis and Cow Island is not unexpected. The degree of differentiation between La Rue Mills and Hill Island is not unlike that observed at the scale of local sub-populations. By contrast, I found significant divergence between the Overpass and Riverside hibernacula, despite the fact that they were separated by only 1.6 km. Because these two hibernacula exist in what is a predominantly urban landscape (multi-lane highway, residential sub-divisions, shopping malls, bowling alleys) snake movements may be restricted, resulting in little opportunity for inter-hibernacula gene flow. Evidence of genetic structure even over fairly short distances (e.g., 2-20 km) implies that gene flow among rat snake populations can be easily disrupted. If so, this pattern could explain the prevalence of sub-species in *Elaphe obsoleta* (5 sub-species currently recognized; Conant & Collins 1991). Habitat interruptions and other ecological discontinuities of seemingly minor extent may be sufficient to isolate snake populations from one another and thus initiate their divergence. This may be particularly true for highly sedentary species and species that center their activities around traditional hibernacula. Human land-use practices have been implicated in the genetic isolation of other herpetofaunal communities (e.g., Reh & Seitz 1990, Madsen *et al.* 1996).

### *Implications for Conservation*

My analysis revealed that a significant component of the total genetic diversity in black rat snakes may be found at the scale of isolated populations. This result indicates that extinction of the Big Creek and Frontenac Axis populations would constitute an important loss of genetic diversity for this species within the eastern half of its range.

From the perspective of conservation of the species within Ontario, my inability to discriminate between Big Creek and Frontenac Axis offers two important implications. First, the apparent lack of genetic structure between southwestern and eastern Ontario might be regarded as evidence that inter-population transfers, aimed at improving the viability of the highly vulnerable Big Creek population, would not result in genetic incompatibles between donors and recipients. However I caution against such an interpretation because RAPD alleles are thought to be neutral genetic markers and it is quite possible that other portions of the genome could be highly differentiated. If so, active mixing of populations could cause "genetic contamination", possibly leading to outbreeding depression (Meffe & Carroll 1994). Second, the lack of genetic distinctiveness of Big Creek and Frontenac Axis implies that the extinction of one of these populations would represent a comparatively minor loss with respect to the diversity of black rat snakes in Ontario. This dispassionate interpretation is akin to the "agony of choice" (e.g., Crozier 1992), in which the evolutionary distinctiveness of taxa is used as a value-weighting factor to direct conservation priorities. Despite the unsavory nature of electing to disregard certain populations as a conservation strategy, pragmatic considerations (financial constraints, logistics) mean that such decisions are likely to be more common in the future and empirical data will be valuable in guiding this process. Nevertheless, I believe it is essential that the results reported here are confirmed with additional genetic markers (e.g., microsatellites) before any decisions regarding the genetic management of these threatened populations are made. Furthermore, genetic novelty should also be weighed against other considerations (e.g., local values, ecological role; Hunter & Hutchinson 1994) in decisions about conserving local populations.

General theory and most empirical evidence support the expectation that isolated and (or) peripheral populations should exhibit reduced heterozygosity and allelic variation (e.g., Lesica & Allendorf 1995). However, following a review of protein variability in 26 populations (mostly *Thamnophis spp.*), Dessauer *et al.* (1987) concluded that snake populations at the geographic periphery of a species' range, on islands, or in other distributional disjunctions do not generally have lower levels of diversity. In contrast, the two isolated and peripheral Ontario populations of black rat snakes tended to be less heterozygous than either Maryland or Arkansas, though the differences were non-significant. Also, Schwaner's (1990) extensive dataset on protein variation in mainland and island populations of tiger snakes (*Notechis scutatus-ater* complex) has revealed a negative correlation between heterozygosity and the degree of isolation (time, distance, or some combination thereof). Schwaner's study is particularly noteworthy in that a strong negative relationship between population heterozygosity and the frequency of scale and skeletal anomalies was found, which may reflect a negative consequence of the loss of genetic diversity. Similarly, observational and experimental work on Swedish adders (*Vipera berus*) supports the view that population isolation, genetic variability, and fitness traits are interrelated in snakes (Madsen *et al.* 1996). While there may not be any immediate cause for concern regarding reduced heterozygosity in populations of rat snakes in Ontario, these data (and those for sub-populations and hibernacula as well) represent a benchmark for the assessment of genetic variability in this species and threatened populations of snakes generally.

As much as 13% of the total genetic diversity in the Frontenac Axis population may be attributable to local sub-population differences. That sub-population divergence may be moderate overall ($F_{ST}$ = 0.058) suggests that local, provincial, and national resource agencies should work toward cooperative management of the entire regional population. In particular, physical or ecological barriers that could impede the natural movements of snakes should be minimized in order to avoid (anthropogenic) sub-structuring of the Frontenac Axis population. To the extent that similar patterns of genetic structure are found in other populations, appropriately-scaled and decentralized management of threatened snakes may be warranted in many jurisdictions. Furthermore, if the pattern of divergence detected between the two hibernacula in Maryland is largely a function of habitat loss and disturbance causing isolation, then we might expect to see similar or more extreme patterns of divergence among sub-populations of snakes occupying human-dominated landscapes. For example, populations of the globally threatened eastern fox snake (*Elaphe vulpina gloydi*) and eastern massasauga rattlesnake (*Sistrurus c. catenatus*), are found in remnant habitat patches within highly urban settings in eastern North America.

In summary, data derived using variable RAPD markers suggest that black rat snake populations are genetically structured across a broad range of spatial scales, including divergence among geographically isolated populations, local sub-populations, and perhaps pairs of neighboring hibernacula. The patterns my results have revealed in conjunction with my review of the existing literature implies that snakes in general may be highly structured genetically. This information will help direct the conservation of populations of black rat snakes within Ontario to the extent that they provide (1) a means of assessing the relative distinctiveness of remnant populations, (2) an empirical basis for identifying appropriate scales for conservation, and (3) a method of testing the efficacy of management practices. The development of hyper-variable taxon-specific genetic markers (e.g., microsatellites) will be useful for confirming the patterns reported here and may reveal even more genetic structure to populations than I have been able to identify.

# Chapter 4.

# Status of the Black Rat Snake in Canada

This chapter formed the basis of a manuscript of the same title
submitted to COSEWIC
and coauthored by Kent A. Prior and Patrick J. Weatherhead.

# Introduction

Over twenty-five years ago Cook (1970) raised issues of concern regarding the conservation status of more than half (14/24) the snake species found in Canada. Many of those highlighted in that list have been defined as peripheral (populations) species whose distribution centers occur south of Canada in the U.S. From the perspective of conservation, peripheral species are locally rare, but globally common, often occurring as geographically isolated or disjunct populations in Canada. In general, peripheral species may have conservation value because they (1) fulfill important ecological roles, (2) act as an umbrella species (e.g., elicit conservation actions that aid other species), (3) represent a significant portion of the genetic diversity of a species (Lesica & Allendorf 1995), and/or (4) represent some local (cultural or heritage) value (Hunter & Hutchinson 1994). Indeed, such cases have been successfully argued for both the blue racer (*Coluber constrictor foxi*; listed as endangered by COSEWIC) and the eastern yellow-bellied racer (*C. c. flaviventris*; listed as vulnerable by COSEWIC) in Canada.

The black rat snake (*Elaphe o. obsoleta*) is yet another peripheral species in need of conservation action in Canada. While the species is found throughout large portions of the eastern U.S., their historically limited distribution in Canada has recently been further restricted and fragmented, primarily due to human activities. This document reviews the decline and current range of the black rat snake in Canada, summarizes their habitat needs, general biology, and potential limiting factors, assesses their conservation value, and evaluates their conservation status.

## Description

Black rat snakes in Ontario regularly attain lengths greater than 130cm snout-vent length (SVL), making it the largest snake native to Canada. Adults are shiny black in colour with faint, irregular-shaped dorsal and lateral blotches of black-brown visible in some individuals upon close inspection. Light-coloured skin between the scales can be white, yellowish, or orange-red. The chin and throat are white or cream with occasional small flecks of black. The ventral surface is a cloudy light grey with irregular black checkerboarding (and occasionally spots of orange-red), on a background of white to yellowish-cream. In contrast to adults, juveniles have a pale grey ground colour overlaid with irregular-shaped, dark-brown to black blotches running dorsally and laterally along the body and tail. This patterning fades with age - the grey ground colour shifting to black and the blotches fading considerably by the time they have reached ca. 100cm SVL. The species exhibits sexual size dimorphism, males having relatively longer tails (ca. 18 vs. 16% of total body length) and achieving a greater maximum SVL (ca. 160 vs. 150cm) than females. Adults have several of the mid-dorsal rows of scales weakly keeled and the others are smooth; keels are only slightly developed or lacking altogether in the young. The anal plate and caudal scutes are divided (see Cook 1984; Ernst & Barbour 1989; Conant 1991).

In Ontario, adult black rat snakes may be confused with (1) northern water snakes (*Nerodia s. sipedon*), (2) melanistic eastern garter snakes (*Thamnophis s. sirtalis*), and (3) blue racers. However, a few key features allow these species to be distinguished from one another. Northern water snakes have dark brown crossbands on the neck and foreparts and alternating dorsal and lateral blotches on the rest of the body, the ground colour may be grey to dark brown, crescent-shaped blotches of black to red-brown occur on the belly, scales are keeled, and they are usually found in aquatic habitats. Melanistic garter snakes are jet-black in colour (though some have a white throat patch), scales are keeled, and they have an undivided anal plate. Blue racers are unpatterned and blue-green to dark-blue on the dorsal surface, they have bluish bellies, a white chin and throat, smooth scales, and in Ontario they are currently thought to occur nowhere other than on Pelee Island in Lake Erie. Young black rat snakes and eastern fox snakes (*Elaphe vulpina gloydi*) have similar colour markings. As such, these species may be easily confused in parts of southwestern Ontario where they are sympatric. However, they can be distinguished by counting the number of ventral scutes - black rat snakes have 221 or more whereas, fox snakes have 216 or less (Conant 1991).

## Distribution

Black rat snakes are widely distributed and can be found where appropriate habitat occurs across much of the eastern half of the U.S. (**Fig. 4-1**). In the southern parts of their range they may be relatively abundant (Ford *et al.* 1991; Durner & Gates 1992). In the east they may be found from southwestern New England south to central Georgia, while in the midwest they occur from southwestern Wisconsin south to southern Oklahoma, northern Texas, and northern Louisiana. Populations of black rat snakes found in Ontario are on the northern edge of the species' distribution and therefore may be regarded as geographically peripheral to the species' central range.

Two distinct regions of Ontario harbor isolated populations of black rat snakes - the Carolinian Forest region, running across the north shore of Lake Erie in southwestern Ontario and the Frontenac Axis region, in southeastern Ontario (see **Fig. 4-1**). I discuss the populations occupying each of these regions in turn.

### *(A) Carolinian populations*

The historic availability of suitable habitat, a broad historic distribution, and the current pattern of population distribution suggest that black rat snakes invaded what is now southwestern Ontario by following the northward advance of mixed deciduous forests (ca. 7,000 years ago), subsequent to the retreat of the Laurentide ice sheet (Smith 1957; Urquhart 1957; Pielou 1991). It is possible that this invasion followed two distinct paths; one across the Niagara Peninsula between Lake Erie and Lake Ontario and another by crossing the Detroit River at the western end of Lake Erie (see **Fig. 4-1**). Vast tracts of deciduous forest interspersed with open savannah communities which formed north of Lake Erie would have provided an abundance of suitable habitat such that black rat snakes were probably relatively common throughout the region prior to European settlement (Yates 1889 cited in Lamond 1994). Historic data implies that rat snakes may have been found across the entire Carolinian Zone of southwestern Ontario. For example, records extend from Point Pelee (41°57'N, 82°31'E) in the west (Logier 1925) to Fonthill (43°02'N, 79°18'E) in the east (Lindsay 1931). However, human-induced landscape changes across the Carolinian region have been severe. Land-clearing reached a peak by 1880 when as much as 80% of the primary forest cover had been removed (Kelly 1990). Reforestation has been minimal since that time, with remnant forests now largely restricted either to valleys or isolated upland blocks, the majority of which are less than 3 ha in size (Pearce 1992). Today the region is dominated by intensive agriculture (Chapman & Putnam 1969) overlaid by an extensive network of paved roads. For rat snakes (and other species), these landscape changes have resulted in major habitat losses and range contraction to such a degree that the entire Carolinian region may now be occupied by as few as four small, habitat-isolated, local populations. This current distribution is in striking contrast to the continuous range portrayed by several authorities (Logier 1958; Cook 1984; Conant 1991). The four provisional Carolinian populations I recognize include the following;

(1) The Skunk's Misery population is located north of the Thames River, between the towns of Bothwell (42°38'N, 81°52'E) and Wardsville (42°39'N, 81°45'E) in Middlesex County. The habitat occupied by the Skunk's Misery population is a complex of upland, open deciduous forest and maple swamp. Recent, verifiable records of rat snakes from Skunk's Misery date from 1984 (Ontario Heritage Information Centre [OHIC] - unpubl. data). Sightings have also been made from south of the village of Rodney (42°34'N, 81°41'E) in 1987 (OHIC - unpubl. data) and 1996 (a pair observed mating on 13 June; S. Thompson, pers. commun.) and from near the village of New Glasgow (42°32'N, 81°37'E), also in 1987 (OHIC - unpubl. data). These locations are 15-20 km south-east of Skunk's Misery and therefore may be representative of a separate population (perhaps associated with Sixteenmile Creek).

(2) The Big Creek population occurs north of the base of Long Point, and appears to extend as far west as Port Burwell (42°35'N, 80°50'E), east to Turkey Point Marsh (42°40'N, 80°20'E) and north to the towns of Tillsonburg and Delhi (42°50'N, including the Counties of Elgin and Haldimand-Norfolk). Among the four Carolinian populations this is probably the largest and most well-defined geographically (covering an area of ca. 700 km$^2$). Local naturalists report opportunistic encounters with members of the Big Creek population on a fairly regular basis (e.g., recent reports from 1996) and the distribution of these sighting/capture records suggest a strong association with (contiguous?) riparian forests along creeks (i.e., Otter Creek, Venison Creek, Deer Creek, Big Creek) and larger forest blocks (OHIC - unpubl. data; M. Gartshore - unpubl. data).

(3) The Oriskany population is associated with the Oriskany Sandstone outcrop (42°57'N, 79°56'E), a regionally rare geological formation (see Fahselt *et al.* 1979), and roughly bounded by the towns of Nelles Corners, Clanbrassil, and Decewsville (Haldimand-Norfolk County). The unique geological, floral, and faunal elements found here have afforded the region "Area of Natural and Scientific Interest" (ANSI) status by the Ontario Ministry of Natural Resources (Fahselt *et al.* 1979; Macdonald 1989). The Taquanyah Conservation Area may serve as a protected habitat area in this region though the species has not been reported from within the boundaries of the conservation area specifically. Members of the Oriskany population are observed infrequently, the most recent confirmed sighting occurring in 1990 (OHIC - unpubl. data). Details of several recent sightings have been recorded by Lamond (1994).

(4) The Niagara population(s) is poorly defined geographically. Several

verified sightings are clustered around the towns of Fonthill (43°02'N, 79°18'E; 1928-1976, see Fahselt *et al.* 1979) and Ridgeville (43°02'N, 79°19'E; 1988, OHIC - unpubl. data) in Niagara County. The predominant land-use in this area includes orchards and vineyards. Forested ravines associated with the nearby tributaries of Twelve Mile Creek may provide suitable habitat for rat snakes. Ministry of Natural Resources staff have also reported sightings from within Shorthills Provincial Park in 1978 (S. Thompson, pers. commun.). A single sighting in 1988 (OHIC - unpubl. data) from near the town of Winger (42°57'N, 79°27'E; ca. 16 km south-west) stands out as a geographic outlier and may be representative of yet another disjunct population.

In addition, I note a fifth area in the Carolinian region that should be surveyed for the presence of black rat snakes. Though I know of no confirmed reports from this area, the expansive forest blocks of the Caradoc/Muncey/Oneida IR (ca. 42°50'N, 81°30'E), ca. 15 km west of the town of St. Thomas and bordering the Thames River, would appear to offer promising habitat for rat snakes.

### *(B) Frontenac Axis population*

The geographically disjunct Frontenac Axis population (**Fig. 4-1**) is confined to an area of approximately 5000 km$^2$ (44°15' — 44°55'N, 75°20' — 76°45'E) in eastern Ontario (Frontenac, Lanark, Leeds and Grenville Counties) and northern New York (Jefferson, St. Lawrence Counties, Weller & Oldham 1989; A. R. Briesch - pers. commun.). I am unaware of any confirmed sightings north of highway 7 in Ontario though I do not regard this line as a definite ecological/physical boundary. Members of the Frontenac Axis population occur at the very northern edge of the species' range and probably represent a relict of a northward advance by the species during the post-glacial hypsithermal period (see Schmidt 1938; Smith 1957). The isolated nature of the population was recognized relatively early (Lindsay 1931; Toner 1934; Toner & Edwards 1938; Logier 1957; Conant 1958). In fact, rat snakes are notably absent from historical herpetofaunal surveys of upper New York (De Kay 1842; Britcher 1903; Weber 1928), suggesting that the disjunction of the Frontenac Axis population may have preceded European settlement and land-clearing for timber and agriculture. Today, the next nearest population is found ca. 120 km to the south, near Syracuse, New York.

The Frontenac Axis population is named for its association with an exposed, southeastern extension of the Canadian Shield of the same name. This geological feature is regarded as an important regional biogeographic link between Algonquin Provincial Park in Ontario and Adirondack State Park and the Adirondack Mountains in New York. The topography of the Frontenac Axis is irregular with a strongly rolling upland terrain and micro-relief that is highly variable (Beschel *et al.* 1962; Rowe 1972). Ridges rise and fall approximately every 500 m, ranging from gentle slopes to vertical cliffs. Coarse-grained outcrops of bedrock (gneiss) occur in nearly every hectare (Beschel *et al.* 1962; Gillespie *et al.* 1966; Hoffman *et al.* 1967). Lake, river, and wetland systems predominate areas of low relief. Extensive second growth, mixed deciduous-coniferous forests (Great Lakes-St. Lawrence Forest Region) cover much of the area (Beschel *et al.* 1962; Rowe 1972). The abandonment of marginal agricultural land over the past 60+ years has also resulted in considerable shrubland. Despite the apparent availability of suitable habitat across most of the region, ecological discontinuities (large lakes, grazed pasture) and physical barriers (highways, urban development) that inhibit movement probably contribute to the maintenance of semi-independent sub-populations across the Frontenac Axis. Indeed, recent analysis of DNA collected from localities distributed across the region provides preliminary evidence of genetic sub-structuring within the Frontenac Axis population (see *General Biology - Population Genetic Structure*).

Interestingly, the presence of rat snakes directly north of Lake Ontario has never been verified, despite intensive surveys by herpetologists and naturalists throughout the area during the early 1900's (e.g., Williams 1913; Logier 1957; although see Johnson 1989). This suggests that the Carolinian and Frontenac Axis populations may never have been contiguous north of Lake Ontario, a southern connection through New York being the more likely possibility (see **Fig. 4-1**).

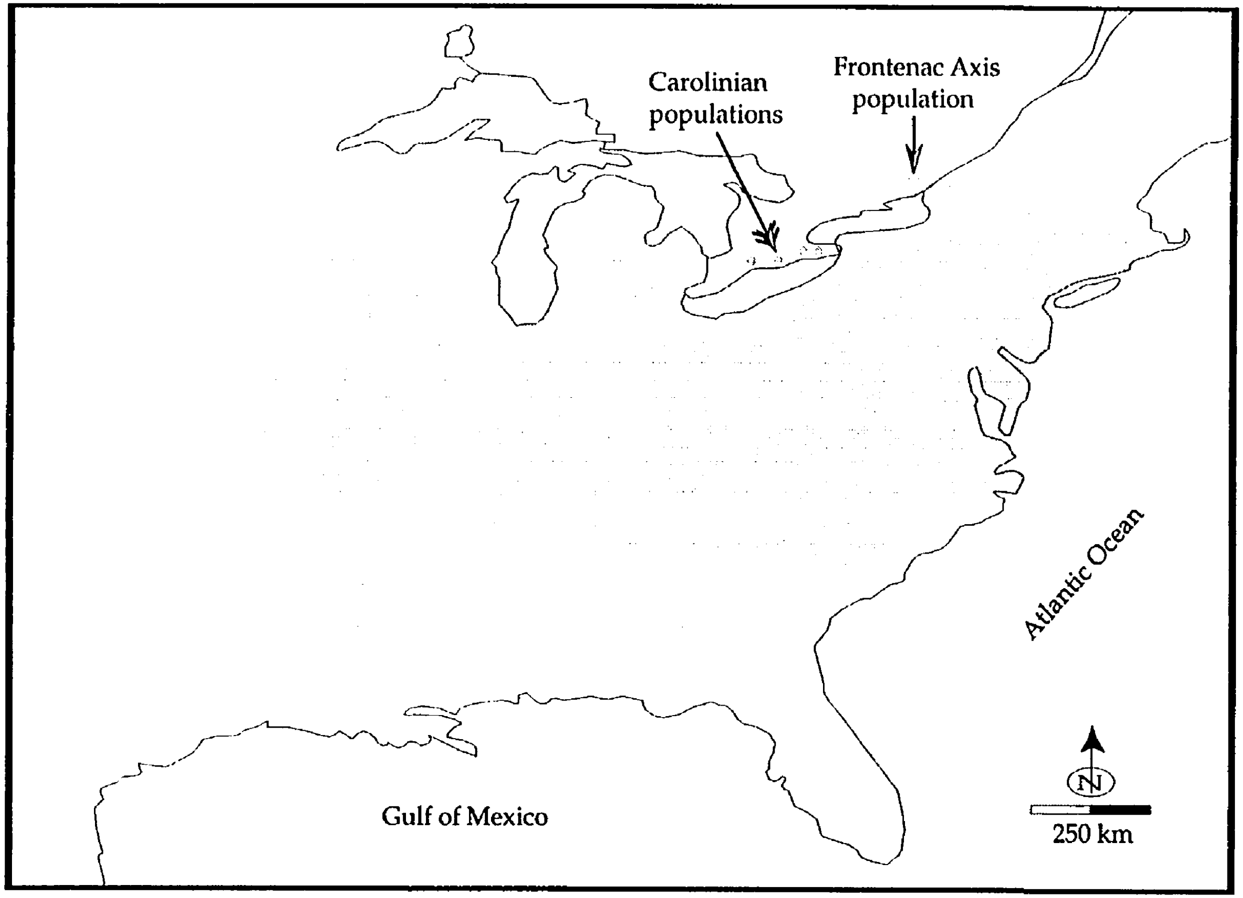

**Figure 4-1. General distribution of the black rat snake (shaded area) in Canada and the U.S. including the relative positions of isolated populations in southwestern and eastern Ontario.**

## Protection

Black rat snakes found in Ontario are legally protected from harassment, taking, or killing under the provincial Game and Fish Act (Regulation 520, Sections 76, 77, 78). That part of the Frontenac Axis population which extends south into New York (see **Fig. 4-1**) receives no legal protection from either the State of New York or U.S. federal authorities (A. Breisch - pers. commun.). Perhaps as little as 5% of the range occupied by this species across the Carolinian and Frontenac Axis regions is protected within conservation areas (e.g., Taquanyah), nature reserves, provincial parks (e.g., Murphy's Point, Charleston Lake), and national parks (St. Lawrence Islands).

## Population Size and Trends

To my knowledge, no quantitative demographic data exist for any of the Carolinian region black rat snake populations. As such, empirical estimates of population size (either historic or current) cannot be made at present. However, evidence of the loss and fragmentation of primary habitat (eastern deciduous forest) within this region indicate that the species has suffered significant range contraction, population isolation, and a major decline over the past 100 years in southwestern Ontario. I conservatively estimate that perhaps as much as 75% of the species historic range north of Lake Erie has been lost due to human landscape alteration. The presumed small population sizes, negative attitude toward snakes by some residents, high incidence of roadkills, and prospects for future land-use suggest that local extinctions across the Carolinian region are likely to occur in the foreseeable future. On the basis of the frequency of sightings and area occupied, the Big Creek population appears to hold the most promise in terms of future viability within the Carolinian region. However, Big Creek has also received relatively more survey/research attention than any other Carolinian population and this fact may bias current expectations for viability.

Recent analyses of long-term capture-recapture data for two local populations (Queen's Biology Station and Hill Island) from the Frontenac Axis found no evidence of any linear trend in either population size or annual survival estimates over the 15-year study period (1981-1995). However, there was a significant correlation between the two sites for both estimates suggesting that rat snake populations may fluctuate synchronously across the Frontenac Axis, phenomenon that perhaps reflects a common response to environmental perturbations (seasonal weather conditions, prey abundance).

The geographic limits of the Frontenac Axis population are moderately well defined on the basis of sighting/capture records. In contrast to the Carolinian populations, the isolation and range limits of the Frontenac Axis population appear to be correlated with natural physiographic and ecological landscape features (e.g., the Frontenac Axis itself) rather than being caused by landscape-scale habitat alteration by humans (although this hypothesis needs to be tested). As such, the range limits of this population may have fluctuated very little since European settlement. However, within this region, habitat availability and quality has varied due to human land-use practices and rat snakes have, undoubtedly, responded accordingly.

It is encouraging to note that the abandonment of agricultural land over the past 60 years across the Frontenac Axis (McKenzie 1967) has probably led to an increase in the availability of suitable habitat through natural succession of vegetation communities. Nevertheless, this pattern is contrasted by a trend of increased residential settlement (country estates, rural sub-divisions) and recreational activity (traffic, boating, camping, hiking, cottage development) across the region. One measure of the trend in recreation is the annual visitation statistics from Murphy's Point, Frontenac, and Charleston Lake Provincial Parks, each of which have resident populations of rat snakes. All three parks have realized increased use through the 1980s and early 1990s (Frontenac opened in 1983). Overall, total visitation increased from ca. 81,000 in 1980 to ca. 161,500 in 1994. There have likely been significant direct negative impacts (e.g., disturbance, incidental mortality) upon local populations of rat snakes resulting from such increases in human presence (see Garber & Burger 1995 for a case study with Wood Turtles, *Clemmys insculpta*). Indirect costs like habitat elimination and the creation of movement barriers caused by infrastructure development (cottages, expansion and upgrading of roads) and high traffic volume are also likely to have resulted from the general increase in recreation across the region. It would be useful to quantify and map (past, present, and future) patterns of landscape-change and human activity across the region in order to help guide population management plans and mitigate future impacts on rat snake populations.

Differences in population density across the species' range and the paucity of comparative data make it difficult to estimate the percentage of the (global) breeding population occurring in Canada (Ontario). With respect to geographic range, the Ontario populations represent < 5% of the global range for this species.

## Habitat

Black rat snakes are considered semi-arboreal and are generally associated with upland, deciduous forest and ecotone habitats (Ernst & Barbour 1989), though they appear to be capable of utilizing a broad range of habitat types (Ford *et al.* 1991; Durner & Gates 1993). Within Ontario, preliminary assessments of habitat use have been conducted across the Frontenac Axis region at the Queen's Biological Station (Weatherhead & Charland 1985), Charleston Lake Provincial Park (McAllister 1995), Murphy's Point Provincial Park (Thompson - unpubl. data), and Mallorytown and Hill Island (May & Andress - unpubl. data). Detailed studies of habitat use are ongoing at Queen's (Blouin-Demers & Weatherhead - unpubl. data). To date, no attempt has been made to characterize habitat use by black rat snakes occupying the agricultural landscapes of the Carolinian region. Given both the qualitative and quantitative differences in habitat found in the Frontenac and Carolinian regions, I expect that populations of rat snakes from these areas might exhibit dissimilar patterns of habitat use and activity.

### *Active season habitat*

Preliminary research conducted on the Frontenac Axis population at Queen's suggests that black rat snakes found there have a strong preference for old field habitats and in particular, the edge created where field and deciduous forest habitats meet (Weatherhead & Charland 1985; Blouin-Demers & Weatherhead - unpubl. data). Interestingly, the preference for edge habitat appears to be most pronounced during the peak of the bird breeding season (15 July and before), suggesting that habitat selection during the spring and early summer may be largely influenced by the distribution of prey (i.e., eggs and nestlings) and individual foraging requirements (though thermoregulatory requirements were not discounted). Such seasonal shifts in habitat use (as effected by sex, reproductive status, and age) are commonly reported in field studies of temperate-zone snakes (reviewed by Reinert 1993).

Overall, data presented by Weatherhead and Charland (1985) imply that black rat snakes may require a mosaic of habitat types (forest, field, edge). This expectation was largely supported by a radio-telemetry study of this species carried out at Remington Farms Wildlife Management Area in Maryland, a landscape composed of 50% mixed forest and 33% cropland (Durner & Gates 1993). This study found a high frequency of movement within, and a disproportionate use of forest edge, hedgerows, and brushy habitats by rat snakes, behaviours which the authors suggest are indicative of the importance of these habitats for foraging. The low frequency of movement in forest interior and residential areas was thought to indicate the use of these habitats as refuges (e.g., during ecdysis and digestion). Crop fields were rarely used by snakes in the Remington Farms study area.

Weatherhead and Charland (1985) hypothesized that the extreme elimination of forest habitat (e.g., > 50% loss) coupled with an increase in the scale of the forest-field mosaic have probably been important factors contributing to the decline of rat snakes across the Carolinian region. In areas where human-induced landscape change has been somewhat less pronounced (e.g., Remington Farms, Maryland), resulting in a mix of agriculture, brushy hedgerows, fallow fields, and numerous small forest blocks with a high edge-to-area ratio, the habitat may be close to ideal for rat snakes (Durner & Gates 1993). This is evidenced by the abundance of black rat snakes (G. Durner - pers. comm.) and their importance as avian nest predators at the Remington Farms study area. Furthermore, their frequent use of residential areas (for foraging and refuge) suggests that rat snakes are able to withstand, or perhaps benefit from, certain associations with humans.

Black rat snakes are clearly familiar with the location of important habitat features within their home ranges since they make directed movements and repeat visits to traditional basking, retreat, oviposition, overwintering, and foraging sites (Weatherhead & Robertson 1990; Durner & Gates 1993; Prior & Weatherhead - unpubl. data) - see below for additional details on specific habitat features.

Females in Maryland make greater use of sheltered retreats (hollow trees, buildings) and choose higher elevated perches than males (Durner & Gates 1993). Such differences in habitat use may reflect differences associated with the reproductive biology of the sexes. Small sample sizes have prevented such inter-sexual comparisons for rat snakes in Ontario, though on-going studies will provide such opportunities (Blouin-Demers - pers. comm.). This work will also help determine the extent to which the findings (and interpretation) of Weatherhead and Charland (1985) are generally representative of rat snakes in the Frontenac Axis.

Technological constraints (i.e., size of radio-transmitter packages) have to date precluded researchers from collecting unbiased data on habitat use (and activity patterns) of neonate and juvenile rat snakes.

### *Hibernacula*

Across much of the northern half of the species range, black rat snakes exhibit a period of seasonal inactivity. In Ontario, overwinter dormancy normally persists for as much as 7 months (October - April) each year (Weatherhead 1989), which the snakes spend underground at traditional, communal sites referred to as hibernacula. At least 15 major hibernacula across the Frontenac Axis region have been identified by following snakes via radio-telemetry. To my knowledge, no hibernacula in the Carolinian region have been located (although *Elaphe vulpina* in the Big Creek area have been found hibernating in what appear to be abandoned mammal burrows, Gartshore & Prior - unpubl. data).

The subterranean structural features of hibernacula in the Frontenac Axis region have yet to be determined though many appear to consist of natural faults, fissures, or rotted tree root systems (pers. obs.). All are relatively stable structures which may be used by snakes for several decades. Data derived from thermal-sensitive radio-transmitters suggest that the internal environment of hibernacula undergo a steady decline in temperature, to a minimum of ca. $3^{o}$ C, from the beginning of the overwinter period (Weatherhead 1989). In addition, hibernacula must provide a sufficiently humid micro-climate to preclude overwinter dehydration (Costanzo 1989; Prior & Shilton 1996).

The surface habitat features (e.g., slope, aspect, canopy closure, ground cover) of 10 communal hibernacula in the Frontenac Axis were recently characterized in an attempt to facilitate the identification and protection of new hibernacula (see *Chapter 1;* Prior & Weatherhead 1996). My demonstrated that hibernacula across the Frontenac Axis are typically situated on relatively rocky, south-facing slopes. The co-occurrence of these features was sufficiently unique as to distinguish hibernacula from a series of random sites, but not from a set of intuitively identified "potential hibernacula" in the surrounding landscape. This implies that additional requisite elements (e.g., underground structure and interior micro-climate), which I was unable to quantify, set actual hibernacula apart from sites that appear to be otherwise suitable (i.e., "potential hibernacula"). I also found that the trees used by snakes for basking at hibernacula tended to be relatively large and decayed or dying with numerous cavities (hollows probably created by fungal infection). Unused trees exhibiting these characteristics were also available at both random sites and potential hibernacula indicating the suitability of those sites for basking.

From a conservation perspective, these findings indicate that both the identification and protection of communal hibernacula in Ontario will have to proceed on a site by site basis. One cannot predictably find new hibernacula by simply looking for characteristic habitat features within a landscape. Therefore, radio-telemetry remains the most effective and practical means of locating hibernacula. The preferential use of particular trees by rat snakes suggests that the availability of arboreal basking sites should be considered in management plans designed to preserve the suitability of communal hibernacula. Specifically, mature and decaying trees and snags should not be cleared from the vicinity of actively used hibernacula. Similarly, where possible, such features might be restored at degraded sites prior to attempts at repatriating extirpated populations (see *Basking and retreat sites*).

### *Basking and retreat sites*

Following emergence from hibernation and prior to dispersal to summer home ranges, black rat snakes typically bask arboreally for several days within the immediate vicinity of their hibernaculum (Prior & Weatherhead - unpubl. data). In most cases, the snakes tend to select relatively large, decaying trees for basking purposes, probably because they provide broad, horizontal perches and numerous cavities into which they may retreat from potential predators and inclement weather (*Chapter 1*; Prior & Weatherhead 1996). As with hibernacula, some basking sites experience long-term traditional use (Stickel *et al.* 1980; Prior & Weatherhead - unpubl. data) and therefore, may be important to identify and protect for local conservation purposes.

Throughout the active season, rat snakes may also remain "inactive" at sheltered locations for extended periods (defined as ≥ 7 days, Weatherhead & Charland 1985) while they undergo ecdysis or during the digestion of large meals (maximum 21 days inactive recorded, S. Thompson - pers. commun.). Sheltered retreat sites used by rat snakes include hollow trunks and branches of trees, logs, stumps, brush piles (Weatherhead and Charland 1985), or artificial structures like buildings, abandoned vehicles, and other machinery (Durner & Gates 1993; Blouin-Demers - pers. commun.). Weatherhead and Charland (1985) found such retreats to be located predominantly along forest-field edges (with open exposure to direct sunlight), although they may also be situated within forest interiors (Durner & Gates 1993; Blouin-Demers - unpubl. data). Depending upon the type of site, retreats may be either long-lasting (e.g., large trees, buildings), resulting in traditional use by many snakes over several years, or more ephemeral (e.g., brush piles), persisting for only few seasons. Where their availability is limited, it may be important to identify and protect retreat sites that are currently used by local populations.

### *Oviposition sites*

The few black rat snake oviposition sites found in the Frontenac Axis, have been located in well-decayed American elm (*Ulmus americana*) stumps (2 separate sites, S. Thompson - pers. commun.), tilled garden soil (A. Forsythe - pers. commun.), and compost and manure piles (P. J. Weatherhead - pers. obs.). Outside Ontario, nests have also been located in the interior of dead trees, piles of sawdust, and mounds of rotting vegetation, including grass clippings and leaves (Fitch 1963; Ernst & Barbour 1989).

As has been found in other parts of the species' range (Lynch 1966; Clark & Pendleton 1995), females on the Frontenac Axis are known to nest communally at traditional sites. For example, over at least 2 seasons, elm stump nests contained multiple clutches at various stages of development (both sites were later destroyed by nest predators, possibly Raccoon, *Procyon lotor*). Communal nesting by black rat snakes suggests either a limited availability of suitable sites or alternatively, that females realize some direct advantage by laying their clutches together.

## General Biology

### *Age and Growth*

Hatchling black rat snakes average 26.03 ± 3.62 cm SVL (N = 27) in Ontario and there is no evidence of sexual-size dimorphism in neonates (Fitch 1963; Clark & Pendleton 1995; S. Mullin pers. commun.). I recently completed a growth rate analysis for rat snakes on the Frontenac Axis (Chapter 2). My analysis indicated that male rat snakes grow relatively faster than females, as has been qualitatively demonstrated by Stickel *et al.* (1980). These sex-specific growth trajectories are responsible for three related demographic attributes. First, relatively fast growth by males results in the male-biased sexual size dimorphism, characteristic of the species. Second, differential growth rates may result in a disproportionate number of members of the faster growing sex in large size-classes, leading to male-biased adult sex ratios. Third, relatively rapid growth by males means that they take less time to reach sexual maturity (i.e., 7y to reach 900mm SVL for males vs. 9y to reach 1000mm SVL for females). Rat snakes in this study also exhibited relatively rapid growth preceding sexual maturity and a subsequent slowing with increasing size in both sexes, a pattern they share with many species (Parker & Plummer 1987; Houston & Shine 1994; Madsen & Shine 1994).

My data indicate that growth rate declines more precipitously with increasing size for females than it does for males in the Frontenac Axis population. This implies that the energy costs associated with maturation and reproductive activity are dissimilar for males and females. Perhaps the ultimate costs of reproduction are higher for females in this population because, the short active season (May-September) limits their ability to recover body reserves lost through reproduction. Females in this population lay eggs in mid to late July, leaving them ca. 1-1.5 mo to acquire energy that must be allocated to growth, overwinter reserves, and possibly subsequent vitellogenesis. In comparison, the mating season ends in mid to late June, allowing males to focus on foraging for as much as 1 mo in advance of females. It may also be significant that males are able to concentrate on foraging during the peak of bird breeding activity, since eggs and nestlings represent major prey items for black rat snakes (Stickel *et al.* 1980; Weatherhead & Charland 1985).

The influence of environmental conditions on growth rate was further illustrated by differences I found between the Frontenac Axis and a population studied in Maryland (Stickel *et al.* 1980). Rat snakes on the Frontenac Axis exhibited relatively slow growth, delayed maturity, and high longevity compared with those in Maryland. Furthermore, the highly variable growth rates exhibited by females on the Frontenac Axis suggest that growth rate is influenced more by environmental conditions than by age *per se*. Differing energy expenditures (e.g., reproduction) and fluctuations in prey availability may have significant effects on inter-annual growth rates of rat snakes on the Frontenac Axis. A shorter growing season may mean that chance plays a much greater role in determining growth rates (e.g., number of meals per summer) for females on the Frontenac Axis than it does in Maryland. Finally, growth rates calculated for the Frontenac Axis indicate that some individuals (both males and females) in this population may be capable of reaching 30+ years.

### *Spatial and Activity Patterns*

Direct observations at communal hibernacula indicate rat snakes on the Frontenac Axis usually begin to emerge from hibernation in mid-April (Weatherhead 1989; Prior & Weatherhead, unpubl. data). The earliest date which I have observed them emerging is 10 April. The emergence period lasts between 5 and 7 weeks, with most snakes having evacuated hibernacula by 1 June. Dispersal from the vicinity of the hibernaculum is somewhat protracted as both males and females often spend 1-3 days basking (usually arboreally) in the immediate area.

Unbiased observations of spatial and activity patterns of black rat snakes have come from radio-telemetry studies conducted on the Frontenac Axis (Weatherhead & Charland 1985; Weatherhead & Hoysak 1989; McAllister 1995) and in Maryland (Durner & Gates 1993). We know nothing about the movement and activity patterns of rat snakes in intensive agricultural landscapes (< 50% natural habitat) like the Carolinian region.

The average active season for rat snakes on the Frontenac Axis extends from about the end of April through to early October (ca. 5 months or 150 days). In the predominantly forested landscape around the Queen's Biological Station some snakes have been found to disperse considerable distances from their hibernaculum (420 m), while others are known to include their hibernaculum in their summer activity ranges (i.e., dispersal distance = 0 m). The shape and size (area) of activity ranges and temporal patterns of activity probably vary among years according to changes in both the distribution of specific habitat types in the local environment and the resource requirements of individuals. For example, sexually mature males are likely to exhibit spatial and temporal patterns of activity during the breeding season that differ considerably from those of immature males. Similarly, during years with low prey densities individuals may have to forage more widely to fulfill their energy needs. Nevertheless, individuals do tend to occupy the same general area in successive years and the use of traditional retreat sites (for pre-ecdysis basking and digestion) serve as important (predictable) activity centres during the summer (Weatherhead & Charland 1985; Weatherhead & Hoysak 1989; Durner & Gates 1993).

Data from the Frontenac Axis also suggests that males and females tend to differ in both the amount of area covered per season (7.57 vs. 1.41 ha, respectively), the average distance per move (69.3 vs. 43.9 m, respectively), and the frequency of activity (36 vs. 31.8% of days spent moving, respectively). These inter-sexual patterns of activity and movement suggested to Weatherhead and Hoysak (1989) that male behaviour may be largely dictated by foraging and mate searching whereas, female behaviour may be influenced by either foraging or thermoregulation depending upon their reproductive status (i.e., gravid vs. non-gravid). However, inter-sexual differences may also be partially contingent on the type of habitat mosaic occupied by the snakes and/or the duration of the active season. For example, Durner and Gates (1993) were unable to detect any difference in home range areas of males and females (mean = 9.49 ha) in an agricultural landscape in Maryland, a region where the active season may be as much as 60 days longer than it is in Ontario. Thus, inter-sexual differences on the Frontenac Axis may result because gravid females in Ontario are burdened by developing eggs for a greater proportion of their active season than are females in Maryland. Whether males and females living in the intensive agricultural landscape of the Carolinian region of Ontario show patterns similar to those in the Frontenac Axis is unknown.

Beyond the fact that neonates and juveniles do not usually attend communal hibernacula, nothing is known about the spatial and activity patterns of young rat snakes.

### *Reproduction*

Black rat snakes are iteroparous (repeated reproductive cycles) and oviparous (egg laying). Females exhibit relatively low reproductive effort per cycle (ca. 34% of body mass into eggs, Seigel & Fitch 1984). Records of reproductive individuals on the Frontenac Axis and in more southern parts of the species' range (Fitch 1963; Stickel *et al.* 1980) suggest that males reach sexual maturity at about 900cm SVL (ca. 7y in Ontario), whereas females may not mature until they reach about 1000cm SVL (ca. 9y in Ontario). On the Frontenac Axis, sexual activity has been observed from mid-June through mid-July, well after dispersal from hibernacula. Mature males are known to combat (wrestle) one another for access to sexually receptive females. While multiple males may be attracted to a single female, individual bouts of courtship and breeding are thought to be restricted to male and female pairs (in contrast to the multi-male/female breeding aggregations of northern water snakes and eastern garter snakes). However, this does not preclude the possibility of multiple paternity in black rat snakes since females may store sperm from sequential matings.

Following a gestation period of 37-51 days the female lays her clutch of eggs at a nest site of decaying wood or vegetation (see *Habitat - Oviposition sites*). The use of traditional communal oviposition sites appears to be a prevalent behaviour throughout the species' range (Lynch 1966; Clark & Pendleton 1995; S. Thompson - pers. commun.). Clutches laid in captivity by wild-bred, Ontario females have ranged in size from 8 to 21 eggs. Clutch size is positively correlated with female size, though we have little data on this relationship for females on the Frontenac Axis. The unattended eggs incubate for a period of 60-75 days. As such, rat snake clutches on the Frontenac Axis may hatch anytime between late-September and early-October. (Incubation period for three artificially incubated clutches averaged 78 days, unpubl. data). Neonates undergo ecdysis at the nest site and may spend their first winter there. I suspect that most mature females on the Frontenac Axis probably reproduce every other year, depending upon their ability to replenish energy reserves following parturition. Having said this, there is anecdotal evidence that some large females on the Frontenac Axis may (occasionally) reproduce annually (S. Thompson - pers. commun.). Furthermore, Diller and Wallace (1996) have presented data indicating that northern populations of female gopher snakes (*Pituophis melanoleucus*), another large oviparous species, are capable of reproducing on an annual basis. Clearly, much more detailed information is required on the reproductive ecology of black rat snakes in Ontario, information that is essential to the development of a thorough understanding of the viability and recovery capacity of local populations.

### *Demographics*

As is typical of the majority of species exhibiting communal overwintering behaviour (reviewed by Gregory 1984), black rat snake hibernacula populations that I have studied on the Frontenac Axis tend to be composed largely of adults (ca. 76%). However, the variation in the percentage of adults associated with the seven hibernacula I studied was quite wide (43-100%). These hibernacula were dominated by mid-sized individuals (≥ 900 - < 1300mm SVL), with relatively few individuals in both the smallest (< 900mm SVL) and largest (≥ 1300mm SVL) size classes. Neonates were not usually found in attendance at the hibernacula I surveyed. This may be largely due to the fact that rat snake oviposition sites, and thus neonate hatching locations, can be quite remote from communal hibernacula. As such, neonate rat snakes often may be unable to commute between birth locations and traditional hibernacula before the onset of their first inactive season. The majority of new recruits to hibernacula on the Frontenac Axis are probably 2-4y old, comparable to the age of recruitment in most other Colubrid species (e.g., Parker & Brown 1973; Parker 1976; Larsen & Gregory 1989).

Sex ratios of hibernacula populations across the Frontenac Axis tend to be highly variable, with roughly equivalent cases of male and female biases. Sex composition was generally even in all size-classes except for the largest (≥ 1300mm SVL), in which males predominated. Though male-biased sex ratios have been documented for other species, it is often the case that sex-biased catchability contributes to such disparities (e.g., Parker 1976; Brown & Parker 1984; Larsen & Gregory 1989). Samples at hibernacula I studied were apparently not plagued by problems of differential catchability (either sex or size; see *Chapter 2*). As such, I am confident that males are truly more prevalent in the large size-classes of black rat snake hibernacula populations of the Frontenac Axis.

Recruitment to hibernacula tends to fluctuate annually, with an average of less than 50% of the population each season being new participants. Given that the majority of recruits may be 2-4y old, recruitment rates serve as crude indices of past reproduction. Because we currently know very little about either the spatial distribution of oviposition (nest) sites in relation to hibernacula or the dispersal patterns of young rat snakes, it is difficult to determine the degree to which local natality and immigration contribute recruits to specific hibernacula.

The choice of a suitable hibernaculum is crucial to overwinter survival for black rat snakes on the Frontenac Axis, since the use of an inappropriate site could prove fatal (e.g., due to freezing temperatures or dehydration). Such high risks suggest that selection should favor high site fidelity (Larsen & Gregory 1989). In keeping with this expectation, hibernacula site fidelity across the Frontenac Axis appears to be on the order to ≥ 95%, indicating that these social groups tend to have relatively consistent adult membership. Fidelity may be relaxed, promoting occasional switches between sites, when an individual's summer range encompasses more than one communal hibernacula. In such cases, changing sites (i.e., lower fidelity) would not be a particularly risky venture since the "new" hibernaculum has been proven suitable by virtue of its occupancy by conspecifics.

Within the Frontenac Axis region I was unable to detect any clear differences in the general size-class distribution of local populations. Averaged over a 15-y study period, the local populations at Queen's Station and Hill Island exhibited very similar patterns of size-structure. However, these similarities belie the fact that the two populations also exhibited *opposing* trends in the proportion of adults present. Queen's exhibited a positive trend, whereas Hill Island followed a negative trend in the percentage of adults sampled. While I am unable to identify a cause for this particular finding, it is perhaps most instructive generally to recognize that contrary trends in the demographic structure of snake populations can occur over relatively short geographic distances (40km).

Data on survivorship for black rat snakes on the Frontenac Axis (Chapter 2) indicate that small (younger) individuals suffer higher mortality rates than larger (older) individuals. I expect that overwinter freezing/dehydration and predation may be the major sources of mortality for juvenile black rat snakes on the Frontenac Axis.

Interestingly, I found no evidence of differential mortality between males and females, implying that the two sexes may incur roughly equal mortality costs due to reproduction. Clarification of the relative importance of the various potential sources of mortality for different size- and sex-classes of black rat snakes will require careful monitoring of the behaviour and actual fates of individuals and field experiments including manipulations of prey availability or food intake so as to control energy reserves (*cf.* Lindell & Forsman 1996; Brown & Weatherhead - in prep.).

Estimates of overall annual survival are relatively high for both the Queen's Station and Hill Island local populations, with no significant longitudinal trends apparent at either site. Neither have I been able to detect any temporal trends (positive or negative) in estimated population sizes. However, Queen's and Hill Island exhibited high correlations in both annual survival and population size over time.

It is possible that these correlations reflect common, population-level responses to ecological perturbation(s). Perhaps region-wide fluctuations in seasonal weather (e.g., wet springs, severe winters) impact the two populations similarly such that survival and capture probabilities vary equally (Parker & Plummer 1987), despite their geographic separation from one another. Correlated changes in population sizes through time by pairs of species (rather than different populations of a single species) at the same study site have been attributed to common, or at least, related responses to human predation (*Masticophis t. taeniatus, Crotalus viridis lutosus;* Parker & Brown 1973), fluctuations in annual precipitation/prey abundance (*Pituophis melanoleucus deserticola, Coluber constrictor mormon;* Parker & Brown

1973), and severe drought (e.g., *Nerodia fasciata*, *Seminatrix pygaea*; Seigel *et al.* 1995). At the very least, my results point to our limited understanding of the dynamics of rat snake populations and the need to explore further the potential reasons why demographic parameters may be synchronous regionally.

### *Population Genetic Structure*

I recently used randomly amplified polymorphic DNA (RAPD) markers to investigate the distribution of genetic variation in populations of black rat snakes sampled in Ontario (Frontenac Axis and Big Creek) and the U.S. (Maryland and Arkansas - see *Chapter 3*; Prior *et al.* in press). Data on the genetic structure of populations is of particular interest because it may reveal evidence of restricted gene flow or genetic isolation that is undetectable through traditional demographic studies (e.g., capture-recapture data). Such knowledge can be used to identify genetically-based "management units" (Moritz 1994), enabling conservation practices to be focused appropriately.

My preliminary analyses revealed that a significant component (11%) of the total genetic diversity in rat snakes may be found within isolated populations like those in Ontario. These results imply that extinction of the Big Creek and Frontenac Axis populations would constitute an important loss of genetic diversity for this *species*. Among the Ontario samples, I have been unable to discriminate genetically between Big Creek and Frontenac Axis. This finding has two important implications. First, it suggests the possibility that inter-population transfers between southwestern and eastern Ontario would not result in genetic incompatibility. That is, the genetic composition of the relatively small Big Creek population might not be compromised were individuals from Frontenac Axis added to it. Although, there are other (non-genetic) reasons why such transfers may not be a sound conservation practice (e.g., inter-population pathogen transfer, life history variation; Reinert 1991; Hindar 1994; Loehle 1995). Second, the lack of genetic structure between Big Creek and Frontenac Axis implies that the potential extinction of one of these populations would represent a comparatively minor loss with respect to the genetic diversity of rat snakes in Ontario. However, genetic novelty should be weighed against other considerations (e.g., local values, ecological role, keystone species; Hunter & Hutchinson 1994) in decisions about conserving local populations of rat snakes. Furthermore, it would certainly be prudent first to confirm the genetic results I have reported with additional genetic markers (e.g., microsatellites) before embarking on active genetic management of the Ontario populations.

I also detected substantial genetic structure among local sub-populations within the Frontenac Axis, with as much as 14% of the total genetic diversity attributable to local population differences. That sub-population divergence may be as low as 6% suggests that conservation and natural resource agencies within this part of Ontario should work toward cooperative management of the entire regional population. In particular, physical or ecological barriers that could impede the natural movements of snakes should be minimized in order to avoid further (anthropogenic) sub-structuring of the Frontenac Axis population.

I was unable to detect genetic structure between adjacent hibernacula in Ontario, suggesting that sufficient gene flow occurs between proximate hibernacula to preclude significant divergence. However, I did find significant genetic structure between two hibernacula sampled in an urban setting in Maryland. If the pattern of divergence detected between these two hibernacula is largely a function of habitat loss and disturbance causing isolation, then we might expect to see similar patterns of divergence among sub-populations of rat snakes occupying the largely agricultural landscapes of southwestern Ontario.

In summary, data derived using variable RAPD markers suggest that black rat snake populations are genetically structured across a broad range of spatial scales including strong divergence among geographically isolated regional populations and internal sub-structuring within regional populations. The patterns of genetic structure identified with RAPD loci will help direct the conservation of threatened populations of black rat snakes within Ontario to the extent that they provide (1) a means of assessing the relative distinctiveness of remnant populations, (2) an empirical basis for identifying appropriate scales for conservation, and (3) a method of testing the efficacy of management practices. The development of hyper-variable taxon-specific genetic markers (e.g., microsattelites) will be useful for confirming the patterns I have reported and may reveal even more genetic structure to populations than I have been able to identify.

### *Diet*

Detailed studies of the diet of black rat snakes in Ontario have not been done although, numerous opportunistic observations of foraging behaviour and inferences made from habitat selection suggest that a diverse array of birds (both eggs and nestlings, Weatherhead & Charland 1985; Rendell & Robertson 1990) and small mammals (S. Thompson - pers. commun.) represent the most important prey items. The list of avian prey taken in

Ontario is quite broad and includes, Anseriformes (e.g., American black buck, *Anas rubripes*), Phasianidae (e.g., ruffed grouse, *Bonasa umbellus*), and a minimum of 12 species of Passeriformes (e.g., American robin, *Turdus migratorius*; northern oriole, *Icterus galbula*; tree swallow, *Tachycineta bicolor*; red-winged blackbird, *Agelaius phoeniceus*; black-capped chickadee, *Parus atricapillus*); the nests of which range from ground-level, to the forest canopy, and include open nests, cavity nests, and artificial nest boxes. Small mammal prey include little brown bat (*Myotis lucifugus*), eastern chipmunk (*Tamias striatus*), red squirrel (*Tamiasciurus hudsonicus*), young snowshoe hare (*Lepus americanus*), and mice (*Peromyscus spp.*). Little is known about the diet of young rat snakes although juveniles has been observed consuming birds eggs (song sparrow, *Melospiza melodia*) and insects.

Weatherhead and Robertson (1990) have reported instances of homing to food (American Robin nests) by black rat snakes following repeated experimental displacement.

*Predators*

Known predators of adult black rat snakes in the Frontenac Axis include mink (*Mustela vison*), raccoon, and red shouldered hawk (*Buteo lineatus*) (S. Thompson - pers. commun.; pers. obs.). Fitch (1963) considered red-tailed hawks (*Buteo jamaicensis*) to be major predators of black rat snakes in Kansas. Eggs and young are potentially susceptible to a broad suite of predators. Nest predators may include, raccoons and skunks (*Mephitis mephitis*), while juveniles may be vulnerable to all of the above plus other mammalian carnivores (e.g., *Mustela spp.*, red fox, *Vulpes vulpes*; domestic cat, *Felis catus*), owls (*Strigidae*), and American crows (*Corvus* *brachyrhynchos*).

In some areas, significant increases in background mortality rates have probably resulted from increased contact with humans (deliberate killing) and human activity (incidental mortality on roads).

## Limiting Factors

A series of intrinsic and extrinsic features serve to limit the persistence and recovery capacity of black rat snakes in Ontario. By intrinsic features I refer to characteristic life history traits and population attributes (e.g., slow growth, delayed maturity, low adult mortality rates, high longevity, large percentage of adults) which make it unlikely that black rat snake populations are capable of recovering quickly from either large overall declines in numbers or even increased mortality rates of adults alone (see Brooks *et al.* 1991 and Congdon *et al.* 1993 for case studies of turtles). High road densities and traffic volume (e.g., as found throughout southwestern Ontario population ranges) probably elevate mortality rates for adult rat snakes above background levels, though this hypothesis should be tested. Furthermore, the wanton killing of even a few individual adult snakes by humans each season could have a severe cumulative impact, particularly in isolated populations where the number of reproductive individuals may already be critically low.

The slow growth rates exhibited by rat snakes also imply that even catastrophic reproductive failures, causing the absence of entire year-class cohorts, may go unrecognized by natural resource managers until several years after the event. This is because, neonates and young juveniles cannot be reliably sampled, since opportunistic captures are biased toward large individuals and young do not attend hibernacula. Therefore, the absence of a given year-class might not be detected until they fail to be recruited into a hibernaculum, 2-4y after they would have been born. Successive years of poor reproduction (e.g., due to cool, wet summers) may have serious negative implications for the viability of small populations.

Because hibernacula are composed largely of (reproductive) adults, the extinction of a single hibernaculum population could have a major negative impact on the local population size and its capacity for recovery. Furthermore, even if the structural integrity and quality of the hibernaculum habitat were to remain high following such an extinction, it could be several years before the site is re-used, since occupation may require a threshold number of individuals in attendance to stimulate conspecific attraction (see Reed & Dobson 1993).

Hibernacula populations across the Frontenac Axis are apparently not uniform, at least in terms of size- and sex-composition. This suggests that monitoring only a few of the several hundred hibernacula populations that may be distributed across the Frontenac Axis would not provide a reliable overview of the status of the larger metapopulation. Furthermore, preliminary evidence suggests that significant genetic structure occurs at the scale of local populations within the Frontenac Axis. As such, natural resource managers who wish to obtain a realistic understanding of populations under their jurisdiction, they need to view local populations within a regional context.

It should be emphasized that these intrinsic features are themselves difficult, if not impossible, to alter directly and therefore should not necessarily be the target of conservation programs. Rather, focus should be placed on addressing those external factors that impact rat snake ecology and population survival.

Two general external features have perhaps the greatest impact on the future viability of rat snake populations in Ontario. The elimination of habitat and the direct killing of snakes can jeopardize populations by both increasing their geographic isolation from one another (affecting demographic and genetic structure) and reducing population size and composition.

As indicated above, direct disturbance or destruction of major traditional hibernacula could eliminate large percentages of local populations, thereby increasing the probability of regional extinctions. This threat may be particularly significant across the Carolinian region where entire local populations may rely on few communal hibernacula. No hibernacula have yet been located and protected directly in the Carolinian region, leaving overwintering populations subject to potential catastrophe. Increasing recreational and residential development across the Frontenac Axis may jeopardize local populations where disturbance of hibernacula results.

Black rat snakes appear to require a variety of habitat elements (forest, field, edge) within their home range (Weatherhead & Charland 1985; Durner & Gates 1993). Presumably, the overall suitability or quality of a habitat landscape is highest where these elements occur in certain proportions. Habitat quality may be commensurate with the relative proportion of requisite elements. If so, then rat snake habitat may be degraded by (1) the absolute loss of specific habitats (e.g., deciduous forest) and/or (2) an alteration in the relative proportions or configuration of the habitat elements

(Weatherhead & Charland 1985; Durner & Gates 1993). Such changes in the landscape composition may affect the spatial and activity patterns of individuals and limit the capacity of a given area to support a viable population. A list of requisite habitat elements for black rat snake populations in Ontario include hibernacula, basking/retreat sites (at hibernacula and within home range), oviposition sites, and a mosaic of relatively mature forest blocks and more open habitats (e.g., edge, old field, beaver meadows, hedgerows).

The Carolinian region populations occupy land areas within predominantly agricultural landscapes. As such, these populations have been subjected to severe landscape-scale habitat alteration including the fragmentation and reduction of forest and the expansion of largely unsuitable habitat (i.e., intensive agricultural crops like tobacco). These changes are the thought to be primary causes of the reduced size and the extreme isolation of populations found there today. It is not known whether the habitat currently available to rat snakes across the Carolinian region is sufficient for the long-term survival of any of the known local populations. That is, some Carolinian populations may be currently inviable.

Agriculture on the Frontenac Axis has always been at best a marginal land-use activity due to the highly variable micro-relief, exposed bedrock, and rocky soils of the region (Chapman & Putnam 1966; McKenzie 1967). These physiographic features have resulted in a heterogeneous habitat landscape, one composed of secondary forest, mixed agricultural land (pasture, hay, oats), successional old fields, wetlands, and rocky outcrops. This habitat mosaic has been regarded as "ideal" for black rat snakes (Weatherhead & Charland 1985) and may be the primary reason for the continued existence of the Frontenac

Axis population. While the abandonment of farmland over the past 60 years has probably increased the quality and availability of suitable habitat in some localities across the region this trend has been countered by a general growth in recreational activity and residential development. Increased disturbance by humans has the potential to reduce habitat quality for snakes (Prior & Weatherhead 1994; C. Parent & P. Weatherhead - unpubl. data) and the building of cottages/homes and roads directly eliminates habitat. Retaining the appropriate habitat composition may be a key to the future persistence of the Frontenac Axis population.

Increased encounters with humans will inevitably lead to higher rates of mortality for rat snakes. All Ontario populations suffer from deliberate killing by humans and incidental mortality on roads, though the relative significance of these sources of mortality may vary among regions. Removal of adults through deliberate or accidental death may have severe negative implications for small, isolated populations. It would be useful to know the degree to which human-related mortality decreases survivorship below baseline levels in different populations. In some regions the relative importance of human-caused mortality may dictate that resource managers directly address this issue with the public through education and other management actions (e.g., property access restrictions, road closures).

The highly isolated nature of each of the Ontario populations means that regional population extinctions will not be compensated by natural re-colonization or immigration. The re-establishment of black rat snakes in areas from which they have been extirpated will require active management (e.g., population translocations).

## Special Significance of the Species

While black rat snakes are widely distributed and enjoy a relatively secure conservation status across much of eastern North America, there are a variety of reasons to regard with special significance the peripheral populations residing in Ontario.

### *Genetic Diversity and Evolutionary Value*

*Elaphe obsoleta* comprise a diverse array of as many as five different subspecies (Conant 1991). Maintaining the full complement of such diversity is a major reason for protecting species throughout their geographic ranges (Meffe & Carroll 1994). Retaining genetic diversity at a coarse, landscape-scale may be important primarily because populations with greater genetic diversity are thought to be better able to adapt to environmental changes (Nunney & Campbell 1993). For example, populations that are genetically adapted to conditions at the edge of a geographic range may be well-adapted to shifting the species' range in response to climatic change (Hoffman & Blows 1994; Lesica & Allendorf 1995). Isolated populations of black rat snakes in Ontario appear to be distinct relative to other regional populations from the U.S. with which they have be compared (see *Chapters 2 & 3*). For example, preliminary estimates suggest that a significant component (11%) of the total genetic diversity in black rat snakes may be found within isolated populations like those in Ontario. Furthermore, evolutionary ecology is often most profitably explored at the extreme limits of a (sub-)species' range (*cf.* Lawson & Lieb 1990). Accordingly, ecological and genetic studies of black rat snakes in Ontario provide an unmatched opportunity to study this species at the very northern edge of its distribution. Such work is likely to yield general insights regarding community structure (Vitt 1987), species diversity (Rosen 1991), and life history variation (Gregory 1982, 1984) in snakes.

### *Cultural Value*

The cultural importance of black rat snakes is perhaps as varied as are the individuals asked to voice an opinion on the subject. To cite but a few examples, I have met farmers from the Big Creek area of southwestern Ontario who are proud to note that rat snakes frequent in their barns and sheds. Some of these farmers view rat snakes as important for controlling rodent populations, while others consider the presence of a healthy population of rat snakes to reflect sound farming practices and land stewardship. Across the Frontenac Axis, I've spoken with both cottagers and permanent residents who enjoy and value the annual return of rat snakes to favored basking sites on their properties. Still others have related to me the wonder and pleasure of having seen trees festooned with large rat snakes during spring emergence. If not by direct experience, many naturalists and students of biology are also familiar with the existence of the Frontenac Axis population. This is because most popular herpetofaunal field guides (e.g., Conant 1991) accurately depict the Frontenac Axis population range as isolated from the species' general distribution in eastern North America. Furthermore, to illustrate the concept of geographic variation, these maps have been reproduced in a widely read evolutionary biology text (see Chapter 4, Fig. 12 in Futuyma 1986).

### *Ecological Value*

The ecological role(s) played by snakes are often unrecognized, in large part due to their inconspicuous nature. However, black rat snakes are known to be major predators of birds and small mammals (Weatherhead & Charland 1985; Durner & Gates 1993) and preliminary density estimates for the Frontenac Axis are relatively high for northern latitudes (2.5-4.0 snakes/ha, see *Chapter 2*). Furthermore, Fitch (1982 cited in Parker & Plummer 1987) considered snake populations to be among the most stable components of an ecological community he studied in Kansas. Thus, direct protection of habitat required to support black rat snake populations in the Carolinian Zone and the Frontenac Axis is likely to afford refuge for many other members of these ecological communities. I estimate that the active season habitats utilized by a hibernating population of rat snakes on the Frontenac Axis may cover as much as 50 ha. Protection of such an area around hibernacula would clearly benefit other species, possibly affording black rat snakes a role as an "umbrella species".

Ontario's black rat snake populations are also representative components of regional ecosystems of high conservation value. The isolated, Carolinian Zone populations occur within the most seriously threatened natural region of Canada and are remnant members of a highly jeopardized Carolinian herpetofaunal community (Cook 1970) which include eastern spiny softshell turtles (*Trionyx s. spinifer*), Lake Erie water snakes (*Nerodia sipedon insularum*), blue racers, fox snakes, Fowler's toads (*Bufo woodhousei fowleri*), and Blanchard's cricket frogs (*Acris crepitans blanchardi*).

The Frontenac Axis is regarded as an important biogeographic link between Algonquin Provincial Park to the north-west and Adirondack State

Park to the south-east, among the largest, (semi-natural) protected areas in eastern North America. Landscape-scale conservation plans are currently being developed for this region (e.g., "The A2A Proposal" coordinated by Canadian Parks and Wilderness Association and Parks Canada). The black rat snakes may serve as a useful indicator species in such efforts because (1) they are widely distributed throughout the region, (2) they serve an ecological role as a top predator, (3) populations can be monitored with relative ease, (4) there exists an extensive baseline dataset on population genetic structure and demography, and (5) the species requires varied habitats including a mosaic of forest and edge, oviposition sites, active season refuges, and traditional communal hibernacula. Beyond their potential utility as an indicators of ecological integrity, the black rat snake may serve as a valuable flagship species for regional conservation efforts. That is, they may act as a representative organism around which conservation management, including public relations, may focus.

## Evaluation and Proposed Status

Black rat snakes have a restricted and insular distribution in Ontario. Habitat loss through intensive agriculture in the Carolinian region appears to have caused a steady range contraction in this region. The four remnant populations found there today (Skunk's Misery, Big Creek, Oriskany, Niagara) are all relatively small and highly isolated. Occurring as they do in a largely agricultural landscape, the Carolinian populations probably suffer from limited habitat availability, mortality on an extensive road network, and deliberate killing by humans during opportunistic encounters. A lack of protected habitat, relatively small population size, and the absence of any inter-population movement make the potential for extinction high among these populations. Indeed, I question the likelihood that any of the Carolinian populations have long-term viability under the current conditions.

The isolated Frontenac Axis population of eastern Ontario is more secure relative to the Carolinian populations. The physiography of the region has limited agricultural activity, and farm abandonment over the past 60 years has produced a mosaic of habitat types suitable for black rat snakes. The range and population size of the Frontenac population is also considerably larger than any of the Carolinian populations. Nevertheless, reproductive constraints due to the life history traits of the species, increases in human recreational activity and residential development (causing habitat loss and direct mortality), the relative lack of protected habitat, and geographic isolation provide reasons for concern about the future viability of the Frontenac population.

The characteristics and distribution of these remnant populations, coupled with the direct threats faced by each, suggest an insecure future for the species in Canada. When considered independent of the Frontenac Axis population, I regard the Carolinian populations of black rat snakes to be seriously "endangered". Regarded collectively, the threat of species extirpation is somewhat reduced. Therefore, I propose that black rat snakes be recognized as a Threatened Species in Canada since they are likely to become endangered if the external factors affecting their vulnerability are not reversed.

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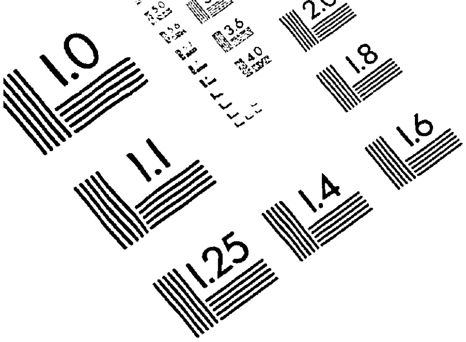

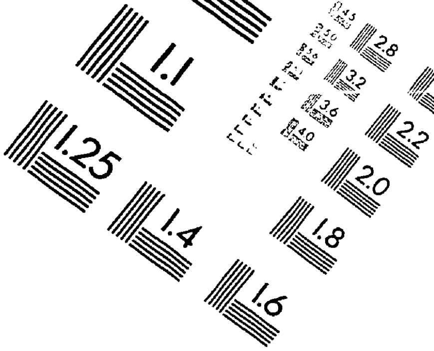

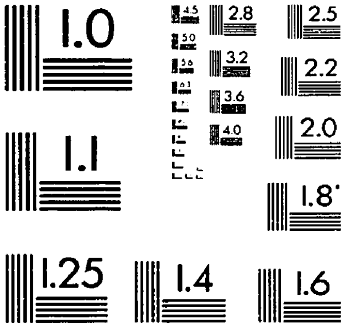

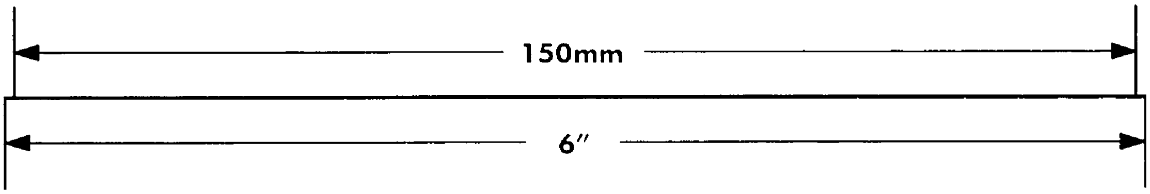

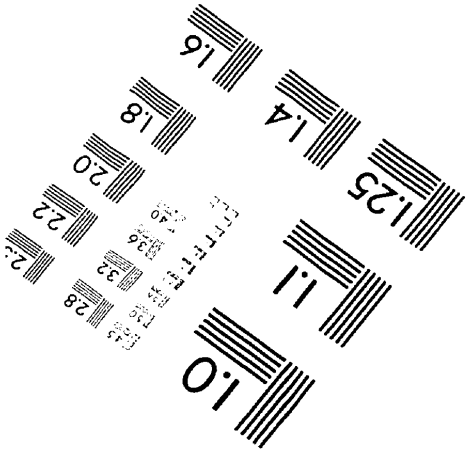

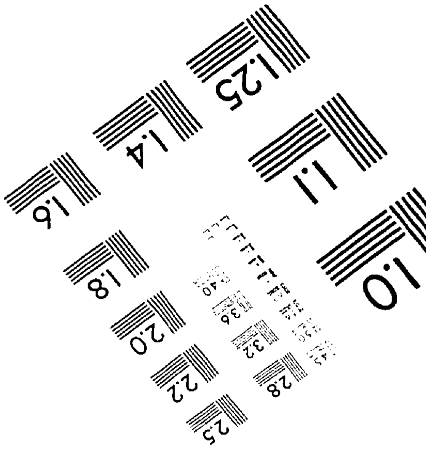

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