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HABITAT SELECTION, CONNECTIVITY, AND POPULATION GENETICS OF A TIMBER RATTLESNAKE (CROTALUS HORRIDUS) METAPOPULATION IN SOUTHWESTERN MASSACHUSETTS AND NEW ENGLAND

Anne Stengle

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A Dissertation Presented

by

ANNE G. STENGLE

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of:

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ORGANISMIC AND EVOLUTIONARY BIOLOGY

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DEDICATION

I dedicate this dissertation to my father, Dr. Thomas “Casey” Stengle (1929-1993). Although he never saw my career path, I know he would’ve been proud of me, and he has always been an inspiration to me.

Gravid Female Timber Rattlesnake, Berkshire County, MA, Sept 2009

"Having gained this intelligence, and recollecting that countries are sometimes represented by animals peculiar to them, it occurred to me that the Rattlesnake is found in no other quarter of the world besides America, and may therefore have been chosen, on that account, to represent her."

Benjamin Franklin, Dec 27, 1775

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ABSTRACT

HABITAT SELECTION, CONNECTIVITY, AND POPULATION GENETICS OF A TIMBER RATTLESNAKE (*CROTALUS HORRIDUS*) METAPOPULATION IN SOUTHWESTERN MASSACHUSETTS AND NEW ENGLAND

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Timber Rattlesnake suffered significant range reduction in the past few centuries. Here, I studied the demographics, movement patterns and habitat use of a metapopulation in Berkshire County, Massachusetts and population genetics of northeastern populations. The metapopulation was split into four subpopulations, based on geographic distance and genetic distance. Differences in gender and color morph (yellow and black) ratios were analyzed by subpopulation. Population size estimates were done for each subpopulation and for the metapopulation. Body condition index (BCI) was compared between individuals exhibiting signs of snake fungal disease (SFD) and with those not exhibiting symptoms. A total of 185 individuals was marked, with 32 recaptures, and a 65:35 (male:female) sex ratio. There was no difference in sex ratio by subpopulation ($P = 0.23$). Color morph did vary significantly among subpopulations ($P < 0.0001$) with yellow being the dominant color in three subpopulations. SFD was observed in 10.3% of individuals, all males. Three of the infected males were radio-tracked and exhibited

healing of lesions with each shed. There was no difference in BCI of individuals due to lesion presence. Six cases of mortality were observed, (three had radiotransmitters) with one predation, one human kill, and four of unknown causes.

Movement patterns can be influenced by many factors, e.g. resource needs that change throughout the year, reproductive condition, and disease. Using radiotelemetry I investigated variation in home range size, 95% kernel density estimates, and maximum distance from a source den. Gravid females moved significantly less often, and used significantly smaller ranges than males and non-gravid females. Individuals used smaller ranges and moved less often during the shedding season than during the active season, supporting a hypothesis that individuals moved farther and more frequently while foraging and mate searching. SFD presence did not affect any movement parameters. Home range size did not vary annually; however, individuals tracked for 4-5 years appeared to use different foraging areas each year, often returning to previously used areas in following years. The results presented here identify key spatial areas, such as basking and foraging areas, for this metapopulation. Habitat selections provide a basis if future management strategies (e.g. headstarting neonates and translocation) are implemented using individuals from this region which should use the same or similar areas for management plans.

Habitat needs often depend on behavior (e.g. foraging, mate searching, gestating), and can vary seasonally and with health condition. I investigated intraspecific variation with regard to health status and sex (male and non-gravid female) using classification tree (CART) analysis, as well as yearly and seasonal variation compared to random available habitat measures using paired logistic regression. Snake fungal disease (SFD) presence

and sex were not correlated with habitat selection. Overall, individuals preferred areas of increased rock cover, decreased canopy cover, lower slope, and increased vegetative cover compared to available random sites. Individuals preferred rock outcrops under open canopies during the shedding season, and used open forested areas with high vegetation cover and tree density during the active season. This population is located in one of the largest intact areas of old growth forest in New England, whereas populations in the region inhabiting other areas where the habitat has been severely altered by humans offer difficult management options.

Understanding how genetic variation is distributed within and among populations of a species produces a basis to make conservation management recommendations. Peripheral populations often have lower genetic diversity than core populations and may need artificial gene flow for future population persistence. I quantified the genetic diversity in 16 peripheral Timber Rattlesnake populations in the northeast using 13 microsatellite loci. These populations were all within the peripheral extent of the species' northeastern range, with several located in the core area of the range in eastern New York and the Appalachian Mountains. Populations were highly differentiated from each other (mean F_{ST} = 0.175). There was no correlation between genetic distance and geographic distance (R = -0.0878, P = 0.67). Seven population level clusters were detected (K = 7), all of which corresponded to single peripheral populations, and suggesting that genetic drift has led to population differentiation. Elevated influence of drift is likely the result of regional loss of over 50% of the rattlesnake population in the past few centuries. Within the largest New England area of occurrence, there appears to be a metapopulation structure, with gene flow among nearby den regions. For future population persistence,

assisted gene flow or ‘genetic rescue’ might provide a viable management action for the most-at-risk populations. If assisted gene flow is implemented, results presented here should serve as a guide for determining which populations are genetically diverse enough to serve as the best donor populations for imperiled populations.

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CHAPTER 1

POPULATION DEMOGRAPHICS OF A METAPOPULATION OF TIMBER RATTLESNAKES IN WESTERN MASSACHUSSETTS

1.1 Abstract

The Timber Rattlesnake has suffered significant range reduction in the past few centuries. Here I studied the demographics of a metapopulation in Berkshire County, Massachusetts. The metapopulation was split into four subpopulations, based on geographic distance and genetic distance (cF Chap 4). Differences due to sex and color morph (yellow and black) ratios were analyzed by subpopulation. Population size was estimated for each subpopulation and for the metapopulation. Body condition index (BCI) was compared with individuals exhibiting signs of snake fungal disease (SFD) with those not exhibiting SFD. A total of 185 individuals was marked, with 32 recaptures. There was no difference in sex ratio (65:35) by subpopulation ($p = 0.23$) whereas color morph did vary among subpopulations ($p < 0.0001$) with yellow being the dominant color in three subpopulations. Lesions were observed in 10.3% of individuals, all male. Three were radiotracked (of Chap 2) and continued to show healing of lesions with each shed. There was no difference in BCI of individuals due to SFD lesion presence. Six cases of mortality were observed in the field, with one due to predation, one to human kill, and four of unknown causes.

1.2 Introduction

Rare species often face many anthropogenic challenges: habitat loss, range fragmentation, and human exploitation. Legal protection, followed by habitat restoration and protection, is often the first step taken in conserving rare species. This approach can secure needed resources for the remaining individuals in the population, but may not result in an adequate population base for further growth. Pressures that may inhibit population growth include mortality due to increased predation or disease. These factors can have especially large effects on long-lived species that are slow to reach sexual maturity and reproduce infrequently. Therefore, it may be possible to protect habitat for a current population, to prevent decreases in the population, and to result loss of genetic variation caused by inbreeding depression.

Resources both biotic and abiotic needed by a species can restrict the habitat patches that are available to a population. If these resources are rare, local populations may exhibit a high degree of philopatry towards them. This effect restricts local populations' geographical ranges, possibly isolating them from other local populations. Connectivity among these local populations, creating metapopulation interactions, is required to maintain genetic variability and provide opportunities for repatriation in areas where a subpopulation has been extirpated (Wade and McCauley, 1988). Gene flow is maintained by individual dispersal or the exchange of genetic material through matings between individuals of separate local populations. These factors counteract the deleterious effects of isolation and small population sizes that lead to inbreeding or extinction (Allendorf and Luikart, 2006). Allopatric populations can genetically diverge without deleterious effects due to ecological barriers (Whiteley et al., 2004). However, the increasing degree of anthropogenic-caused isolation (e.g. bisecting summer and

winter habitat) may produce deleterious effects to small populations that require access to scarce resources.

Protecting and managing habitats are commonly used techniques in conservation biology. Some approaches used to enhance population growth include closing trails and sensitive areas seasonally, providing public education on rare species, or using game wardens to protect sites. Unfortunately, these methods may not always be politically or financially possible if habitat corridors cannot be protected between subpopulations; thus genetic diversity may still decrease. In such cases, it may be necessary to increase genetic diversity artificially via genetic rescue. Other approaches include population supplementation, captive breeding, and translocaing individuals. For all of these methods it is necessary to have an understanding of the genetic and population structure of the affected populations to determine appropriate donor and recipient populations.

A critical limiting resource for many non-migratory ectotherms is overwintering habitat, which is especially true for the Timber Rattlesnake (*Crotalus horridus*) which exhibit a high degree of philopatry towards communal den sites, or hibernacula, in the northern parts of its range (Brown, 1993). These hibernacula are disintictive habitat features, typically south-southwest facing talus slopes with access to subterranean crevices (Brown, 1993, Browning et al., 2005). Local populations are centered on these hibernacula, and when they are clustered together, the population exhibits a metapopulation structure, with each hibernaculum acting as a subpopulation (Clark, 2008). Connectivity between subpopulations is needed to maintain genetic diversity. Radiotelemetry data show that male Timber Rattlesnakes travel long distances in search of females and that individual snakes invariably return to their maternal hibernaculum

throughout their long lifespans (Brown, 1993). Recent genetic work has demonstrated that male mate searching facilitates genetic exchange within a Timber Rattlesnake metapopulation, without individuals actually migrating to other local populations (Clark, et al., 2008).

The Timber Rattlesnake (*Crotalus horridus*, Linnaeus, 1758) has been declining throughout its range over the past few centuries (Brown, 1993). Facing many of the same threats as other rare species, including habitat loss and intentional eradication, this species also faces a possible new disease pathogen that results in facial lesions (McBride et al., 2015). In the central part of its range in the Appalachians, the species still exists in relatively large numbers, but many of the peripheral populations in the northeast have been extirpated in the past century (Martin et al., 2008). Historically in New England, the species existed in large metapopulations, as it currently does in the Appalachians, but all remaining populations in New England are now dramatically smaller and isolated from each (Furman, 2007). Recent population genetic studies of Timber Rattlesnakes indicate that in healthy populations there is exchange of genes among subpopulations (Clark et al., 2008, Bushar et al., 1998) but where subpopulations become isolated, genetic diversity declines rapidly (Clark et al., 2011). In larger metapopulations found in continuous habitat, there is less genetic differentiation among den sites (Anderson, 2010), and high genetic diversity for the metapopulation as a whole.

The Timber Rattlesnake is listed as 'Endangered' under the Massachusetts Endangered Species Act (MESA; M.G.L c. 131A; CMR 10.00) and protected in all New England states; however, many of the existing populations are declining (Tyning, 1991). In Massachusetts the species was likely historically ubiquitous and now only exists in

five isolated populations, four of which are surrounded by urbanization (Tyning, 2005), and one population apparently exists at a size likely less than 10 individuals (B. Butler, pers comm, 2014). Only one of these populations, located in the Berkshire Taconic Range of southwestern Massachusetts, exhibits a metapopulation with subpopulations located at different den sites, thus representing probable historic pattern.

Recently there have been observations of an increased frequency of facial lesions in an isolated population of Timber Rattlesnakes in New Hampshire (Clark et al., 2011). In other New England populations of Timber Rattlesnakes, a new fungal species has been described from lesions with a similar appearance (Rajeetv et al., 2009; McBride et al., 2015). The same fungus has been found in Massasauga Rattlesnakes (*Sistrurus catenatus*) in Illinois, where three of four infected individuals died in captivity (Allender et al., 2011), although severity and frequency of these lesions now seems to be declining (Allender et al., 2013). The fungus may be either a primary or secondary pathogen, and is likely correlated with increased precipitation at some sites (Clark et al., 2011).

Historically, it is believed that most regional and local Timber Rattlesnake populations throughout their range from northern Florida to Maine existed as metapopulations with connected subpopulations and few natural barriers. Glacial events have produced naturally isolated populations in glacial refugia, mainly at the northern edge of the range (Tyning, 2005). Populations across the geographic range have been further isolated due in part to hibernaculum separation within metapopulations caused by habitat fragmentation and persecution (Brown, 1993). Timber Rattlesnake populations are viewed as having have declined significantly over the past 200 years (Stechert, 1982; Brown, 1993) as evidenced by their extirpation in Maine, Rhode Island, and Ontario, and

their current listing as endangered in Connecticut, Massachusetts, New Hampshire, and Vermont, among other states. Intentional killings and community harvests resulted in a great decline of this species (Furman, 2007). Bounties were paid for killed specimens by most New England states from the 1890s through the 1960s and 1970s (Palmer, 2004; Furman, 2007). New Hampshire has only one documented remaining population, with a census size estimate of less than 50 individuals (Clark et al, 2011), although one individual, Frank Young, collected hundreds from the state for bounties through the 1960s (Young, 1963). In New York, where the species is more prevalent than in New England, one bounty hunter reportedly turned in more than 5,000 rattlesnakes for bounty throughout his career, ending with the state ceasing the bounty in 1973 (Furman, 2007). Current estimates suggest that New York State now contains only 7,000-10,000 rattlesnakes (R. Stechert, pers. comm.). Snakes were also harvested in the 1800s to render snake oil, with one report of two men in Warren County, New York, harvesting 1,104 individuals in three days (Furman, 2007). Other more modern causes of decline include population fragmentation, habitat loss, and increased mortality due to roads. Although illegal, malicious killings do still occur in all New England states (T. French pers. comm., B. Butler, pers. comm.).

Conservation actions needed to remedy and reverse current decline must take into consideration the year-long habitat needs of local populations, their connectivity to other den sites (e.g. local populations), and the minimum values of demographic parameters required for a stable or growing metapopulation. Habitat needs of Timber Rattlesnakes include the main focal feature, the den site for overwintering, in addition to surrounding habitats for birthing, basking, and foraging. Den sites are frequently found on south,

southeast, or southwest facing rocky slopes where snakes are believed to move through crevices in order to reach subterranean crevices (Petersen and Fritsch, 1970). Birthing rookeries are typically found within 200m of a den site, have little canopy cover, and are found on steep south or west-facing rocky slopes (Brown, 1991; Reinert, 1984; Tyning, 2005). These areas are critical to gravid females occupying these sites from spring emergence until giving birth in late summer (in Massachusetts from late August through September). Additional habitat critical to adults, used by both males and non-gravid females, are basking sites and areas used for foraging. Spatially, males typically use areas that are larger than those used by non-gravid females (Brown et al., 1982; Tyning, 2004), and both categories select sites that have higher canopy cover (Reinert and Zappalorti, 1988). Because of their small size and secretive nature, little is known about the habitat selection of neonates (or young of the year) snakes, but they typically disperse away from the birthing site at about 10 days of age, and then follow the cent trail of their mother to her den site for the winter (Brown and MacLean, 1983; Reinert and Zappalorti, 1988; Cobb et al., 2005).

Fragmentation of Timber Rattlesnake habitat negatively influences metapopulations by blocking gene flow between the component den sites (Bushar et al., 1998; Clark et al., 2008; Clark et al., 2010), the increasing adult mortality rates (Aldridge and Brown, 1995), and modifying the behavior of individual animals (Brown, 1993). Gene flow between local populations rarely occurs by direct exchange of individuals between den sites due to the high natal philopatry of this species (W.S. Brown, R. Stechert, pers. comm.). Instead, indirect gene flow occurs as a result of males from different den sites mating with females at basking sites scattered between hibernacula

(Bushar et al., 1998; Clark et al., 2008). Fragmentation of habitat can interrupt this indirect flow of genes by preventing mixing of animals at shared basking locations, and thus may lead to inbreeding depression (Clark et al., 2010). In addition to restricting gene flow, habitat fragmentation can lead to increased adult mortality, especially for males due to more frequent crossing of roads, and direct interactions with humans (Aldridge and Brown, 1995). For a highly K-selected species, such as Timber Rattlesnakes increases in adult mortality rates may quickly endanger local populations (Brown, 1991; Aldridge and Brown, 1995). Finally, habitat fragmentation can lead to humans contacting Timber Rattlesnakes more frequently, as urbanization and recreational use encroaches on their habitat, thus changing the habitat use of individual snakes (Brown, 1993). Changes in habitat use may lead to decreased prey capture by snakes, and therefore reduced energy intake and growth of individuals (Beaupre et al., 2017).

Here I describe the population demographics of a Timber Rattlesnake population in Berkshire County, Massachusetts. Differences in color morph ratios and sex ratios are compared among different den (subpopulation) sites, and with regard to gender and snake fungal disease (SFD) presence. Differences in body condition for snakes with and without SFD are analyzed. Population estimates for each subpopulation are provided. Average ingress and egress dates are reported and analyzed for potential difference due to SFD. Mortality observations are reported. Information from this study and others are used for population viability analysis to determine the probability of extinction for this metapopulation over the next 100 years.

These observations will guide management decisions in the future, particularly how SFD affects the population. These results are compared to other studies in the

region, and indicate how population demographics may affect in this population. By providing subpopulation size estimates, the subpopulation with the smallest size can be identified and recommended for future management to protect it. Sex ratio and SFD occurrence could affect the reproductive success of a subpopulation, and if, greatly skewed, additional management may be required.

1.3 Materials and Methods

1.3.1 Study Area

The southern Berkshire Taconic region is part of the Berkshire Plateau, in the southwestern corner of Massachusetts, adjacent to both New York and Connecticut. The Timber Rattlesnake target population extends into the New York's Taconic State Park. The area is heavily trafficked by humans, with the Appalachian Trail running through it, along with dozens of public camping areas. The town of Mt. Washington, Massachusetts, contains 57.9 km^2 of land and 167 residents, and has the lowest human population density in the state, with 5.8 people per square mile (United States Census Bureau, 2008). Much of the land in the town and surrounding areas is owned by state agencies. The Mt. Everett State Forest on the eastern edge of Mt. Washington is 5.5 km^2 in area, with the Appalachian Trail transecting it. The Mt. Washington State Forest on the western edge of town is composed of 16.87 km^2 and 50 km of trails, and is bounded on the west by the adjacent New York Taconic state park (20.23 km^2), which borders western Mt. Washington. This area includes Bash Bish Falls, a popular tourist attraction. On the northern end of town is the Jug End State Reservation, which is managed both by the Massachusetts Department of Conservation and Recreation and the Fisheries and

Wildlife Department and is comprised of 4.69 km^2. Nearby is a 2.94 km^2 parcel owned and managed by The Nature Conservancy. White tailed deer and wild turkey hunting is seasonally allowed only within the Taconic State Park in New York, and only on private property in Massachusetts, with consent of the landowner.

Much of this region was clear-cut in the late 1700s to mid 1800s to provide charcoal fuel for nearby iron forges. The area within the Mt. Everett State Forest is one of the largest areas of old-growth forest in New England (Davis, 1996). The area consists of mostly northern hardwood forest species, with Eastern hemlock (*Tsuga canadensis*), American beech (*Fagus grandifolia*), American chestnut (*Castanea dentata*), striped maple (*Acer pensylvanicum*) and American hazelnut (*Corylus americana*). Dominant shrubs include mountain laurel (*Kalmia latifolia*), scrub oak (*Quercus ilicifolia*), and low bush blueberry (*Vaccinium angustifolium*). Bedrock geology is primarily granite, phyllite, and quartzite. The average elevation for the region is about 609 m, with Mt. Everett reaching 793 m.

The Mt. Washington population of Timber Rattlesnakes is the largest known population in Massachusetts (Tyning, 1991). The study area consists of two parallel north-south mountain ridges, with several locations containing historic rattlesnake den sites. Private homes, cultivated fields, and protected lands dominate the area, with two main town roads bisecting the site. These roads have low traffic, with one being a dirt road. Historic records of Timber Rattlesnakes in this region include reports and collections by Raymond L. Ditmars (1936), and the Whitbeck family from the mid-1800s to the present. A notorious poacher is known to have taken a minimum of forty adults as recently as the 1970s (R. Stechert, pers. comm.) from a single den. Current residents

annually report rattlesnakes on town roads, yards and school grounds (T. Tyning and E. Tilinghast, pers. comm.b).

1.3.2 Study Species

The Timber Rattlesnake (*Crotalus horridus*) was first officially described by Linnaeus (1758) and ranges throughout much of the eastern United States (Fig 1.1), but historically had a much broader range (Fig 1.2). Historically *C. horridus* was classified as containing two subspecies, the Northern Timber Rattlesnake (*Crotalus horridus horridus*), and in southern coastal areas, the Canebrake Rattlesnake (*Crotalus horridus atricaudatus*) (Gloyd, 1940). Recent genetic research demonstrates no evidence supporting a taxonomic subdivision (Clark et al., 2003), despite coloration and behavioral differences.

In northern populations, female reproduction typically begins at about 8 years of age, with many females only birthing once every 3-5 years (Brown, 1993). Litter sizes range from 8-12 offspring (Brown, 2016). The offspring are philopatric to their mother's den, scent trailing her back to the den after the first shed, about 7-10 days post birth (Brown, 1993). Active maternal care has been documented in a closely related species, Arizona Black rattlesnake (*Crotalus cerberus*) (Amarello et al. 2011), and has been observed in *C. horridus* in captivity (L. Perrotti, pers. comm.). Long-term studies in northern New York show that this species has a natural life span in excess of 40 years (Brown, 2016).

Across its range, the species inhabits very different habitat types, from Coastal Plain woodlands to mountainous deciduous forest (Odum, 1979). In the northeast the

species prefers old-growth forest, but upland forested mountains of various community types are used. The snake's diet consists mainly of mice (*Peromyscus* spp.), voles (*Microtus* spp.), and eastern chipmunks (*Tamias* striatus), although the diet differs geographically (Clark et al., 2003). Southern populations have even exhibited possible different prey selection, and therefore different habitats selectioned, based on sex (Waldron et al., 2006). One MA population heavily affected by urbanization and deforestation appears to consume more avian prey then other MA populations (A. Stengle, unpub data) indicating that this species may be plastic with respect to prey selection based on availability.

1.3.3 Sampling and Marking

Visual searches of previously known den areas were conducted during spring emergence and fall ingress, and basking sites (NHESP, MA, T. Tying, pers. comm.) from May 2009 to October 2014. Radiotelemetry (cF Chap 2) of some individuals led to discovering several new areas within den sites and finding more individuals throughout the summer opportunistically. Based on geographic distances between dens (average 6.08 km), radiotelemetry data (cF Chap 2) and genetic distances (cF Chap 4) the population was considered to be a metapopulation, comprising of four distinct denning areas (noted as MBER1-MBER4). A map of the den areas is not provided due to potential human pressures on this metapopulation.

Snakes were handled using appropriate equipment (e.g. tongs, hook, tubes, bagging system [Midwest Tongs, Inc, Greenwood, MO]), to increase safety for both snake and researcher. Measurements of ventral scale counts, snout-vent length (SVL,

cm), tail length (cm), weight (g), and number of rattle segments were done in the field at the site of capture. If the animal was not receiving a transmitter, it was then immediately released. If the animal was receiving a radio transmitter, these measurements were taken during the implantation procedure. Sex was determined by tail lengths, subcaudal ventral scale counts, or cloacal probing (Schaefer, 1934, Brown, 2008). For females reproductive condition (gravid, postpartum, non-gravid) was assessed by presence of lateral folds (post partum), increased swelling in the posterior abdominal area, and weight gain (gravid) or normal body condition (Brown, 2016). Each snake received a passive integrated transponder tag (PIT tag, Biomark, Boise, Idaho), injected subcutaneously approximately 8 cm lateral and anterior to the cloaca, using a 12-gauge sterile syringe.

All individuals were scored for presence or absence of evident skin lesions indicating disease, and if present, lesions were usually photographed. Individuals weighing over 200 g in 2013-2014 are included in the Roger Williams Park Zoo (RWPZ) RCN Health Survey (McBride et al. in rev.) of this species. This study describes possible disease agents, disease frequencies in populations throughout New England, and assessing overall health by examining standard blood values, heavy metal exposure, and paramyxovirus exposure. Snakes with a poor prognosis (e.g. low body weight, listlessness, or severe facial deformity), were treated before release, as recommended by the veterinary staff at RWPZ. If an individual with lesions was encountered in the field a second time, its condition was recorded as having improved or declined in the interim.

1.3.4 Analyses

Population estimation was conducted assuming the metapopulation was closed, (i.e. lacking additions from reproductions or immigration) and using the package Rcapture (Rivest and Baillargeon, 2015) for R version 3.1.3 (R Development Core Team, 2006) with the function **closed.** Each year was defined as one sampling period, although only individuals encountered in the immediate area of the den site were included in the model, occurring during ingress and egress of that year. Although new individuals were encountered while tracking throughout the year, to avoid any bias from radio-tracking if they were not located during ingress or egress they were not included in the abundance analysis. Variation in color morph frequencies and sex ratios among den sites were evaluated with Fisher's exact test.

Population viability analysis (PVA) was done with program Vortex 10.0 (Lacy and Pollack, 2014), using the estimated mean, initial population size, and both bounds of the confidence interval. Species-specific parameters used were either results from this population study, or from previous studies (Table 1.1, Brown, 1993; Brown et al., 2007; Brown, 2016). Fifty iterations with a 100-year time span were run. Extinction was defined when only one sex remained. Inbreeding was included with the default settings of 6.29 lethal equivalents and 50 percent due to recessive lethal alleles, with environmental concordance of reproduction and survival and a polygynous reproductive system. Survival by age class was assumed to be the same for both males and females, with no harvest or catastrophes. Carrying capacity at this site is unknown; K = 3,000 was chosen arbitrarily. If any parameters here were found to vary from published parameters, PVAs were run again with the published parameters, as well as with those found here.

Body condition index (BCI) was calculated by the residual score from the general linear regression of log-transformed mass against the log transformed SVL (Weatherhead and Brown, 1996; Way and Mason, 2008). BCI was calculated separately for adults with and without lesions, and these states were compared using ANCOVA. BCI was expected to be lower for individuals with SFD. As the lesions typically are observed to affect major sensory organs, (eyes and pit organ) it was expected that foraging ability would be decreased. Also, snakes inoculated with disease typically show a fever response (Burns, 1996), accompanied by more frequent basking. This effect would lead to an individual favoring basking habitat rather than foraging habitat, therefore feeding less often and having a lower BCI.

1.4 Results

1.4.1 Total Captures, Behavior, Color Morph, and Sex

From May 2009 through October 2015, 185 individuals were marked, with 32 recaptures (Table 1.2). No individual was captured more than three times. Of the 185 individuals, there were 113 males, and 64 females, resulting in a 66:34 male:female sex ratio (for 8 neonates sex was not determined). Sex ratio did not vary significantly among subpopulations ($p = 0.23$, Fisher's exact test). Color morph ratio varied significantly among subpopulations (den regions) ($p < 0.0001$, Fisher's exact test), with three predominantly yellow, and one predominantly black (Table 1.3). Size estimates were not possible for individual den areas, due to low sample sizes. The overall population size was estimated at $n = 995$ (95% CI 560– 2114). Results from PVA indicated that this population has a probability of extinction of zero over the next 100 years, for $n = 560$,

995, and 2114, the mean and confidence bounds of the population with a 50:50 sex ratio (Fig. 1.3), as found in previous studies (Brown, 1993). Sex ratio, however, did differ from previous studies in the region and PVA of the three census sizes with the observed sex ratio (66:34) did increase the probability of extinction, with a probability of extinction of 0.04 for n = 560, 0.02 for n = 995, and 0.00 for n = 2114 (Fig. 1.4).

The earliest observation of an individual was 5 April (2010), with the latest 25 October (2010). Average date of individuals observed (n = 61) at the den during emergence was 7 May, with the latest observation of 30 May, excluding radio-implanted individuals. Average ingress date (n = 74) was 4 October with the earliest observation on 16 September. The first shed typically occurred in June (range 5 June – 3 July), and second shed (if occurred) typically occurred in August (range 16 July – 29 August), with one additional observation on 16 September, 2013, at the den site. Breeding and courtship were observed between 14 July through 24 August. Foraging behavior, defined as the individual coiled alongside a log with the head resting on it (Reinert et al., 1984) was observed from 28 June through 11 September, with one additional observation on 22 May, 2013. Both breeding and foraging behaviors were most frequently during the month of August.

1.4.2 Reproductive Efforts

Few gravid females were observed during the study, although only birthing sites used by radio-tracked females were monitored. Three gravid females were observed, with one giving birth in 2009, one in 2009 and 2012, and one in 2011. The two in 2009 were from the same den, and returned to the den in September to give birth, after spending the

active season at two different birthing sites. Litter size could only be determined for the 2011 female, as she was brought into captivity to Roger Williams Park Zoo, Providence, RI, so the offspring could be marked. The female gave birth 24 August, 2011, to $n = 11$ offspring (6M:5F) with an average mass of 29.2g per neonate. The female was captured on 24 June, 2010, and appeared to be post partum, indicated by the presence of lateral folds (Brown, 2016), indicating that she also had birthed in 2009. The female that gave birth only in 2009 was radio-tracked through 2013, and did not become gravid again during this period. These limited data indicates that females can reproduce once every 2 — 5+ years in this population. Methods used in the field using spring scales and snake bags were not accurate enough to determine average neonate size for other neonates encountered.

A radioed male was observed mating on 3 August, 2011, at MBER4. Mating was also observed at a basking area at MBER1 on 17 August, 2011 with a non-radioed pair. Male combat was observed at MBER1 on 2 August, 2011. A radioed male from MBER1 was observed mate guarding on Aug 18, 2011, observed as a male following a female while she was pre-shed (Merrow and Auberton, 2005). The mate-guarding site was revisited on 26 August, 2011 and both snakes were not in the immediate area, but the male's rattle string was found where the female had been coiled, indicating that vigorous activity had occurred, thus courtship and/or mating was assumed. Courtship was observed on 29 July, 2011 at MBER3 between a radioed male and a female that had been marked previously in 2010 at a basking site.

1.4.3 Radio Failures and Expulsions

One male was tracked starting 3 May, 2010 and was last observed on 16 July, 2010 before the signal disappeared. My initial assumption was that the radio had failed until only the radio was found 3.35 km west of the snake's last relocation on 16 July, 2010. A conclusion was that the individual had perished, and scavengers had moved the radio was not confirmed when the following spring (on 8 May, 2011) the individual was recaptured emerging from the den. Its incision was completely healed, and a new radio was implanted. I assumed that the snake expelled the radiotransmitter through the gastrointestinal tract. Another male was tracked beginning on 15 May, 2009 and its radio was subsequently located on 24 August, 2009. A seven-segment rattle was located next to the radio, so it was inferred that the male was at this location when its radio was expelled. Another male's signal was lost after 25 September, 2009. The male was relocated on 6 August, 2011 while courting another radioed female. The failed radio (still inside this male) was replaced with a new one. One male tracked from 2009 to 2010 when the signal died during the 2010 wintering season, was located, after entering the den. This male exhibited severe lesions that were improved greatly after shedding the previous season (Fig 1.5). All four males were located in the MBER3 population. A female was tracked from 17 September, 2011 through 23 August, 2011, when only the radio was found, in MBER1 with the last prior location on 12 August, 2013. The status of this snake is unknown; her radio was located in an area consistent with her prior movement path when tracked returning to the den.

1.4.4 Mortality

Six deaths were directly observed during the study (Table 1.4). More males were found dead, although sex was not a significant effect ($X = 0.199$, $p = 0.656$). One male tracked from 2009 to 2013 was found as a partial skeleton on 20 November, 2013, with its last observed relocation on 25 September, 2013. One female was located at a basking area on 6 June, 2011, and subsequently was located there two more times and finally found as a complete skeleton on 1 July, 2011. Both appeared healthy at their last known previous observed location, but cause of death is unknown, as only skeletons were located. Another radioed male tracked from 6 June, 2011 was found dead on 5 August, 2012. When first observed, he exhibited lesions; however, the lesions were no longer present after his first shed (Fig 1.6). When located after death the hemipenes were found extroverted and a small hole in the skin in the lateral caudal area. The body was found near an illegal dirt bike trail, so initially it was concluded that the snake had been run over, but subsequent radiographs showed no broken bones; therefore cause of death is unknown. One male tracked from 2 May, 2011 was found killed by a Red Tailed-Hawk on 9 July, 2011. The individual was located at a basking site while the hawk was feeding on him, and the snake was still alive. All of these individuals were in the MBER1 subpopulation. Two MBER3 females, one radioed throughout 2009 and the other from 2009 until 2010, entered their dens, but they (and their radios) never emerged the following spring. A rattlesnake skeleton was also found at the den entrance in spring 2010, but could not be identified. Another skeleton was found on Jul 19, 2012 in the middle of a trail in MBER4. It could not be identified, as no PIT tag was present in the leaf litter; however the rattle and skull were missing (despite the vertebral column and ribs being intact) so human killing is assumed.

1.4.5 Snake Fungal Disease

Fungal disease was observed in 19 individuals, a 10.3% population prevalence. Lesion presence differed by sex and was only observed in males ($p = 0.00022$, Fisher's exact test). Three of these individuals were recaptured, with an additional three subjected to radiotelemetry (of Chap 2). Five, including both radioed individuals, later showed complete healing of lesions, although scarring was prominent (Fig 1.5-1.7). Only the skeleton of the third was found 23 days after the last observation, at the basking site where the individual was previously in a pre-shed condition. As only the skeleton was found, identification was confirmed by locating the PIT tag but cause of death could not be determined. Lesions were observed more frequently earlier in the year, prior to the first shed, than later in the year ($p = 0.0034$, Fisher's exact test), with only three of the 19 having lesions that were discernible after the first annual shed. Snakes with and without lesions did not differ in average emergence date ($t = 0.39$, $df = 13.59$, $p = 0.70$). Snakes with lesions had a later average ingress date, than snakes without lesions, (11 October and 4 Oct ober, respectively, $t = 3.44$, $df = 6.03$, $p = 0.013$). BCI did not differ between individuals with and without lesions ($p = 0.44$, Fig. 1.8). There were no known mortalities due to SFD presence in this population.

1.5 Discussion

1.5.1 Color Morph, Sex Ratio, and Population Size

The ratio of black to yellow morphs differed by den site. Difference in color morphs among subpopulations could be a result of genetic separation. The most isolated

population in New England exhibits only black morphs (Clark et al., 2011), and dens closer geographically have similar color ratios (W. Brown, pers. comm.). MBER1-3 had mostly yellow morphs, and MBER4 had mostly black morphs (Table 1.3). Geographic and genetic distance is greatest for MBER4 to the other subpopulations (of Chap 4), supporting the hypothesis of some genetic isolation among subpopulations within a metapopulation rather than resulting from exchange within.

Three of the four subpopulations exhibited a male-skewed sex ratio, despite most studies of this species reporting a near 50:50 ratio (Brown, 1993). One den was predominantly male (MBER3), with only 21% of those marked being female. This disparity could be due to sample bias, i.e., males could be more prominent at basking and den areas, or it could indicate historic poaching of gravid females at birthing rookeries. With fewer females than males, such a bias could lead to a population decrease and inbreeding depression in the future; if an increase in probability of population extinction results from the skewed sex ratio (Fig. 1.3 and 1.4). As females only reproduce once every 3-5 years in this population, the effect could be significant. If there truly is a surplus of males, they would be good source population candidates for translocation, population supplementation or captive breeding for males, as losing a few males from this den site likely will have minimal effect on reproductive output.

Analysis estimates an overall population size of $N = 995$ (95% CI 560 – 2114). This could be an overestimation, due to violations of the assumptions of the model: that all areas were sampled evenly across all sampling events, and that no mortality occurred during the sampling period. Not all dens were sampled every year, therefore not all individuals had an equal probability of detection. With the broad confidence interval

calculated, the point estimate cannot be assumed to be precise, estimate, although with the low end of the interval at 560 individuals, this indicates it's the largest known population in MA, and a stable population. Population viability modeling for this population at the mean estimate and both bounds of the confidence interval resulted in a probability of extinction equal to 0.00. This suggests that although the broad confidence interval is difficult to interpret with regard to the actual census size, the population may be viewed as stable.

1.5.2 Reproductive Effort

Courtship and mating were observed between 29 July and 18 August across all years. This is consistent with the breeding season for this species in the northeast (Brown, 1993). The majority of females in the northeast often mate and reproduce on a triennial cycle (Brown, 2016). This metric is supported here with two females birthing in 2009, one of these two birthing again in 2012, the majority of matings observed in 2011, along with a postpartum female located in 2010 indicating she birthed in 2009 and birthed again in 2012. One female birthed in 2009, and did not do so again during the study through 2013, also indicating the time span between births can be at least five years.

In the northeast with a limited foraging season, birthing is likely constrained by adequate foraging time and success in regaining the weight lost by gestating females (Brown, 2016). There could be great variation among areas chosen by females for foraging success and/or yearly prey availability, which would likely result in differences in reproductive frequency. Because this metapopulation is male skewed (males can mate

every year) and has a large estimated population size, it is unlikely that a lack of available males or the Allee effect is causing this variation in length between births.

1.5.3 Radio Failure and Expulsion

Several radiotracking studies with snakes have found radios with no animal remains or explanation (C. Smith, T. Tyning, pers. comm., Fitch and Pisani, 2005.). Usually the;conclusion is that death intervened, likely due to a predator that consumed the body and either left the radio, or passes it through the predator's gastrointestinal tract. Radio expulsions have been documented in some catfish species (Baras and Westerloppe, 1999, Summerfelt and Mosier, 1984), but has been less frequently documented in snakes (Bryant et al., 2010). However Pearson and Shine (2002) reported frequently finding expelled radios in fecal matter in Carpet Pythons, indicating the radio was absorbed through the gastrointestinal tract and expelled with bowel movements. In my study lone radio were not found with fecal matter, but fecal matter could have eroded prior to location of the radios. The MBER1 female whose radio was found was never relocated, although the radio was relocated in a pathway consistent with her prior movements. Transmitter expulsion is consistent with a MBER1 female moving consistently east towards the den when the radio was found. The MBER3 male radio was found 3.35 km west of his last relocation when he was actively moving north, and the male was relocated the following year at the den radio expulsion occurred. His last relocation he was moving north, so how the radio was found far to the west is inexplicable.

The MBER3 site had more complications with radios than the other sites. One was a known radio expulsion, with the individual found lacking its radio the following

spring. Two individuals appeared to have radio failures, with one snake found two years later with the expired radio still inside, and the fourth being a likely expulsion. Only one other radio appeared to have been expelled in MBER1, possibly because the majority of radios were deployed at MBER3.

1.5.4 Mortality

Red Tailed-Hawks are known prey on Timber Rattlesnakes in some areas (R. Stechert, N. Smith, pers. comm.) a finding also in the snakes studied here. A MBER1 male preyed upon by a Red Tailed-Hawk is the only known case of predation observed in in this study and suggesting that predation is not a major threat of mortality in this population. For all other cases of mortality, there were no signs of predation (e.g. lacerations, puncture wounds) and cause of death could not be determined. In these cases where either the body or skeleton was found, predation is unlikely, because a possible predator would likely move the snake away from the last observed location.

Road mortality can have a major effect on mortality in a rattlesnake population (Clark et al., 2010) and Timber Rattlesnakes are more susceptible to road mortality than many other sympatric species (Andrews and Gibbons, 2005). This finding does not appear to be the case here. Road kills are not likely because there are few roads in area. Approximately 50% of the roads are dirt roads, which receive little traffic. Road mortality could become a concern, however if the area were to become more developed.

Two MBER3 females whose signal never left the den during emergence in 2009 and 2010 cannot be judged positively as mortalities, as radio expulsion is possible. A

skeleton was found at the den entrance in the spring of 2009, but no radio or associated PIT tag was located, so it is unlikely this skeleton was that of a radioed female.

Male Timber Rattlesnakes have been observed to have a higher mortality rate attributed to their longer and more frequent movements in search of females during the breeding season (Brown and Aldridge, 2005). Moving more frequently and over greater distances exposes males more often to roads and predation. My observations show that the majority of mortalities were males, a finding consistent with a mating season hypothesis.

Mortality was most frequently observed at MBER1 (Table 1.4). One had minor lesions that resolved after its first shed a year prior to its death, and the cause of death was ruled as unknown, with the other two appeared healthy prior to death. The fourth mortality at this site was caused by predation. As the cause of the majority of these individuals is unknown, no definitive source of its increased mortality can identified.

1.5.5 Snake Fungal Disease

In this study only males exhibited fungal lesions, a finding that may indicate gender specific variation in disease susceptibility. This finding could be the result of male bias in this metapopulation, with a greater number of males than females, or there may be another factor leading to an increase in lesion rate in males. Lesions have been documented in female Timber Rattlesnakes in only one other study (McBride et al., 2015), with two females, five males, and one of unknown sex being infected, and still suggesting lesions are more common in males. Increased susceptibility of males may be due to: 1.) reduced immune response to pathogens, 2.) a higher use of different habitats

3.) occupying larger spatial areas containing pathogens compared to females, 4.) or male aggressive behavior that leads to wounds that lead to secondary infections (as been observed in some species of snakes (e.g. Shine et al., 1981)). Other researchers have found that injuries in squamates increase with age, and are more common in males (Schoener and Schoener, 1980; Hudson, 1996), consistent with my finding of lesions only on adult males at the primary study site. Male *C. horridus* travel farther and more frequently than females (Brown, 1993), and also engage in combat with other males over females (Klauber, 1956). Combat behavior has been correlated with injury in male rattlesnakes (McGowen and Madison, 2008), and injury rates from male combat do increase with age and body size in other snake species (Tolson, 1992; Fearn et al., 2006). Prey animals can also cause injury to snakes during feeding (Klauber, 1956), but it is not known whether feeding injuries are more prevalent in males.

Lesions were more common in the spring, (post emergence and preshed) compared to their appearance later in the year. This is consistent with the occurrence of hibernation blisters, which were documented by Fitch (1963) in racers, which occur more frequently in the spring, and are observed more frequently during years with increased precipitation (Fitch, 1963). This result could indicate that these lesions are not caused by a novel pathogen, but rather from a pathogen that has historically occurred in snake populations. Fungal lesions may not have a large effect on stable populations, but may more strongly affect small or declining populations as documented in NH where higher lesion prevalence prevailed in 2006, which was also the year that received the most precipitation in the previous 100 years (Clark et al., 2011). Clark et al. (2011) also suggest that inbreeding depression may lead to increased disease susceptibility, as has

been observed in other taxa (Savage and Zamudio, 2011). Clark et al. (2011) does not report a decrease in fitness, which is required to conclude inbreeding depression is occurring, and assumes inbreeding depression is occurring based on color morphologies only seen in this population. There is no photographic documentation provided to public demonstrating the observed changes in color morphology.

Snakes with lesions did not differ in average emergence date, but did differ in average ingress date, as snakes with lesions moved into the dens later in the year. This could indicate that snakes with lesions basked around the den longer prior to ingress. The earliest and latest annual observations of individuals were also in the same year (2010) and these individuals did not have lesions. Variation in ingress-egress dates could also be accounted by annual weather variation.

1.5.6 Management Implications

Population estimates indicate that this is the largest extant population in Massachusetts, although some of the metapopulation dens in New York. The majority of the land that the population uses is protected by state parks in both states. Habitat in both states needs to continue to be protected if this metapopulation is to remain viable.

Human traffic in den areas increased during the span of this study (T. Tyning, pers comm, A. Stengle, unpub data). Some individuals encountered were known poachers, while others claim to be visiting the area only to take photos. These human often manipulate the rattlesnakes for their photographs (R. Stechart, pers. comm.). Brown et al. (2007) noted an intimidation effect, defined as a behavioral inhibition among snakes that are manipulated, such that they prefer not to return to an area where a disturbance

occurred. This effect could lead to a change in behavior e.g. snakes ceasing to bask near the den or shifting to other basking areas. This effect was not quantified in the study, but in the final years of the study it became increasingly difficult to locate individuals at the den, despite decreased handling of the snakes by researchers in the final years. As most of these dens are located on state property, state agencies are advised to develop deterrent methods to reduce human traffic in these sensitive areas. Snakes in the study region do occasionally move through residential areas, where fortunately, the majority of residents looks favorably on the snakes and wish to protect them. Having a strong network of local individuals who are willing to remove rattlesnakes in residential areas has been essential to preserving a relationship of cooperation.

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Figure 1.1. Current extant range of the Timber Rattlesnake (*Crotalus horridus*) in the United States. Dots represent locality records and shading shows presumed continuous distribution (Martin et al. 2008).

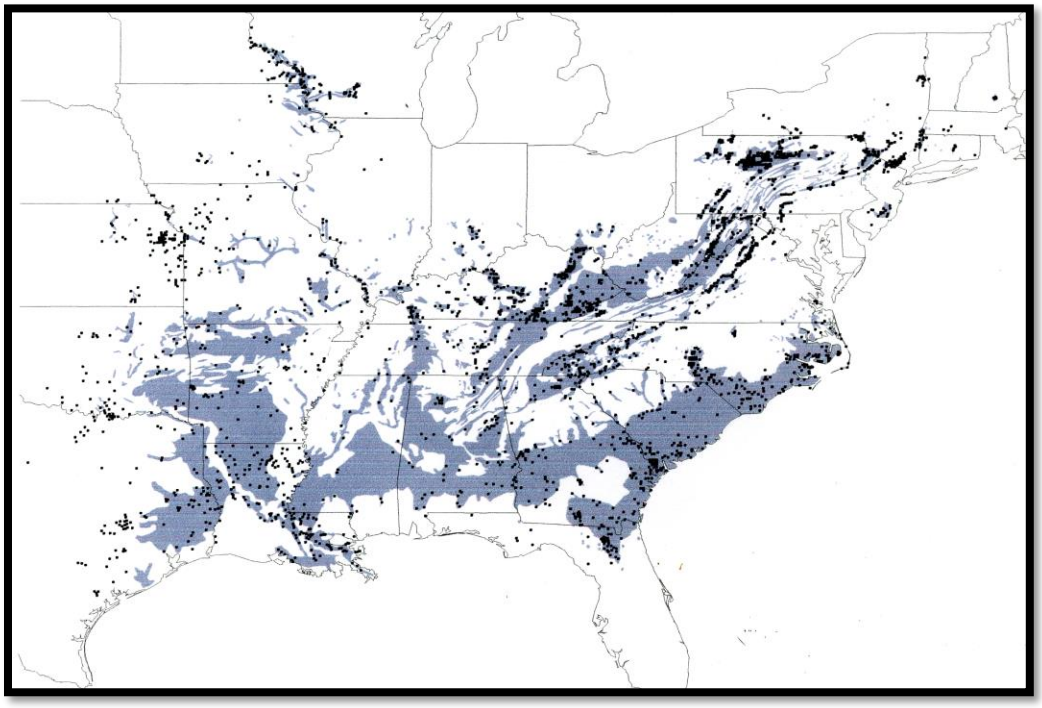
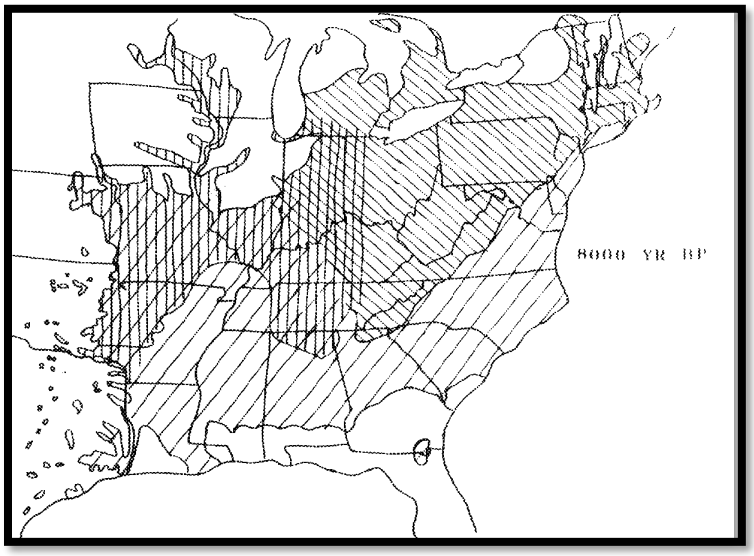


Figure 1.2. Historic range of the Timber Rattlesnake ca. 8,000 years before present (a.) and 400 years before present (b.) from Martin (unpubl data). Different slash marks correspond to what was previously thought to be different subspecies at the time these maps were created, but more recent work confirms there is no subspecies differentiation taxonomically recognizable (Clark et al. 2003).

a.)



b.)

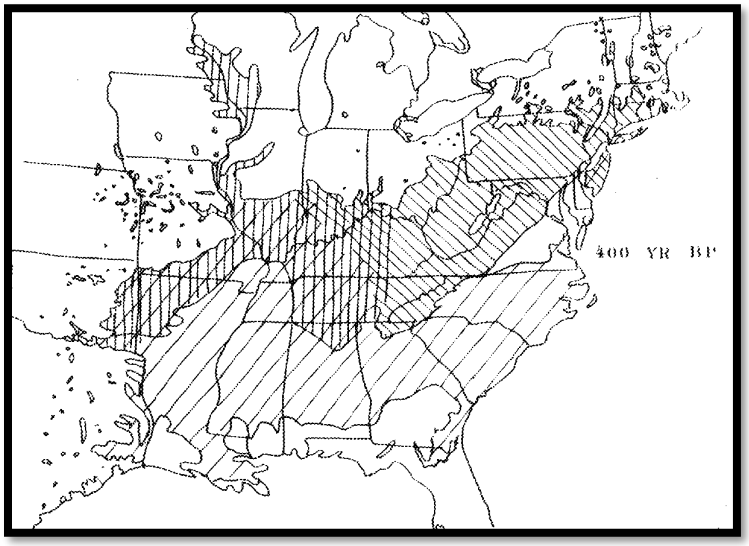


Table 1.1. Parameter estimates used for population viablity estimates using program Vortex for a Timber Rattlesnake population in Berkshire County, MA.

Parameter	Parameter Estimate	Source
Initial population size	995	This study
Age of first offspring – F	7 years	Brown, 2016
Age of first offspring – M	6 years	Brown, 1993
Maximum lifespan	45 years	Brown, unpubl data
Maximum broods per year	1	Brown, 1993
Maximum offspring per brood	14	Brown, 2016
Sex ratio M:F	33:67	This study
Maximum age of reproduction – F	50	Brown, 2016
Maximum age of reproduction – M	45	Assumed
Percent breeding adults – F	33%	Brown, 1993, 2016
SD percent breeding adults due to EV	0	Unknown value
Mortality age 0 – 1 year	0.348	Brown et al., 2007
SD of mortality age 0 – 1 year	0.1537	Brown et al., 2007
Mortality age 2 -4 year	0.078	Brown et al., 2007
SD of mortality age 2 – 4 year	0.0836	Brown et al., 2007
Mortality age 5 and over	0.110	Brown et al., 2007
SD of mortality age 5 and over	0.0365	Brown et al., 2007

Table 1.2. Meristic data for all Timber Rattlesnakes captured and recaptured from 2009-2015. Status indicates if the individual was a first capture (New), a recapture (Recap), or a radioed individual with years tracked given. Subpopulation (SubPop) is given as the MBER numbers listed in text. ID is the last five digits of the PIT tag number, with unmarked neonates (NEO) and adults (MARK), where a marker was applied to the rattle with no PIT tag. Lesion presence is noted as yes (Y) or no (N). Morph is abbreviated as yellow (Y) and black (B).

ID	Date	Sex	SVL (cm)	Tail (cm)	Total (cm)	Mass (g)	Ventrals	Subcauds	Morph	SubPop	Lesion	Status
C1204	5/15/09	M	106	10.4	116.4	1100	175	28	Y	2	Y	2009-2010
47B77	5/15/09	F	102	7.5	109.5	779	171	23	Y	2	N	2009
77228	5/15/09	F	87.5	6.5	94	679	168	21	B	2	N	2009-2011
52D10	5/15/09	M	112.5	10.7	123.2	1679	163	24	Y	2	N	2009
D4B47	5/15/09	M	112	9.8	121.8	1379	169	28	Y	2	N	2009-2013
94B63	5/15/09	F	96.5	7.5	104	879	169	23	Y	2	N	2009-2013
83B74	5/17/09	F	65	7.2	72.2	900	174	23	Y	2	N	2009-2013
D340B	6/8/09	M		13.5			173	28	Y	2	N	New
B5342	8/6/09	M	124	10	134	1630.4	165	27	Y	1	N	2009-2013
A3F16	9/19/09	M	110	11.3	121.3			25	Y	1	N	New
56636	9/20/09	M	79	7	86		169	27	Y	2	N	2011-2013
D1166	4/5/10	M	123	10.5	133.5	1631.8	168	24	Y	1	Y	2010-2013
71518	5/3/10	M	106	9.3	115.3	1210	169	26	B	2	N	2010-2013
02812	5/3/10	F		7.8			172	22	Y	2	N	New
56636	5/3/10	M							Y	2	N	Recap
53B6A	5/16/10	F	83.7	6.3	90	763.7	174	20	Y	2	N	New
53B6A	5/17/10	F							Y	2	N	New
53B6A	5/20/10	F								2	N	Recap
E5112	6/14/10	F	104.5	6.4	110.9	742	175	18	B	4	N	2010-2012
D1514	6/15/10	M	103.5	7.8	111.3	1082	165	24	B	4	N	2010-2012
46E3E	6/15/10	M	103	9.4	112.4	1182	167	24	B	4	N	New
53137	6/22/10	M	106	4.8	110.8	159.7	154	23	Y	2	N	New
90B05	6/23/10	M	113	9.4	124.4	1382	173	27	B	4	N	2010-2012
D1166	6/29/10	M				1791				1	Y	Recap
9627F	7/31/10	M	66	7	73	372	177	29	Y	2	N	New
97754	7/31/10	F	108	9.8	107.8	1381	168	24	Y	2	N	New

8326B	8/3/10	M	115	9.2	124.5	1278	168	24	Y	4	N	2010-2012
C370E	8/9/10	M	109	10.5	119.5	1250	166	25	B	4	N	New
C1166	9/4/10	M	121	9	130	2000	171	26	Y	2	N	New
B1F23	9/17/10	F	81	5.6	86.6	487	174	20	B	1	N	2011-2013
B102E	10/8/10	M	118	10.4	128.4	1684	173	26	Y	4	N	New
02043	10/8/10	M	88	8.5	96.5	700	165	27	B	4	N	New
C2327	10/8/10	F	50	3.2	53.2	104	180	19	B	4	N	New
B635B	10/8/10	M	90	8.7	98.7	809	170	25	B	4	N	New
F551B	10/8/10	M	129	10.8	139.8	2000	173	27	B	4	N	New
NEO	10/8/10	F	33	2.3	35.3	24	173	18		4	N	New
F303E	10/9/10	F	89.5	6.4	95.9	805	174	21	B	4	N	New
32C2D	10/18/10	M	92	8.3	100.3	697	165	25		1	N	New
70323	10/25/10	M	109	9.2	118.2		170	25	B	4	N	New
48254	4/30/11	F	101	6.5	107.5	1075	159	20	B	1	N	New
47741	5/7/11	F	85	6.6	91.5		177	21	B	4	N	New
73694	5/7/11	F	89	6	95		173	16	Y	4	N	New
71518	5/8/11	M								2	N	Recap
75074	5/8/11	F	70	5.3	75.3		173	16	B	2	N	New
71357	5/9/11	M	64	5.8	69.8		163	25	Y	3	N	New
47172	5/9/11	M	115	10	125	1725	167	22	Y	3	Y	New
48318	5/9/11	M	100	9	109	1175	170	23	Y	3	N	New
49661	5/9/11	F	86	6.2	92.2		174	21	Y	3	N	New
51084	5/9/11	M	100	9.5	109.5	1125	168	24	Y	3	N	New
52070	5/9/11	F	83	5.5	88.5	717	172	19	Y	3	N	New
72712	5/9/11	F	68	5	73	265	167	19	Y	3	N	New
12716	5/9/11	M	122	10	132	1750	170	24	B	3	N	New
13663	5/9/11	M	134	10	144	2075	168	24	Y	3	N	New
16646	5/9/11	M	79	6	85	305	168	23	Y	3	N	New
18272	5/9/11	M	114	9.5	123.5	1625	170	19	Y	3	N	New
MARK	5/9/11	M	125	10	135	2165	170	25	Y	3	N	New
75146	5/12/11	M	108	9.4	117.4	1429		25	B	1	Y	2011-2013
49911	5/19/11	M	109.5	10.8	120.3			26	Y	1	Y	2011-2012
56636	5/19/11	M							Y	2	N	Recap

75491	5/21/11	F				405	176	23	Y	2	N	New
71693	5/31/11	M	106	10.5	116.5	1200	171	25	B	1	Y	New
97620	6/6/11	F	94	7	101	950	170	19	Y	1	N	New
98779	6/6/11	F	101	7	108	990	174	21	B	1	N	New
98924	6/6/11	M	115	9.8	124.8	1285	165	25	Y	1	N	New
99084	6/6/11	F	92	6.8	98.8	960	167	19	Y	1	N	New
71693	6/6/11	M					171	25	B	1	Y	Recap
71693	6/7/11	M					171	25	B	1	Y	Recap
98447	6/8/11	M	75	7.4		362	171	25	Y	1	N	New
71693	6/8/11	M					171	25	B	1	Y	Recap
98779	6/13/11	F							B	1	N	Recap
99084	6/13/11	F							Y	1	N	Recap
47802	6/15/11	M	118	9.9		1555	172	25	B	4	N	New
B1F23	6/27/11	F	81.3	6	87.3	400			B	1	N	Recap
49652	6/27/11	F	85	6		732	171	20	Y	1	N	New
74986	6/27/11	M	96	8.5		865	161	25	B	1	N	New
97540	7/1/11	F	86	6.8		750	170	21	Y	1	N	New
97620	7/1/11	F							Y	1	N	Recap
98214	7/1/11	F	83	6.5		325	174	19	Y	1	N	New
71693	7/1/11	M					171	25	B	1	Y	Recap
98212	7/5/11	F	96	7.5		1095	185	19	B	4	N	New
98212	7/26/11	F				1055			B	4	N	Recap
02812	7/29/11	F								2	N	Recap
98685	7/29/11	M	105	10	125	1680	170	24	B	4	N	New
97154	8/2/11	F	95	6.5	101.5	770	175	19	Y	1	N	New
98151	8/2/11	M	74	6.5	80.5	415	168	22	Y	1	N	New
98796	8/2/11	M	114	11	125	1440	162	25	Y	1	Y	New
98890	8/3/11	M	116	10	126	1580	166	22	B	4	N	New
98685	8/3/11	M							B	4	N	Recap
98902	8/8/11	F	96.5	7	103.5	1015	178	22	Y	1	N	New
97134	8/17/11	F	88	8.1	97.1	735	176	23	B	1	N	New
97817	8/17/11	F							B	1	N	New
98796	8/17/11	M							Y	1	Y	Recap

98902	8/18/11	F							Y	1	N	Recap
97663	8/24/11	M	115	10.7	125.7	1320	171	25	Y	2	N	New
98629	10/3/11	M	112	10.1	122.1		169	25	Y	1	N	New
26342	10/3/11	M	27.5	2.8	30.3	24.1	172	26	B	1	N	New
26391	10/3/11	M	27	2.9	29.9	20.6	173	23	Y	1	N	New
74986	10/3/11	M							B	1	N	Recap
97284	10/10/11	M	108	9.4	117.4	1460	170	25	B	4	N	New
97687	10/10/11	M	112	10.4	122.4	1535	169	26	Y	4	N	New
97901	10/10/11	M	73	5.7	78.7	255	168	26	Y	4	N	New
97927	10/10/11	M	116	10.4	126.4	1652	166	27	B	4	N	New
98194	10/10/11	M	72	6.9	78.9	370	168	26	Y	4	Y	New
98459	10/10/11	F	66	4.4	70.4	260	167	22	B	4	N	New
98763	10/10/11	M	102	8.6	110.6	1240	169	23	B	4	N	New
98868	10/10/11	M	115	11	126		172	26	Y	4	Y	New
99010	10/10/11	M	116	10	126	1560	179	23	B	4	N	New
26347	10/10/11	M	32.5	2.7	35.2		167	24	B	4	N	New
73694	10/10/11	F				814		16	Y	4	N	Recap
0164B	10/11/11	M	105	9.5	104.5		172	25	B	4	N	New
F6D4B	10/11/11	M	107	10	117	1260	169	26	B	4	N	New
F757E	10/11/11	M	107	9.1	116.1	1530	169	24	B	4	N	New
0044D	10/11/11	F	72	5.2	77.5	380	167	20	B	4	N	New
47866	10/11/11	M	100	9.3	109.3	1280	167	25	B	4	N	New
52202	10/11/11	M	112	9.5	121.5	1405	162	25	B	4	N	New
10E30	10/11/11	F	83	5.2	88.2	465	171	19	B	4	N	New
A344D	10/11/11	F	102	7	109	920	172	23	B	4	N	New
26321	10/11/11	F	30	2.4	32.4		172	20	B	4	N	New
61064	10/11/11	M	110	10	120	1205	163	25	Y	4	N	New
98873	10/12/11	M	85	8.4	93.4	405	162	25	B	4	N	New
97438	4/15/12	M	105	11.5	116.5	1290	182	26	Y	4	N	New
98210	4/15/12	M	107	10.1	117.1			27	B	4	N	New
98743	4/15/12	M	121	12	135	1745	174	26	B	4	N	New
99011	4/15/12	M	118	8	126				Y	4	N	New
98096	4/16/12	M	94	9.8	103.8	1155	168	27	Y	1	N	New

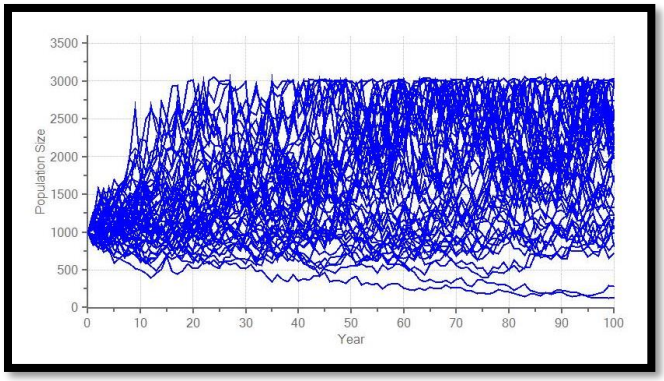
98370	4/16/12	M	110	10.2	120.2	1920	167	24	B	1	N	New
98733	4/16/12	M	107	9.9	106.9	1255	168	23	B	1	Y	New
98993	4/16/12	M	90.5	9.4	99.9	680	170	26	Y	1	N	New
26414	5/7/12	F	30	2.2	32.2	20	172	21	N	1	N	New
48254	5/7/12	F				320			B	1	N	Recap
98603	5/11/12	F	83	6	89	370	171	20	Y	1	N	New
D340B	5/12/12	M							Y	2	Y	Recap
98316	5/12/12	F	73	5.2	78.2	320	170	19	Y	2	N	New
98733	5/17/12	M				1180			B	1	N	Recap
23646	5/17/12	M	57	4.8	61.8	175	168	24	B	1	N	New
23433	5/29/12	M						25	Y	1		New
98924	6/10/12	M				1555			Y	1	N	Recap
26414	6/12/12	M	32.5	2.9			174	23		1	Y	New
23847	7/3/12	M	71	6.1		250	166	23	B	1	N	New
23077	7/19/12	F	85	6.6		740	165	20	B	4	N	New
23234	7/26/12	M	93	7.6		800	173	22	Y	3	N	2012-2013
98518	8/2/12	M	107	8.3	115.3	1040	173	27	Y	4	N	New
98368	8/6/12	M	89	8.5		510	169	25	B	1	N	New
97148	8/17/12	M	102	10.2	112.2	1300	167	22	Y	3	N	2012-2013
26323	8/24/12	M	32	2.6	34.6	32.9		23	B	4	N	New
26328	8/24/12	F	32	2.2	34.2	27.2		20	B	4	N	New
26358	8/24/12	M	32	2.7	34.7	29.4		25	B	4	N	New
26360	8/24/12	M	32	2.9	34.9	29.4		26	B	4	N	New
26363	8/24/12	M	32	3.1	35.1	30.8		25	B	4	N	New
26364	8/24/12	F	32.5	2.4	34.9	26.4		20	B	4	N	New
26374	8/24/12	F	28.3	2.2	30.3	28.3		20	B	4	N	New
26382	8/24/12	M	32	2.9	34.9	28.5		24	B	4	N	New
26386	8/24/12	M	33	2.5	35.5	29.2		23	B	4	N	New
26399	8/24/12	F	30	2.4	30.4			18	B	4	N	New
26405	8/24/12	F	33	2.3	35.3	29.7		19	B	4	N	New
23812	9/6/12	F	92	7	99	800	175	22	Y	4	N	New
22336	9/27/12	M	114	10	124	1430		25	B	4	N	New
22784	9/27/12	M	101	9	110	855	166	24	Y	4	N	New

22902	9/27/12	M	102.8	9.2	112	1240	176	26	B	4	N	New
23442	9/27/12	F	58.7	3.8	62.5	220	177	19	B	4	N	New
23450	9/27/12	F	88.2	5.8	94	600	172	18	B	4	N	New
23715	9/27/12	M	115	9	124	1420	178	24	B	4	N	New
23798	9/27/12	M	74	3	77	255	170	26	B	4	N	New
23979	9/27/12	M	101	9.3	110.3	860	167	26	B	4	N	New
24160	9/27/12	M	90.5	7.5	98	645	165	23	Y	4	N	New
26324	9/27/12	F	40	2.5	42.5		170	19	B	4	N	New
26415	9/27/12	F	30.5	2.5	33		175	21	B	4	N	New
99084	9/29/12	F							Y	1	N	Recap
22431	9/29/12	F	88	6	94	765	171	20	B	1	N	New
24027	9/30/12	M	33	2.7	35.7		161	23	Y	1	N	New
97611	10/5/12	F	43.5	2.8	46.3	65	174	20	Y	4	N	New
21287	10/5/12	F	93	6.5	99.5	705	174	19	B	4	N	New
22348	10/5/12	F	55	4.2	59.2	125	171	18	B	4	N	New
23736	10/5/12	M	56	4.8	60.8	125	183	25	Y	4	N	New
26418	10/5/12	M	25.5	3.5	29		168	24	Y	4	N	New
B102E	10/11/12	M	124	10.2		1700			Y	4	N	Recap
98229	10/11/12	F	41	2.9	43.9		167	19	Y	4	N	New
23001	10/11/12	F	35	2.1	37.1		174	22	B	4	N	New
23711	10/18/12	M	45	4.3	48.3		167	24	Y	1	N	New
26383	10/18/12	F	34	2.5	36.5		169	19	Y	1	N	New
22848	4/19/13	M	71.5	6	77.5	260	164	22	B	1	Y	New
99010	4/30/13	M				1470			B	4	N	Recap
22418	4/30/13	F	94	6.2	98.2	505	175	20	B	4	N	New
22427	5/3/13	F	93	6	99	670	171	19	Y	3	N	New
22452	5/3/13	M	117	10.2	127.2	1500	165	25	B	3	N	New
23815	5/4/13	M	120	11.1	131.1	1121	157	24	B	4	Y	New
24098	5/4/13	M	109	9.8	118.8	1350	177	24	B	4	N	New
98151	5/16/13	M	85	8.3	93.3	580				1	N	Recap
22606	5/16/13	M	108	10.8	118.8	1450	163	23	B	1	N	New
23622	5/17/13	M				1930			B	3	Y	New
24547	5/22/13	F	87	6	93	555	167	18	B	2	N	New

23983	5/28/13	M	120.2	10.2	130.2	1245	164	25	Y	1	Y	New
43081	7/14/13	M	104	8.3	112.3	1100	164	23	Y	2	N	New
97438	9/12/13	M	105	11.5	126.5	1280			B	4	N	Recap
41666	9/12/13	M	91	8	99	645	163	23	B	4	N	New
42612	9/12/13	M	38.5	3.6	45.1	50	165	25	B	4	N	New
42844	9/20/13	F	79.5	6.5	86	440	171	20	Y	1	N	New
22696	9/20/13	F	52	4.6	56.5	225	175	20	B	4	N	New
41726	9/20/13	F	80	5	85	485	172	17	Y	4	N	New
41314	10/16/13	M	42	2.8	44.8	60	182	21	Y	1	N	New
42495	5/7/14	M	138	11.5	149.5	2035		27	Y	3	Y	New
D340B	5/30/14	M				1450			Y	2	N	Recap
22301	6/21/14	M	70	7.5	77.5	345			B	4	N	New
41731	6/21/14	M	97.5	8	105.5	820			B	4	N	New
42141	6/21/14	M	65.2	5.3	68.5	130		24	Y	4	Y	New
43197	6/21/14	M	68	5	73	130		24	B	4	Y	New
99084	6/25/14	F				700			Y	1	N	Recap
marker	10/2/14	U							Y	2	N	New
13663	10/9/14	M	125	10	135	2000	168	24	Y	3	N	Recap
98796	10/9/14	M	112	12	124	1160		25	Y	1	Y	Recap
00093	10/14/14	M							Y	3	N	New
41866	10/17/14	M	55.5	5	60.5	180		24	Y	1	Y	New
98993	9/22/15	M				1000			Y	1	N	Recap
41866	9/22/15	M				350			Y	1	N	Recap

Figure 1.3. Population viability analysis of a Timber Rattlesnake population in Berkshire County, MA, using the mean estimated population size (a.) (*n* = 995) and confidence interval bounds (b.) (*n* = 560), (c.) (*n* = 2114) with a sex ratio of 50:50. Probability of extinction for all three scenarios was 0.00.

a.)



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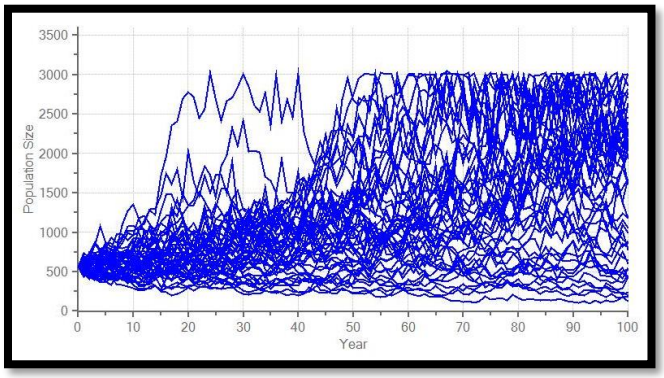
b.)



c.)

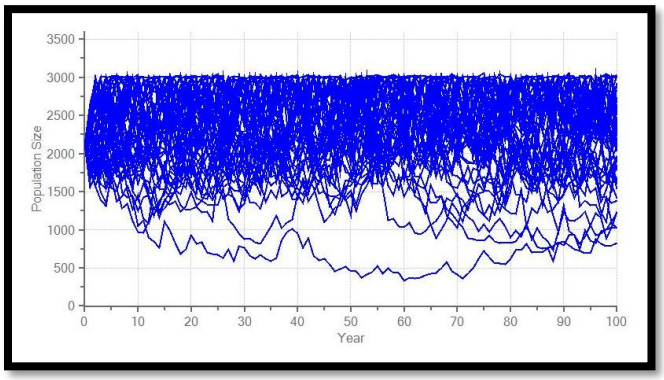
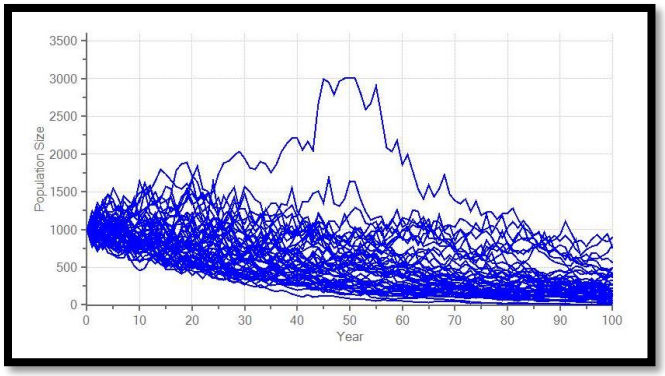
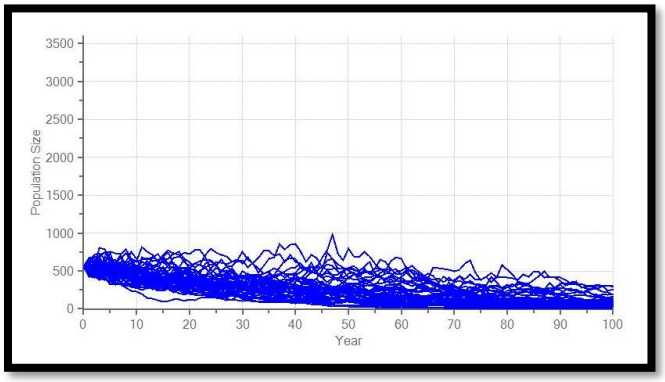


Figure 1.4. Population viability analysis of a Timber Rattlesnake population in Berkshire County, MA, using the mean estimated population size (a.) (*n* = 995) and confidence interval bounds (b.) (*n* = 560), (c.) (*n* = 2114) with a sex ratio of 66:34. Probability of extinction for all three scenarios was 0.02 (a.), 0.04 (b.) and 0.00 (c.).

a.)



b.)



c.)

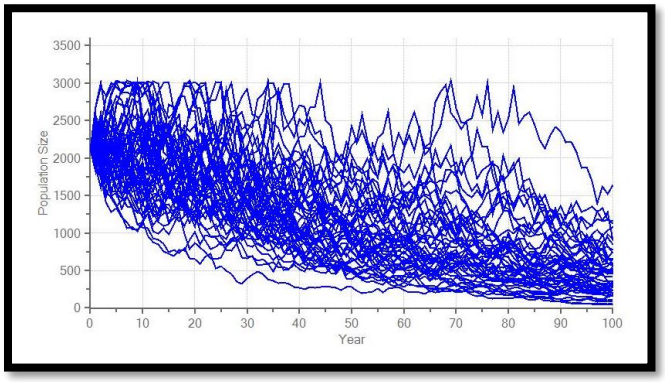


Table 1.3. Color morph (yellow:black), sex ratio (M:F), individuals with lesions present (percent) and number of males with lesions for each subpopulation. Sex and color morph was not possible to determine for all neonates.

Subpopulation	Color Morph Y:B	Sex Ratio M:F	Lesions (%)	Lesion Male
MBER1	30:17	30:17	21.3% (10)	10
MBER2	20:4	11:12	4.1% (1)	1
MBER3	16:3	15:4	15.7% (3)	3
MBER4	20:68	57:31	5.7% (5)	5
Total	86:86	113:64	10.3% (19)	19

Table 1.4. List of known mortalities, cause of death (if known) and site location for Timber Rattlesnakes in a Massachusetts metapopulation from 2009-2013 (*individuals with radio transmitters). Individuals listed are those that are confirmed dead and does not include radio failures or possible radio expulsions.

Ind ID	Site	Sex	First Date	Date of Death	Day Interval	Cause of Death
5342*	MBER1	M	8/6/09	11/20/13	1567	Unk-skeleton
1693	MBER1	F	6/6/11	7/1/11	25	Unk-skeleton (PIT tag found)
5146*	MBER1	M	6/6/11	8/15/12	433	Unk-whole body, no major injuries
9911*	MBER1	M	5/2/11	7/9/11	68	Red tailed hawk predation
N/A	MBER3	U	N/A	5/3/10	N/A	Unk-skeleton
N/A	MBER4	U	N/A	7/19/12	N/A	Human-skeleton found in trail

Figure 1.5. Male Timber Rattlesnake radiotracked in 2009 through 2010 at MBER3 exhibiting facial lesions increasing in severity, and then decreasing in severity. Photos taken (a.) May, 2009 (b.) June, 2009 (c.) July, 2010 (photo credit: Chris Camacho) (d.) June, 2010 (e.) August, 2010. Photos represent condition after each shed.

a.)

b.)

c.)

d.)

e.)

Figure 1.6. Male Timber Rattlesnake radio tracked in 2011 exhibiting lesions after emergence (a,b.) and after the first shed (c.). Photo a.) was taken in the lab during radiotransmitter implantation surgery, May, 2011, and photo b.) was taken in the field, June, 2011. Note the rostral swelling and disfiguration seen in (a. and b.) is not present in c.).

a.)

b.)

c.)

Figure 1.7. Male Timber Rattlesnake radiotracked from 2009 through 2013 exhibiting lesion improvement, and eventual healing. This individual tested positive for SFD. Photos taken (a.) April, 2010 (b.) June, 2010 (c.) August, 2010 (pre-shed) (d.) August, 2011 (e.) June, 2012.

a.)

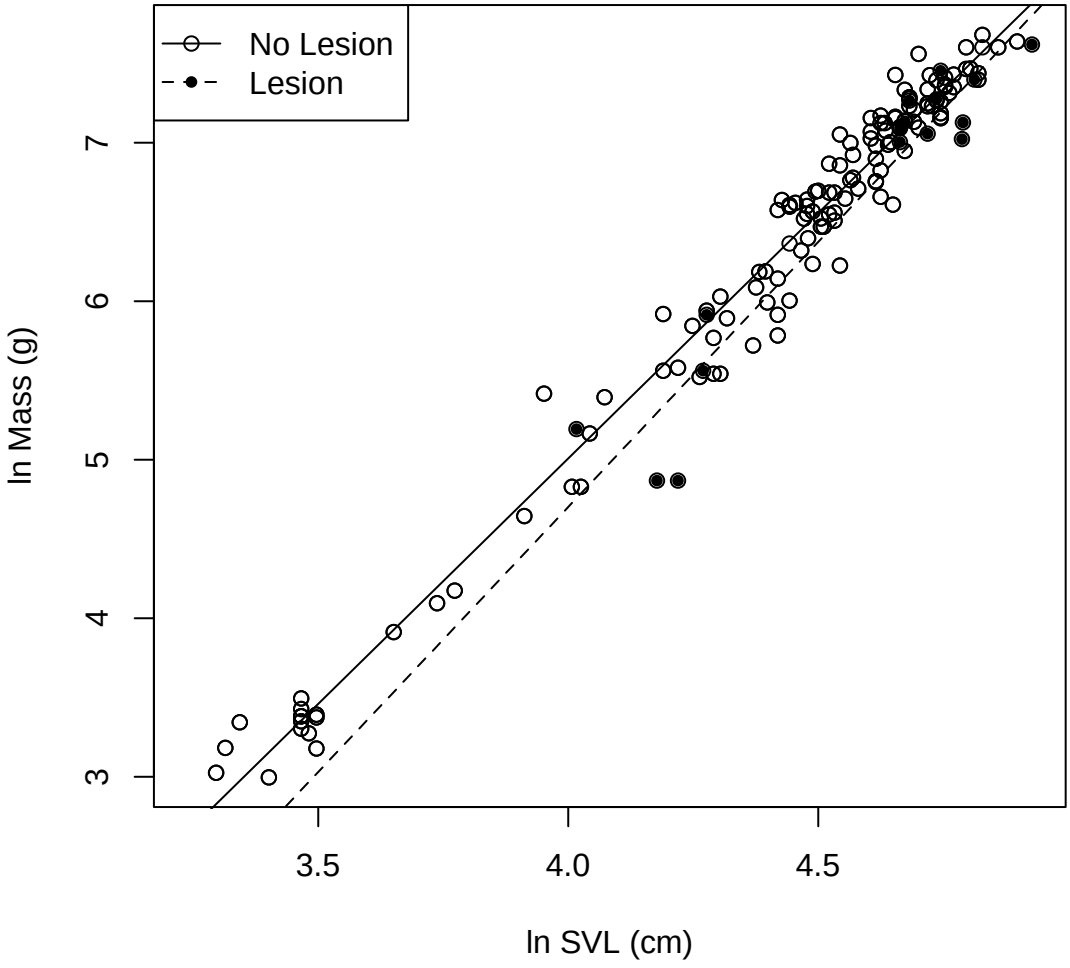
b.)

c.)

d.)

e.)

Figure 1.8. Body condition index (BCI), regression of log-n mass (g) and log-n snout vent length (SVL, cm) for Timber Rattlesnakes with and without lesions captured from 2009-2014.



CHAPTER 2

MOVEMENTs AND SPATIAL BIOLOGY OF A TIMBER RATTLESNAKE METAPOPULATION IN WESTERN MASSACHUSETTS

2.1 Abstract

Movement patterns of a vertebrate species can be influenced by many factors, e.g. resource needs that change throughout the year, reproductive condition, and disease.. Here I used radiotelemetry to investigate variation in home range size, 95% kernel density estimates, and maximum distance from a den in a metapopulation of Timber Rattlesnakes (*Crotalus horridus*) in southeastern Massachusetts. Timber Rattlesnake gravid females moved significantly less, and used significantly smaller ranges than males and non-gravid females. Individuals used smaller ranges and moved less often during the shedding season than during the active season, supporting a hypothesis that individuals move farther and more frequently while foraging and mate searching. Snake Fungal Disease (SFD) presence did not affect any movement parameters. Home range size did not vary annually, but individuals tracked for 4-5 years appeared to sometimes use different foraging areas each year, often returning to these areas in subsequent years. The results presented here identify key locations, such as basking and foraging areas, for a metapopulation. If future management strategies are implemented these results provide a basis for comparison to provide guidelines for using headstarted or translocated snakes to ensure that the management plan is successful.

2.2 Introduction

Animals are known to use different types of habitat to obtain required resources for survival, foraging, birthing, and thermoregulation. Protecting these areas is required for many threatened and endangered species to insure their future persistence. Determining where these areas are located can be difficult, especially with species that use camouflage as their primary defense. Radiotelemetry is a useful tool for studying movement patterns and habitat usage of cryptic animals, such as snakes, especially in determining areas to protect, or potential barriers to movement, such as roads. Radiotelemetry proved useful in determining areas and habitat types to protect for the federally endangered Copperbelly Water Snake (*Nerodia erythrogaster neglecta*) (Roe et. al., 2003). Spatial movement patterns of individuals often vary yearly, or with reproductive condition and gender, as seen in southern populations of Timber (Canebrake) Rattlesnakes (Waldron et. al., 2006). Quantifying variation in habitat and spatial use from these predictors is required when evaluating areas that may be in need of protection. Understanding spatial requirements and movement patterns can be critical to conserving threatened and endangered species and populations of amphibians and reptiles.

Radiotelemetry is often used in mitigation projects to determine whether an endangered species is using an area proposed for development. Species that have a low detectability rate call out for the most efficient way to determine whether the species is using a particular area of habitat. The Timber Rattlesnake and their habitat are protected in Massachusetts under the Massachusetts Endangered Species Act (MESA, 321 CMR 10.18), so understanding spatial needs is critical to protecting the species and developing

a management plan. Timber Rattlesnake habitat in the northern extent of the species' range consists of two primary aspects, the overwintering habitat (hibernaculum, or den) and the summer range area. Dens usually consist of rock or talus slopes, and typically are south-facing. Summer range typically consists of deciduous-coniferous forest, primarily for foraging and mate-searching (Brown, 1993). The results provided here will create guidelines for how much space is needed for one of the largest metapopulations in New England in order to guide land protection decisions for this and other populations. Spatial parameters will also identify areas that appear to be essential to a metapopulation (i.e. for denning areas, birthing, shedding). Although most of this region (ca. 83%) is protected land, individuals own private property at some of these areas used by the snakes. Results from this research will also provide guidelines for future headstarting and translocation efforts that may be proposed in the northeast region. Here, I examine the spatial needs of the state-endangered Timber Rattlesnake, a species that has been declining throughout its range over the past few centuries (Brown, 1993).

I estimated home range size (with minimum convex polygon and 95% fixed Kernel Analysis), maximum distance moved from a den, and daily distance moved. These measures were evaluated as to how these varied with respect to year, reproductive condition, and Snake Fungal Disease (SFD) presence. SFD was identified initially by inspecting a snake, typically characterized by facial lesions (see Chapter 1 for complete description and disease history). Male and non-gravid female Timber Rattlesnakes typically have larger home ranges than gravid females (Brown, 1993, Reinert and Zappalorti, 1988). Individuals with SFD infections are expected to bask more frequently, and to exhibit a fever response (Burns, 1996), both of which might increase shedding

frequency (Lorch et. al., 2015). Shedding more frequently facilities faster healing of SFD lesions (Lorch et. al., 2015). Typically there are basking areas near rattlesnake dens where individuals undergo shedding shortly after emergence. Individuals that were negatively affected by SFD are predicted to travel less frequently and cover distances, thereby remaining at these basking sites and also might expend less energy in the presence of SFD.

2.3 Materials and Methods

2.3.1 Study Area

The southern Berkshire Taconic region in southwestern Massachusetts extends into adjacent New York and Connecticut. The area is approximately 60 km^2, with an average elevation of about 600 m, and has one of the Northeast's largest areas of old-growth forest (Davis, 1996). The area consists of mostly northern hardwood species, dominated by Red Oak (*Quercus rubra*), Eastern Hemlock (*Tsuga canadensis*), Chestnut Oak (*Quercus prinus*), American beech (*Fagus grandifolia*), Striped Maple (*Acer pensylvanicum*), American Hazelnut (*Corylus americana*), and along with an occasional American Chestnut (*Castanea dentata*). Dominant shrubs include Mountain Laurel (*Kalmia latifolia*), Scrub Oak (*Quercus ilicifolia*), and Low Bush Blueberry (*Vaccinium angustifolium*). Other features include small wetlands, bogs and natural ponds. The area is primarily used for recreation (e.g. hiking, camping, geocaching), and has a low density of human residents (ca. 0.5 persons/km^2).

2.3.2 Radiotelemetry

Visual searches for Timber Rattlesnakes were conducted at known den areas during spring emergence and fall ingress, and at basking sites. Nineteen adult rattlesnakes (13 males, 6 females) were captured opportunistically during den and basking area surveys between 2009-2013, and these snakes were then implanted with tracking radio transmitters (see below). On average each snakes was tracked for two years, although three individuals were tracked over the entire study period (five years), and one was observed for four years. Measurements of ventral scale counts, snout-vent length (SVL, cm), tail length (cm), weight (g) and rattle segments were done on site and time of capture, if the animal was not receiving a transmitter. Subcaudal ventral scale counts, or cloacal probing (Schaefer, 1934), were used to determine sex. All snakes received a passive integrated transponder tag (PIT tag, Biomark, Boise Idaho) that was injected subcutaneously laterally approximately 8 cm anterior to the vent with a 12 gauge sterile syringe. Individuals were scored for presence or absence of SFD (lesions covering ~>10% of the head region), and all lesions were photographed. Snakes were surgically implanted with Holohil SI-2T (13 g) transmitters (Holohil Systems Ltd., Carp, Ontario, Canada), weighing <5% of body mass following Blouin-Demers et al. (2000). All surgical procedures were performed in the veterinary laboratory at Holyoke Community College, Holyoke, Massachusetts, and followed approved protocols of the University of Massachusetts Institutional Animal Care and Use Committee (#29-02-03R, and #2012-0009). Snakes were kept in captivity and observed for at least 24 hours post-surgery, and released at their point of capture. Snakes were subsequently relocated 1-2 times per week using a R-1000 (Communication Specialists, Inc., Orange, CA) telemetry receiver and 3-

element Yagi antenna. GPS locations were recorded with a Garmin Oregon 550T (Garmin International, Inc, Olathe, KS) hand-held unit.

2.3.3 Seasonal Variation

Snake behaviors were placed into 5 categories: shedding, mating (includes copulation and courtship), foraging (classified by ambush posture, Reinert et al., 1984), transient (actively moving), and coiled/exposed (basking). The first shed occurred on 5 June with the latest occurring on 3 July, including the entire time the eyes were opaque. The shedding season began once individuals moved away from the den and into the areas (basking sites) occupied during the first shed of the year (approximately 150 m from the den). After 3 July, individuals exhibited different and temporally overlapping behaviors, so foraging, breeding, and the second shedding event could not be subdivided into separate seasons. These behaviors occurred from 4 July to 11 September, after which individuals began returning to the den. This period is therefore referred to as the core active season. Gravid females were excluded from seasonal variation analyses since they were sedentary, using the same location during both the shedding and core active season.

2.3.4 Movement Parameters

Movement analyses were conducted for individuals tracked for at least one complete field season. Home range size via minimum convex polygons (MCP), maximum distance from the den site, and average daily movement distance were analyzed using ARC View 10.2 and Geospatial Modeling Environment 0.7.1 (Beyer, 2012). Fixed 95% Kernel Analysis (Worton, 1987, 1989), was analyzed using the

adehabitat package (Calenge, 2007) for R version 3.1.3 (R Development Core Team, 2006). Maximum distance was the straight-line distance from the den to the farthest location. Average daily movement distances were calculated by dividing total the distance moved between relocations by the number of days between relocations. Data from recaptured individuals without radios were used only if repeated relocations were made within the average time between measured telemetry relocations for radiotracked snakes.

2.3.5 Analyses

Statistical analyses were performed using R version 3.1.3 (R Development Core Team, 2006). I used a repeated measures two-factor ANOVA to account for multiple measures of home ranges (MCP and 95% Kernel), maximum distance from the den site and average daily distance moved, for the same individual across multiple years. Predictor variables included presence of SFD, reproductive condition, and year. The analysis was pooled across all years to allow inclusion of differences between reproductive condition and SFD presence. Averages and standard errors for these values are given for reproductive condition and SFD presence by year.

2.4 Results

2.4.1 Home Range Variation-MCP and Kernel Density Estimates

Sex, including reproductive condition and SFD presence (only males were observed with lesions) did not have a significant effect on MCP ($F_{1,12} = 0.38$, $p = 0.054$,

$F_{1,12}$ = 3.77, p = *0.55*), or kernel density estimates ($F_{1,12}$ = 2.236, p = *0.150*, $F_{1,12}$ = 0.214, p = 0.652). Gravid females had the smallest MCP and kernels (Table 2.1, Fig 2.1).

Individuals that were followed for two or more seasons did not differ in MCP size ($F_{1,11}$ = 0.56, p = 0.47) or kernel estimates ($F_{1,11}$ = 0.56, p = 0.47) between years. Average and standard errors of MCP and kernel estimates by year are reported in Table 2.2. For individuals followed 4-5 years, there were some differences in areas chosen during the active season. Males differed yearly in active areas chosen, often returning to the previous areas a few years later (Fig 2.2), but did not vary by movement parameters (Table 2.1). Females typically used the same areas each year, except when gravid (Fig 2.2). Two females followed for two gravid years each used the same birthing rookery in both years.

2.4.2 Maximum Distance from Den

Maximum distance moved from the den was related to sex, with gravid females moving the shortest distance ($F_{2,11}$ = 4.21, p = 0.041, Fig.2.3). SFD presence did not have an effect *(*$F_{1,12}$ = 0.45, p = 0.584). Maximum distance moved from a den across sex and reproductive condition did not differ significantly across years ($F_{1,11}$ = 0.38, p = 0.548) with average and standard errors reported in Table 2.2. The farthest distance of all individuals (males and females) was for a postpartum female, at 4.78 km (in 2010). She also moved an extremely long distance when she was gravid, 3.42 km (in 2012), a distance likely accounting for a lack of significant effect. This female had the shortest timespan between gravid years, with a 3-year interval (2009 and 2012). and moved farther than average reported distances for this species (Brown, 1993).

Females followed for five years (n = 2) used the same areas each year when they were not gravid. One gravid female switched rookeries in 2012. She was located at the same rookery she used in 2009 on 27 August, 2012. She was not approached and was observed from approximately 3 m away, to prevent undue disturbance. On 3 September, 2012 she was found at a different rookery at a distance of 660.8 m from her 2009 rookery. Males followed for 4 — 5 years appeared to use different areas during the active season, though there was some range overlap. Despite using different areas during the active season, males returned to the same core areas in subsequent years (Fig 2.2). These observations were only possible because I was able to collect locations for the same individuals over an unusually long period (5 years) as compared to other studies.

Only two individuals (1 male, 1 female) moved early in the active season directly south and downward from the mountain where the den is located (the same south-facing den [MBER3]). All others moved to higher elevation from the den, either east or north, depending on whether the den was west-or south-facing respectively. One such individual was an older female, as indicated by 16 rattle segments with no taper (likely 20+ years), who spent one summer in an early successional field. Another such individual was a male not included in this study, as he expelled his radio midway through the active season. Both individuals did move to a basking site for the first shed that was located south of the den. MBER3 was the only den that appeared to have a suitable basking area below the den. All other individuals that moved downward off the mountain did so by moving up and over the mountain range, to the east or north. Individuals at south-facing dens (3 den sites) typically moved north of the den during the active season. Individuals at west-facing dens (4 den sites) moved east of the den during the active season.

2.4.3 Average Daily Movement Distance

Daily movement distances were estimated for 614 pathways in 32 individuals, with an average of 11.8 days separating each relocation. There was no effect of SFD ($F_{2,579} = 1.07$, $p = 0.309$) or sex ($F_{3,579} = 4.35$, $p = 0.128$) on daily movement distances (Table 2.1). Individuals moved significantly shorter distances daily during the shedding season (24.36 m ± 2.17) than during the core active period (44.64 ± 4.75 m, $p = 0.021$, $F_{2,579} = 6.65$). In general, individuals moved away from the den after emergence, remained sedentary during shedding (peaking at week 27, Fig 2.4), and then dispersed during July and August (weeks 28 – 35, Fig 2.4). There was no effect of year on average daily distance moved ($F_{1,567} = 0.015$, $p = 0.90$, Table 2.2). Individuals were relocated having not moved from their previous location 48 times, events which mostly occurred during the shedding season, and in gravid years.

2.5 Discussion

The first radiotelemetry study of Timber Rattlesnakes was conducted by Fitch in 1966 in Kansas (Fitch, 1999). At that time transmitters were forced into the snakes' stomachs, and rarely lasted longer than a month before being egested. Snakes were found to have an average daily movement of 57.5 m. The snakes did not move on thirty percent of the days monitored. In Massachusetts, this lack of movement between relocations was not observed as frequently, although snakes here were not detected every day as in (of Fitch, 1999). Brown et. al. (1982) followed five individuals in New York, and documented that males travel farther than females, and that non-gravid females traveled

farther than gravid females. Extensive movements have been seen after the first shed of the season, and snakes have transient routes after the active season; these movements represent migration from the core active area to the den (Reinert et. al., 1984, Reinert and Zappalorti, 1988, Waldron et. al., 2006). Many studies of this species have found this trend over the entire species' range (Reinert et al. 1984; Reinert and Zappalorti 1988; Waldron et. al. 2006), including this study, although in contrast to others, I found little variation in movement patterns related to reproductive condition.

2.5.1 Home Range Variation and Kernel Density Estimates

Home range size did not vary significantly with reproductive condition, SFD presence, or year. The lack of a reproduction-condition effect is at odds with most studies (Brown, 1993; Waldron et. al. 2006; Fitch, 1999), where males typically have been shown to have larger home ranges, and gravid females to have smaller home ranges than non-gravid females. My study population may truly differ from other sites, but it is also possible that I did not detect an effect due to my sample being heavily male-biased, and having one gravid female moving much farther than is reported in previous studies (Brown, 1993). The lack of SFD effect suggests that fungal lesions do not influence movements of individuals. If infected snakes did bask more, they would probably move less frequently and would remain at the basking site; therefore, infected snakes apparently did not bask more frequently (see Chapter 3 for further habitat analysis).

Movement direction from dens could prove useful in predictions for management in regions around unstudied dens, although it is likely that most snakes move upward

from the den to find suitable open basking areas, rather than the movement being driven by a specific cardinal direction.

2.5.2 Maximum Distances and Daily Movements

During the shedding season, individuals did not move as far as in the core active season. Individuals shed soon after emergence, and typically did so near the den area, and these shedding sites were typically in open rocky areas above the den site (Brown, 1993). It seems likely that individuals with SFD would be inclined to shed more frequently during the year to increase the healing process, thus limiting their movements. I did not observe this trend, therefore it seems that the observed fungal lesions did not influence shedding frequency, or movement patterns. For individuals followed for two or more seasons, maximum distance from the den and average daily distance moved did not vary among years. Females typically used the same foraging areas among years, but males typically used different areas each year. Males were often following the scents of females during the mating season, which could lead to the variation in foraging and second annual shedding areas (Brown, 1993).

The reason there was no effect of sex and reproductive condition could be due to one gravid female switching rookeries in 2012 and another female (postpartum) having the greatest maximum distance from the den by any individual, traveling 4.78 km in 2010, and 3.42 km in 2012 while gravid. Brown (1993) reports a maximum distance of 3.7 km for non-gravid females. These two females I recorded increased the variation and mean of the analyses, and their movements are probably why there was no difference in distance between males and non-gravid females.

2.5.3 Seasonal Variation

Individuals moved less often daily and used smaller areas during the shedding season compared to the core active season. This finding is consistent with the hypothesis that a majority of mate0searching and foraging occurs during the active season, and is also consistent with a Timber Rattlesnake study in South Carolina (Waldron et. al., 2006). Most individuals in my area moved upslope from the den and remained in that higher area during the first shed. These shedding regions were either north of the den, (for south-facing dens) or east of the den (for west-facing dens). These directional movements are likely due to the available basking areas being above the den, rather than to a pre-determined cardinal directional movement. Also, most foraging areas appear to be above the basking areas, at the peak of the mountain, again suggesting snakes chose to move uphill to locate seasonal home range habitat. When they dispersed, individuals also congregated more often during the shedding season than during the active season, suggesting that these shedding areas are critical habitat areas that should be protected, as these sites are limited spatially because they usually are near the den sites. Thus, active season areas appear to be more flexible and individuals may be more resilient to natural disturbances in these core areas. Protecting rock outcrops above the dens should be a priority, and if increased canopy cover occurs through succession, habitat management, (e.g. cutting back the canopy or removing encroaching trees and shrubs) should be implemented to assure that these critical sites are available (Brown, 1993).

2.5.4 Management Implications

Mapping seasonal spatial use of a species allows management practices to be implemented in a more effective way to protect areas that are critical to a population. In this metapopulation roads do not appear to be barriers as most are narrow and unpaved. Individuals typically moved uphill from their den, and roads are often located at lower elevations between mountains. Behavioral patterns observed here may apply to other, more urbanized, populations. For example, in areas with less available habitat, the focus should be on roads located north and east of den as possible sources of morality. In a more urbanized settings, where roads can act as a barrier (Clark et. al., 2010), closing such roads can be both a helpful act and a political problem, even if only done at night. Considering the observations here, however, I suggest that closing roads only during the active season (approximately July-August) might be effective.

The majority of individuals moved upslope after emerging from the dens. Therefore, protecting land at a higher elevation than the den should take priority over protecting areas below. Farthest distances moved from the den varied widely, and ideally the farthest distance would be used as a radius from the den to create an area of maximum protection. The farthest distance moved for this species was 7.2 km in New York (Brown, 1993), but protecting this much land around a den site may not be possible, especially since every Timber Rattlesnake den in Massachusetts is within 4.5 km of some type of urbanization. Mapping out areas of frequent use by the population with radiotelemetry studies is a more efficient way to determine which areas of habitat are used by a population and as critical to the species.

Results reported here should be useful for future conservation efforts, as the Massachusetts Natural Heritage and Endangered Species Program explores the option of

captive breeding for the purpose of headstarting for population supplementation, as well as and re-introduction in two other smaller populations. Knowing what types of habitat are needed by an apparently stable population can reform future headstartings efforts evaluating these methods as to whether the introduced snakes are moving and using habitat in ways similar to those of this study, or if their movement and habitat choices are erratic, can suggest whether they are acclimating to their adopted population. One headstarting study by Conner et. al. (2003) in a Texas population reported that headstarted snakes had a 50% survival rate (range 44 — 77%) over the course of the study, and successful reproduction was observed. The range of survivorship is reported because they could not differentiate between radio failure vs. individuals leaving the field site. This range of survivorship is consistent with survivorship for wild born neonates reported for this species in a New York metapopulation (Brown et. al., 2007). Snakes studied by Conner et. al. (2003) were able to locate dens as well as basking sites used by resident snakes. Reinert and Rupert (1999) support this, with a radiotelemetry study in New Jersey comparing movements and habitat use by adults translocated into a new population to the local adults. Translocated snakes at first made larger, more frequent movements than residents, but then settled into home ranges that did not differ in area from those of local adults. Translocation was also critical in saving an adder population suffering from inbreeding depression (Madsen, 1999). Although headstarting is not seen as necessary or desirable for this stable metapopulation, the results here can be helpful in evaluating these efforts in other populations. Distances moved and home range sizes of native snakes will likely vary among populations, based on the available habitat, but overall patterns of short uphill movements during the shedding season, and larger

movements during the active season as observed here, could be informative when analyzing the movement patterns of headstarted and translocated individuals elsewhere. Without knowing the movement patterns of native snakes, any other movement patterns cannot be compared to translocated and headstarted snakes, and it therefore one cannot know if the introductions was successful.

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Table 2.1. Mean movement variables measured for Timber Rattlesnakes from 2009 to 2013 with regards to reproductive condition, gender, and SFD presence, with minimum convex polygon (MCP), kernel (95%), maximum distance from den site (Max Dist), and average daily distance moved (Daily Dist). Standard error is given in parentheses.

	SFD absent			SFD present
	Male (n = 22)	Non-gravid Female (n = 13)	Gravid Female (n = 5)	Male (n = 9)
MCP (km^2)	0.87 (±0.14)	0.42 (±0.09)	0.06 (±0.04)	0.79 (±0.15)
Kernel (ha)	986.64(±233.17)	530.56(±178.03)	85.53(±39.67)	600.56(±188.93)
Max Dist (km)	2.18 (±0.16)	1.62 (±0.30)	1.29 (±0.72)	1.69 (±0.17)
Daily Dist (m)	45.74 (±4.74)	38.73 (±6.64)	11.78 (±3.35)	30.25 (±3.27)

Table 2.2. Mean movement variables measured for Timber Rattlesnakes from 2009 to 2013 with regards to reproductive condition, gender, and year, with minimum convex polygon (MCP), kernel (95%), maximum distance from den site (Max Dist), and average daily distance moved (Daily Dist). Standard error is in parentheses.

Year	Gender	*n*	MCP (km^2)	Kernel (ha)	Max Dist (km)	Daily Dist (m)
2009	Male	4	0.329(±0.227)	322.72(±135.37)	1.451(±0.382)	9.35 (±2.05)
	Female	1	0.719(n/a)	1188.43(n/a)	1.810(n/a)	81.34 (±28.57)
	Gravid	3	0.0269(±0.004)	48.65(±20.68)	0.499(±0.320)	10.64 (±3.53)
2010	Male	6	1.078(±0.313)	1372.00(±226.48)	2.275(±0.462)	13.37 (±7.80)
	Female	4	0.393(±0.260)	656.18(±514.65)	1.968(±0.80)	28.83 (±5.27)
2011	Male	8	0.796(±0.184)	937.82(±523.54)	2.171(±0.273)	32.44 (±4.24)
	Female	3	0.375(±0.115)	240.03(±13.49)	1.425(±0.313)	48.15 (±25.86)
	Gravid	1	0.208(n/a)	n/a	3.418(n/a)	n/a
2012	Male	8	0.924(±0.235)	763.81(±388.64)	2.173(±0.119)	48.09 (±8.10)
	Female	2	0.551(±121)	552.63(±284.24)	1.412(±0.292)	34.57 (±8.06)
	Gravid	1	0.0363(n/a)	196.15(n/a)	0.758(n/a)	17.47 (±9.97)
2013	Male	5	0.785(±0.148)	498.03(±136.15)	1.804(±0.149)	30.71 (±4.59)
	Female	3	0.298(±0.216)	322.75(±89.67)	1.455(±0.198)	28.31 (±4.52)

Figure 2.1. Home range size, as measured by minimum convex polygon (MCP) associated with reproductive condition (F = non reproductive female, *n* = 13, G gravid female, *n* = 5, M = male, *n* = 25) for Timber Rattlesnakes from 2009-2013.

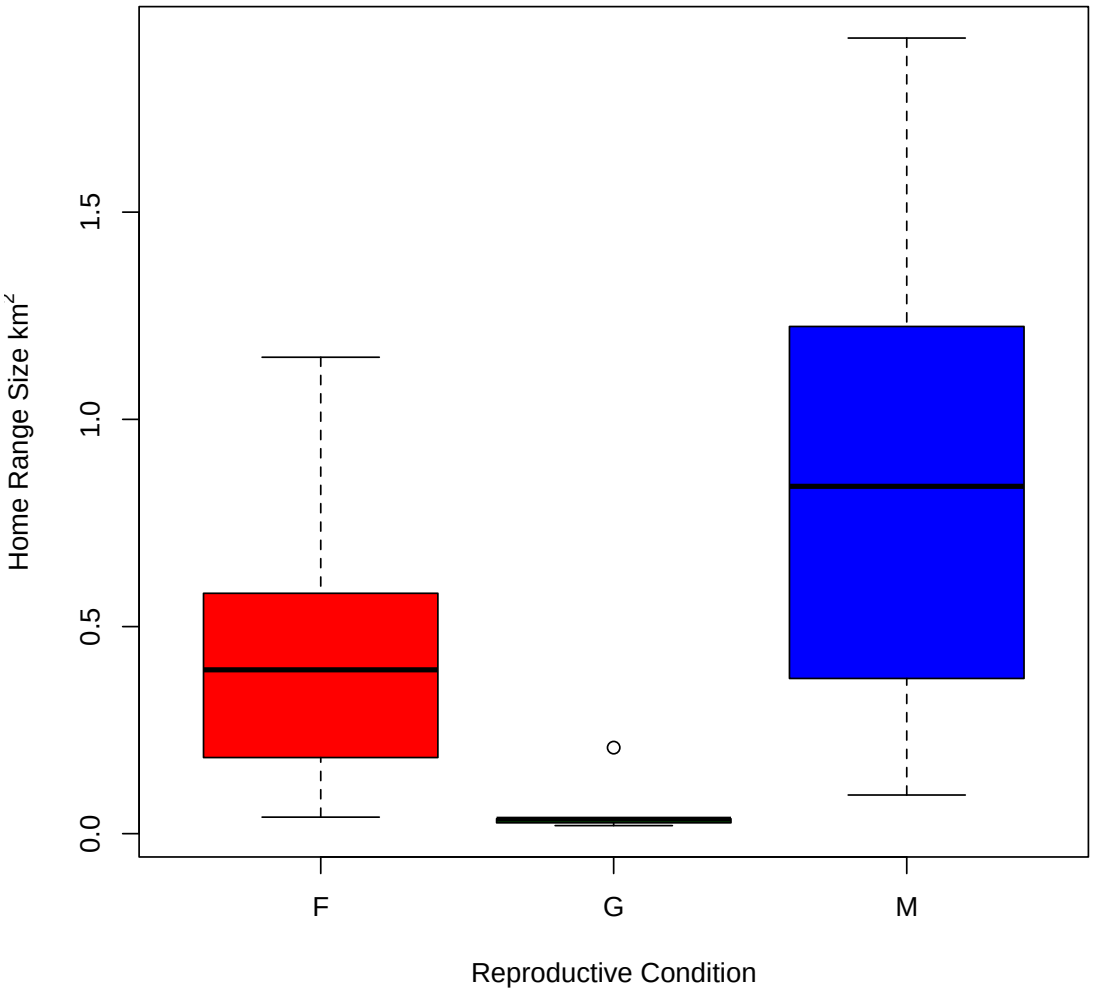


Figure 2.2. Annual home range variation for three adult Timber Rattlesnakes tracked for at least four years. Shown are two males (a.) and (b.) and one female (c.). She was gravid in 2009 and 2012. Den sites are located where all polygons converge.

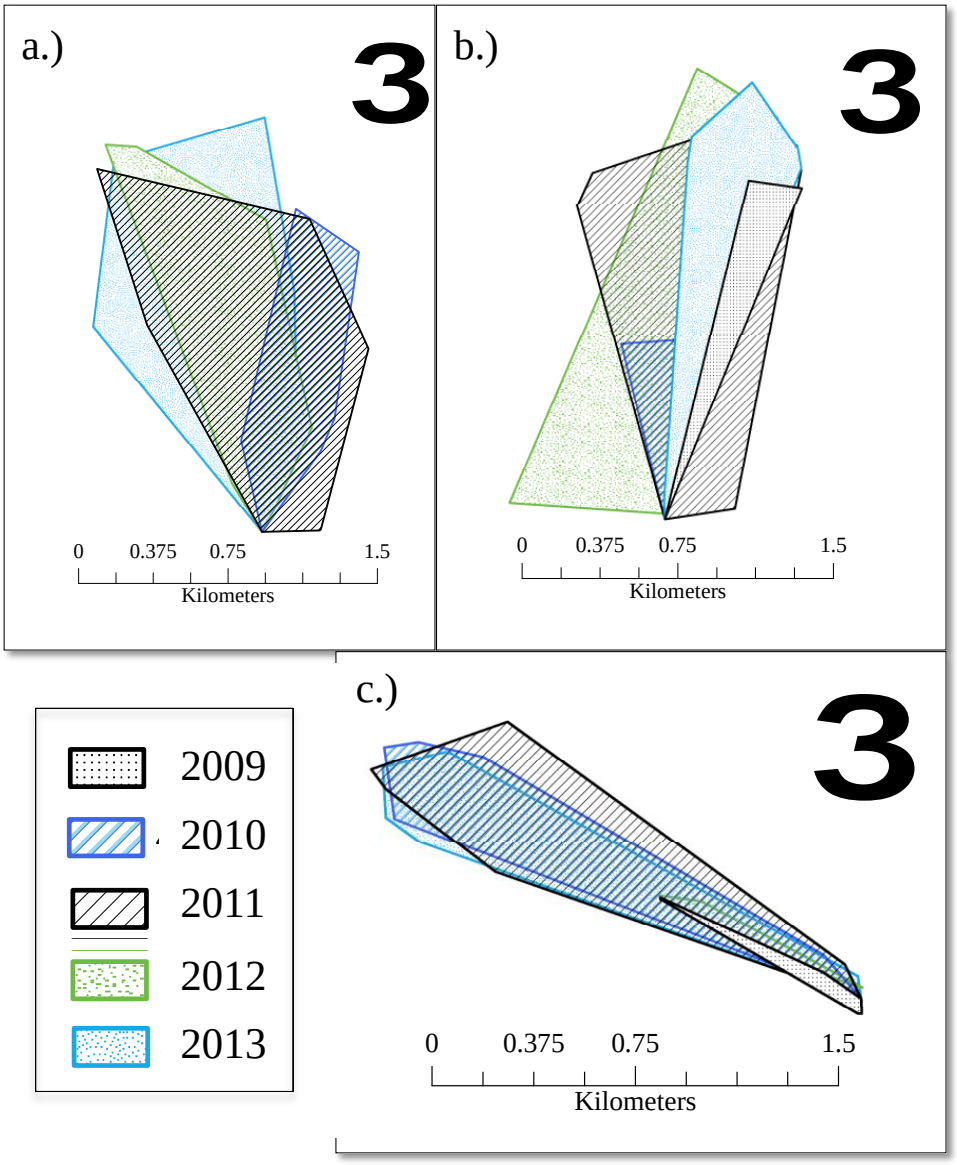


Figure 2.3. Maximum distance (km) located from the den site for Timber Rattlesnakes tracked 2009-2013 associated with reproductive condition (F = non reproductive female, G = gravid female, M = male).

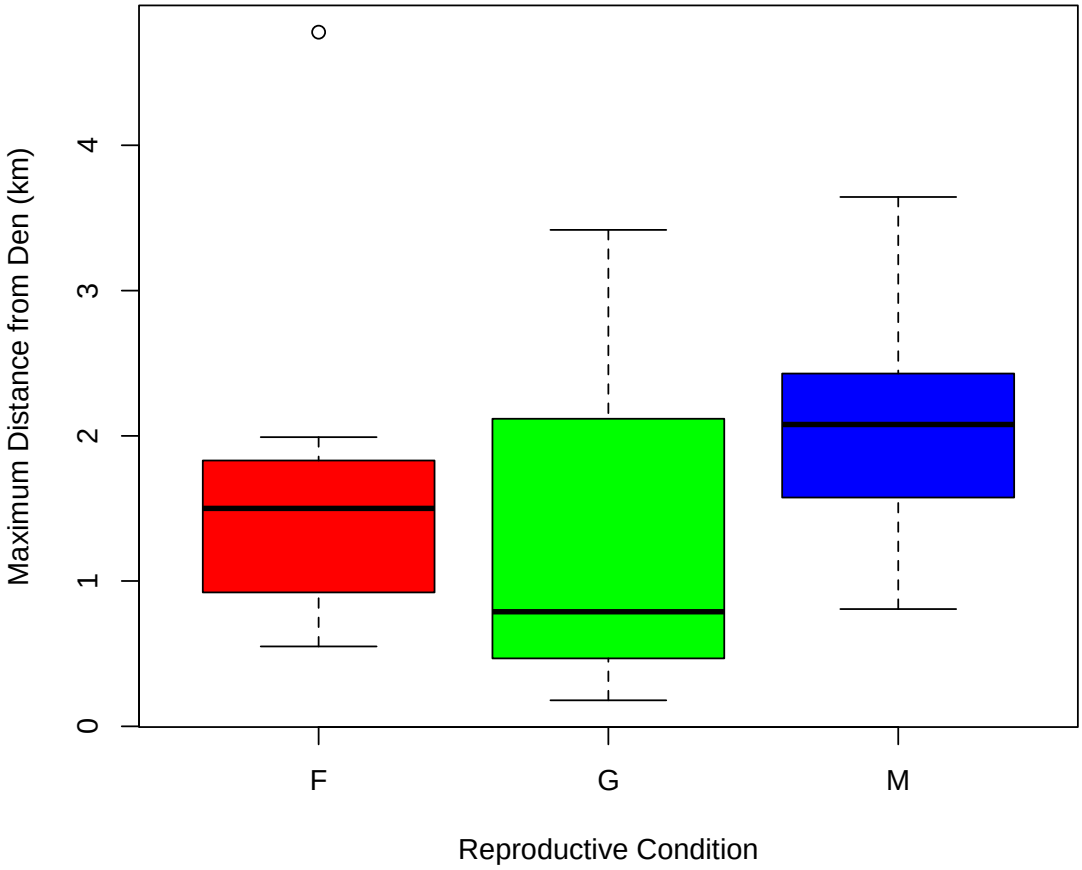
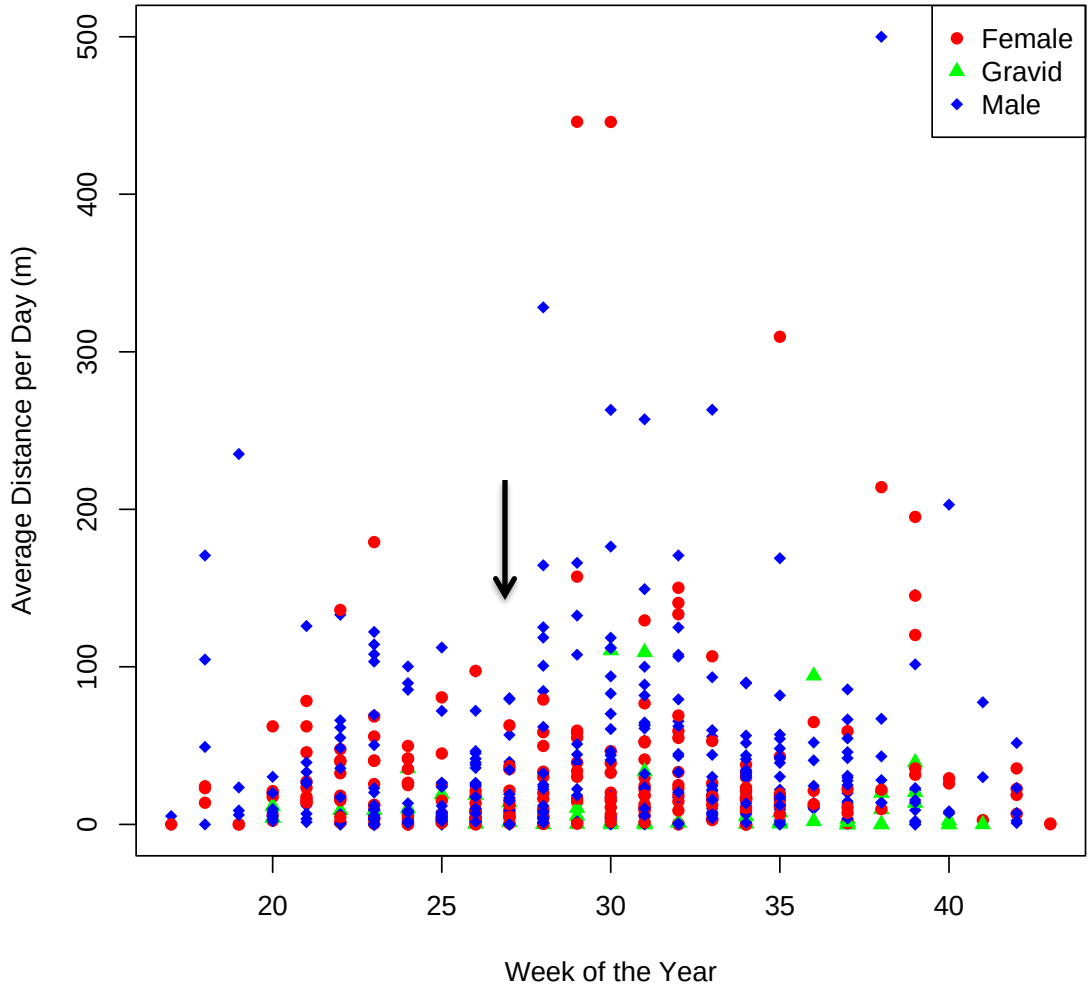


Figure 2.4. Average daily movements for Timber Rattlesnakes from 2009-2013 plotted by week of the year. Arrow shows the shift from the shedding season to the active season. Actual shedding took place during weeks 24-27.



CHAPTER 3

HABITAT SELECTION IN A TIMBER RATTLESNAKE POPULATION IN WESTERN MASSACHUSSETTS, AND EFFECTS OF DISEASE, GENDER, AND SEASONAL AND YEARLY VARIATION

3.1 Abstract

Habitat needs of snakes often depend on behavior (e.g. foraging, mate searching, gestating, shedding), and can vary seasonally and with health condition. Here, I investigate intraspecific variation in a Timber Rattlesnake (*Crotalus horridus*) population in western Massachusetts, with regard to health status and gender (male and non-gravid female) using classification tree (CART) analysis, and yearly and seasonal variation compared to random available habitat using paired logistic regression. Snake fungal disease (SFD) and sex were not correlated with habitat selection. Overall, individuals preferred areas of increased rock cover, decreased canopy cover, lower slope, and increased vegetative ground cover compared to available random sites. Individuals preferred rock outcrops under open canopies during the shedding season, and used more open forested areas with high vegetation cover and tree density during the active season. Because this population is located in one of the largest intact areas of old-growth forest remaining in New England, results can be used to guide management plans for populations in the region where the habitat has been altered or affected by humans.

3.2 Introduction

Protecting its habitat needs is critical to protecting any species, because animals often require different types of habitat for different behaviors (e.g. foraging, mate

searching, gestating), and seasonal variation (Waldron et. al., 2006; Timmerman, 1995). Habitat needs may also vary by body size differences in sex, such as foraging in different habitat types for different species of prey based on size; for example Timber Rattlesnake (males are larger than females (Brown, 1993), habitats used could also vary depending on the health status of the individual (Madsen, 1984; Timmerman, 1995; Reinert and Zappalorti, 1988). Males and gravid females of many snake species exhibit different habitat preferences (Reinert, 1984*a*). A relationship between individual health and habitat use in snakes may also be expected, but remains largely unexplored (Reinert, 1993). In several North American snake species experimentally inoculated with pathogens (Burns et al., 1996; Funk, 2006), infected individuals chose warmer locations and actively raised their body temperatures. This behavioral response would likely cause a change in habitat use, as habitat selection is often influenced by thermoregulation (Blouin-Demers and Weatherhead, 2001). Basking sites typically consist of areas with increased rock coverage, open canopy, greater sun exposure, and less shrub vegetation (Brown, 1993). Individuals with snake fungal disease (SFD) have been observed basking more often than healthy individuals (McBride et al., 2015). SFD presence was defined as the physical appearance of the infected skin lesions that are typically characterized malformation, blisters or necrotic depressions (see Chapter 1 for complete description and disease history). Snakes also often shed more frequently when afflicted with SFD or injury (Lorch et. al., 2015). Individuals adversely affected by SFD would likely use these habitat types more frequently than apparently healthy individuals.

The purpose of this study was to investigate intraspecific variation in a Timber Rattlesnake population in western Massachusetts, with regard to health status, gender,

and annual and seasonal variation compared to paired available random site not occupied by the individual. Many habitat models assume random points within the home range of an individual are available to the individual at all times, using approaches such as grid surveys (Arther et. al., 1996). This approach does not, however, analyze the habitat actually available to the individual at a given moment in time. By using random points paired to the individual's location, on a spatial scale of reasonable distance traveled within a day, this assumption is avoided. Several outside variables are also eliminated, such as weather condition, time of day, and resource availability, all of which affect habitat selection on a daily temporal scale (Compton et. al., 2002).

The Timber Rattlesnake has been declining throughout its range over the past few centuries (Martin et. al. 2008). Its corresponding habitat is protected in Massachusetts under MESA (321 CMR 10.18). Therefore, understanding habitat needs is critical to protecting the species and developing management plans. Timber Rattlesnake habitats in the northern extent of the range consist of two primary components: (1) the overwintering habitat (hibernaculum, or den) and (2) the summer range area. The majority of Timber Rattlesnake habitat in Massachusetts is protected by various agencies (United States Fisheries and Wildlife, Massachusetts Department of Conservation and Recreation, New York Department of Environmental Conservation, The Nature Conservancy, the Massachusetts Audubon Society, and town municipalities.), and future development is unlikely. However, habitats can vary greatly across the few geographically constrained populations in Massachusetts, and by analyzing habitat selection in the largest, healthiest population in Massachusetts, more realistic management strategies can be suggested for

small and fragmented populations, and those where habitat has undergone anthropogenic change.

3.3 Materials and Methods

3.3.1 Study Area

This study was conducted in the southern Berkshire Taconic region in southwestern Massachusetts, adjacent to both New York and Connecticut. The area is approximately 60 km^2, with an average elevation of about 610 m, and has one of the Northeast's largest areas of old growth forest (Davis, 1996). The area consists of mostly northern hardwood forest, dominated by Red Oak (*Quercus rubra*), Eastern Hemlock (*Tsuga Canadensis*), Chestnut Oak (*Quercus prinus*), American Beech (*Fagus grandifolia*), Striped Maple (*Acer pensylvanicum*), stands of American Hazelnut (*Corylus americana*) ,and occasional American chestnut (*Castanea dentata*). Dominant shrubs include Mountain Laurel (*Kalmia latifolia*), Scrub Oak (*Quercus ilicifolia*), and Low Bush Blueberry (*Vaccinium angustifolium*). Other features include small wetlands, bogs and natural ponds. The area is primarily used for recreation (i.e. hiking, camping, etc.), with approximately 0.5 permanent residents per km^2.

3.3.2 Radiotelemetry

Visual searches for Timber Rattlesnakes were conducted at known den areas during spring emergences and fall ingress, and at basking sites previously mapped by the Massachusetts Natural Heritage and Endangered Species Program (MANHESP). Measurements of ventral scale counts, snout-vent length (SVL, cm), tail length (cm), weight (g) and rattle segment counts were done at the capture sites, if the animal was not

receiving a transmitter. Subcaudal ventral scale counts or cloacal probing (Schaefer, 1934) determined sex. Each snake received a 128 Khz passive integrated transponder tag (PIT tag, Biomark, Boise Idaho), injected subcutaneously laterally approximately 8 cm anterior to the cloaca with a 12 gauge sterile syringe. Individuals were scored as SFD present if a lesion covered at least 10% of the facial area, and photographed. Nineteen individuals were selected for radiotransmitter implantation from July 2010 through 2013. Snakes were surgically implanted with Holohil SI-2T transmitters (Holohil Systems Ltd., Carp, Ontario, Canada), weighing <5% of body mass following Blouin-Demers et al. (2000). All surgical procedures were performed in the veterinary laboratory at Holyoke Community College, Holyoke MA, and followed approved protocols of the University of Massachusetts Institutional Animal Care and Use Committee (#29-02-03R, and #2012-0009). Snakes were observed for at least 24 hours post surgery and released at the point of capture. All individuals appeared normal after the anesthesia wore off. Using a R-1000 (Communication Specialists, Inc., Orange, CA) telemetry receiver and 3-element Yagi antenna, snakes were located once to twice weekly. GPS locations were recorded with a Garmin Oregon 550T (Garmin International, Inc, Olathe, KS) hand-held unit.

3.3.3. Habitat Variable Measurements

Eleven habitat variables were measured at each snake telemetry location (Table 3.1), with the telemetry study described in Chapter 2. These variables were previously identified as important habitat selection variables to Timber Rattlesnakes by Reinert, (1984a.). The 11 variables were also measured at a paired random point, within 10-200 paces (approximately 10-200 m) in a random direction from the snake. This is a reasonable estimation of the distance a snake might move within a day (Keller and

Heske, 2000). Direction and distance of the random point were determined with a random number generator. Habitat variables were only measured once individuals moved away from the den entrance (at least 150 m), to avoid biasing analyses with repeated locations at den sites. Aspect and slope were measured directly at the site where the individual was located, rather than using data extracted from GIS layers, with a compass and clinometer. Canopy openness was measured with a Model C densitometer (Forestry Suppliers, Jackson MS). Distances and heights were measured using a meter tape. Percentage vegetation, rock cover, and woody debris cover were estimated after measuring a 1 m radius area around from the individual's location.

3.3.4 Statistical Analyses

Statistical analyses were preformed using R version 3.1.3 (R Development Core Team, 2006). Slope aspect was cosine transformed so northern values = 1 and southern values = -1. Effect of SFD presence and gender on habitat use was evaluated first, and once no effect was found, individuals were pooled for further analyses.

3.3.4.1 Snake Fungal Disease and Gender

A classification and regression tree (CART) was built to examine the effects of gender and SFD presence on habitat use using the R package cartware.R (Compton, 2006). CART analysis has the advantage of being non-parametric and non-linear, which is often the case with environmental data. Classification used a tree-building algorithm based on the strongest predictor value for each "if-then" logical binary split. No gravid females exhibited SFD and were excluded due to low sample size (n = 12) from both the SFD-present and SFD-absent variables, and from the gender variation variables. This was

done because gravid females are known to use birthing rookeries for the entire year of gestation, not when when hibernating, or traveling to and from the den. These areas were defined as areas with more rock cover and less canopy then areas used by non-gravid females and males; thus including them in these analyses could bias the results, as males do not exhibit seasonal variation (Brown, 1993). CART analysis is also sensitive to extremely unbalanced sample sizes, and the values will result in false positive classifications (Cieslak and Chawla, 2008). There were four categories of classification used: (1) male-SFD absent; (2) male-SFD present; (3) non-gravid female-SFD absent; (4) non-gravid female-SFD present. The tree was trimmed using the 1-SE rule (Breiman et al. 1984), with 10-fold cross-validation and using the splitting index "gini". Cohen's kappa (Cohen, 1960) evaluated model classification rates, and a corrected classification above random was calculated using Monte Carlo resampling rates. To evaluate variable importance and effectiveness of splitting the data, a random forest approach with 1,000 runs was used (Breiman, 2001).

3.3.4.2 Selected vs. Random Sites

Timber Rattlesnakes exhibit strong site fidelity and often only congregate during hibernation, shedding, and gestation (Brown, 1993; Martin, 2002), so all random sites are seldom available to all individuals. Paired logistic regression was used to analyze differences between chosen and available sites. Pairing the selected site with a random site eliminates the assumption that all random sites are available to all individuals. Matched-pairs logistic regression is more appropriate for analyzing paired data points than standard logistic regression (Breslow and Day, 1980, Hosmer and Lemeshow, 1989). This nonparametric approach is robust to violations of assumption of normality,

which are often the case with ecological (specifically habitat use) data (Morrison et. al., 2006). It is cautioned here that this approach is not a predictive model (Keating and Cherry, 2004), but explains only the strength of relationships of the variables at the selected site. Random values were subtracted from the present site values to fit the regression model using standard logistic regression to fit a response vector of all 1's to a matrix of predictor variables with a constant term excluded. The final model was chosen using step-wise Akaike information criterion (AIC) deletion of variables from the model. To explore the relationship of contributing variables, a classification tree was built and trimmed using the 1-SE rule (Breiman et al. 1984). Cohen's kappa (Cohen, 1960) was used to evaluate model classification rate, and correct classification above random was calculated using monte carlo resampling rates. A random forest approach was used to evaluate variable importance and the effect of splitting the data (Breiman, 2001).

3.3.4.3 Seasonal Habitat Variation

Snake behavior was divided into five categories: (1) shedding, (2) mating (includes copulation and courtship), (3) foraging (classified by ambush posture, Reinert et al., 1984), (4) transient (actively moving), (5) coiled and exposed. The timing of the first shed was consistent across individuals (5 June through 3 July). The second shed, breeding, transient, and foraging primarily occurred between 13 July and 11 September. Based on temporal overlap of behavioral observations other than the first shed, distinct behavioral seasons could not be separated, resulting in only two seasons. The shedding season began once individuals moved away from the den and into nearby areas occupied during the first shed (approximately 150 m). The core active season includes foraging, transient movements, mating, and the second shed, as all behaviors overlap during this

time period. The average date of 1 July demarcated the two behavioral seasons, though observations were included in the appropriate season if there was overlap around this date (± 2 days). Statistical methods used were the same as described for selected vs. random points.

3.4 Results

Habitat observations (n = 509) were recorded from 18 telemetry individuals and 48 new and recaptured individuals. For two observations sex of the individual was unknown and these snakes were omitted from analyses of sex differences, but were included in all other analyses. Average values for all habitat variables (with standard errors) for all treatments are reported in Table 3.2. Canopy openness and slope were the leading predictor variables in all CART analyses. Across all tests, all individuals preferred more open canopy and lower slope throughout the year.

3.4.1 Snake Fungal Disease and Gender

One individual with SFD was biopsied in collaboration with a health survey conducted by Roger Williams Park Zoo, Providence, RI. The sample tested positive for *Ophidiomyces ophiodiicola,* the causative agent for SFD (Lorch et. al., 2015). Only one radiotracked female had SFD, and didn't exhibit symptoms until after the study was completed, while all others were males (n = 3). Of the 509 observations across 22 individuals, 376 were SFD-absent, and 133 SFD-present. Observations were for 170 non-gravid females (n = 6), 325 males (n = 13), and 12 gravid females (n = 3). Gravid females were omitted from analyses due to low sample size. For SFD-present and SFD-absent analysis, a CART tree could not be created, as only one node was present, with no leaf

branches. This indicates that the model could not differentiate any of the habitat variables between snakes with SFD-present and SFD-absent, and results in an intercept only model (Table 3.3). Canopy openness and percentage rock coverage were higher for SFD-absent snakes, although not significantly (Table 3.2). This result was contrary to the hypothesis that SFD-present snakes would bask more frequently to increase their body temperature and shedding rate. With this result, SFD-present and SFD-absent groups were pooled for further sex, annual, and seasonal tests. Gravid females were observed here to use areas of increased rock cover and less canopy coverage than non-gravid females, males, and individuals with and without SFD (Table 3.2).

3.4.2 Selected vs. Random Sites for Annual Variation

Step-wise model selection of all (n = 509 treatments pooled) observations tested with AIC found that eight of the 11 original variables were useful in predicting selected sites compared to not used random sites (Table 3.4) throughout the year. These data indicate individuals preferred southern facing areas with a lower slope and open canopy as leading predictor variables. Individuals also preferred areas with either increased rock cover, and increased vegetation, or more woody brush cover (Table 3.5). CART analysis shows open canopy and lower slope as leading predictor values for a site selected by an individual (Table 3.3, Fig. 3.1), in addition to increased rock cover and vegetation cover.

3.4.3 Selected vs. Random Sites for Seasonal Habitat Variation

Due to low sample size, gravid females were not analyzed by season. The shedding season consisted of 194 locations (M = 123, F = 71). SFD-absent and SFD-present groups and male and females were all used for seasonal variation analyses. Using

a step-wise model selection testing with AIC found that nine of the 11 original variables discriminated between used sites and random sites (Table 3.4) during the first shedding season. Open canopy, and increased vegetation and rock cover areas, are the most explanatory values during the shedding season (Table 3.5) as described by CART analysis (Table 3.3, Fig. 3.2).

The active season consisted of 288 locations (M = 105, F = 183). During the active season, ten habitat variables were included in the final model selection (Table 3.4). During the active season open canopy, increased woody debris cover, and areas with fewer understory trees were preferred, as well as lower slope and southern aspect (Table 3.5). CART analysis indicates open canopy and lower slope as leading predictors, with individuals also selecting areas farther from over story trees and having increased vegetation cover (Table 3.3, Fig.3.3).

Open canopy and lower slope were the strongest predictor values for both seasons, though individuals selected more open canopy sites during the shed season. Rock cover was also greater during the shed season, consistent with known basking sites used while shedding (Brown, 1993). These results also suggest that during the active period, individuals use areas of open forest with farther distances from trees, compared to available random sites, despite often shedding a second time during this period.

3.5 Discussion

Timber Rattlesnakes in the Northeast typically prefer older growth forests, with dense vegetation ground cover when not basking (Reinert, 1984*a*; 1984*b*; Brown, 1993), traditionally described as: oak lands, oak-pine woods, or oak-laurel-poplar-chestnut hills (Wright and Wright, 1957). My results are consistent with these previous studies in the

Northeast, with preference (other than gravid females) for areas of increased vegetation cover, and canopy openness of less than 50% (Brown, 1993). Individuals had a strong fidelity toward basking, denning, and birthing sites (Martin, 2002; Brown, 1993). Reinert et al. (2011) demonstrated the repeated use of the same areas by individuals over several years, despite habitat alteration caused by logging that resulted in significant differences in vegetation cover and canopy closure in their study area in Pennsylvania. This suggests strong site fidelity throughout the year. Thus, determining what role microhabitat selection plays in site selection may not depend solely on actual habitat variables, chosen for measurement.

3.5.1. Snake Fungal Disease Presence

Lorch et al. (2015) found Corn Snakes (*Pantherophis guttata*) inoculated with *Ophidiomyces ophiodiicola,* the fungal causative agent of SFD, shed their skin more frequently and remained more exposed in a laboratory setting. Shedding and being more exposed, if applied to natural behavior, would result in an increase in the use of basking areas (categorized by more rocky areas with less canopy cover) in SFD-present snakes. This was not seen here; however, Lorch et al. (2015) repeatedly reinfected individuals, even after an individual appeared to overcome SFD. This extreme rate of reinfection likely would not represent a natural condition, and behavioral changes seen in these Corn Snakes cannot be applied to wild populations. In the current study individual Timber Rattlesnakes with SFD preferred areas of lower canopy openness and less rock cover, contradicting the hypothesis that they would use open basking areas more frequently (Lorch et al., 2015).

3.5.2 Habitat Selection, Sex and Reproductive Condition

With many snake species, including Timber Rattlesnakes, there are often no detectable habitat selection differences between males and non-gravid females (Reinert and Zappalorti; 1988, Reinert, 1993), as seen with the results presented here. In contrast, to this, in coastal South Carolina, males and non-gravid females did occupy different forest types while foraging, with foraging males associated with hardwood bottoms and foraging females associated with pine hardwood forests. This result was attributed to foraging for different prey species of different sizes, as females are smaller than males (Waldron et. al., 2006; Brown, 1993). Males and females overlapped in hibernating and basking areas, consistent with other northeastern studies. Gravid females differed in habitat selection compared to non-gravid females and males (Reinert, 1984*b*; Keenlyne, 1972) using areas of increased rock cover and canopy openness, and decreased shelter rock distance, reflecting the snakes' selection of birthing rookery site (Reinert and Zappalorti, 1988; Brown, 1993). This pattern was observed here too, but is not included in my analyses due to low sample sizes (Table 3.2).

3.5.3 Selected vs. Random Sites

Overall, individuals preferred areas with open canopy, increased rock cover and surface vegetation, and lower slope compared to available random sites, consistent with previous studies (Reinert, 1984*a*, Reinert and Zappalorti, 1988). Slope was the most predictive variable in individual habitat selection across most analyses here. Many studies measure slope using GIS layers (Browning et al., 2005), which does not assess slope selection on the microhabitat scale, and therefore is not often the strongest predicative

variable. When using areas with steep regional slope, individuals in this study were often coiled in small flat areas, often of a size only the diameter of the coiled snake. Extracting slope from GIS layers in these situations would grossly overestimate the steepness of the slope of the area the individual is actually using.

3.5.4 Seasonal Variation

Timber Rattlesnakes in other parts of the species' range exhibit seasonal variation in habitat use (Waldron et. al. 2006). A similar trend was observed in this study, although many behavioral categories overlapped temporally and could not be separated into as many distinct behavioral groups as was the case in South Carolina (Waldron et. al., 2006). Instead, only this first shedding period and the active season were defined outside of hibernation as has been done with other northeastern snake species (Weatherhead and Charland, 1985) where more specific behavioral temporal breakdowns could not be made. During the first shedding season, individuals used areas of increased rock cover and higher canopy openness compared to random sites. Thirteen individuals were observed shedding a second time during the active season, with females receptive to breeding often exhibiting a pre-receptive shed, and all others either foraging or mate searching during this second shedding time. During the active season, individuals used more forested habitats than during the shedding season despite several individuals shedding a second time during the active season. Second sheds occurred in forested areas, rather than the rocky outcrops used during the first shedding season. This could be due to warmer environmental temperatures during the second shedding period of the active season, and if rock outcrops are too hot during the warmest part of the summer. Prey availability is greater in more forested areas, which is also likely to be driving this trend

toward forested habitat use during the active season. During the first shedding season, the rock outcrops that are used are often near and above the dens (Brown, 1993) and individuals are in closer proximity to each other than during the active season. Competition for prey and mates likely drives individuals away from these high-density areas, and leads to movements that are more frequent and with increased habitat variation.

It is not fully understood how snakes navigate through their environment, although chemical cues and light appear to provid strong directional cues (Lawson et. al. 1991; Landreth, 1973; Brown and MacLean, 1983; Noble and Clausan, 1936). However, blinded snakes appear to navigate just as well as their normal sighted conspecifics (Smith, 2002; Bonnet et al., 1999). Reinert (2011) found individuals tracked before and after logging used the same areas and migration routes despite major changes in the habitat. This finding suggests that they are not solely using immediate habitat cues (i.e. light exposure, chemosensory) to reach desired locations, and may be resistant to some habitat alterations. Garter snakes have responded to geomagnetic cues (Smith, 2002), and this is likely a key factor in navigation in other snake taxa, including Timber Rattlesnakes.

This species exists in a variety of ecosystems throughout its range that vary in vegetation species composition and structure, e.g. southern coastal plain forests, northeastern old-growth forests. Comparing this species across two different habitat types, Reinert (1993) concluded that although the ecosystems appear very different, Timber Rattlesnakes were using the structure of the habitat in similar manners, as when, for example canopy closure and fallen log cover were very similar at snake locations at

both sites. The habitat values here are also similar to those compared by Reinert (1993). Reinert (1993) studied populations in New Jersey, still within the northeast, however the populations studied here is primarily old-growth forest, also a different ecosystem from the two studied by Reinert (1993). Results here support Reinert's (1993) conclusion that ecosystem types inhabited by this species may vary, individuals all select the same microhabitat.

3.5.5 Management Implications

The preference during the shedding season towards rock outcrops with open canopy suggests habitat management plans should assure that the canopy stays open in these areas. Basking areas used during the shedding season had the most significant effect of habitat variables compared to all other factors. Canopy clearing through the action of cutting down encroaching vegetation, would maintain these areas, but may need to be performed on a routine basis, as vegetation more rapidly rebound after removal. These results can be applied to other populations. For example, in an eastern Massachusetts population, eastern white pine (*Pinus strobus*) quickly fills in rock outcrops (N. Smith, pers comm.; A. Stengle, unpubl data). These sites are typically above a den (Chap 2, A. Stengle, unpubl data), and could similarly be identified in other populations with visual surveys, without radiotelemetry. Where den sites are known and the timing of the shedding season is very predictable, individuals congregate in high density and can be monitored directly.

The preference during the active season for large tracks of open forest with dense low vegetation forest is critical for Timber Rattlesnakes. Management strategies should aim to protect uninterrupted tracks of forest, particularly north or east of mountainous

dens, depending on whether the den is south, or west-facing (respectively), as based on movement patterns analyzed (cf. Chapter 2). These results can be applied, again, to an endangered eastern Massachusetts population, where over-population of white tailed deer (*Odocoileus virginianus*) that is 6.5 times higher than the state-wide average, has led to a serious decrease in understory vegetation (Massachusetts Department of Conservation and Recreation, 2015), thus limiting the availability of this habitat type. Recent efforts by Massachusetts Department of Conservation and Recreation (DCR) to decrease deer numbers will likely benefit the local rattlesnake population, as the individuals in the eastern Massachusetts population appear to be using the habitat very differently from those at my study site (A. Stengle, unpubl data).

Reinert's (2011) study demonstrating a lack of effect of logging on Timber Rattlesnake behavior, and my study population having been historically exposed to logging, suggests that some populations do tolerate some degree of habitat disturbance. Within my Berkshire county study site, two known wildfires caused by lightning strikes, occurred within the past ten years (A. Stengle, pers obsv, S. Winters, pers comm). One opened up a basking area often used by the snakes in this study. This may suggest that not only is habitat disturbance tolerated, but that it is required to some degree to maintain the viability of the community, as has been noted in southern populations of this species (Beaupre and Douglas, 2012). In the Berkshire area, fortunately the habitat surrounding basking areas is forested and undisturbed by humans.

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Table 3.1. Description of habitat variables, and abbreviations used in analyses for Timber Rattlesnakes (*Crotalus horridus*) measured from 2010-2013 in Berkshire County, Massachusetts.

Habitat Variable	Abbreviation	Description
Canopy openness(%)	Canopy	Canopy closure opening using spherical densitometer
Distance to over story trees (m)	Otree	Distance to nearest over story tree (>7.5 cm dbh and 2 m ht)
Distance to understory trees (m)	Utree	Distance to nearest understory tree (<7.5 cm dbh)
Surface vegetation cover (%)	VegCover	Ground cover vegetation within 1 m^2
Vegetation height (m)	VegHeight	Vegetation height of understory within 1 m^2
Surface rock cover (%)	RockCover	Rock cover within 1 m^2
Distance to rocks (m)	RockDist	Distance to nearest rock >5 cm diameter
Surface fallen log cover (%)	LogCover	Fallen log cover within 1 m^2
Distance to log (m)	LogDist	Distance to nearest fallen log >5 cm diameter
Slope (°)	Slope	Surface slope at exact location using clinometer
Aspect (°)	Aspect	Surface aspect at exact location using compass
Cosine Aspect	AspectCOS	Cosine transformation of aspect

Table 3.2. Average habitat values for all categories of all individual Timber Rattlesnakes tracked from 2010-2013 with regards to Snake Fungal Disease (SFD) presence and absence; gender and reproductive condition, Female (non-gravid), Gravid, and Male; Yearly present and Yearly paired random (absent) locations; shed season present and shed season paired random (absent) locations; active season present and active season paired random (absent) locations. See Table 3.1 for variable definitions.

	n (ind)	*n* (loc)	Canopy	Slope	VegCover	VegHeight	LogDist	LogCover
SFD Absent	55	376	25.75	6.25	42.45	0.50	2.43	18.11
SFD Present	12	133	21.80	7.16	38.98	0.45	1.67	20.76
Female	25	170	25.97	7.30	42.86	0.51	2.38	18.85
Gravid	3	12	58.19	4.36	17.33	0.20	4.51	9.75
Male	37	325	22.72	6.10	41.80	0.49	2.08	19.31
Year Present Locations	74	509	24.71	6.48	41.55	0.49	2.23	18.85
Year Random Locations	74	509	14.24	11.02	36.30	0.50	1.90	14.98
Shed Present	76	194	29.94	7.22	42.54	0.44	2.52	17.14
Shed Random	76	194	19.54	10.40	39.27	0.49	1.85	14.25
Active Present	46	288	23.51	6.06	42.35	0.52	2.21	20.07
Active Random	46	288	12.09	11.36	35.85	0.51	2.02	15.30

	n (ind)	*n* (loc)	RockDist	RockCover	Otree	Utree	Aspect	AspectCOS
SFD Absent	55	376	3.46	20.74	2.01	1.86	173.60	-0.36
SFD Present	12	133	4.34	13.21	1.94	1.78	194.51	-0.32
Female	25	170	2.94	25.37	1.88	1.94	175.70	-0.50
Gravid	3	12	0.61	61.25	1.99	2.28	197.50	-0.82
Male	37	325	4.22	13.46	2.04	1.77	179.82	-0.26
Year Present Locations	74	509	3.69	18.64	1.99	1.84	179.21	-0.35
Year Random Locations	74	509	3.53	9.49	2.14	1.59	181.62	-0.28
Shed Present	76	194	3.09	24.18	2.31	2.20	188.54	-0.48
Shed Random	76	194	3.33	8.67	3.15	1.68	177.01	-0.40
Active Present	46	288	4.04	14.61	1.96	1.69	171.59	-0.24
Active Random	46	288	3.64	9.95	1.48	1.50	184.71	-0.16

Table 3.3. Classification trees (CART) for each model evaluated for Timber Rattlesnakes (*Crotalus horridus*) radio-tracked 2010-2013, based on habitat selection variation caused by SFD presence and gender and selected sites compared to paired random not used sites with regards to annual, shed season and active season variation.

Model	Leaves (using 1-SE rule)	Kappa	Correct Classification Rate	P	Important Variables
SFD/Sex	node only	0	0	N/A	N/A
Yearly	6	0.417	70.95	<0.01	Canopy, Slope, Rock Cover, VegCover, Otree
Shed	6	0.484	74.52	<0.01	Canopy, Slope,, RockCover, RockDist,Aspect
Active	7	0.473	73.69	<0.01	Canopy, Slope, Otree, VegCover,

Table 3.4. Paired logistic regression model results using stepwise AIC model selection for yearly and seasonal habitat use.

Yearly Logistic Regression Model	AIC	Deviance	DF
Canopy+Slope+VegCover+VegHeight+LogCover+RockDist+RockCover+Aspect	319.67	303.67	364
Canopy+Slope+VegCover+VegHeight+LogDist+LogCover+RockDist+RockCover+Aspect	320.72	302.72	363
Canopy+Slope+VegCover+VegHeight+LogDist+LogCover+RockDist+RockCover+Utree+Aspect	322.14	302.14	362
Canopy+Slope+VegCover+VegHeight+LogDist+LogCover+RockDist+RockCover+Otree+Utree+Aspect	324.11	302.11	361
Shed Season Logistic Regression Model			
Canopy+Slope+VegCover+VegHeight+LogCover+RockDist+RockCover+Utree+Aspect	125.33	107.53	155
Canopy+Slope+VegCover+VegHeight+LogDist+LogCover+RockDist+RockCover+Utree+Aspect	126.52	106.52	154
Canopy+Slope+VegCover+VegHeight+LogDist+LogCover+RockDist+RockCover+Otree+Utree+Aspect	128.27	106.27	153
Active Season Logistic Regression Model			
Canopy+Slope+VegCover+VegHeight+LogCover+RockDist+RockCover+Otree+Utree+Aspect	150.71	130.71	171
Canopy+Slope+VegCover+VegHeight+LogDist+LogCover+RockDist+RockCover+Otree+Utree+Aspect	152.52	130.52	170

Table 3.5. Parameter estimates for difference between paired used and not used timber rattlesnakes using paired logistic regression. Sign of parameter estimate reflects the relationship of probability of snake presence to the variable.

Yearly					Residual Deviance
	Estimate	SE	z	*P*	303.67
Canopy	0.05	0.01	4.86	<0.00001	
Slope	-0.14	0.02	-7.04	<1.0E-12	
VegCover	0.01	0.00	2.93	0.003	
VegHeight	-0.95	0.36	-2.66	0.008	
LogCover	0.03	0.01	3.42	<0.001	
RockDist	0.10	0.05	2.19	0.028	
RockCover	0.03	0.01	4.05	<0.001	
Aspect	-0.51	0.22	-2.38	0.018	

Shed Season					
	Estimate	SE	z	*P*	107.53
Canopy	0.03	0.02	2.17	0.030	
Slope	-0.13	0.03	-4.13	<0.0001	
VegCover	0.02	0.01	2.82	0.005	
VegHeight	-2.30	0.73	-3.16	0.002	
LogCover	0.04	0.01	3.04	0.002	
RockDist	0.17	0.09	1.88	0.060	
RockCover	0.05	0.01	3.54	<0.0001	
Utree	0.57	0.17	3.31	<0.001	
Aspect	-0.96	0.39	-2.48	0.013	

Active Season					
	Estimate	SE	z	*P*	130.71
Canopy	0.08	0.02	3.68	<0.001	
Slope	-0.17	0.04	-4.54	<0.00001	
VegCover	0.01	0.01	1.06	0.288	
VegHeight	-0.76	0.61	-1.25	0.211	
LogCover	0.04	0.02	1.97	0.049	
RockDist	0.09	0.08	1.07	0.285	
RockCover	0.01	0.01	0.78	0.433	
Otree	0.24	0.19	1.29	0.201	
Utree	-0.25	0.15	-1.67	0.094	
Aspect	-0.23	0.35	-0.66	0.509	

Figure 3.1. Yearly present and absent (paired random point) points of habitat use in Timber Rattlesnakes, excluding hibernation locations, using CART.

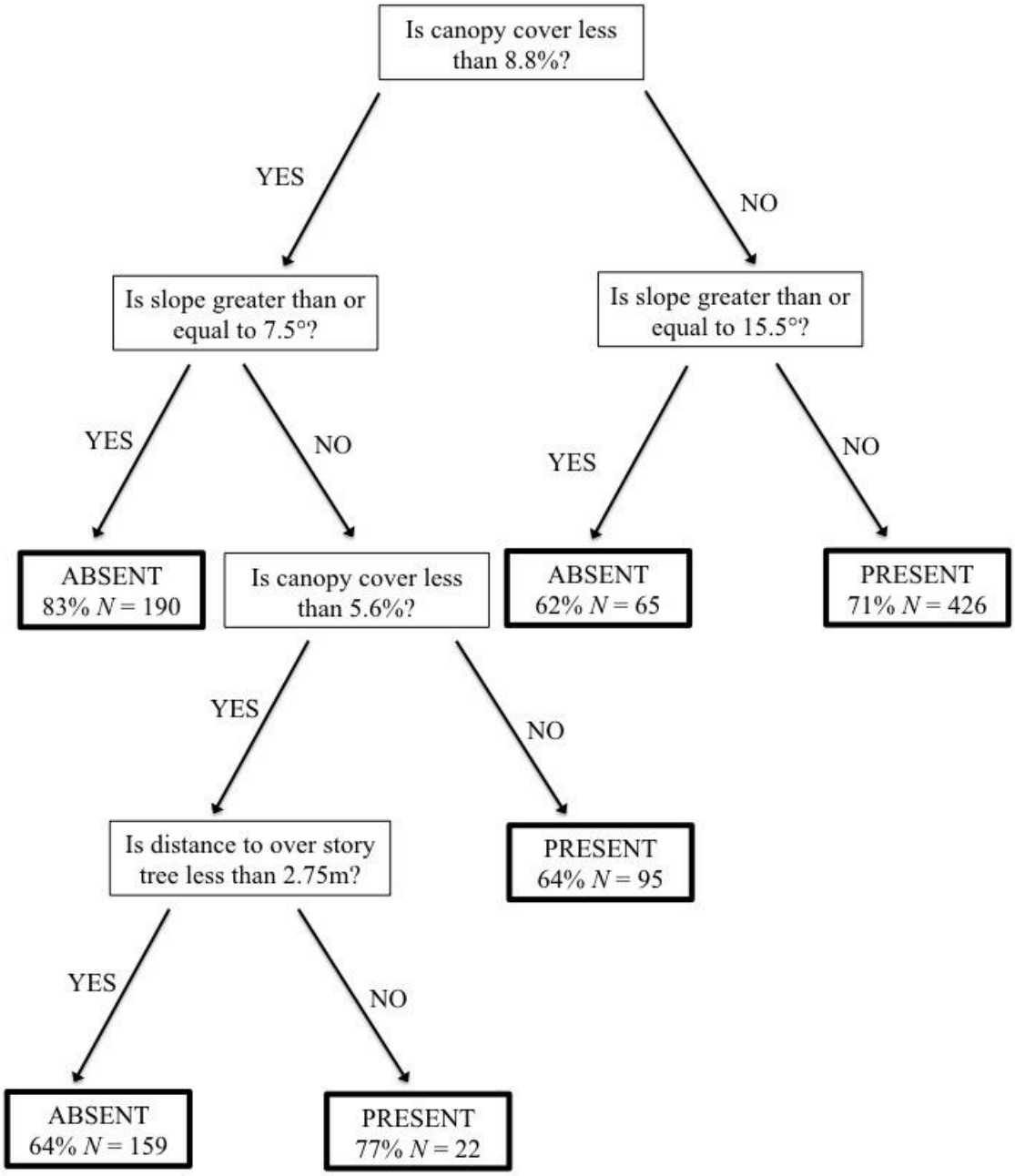


Figure 3.2. Shedding season present and absent points of habitat use in Timber Rattlesnakes, excluding hibernation locations, using CART.

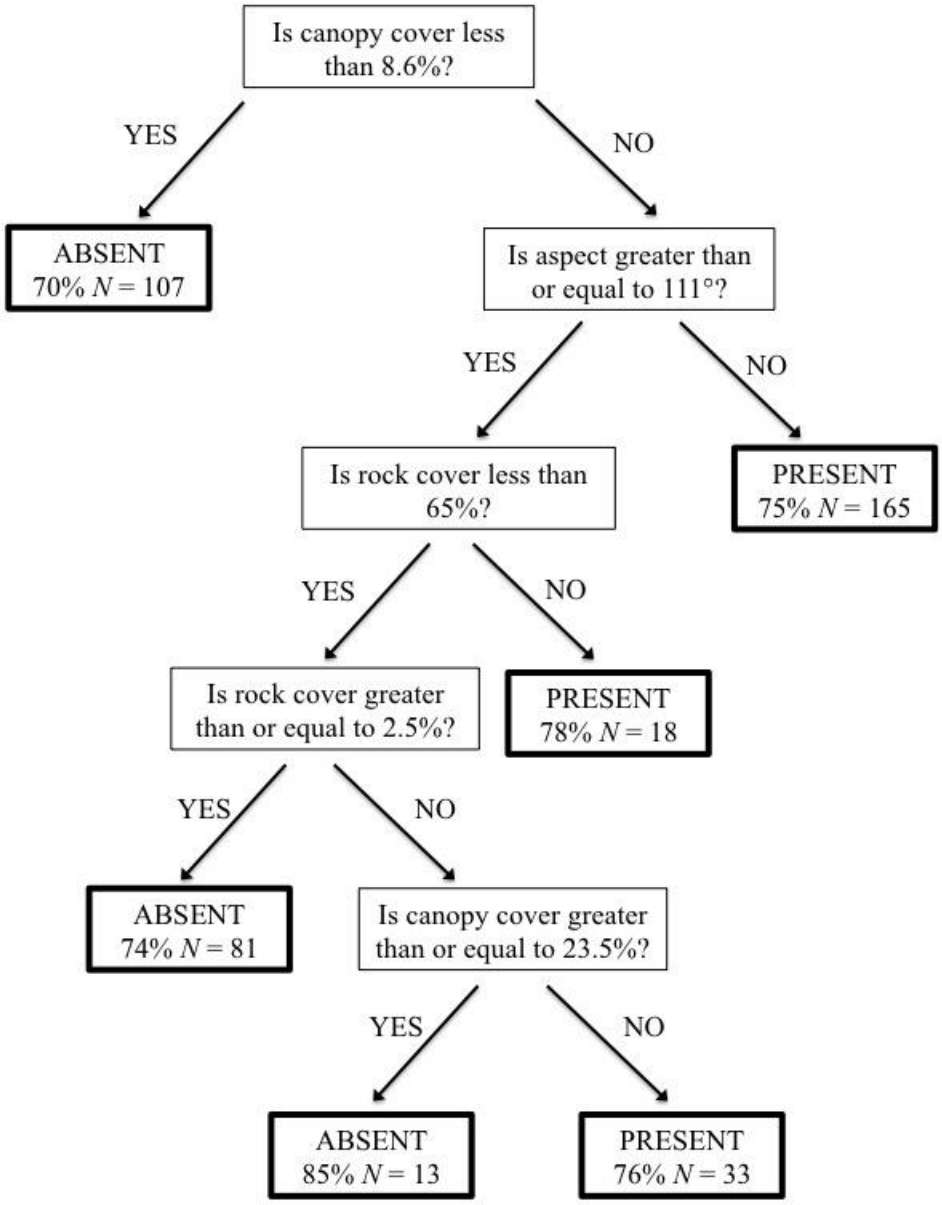
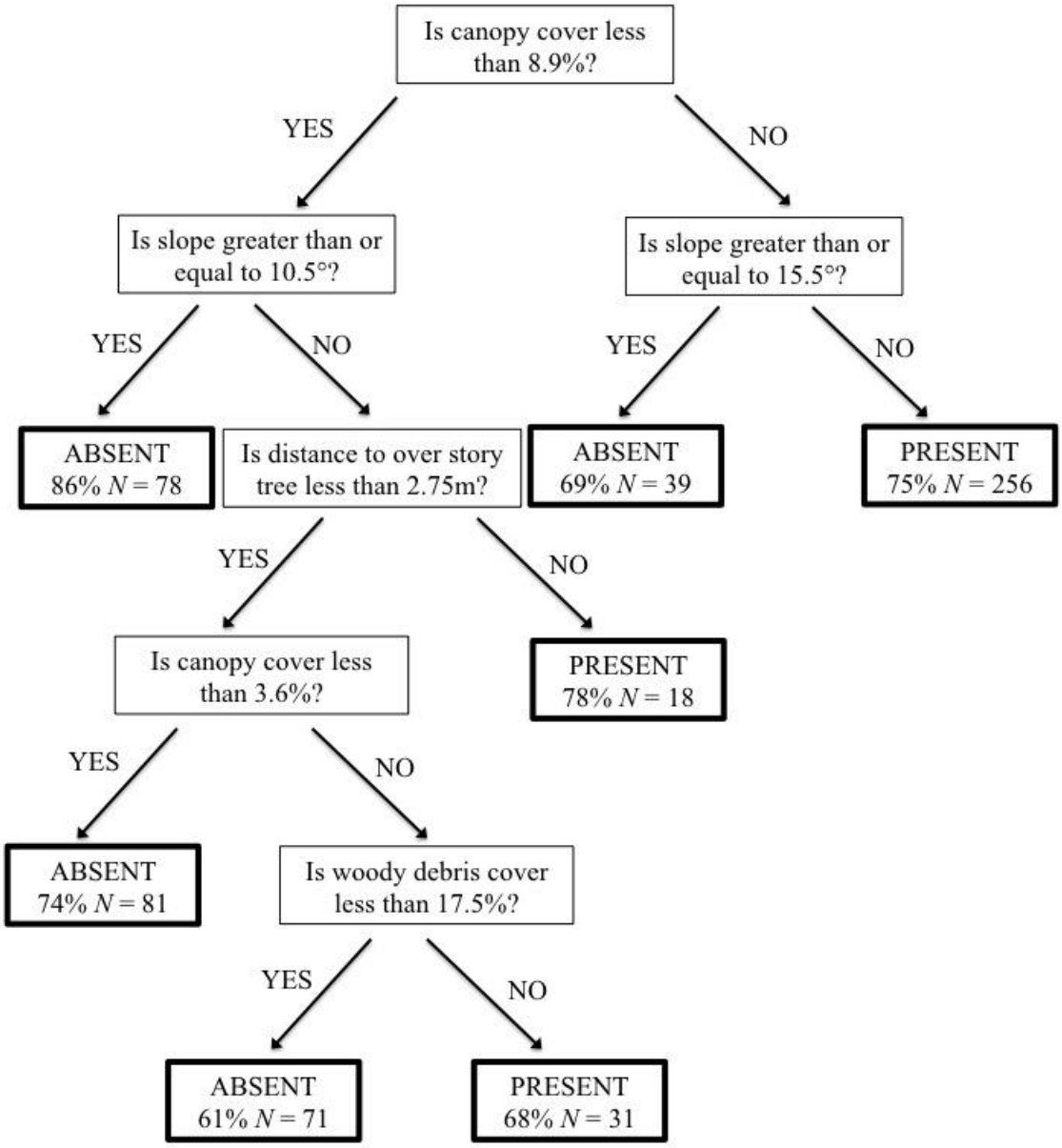


Figure 3.3. Active season present and absent points of habitat use in Timber Rattlesnakes, excluding hibernation locations, using CART.



CHAPTER 4

POPULATION GENETICS OF NORTHEASTERN TIMBER RATTLESNAKE POPULATIONS

4.1 Abstract

Understanding how genetic variation is distributed within and among populations of a species is widely used to make conservation management recommendations. Peripheral populations often have lower genetic diversity than core populations and may benefit from artificial gene flow for future population persistence. Using 13 microsatellite loci I quantified genetic diversity in 16 peripheral Timber Rattlesnake (*Crotalus horridus*) populations in the northeast, including all of the ten populations in New England. Most of these populations are all within the geographic periphery of the range, with some in the core area of the range in eastern New York and the Appalachian Mountains. Populations were highly differentiated from each other (mean F_{ST} = 0.175). There was no correlation between genetic distance and geographic distance (R = -0.0878, P = 0.67). Seven clusters of individuals were identified (K = 7), with each cluster corresponding to a geographic region. This finding suggests that genetic drift has led to population differentiation, and likely overwhelmed natural selection. Within the largest New England population, there appears to be a metapopulation structure, with gene flow among nearby den regions. For future population persistence, assisted gene flow or 'genetic rescue' might provide a viable management action for the most-at-risk populations. If assisted gene flow were to be implemented, results presented here should

serve as a guide for determining which populations are genetically diverse and large enough to serve as the best donor populations and which are most imperiled.

4.2 Introduction

Small and isolated populations are often susceptible to increased rates of extirpation (Blows and Hoffman, 2005). Genetic exchange among populations is required to maintain genetic diversity within subpopulations, along with evolutionary potential, or a population's ability to evolutionarily respond to a changing environment (Wright, 1969; Slatkin, 1987). The rate of gene flow needed for diversity can be relatively low, as little as one migrant per generation in stable populations (Speith, 1974; Mills and Allendorf, 1996). Genetic exchange lowers the risk of inbreeding depression, defined as a decrease in individual fitness that occurs with matings among close relatives (Keller and Waller, 2002; Savage and Zamudio, 2011; Spielman et al. 2004). Inbreeding depression is most likely to occur in small, isolated populations and can be associated with reduced population viability (Keller and Waller, 2002). In this situation a stochastic event, such as introduction of a new disease pathogen, habitat loss, or climate change, could extirpate the population. This leads to an extinction vortex, where these negative effects work in a feedback loop in small populations, further reducing the population size (Brooks et. al., 2008).

Within core areas of a species' range, genetic diversity is often high and stable (Hoffman and Blows, 1994; Hampe and Petit, 2005) whereas peripheral populations are often isolated from each other and can be highly genetically divergent, exhibit lower genetic diversity, resulting from small effective population sizes and strong genetic drift (Safriel et al., 1994). In the periphery, the lower evolutionary potential, the ability of a population or

species to adapt to a future changing environment, increases the chance of extirpation due to stochastic events (Blows and Hoffman, 2005). However, these geographically proximate populations can be highly genetically divergent (Eckert et. al., 2008; Safriel et. al., 1994). Historically, when these peripheral populations were connected, their genetic pattern would likely have been similar to the populations in the core range. Isolated, peripheral populations present a conservation dilemma. One argument has been that isolated and genetically distinct populations should be protected as separate units and treated as separate MUs to avoid the risk of outbreeding depression, because the genetic differentiation among populations could be due to local adaptations. (Templeton, 1986; Thornhill, 1993). Alternatively, because isolated peripheral populations are more prone to genetic drift, observed differentiation is unlikely to be the result of local adaptation (Vucetich and Walte, 2003). Protecting these isolated population units individually will only continue to decrease genetic variation, as the effects of drift increase within each populations, a dilemma which has been referred to as 'managing drift' (Coleman et al., 2013).

Defining the population genetic structure of a species across its range can help guide conservation management (Ryder, 1986). This approach can be used to identify small and isolated populations. It can also be used to define conservation units below the species level (Moritz, 1994). Waples (1991) described these within-species units as evolutionarily significant units (ESUs) as population units reproductively isolated from other conspecific population units and representing important evolutionary legacies of the species, with evolutionary legacy referring to adaptive divergence. These geographic groups are recognized by the Federal Endangered Species Act, and are referred to as Distinct Population Segments (DPS) (USFW and NOAA, 1996). Within a DPS, there can be smaller groups of

populations, or Management Units (MU), which are groups of geographically nearby populations that exhibit gene flow among them (Mortiz, 1994; Palsboll et. al., 2006). Management Units do not differ in local adaptations, as compared to ESUs (Mortiz, 1994) but MUs are distinct different genetic groups of populations.

Genetically diverse systems of populations increase the probability of species persistence. Many species exhibit metapopulation structures, defined as a system of subpopulations that are geographically in close by proximity and are connected by gene flow (Levins, 1970). Metapopulations tend to have higher resilience than large continuous genetically homogenous populations (Schindler et al., 2010). However, anthropogenic change can quickly isolate subpopulations within a metapopulation. Extirpation of these subpopulations can happen quickly after isolation, revealing the playing out of extinction vortices (Palomares, et. al., 2012).

It is important to ensure that peripheral populations maintain adaptive potential (Safriel, et. al., 1994). Populations are often stable in the central core of the species' range however, future climate change conditions could threaten the peripheral areas, already at risk from isolation, small population size and inbreeding depression. One management option that could help alleviate inbreeding depression and maintain adaptive potential in peripheral populations is genetic rescue, a form of assisted gene flow where individuals are translocated into an isolated population in an effort to restore fitness and genetic diversity (Madsen, et. al., 1999). Genetic rescue appears to have beneficial fitness effects even when source and recipient populations have elevated levels of inbreeding (Heber, et. al., 2012). In the absence of attempts to restore gene flow, remaining isolated populations might become extinct even if

other non-gene flow management plans (e.g. habitat remediation) are implemented (Coleman, et. al., 2013).

Most species in the northeastern United States first colonized the region after the end of the Wisconsin ice age during the Pleistocene, 11,700 years ago. Many species in this area are at their range peripheries (DeGraaf, 1983; DeGraaf and Yamasaki, 2000). With historical species range expansion, which occurred after the end of the Wisconsin Ice Age, approximately 10,000 years ago, along with more recent anthropogenic fragmentation, many small and fragmented populations occur. Among reptiles the Timber Rattlesnake provides an example of this pattern. Its range covers most of the eastern United States, and there are genetically and phenologically distinct units within different geographical and environmental regions (Clark, et. at. 2003). The core of this species' range extends from Tennessee to Pennsylvania. The northeastern region of the species range is on the periphery of the species range (Brown, 1993). These peripheral populations tend to have low snake abundances in small populations distant from one another (Brown, 1993). In Timber Rattlesnake populations, population subdivision is associated with the den sites (Clark, et. al., 2008). Subpopulations tend to correspond to single den sites (Clark, et. al., 2008). Core areas (e.g. in Pennsylvania) tend to have high within-population genetic diversity and exhibit metapopulation structures associated with the number and spatial proximity of den sites (Bushar, et. al., 2015). In the Northeastern US, small and geographically distant populations tend to contain only one den site. These populations could be both genetically depauperate and highly genetically divergent. Isolated peripheral populations in the northeast often do not have enough non-fragmented habit available to sustain a metapopulation, with individuals from the different hibernacula not likely to overlap spatially during the early active season.

Without enough habitat available some small populations exist with only a single den site. These isolated populations are at greater risk of extirpation due to genetic drift and inbreeding depression (Wright, et. al., 2007).

In this study, I used microsatellite loci to examine the genetic structures of 16 northeastern Timber Rattlesnake populations from West Virginia to Vermont. I examined within-population genetic diversity and among populations. I estimated the potential risk of future inbreeding by estimating of the effective number of breeders and genetic distinctiveness. Within one subset of nearby populations for which demographic analyses have provided evidence of connectivity, I tested for evidence of metapopulation genetic structure. These results will determine whether the remaining northeastern populations should be managed as a single genetic unit (Martin, 2008), or as distinct genetic units that should be managed separately.

4.3 Materials and Methods

4.3.1 Sample Collection

A total of 1,020 tissue samples were collected from 33 sites, including all ten remaining New England populations along with several populations in adjacent eastern New York, Virginia, Kansas and Pennsylvania in conjunction with other research efforts (from 2009-2014). Samples of extirpated populations were included when available (Fig 4.1, Table 4.1) including samples from historic Massachusetts' collections (1880-2007) Populations with low sample sizes ($n < 12$) were excluded from analyses, to prevent bias due to low sample size, specifically with regards to allelic richness (Leberg, 2002), resulting in 16 populations, that were used in statistical analyses. Populations are abbreviated by the first

letter of the state, followed by the first three letters of the county name. If there were multiple populations in a county, a number was added (Table 4.1). Further location specificity of sites is not provided to protect each population from poaching. Tissue samples, in the form of 2 mm scale clips, were collected from each individual. The tissues were either stored in 95% ethanol or dried. Shed skins found opportunistically were also used in analyses. Larger muscle tissue samples were collected from individuals (n = 8), previously collected and stored frozen, from Massachusetts Wildlife (Westborough, Massachusetts). The Museum of Comparative Zoology (MCZ), Harvard, Massachusetts, provided 22 samples stored in ethanol.

Samples were collected in collaboration with other projects including the following: Virginia (courtesy of W. H. Martin), Kansas, (courtesy of George Pisani), Massachusetts (courtesy of Tom Tyning, Kay Sadighi, Bill Hoffman, John Corey and Brett Trowbridge), New York (courtesy of Randy Stechert, Ed McGowan, William Brown, Ted Levin, and Matt Simon), Connecticut (courtesy of Dennis Quinn and Bob Fritsch) and Vermont (courtesy of Doug Blodgett and Kiley Briggs), with additional New England samples provided by Roger Williams Park Zoo (RWPZ), Providence, Rhode Island, in conjunction with their New England Timber Rattlesnake Health Survey, 2012-2014 (courtesy of Mike McBride). In 2012 and 2013, four individuals were located where there is no known population in Rockingham County, New Hampshire, and treated as a separate unknown population (UNK). Thirty-four New Hampshire samples used by Clark, et. al. (2011) were provided (courtesy of Mike Marchand, New Hampshire Fish and Game Commission). Their study yielded 20 usable samples of the 34, and here I was able to amplify 29 of them. From 2011-2012, New Hampshire provided an additional 44 samples from their population in conjunction with an

ongoing study. Thirty samples were from the Adirondacks, New York, (courtesy of Matt Simon and William Brown), the same population also analyzed by Clark, et. al. (2008). The samples used here were collected after Clark, et. al. (2008) study, so it is unknown if any of the same individuals were sampled.

4.3.2 DNA Amplification and Microsatellite Analyses

The DNA extraction protocol followed King, et. al., (2005), with tissue samples incubated for 24 hr and shed skin samples for 48 hr prior to extraction, in cell lyses at 55°C. The eluted product was used as a template in Polymerase Chain Reactions (PCR). Seventeen microsatellites previously described for this species were amplified: Scu01, Scu05, Scu07, Scu11, Scu16, and Scu26 (Anderson 2006), C*w*A29, C*w*B6, C*w*B23, C*w*C24, and C*w*D15 (Holycross et al. 2002), and 5A, 7-150, 5-183, 7-144, 7-87, and 3-155 (Vilarreal et al. 1996). PCR reactions were performed in 10 μL reaction volumes using 5 μL Qiagen multiplex buffer, 1μL of primer mix, and 4 μL of 50:50 dilution of extracted DNA product. The PCR temperature profile was 95°C for 5 min; 32 cycles of 94°C for 30 sec, 57°C, and 72°C for 1 min; followed by a final 30 min extension at 60°C. An Applied Biosystems 3130xl capillary sequencer was used to amplify 1μL of PCR product. Allele sizes were initially recorded using GENEMAPPER 3.0 (Applied Biosystems), and confirmed with GENEIOUS (Biomatters Limited). Individuals missing more than 50% of loci, after re-extraction and re-amplification, were excluded from analyses. Of historic samples (n = 22), only 5 yielded usable genotypes. Samples stored prior to 1880 (n = 2) and after 1988 (n = 3) amplified well, while the remaining samples did not amplify due to prolonged storage in formalin.

4.3.3 Within-Population Diversity

Duplicate genotypes were possible if a shed skin was collected from an individual that was previously or subsequently scale clipped at a different date. I used GIMLET (Valiere, 2003) to identify duplicate multi-locus genotypes that possibly represented samples taken from the same individual. Collection notes regarding age, sex, color phase, and location, were used to determine whether duplicate genotypes likely represented the same individual. When multiple samples were available from one litter of neonates, one individual was randomly chosen to represent the entire litter, to avoid sibling bias (Rodriguez-Ramilo, 2012). When multiple neonate sheds were collected from the same communal rookery, COLONY 1.2 (Wang, 2004) was used to estimate the number of litters.

F_{IS} was calculated for each locus in each population to test for conformation to Hardy-Weinberg equilibrium proportions using GENEPOP 4.0.10 (Rousset, 2008). Loci repeatedly in violation of Hardy-Weinberg proportions across more than half of populations were excluded from further analyses. GENODIVE 2.0b22 (Meirmans and Van Tienderen, 2004) was used to estimate the following allele frequencies: (1.) observed heterozygosity (H_O), (2.) expected heterozygosity (H_E), and (3.) mean within-population expected heterozygosity (H_S) per locus and per population. Allelic richness (mean number of alleles scaled to the smallest sample size, $n = 12$), F_{IS}, and F_{ST} were estimated using FSTAT 2.9.3.2 (Goudet, 2001).

Effective number of breeders (N_b) was calculated with LDNE 1.31 (Waples and Do, 2008). This program gives an estimate for effective population size (N_e), but with species with overlapping generations N_b is often lower than N_e (Waples, et. al., 2013). Estimates obtained from mixed-aged samples are more correctly referred to as estimates of the effective

number of breeders (N_b) that gave rise to the cohorts included in a population sample, rather than effective population size per generation (Luikart, et. al., 2010), and are reported as such. A random mating model was used, with a minimum allele frequency cutoff (P_{crit}) of 0.02, which tends to provide a balance between bias and precision (Waples and Do, 2008). The jackknife approach was used to calculate 95% confidence intervals.

4.3.4 Among-Population Diversity

Pairwise F_{ST} and F'_{ST} values were calculated across all paired sampled populations for sample sizes greater than or equal to 12 with GENODIVE 2.0b22. Population genetic distinctiveness (mean population-specific F_{ST}, akin to "genetic uniqueness"; cF Coleman, et. al., 2013) was assessed by plotting, for each locus, the mean population-specific F_{ST} against allelic richness, expected heterozygosity, and mean number of alleles. Migration among populations is unlikely given the geographic distances among them, therefore F_{ST} coalescence analysis was not applied. A negative relationship between genetic distinctiveness parameters and mean population-specific F_{ST} would indicate that populations with the lowest genetic diversities are also the populations with greatest mean genetic divergence from other populations, indicating genetic drift is largely responsible for the observed divergence. The relationship between genetic distance (population pairwise F_{ST}) and great circle geographic distance (isolation by distance, IBD) was analyzed with a Mantel test, using R version 3.1.3 (R Development Core Team, 2015). Pairwise F_{ST} was transformed as $F_{ST}/(1- F_{ST})$ (Slatkin, et. al. 1995), and geographic distances (km) were log transformed.

Geographic groupings of populations were analyzed with the Bayesian modeling approach implemented by STRUCTURE 2.3.1 (Pritchard, et. al., 2000), and discriminant

analysis of principal components (DAPC; Jombart, 2008) in populations with a sample size $N \geq 12$. Both the STRUCTURE and DAPC software packages were used because STRUCTURE can fail to identify complex spatial structures (Schwartz, et. al., 2007). To estimate the number of population clusters (*K*) with the highest log likelihood STRUCTURE analyses used 100,000 replicates, with 50,000 burn-in cycles. An admixture model was used, with correlated allele frequencies. Ten runs were performed for $K = 1$ to $K = 20$, the maximum number of populations sampled. Analysis did not include a prior location of origin for each individual, so analyses were not biased due to geographic location of the individual. I used STRUCTURE HARVESTER (Earl and von Hold, 2012) to visualize STRUCTURE results and I inferred the number of clusters (*K*) based on interpretation of the relationship of estimated log probability of the data (LnP(D)) with *K*. The program CLUMPP (Jakobsson and Rosenberg, 2007) was used to achieve permutations of all 10 iterations for each *K*, using the "greedy" algorithm. The program DISTRUCT version 1.1 (Rosenberg, 2004) was used to create bar plots for each value of *K*. Four individuals an unknown site in NH were included in STRUCTURE analyses, even though this 'population' has a sample size below the minimum cut off, because their population of origin is unknown. Genetically divergent populations with few representatives included in a STRUCTURE analysis can cause misleading results, however, in this case we suspected that the four unknown individuals originated from other wild populations I included in the analysis. There is no known population in Merimack County where the individuals were found, and snakes have only been observed in this area in 2012 and 2013. The state of New Hampshire also has individuals held in captivity from West Virginia and Pennsylvania (W.H. Martin, pers comm). I used assignment tests to further test for the origin of the four unknown individuals

from New Hampshire. I used GENECLASS version 1.0.02 (Piry, et al. 2004) to conduct assignment tests, using methods described by Rannala and Mountain (1997). All sampled populations were used as references, treating the individuals as unknown. Probability computation was not enabled, giving an assignment score for each individual.

Genetic clustering by DAPC used successive *K*-means clusters in the *find.cluster* function with the R package *adegenet* (Jombart et al. 2010). The *K* with the lowest Bayesian Information Criterion (BIC) was considered the optimal number of clusters, using $K = 1$ — 30, with ten runs for each *K*. The *dapc* function was executed using optimal grouping, retaining the Principal Components Analysis axes and explaining >90% of variation in the data.

Radiotelemetry data (cF Chap 2) suggest the largest Massachusetts (MABER) population could be a metapopulation. Individuals from dens closer together overlapped spatially during the active season. Individuals at dens at a greater distance apart did not show spatial overlap during the active seasons, however all den sites are within the maximum known distance (7.2 km) traveled by other Timber Rattlesnake individuals in other northeastern regions (Brown, 1993). The telemetry data indicate that four possible subpopulations, with nine den sites total, are involved. Six of the den sites are all located within 1.22 km of each other, and movements of individuals here overlapped spatially to a large extent. Individuals from the other three dens did not overlap spatially with individuals from the remaining six dens. These four putative subpopulations, the group of six proximate dens, and three more distant dens, had distances ranging from 3.17 – 9.71 km (mean = 6.51 km) between dens, with only low traffic (some dirt) roads and valleys separating them. I

examined individuals sampled in this sub-region separately with STRUCTUE, using the above parameters and tested $K = 1$ to $K = 9$. No location prior was included in analysis.

4.4 Results

4.4.1 DNA Amplification

Eight samples were excluded as duplicates across three populations, MABER ($n = 5$), MANOR ($n = 1$), MAHAD1 ($n = 2$). These were likely to be from the same individuals of each site, because they were all sheds matching a tissue sample from the same site, and only one genotype from that individual was included Amplification from shed skins of all samples was successful in 398 of 400 cases (99.50%).

Over all populations, an estimated seven litters were sampled across four populations (CTHAR, MANOR, MAHAD1, MAHAM). Timber Rattlesnakes in the northeast give birth to litters at communal sites, birthing rookeries, near the den, therefore if only the sheds of the neonates are found, they could be from multiple litters. Litter and mother identity (based on field observations) for three litters was known, as they were birthed in captivity at RWPZ, from the populations MANOR (2014) and two from MAHAM (2011 & 2013). Of the other four litters birthed in the wild in populations CTHAR and MAHAD1, both were estimated to be 2 litters per population. I retained one randomly selected individual per litter for subsequent analyses. After removing these individuals, 956 individuals remained.

4.4.2 Within-Population Diversity

Four loci (C*w*A29, CwB6, CwC24 and Scu16) were excluded due to inconsistent success in amplification and strong evidence of violation of Hardy Weinberg proportions,

primarily because the presence of null alleles was likely, resulting with 13 loci for analyses. F_{IS} testing of the remaining loci for conformation to Hardy-Weinberg equilibrium resulted in 364 tests, with 127 significant ($P < 0.05$), with 18 expected by chance ($\alpha = 0.05$). After table wide Bonferroni correction ($\alpha = 0.05$), 31 tests remained significant, but were not eliminated because this is often seen in northeastern populations of this species, and eliminating these have previously not yielded a difference in results (Bushar, et. al., 2014). Significant linkage disequilibrium was detected in 121 of 1091 (11.1%) tests performed ($P < 0.05$), where 54.5 were expected by chance ($\alpha = 0.05$). Following table wide Bonferroni correction, 18 of the tests remained significant (Table 4.2). Average per population F_{IS} ranged from 0.094-0.217. Mean allelic richness (A_R) ranged from 1.9 - 5.8, scaled to $N = 12$. Average number of alleles per locus per population (A) ranged from 4.1 - 10.3. Effective number of breeders (N_b) varied greatly among populations, with point estimates ranging from 2.0 to 893.9 (Table 4.3). Two of the confidence intervals included infinity. Only two of the New England populations exhibited an N_b greater than 50 (MABER and VTRUT2). The population with the lowest N_b, NH, also exhibited the lowest genetic diversity.

4.4.3 Genetic Differentiation Among Populations

Pairwise F_{ST} estimates were significant for all population pairs (171 tests), based on Fisher's method. Overall F_{ST} was 0.173 (95 % CI 0.141 – 0.205). There were negative relationships between genetic diversity (A, H_E and A_R) and genetic distinctiveness (mean population-specific F_{ST}) and was not significant. For the regression of mean population-specific F_{ST} on mean number of alleles, R^2 =-0.416; $P = 0.068$. For the regression of mean population specific F_{ST} and mean heterozygosity, $R^2 = -0.789$; $P < 0.00001$. For the

regression of mean population specific F_{ST} and mean allelic richness, R^2 = -0.846; $P < 0.0001$ (Fig 4.2). Population pair-wise F_{ST} and F'_{ST} values are presented in table 4.4. There was no correlation between population pairwise genetic and geographic distances (Mantel test, R = -0.325, P = 0.951). Variance in genetic differentiation was particularly pronounced at small geographic distances, with the highest F_{ST} values pertaining to pairs of nearby populations. For example, the two closest pairs of populations (<15km) had some of the highest pairwise F_{ST} values (Table 4.5, Fig 4.3).

In the STRUCTURE analysis, K = 7 had the greatest support (Fig 4.4,4.5). LnP(D) increased with each increase in K value and plateaued after K = 8, and variance increased after K = 10. With K = 5, NH and MANOR each formed a separate cluster. VTRUT1, VTRUT2, and NYWAS clustered together, as did all the MABER subpopulations. At K = 5 and K = 6, MAHAD1 and MAHAD2 fell into separate clusters. At K = 7 VTRUT1, MAHAD1, and MAHAD2 fell into separate clusters. The clusters of VTRUT1, VTRUT2, and NYWAS correspond to three geographically near populations in western Vermont and northeastern New York. Other distinct clusters did not appear to have distinct geographic relationships.

For the DAPC analysis, examination of BIC did not provide a useful basis for inferring K. BIC continued to decrease with increasing K from K = 1 through 30 (Fig 4.6). For further DAPC analyses, K = 7 was used based on the STRUCTURE results. Results from DAPC were similar to STRUCTURE. The populations most distinct were the peripheral New England populations, NHNA, MANOR, MAHAD1, VTRUT1 and VTRUT2 (Fig 4.7), consistent with STRUCTURE results. In regions where populations were larger, (PACEN, VASHE, and most of New York) there was less evidence for population differentiation. The

New Hampshire population was the most genetically divergent with DAPC. This population also had the highest population specific F_{ST} values (mean F_{ST} = 0.401).

4.4.5 Assignment Testing

The four individuals from a previously unknown Rockingham County, New Hampshire region do not assign to the single known New Hampshire population. Three of the four were assigned to the central Pennsylvania population (assignment scores = 99.56, 99.88, and 94.98). The fourth individual was assigned to the Orange County New York population (assignment score = 96.29), which borders Pennsylvania (table 4.6). Subsequent scores for other populations ranged from 3.694 to 0.018, with none corresponding to the New Hampshire population or other geographically proximate populations.

4.4.4 Metapopulation Genetic Structure

253 individuals from the putative Massachusetts metapopulation (MABER), were sampled, from four potential subpopulations separated by valleys. Sample sizes were n = 124, 20, 28 and 79. One of these regions contained multiple dens. STRUCTURE results, run separately on these subpopulations, provided greatest support for K = 4 (Fig. 4.8,4.9). LnP(D) increased with each increase in K value and plateaued after K = 5; variance increased after K = 5 (Fig. 4.8). With K = 3, the most geographically proximate MABER2 and MABER3 were clustered together, with MABER1 and MABER4 formed different clusters. K = 4 separated MABER2 and MABER3. K = 5 did not provide additional biologically meaningful clustering.

Estimated pairwise F_{ST} (mean = 0.081, range: 0.042-0.123) indicated that there was some genetic differentiation among the four subpopulations, with the least differentiation between the two most geographically proximate subpopulations (F_{ST} = 0.042). MABER1 was the most genetically differentiated from other den regions. MABER4, the largest site, was the least genetically differentiated. Point estimates of N_b varied from 3.7 to 83.7 (Table 4.3).

4.5 Discussion

4.5.1 DNA Amplification

DNA amplification from shed skins was very successful. Contrary to Bricker et al. (1996), storing skins at -20°C -80°C, did not appear to be necessary. They reported a lower success rate (93.94%, *n* =33) than that achieved here (99.50%, *n* = 400) even though the current samples were stored at room temperature for up to 20 years. One shed skin, collected by a private individual, was found in a mud puddle and then washed with dish detergent. Despite this treatment the sample still yielded usable DNA. I incubated skin samples for 48 hours, much longer than the 30-minute incubation period of Bricker et al. (1996), and this probably led to more successful extraction.

4.5.2 Hardy-Weinberg and Effective Number of Breeders

Populations of Timber Rattlesnakes in the northeast often exhibit repeated Hardy-Weinberg heterozygote deficits (Villareal, et. al., 1996; Clark, et. al.; 2008, Bushar, et. al., 2014; Bushar, et. al., 2015). Deviations can be attributed to population substructure (Chakraborty and Jin, 1992), as seen with MABER, or may be attributed to the presence of null alleles (Callen, et. al., 1993). Population substructure occurs within some Timber

Rattlesnake populations (Clark, et. al., 2008). If deviations were solely due to population substructure, the majority of loci within a population would also deviate. Bushar, et. al. (2014) performed analyses with their original data set, and with loci adjusted for possible null alleles. Results from both of their analyses were similar, and these authors chose to include deviating loci in final analyses.

All loci were polymorphic, though the 7-150 locus was fixed for a single allele with the exception of Kansas samples and one Virginia sample. This result is consistent with most other northeastern population genetic studies (Bushar, et. al. 1998). Villareal, et. al. (1996) found the locus to be polymorphic (A = 2) in eastern Pennsylvania (Berks County). I did not obtain samples in this area of Pennsylvania studied by Villareal, et. al. (1996), and it was monomorphic in the central Pennsylvania population sampled here. This locus was found to be polymorphic in Missouri, although low in allelic diversity compared to other loci (Anderson, 2010). This suggests this locus has an allele that is rare in the northeast but more common in western populations.

Effective number of breeders overall was relatively low for most populations. NHNA and MAHAM have effective numbers of breeders of less than three. NHNA appears to have low relative genetic diversity, and MAHAM currently has fewer than 5 known individuals in the population. NYWAS is believed to be one of the largest populations in New York, and only has an N_b of 18.8 (CI: 12.7-30.3). This population exhibits only black morphs, instead of both color morphs, yellow and black. The only other population exhibiting one color is NHNA, and Clark, et. al. (2011) suggested this could be correlated with inbreeding and an increase in homozygosity. NYWAS has never been studied, so age structure and sex ratio are unknown, and if skewed, could cause a lower than expected N_b compared to census size.

NWAR is also a large population, with an N_b estimate of 893 with a very broad CI (45.9-INF). Large estimates of N_b are often imprecise unless sample size approaches the true N_b (Ackerman, et. al. 2016). This population was more intensively sampled by Clark et al. (2008). These authors used a coalescent-based estimator (program MIGRATE) on individuals pooled across subpopulations to obtain effective size of 796 (CI: 452-1,020). It is difficult to directly compare contemporary mixed-aged estimates of N_b with coalescent-based N_e , but both estimates are consistent with large effective size in this population. The four subpopulations in MABER had an N_b ranging from 3.7-83.7. MABER4 was the largest subpopulation sampled, with an N_b of 83.7. MABER3 had the lowest N_b, 3.7, and is skewed male, 80:20 (cF Chap 1), which is consistent with a low N_b if there are significantly fewer females than males.

Franklin (1998) suggested that an effective size of 50 is needed for short-term population persistence, and 500 for long-term range wide persistence and retention of evolutionary potential. These guidelines, though heavily debated (Frankham, et. al. 2014, Allendorf and Jamieson, 2012), provide a useful starting point when ranking populations that may need more intensive management for future persistence. I did not estimate global N_e, to which the value of 500 applies. However, the single-sample N_b estimates can be compared to the value of 50, although N_b are often lower then N_e and are often biased low in samples with mixed age groups (Waples, 2014), therefore the cut-off of 50 should not be applied here as an absolute rule. As sample size approaches or exceeds the true value of N_b, the estimate for N_b, is more accurate (Ackerman, et. al. 2016). With this species in the northeast only approximately a third of the population successfully breeds each year on average (Brown, 1993), therefore with the sample sizes provided with this study, the estimates of N_b provided

here should represent true $N_{b.}$ Seven populations have effective number of breeders of less than 50.

4.5.3. Genetic Differentiation Among All Populations

Results of this analysis here suggest that differentiation among northeastern (New England and New York) populations is the result of genetic drift rather than local adaptation. Populations exhibited high population distinctiveness, as estimated with methods described by Coleman, et. al. (2013). The negative relationship between genetic distinctiveness (population-specific pairwise F_{ST}) and genetic diversity indicates that more distinct populations have lower genetic diversity, with fewer unique alleles (Coleman, et. al., 2013). The lack of isolation by distance was also consistent with strong genetic drift being responsible for population differentiation. There was no isolation by distance (IBD) relationship seen here among populations, despite some populations being geographically close enough for possible gene flow (<15 km, 3 pairs). Peripheral populations appeared to be most prone to genetic drift. Both STRUCTURE and DAPC revealed strong single population clusters of the most peripheral populations,, with greater interpopulation clustering of those closer to the core range. Some population pairwise F_{ST} values were high, with the geographically closest population pair (MAHAD1 and MAHAM) having one of the highest values. This suggests that gene flow is overwhelmed by drift even in geographically nearby populations.

My results differ from those of previous population genetic studies of Timber Rattlesnakes (Clark, et. al., 2008; Bushar, et al., 1998; 2014; 2015; Anderson, 2010). These previous studies have found evidence for IBD, strong K clustering and high population

pairwise F_{ST} values of nearby populations. Highways, which are barriers to gene flow (Bushar, et. al. 2015; Clark, et. al. 2010; Andrews, 2008), separate all but one of the population pairs I examined. The lack of a positive correlation between population genetic differentiation and geographic distance contrasts with other Northeastern (New York, New Jersey and Pennslyvania) genetic studies of this species (Bushar, et. al. 2014; 1998; Clark, et. al. 2008). Genetic drift is less likely to have an effect in these areas, as they are larger populations closer to the core region where higher gene flow is likely.

The data reported here can be compared to past efforts to define conservation units for Timber Rattlesnakes. Martin, et. al. (2008) proposed five range-wide Timber Rattlesnake management units (MUs) based on life history traits, morphology, and genetic variation (Clark et al., 2003). MUs were designated based on habitat variation and natural geographical barriers. Martin, et. al.'s (2008) MUs are equivalent to more generally defined evolutionarily significant units (ESU). The definition of ESU used here is a group of populations separated geographically, genetically differentiated at neutral markers, and having locally adapted traits caused by natural selection (Waples, 1991; 2005). Martin (2008) defined geographical subunits, which are equivalent to the more typical definition of MUs, that is, demographically independent and geographically separated subpopulations (Palsboll et al., 2007). MUs tend to correspond to single subpopulations or sets of subpopulations with correlated demographic rates. The remaining New England populations are currently more genetically distinct from each other than those in Pennsylvania and New Jersey (Bushar, et al., 2014). My results suggest that each of the subpopulations in New England is likely a separate MU and New England might represent a single ESU. However, definition of an

ESU would require additional evidence on possible adaptive divergence from other portions of the species' range.

Martin, et. al. (2008) described New England as one subunit (more commonly, and subsequently referred to as an MU). The remaining New England populations are currently more genetically distinct from each other than those in Pennsylvania and New Jersey (Bushar, et. al., 2014) despite a smaller spatial scale, this finding does not support Martin, et. al.'s (2008) suggestion of a single New England MU. Historically however, there was likely a single MU when now extirpated populations provided connectivity among extant populations. In the past few centuries this species has declined by over 50% (Furman, 2007) and the creation of anthropogenic barriers impede gene flow. I suggest that anthropogenic barriers are increasing the effects of drift, relative to adaptive divergence; therefore these results do not contradict Martin, et. al.'s (2008) suggestion of a single historic New England MU. With peripheral Northeast populations so genetically distinct from each other, protecting each populations' genetic signature could lead to a decrease in overall genetic diversity for the region, as the effects of genetic drift will only increase in the future in these isolated populations (Coleman et. al. 2013). By protecting unique allele complexes resulting from drift and not natural selection, genotypes in future generations are limited to those produced by drift and this doesn't protect the genetic diversity of the region. Populations that were historically connected would benefit from artificially introducing new genotypes with strategies such as genetic rescue (Coleman et. al. 2013).

4.5.4. Genetic Differentiation and Diversity Among the Most Peripheral Populations

I identified populations most likely to be at risk from extirpation from small population effects such as inbreeding and loss of genetic diversity. NHNA was consistently an outlier, with respect to all tests of genetic diversity, and is most at risk for inbreeding depression. This population appears to have declined in abundance. Clark et. al. 2011 sampled only 19 individuals from 2006-2010. Clark et. al. (2011) report not seeing some individuals for six years between recaptures, and conclude a fungal disease is depleting the population. It is common not to see individuals for this length of time, or even 20 years between relocations with intensive survey efforts (Brown, pers comm). From 2010-2011, an additional 43 adult individuals were sampled. This could indicate that the population size is increasing, however, this is unlikely in a four-year time span for a species that takes 6-8 years to reach sexual maturity. Alternatively the population may have been stable through this period, but sampling efforts have become more efficient or environmental conditions more favorable after 2010. Clark et. al. (2011) report above average precipitation during their sampling period, which would result in lower sampling success. Clark et. al. (2011) suggested that coloration patterns seen only in this population indicated of a high homozygosity, although this coloration does not appear to have had direct effects on individual fitness. Low genetic diversity, reduced population size, and possible inbreeding effects led Clark et. al. (2011) to recommend genetic rescue, defined as an increase in absolute population fitness (i.e. population growth rate), due to the introduction of new alleles (Tallmon et. al. 2004; Whiteley et. al. 2015), for this population.

VTRUT2 and MAHAD1 were also genetically divergent, had low genetic diversity, and high genetic distinctiveness. These two populations may also be good candidates for genetic rescue. Nearby NYWAS might be the most suitable source of transplants for

VTRUT2, and MAHAD2 for MAHAD1, in terms of minimizing risks of outbreeding depression, defined as when the hybrid progeny have a lower fitness, due to local adaptation to different environments (Lynch and Walsh, 1998).

The MANOR population is geographically distant and even though I expected this population to be isolated from other sites, I observed high genetic diversity with the population's H_E 113.64% higher than average population H_E, and an AR 104.58% higher than average population AR. Since 1965, five known adult rattlesnakes were released in the MANOR population by the state of Massachusetts (Smith, N. pers comm.). The transfer of these five snakes represents an unmonitored and unreplicated form of genetic rescue. The five individuals were confiscated by the state from private citizens keeping them illegally as pets. This introduction could be responsible for the high genetic diversity observed in MANOR. The mean generation interval for this species is 18 years (Martin, 2002), so the interval from 1965 to 2007 when samples were collected represents 2.3 generations. MANOR appears to have lower abundance than most New England populations (cF Chap1); but it is possible that abundance is larger than perceived. Individuals at this location are less visible, hiding more in leaf in litter, than other New England populations (Stengle, unpubl data), thus making sampling more difficult. This population has the third highest effective population size in New England, suggesting this population could be larger than originally thought, and stable at the current size. Seven MANOR dens are known but there is no evidence to support a metapopulation structure based on movement patterns (Stengle, unpubl data). Individuals from different dens typically overlapped spatially, and the distances between den sites are shorter then the maximum distance moved by a Timber Rattlesnake during one year (Brown, 1993; Stengle, unpubl data). Surrounded by urbanization, the

population has only approximately 1.6 km^2 of available habitat, and has the highest number of individuals killed by automobiles in New England (Smith, N. pers comm.). Timber Rattlesnakes in the northeast often travel farther than 1.6 km in a given year, indicating this population is likely panmictic. Although this is a small population isolated by highways, the panmictic multiple den structure may be sustaining the population's genetic diversity, whereas in NH, the population may suffer from inbreeding depression, with only a single den present. Because the MNOR population was never formally studied until 2014-2015 (Stengle, unpubl data), it is not possible to compare population sizes before or after inadvertent genetic rescue occurred, and therefore I cannot state if this genetic rescue resulted in the high genetic diversity of this population. As the origins of the individuals released in the population are unknown, outbreeding depression cannot be ruled out, and even if outbreeding depression is occurring, this would still increase the current genetic diversity. Southern populations differ phenotypically, if these individuals were from these populations, there could be out breeding depression, or possibly not survived northeastern winters. Therefore the high levels of genetic diversity of the MANOR population should not be assumed to indicate a stable population, especially given the small area of habitat the population is restricted to.

4.5.5. Assignment Test

The UNK individuals from New Hampshire assigned strongly to the Pennsylvania population. The collection site of these individuals was not provided by the state until one individual was located in a garage in Raymond, New Hampshire, 1 October, 2012, and broadcasted by the local news station. The state has not announced any captive breeding

projects for this species, however the New Hampshire Fish and Game Department possesses legally collected individuals from Pennsylvania and West Virginia for future potential captive breeding (Martin, W.; McCurly, K. pers comm). The individuals in this previously unknown site are not migrants from the known population, and do not appear to be from New England. It is possible that these four individuals represent a previously unknown population that is genetically similar to the Pennsylvania population. I consider this unlikely because there is no known historic record for the area, and New Hampshire Fish and Game, and others, have searched the state extensively looking for other populations. One other individual was located in the area in 2013 and not included in this study, but none subsequently. It is also important to note that assignment tests can be limited if the true population of origin of individuals is not among baseline populations examined (Piry et. al., 2004). If the population of origin is not represented in the analyses, the individual(s) will be assigned weakly to one or multiple populations. Here, this was not the case, given the strong assignments of the UNK to Pennsylvania, the most likely explanation for these results is introduction by humans of snakes from Pennsylvania to New Hampshire.

4.5.6. Metapopulation Genetic Differentiation

Even though mark recapture data (cF Chap 1) and radiotelemetry data (cFChap 2) did not show overlapping of home ranges among subpopulations of MABER, there appears to be genetic exchange within subpopulations based on F_{ST}, STRUCTURE, and DAPC results. Distances between subpopulations were within the distances traveled by adult Timber Rattlesnakes in the northeast within the active season (Brown, 1993), with an average of 6.50 km (range 3.13 – 9.51 km) between subpopulations. Observed heterozygosity was similar

across all subpopulations, exhibiting the same alleles, also suggesting regular gene flow among them. Valleys between regions could limit gene flow among subpopulations, consistent with other genetic studies in the Northeast, with metapopulations connected through suitable basking habitats between den areas (Bushar et. al., 1998; Clark et. al., 2008). Although roads separate most of the subpopulations in MABER, roads do not appear to be barriers to gene flow here, in contrast to other studies (Clark et. al. 2010; Bushar et. al. 2015). Roads at this site currently have little traffic; with approximately one road mortality per year (Whitbeck, D.; Matthews, R.; Tillinghast, E.; pers comm). It appears that small, low-traffic town roads pose no additional negative effects on gene flow, however an increase in traffic could cause them to became barriers in the future.

Estimated effective number of breeders ranged from 3.7 to 83.7 within these four subpopulations. The largest subpopulation, MABER4, had the highest N_b. MABER3 has the lowest N_b, which could be due to a male skewed sex ratio (n = 16:4) likely from historic collecting of gravid females, which are more vulnerable at communal birthing rookeries (Brown, 1993). This species typically has an even sex ratio (Brown, 1993), and this uneven sex ratio does increase the probability of population extinction (cF Chap 1), so it may be beneficial to consider management plans aimed towards the protection and/or increasing the number of females. The MABER metapopulation is one of the largest in New England, and may be the only one exhibiting metapopulation structure. This region has little anthropogenic change compared to other populations and could serve as a model of historic genetic structure.

4.5.7. Management Implications

Most genetic studies of Timber Rattlesnakes have argued for protection of separate populations (Bushar et. al., 2014; Clark et. al., 2003); however, in New England populations appear to be differentiated, but were likely historically one MU, and anthropogenic changes in the landscape have caused this differentiation, and not natural causes. Because of their anthropogenic isolation from each other despite relative close proximity, genetic drift appears to be largely responsible for differentiation. Rather than keeping each New England population as a separate genetic unit, these populations would appear to benefit from artificial gene flow between them (Coleman et. al. 2013), as would have likely occurred historically when populations were connected and acting as a single MU.

Genetic rescue may be the best management strategy for reducing the effects of genetic drift and inbreeding in isolated populations. Clark et. al. (2011) have recommended genetic rescue for New Hampshire. Genetic rescue appears to have helped forestall extirpation in populations of many species across many taxa, including the Sage Grouse, Florida Panther, and Mexican Wolf. Genetic rescue reversed the effects of inbreeding depression in an isolated European adder population (Madsen et. al. 1999). Here 20 males were transplanted into the rescue population for four breeding seasons and returned to their native population. This action increased fitness and genetic diversity. Unfortunately, the adder population is declining due to anthropogenic habitat alteration (Madsen and Ujvari 2011), emphasizing the need for simultaneous habitat protection. It is unknown whether this method would work with New England Timber Rattlesnakes since transplanted individuals have been found to move more frequently and sporadically before settling into normal home ranges (Reinert et. al., 1999). In some New England populations, such as MANOR, individuals will encounter either large highways or urbanization within less than mile from

the den site, far less than the average distance traveled by an adult in larger more stable populations (Reinert et. al. 1999; Reinert et. al. 1988; Brown et. al. 1982). Individuals translocated into geographically small populations would have greater risks of road mortality then originally suggested by Reinert et. al. (1999). Properly monitoring genetic rescue via translocation would be labor intensive and require monitoring through radiotelemetry and ideally pedigree construction to test for fitness effects. With the Swedish adder population, sampling was so effective that all individuals could be relocated every year, and translocated males could be located without telemetry. An increase in genetic diversity could be detected in four years because adders reach reproductive age at four years and reproduce every other year (Madsen et. al., 2004). Timber Rattlesnakes in the northeast first reproduce at 6 -11 years, and reproduce every three to five years (Martin, 2002; Madsen and Shine, 1992). Juveniles are more difficult to locate than adults (Brown, 1993) therefore it would likely be more than ten years before there is a detectable change in genetic diversity, assuming adult offspring from translocation breedings could be differentiated from native adults. Future telemetry or mark re-capture studies could indicate survival, body condition, and reproduction (i.e. locating a gravid female) of translocated individuals in a shorter time period.

Another approach to increasing genetic diversity across populations is captive breeding, which eliminates threats of increased of road mortality of translocated adults, as seen by Reinert et. al. (1999). Captive individuals could be returned to their native population to decrease negative effects on the native population. Future captive breeding could rely mostly on captive bred snakes that are not released and held in captivity, thus further reducing the effects on native populations. New alleles would need to be brought in

periodically. Individuals would be housed prior to breeding and while gestating, likely for several years. Captive breeding of several snake species has been practiced in the private pet trade for decades, though it has rarely been applied to conservation and reintroduction plans. Newborn offspring could be released after birth, or held in captivity for headstarting. Headstarting has mixed success with snakes (Conner et. al., 2003), with some studies suggesting that long periods in captivity decrease survival (Roe et. al., 2010; Blouin-Demers and Weatherhead, 2001). Due to long-term husbandry requirements captive breeding and headstarting are also labor and resource intensive.

Artificial insemination of females from other populations may be less labor intensive and require less time in captivity. This could potentially be performed in the field, eliminating the need to remove snakes from the population. Road mortality would not be increased, as it might if translocations were conducted (Reinert et. al. 1999). This technique has been successful in the lab with other rattlesnake species (de Langlada et. al. 1994), although sperm were collected post mortem. Artificial insemination has been successful with other snake species without euthanasia (Mattson et. al. 2007).

Whichever assisted gene exchange method is chosen, individuals from within the Northeastern MU (Martin et. al., 2008) should be used to avoid outbreeding depression, resulting in loss of local adaptation. Most New England populations are small and should not contribute individuals to other populations because this would lower census size of already small populations. However, genetic rescue can be successful with only a single or a few individuals (Vila et al., 2003; Zajitschek et. al., 2009) so a stable population could donate or loan a few males without risking population viability. Specifically, MABER3 has a very biased male sex ratio, likely causing the low effective populations size, and removing a few

males should have no negative effect. Overall, the New York populations appear to be the most genetically diverse, with higher overall census size (Stechert, R. pers comm), indicating stable eastern New York populations would be the well-suited donor populations if individuals are needed for New England. Long term genetic monitoring should continue, no matter which method is chosen. However, with this species' low reproductive rate and long age to maturity, it could be 10 to 20 years before any change in genetic diversity is detected.

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Figure 4.1. Map of Northeastern Timber Rattlesnake populations sampled, with larger circles represent larger connected metapopulations sampled.

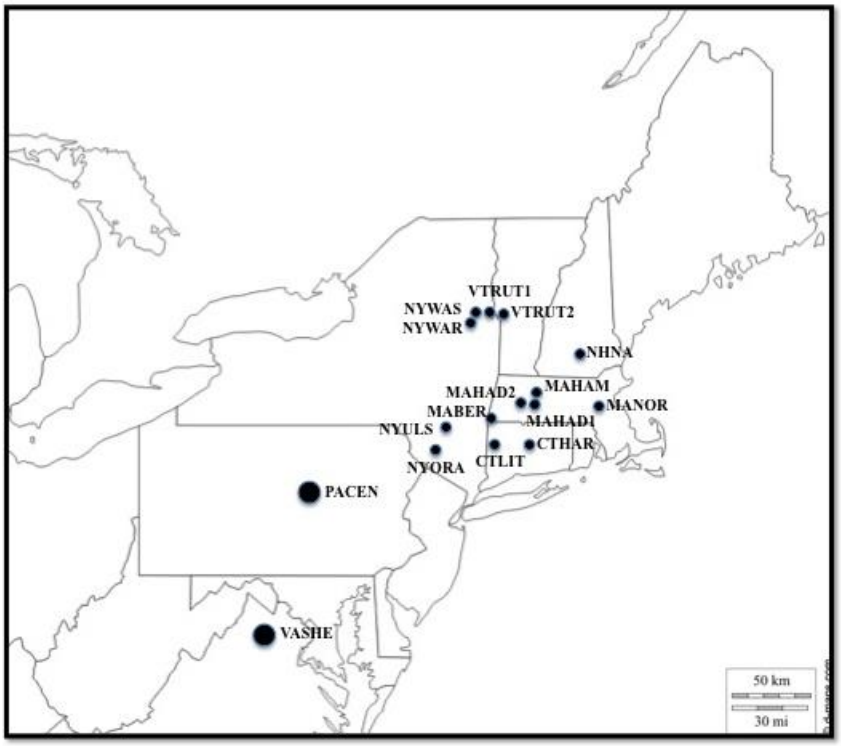


Table 4.1. Population abbreviations of sampled Northeastern Timber Rattlesnake populations.

Abbreviation	State	County
CTHAR	Connecticut	Hartford
CTLIT	Connecticut	Litchfield
KSJEF	Kansas	Jefferson
MAESS	Massachusetts	Essex
MABER	Massachusetts	Berkshire
MAHAD	Massachusetts	Hampden
MAHAM	Massachusetts	Hampshire
MANOR	Massachusetts	Norfolk
NCBUN	North Carolina	Buncombe
NYCHE	New York	Chemung
NCMON	North Carolina	Montgomery
NYWAS	New York	Washington
NYDUT	New York	Duchess
NYESS	New York	Essex
NHNA	New Hampshire	N/A
NJWAR	New Jersey	Warren
NYORA	New York	Orange
NYROC	New York	Rockland
NYSUL	New York	Sullivan
NYULS	New York	Ulster
NYWAR	New York	Warren
PACEN	Pennsylvania	Multiple, Central PA
RINEW	Rhode Island	Newport
SCCOL	South Carolina	Colleton
VASHE	Virginia	Multiple –Shenandoah National Park
VTRUT	Vermont	Rutland

Table 4.2. For each locus the number of samples (N), number of alleles (*A*), observed heterozygosity (H_o), expected heterozygosity (H_E), departure from Hardy-Weinberg (F_{IS}), and the p-value associated with test for departure from Hardy Weinberg Equilibrium (HWE). Bold values represent significant departures from HWE before Bonferroni correction and grey highlighted cells indicate significant departures after Bonferroni correction. Here the Berkshire County metapopulation is considered as one population.

Population	Locus	3-155	5-183	5A	7-144	7-150	7-87	CwB23	CwC24	CwD15	Scu01	Scu05	Scu07	Scu11	Scu26
MABER1	***N***	75	79	79	79	79	78	79	74	79	78	75	79	75	78
	A	5	4	6	4	1	4	11	18	2	4	9	4	9	6
	H_o	0.31	0.25	0.44	0.44	-	0.27	0.56	0.52	0.16	0.10	0.42	0.01	0.49	0.49
	H_E	0.39	0.26	0.46	0.42	-	0.30	0.57	0.65	0.22	0.15	0.58	0.06	0.56	0.55
	F_{IS}	0.03	-0.07	0.20	0.33	-	0.20	0.24	0.08	0.002	0.43	0.43	-0.10	0.11	0.02
	HWE	0.48	0.21	0.10	0.013	-	0.34	0.02	0.14	0.55	0.14	0.001	0.73	0.21	0.52
MABER2	***N***	27	28	28	28	28	28	28	26	28	28	27	28	28	28
	A	4	3	5	4	1	2	7	170.24	3	2	11	2	7	7
	H_o	0.18	0.06	0.15	0.10	-	0.05	0.15	0.22	0.08	0.08	0.13	0.06	0.18	0.17
	H_E	0.19	0.06	0.19	0.15	-	0.06	0.20		0.08	0.14	0.22	0.05	0.20	0.17
	F_{IS}	0.21	0.05	0.05	-0.40	-	0.11	0.01	0.21	0.27	0.33	0.25	0.85	0.13	0.11
	HWE	0.001	0.38	0.30	0.35	-	0.20	0.49	0.001	0.03	0.007	0.001	0.001	0.028	0.7
MABER3	***N***	20	22	21	22	22	22	19	20	22	21	18	22	22	20
	A	3	2	6	5	1	3	6	15	5	3	8	3	9	7
	H_o	0.10	0.03	0.11	0.12	-	0.08	0.13	0.18	0.11	0.11	0.06	0.05	0.11	0.15
	H_E	0.10	0.05	0.12	0.15	-	0.09	0.16	0.18	0.13	0.11	0.15	0.06	0.19	0.16
	F_{IS}	0.02	0.34	0.07	0.23	-	0.15	0.18	0.03	0.0.21	-0.005	0.61	0.21	0.41	0.06
	HWE	0.57	0.23	0.40	0.06	-	0.31	0.11	0.001	0.12	0.58	0.001	0.25	0.001	0.36
MABER4	***N***	118	121	122	121	122	123	119	114	123	123	120	124	120	122
	A	4	3	8	7	1	5	9	23	5	3	13	4	11	8
	H_O	0.64	0.09	0.73	0.81	-	0.57	0.69	0.91	0.56	0.56	0.78	0.04	0.85	0.79
	H_E	0.68	0.10	0.78	0.88	-	0.56	0.94	1.00	0.62	0.62	0.97	0.10	0.87	0.82
	F_{IS}	0.06	0.16	0.06	0.08	-	-0.02	0.27	0.9	0.10	0.10	0.20	0.59	0.02	0.04
	HWE	0.19	0.10	0.12	0.10	-	0.45	0.001	0.004	0.10	0.10	0.001	0.001	0.35	0.27
MANOR	***N***	93	105	104	107	106	103	100	84	106	105	95	103	94	96
	A	4	5	8	6	1	3	9	19	4	4	8	3	10	10
	H_o	0.52	0.50	0.57	0.57	-	0.41	0.71	0.77	0.67	0.59	0.58	0.15	0.71	0.58
	H_E	0.54	0.56	0.72	0.73	-	0.45	0.80	0.89	0.72	0.68	0.81	0.14	0.75	0.63
	F_{IS}	0.04	0.10	0.21	0.22	-	0.10	0.11	0.12	0.07	0.13	0.28	-0.07	0.05	0.07
	HWE	**0.0000**	**0.0060**	**0.00000**	**0.00000**	NA	0.4267	**0.0016**	**0.0000**	0.1345	**0.0027**	**0.00000**	1.00000	**0.0009**	**0.0070**
CTHAR	***N***	36	37	38	37	37	38	38	36	38	38	36	38	38	38

	A	3	2	5	5	1	5	7	17	3	3	8	4	8	7
	H_o	0.42	0.08	0.55	0.62	-	0.42	0.63	0.75	0.58	0.63	0.53	0.21	0.87	0.63
	H_E	0.56	0.08	0.58	0.65	-	0.55	0.74	0.90	0.52	0.65	0.82	0.24	0.84	0.73
	F_{IS}	0.26	-0.03	0.05	0.04	-	0.24	0.15	0.17	-0.11	0.02	0.36	0.13	-0.04	0.14
	HWE	0.1205	1.0000	0.3513	**0.0188**	NA	**0.0084**	**0.0093**	**0.0000**	0.9302	0.0782	**0.0000**	**0.0012**	0.1244	0.2203
CTLIT	***N***	13	11	13	12	12	13	13	13	13	13	13	13	13	13
	A	3	3	6	8	1	3	7	12	4	3	10	2	9	5
	H_o	0.31	0.09	0.62	0.54	-	0.62	0.77	0.85	0.54	0.62	0.62	0.38	0.85	0.62
	H_E	0.57	0.36	0.67	0.76	-	0.60	0.83	0.89	0.58	0.53	0.84	0.41	0.86	0.70
	F_{IS}	0.47	0.65	0.09	0.30	-	-0.03	0.07	0.05	0.07	-0.18	0.27	0.06	0.01	0.13
	HWE	**0.0209**	0.0488	0.3486	**0.0058**	NA	0.6388	0.0676	0.2702	0.0432	1.00000	**0.0173**	1.00000	**0.0332**	0.6461
MAHAD1	***N***	52	55	54	55	55	54	51	50	54	55	53	53	53	51
	A	4	2	6	6	1	3	7	14	4	3	7	2	4	4
	H_o	0.12	0.04	0.44	0.51	-	0.06	0.55	0.62	0.35	0.51	0.68	0.30	0.57	0.51
	H_E	0.13	0.04	0.62	0.65	-	0.16	0.67	0.66	0.34	0.64	0.82	0.28	0.60	0.53
	F_{IS}	0.11	-0.01	0.28	0.22	-	0.65	0.19	0.06	-0.03	0.20	0.17	-0.06	0.05	0.03
	HWE	0.0226	1.0000	**0.0000**	**0.0001**	NA	**0.0001**	**0.0445**	**0.0048**	0.7105	**0.0110**	**0.0000**	1.0000	**0.0002**	**0.0240**
KSJEF	***N***	7	8	8	7	8	8	8	7	8	8	8	8	8	7
	A	5	2	5	4	3	2	6	10	3	2	6	1	8	6
	H_o	0.43	0.50	0.50	0.29	0.14	0.5	0.86	0.00	0.50	0.00	0.63	-	0.88	0.86
	H_E	0.76	0.40	0.60	0.71	0.52	0.5	0.90	1.00	0.54	1.00	0.80	-	0.83	0.81
	F_{IS}	0.45	-0.27	0.18	0.62	0.74	0.00	0.05	-0.06	0.08	1.00	0.23	-	-0.07	-0.06
	HWE	**0.0127**	1.0000	**0.1213**	**0.0046**	0.0757	NA	0.7496	0.3711	1.0000	0.0655	0.7256	NA	0.8443	**0.0035**
NYBUN	***N***	2	2	2	2	2	2	2	2	2	2	2	2	1	2
	A	2	1	2	2	1	1	2	2	3	3	3	1	2	2
	H_o	1.00	-	1.00	1.00	-	-	1.00	1.00	1.00	1.00	1.00	-	-	1.00
	H_E	0.67	-	0.67	0.67	-	-	0.67	0.67	0.67	0.67	0.67	-	-	0.67
	F_{IS}	-1.00	-	-1.00	-1.00	-	-	-1.00	-1.00	-1.00	-1.00	-1.00	-	-	-1.00
	HWE	1.0000	NA	1.0000	1.0000	NA	NA	1.0000	1.0000	1.0000	1.0000	1.0000	NA	NA	1.0000
NYWAS	***N***	25	25	25	26	26	26	26	21	26	24	23	26	25	25
	A	3	3	6	7	1	3	9	15	3	2	8	2	6	5
	H_o	0.64	0.32	0.36	0.46	-	0.62	0.38	0.95	0.38	0.13	0.52	0.04	0.64	0.36
	H_E	0.65	0.55	0.47	0.63	-	0.60	0.56	0.93	0.32	0.12	0.79	0.11	0.73	0.70
	F_{IS}	0.01	0.42	0.24	0.27	-	-0.02	0.32	-0.02	-0.19	-0.05	0.34	0.66	0.12	0.49
	HWE	0.7237	**0.0128**	0.0531	0.0018	NA	0.5986	**0.0017**	0.0646	0.6324	1.0000	**0.0023**	0.0592	0.0713	**0.0002**
NYESS	***N***	1	3	3	3	3	3	3	3	3	3	3	3	3	3
	A	2	1	2	2	1	3	2	3	1	1	3	1	2	2
	H_o	-	-	0.00	0.33	-	0.66	0.66	0.33	-	-	0.33	-	0.33	0.33
	H_E	-	-	0.53	0.33	-	0.53	0.53	0.73	-	-	0.73	-	0.33	0.33

	F_{IS}	-	-	1.00	0.00	-	-0.33	-0.33	0.6	-	-	-.60	-	0.00	0.00
	HWE	NA	NA	0.2008	1.0000	NA	1.0000	1.0000	0.1962	NA	NA	0.2024	NA	1.0000	1.0000
NHNA	***N***	64	69	68	68	69	68	68	64	68	68	67	68	68	66
	A	3	1	3	2	1	3	8	5	4	3	3	3	4	4
	H_o	0.02	-	0.37	0.00	-	0.19	0.07	0.63	0.56	0.01	0.06	0.32	0.43	0.30
	H_E	0.05	-	0.43	0.03	-	0.20	0.13	0.55	0.48	0.04	0.01	0.51	0.44	0.46
	F_{IS}	0.66	-	0.15	1.00	-	0.05	0.43	-0.14	-0.16	0.67	0.75	0.37	0.04	0.34
	HWE	0.0067	NA	0.1366	**0.0074**	NA	**0.0121**	**0.0000**	**0.0098**	**0.0122**	**0.0083**	**0.0020**	**0.0000**	**0.0054**	**0.0000**
NYORA	***N***	18	19	19	19	19	19	19	17	19	19	18	19	19	19
	A	4	3	7	5	1	3	10	15	2	4	9	2	8	7
	H_o	0.44	0.42	0.42	0.52	-	0.63	0.74	0.71	0.37	0.58	0.67	0.37	0.74	0.68
	H_E	0.65	0.50	0.54	0.75	-	0.54	0.86	0.94	0.37	0.62	0.78	0.31	0.84	0.77
	F_{IS}	0.32	0.16	0.24	0.30	-	-0.18	0.15	0.25	0.01	0.06	0.15	-0.20	0.12	0.11
	HWE	**0.0252**	0.7434	**0.0391**	**0.0455**	NA	0.4827	**0.0136**	**0.0000**	1.0000	0.3770	**0.0160**	1.0000	0.3786	0.0861
NYROC	***N***	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	A	3	3	3	2	1	2	4	7	2	2	5	2	6	4
	H_o	0.50	0.50	0.50	0.50	-	0.50	1.00	0.75	0.25	0.25	0.50	0.25	1.00	0.75
	H_E	0.47	0.68	0.47	0.57	-	0.43	0.82	0.97	0.54	0.25	0.86	0.25	0.93	0.79
	F_{IS}	-0.09	0.29	-0.09	0.14	-	-0.20	-0.26	0.25	0.57	0.00	0.45	0.00	-0.09	0.05
	HWE	1.0000	1.0000	1.0000	1.0000	NA	1.0000	1.0000	0.1531	0.4306	1.0000	0.0825	1.0000	1.0000	1.0000
NYULS	***N***	10	14	13	14	14	14	12	9	14	13	12	14	13	12
	A	3	4	4	4	1	3	6	12	3	5	4	2	6	4
	H_o	0.50	0.21	0.54	0.43	-	0.64	0.50	0.89	0.43	0.46	0.42	0.07	0.69	0.67
	H_E	0.54	0.33	0.74	0.47	-	0.65	0.77	0.95	0.37	0.62	0.49	0.20	0.74	0.69
	F_{IS}	0.08	0.35	0.28	0.09	-	0.01	0.36	0.07	-0.16	0.27	0.15	0.65	0.06	0.04
	HWE	0.1835	0.0910	0.3676	0.2404	NA	0.5971	0.1656	0.5553	1.0000	0.1287	0.1884	0.1098	0.4157	1.0000
NYWAR	***N***	20	29	28	28	29	29	26	21	29	29	22	28	24	26
	A	4	2	7	7	1	3	7	18	4	5	8	3	9	7
	H_o	0.35	0.28	0.61	0.61	-	0.45	0.69	0.76	0.14	0.41	0.68	0.18	0.67	0.69
	H_E	0.68	0.33	0.80	0.84	-	0.54	0.77	0.95	0.34	0.53	0.78	0.23	0.80	0.71
	F_{IS}	0.49	0.18	0.25	0.27	-	0.17	0.11	0.20	0.59	0.22	0.13	0.22	0.16	0.21
	HWE	**0.0012**	0.5629	0.1711	**0.0021**	NA	0.0978	**0.0089**	**0.0034**	**0.0002**	**0.0167**	0.0804	**0.0320**	0.1453	0.2045
PACEN	***N***	29	33	33	32	33	32	30	27	33	32	31	32	28	30
	A	6	5	9	6	1	4	11	26	5	3	10	2	16	10
	H_o	0.45	0.39	0.67	0.53	-	0.50	0.77	0.96	0.55	0.34	0.68	0.16	0.75	0.73
	H_E	0.75	0.38	0.82	0.79	-	0.60	0.79	0.95	0.64	0.50	0.86	0.15	0.83	0.85
	F_{IS}	0.41	-0.06	0.19	0.33	-	0.17	0.03	-0.01	0.15	0.31	0.19	-0.07	0.10	0.14
	HWE	**0.0012**	0.3813	**0.0352**	**0.0073**	NA	0.3562	0.6462	0.7353	0.1863	**0.0178**	0.0671	1.0000	0.0850	**0.0281**
RINEW	***N***	2	2	1	2	2	2	2	2	2	2	1	2	2	1

	A	3	2	1	3	1	2	2	2	2	3	1	1	1	1
	H_o	0.50	0.00	-	0.50	-	1.00	0.00	1.00	0.50	0.50	-	-	-	-
	H_E	0.84	0.67	-	0.84	-	0.67	0.67	-	0.50	0.56	-	-	-	-
	F_{IS}	0.50	1.00	-	0.50	-	-1.00	1.00	-	0.00	0.50	-	-	-	-
	HWE	0.3667	0.3337	NA	0.3236	NA	1.0000	0.3366	NA	1.0000	0.3280	NA	NA	NA	NA
NYMON	***N***	2	3	2	3	3	3	3	2	3	3	2	3	3	3
	A	2	2	3	2	1	3	4	3	2	3	3	1	6	4
	H_o	0.5	0.50	0.50	0.67	-	0.50	0.75	0.50	1.00	0.66	0.50	-	1.00	0.5
	H_E	0.50	0.50	0.84	0.53	-	0.73	0.65	0.84	0.60	0.73	0.84	-	1.00	0.65
	F_{IS}	0.00	0.00	0.50	-0.33	-	0.11	-0.20	0.50	-1.00	0.11	0.50	-	0.00	0.27
	HWE	NA	NA	0.3276	1.0000	NA	1.0000	1.0000	0.3391	0.3996	1.0000	0.3377	NA	1.0000	0.4631
MAHAD2	***N***	27	28	27	28	28	27	28	26	27	27	26	27	26	27
	A	4	1	7	5	1	3	8	10	3	3	7	2	6	5
	H_o	0.37	-	0.78	0.75	-	0.67	0.61	0.65	0.37	0.52	0.54	0.15	0.65	0.33
	H_E	0.58	-	0.78	0.70	-	0.67	0.74	0.71	0.37	0.53	0.74	0.14	0.65	0.43
	F_{IS}	0.36	-	0.00	-0.08	-	0.01	0.18	0.09	0.00	0.02	0.28	-0.06	0.00	0.23
	HWE	**0.0166**	NA	**0.0006**	0.4096	NA	**0.0283**	0.0785	**0.0085**	0.1655	1.0000	0.0000	1.0000	**0.0075**	**0.0002**
MAHAM	***N***	19	17	19	19	18	19	18	17	19	19	17	18	18	5
	A	5	2	4	7	1	4	6	4	4	5	9	2	5	18
	H_o	0.36	0.36	0.63	0.53	-	0.21	0.44	0.65	0.58	0.32	0.76	0.00	0.50	0.78
	H_E	0.45	0.26	0.72	0.81	-	0.37	0.50	0.50	0.59	0.52	0.86	0.11	0.52	0.71
	F_{IS}	0.18	-0.14	0.12	0.36	-	0.44	0.11	-0.31	0.01	0.40	0.11	1.00	0.05	-0.10
	HWE	0.1026	1.0000	**0.0171**	**0.0031**	NA	**0.0073**	**0.0101**	0.5923	**0.0066**	**0.0024**	0.0954	**0.0278**	0.1634	**0.0260**
VASHE	***N***	62	64	64	64	63	65	63	53	65	65	55	65	63	63
	A	6	5	9	8	2	5	14	28	5	5	16	3	20	13
	H_o	0.48	0.42	0.53	0.53	0.16	0.49	0.71	0.87	0.58	0.57	0.73	0.25	0.77	0.66
	H_E	0.60	0.52	0.78	0.71	0.16	0.57	0.87	0.95	0.63	0.53	0.90	0.36	0.93	0.84
	F_{IS}	0.20	0.17	0.33	0.25	0.00	0.13	0.0.18	0.07	0.07	-0.06	0.20	0.32	0.16	0.21
	HWE	0.0525	0.20	**0.0000**	**0.0000**	1.00	**0.0475**	**0.0000**	0.1914	0.4278	0.0741	**0.0000**	**0.0382**	**0.0000**	**0.0000**
VTRUT1	***N***	30	35	34	35	35	36	34	32	36	35	31	36	35	33
	A	4	2	3	6	1	3	5	10	2	3	6	2	6	4
	H_o	0.43	0.46	0.41	0.57	-	0.58	0.15	0.75	0.11	0.57	0.44	0.39	0.18	0.76
	H_E	0.63	0.50	0.34	0.71	-	0.64	0.34	0.73	0.115	0.52	0.58	0.51	0.48	0.73
	F_{IS}	0.32	0.09	-0.22	0.19	-	0.09	0.57	-0.03	0.29	-0.11	0.23	0.23	0.63	-0.06
	HWE	**0.0340**	0.4626	0.4356	0.0944	NA	0.2817	**0.0054**	0.1911	**1.0000**	0.7035	**0.0175**	0.1356	**0.0000**	0.1131
VTRUT2	***N***	75	94	93	93	94	94	91	93	94	94	91	93	91	92
	A	3	1	5	4	1	3	5	13	3	3	4	1	6	5
	H_o	0.40	-	0.52	0.62	-	0.20	0.71	0.86	0.12	0.48	0.33	-	0.70	0.65
	H_E	0.52	-	0.57	0.65	-	0.22	0.76	0.82	0.11	0.38	0.33	-	0.74	0.68

F_{IS}	0.23	-	0.09	0.05	-	0.08	0.06	-0.05	-0.04	-0.25	-0.01	-	0.05	0.04
HWE	**0.0124**	NA	0.2829	**0.0130**	NA	**0.0245**	**0.0049**	0.5779	1.0000	**0.0402**	0.7572	NA	0.0866	0.3764

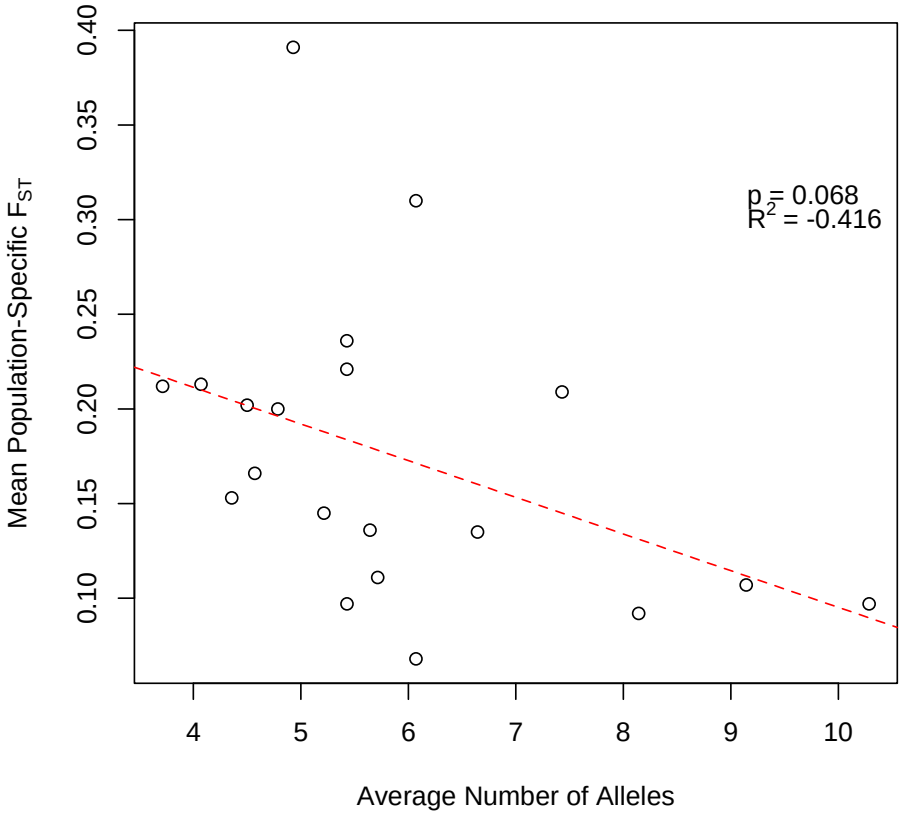
Table 4.3. Population genetic parameter estimates for all populations sampled. Sample size *(N*), mean number of alleles per locus (*A*), allelic richness (*AR*), expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), genetic divergence (F_{ST}), and effective population size (N_b) with 95% confidence intervals *AR*, F_{IS}, and N_b are calculated for populations with $n > 10$.

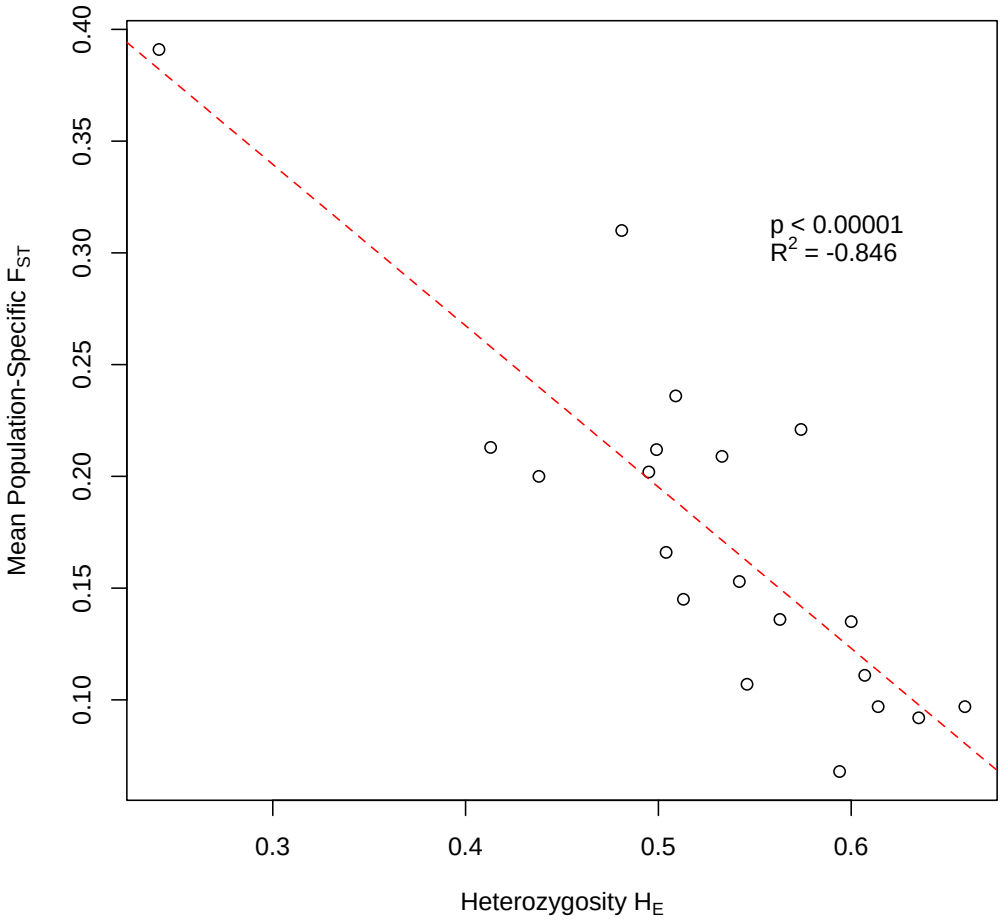
Population	*N*	*A*	*AR*	H_E	H_O	F_{IS}	F_{ST}	N_b
NHNA	69	4.929	1.896	0.241	0.207	0.14	0.391	2 (1.3-3.1)
MANOR	110	6.643	4.201	0.600	0.524	0.128	0.135	47.5 (36.2-64.2)
MAHAD1	55	4.786	3.164	0.438	0.375	0.145	0.200	11 (6.4-17.8)
MAHAM	19	4.500	3.723	0.495	0.433	0.125	0.202	2.2 (1.7-2.8)
MAHAD2	28	4.571	3.495	0.504	0.456	0.094	0.166	9.2 (5.3-15.5)
CTHAR	38	5.643	4.117	0.563	0.495	0.122	0.136	26.8 (16.3-50.7)
CTLIT	13	5.429	4.800	0.614	0.532	0.134	0.097	6.4 (3.6-9.5)
MABER1	79	6.071	3.640	0.481	0.414	0.139	0.107	29.5 (21.5-41.5)
MABER2	28	5.428	4.061	0.509	0.418	0.178	0.310	23.7 (14.6-45.9)
MABER4	124	7.429	4.077	0.533	0.477	0.106	0.236	83.7 (59.8-125.5)
MABER3	22	5.428	4.367	0.574	0.459	0.201	0.209	3.7 (2.7-6.3)
VTRUT1	39	3.714	3.065	0.499	0.425	0.148	0.221	25.6 (13.7-62.6)
VTRUT2	94	4.071	3.190	0.413	0.399	0.034	0.212	107.9 (54.4-406.6)
NYWAS	26	5.214	4.014	0.513	0.415	0.192	0.213	18.8 (12.7-30.3)
NYWAR	29	6.071	4.656	0.594	0.465	0.217	0.145	893.9 (45.9-INF)
NYULS	14	4.357	4.129	0.542	0.461	0.150	0.068	24.5 (9.6-INF)
NYORA	19	5.714	4.568	0.607	0.521	0.142	0.153	43 (25.4-108.0)
PACEN	33	8.143	5.408	0.635	0.534	0.159	0.111	60 (35.8-150.5)
VASHE	65	10.286	5.750	0.659	0.546	0.170	0.092	184.1 (106.1-539.1)
MAESS	1	1.1	NA	NA	NA	NA	NA	NA
SCCOL	1	1.429	NA	NA	NA	NA	NA	NA
NCBUN	2	1.714	NA	0.346	0.692	-1	NA	1.7 (-0.6-INF)
RINEW	2	1.929	NA	0.667	0.333	0.5	0.500	1.6(-.8-INF
NCYHE	3	2.786	NA	0.649	0.571	0.119	0.119	2.5(-1.7-INF)
NYESS	3	1.786	NA	0.333	0.231	0.308	0.308	2.8(-3.1-INF)
NYROC	4	3.286	NA	0.580	0.518	0.108	0.108	4(-22.9-INF)
KSJEF	8	4.429	NA	0.579	0.469	0.189	NA	7.3(21.4-INF)
Avg $n > 10$	33	5.879	4.017	0.528	0.4503	0.145	0.175	84.39

Table 4.4. F_{ST} and F'_{ST} for population pairs of the Timber Rattlesnake in the Northeastern United States. F_{ST} is above the diagonal and F'_{ST} is below the diagonal.

	NHNA	MANOR	MAHAD1	MAHAM	MAHAD2	CTHAR	CTLIT	MABER	VTRUT1	VTRUT2	NYWAS	NYWAR	NYULS	NYORA	PACEN	VASHE
NHNA	0	0.297	0.407	0.437	0.431	0.352	0.351	0.280	0.476	0.417	0.407	0.377	0.471	0.372	0.353	0.329
MANOR	0.563	0	0.181	0.181	0.168	0.123	0.080	0.099	0.220	0.208	0.158	0.113	0.138	0.100	0.075	0.073
MAHAD1	0.660	0.353	0	0.302	0.186	0.176	0.150	0.152	0.229	0.282	0.230	0.152	0.231	0.191	0.182	0.147
MAHAM	0.710	0.371	0.532	0	0.225	0.172	0.178	0.152	0.280	0.281	0.249	0.135	0.168	0.164	0.131	0.145
MAHAD2	0.714	0.349	0.327	0.424	0	0.122	0.134	0.146	0.233	0.253	0.208	0.104	0.150	0.126	0.129	0.108
CTHAR	0.633	0.270	0.333	0.354	0.251	0	0.092	0.080	0.224	0.215	0.169	0.083	0.101	0.050	0.081	0.089
CTLIT	0.618	0.160	0.277	0.368	0.276	0.209	0	0.049	0.185	0.134	0.096	0.046	0.073	0.064	0.054	0.025
MABER	0.555	0.216	0.298	0.312	0.302	0.170	0.102	0	0.382	0.188	0.125	0.062	0.077	0.057	0.052	0.065
VARUT1	0.586	0.424	0.487	0.508	0.465	0.429	0.243	0.382	0	0.155	0.153	0.123	0.225	0.217	0.172	0.184
VARUT2	0.696	0.420	0.376	0.474	0.399	0.407	0.329	0.295	0.385	0	0.088	0.124	0.210	0.196	0.171	0.157
NYWAS	0.677	0.325	0.408	0.473	0.398	0.352	0.192	0.257	0.139	0.256	0	0.068	0.047	0.135	0.101	0.111
NYWAR	0.651	0.234	0.270	0.249	0.192	0.170	0.069	0.129	0.212	0.208	0.114	0	0.047	0.051	0.037	0.058
NYULS	0.764	0.269	0.398	0.289	0.269	0.202	0.123	0.154	0.351	0.375	0.269	0.037	0	0.073	0.057	0.079
NYORA	0.662	0.216	0.365	0.345	0.265	0.111	0.151	0.122	0.387	0.393	0.283	0.090	0.132	0	0.040	0.040
PACEN	0.650	0.162	0.357	0.276	0.275	0.186	0.117	0.114	0.348	0.318	0.211	0.060	0.093	0.086	0	0.037
VTSHE	0.573	0.173	0.312	0.336	0.248	0.220	0.053	0.150	0.351	0.369	0.257	0.132	0.173	0.102	0.090	0

Figure 4.2. Two-dimensional plots comparing genetic diversity to genetic distinctiveness for northeastern Timber Rattlesnake populations. Two dimensional plots comparing genetic distinctiveness (mean population-specific F_{ST}) to three measures of within-population genetic diversity: (a) average number of alleles per locus, (b) average number of alleles per locus, and (c) heterozygosity for all populations with sample sizes greater than 10 individuals.




Mean Population-Specific F_{ST}
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.3
0.4
0.5
0.6
Heterozygosity H_E
p < 0.00001
R^2 = -0.846

b.)

c.)

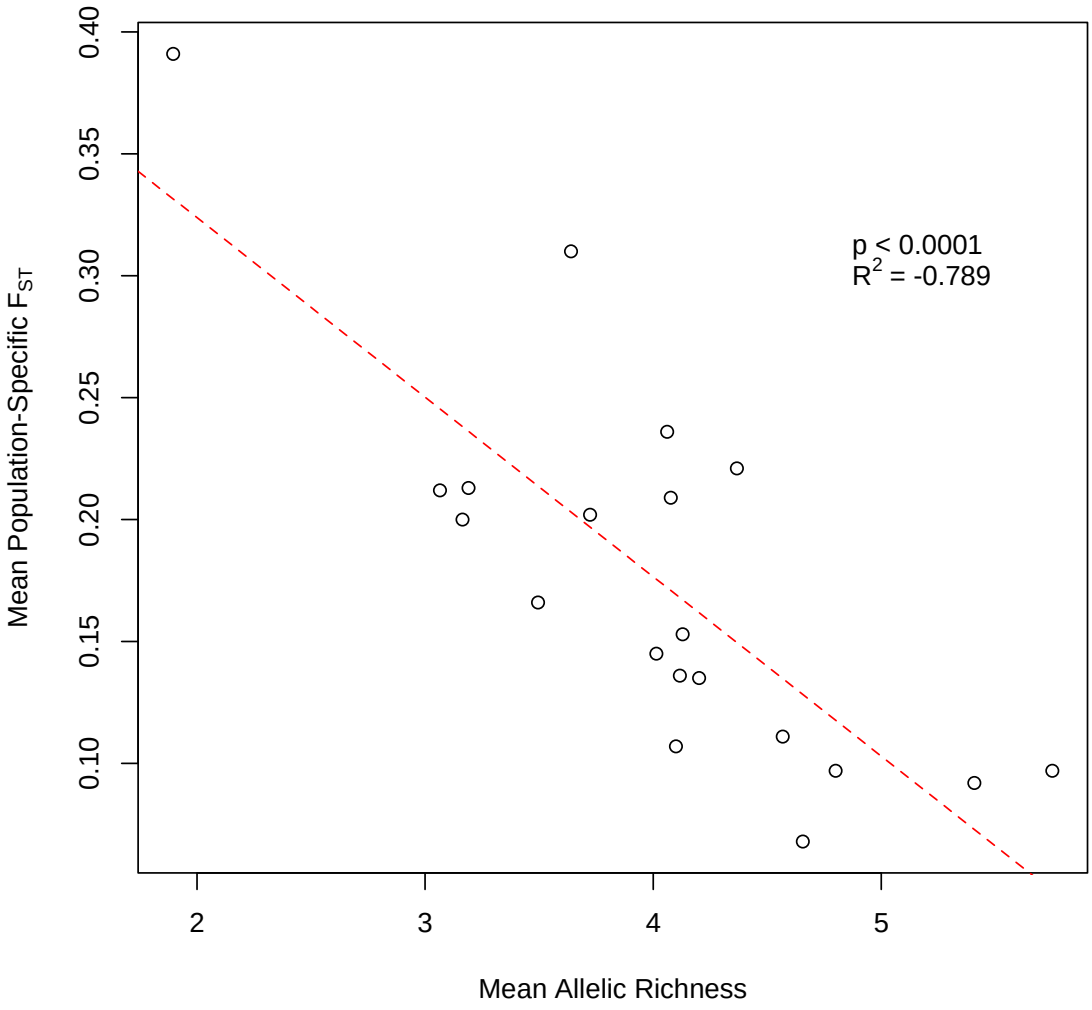

Mean Population-Specific F_{ST}
0.10
0.15
0.20
0.25
0.30
0.35
0.40
2
3
4
5
Mean Allelic Richness
p < 0.0001
R^2 = -0.789

Figure 4.3. Geographic distance (km) compared to genetic distance (F_{ST}/1-F_{ST}) for all Northeastern Timber Rattlesnake population pairs sampled (n = 20). Filled circles indicate the New Hampshire population, the most geographically isolated population.

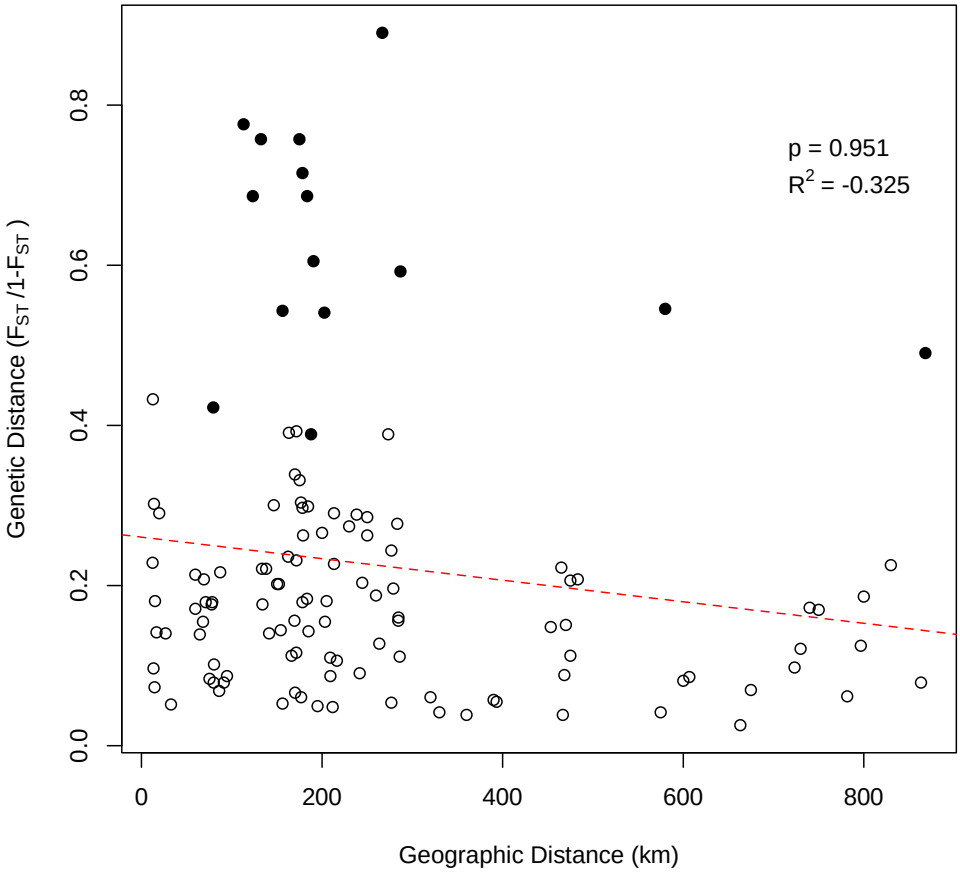


Figure 4.4. An Evanno log likelihood probability plot depicting the change in the number of genetic clusters (*K*), for 20 Timber Rattlesnake populations, with *K* = 1 to 20, from STRUCTURE, a Bayesian analysis of population structure.

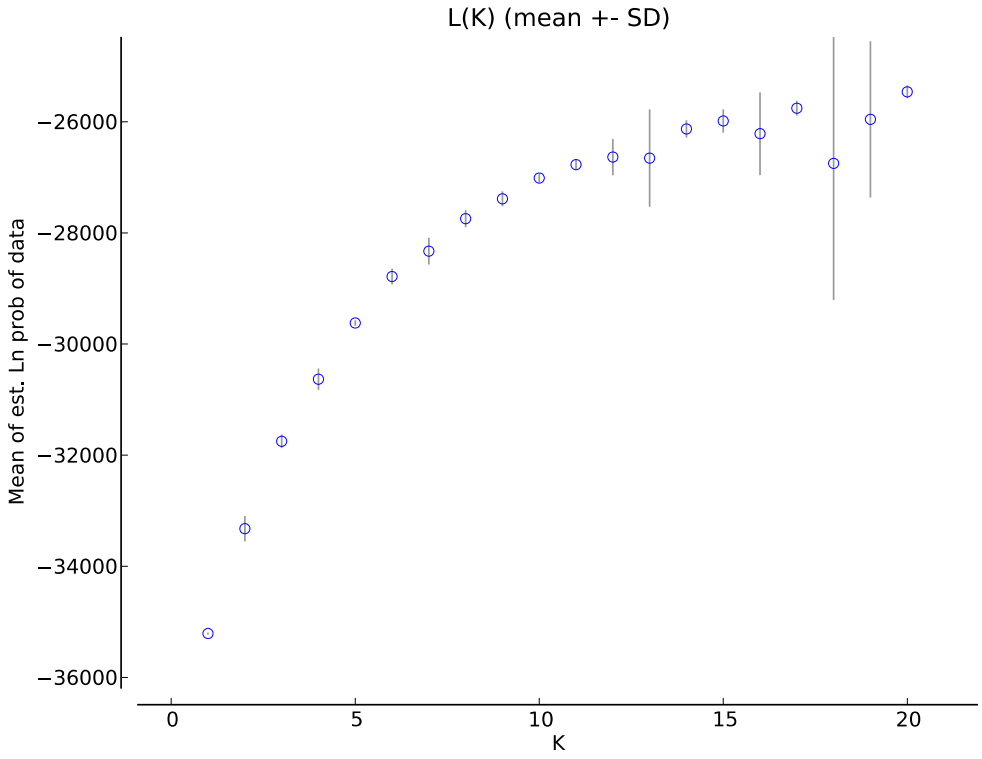


Figure 4.5. Proportion of the genome (*Q)* of each individual assigned by STRUCTURE to each population sample of northeastern Timber Rattlesnakes. Each column corresponds to an individual and a horizontal black bar separates sample locations. Each cluster (*K*) corresponds to a separate color. STRUCTURE plot results are shown for *K* = 5 (a), 6 (b), and 7 (c) for 20 populations.

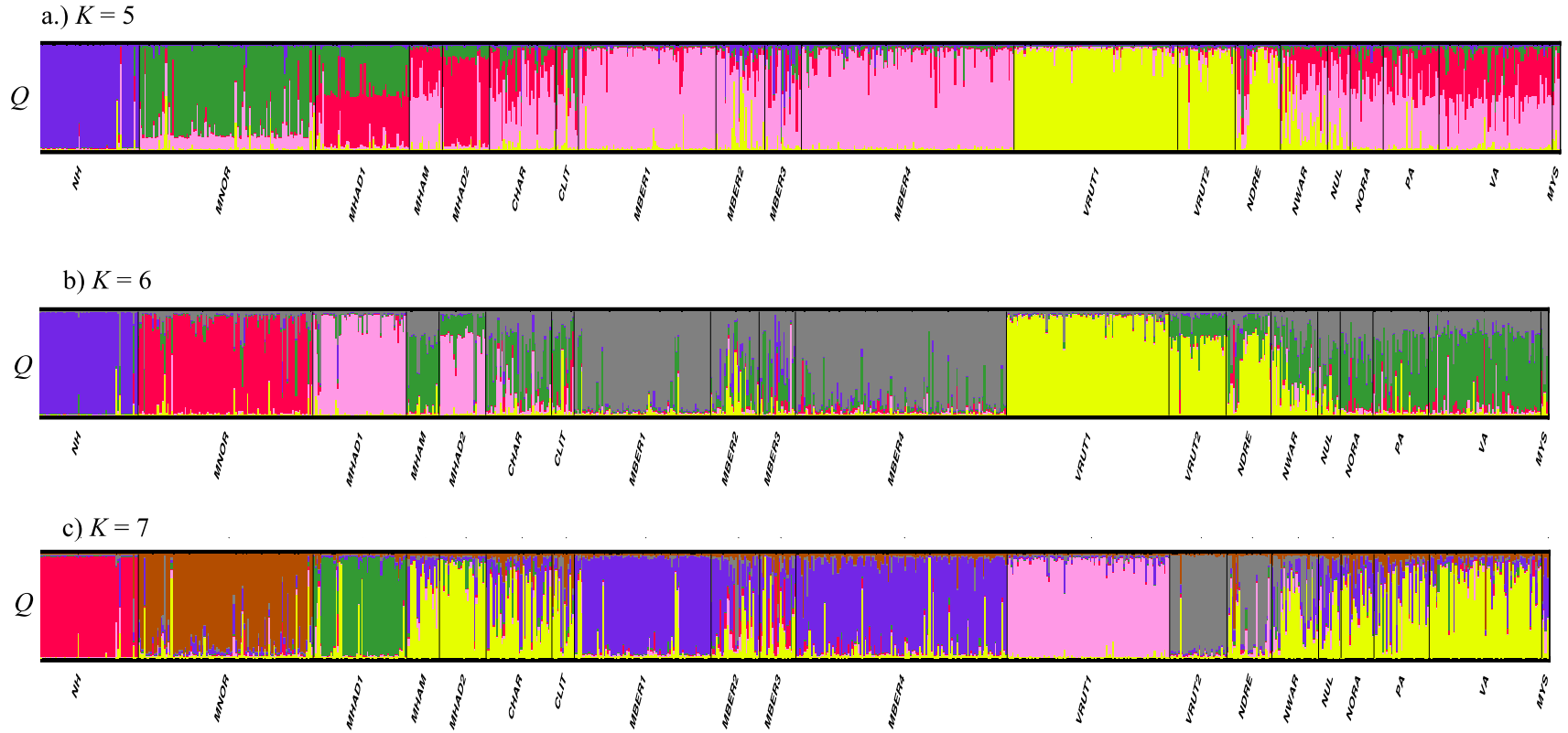


Figure 4.6 Value of Bayesian Information Criterion (BIC) versus the number of population clusters using discriminant analysis of principal components (DAPC) for *K* = 1 to 30 for 20 Northeastern Timber Rattlesnake populations.

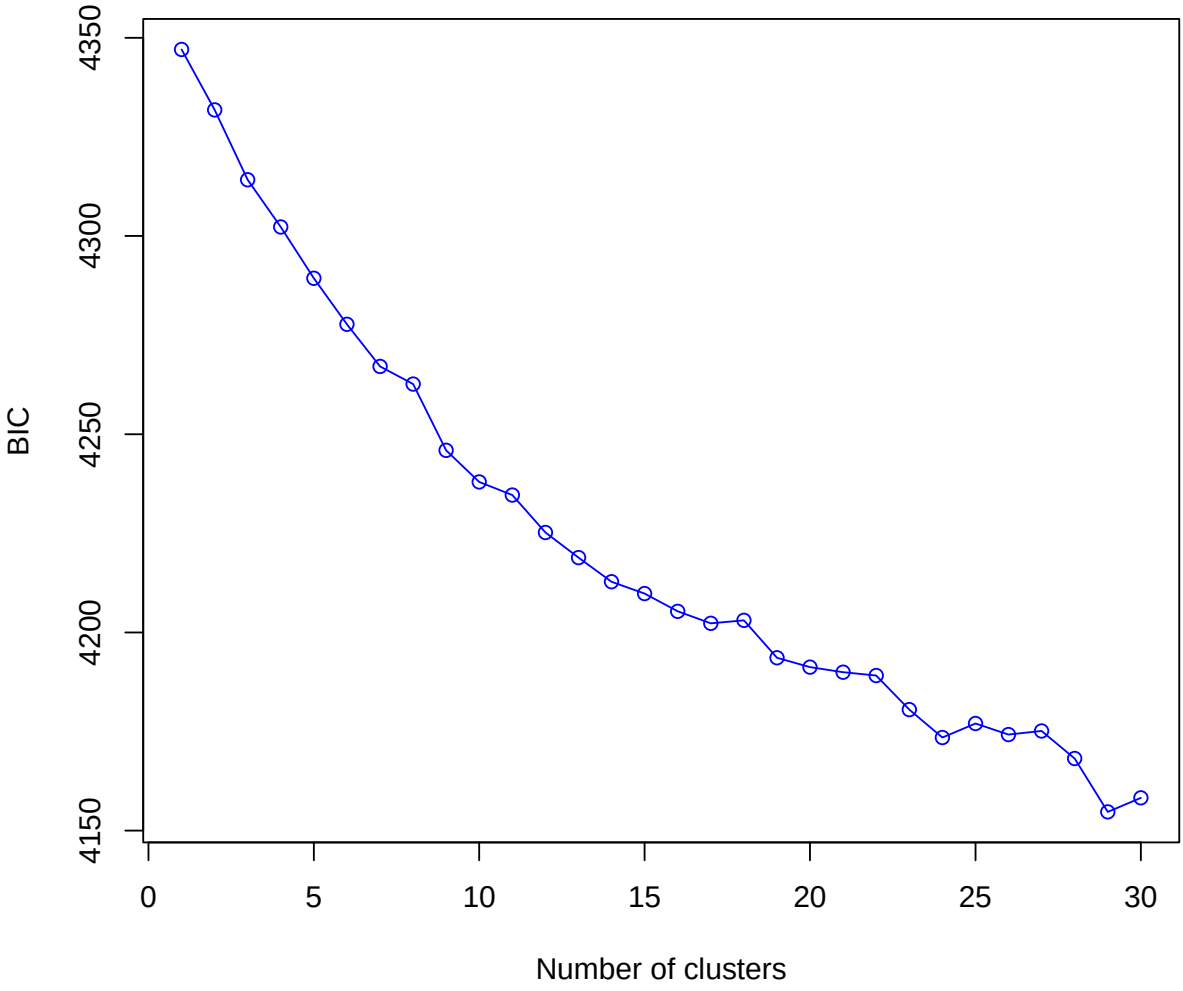


Figure 4.7. Discriminant analysis of principal components (DAPC) of the first two principal components for population clusters (*K*) for *K* = 7. Twenty northeastern Timber Rattlesnake populations were sampled. Distinct single population clusters are labeled, with all other assigning equally in clusters 5 and 6.

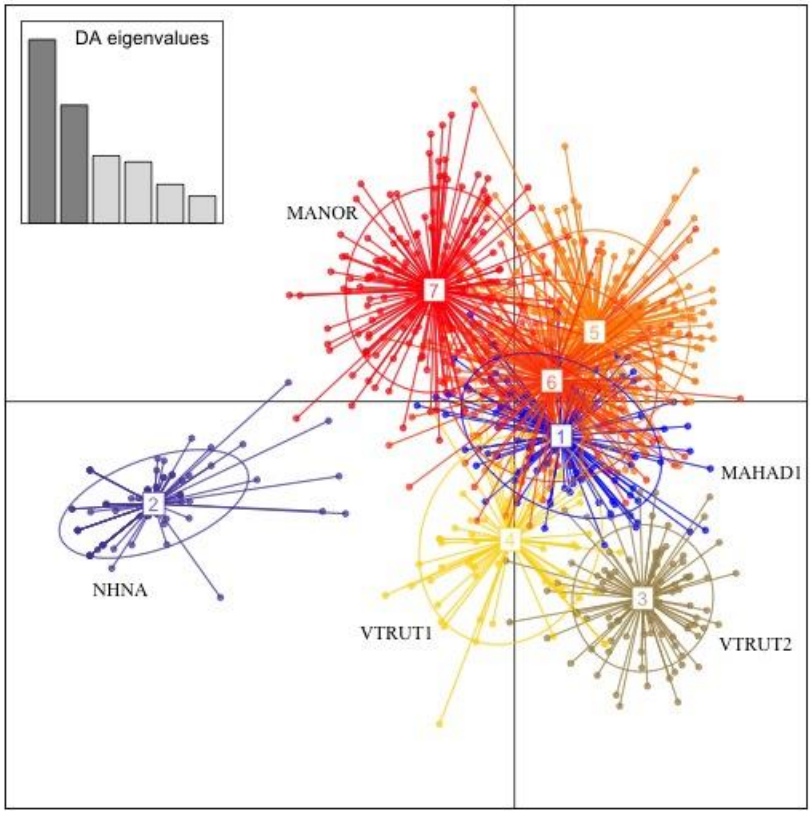


Table 4.5. Pair wise population F_{ST}, above the diagonal, and Euclidian distance (km), below the diagonal, for Northeastern Timber Rattlesnake populations.

	NHNA	MANOR	MAHAD1	MAHAM	MAHAD2	CTHAR	CTLIT	MABER	VTRUT1	VTRUT2	NYWAS	NYWAR	NYULS	NYORA	PACEN	VASHE
NHNA	0	0.297	0.407	0.437	0.431	0.352	0.351	0.280	0.476	0.417	0.407	0.377	0.471	0.372	0.353	0.329
MANOR	79.6	0	0.181	0.181	0.168	0.123	0.080	0.099	0.220	0.208	0.158	0.113	0.138	0.100	0.075	0.073
MAHAD1	123.3	138.1	0	0.302	0.186	0.176	0.150	0.152	0.229	0.282	0.230	0.152	0.231	0.191	0.182	0.147
MAHAM	113.2	133.5	12.7	0	0.225	0.172	0.178	0.152	0.280	0.281	0.249	0.135	0.168	0.164	0.131	0.145
MAHAD2	132.4	150.2	12.4	19.6	0	0.122	0.134	0.146	0.233	0.253	0.208	0.104	0.150	0.126	0.129	0.108
CTHAR	156.3	141.4	59.6	69.1	64.6	0	0.092	0.080	0.224	0.215	0.169	0.083	0.101	0.050	0.081	0.089
CTLIT	202.5	209.3	77.7	87.2	68.2	80.3	0	0.049	0.185	0.134	0.096	0.046	0.073	0.064	0.054	0.025
MABER	187.8	209.0	71.1	78.4	59.7	94.7	32.7	0	0.155	0.188	0.125	0.062	0.077	0.057	0.052	0.065
VTRUT1	178.3	250.0	171.7	163.3	170.0	230.0	203.3	171.7	0	0.155	0.153	0.123	0.225	0.217	0.172	0.184
VTRUT2	175.0	250.0	178.3	273.3	176.7	238.3	213.3	183.3	13.8	0	0.088	0.124	0.210	0.196	0.171	0.157
NYWAS	183.5	259.8	184.5	175.2	179.0	244.5	216.5	185.0	13.3	15.0	0	0.068	0.047	0.135	0.101	0.111
NYWAR	190.5	263.4	178.3	169.4	171.5	241.7	211.7	170.2	16.7	26.7	14.5	0	0.047	0.051	0.037	0.058
NYULS	266.7	284.7	146.5	152.3	134.0	166.2	80.0	75.3	200.0	213.3	205.0	195.0	0	0.073	0.057	0.079
NYORA	286.8	286.0	162.5	279.0	154.2	156.2	86.0	176.8	276.7	283.3	284.5	276.7	91.3	0	0.040	0.040
PACEN	580.0	600.0	465.0	470.0	453.3	468.3	390.0	393.3	475.0	483.3	475.0	466.7	320.0	330.0	0.0	0.037
VASHE	868.3	863.3	740.0	750.0	730.0	723.3	663.3	675.0	800.0	830.0	796.7	781.7	606.7	575.0	360.0	0

Table 4.6. Assignment testing results for four Timber Rattlesnakes sampled in New Hampshire of unknown population origin. Ordered populations represent the population is assigned to and relative score.

Individual	Population 1	Score 1	Population 2	Score 2	Population 3	Score 3
1	PACEN	99.561	VASHE	0.4	MABER4	0.037
2	PCAEN	99.884	NYULS	0.07	VASHE	0.045
3	PACEN	94.982	VASHE	3.643	MABER3	1.278
4	NYORA	96.287	PACEN	3.694	VASHE	0.018

Figure 4.8. Log likelihood probability plot of population clusters (*K*) values for a Massachusetts Timber Rattlesnake metapopulation (MBER), with *K* = 1 to 9, from STRUCTURE.

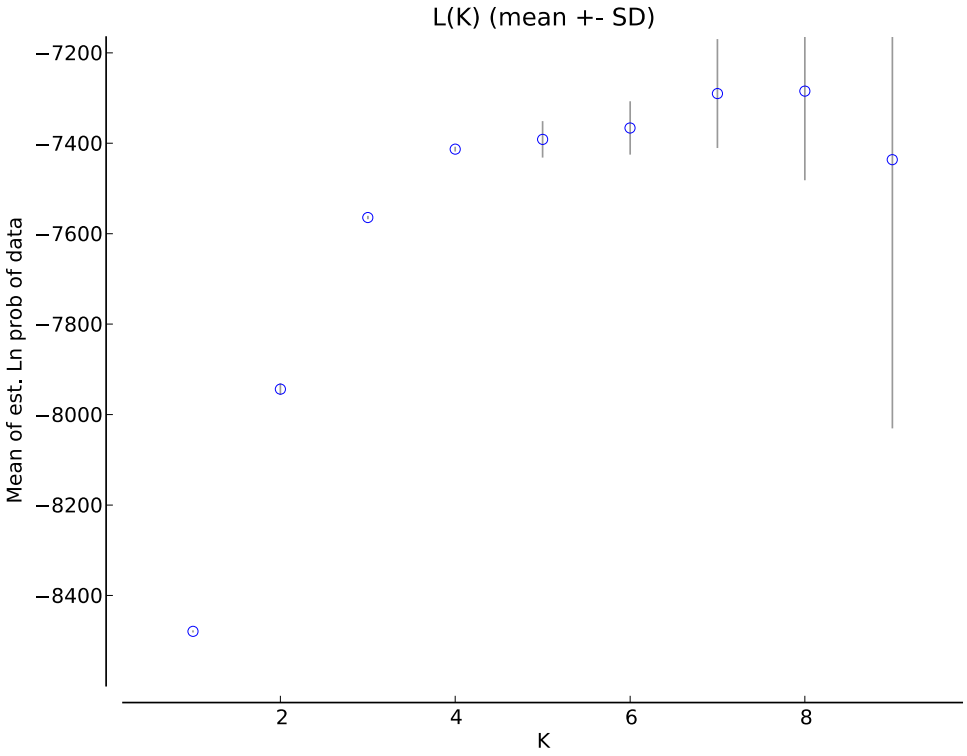
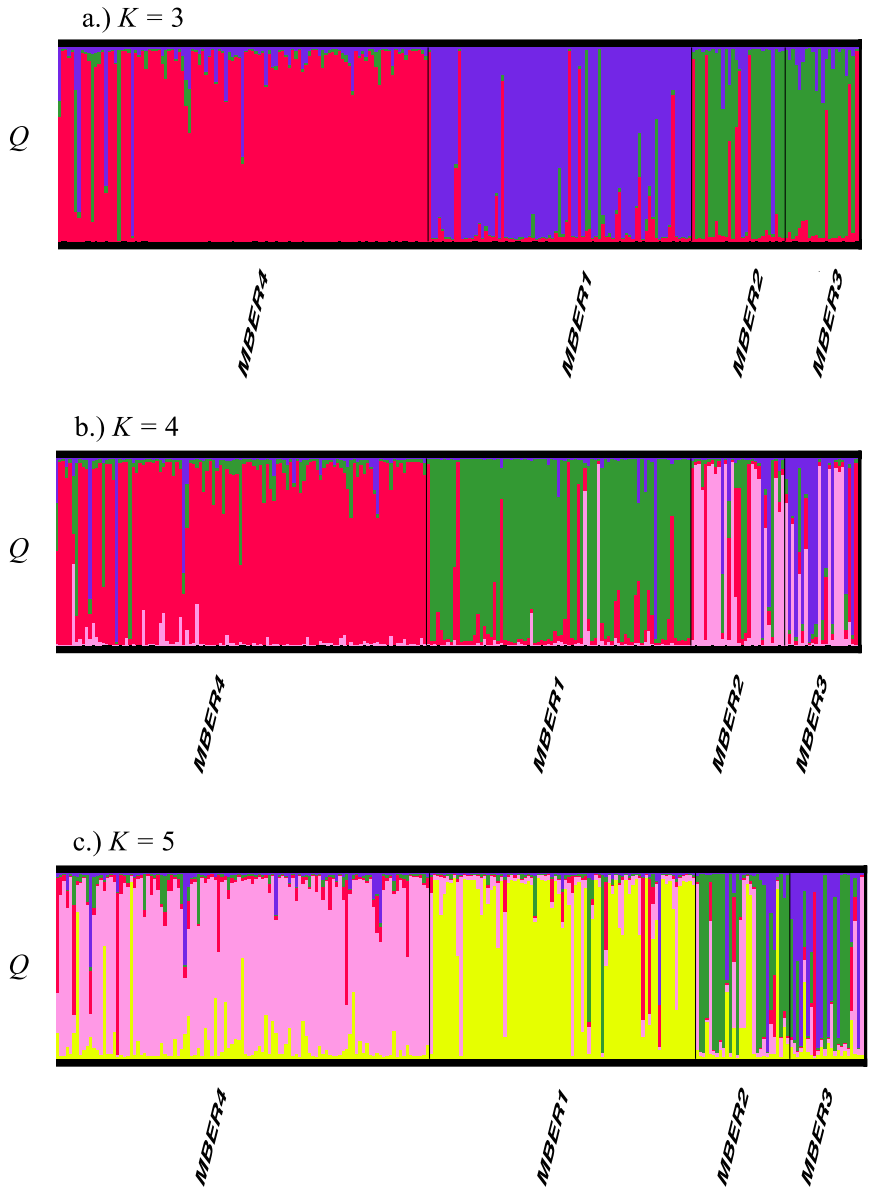


Figure 4.9. Proportion of the genome (*Q)* of each individual assigned by STRUCTURE to each population sample of northeastern Timber Rattlesnakes. Each column corresponds to an individual and a horizontal black bar separates sample locations. Each cluster (*K*) corresponds to a separate color. STRUCTURE plot of population clusters for *K* = 3 (a), 4 (b), and 5 (c), for a Massachusetts Timber Rattlesnake metapopulation.



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