Population Demographics, Monitoring, and Population Genetics of Timber Rattlesnakes in New England

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Executive Summary

The timber rattlesnake (*Crotalus horridus*) was historically widespread throughout eastern North America but has undergone declines throughout its range due to anthropogenically-induced habitat loss and fragmentation and human persecution. Timber rattlesnakes currently persist in four New England states in small, isolated populations. These populations are at increased risk from the effects of environmental, demographic, and genetic stochasticity leading to increased extinction risk. The conservation of this species in New England therefore requires an accurate assessment of population demographics and viability, the genetic structure and connectivity within and among populations, and standardized protocols for monitoring population-specific state-variables (e.g., occupancy, abundance, demographic stability, and genetic diversity) over time. The goal of this study was to address each of these components through three broad objectives: 1) assess the population viability of New England timber rattlesnake populations, 2), describe the population genetics structure of timber rattlesnakes in New England and provide recommendations for genetic management and monitoring, and 3) develop a standardized protocol for monitoring timber rattlesnake populations informed by model-based estimates of occupancy and abundance. An additional objective was to facilitate the planning and implementation of a New England Inter-Agency Rattlesnake Conservation Group.

To address Objective 1, we estimated demographic parameters, including survival, reproductive metrics, and abundance, using existing mark-recapture and radio telemetry data. We used our empirical estimates and values from the literature to conduct a population viability analyses (PVA). Survival estimates were largely consistent with those from other north-temperate communally-denning rattlesnakes and were highest for the Berkshire County metapopulation in western Massachusetts. We found no evidence that survival rates were lower in radio-tracked individuals. Recapture probabilities were generally low (≤0.20) but were highest during joint radio-telemetry and mark-recapture studies at single populations. We found no difference in recapture probabilities between males and females. Small sample sizes prevented us from accurately estimating reproductive parameters although our observations were consistent those from other northeastern populations. Model-based estimates of population growth and PVA results suggest that populations in Vermont, New Hampshire, and Connecticut may be declining while the Berkshire Mountains metapopulation does not appear to be declining under current conditions. In all cases, population persistence was highly sensitive to survival

suggesting that reducing anthropogenically-induced mortality is important for population persistence. We encourage collecting additional data to provide more accurate estimates of age-specific survival, reproduction frequency, fecundity, and population size, and note that our model-based estimates do not account for the potential population-level effects of catastrophic or stochastic mortality events.

To address Objective 2, we analyzed microsatellite DNA data from 14 populations in New England and New York and reviewed the literature to identify genetic-based thresholds for initiating genetic rescue. We found significant genetic structure among populations and no evidence of isolation-by-distance suggesting a strong effect of genetic drift. Populations in New Hampshire, Vermont, and 1–2 populations in Massachusetts were most genetically isolated and had lowest genetic diversity. Our literature review found no consistent genetic-based threshold for initiating genetic rescue suggesting instead that phenotypic traits (e.g., abnormal color morphs) may be more reliable indicators of the need for genetic rescue. Moreover, differences among genetic rescue studies in taxa, genetic markers and metrics, and sampling methods suggest caution in developing genetic-based thresholds. We therefore recommend a multi-facetted approach incorporating genetic, phenotypic, and demographic data to determine if and when genetic rescue is warranted. Available data strongly suggest that some timber rattlesnake populations in New England will benefit from genetic rescue. Given the potential benefits and previous success of genetic rescue in other taxa, we discuss alternative approaches for implementing genetic rescue for timber rattlesnakes in New England. We discuss approaches for identifying timber rattlesnake conservation units in New England but strongly recommend integrating an understanding of historical distribution and connectivity and the potential for local adaptation into any such exercise. While long generation times and logistical considerations may make genetic and phenotypic monitoring difficult, we recommend that it accompany any genetic rescue efforts.

To address Objective 3, we used simulations to evaluate the use of presence-absence and count data to provide estimates of occupancy and abundance using hierarchical models and identify optimal sampling designs. We also discuss the implementation of different monitoring protocols for timber rattlesnakes. We found that counts of individuals at dens cannot accurately estimate abundance. While data collected at gestation or shedding sites could potentially meet model assumption, our results show that obtaining sufficient sample sizes to detect trends may be difficult to impossible although statistical power was greater for abundance than occupancy. Maximizing the number of sites led to the greatest increases in statistical power and we recommend pooling sites across populations where feasible. Field observations suggest that temporary emigration is present at gestation and shedding sites. While this is unlikely to bias occupancy and abundance estimates, temporary emigration affects the interpretation of these estimates and we recommend that temporary emigration initially be estimated using appropriate survey protocols. We suggest that mark-recapture provides the greatest level of information for population monitoring although note that it may be impractical in some situations due to limited financial and logistical resources and potential impacts to individual rattlesnakes. In these cases, we recommend that researchers not rely primarily upon counts of individuals at dens for monitoring but instead focus on monitor population status using the occupancy and abundance at gestation and shedding sites, where they are known. This project represents a key step towards the formation of an inter-agency New England Rattlesnake Conservation Group to implement the recommendations provided in this report. Ongoing efforts towards implementation include regular conference calls among project participants, capacity building efforts to purchase field supplies and equipment to initiate monitoring work in the spring of 2018 and the compilation of a spatially-referenced database of potential monitoring sites for select populations.

Preface

The timber rattlesnake (*Crotalus horridus*) is a large-bodied venomous snake that historically occurred throughout eastern North America (Brown 1993; Martin et al. 2008). However, this species has declined throughout much of its range due to habitat loss and fragmentation and human persecution particularly along the northern edge of its range (Martin et al. 2008). These declines have been particularly acute in New England. Formerly occurring in all six New England states, timber rattlesnakes are extirpated in Maine and Rhode Island, persist in one and two populations in New Hampshire and Vermont, respectively, and are endangered in Massachusetts and Connecticut (Martin et al. 2008). These remaining populations are largely isolated due to anthropogenic landscape changes which, combined with presumably small population sizes, increases their susceptibility to demographic, environmental, and genetic stochasticity and, ultimately, population extinction (Brook et al. 2008; Shaffer 1981). The conservation of timber rattlesnakes in New England, therefore, represents an important regional conservation priority.

Despite its imperiled status, relatively few formal studies of timber rattlesnakes have occurred in New England (Clark et al. 2011; Stengle 2017; Tyning 2005) although an adjacent metapopulation in the Adirondacks of eastern New York has received considerable research attention (Aldridge & Brown 1995; Brown 1991; Brown 2008; Brown 2016; Brown et al. 2007). Population-specific estimates of demographic parameters and population persistence are essential for informed conservation, particularly since demographic parameters in snakes may show substantial within- and among-population variability (Bronikowski & Arnold 1999; Brown 2016; Jenkins et al. 2017; Jenkins et al. 2009). Additionally, understanding population genetics structure and connectivity becomes increasingly important for the conservation of small, isolated populations which are more at risk from inbreeding depression (Keller & Waller 2002). Where natural dispersal is absent, assisted dispersal (i.e., genetic rescue) may be required to reduce the impacts of inbreeding depression (Hedrick et al. 2011; Whiteley et al. 2015). An accurate assessment of timber rattlesnake population persistence in New England therefore requires consideration of both demographic and genetic factors.

To accomplish this, we initiated this project as a multi-state cooperative effort involving all New England states supporting timber rattlesnakes. The overall goal of this project is to assess and ensure the persistence, connectivity, and evolutionary potential of timber rattlesnakes in New England while minimizing the loss of genetic diversity caused by genetic drift and inbreeding. We address this goal through four project components. Under the first component (Chapter 1), we use existing data from state agencies and project partners to obtain population-specific estimates of key demographic parameters and conduct formal population viability analyses evaluating the probability of population persistence and the factors influencing that persistence. Under the second component (Chapter 2), we evaluate the population genetics of timber rattlesnakes in New England to evaluate the potential need for and benefits of genetic rescue. We use our results and examples from the peer-reviewed literature to develop a comprehensive genetic management plan for timber rattlesnakes in New England with particular emphasis on a framework for determine when and how genetic rescue should be implemented. Under the third component (Chapter 3), we evaluate different approaches for monitoring the population status of timber rattlesnakes in New England and provide specific recommendations for standardized, region-wide monitoring protocols. Our final component (Conclusion) discusses ongoing steps by the New England Rattlesnake Conservation Group to implement the recommendations provided in this report. Together, these components represent substantial contributions toward the development of state-specific and region-wide timber rattlesnake conservation plans.

Chapter 1—Population Demographics of Timber Rattlesnakes in New England

Abstract

Timber rattlesnakes (*Crotalus horridus*) historically occurred in all six New England states but currently persist in only four of these states in relatively small, isolated populations. At northern latitudes, timber rattlesnakes display slow life histories characterized by late maturation, multi-year reproductive intervals, relatively low fecundity, and high adult survival. While these traits may prove adaptive in response to environmental variation, they also increase population susceptibility to anthropogenically-induced mortality. Furthermore, demographic parameters in snakes can vary markedly within- and among-populations making population-specific estimates of demographic parameters essential for an accurate understanding of population viability. Our objectives were to provide population-specific estimates of demographic parameters including survival, reproductive traits, and abundance and use those estimates to conduct population viability analyses (PVA) for timber rattlesnakes in New England. We used existing mark-recapture and radio telemetry data from six populations and conducted PVA for three populations. Our estimates of annual survival (0.77–0.95) were largely consistent with estimates reported for communally-denning north-temperate rattlesnakes. Despite small sample sizes, reproductive metrics were similarly consistent. The PVA gave different estimates of population persistence among populations which were largely consistent with our demographic estimates. The Vermont population showed relatively low female survival, a male-biased sex ratio, a negative model-based estimate of population growth, and low probability of persistence. Survival estimates from the New Hampshire population were relatively low but varied interactively by time, season, and age, likely due to outbreaks of snake fungal disease in 2006 and 2007. This population also had a low probability of persistence. The Connecticut population also showed relatively low survival and probability of persistence although counts of gravid females showed no declines over 27 years. The Massachusetts population showed relatively high survival, a stable model-based estimate of population growth, and high probability of persistence. While population persistence was most sensitive to survival rate, our estimates of juvenile survival and reproductive traits were poorly estimated and we suggest that future research focus on obtaining more accurate estimates.

Introduction

Timber rattlesnakes (*Crotalus horridus*) historically ranged throughout eastern North America from southern Canada to the Gulf of Mexico and the Atlantic Coast to the eastern Great Plains (Brown 1993; Martin et al. 2008). However, timber rattlesnakes have declined in many portions of their range, primarily due to anthropogenically-induced habitat loss and fragmentation and human persecution. These declines have been particularly acute in the six New England states of the northeastern United States. Timber rattlesnakes were extirpated from Maine and Rhode Island, persist in one to two populations in New Hampshire and Vermont, respectively, and are endangered in Massachusetts and Connecticut. These remaining populations are largely isolated due to anthropogenic landscape changes which, combined with presumably small population sizes, increases their susceptibility to demographic, environmental, and genetic stochasticity (Brook et al. 2008; Shaffer 1981). Small, isolated populations may also be at greater risk if disease outbreaks (Clark et al. 2011).

Timber rattlesnakes in the northern parts of their range utilize communal hibernacula (i.e., dens) for overwintering and generally show high den fidelity (Anderson 2010; Brown 1993, 2008; Clark et al. 2008; Martin et al. 2008). During the activity season, males and non-gravid females migrate into surrounding habitats for foraging and may travel up to 7.2 km from their den (Brown 1993; Reinert & Zappalorti 1988b; Stengle 2018, J.M.B. & D.B., unpublished data). Males often undertake extensive mate-searching movements during the late summer to locate females (McGowan & Madison 2008; Waldron et al. 2006). In contrast, gravid females generally move shorter distances towards discrete gestation sizes with open microhabitats and show precise behavioral thermoregulation to assist embryo development (Anderson 2010; Brown 1993; Gardner-Santana & Beaupre 2009). In forested landscapes, open rocky outcrops, balds, and ridge-lines with southerly aspects are often utilized. Individuals of all reproductive condition may also utilize similar open microhabitats during ecdysis (Brown 1993; Stengle 2018). At northern latitudes or high elevations, gestation and shedding sites may represent limiting resources. The large spatial and specific habitat requirements of timber rattlesnakes creates additional conservation challenges and makes understanding the local spatial and habitat ecology of timber rattlesnakes essential for conservation efforts.

Ectotherms at high latitudes and elevations exhibit comparatively slower and longer life history traits compared to populations in more mild climates. These differences have been illustrated in multiple rattlesnake species (e.g., Diller & Wallace 2002; Hileman et al. 2017; Jenkins et al. 2017; Jenkins et al. 2009; Macartney & Gregory 1988; Schuett et al. 2011), particularly timber rattlesnakes (Brown 2008; Brown 2016; Martin 2002; Martin et al. 2008). At high latitudes or elevations, such as the Appalachian Mountains or the northeastern United States, timber rattlesnakes show high (> 0.80) adult survival, take 7–14 years to reach sexual maturity, reproduce every 2–7 years, and live over 40 years (Brown 1991, 2016; Brown et al. 2007; Martin 2002). Under such circumstances, females may exhibit as little as 1–2 reproductive events in their lifetime (Brown 2016). While such life history traits may buffer populations against temporal variation in recruitment and other effects of environmental stochasticity, they may also increase population vulnerability because relatively small decreases in survival, particularly for adults, may disproportionately affect population viability (Gregory 2009). Furthermore, such traits may make it difficult to detect population declines because populations may be sustained by a small number of older individuals (Shoemaker et al. 2013).

Within a single species, snake populations often show marked variation in ecological and demographic traits, sometimes over relatively small spatial extents (Shine & Bonnet 2009). For example, Jenkins et al. (2009, 2017) found differences in multiple demographic traits among three populations of great basin rattlesnake (*Crotalus oreganus lutosus*) in eastern Idaho 24–42 km apart which led to differences in estimated population growth rates. These differences were driven by variation in anthropogenic disturbance and prey abundance. Similarly, Bronikowski & Arnold (1999) demonstrated differences in life history strategies in western terrestrial gartersnake (*Thamnophis elegans*) populations 5–15 km apart and found that these differences had an adaptive genetic basis (Bronikowski 2000). Other studies have demonstrated similar fine-scale inter-population variation in behavior, movement patterns, morphology, and reproductive traits (Gomez et al. 2015; Shine et al. 2012; Shine et al. 2003; Siers et al. 2017). Therefore, effective conservation of timber rattlesnakes in New England requires an accurate understanding of population-specific estimates of abundance, population structure, and demographic parameters.

Despite its conservation concern within New England, relatively few formal studies of timber rattlesnake ecology, demographics, and population viability have been conducted within this region (Clark et al. 2011; Stengle 2018; Tyning 2005) and population-specific estimates of demographic parameters remain lacking for most populations. The overall goal of this study, therefore, was to analyze existing data sets from multiple populations in New England and evaluate the utility of these data for assessing population viability. Data were collected as part of past or ongoing state monitoring programs or graduate student research and the quality and quantity of data varied among populations. Our specific objectives were to, where possible, 1) obtain model-based estimates of age- and sex-specific survival and recapture rates, 2) estimate additional demographic parameters such as sex ratios, frequency of reproduction, litter size, and age at maturity, 3) estimate population abundance and growth, and 4) conduct formal population viability analyses.

Methods

Study Areas and Data Collection

Vermont

We obtained data from the last two confirmed populations of timber rattlesnake in Vermont, both located in Rutland County. These populations are on the edge of the Taconic Mountains with metamorphic bedrock geology. Dominant vegetation communities include mesic maple-ash-hickory-oak forests and xeric oak-hickory-hophornbeam forests (Thompson & Sorenson 2005). Populations are approximately 12 km apart (Euclidean distance). The first population, VRUT1, consists of two separate denning areas separated by approximately 1.1 km, although given their proximity and contiguous habitat between them we consider them one population for research and management purposes. We refer to the second population at VRUT2. Within each population, denning areas consist of rocky soil, talus, and exposed rock outcrops and include multiple openings or entrances. The denning areas and the surrounding habitat are owned entirely or in part by The Nature Conservancy.

A joint mark-recapture and radio telemetry study was conducted at VRUT2 from 2011 through the spring of 2013. During this time, researchers searched for rattlesnakes throughout the denning area during spring egress and fall ingress and around known or suspected shedding and gestation sites throughout the summer. Several individuals were also captured opportunistically while monitoring radio-tracked individuals. Additional mark-recapture efforts continued through

2014 after which efforts to mark new individuals were replaced by visual encounter surveys conducted around the denning area and known basking/gestation sites through 2017. Sporadic recaptures of previously marked individuals were recorded in 2015 and 2017. Individuals were marked using PIT tags (Biomark, Inc., Boise, Idaho) and researchers recorded sex, SVL, tail length, weight, and the number of rattles. The basal rattle segment was painted to estimate shedding rates and the reproductive condition of females determined via palpation, during which researchers estimated the number of follicles/embryos. The presence of skin lesions or blisters was also noted.

A professional veterinarian surgically implanted radio transmitters (9 g SI-2T transmitters, Holohil Systems Ltd., Carp, Ontario, Canada) into 22 individuals at VRUT2 following standard surgical procedures (Reinert & Cundall 1982). Transmitters were <3% of an individual’s body mass and individuals were released after 24–48 hours in captivity. All surgeries were conducted in the spring or early summer and all transmitters were removed in the spring after approximately one year of use. Researchers also attached external radio transmitters (3.6 g R1680 transmitters, ATS, Isanti, Minnesota) using Tegaderm ™ tape (K. Michelle, personal communication). Radio-tracked individuals were monitored approximately twice per week throughout the 2011 and 2012 activity seasons although some individuals were not located for longer periods of time due to extreme movements in difficult terrain.

No systematic mark-recapture was conducted at VRUT1 to minimize disturbance to individuals at this population. However, a small number of individuals were captured and marked while collecting genetic samples or conducting health assessments.

New Hampshire

We obtained data from the last confirmed population of timber rattlesnake in New Hampshire (hereafter NH). Population monitoring suggests that the New Hampshire population has maintained approximately 40 individuals from 1995–2005 (Taylor & Marchand 2006). Population surveys after 2006 suggest that the population may have declined by as much as 50% between 2006 and 2007 due to a bacterial-fungal skin infection (i.e., snake fungal disease [SFD], Lorch et al. 2016) presumably facilitated by unusually wet weather from 2005–2006 (Clark et al. 2011). Additionally, this population exhibited markedly lower genetic diversity relative to populations in eastern New York and individuals were found with unusual phenotypes (e.g., piebald coloration and pink tongues, Clark et al. 2011).

Population monitoring occurred at varying intensities from 1992–2016. Prior to 2006, surveys were informal and mark-recapture data were not collected although some individuals were marked by painting their rattles. Formal mark-recapture data were collected beginning in 2006 at which time radio telemetry was also used to monitor select individuals. Multiple visual encounter surveys were conducted throughout the activity season (April–November) focusing around the den entrance and at known basking and gestation sites. Unmarked individuals were captured, if needed, and marked by painting rattles and/or marked with PIT tags (Avid Identification Systems, Inc., California). Rattles were often painted without capturing the individual. Marked individuals were identified from either their painted rattle or by scanning the individual with a PIT tag reader on an extended pole to minimize disturbing the individual. The health of all marked individuals was visually assessed when possible and select individuals in poor health or with noticeable injuries were brought into captivity for treatment. Marked individuals were classified individuals as “Adult” or “Sub-Adult” based on approximate total length. Additional details on field procedures are provided in Clark et al. (2011).

Select individuals were monitored over the course of the study using surgically implanted radio transmitters (5 g SB-2T and 9, 11, and 13 g SI-2B transmitters, Holohil Systems Ltd., Carp, Ontario, Canada) or external radio transmitters (3.6 g R1680 transmitters, Advanced Telemetry Systems, Isanti, Minnesota). Transmitter surgeries followed standard surgical procedures (Reinert & Cundall 1982). External transmitters were attached using Tegaderm™ surgical tape. Radio-tracked snakes were visually located every 3–6 days. Internal transmitters for some individuals were replaced prior to battery failure to extend their tracking duration across multiple years.

Massachusetts

We obtained data from three populations of timber rattlesnake in Massachusetts. The first population was a meta-population in the Berkshire Mountains of southwestern Massachusetts, Berkshire County (hereafter MBER). This metapopulation is continuous with denning areas in New York and therefore some individuals within this metapopulation had activity season home ranges within New York. Within this metapopulation we identified four major denning areas (hereafter subpopulations) from which we collected data. We refer to these subpopulations as MBER1, MBER2, MBER3, and MBER4 following Stengle (2018, see Chapter 2 for additional details). Most of the land used by the population is within state parks and private property, with approximately 80 km^2 of habitat available. This region is one of the largest tracks of old growth forest in New England most of which is unused by the public with few low-traffic (mostly dirt) roads.

The second population is located in eastern Massachusetts in Norfolk County (hereafter MNOR). This population consists of about 2.5 km^2 of available habitat bounded on all sides by paved highways and urban development. This site is second growth mixed deciduous forest and subject to heavy recreational use with approximately 200,000 visitors per year. The third population is located in central Massachusetts in Hampden County (hereafter MHAD1).

We used data collected at MBER from 2009–2014 using a combination of mark-recapture (all years) and radio telemetry (2009–2013). Researchers searched for rattlesnakes around known denning areas during spring egress and fall ingress and around gestation and shedding sites throughout the year. Several individuals were encountered opportunistically during radio telemetry field work. Individuals were marked with PIT tags (Biomark, Inc., Boise, Idaho) and researchers recorded sex, snout-vent length (SVL), tail length, weight, and the number of rattle segments. Researchers assessed the reproductive condition of females via palpation and estimated the number of follicles/embryos. We used data collected from MNOR from 2014–2015 using both mark-recapture and radio-telemetry. Data from MHAD1 were collected using mark-recapture from 2010–2017.

A certified veterinary technician surgically implanted radio transmitters (SI-2T transmitters, Holohil Systems Ltd., Carp, Ontario, Canada) into 20 individuals at MBER and 13 individuals at MNOR following standard surgical procedures (Reinert & Cundall 1982). Transmitters were <5% of an individual's body mass and individuals were released after 24–48 hours in captivity. All surgeries were conducted in the spring or summer and attempted to remove transmitters in the spring prior to battery expiration. Radio-tracked individuals were monitored approximately weekly throughout the activity season although some individuals were not located for longer periods of time due to extreme movements in difficult terrain. See Stengle (2018) for additional details on radio telemetry methods for MBER.

Connecticut

We used two data sets collected from a timber rattlesnake population in Hartford County, Connecticut (hereafter CHAR) separated by a roadway. This population consists of two major den openings on the southern side of the road and one major den opening on the northern side. The first data set comes from a mark-recapture and radio telemetry study conducted on the south side of the road from 1998–2000. During this time, individuals were captured and marked with PIT tags (Biomark, Boise, Idaho) and the individuals' sex, snout-vent length, weight, and pregnancy status was recorded. Individuals were classified as adults, juveniles, or neonates. For neonates, the identity of the mother was recorded when possible. Two individuals originally classified as neonates were captured in the spring so we reclassified them as juveniles to ensure that neonates were individuals captured prior to their first winter. Select individuals received surgically implanted radio transmitters (Advanced Telemetry Systems, Isanti, Minnesota) and were located multiple times during the activity season. Transmitters were removed at the end of the study in the spring of 2001.

The second data set consisted of counts of gravid females from the den opening on the northern side of the road from 1991–2017. Counts were made approximately once per month during the active season and represented the estimated total number of gravid females in the population each year.

Survival Analysis

Combined Mark-Recapture and Radio Telemetry Data

We estimated annual survival for NH using use Burnham's joint recapture-recovery model (Burnham 1993). This model incorporates recoveries of dead individuals and recaptures of live individuals to provide an estimate of true survival (S). Burnham's model uses a live-dead (LD) capture history where a one in the first entry indicates a live recapture/recovery and a one in the second entry indicates a dead recovery. Live recaptures are assumed to occur over short durations relative to the intervals between capture occasions. Burnham's model also estimates the recapture or resighting probability conditional upon being alive and within the study area (p), fidelity (F), which is the probability of remaining within the study area, and recovery probability (r), which is the probability of recovering a dead individual during intervals between capture occasions. Estimates of *F* are only valid if the spatial area in which individuals are marked is a subset of a larger area wherein individuals can be recovered. Because individuals could be marked and recovered throughout our study area we fixed *F* = 1 and interpreted *S* as apparent, rather than true, survival (Burnham 1993). Individuals whose transmitters did last the winter were identified in the data as those with subsequent LD encounters as either 10 (the radio-tracked individual did emerge and was recaptured the following spring) or 11 (the radio-tracked individual did not emerge and was therefore considered to have died over the winter).

We used the Cormack-Jolly-Seber model (CJS) to estimate annual apparent survival (Φ) and probability of recapture (*p*) for VRUT2, MBER, and CHAR (Cormack 1964; Jolly 1965; Seber 1965). We did not use Burnham's joint recapture-recovery model because of small numbers of dead recoveries. The CJS model uses LL capture histories where a one indicates an individual was observed and a zero indicates that it was not observed on a given capture occasion. The CJS model cannot distinguish between permanent emigration and mortality but given the isolation of our populations and the high den fidelity of timber rattlesnakes (Anderson 2010; Clark et al. 2008) we suspect that emigration is negligible.

We fixed *p* and *r* = 1 for capture occasions and intervals, respectively, when an individual carried a functioning radio transmitter (we assumed that we would always recovery a dead individual with a functioning transmitter) and fixed *S* = 1 for NH individuals while in captivity. However, because some individuals were recaptured prior or subsequent to receiving radio transmitters we needed to fix these parameters in a temporally-varying manner. We used two different approaches for the Burnham and CJS models. For the Burnham model, we used multiple binary time-varying individual covariates (TVIC, Dinsmore et al. 2002; Rotella 2004; Terhune et al. 2007). These approaches do not truly fix real parameter estimates but rather force a large numerical beta estimate on the logit scale which corresponds to a real parameter value of one. We used Fixed_p to force *p* = 1 on capture occasions where an individual was located using radio telemetry or in captivity, Captivity to force *S* = 1 for intervals where an individual was in captivity, and Fixed _r to force *r* = 1 for intervals where an individual was located using radio telemetry. We did not set Fixed_r for intervals with external transmitters unless the transmitters remained attached to the individual and active throughout the entire occasion (snake #23 only). We set Fixed_r = 0 for intervals prior to the first occasion where the transmitter died because internal transmitter lasted the majority of the interval between sampling occasions. An example of this would be where a transmitter died over the winter and therefore we were unable to determine if the individual emerged from the den the following spring. To test for differences in apparent survival between radio-tracked and PIT-tagged individuals, we created a third binary TVIC, Transmitter, to denote intervals where an individual carried a radio transmitter regardless of whether the transmitter was functioning or not. Because of transmitter failures we were unable to remove the transmitters from some individuals and we therefore assumed that these individuals carried their transmitters through the duration of the study. Finally, to test for differences in recapture probability before and after an individual was radio-tracked (e.g., Breininger et al. 2012), we created a fourth binary TVIC, PostTrans, to denote capture occasions after an individual was radio tracked. If an individual's transmitter died and was replaced on a subsequent occasion, we set PostTrans = 0 during occasions in which the individual was located using the second radio transmitter.

As an example, consider an individual first captured on occasion one, recaptured and brought into captivity on occasion two, released with a radio transmitter on occasion three, tracked until occasion six after which its transmitter battery died, recaptured on occasion eight but then found dead prior to occasion nine, its LD encounter history and corresponding TVIC would be:

ch	Fixed_p	Fixed_r	Captivity	Transmitter	PostTrans
1010101010100011	00111100	01111100	01000000	00111111	00000011

As another example, an individual captured on occasion one, recaptured on occasions two and three, released with a radio transmitter on occasion three, tracked through occasion four after which its transmitter expired, opportunistically capture on occasion five and given a new transmitter, tracked through occasion six after which its transmitter expired, and then opportunistically located on occasion eight would have an LD encounter history and corresponding TVIC as follows:

ch	Fixed_p	Fixed_r	Captivity	Transmitter	PostTrans
1010101010100010	00010100	00101000	00000000	00111111	00001011

We were unable to use TVIC to fix real parameter values in the CJS models so we split the capture histories of these individuals into multiple histories to denote the capture occasions where the individual carried a transmitter and those where it did not and created a binary covariate (Capture) to denote whether an individual was radio tracked on a given capture occasion. We then manually fixed *p* = 1 for the capture histories of radio-tracked individuals. We also created a three-level covariate (PostTrans) to identify individuals that were recaptured after their radio transmitters had expired. For the VRUT2 analysis, we also created a four-level covariate (PostTrans2) to further identify post-transmitter recaptures of individuals that received a radio transmitter on their first capture and individuals that were recaptured at least once prior to receiving a radio transmitter. We created another two-level covariate (Transmitter) do denote capture histories during which individuals carried radio transmitters to test for differences in Φ between radio-tracked and non-radio tracked individuals. If we were unable to confirm that a transmitter was removed we assumed individuals carried transmitters for the remainder of the study. For CHAR, we assumed that individuals radio-tracked for two consecutive years were recaptured on the second spring occasion with functioning radio transmitters and fixed *p* = 1. We also assumed that all recaptures after an active season where an individual was radio tracked were made without the assistance of radio telemetry (i.e., were equivalent to recaptures of PIT-tagged individuals) and did not fix *p*. For each individual, we censored all capture histories up to and including the capture history where the individual had its transmitter. For example, if an individual was captured without a transmitter on occasions two and four, received a transmitter on occasion four, carried a transmitter until occasion six, and then was not recaptured, the original encounter history would be:

01011100

and the “split” capture history and corresponding value for Capture, PostTrans, PostTrans2, and Transmitter would be (where -1 denotes censored histories):

ch	freq	Capture	PostTrans	PostTrans2	Transmitter
01010000	-1	PIT	PIT	PIT	None
00011100	-1	Telemetry	Telemetry	Telemetry	Transmitter
00000100	1	PIT	PostTrans	PostTrans2	Transmitter

Similarly, an individual radio tracked on occasions one and two whose transmitter failed, then was recaptured on occasion four and radio tracked until occasion six would have “split” encounter histories and Capture, PostTrans, and Transmitter values of:

ch	freq	Capture	PostTrans	PostTrans2	Transmitter
11000000	-1	Telemetry	Telemetry	Telemetry	Transmitter

01010000	-1	PIT	PostTrans	PostTrans1	Transmitter
00011100	-1	Telemetry	Telemetry	Telemetry	Transmitter
00000100	1	PIT	PostTrans	Not observed	None

For each study we grouped captures into spring and fall capture occasions. Because of population-specific differences in activity season length we allowed the duration of each capture occasion and the corresponding summer and winter intervals to vary as follows: VRUT2, spring = 17 April–18 July, fall = 19 July–18 October; NH, spring = 15 April–27 July, fall = 28 July–8 November; MBER, spring = 15 April–16 July, fall = 17 July–25 October; CHAR, spring = 15 April–18 July, fall = 19 July–19 October. This resulted in 22 capture occasions for NH, 10 capture occasions for MBER, and six capture occasions for CHAR. For VRUT2, after the fall of 2014 only two recaptures were made in the spring of 2015 and four recaptures were made in the spring of 2017. To retain these observations, we collapsed the intervals between the spring 2015 and fall 2016 occasions into a single 2-year interval. We used spring 2017 as the final capture occasion resulting in a total of 10 capture occasions. We calculated the length of the summer and winter interval using the midpoint of each capture occasion. We then incorporated the length of these intervals into the survival analysis so that all estimates of S/Φ represent annual estimates of apparent survival. We chose to report annual estimates rather than monthly or seasonal estimates both for consistency with previous studies of snake survival and because most populations showed little evidence of season-specific survivorship (except NH, see Results). Because of insufficient recaptures we excluded all individuals ≤1-year old or captures made of individuals when they were ≤1-year old for all populations except CHAR. We identified ≤ 1 year old individuals as those ≤2 rattle segments including the button. Our survival estimates therefore apply to individuals >1-year old.

Because the CJS model cannot directly incorporate mortalities, we specified mortalities of radio-tracked individuals by using a single capture history and keeping p fixed at one. We observed several probable mortalities from individuals that did not emerge in the spring or whose transmitters were found on the ground. While one individual at MBER was confirmed to have expelled its transmitter, we thought it far more probable that the aforementioned examples represented mortalities rather than transmitter expulsions. Furthermore, we reasoned that, for a species of conservation concern, it is more conservative to assume that these individuals died and to underestimate survival than to censor those individuals after their last confirmed capture and overestimate survival. Two radio-tracked individuals in MBER were not located during one capture occasion (PIT # 4A630E5112 and 4A6018326B, both in Spring 2012) while they were radio-tracked due to a combination of extensive movements and logistical constraints. Both individuals were located using radio-telemetry in Fall 2012. To account for the non-detection while both individuals were radio-tracked and *p* fixed to one, we split the encounter history during which each individual was radio-tracked and censored the capture history prior to Spring 2012.

Because of data limitations we evaluated a limited number of covariates within our models. For each population, we modeled *p* as a function of Year, Season (spring and fall), and a linear trend effect of time. We also used a fully time-dependent term (time) to model *p* in VRUT2 and an effect of subpopulation (Den) for MBER. We also used SVL and Sex for MBER and VRUT2 but were unable to do so for NH or CHAR because these data were not collected for a sufficient number of individuals. Instead we modeled *p* as a function of age for CHAR using multiple covariates. For NH, we also modeled *p* as a function of binary covariate denoting whether or not an individual had ever been brought into captivity (Captive) and age (Age). We used a covariate denoting age at first capture with ≤1-year olds combined with neonates (Age). Because numbers of recaptures of juveniles and neonates were relatively low we also used a binary covariate to denote adults and juveniles/neonates (Adult). We also used two age-dependent structures for neonates that allowed neonates and ≤ 1 year olds to transition into juveniles. The first age-dependent structure allowed neonates and ≤1-year olds to transition into juveniles after reaching 1 year in age (Neo_AgeDep_p) while the other simply allowed them to transition to juveniles on the third capture occasion following their initial capture (Neo_age_p). Because we could not accurately age all juveniles we assumed that juveniles would remain juveniles for the duration of the study. We included terms with additive and/or interactive effects of our temporal and age covariates although data sparseness prevented us from evaluating some complex interactive effects. We modeled *r* for NH as a function of Age, Season, and Time.

For each population, we modeled Φ as a function of Transmitter, Season, and Time. For VRUT2, we used a three-level Season covariate where the 2 year interval between the spring of 2015 and 2017 represented its own level. We also modeled Φ as function of Sex and SVL for MBER and VRUT2, Age for NH, and Year and age for CHAR. For CHAR, we again evaluated multiple age-dependent structures to estimate Φ for adults, juveniles, neonates (including ≤1-

year olds), adults and juveniles combined, and juveniles and neonates combined. Where possible, we evaluated additive and interactive effects of temporal and age covariates. For all analyses we *z*-score standardized SVL to have mean = 0 and SD = 1.

We fit our models using MARK v.8.2 (White and Burnham 1999) through the package *RMark* (v.2.2.4 , Laake 2013) in R (R Core Team 2017). We evaluated model goodness-of-fit using the median c-hat ($\hat{c}$) procedure and a global model (Cooch & White 2017). Because we had insufficient data to estimate fully-time dependent models we used a highly parameterized model as the global model. Because the median $\hat{c}$ procedure does not accommodate individual covariates or TVIC we reclassified SVL into two- or three-level categorical covariates and, for NH, used a binary covariate denoting whether or not an individual ever carried a radio transmitter to approximate the effect of fixing p and $r = 1$. We first estimated $\hat{c}$ using a large upper bound with few intermediate and replicate points and then used our preliminary estimate of $\hat{c}$ to estimate our final $\hat{c}$ using a lower upper bound with more intermediate and replicate points. If $\hat{c} > 1$ we compared candidate models using AIC adjusted for small sample sizes and overdispersion (QAIC$_c$) and adjusted our standard errors by $\hat{c}$ (Burnham & Anderson 2002). Otherwise we compared models using AIC$_c$.

We used a multi-step approach to select the best covariate structure for each model parameter. First, we held Φ constant using a global term and modeled p as a function of multiple *a priori* terms while fixing $p = 1$ for radio-tracked individuals. We retained the terms for p with Δ(Q)AIC$_c$ ≤ 4 (ΔQAIC$_c$ ≤ 2 for CHAR, see Results) and then modeled Φ as a function additional *a priori* terms. For NH, we added a step whereby S and p were held constant using global terms and modeled r as a function of covariates. We then retained the terms for r with Δ(Q)AIC$_c$ ≤ 4 and modeled p and S as described above. We calculated model-averaged estimates of r, p, S, and Φ and their 95% CI across the final model set. For populations other than NH, we used *RMark*'s default drop = TRUE argument when model averaging to exclude models with non-positive variances (i.e., models with convergence issues) although the results were virtually identical to those using drop = FALSE. We used drop=FALSE for NH because all non-positive variances were due to our use of TVIC to fix r and p.

To obtain annual survival estimates for the population viability analyses we re-ran our models excluding temporal effects on apparent survival and calculated model-averaged estimates of annual apparent survival. We modeled p and r using the terms with Δ(Q)AIC$_c$ ≤ 4 (ΔQAIC$_c$ ≤ 2

for CHAR). Because the variance of survival estimates is comprised of both the variance due to sampling error and random environmental variation (i.e., process variance, Burnham et al. 1987; Gould & Nichols 1998), using the estimated variance in population viability analyses can overestimate the probability of extinction (Gould & Nichols 1998). While the process variance can be estimated using variance components analyses (Burnham 2012; Burnham & White 2002), this approach requires relatively long-term data sets (>10–15 sampling occasions) and cannot be implemented with unequal sampling intervals (Burnham 2012). We therefore used the standard errors of model-averaged point estimates as estimates of survival variance and emphasize that these estimates are likely to overestimate the variance in annual survival.

Radio Telemetry Data

We estimated survival for 13 radio-tracked adults at MNOR using known-fate models (Cooch & White 2017). Individuals were monitored between May 2014 and May 2016. Known-fate models use LD capture histories where 10 indicates the individual survived the occasion and 11 indicates the individual died sometime during the occasion. Occasions where individuals were not located or their fates otherwise unknown are censored from the estimation using 00. We divided our sampling period into 8 one-month occasions, one winter occasion of 7.5 months (1 October 2014 through 15 May 2015), and one winter occasion of 8.5 months (15 September through 1 June 2016) to account for staggered entry of individuals into the study and occasional lapses in active season monitoring. Because known-fate males assume the fates of individuals are known at the beginning and the end of each occasion we considered an individual to have survived an occasion if it was located alive in both the first and last halves of a month. Because of our small sample sizes we only evaluated models with constant survival (S) and survival differing between the activity season and winter. Two individuals (radio #533 and #945) were held in captivity prior to release for extended periods of time receiving medical treatment. We therefore reran our analyses excluding these individuals.

Population Demographics

Activity Season Length

We calculated activity season length following Brown (2008). Specifically, we calculated the day-of-year for each observation (1–365) and took the difference between a lower and upper quantile as activity season length. We used the 2.5th and 97.5th quantiles (95th range) following Brown (2008) and also the 1st and 99th quantiles (98th range) to examine the sensitivity of our results to quantile value. Including neonates caused quantile dates to vary by ≤ 1 day so we included neonates following Brown (2008). We calculated bootstrapped 95% CI for activity season length, egress date, and ingress date using 10,000 iterations sampled with replacement. Because most observations were from radio-tracked individuals, we also calculated activity season length excluding all observations of radio-tracked individuals except their first observation. We used Spearman's rank correlations to test for a correlation between activity season length, egress date, and ingress date and latitude.

Sex Ratio and Color Phase

We report sex ratios as the ratio of females to males and tested for unequal sex ratios using chi-square goodness-of-fit tests. Because field effort at VRUT2 was greater during the radio telemetry study from 2011 through the spring of 2013, we also report sex ratios from this time period. Where possible, we report sex ratios by age classes. However, because we were only able to confidently age individuals ≤1-year old in most populations (except CHAR), we refer to all individuals >1-year old as adults unless otherwise noted. We also tested for unequal ratios of yellow- and black-phase individuals using chi-square goodness of fit tests. For populations including multiple den openings or subpopulations, we also tested for unequal sex and color phase ratios for each den opening or subpopulation separately.

Body Size and Condition

Within each population, we tested for differences in SVL and weight between sexes using either t-tests or non-parametric Wilcoxon signed rank tests. Unless otherwise noted, we report means and one standard error (SE) and the inter-quartile range (IQR). We modeled log-transformed weight as a function of log-transformed SVL as a measure body condition and used a general linear model to test if body condition (i.e., slopes) differed between sexes (e.g., Jenkins et al. 2009). Because SVL or weight was not recorded for all individuals sample sizes varied among tests.

To compare body size and condition among populations, we calculated a body condition index (BCI) by taking the residuals of a simple linear regression of log-transformed weight and log-transformed SVL. We then tested for sex- and population-specific differences in SVL and body condition using general linear models. We excluded all neonates and individuals with ≤2 rattle segments and the button (i.e., all individuals ≤1-year old). We also excluded individuals with SVL <50 cm which included two individuals from VRUT2 and one individual from MHAD1. We evaluated five candidate models including additive and interactive effects of sex and population on SVL and BCI as well as a null (intercept-only) model. We compared models using AIC_c and calculated model-averaged predicted values of SVL and BCI for each sex and population. The variances of our model residuals were approximately equal among covariate classes but our model residuals were consistently non-normal. We therefore calculated bootstrapped 95% CI around our model-averaged predicted values by resampling our data with replacement 10,000 times, fitting our candidate models, calculating model-averaged predictions, and then taking the 2.5th and 97.5th quantiles. We also compared neonate SVL and weight among populations. We compared a model with a population effect and a null model using AIC_c and report model-averaged predicted values for each state with bootstrapped 95% CI.

Shedding Rates

Where researchers painted basal rattle segments (VRUT2), we estimated shedding rates using field notes recording the number of rattle segments since the last painting. Where basal rattle segments were not painted or where it was unknown if researchers painted basal segments (CHAR), we estimated shedding rates from the data notes providing the dates of observed or inferred sheds for marked or radio-tracked individuals. Because of evidence at CHAR that individuals would shed in the spring, we only included shedding information if the individual was observed prior to July of a given year and either the fall (September or later) of the same year or in the early spring (April or May) of the following year. We also included an individual at CHAR that shed once between July 1998 and July 1999 because this would represent one shed in approximately one activity season.

Reproduction Frequency

We report the SVL of the 10 largest females and 10 smallest gravid females following Jenkins et al. (2009). When possible, we used the SVL of the smallest gravid female as the minimum size at reproduction. For CHAR, we considered all adult females potentially reproductive and for NH we used number of female captures provided by the state as the number of potentially reproductive females. At CHAR (southern major den openings), researchers recorded the reproductive status of females when possible. However, they did not report how reproductive status was determined and the reproductive status of several females was marked as uncertain (i.e., with a question mark, “?”). We therefore only used observations of females where reproductive status was not marked as uncertain. We used the captures of neonates with known mothers to estimate fecundity.

We used two approaches, when possible, to estimate reproduction frequency. First, we calculated the ratio of gravid to non-gravid females for each year and for all female-years. For example, if 10 gravid females and 30 non-gravid females were observed one year and 5 gravid females and 20 non-gravid females another year, the yearly ratios would be 1:3 and 1:4 and the overall ratio would be 1:3.33 suggesting reproduction intervals of 3, 4, and 3.33 years, respectively. However, this approach may underestimate the frequency of reproduction (Brown 1991; Macartney & Gregory 1988) particularly for during short-term studies. Our second approach was to estimate reproduction frequency directly by recording the reproductive status of females over multiple years.

Where researchers assessed reproductive status using palpation we report the mean number of follicles/embryos (SE) and the range. When possible, we estimated litter sizes from observations of neonates with a female when we were confident that all neonates were from the same litter.

Gravid Female Counts

We tested for a linear trend effect of year on counts of gravid females from the northern major den opening in CHAR using generalized least squares regression. Because time series data is temporally correlated we tested to see whether including correlation structures improved model

AIC. We evaluated compound symmetry, first-order autoregressive, and autoregressive moving average with 1, 2, and 3 parameter correlation structures (Zuur et al. 2009).

Movement Metrics

While our objective was not to describe timber rattlesnake spatial ecology in our study populations we calculated and report select movement metrics from VRUT2 to help understand the relationship between body size and movement metrics. For each individual with an internal radio transmitter, we calculated the total distance moved and maximum displacement as the straight-line distance between the individual's den and its furthest point from the den. We compared these metrics between males and non-gravid females using Wilcoxon signed rank tests and used general linear models to test for relationships between movement metrics and SVL. Detailed movement analyses for MBER are provided in Stengle (2018).

Population Growth Rate and Abundance

The Jolly-Seber (JS) model (Jolly 1965; Seber 1965) and its several derivations (reviewed in Cooch & White 2017) can estimate population abundance and growth rate using data from open populations. These models assume equal capture probabilities of marked and unmarked animals and this assumption is violated in the presence of behavioral responses to capture and marking (e.g., "trap-shy" responses, Pollock et al. 1990; Williams et al. 2002). The presence of negative behavioral responses by timber rattlesnakes to capture and marking (i.e., the "spook" factor) hypothesized by Brown (2008; Brown et al. 2007) could potentially violate this assumption. Since we are currently unable to robustly assess the validity of the assumption of equal capture probabilities in our study populations we recommend caution when drawing inferences from our analyses using JS models. Additionally, JS models assume that the size of the study area remains constant throughout the study which was likely violated to some extent within each population because some individuals were captured away from the den during the summer.

To fit JS models we used data from PIT-tagged individuals because the inclusion of radio-tracked individuals violates the assumption of equal capture probabilities. Because most JS models cannot accommodate age-dependence we accounted for differences in age among individuals at CHAR by assigning all neonates and ≤1-year-olds into one of three cohorts based on their birth year (i.e., 1998, 1999, 2000). We then gave all individuals ≤ 1 year old the same capture probability after which they were allowed to transition into the juvenile age class. We left-censored all neonates prior to their capture occasion of birth to ensure those individuals were not present in the population prior to their birth. We also modeled data from CHAR using only PIT-tagged juveniles >1-year old and adults.

We attempted to fit our data using the original JS model formulation in the program JOLLY (https://www.mbr-pwrc.usgs.gov/software/jolly.shtml, Pollock et al. 1990), the POPAN model (Schwarz & Arnason 1996), and Pradel's model for estimating population growth rate (Pradel 1996). JOLLY allows users to fit a fully time-dependent model for apparent survival and capture probability and a model with constant apparent survival and time-dependent capture probability The POPAN model assumes the presence of a "super-population" (*N*) consisting of all individuals that enter the population and estimates the probability of an individual entering (e.g., being born) the population during each interval (*b*). In addition to *N* and *b*, the POPAN model estimates apparent survival (Φ) and capture probability (*c*). Estimated population size for each capture occasion is calculated as a derived parameter. Pradel's model estimates population growth rate (λ) based on the net effects of survival and recruitment although this model does not provide abundance estimates (Cooch & White 2017; Pradel 1996).

We were unable to fit the JS or POPAN model to any of our data sets, presumably due to small sample sizes and low recapture rates. We were unable to fit Pradel's model to the CHAR data but were able to do so for the VRUT2 and MBER data. We therefore modeled apparent survival (Φ), capture probability (*p*), and population growth rate (λ) in *RMark* (Laake 2013) using a multi-step process similar to that used in the survival analyses. Specifically, we held Φ constant using a global term (Φ(Sex+Time) for MBER and Φ(Sex*Time) for VRUT2) and modeled *p* as a function of Sex, Year, Time, Season, and Den (MBER only). We then retained the terms for *p* with ΔAICc ≤4 and then modeled Φ as a function of Sex, Time, Season, and Study (VRUT2 only). We either held λ constant or allowed it to vary by sex. We calculated model-averaged estimates of λ.

Closed population models assume demographic and spatial closure (i.e., no births/deaths and no immigration/emigration, respectively, Otis et al. 1978; Pollock et al. 1990) and consequently are generally unsuitable for multi-year data. An additional complication using data from timber rattlesnakes is that most of our captures were made around dens during spring egress and fall ingress. As a result, the proportion of the total population within the sampling area is not constant representing a violation of the assumption of spatial closure. Nevertheless, because of the need to specify starting population size in population viability analyses, we used closed-population models to estimate total population abundance. We excluded radio-tracked individuals and individuals ≤ 1 year old. We used full likelihood and conditional likelihood (Huggins 1989) models with two finite mixtures (model type “FullHet” and “HugFullHet” for full and conditional likelihoods, respectively) in *RMark* (Laake 2013) and evaluated models with and without mixtures, behavioral responses (i.e., $p \neq c$), and time-varying capture probabilities. When finite mixture models did not converge we used models without mixtures (model type “Closed” or “Huggins”). We obtained model-averaged estimates and 95% CI of the number of unmarked individuals in the population (f_0) and added the number of marked individuals to obtain estimates of total population size. We stress that these estimates are only intended to provide rough estimates of initial population size for population viability analyses and should not be interpreted as absolute estimates of population size.

Population Viability Analyses

We conducted population viability analyses for VRUT2, NH, MBER, and CHAR using Vortex 10.2.15 (Lacy 1993; Lacy & Pollak 2017). Vortex is an individual-based stochastic modeling software that incorporates environmental and demographic stochasticity and inbreeding depression when simulation populations. We used demographic parameters estimated from this study when possible to parameterize our simulations (Appendix 1). However, because we lacked the data to reliably estimate many parameters, we substituted parameter values from a nearby metapopulation of timber rattlesnakes in the Adirondack Mountain of eastern New York (Aldridge & Brown 1995; Brown 1991; Brown 2008; Brown 2016; Brown et al. 2007). In particular, and because our survival analyses were dominated by adults, we used estimates of survival from Brown et al. (2007) for all 0–4-year old individuals. Because Brown et al. (2007) reported survival estimates separately for black- and yellow-phase individuals, we used the median survival and SE for each age class. We acknowledge that this assumes transferability of parameter estimates across our study region.

Because our estimates of abundance were for individuals > 1 year old and most of our captures were of adults, we used initial population sizes of 100%, 250%, and 500% of our closed population model estimates of abundance and assumed a stable age distribution in all cases. We assumed a carrying capacity of ten times the lowest value of initial population size. We simulated MBER and CHAR as single populations because we lacked data on dispersal among subpopulations. Because of the small size and genetic isolation of our populations, we ran simulations with and without the effects of inbreeding depression using the default parameter settings. We retained the default parameters for lethal equivalents (6.29) and % due to recessive alleles (50%), a practice used in other recent studies (Harrisson et al. 2016; Jensen et al. 2017). However, other authors have recommended the use of higher lethal equivalents for wild populations so we re-ran our simulations using 12 lethal equivalents (Frankham et al. 2014). The use of lethal equivalents in our simulations or the number of lethal equivalents did not have a strong influence on population persistence so we only report results including inbreeding depression in detail. We assumed a polygynous mating system, that all males were available for breeding each year, an equal sex ratio at birth, and no correlation in environmental variation between reproduction and survival. We also excluded catastrophes from our simulations because of a lack of empirical data on such events. We used 1000 iterations for each population/parameter set with 100 years for each simulation except for sensitivity tests where we used 500 iterations to reduce computational times. Because timber rattlesnakes can live over 35 years, we considered a population to have gone extinct if only sex remained. Finally, we also performed a PVA using parameters from Brown’s eastern New York metapopulation. We report the mean simulated rate of population growth (*r*) and its SD, the proportion of runs where the population went extinct, and the median time until extinction.

Rather than conduct a full sensitivity analysis we focused on examining how changes in survival affect population persistence. Other studies (Gregory 2009) and our preliminary analyses showed that reproduction frequency and fecundity had little impact on population persistence compared to survival. We therefore ran Vortex after adjust adult and/or juvenile survival while holding all other parameters constant.

Results

Mark-recapture and Radio Telemetry

Vermont

Twenty-two individuals were marked at VRUT1 between 7 July 2012 and 13 June 2014, including four neonates, none of which were recaptured. A total of 151 individuals were marked at VRUT2 between 7 May 2011 and 24 September 2014. Of 123 marked individuals that never received radio transmitters, seven (6%) were recaptured at a later capture occasion. Two individuals were recaptured on 4 May 2015 and nine individuals were recaptured between 12 May and 3 July 2017. Twenty-four neonates were marked, 18 of which were marked between 21 September and 10 October 2011, as well as four 1-year olds. Researchers monitored six males in 2010 and 11 males, three non-gravid females, and two gravid females in 2011 using radio telemetry and internal radio transmitters for a single activity season (median number of days per activity season 148 days, range 108–171 days) and collected a median of 30 observations per individual (IQR 27–33 days, range 23–42 days). Mean SVL for radio-tracked individuals with internal transmitters was 109.1 cm (SE 2.16, 94.2–125.0 cm) and mean weight was 1,021 g (SE 48, 710–1,327 g). Researchers attached external radio transmitters to four adults in 2011 and two adults in 2012. Only two of these individuals were monitored for more than 10 days (60 and 64 days with 14 and 15 observations, respectively).

New Hampshire

A total of 100 individuals were captured and marked between 2006 and 2016 of which 67 (67%) were recaptured on a later capture occasion (i.e., a later spring or fall capture occasion). Of non-radio tracked individuals (*n* = 66), 33 (50%) were recaptured at a later capture occasion. Researchers also made 42 observations of unmarked snakes or snakes whose mark could not be identified. Researchers marked 30 adults, 68 sub-adults, and 2 neonates of which 23, 43, and one individuals were recaptured at a later capture occasion. Two sub-adults were initially captured as ≤1-year olds (i.e., captured in the spring with one rattle and the button) but both were recaptured >1 year later. Two neonates were observed in 2008, 46 in 2011, nine in 2013, 47 in 2014, nine in 2016, and no neonates in the other years from 2006–2016. Three individuals (two females and one male) were monitored for 2–264 days (range 2–12 fixes) using externally attached transmitters. Thirty-one individuals (12 females and 19 males) were monitored with surgically implanted radio transmitters. Researchers obtained 3–107 telemetry fixes per individual and monitored individuals over a total of 91–1,590 days although radio tracking was not continuous for some individuals due to transmitter failures.

Massachusetts

A total of 178 individuals were marked at MBER between 2009 and 2014 of which 20 received surgically implanted radio transmitters (Fig. 1). Of 158 PIT-tagged individuals, 15 (9%) were recaptured on a subsequent capture occasion. Twenty-five neonates and seven individuals ≤1-year old were also captured and marked although none were recaptured. Radio-tracked individuals were monitored for between one and five activity seasons (median = two activity seasons) over a median of 134 days per activity season (range 79–189 days) with a median of 12 locations per individual per activity season (IQR = 9–16 locations, range 1–24 locations). Mean SVL for radio-tracked individuals was 102.2 cm (SE 3.33, range 65.0–124.0 cm) and mean weight was 1,134 g (SE 87, range 527–1,711 g). Seventy-seven timber rattlesnakes observations were made at MAHD1 between 2010 and 2017 and 60 individuals were captured and marked. At MNOR, 42 individuals were captured and marked in 2014 and 2015, 13 of which received radio transmitters and were monitored for a median of 125 days per activity season (range 1–151) with a median of 29 locations per individual per activity season (range 1–36). Of these 13 individuals, six were monitored during both years. Six individuals were monitored over the winter of 2014 and two individuals were monitored over the winter of 2015.

Connecticut

A total of 177 timber rattlesnake observations were recorded and 147 individuals marked from the southern population from 1998–2000 (Fig. 1). Seventy-five individuals were marked in 1998, 51 in 1999, and 21 in 2000. Eighty-nine individuals (55%) were observed from the first major den opening and 46 were observed from the second major den opening (29%, $p < 0.0001$). Two individuals, one radio-tracked adult and one neonate/juvenile were recorded as switching den openings. Den opening was unknown for the remaining 24 individuals (15%).

Thirty-four individuals (17 males and 13 females) from the southern population received surgically implanted radio transmitters. Nine individuals were radio-tracked for two activity seasons and 25 individuals were tracked for one activity season with a median of 139 days per activity season (range 1–185 days) and 65 locations per individual per activity season (IQR 40–78 locations, range 1–101 locations). Mean SVL for radio-tracked individuals was 107.8 cm (SE 2.16, range 87.0–128.0 cm) and mean weight was 1,178 g (SE 83, range 527–2,075 g).

Survival Analysis

VRUT2

We included 122 individuals >1-year old from VRUT2 in our survival analysis including 22 and six individuals with internal and external radio transmitters, respectively. Two probable mortalities were observed at VRUT2. One non-pregnant female radio tracked rattlesnake was found dead of unknown causes in September 2012. Another male was found with severe degenerative scale rot on the ventral scales and was brought into captivity where it subsequently died. We considered this individual a mortality conservatively assuming that it would have died in the wild. Mean SVL of individuals included in the analysis was 102.4 cm (SD 15.64 cm, range 37.8–136.0 cm).

Our $\hat{c}$ using the model Φ(Sex*Time+Capture+Size)*p*(PostTrans2+Sex*Time*Size) as the global model was 1.06. The term for *p* containing Capture and an additive effect of SVL and Year had relatively high model support (*w* = 0.43, Table 1). All of the models in the 95th model set contained SVL and a temporal covariate (Year, Time, or time). Recapture probability increased with increasing SVL and was highest in 2011, 2012, and 2017 (Fig. 2). However, the 95% CI for *p* in Spring 2017 were very broad. For PIT-tagged individuals, *p* was generally low (<0.20) although it exceeded 0.20 in 2011 and 2012 for individuals ≥ 108.6 cm SVL and in Spring 2017 for individuals ≥49.18 cm. However, *p* only exceeded 0.15 for average-length individuals (SVL = 102 cm) in Fall 2011 and Spring 2017 and only exceeded 0.20 in Spring 2017 (Fig. 2). Recapture probability generally declined over the course of the study consistent with the greater level of field work associated with the radio telemetry study (Fig. 3). Recapture probabilities were very similar between individuals never receiving a radio transmitters and individuals captured after receiving a radio transmitter (maximum difference in model-averaged estimates = 0.008). Recapture probabilities were slightly higher for females than for males but not appreciably so (maximum difference in model-averaged estimates = 0.05).

Model support was relatively low across all models for Φ (max. $w = 0.05$, Table 2). Of the 42 models with $\Delta QAIC_c \leq 4$, 30 included SVL, 30 included a temporal covariate (Study or Time), 25 included Sex, and 15 included Capture. Apparent survival was highest for radio-tracked individuals and highest for males (Fig. 4). Model-averaged estimates for Φ were ≥0.74 (median = 0.83, max. = 0.95) for average-length individuals across all groups. The median model-averaged annual apparent survival for an average-length individual was 0.77 (range 0.74–0.80) and 0.86 (0.79–0.88) for PIT-tagged females and males and 0.85 (0.83–0.88) and 0.94 (0.94–0.95) for radio-tracked females and males. While there was support for temporal variation in Φ, the magnitude of variation in model-averaged estimates Φ for average-length individuals over time was relatively minor and did not vary consistently over time (Fig. 5). While model-averaged estimates for Φ indicated that Φ declined with increasing SVL, the 95% CI were very broad beyond approximately the mean SVL (Fig. 5). Size-specific variation in Φ for PIT-tagged individuals during 2011 and 2012 (range 0.48–0.84 and 0.54–0.90 for females and males, respectively) was less than later in the study (range 0.27–0.83 and 0.24–0.88 for females and males, respectively). Model-averaged estimates for Φ across all SVL for radio-tracked individuals in 2011 and 2012 ranged from 0.56–0.92 and 0.62–0.95 for females and males, respectively.

Model-averaged annual apparent survival estimates for PIT-tagged males and females (of average length) from models with no temporal effects were 0.719 (SE 0.1595) and 0.662 (SE 0.1503), respectively. Model-averaged annual apparent survival estimates for radio-tracked male and female from models with no temporal effects were 0.926 (SE 0.0680) and 0.893 (SE 0.0902), respectively. Because our time-invariant point estimates of survival for PIT-tagged individuals were less than any point estimates obtained from the complete analysis, we reran this analysis including Time and took the model-averaged annual apparent survival estimates from the midpoint of the study which were 0.776 (SE 0.2390) and 0.856 (SE 0.2032) for females and males, respectively. Model-averaged estimates for radio-tracked males and females were 0.925 (SE 0.1280) and 0.860 (SE 0.1741), respectively.

NH

We used data from 98 individuals >1-year old in our survival analysis. We observed six confirmed or highly probable mortalities and an additional 12 suspected mortalities. Number 3 was observed on 26 May and 14 June 2006 with a severe fungal skin infection and gaping mouth. This individual was never recovered but we assumed it died given the severity of its skin infection so we considered it a dead recovery in Spring 2006. Number 21 was last seen alive on 1 November 2006 and a skeleton was found on 1 May 2007 on the denning slope where it had repeatedly basked September and October 2006. We therefore assumed that the skeleton belonged to Number 21 and considered it a dead recovery in Spring 2007. Numbers 23 (radio-tracked) and 31 were last seen alive at the den on 21 Sep and 5 October 2006 and were confirmed predated on 22 and 30 May 2007, respectively. We also considered them dead recoveries in Spring 2007. Numbers B13 and F9-14 (both radio tracked) were observed May through July 2016 and confirmed dead of unknown causes on 18 and 16 August 2016, respectively, so we considered them dead recoveries in Fall 2016. While there may be some uncertainty as to the fates of Numbers 3 and 21 we felt confident that these individuals did die. An additional 12 radio-tracked individuals (Numbers 6, F6, WB9, F1, C4, WB14, F5-14, F6-14, WB2, T5-14, T6-14, and F7-14) did not emerge from the den in the spring. Because these individuals likely died during the winter (i.e., the interval between the fall and spring sampling occasions), we coded these individuals as dead recoveries in the fall with their last observed capture. Numbers F6 and WB9 died in the winter of 2012, F1 in the winter of 2013, 6, C4, F5-14, F6-14, WB2-14, T5-14, T6-14, and F7-14 in the winter of 2015, and WB4-14 in the winter of 2016. We reasoned that, for a species of conservation concern, it is more conservative to assume that these transmitters represented mortalities and to under-estimate survival than to censor those individuals.

We initially used the model *S*(Age*Time+Telemetry+Captivity)*p*(Age*Season+Year+Telemetry+Captivity)*r*(Age*Season+Telemetry) as our global model but the observed $\hat{c} = 1.00$ so we could not estimate the median $\hat{c}$ (Cooch and White 2017). Our estimated median $\hat{c}$ using the model *S*(Age*Time+Telemetry+Captivity) *p*(Age*Time*Season+Telemetry+Captivity)*r*(Age*Season+Telemetry) was 0.68. Given this consistent lack of evidence for over-dispersion and the high likelihood that the TVIC would greatly improve model fit, we compared candidate models using AIC_c. Six of the seven terms we considered for *r* had $\Delta\mathrm{AIC}_c < 4$ but the null model (containing only Fixed_r) had the greatest support ($w = 0.35$, Table 3). We retained these six terms for subsequent analysis. Recovery rate was relatively invariant (range 0.13–0.17 across the study) and similar between adults and sub-adults (maximum difference in model-averaged estimates = 0.026).

Year had the strongest effect on *p* with year being in the top 96 models (cumulative *w* = 1.00, Table 2). Recapture probability varied strongly and non-linearly amongst years (0.17–0.82, Fig. 6). Recapture probability was <0.20 only during 2012 when it was 0.17–0.19. Recapture probabilities were similar between adults and sub-adults (maximum difference in model-averaged estimates = 0.024) and between spring and fall (maximum difference in model-averaged estimates ≤ 0.001). Recapture probabilities were also very similar prior and subsequent to radio tracking (maximum difference in model-averaged estimates = 0.0005).

We found strong evidence for interactive effects of Age, Season, and Time on *S* (Table 5). Either an interactive effect of Age and Season with an additive effect of Time or an interactive effect of Season and Time were within the top 63 models with a cumulative *w* = 0.93. When only models with the terms *p*(Fixed_p+Year)*r*(Fixed_r) were considered, only three terms for *S*, *S*(Captivity+Age*Season+Time), *S*(Captivity+ Season*Time), and *S*(Captivity+Age*Season+Time+Transmitter), had ΔAIC_c <7 and these three models had a cumulative *w* = 0.93. There was no appreciable difference in *S* between PIT-tagged and radio-tracked individuals (maximum difference in model-averaged estimates = 0.002). Apparent survival during the summer increased for both age classes over the course of the study and was highest for sub-adults but winter apparent survival decreased for both age classes and was lowest for sub-adults (Fig. 7). Annual apparent survival was 0.28 (95% CI = 0.02–0.90) and 0.19 (0.02–0.74) for PIT-tagged sub-adults and adults in the summer of 2006 and 0.69 (0.14–0.97) and 0.84 (0.53–0.96) for PIT-tagged sub-adults and adults in the winter of 2006. By 2016, annual apparent survival was 0.98 (0.13–0.99) and 0.94 (0.20–0.99) for PIT-tagged sub-adults and adults in the summer and 0.59 (0.17–0.91) and 0.50 (0.26–0.74) in the winter.

Because we were not expecting such low summer adult survival and winter sub-adult survival, we conducted a *post-hoc* analysis exploring whether more complex interactions among Age, Season, and Time improved model fit. Specifically, we included the term *S*(Captive+Age*Season+Age*Time) in our list of candidate terms for *S*. Models with this *post-hoc* term had some support (min. $\Delta\text{AIC}_c = 2.77$, Table 5). However, the overall patterns for *S* with respect to age, season, and time remained consistent. With the post-hoc term for *S*, annual apparent survival was 0.40 (95% CI = 0.03–0.94) and 0.21 (0.03–0.71) for PIT-tagged sub-adults and adults in the summer of 2006 and 0.62 (0.12–0.95) and 0.83 (0.49–0.96) for PIT-tagged sub-adults and adults in the winter of 2006. By 2016, annual apparent survival was 0.97 (0.31–0.99) and 0.94 (0.27–0.99) for PIT-tagged sub-adults and adults in the summer and 0.51 (0.28–0.74) and 0.68 (0.15–0.96) in the winter.

For models with seasonal but no temporal effects, summer survival was 0.967 (SE 0.0353) for radio-tracked sub-adults, 0.961 (SE 0.0456) for PIT-tagged sub-adults, 0.870 (SE 0.0602) for radio-tracked adults, and 0.858 (SE 0.0589) for PIT-tagged adults. Winter survival was 0.606 (SE 0.0701) for radio-tracked sub-adults, 0.588 (SE 0.0768) for PIT-tagged sub-adults, 0.894 (SE 0.0765) for radio-tracked adults, and 0.891 (SE 0.0665) for PIT-tagged adults. For models with no temporal effects, annual apparent survival for PIT-tagged sub-adults was 0.572 (SE 0.0682) and 0.753 (SE 0.0498) for PIT-tagged adults. Annual apparent survival for radio-tracked sub-adults was 0.613 (SE 0.0682) and 0.775 (SE 0.0702) for radio-tracked adults.

MNOR

Two probable mortalities were observed at MNOR. One individual (radio #682) received a radio transmitter on 12 June 2014 and its transmitter was found several hundred meters from its last known location on the edge of a highway exit ramp. Another individual (radio #968) was confirmed to have survived the 2014 winter at the den but its transmitter was found 5 May 2015. The model with constant survival had more AIC_c support than the model with survival varying between the activity season and winter ($\Delta AIC_c = 2.08$). Annual survival from the null model was 0.805 (SE 0. 124, 95% CI = 0.47–0.95). Model-averaged monthly survival from activity season was 0.982 (SE 0.014, 95% CI = 0.92–0.996) and model-averaged monthly winter survival was 0.982 (SE 0.014, 95% CI = 0.92–0.996). When we excluded the two individuals that were kept in captivity for health treatments, the model with constant survival had more AIC_c support than the model with survival varying between the activity season and winter ($\Delta AIC_c = 2.13$). Annual survival from the null model was 0.798 (SE 0.127, 95% CI = 0.46–0.95). Model-averaged monthly survival from activity season was 0.981 (SE 0.015, 95% CI = 0.91–0.996) and model-averaged monthly winter survival was 0.982 (SE 0. 014, 95% CI = 0.91–0.996).

MBER

We included 145 individuals >1-year old in our analysis, including 20 radio-tracked individuals. Of 125 non-radio tracked individuals, 15 (10%) were recaptured. Mean SVL of MBER individuals used in the survival analysis was 96.2 cm (SE 1.54, range 52.0–138.0 cm). Seven confirmed or probable mortalities of radio tracked individuals were observed. Three radio tracked males from subpopulation MBER1 were found dead. Two individuals (PIT # 49392B5342 and PIT # 98517-475146) were found dead from unknown causes on 20 November 2013 and 5 August 2012, respectively. The third individual (PIT # 98517-449911) was apparently killed by a Red-Tailed Hawk (*Buteo jamaicensis*) on 8 Jul 2011. Two radio tracked individuals (PIT # 493E047B77, a female from MBER2; PIT # 4A065C1204, a male from MBER2) did not emerge from their dens in the spring. We also recovered the transmitters of two individuals (PIT # 4963252D10, a male from MBER2; PIT# 4A660B1F23, a female from MBER1) on 24 August 2009 and 23 August 2013, respectively.

Our $\hat{c}$ using the model Φ(Time+Size)*p*(Capture+Den+Sex+Size*Time) was 0.72 and other potential global models estimated $\hat{c} \leq 0.72$. Time had the greatest influence on *p* appearing in 10 of the top 11 models (Table 6). Snout-vent length and an interactive effect of Time and SVL appeared in six and five of the top 11 models, respectively, including the top two models. Recapture probability was greatest for below-average length individuals through approximately 2011 and then either remained constant across body sizes or showed a slight increase with increasing SVL towards the end of the study (Fig. 8). Den appeared in five of the top 11 models although the difference in *p* between the subpopulations with the highest and lowest *p* (MBER3 and MBER2, respectively) exceeded 0.10 only for individuals with SVL ≤97 cm in 2009, 2010, and Spring 2011 and for individuals ≥134 cm in 2014. The median difference in *p* between these two subpopulations was 0.04. Except for below-average length individuals at the beginning of the study, recapture rates were generally low. Median recapture rates at subpopulations MBER3 and MBER2 were 0.04 (range 0.005–0.57) and 0.08 (range 0.01–0.70), respectively, and only exceeded 0.20 for average-length individuals (96.7 cm) at these two subpopulations during Fall 2009 (Fig. 9). The differences in *p* between radio tracked individuals after their transmitter had expired or been removed and individuals that had never received a radio transmitter and the differences in *p* between males and females were small (max. difference in model-averaged estimates = 0.008 and 0.004, respectively).

There was very little support for any single model for Φ (max. $w = 0.04$) and null models (i.e., Φ(.)) were included in three of the top six models including the top-ranked model (Table 7). Model-averaged point estimates for annual apparent survival were consistently high (median = 0.87, range 0.82–0.91, Fig. 10). For averaged-sized individuals, model-averaged point estimates for Φ were virtually identical between PIT-tagged and radio-tracked individuals and between males and females (max. difference in model-averaged estimates = 0.005 and 0.002, respectively). Annual apparent survival was slightly higher in summer (median = 0.89, range 0.88–0.89) than in winter (median = 0.85, range 0.84–0.86). Annual apparent survival was relatively consistent across body sizes; never varying by more than 0.04 across the range of SVL we included (Fig. 11).

Model-averaged estimates of PIT-tagged male and female annual apparent survival with no temporal covariates were 0.869 (SE 0.122) and 0.868 (SE 0.127), respectively. Model-averaged estimates of PIT-tagged summer and winter apparent survival with no effect of sex were 0.969 (SE 0.0334) and 0.895 (SE 0.0935), respectively.

CHAR

We included 147 individuals in our survival analysis of which 45 were recaptured at least once including 32 radio-tracked individuals. Five probable mortalities were observed. Two radio tracked females died of unknown causes, one in May 1998 (Radio #232/PIT #414D1D3C41) and another in July 2000 (PIT #414D0C7525). Two radio-tracked males died, one (PIT #411B242621) in the spring of 2000, presumably due to a facial inflammation, the other (PIT# 414D1E4149) from vehicular-induced mortality in May 1999. A fifth radio-tracked female (PIT #414D0A1E65) did not emerge from the den in the spring of 1999. Thirteen individuals receiving transmitters on their first capture were not recaptured after their transmitter batteries expired compared to 10 individuals that received transmitters on their first capture and were recaptured after their transmitter batteries expired.

Our $\hat{c}$ using the model Φ(AdultJuv:Year+Season) *p*(Capture+Adult:Year2+Neo_age_p+Year2+Season) as the global model was 1.70. Year2 combined 1998 and 1999 capture occasions while AdultJuv combined captures of adults and juveniles. Model support among candidate models for *p* was relatively low (max. $w = 0.0284$) and 38 models had $\Delta QAIC_c \leq 2$ (Table 8). Nevertheless, there was strong support for a temporal and age effect on p. Recapture rates were highest for adults and increased over time while recapture rates for juveniles and neonates were relatively low and consistent over time (Fig. 12). Median p was 0.24 (range 0.14–0.46), 0.07 (range 0.07–0.08), and 0.06 (range 0.05–0.07) for adults, juveniles, and neonates, respectively. There was relatively little support for differences in p for PIT-tagged snakes prior or subsequent to monitoring with radio telemetry (Table 8). Median p was 0.28 (range 0.17–0.46) for PIT-tagged only individuals and 0.22 (range 0.14–0.39) for PIT-tagged individuals recaptured after radio tracking.

Model support was lower among candidate models for Φ (max. $w = 0.0052$) and 59 models had $\Delta\text{QAIC}_c \leq 2$ (Table 9). The constant term for Φ (i.e., $\Phi(.)$) was in the top three models and in 18 of the top 59 models. Median annual apparent survival was 0.77 (range 0.62–0.84), 0.77 (range 0.70–0.78), and 0.77 (range 0.63–0.78) for adults, juveniles, and neonates, respectively (Fig. 13). While there was some support for differences in Φ between PIT-tagged and radio-tracked individuals (Table 9), these differences were negligible (maximum difference in model-averaged point estimates = 0.03).

Model-averaged annual apparent survival estimates from models with no temporal effects were 0.627 (SE 0.4169) for neonates, 0.777 (SE 0.2653) for juveniles, 0.831 (SE 0.1624) for PIT-tagged adults, and 0.758 (SE 0.1183) for radio-tracked adults.

Population Demographics

Activity Season Length

Activity season length varied among populations from 159–191 days using the 95th range and 175–198 using the 99th range (Table 10). Activity season length tended to increase as latitude decreased and this pattern generally held between quantiles (Table 10 and Fig. 14). The bootstrapped 95% CI generally overlapped among populations with the exception of VRUT2 and CHAR, the northernmost and southernmost population, respectively. The relationship between egress and latitude was more variable but ingress date also tended to increase with decreasing latitude (Table 10 and Fig. 14).

Sex Ratio and Color Phase

Of the 18 marked adults at VRUT1, researchers confirmed the sex of 17 individuals (seven females and 11 males) and the sex ratio was not significantly unequal (1:1.57, $p = 0.35$). Of the

123 marked individuals >1-year old at VRUT2, 46 were females and 77 were males (1:1.67, p = 0.0052). Of marked individuals ≤1-year old, 12 were females and 13 were males (1:1.08, p = 0.84). Researchers marked 140 individuals during the radio telemetry study from 2011 through the spring of 2013 including 117 adults, four 1-year olds, and 19 neonates. Of these marked individuals >1-year old, 45 were females and 72 were males (1:1.60, p = 0.0126) while of the 23 marked individuals ≤1-year old, 10 were marked of each sex. Of individuals whose color phase was recorded, three were yellow-phase (2%) and 34 were black-phase (23%, 1:11.33, p < 0.0001). Color phase was unknown or unrecorded for 114 individuals (75%).

Of marked individuals of known sex at NH, 39 were females and 44 were males (p = 0.58, n = 17 of unknown sex), and of adults of known sex, 11 were males and 11 were females (n = 8 of unknown sex). Of sub-adults of known sex, 28 were females and 32 were males (p = 0.61, n = 8 of unknown sex). All individuals at NH were black-phase.

Of the 59 marked individuals at MHAD1, 36 were adults, 13 were sub-adults, six were first-years, and two were neonates. Researchers marked 27 females and 20 males >1-year old (1:0.74, p = 0.31) and 31 females and 24 males in total (1:0.77, p = 0.35). Thirty-nine (66%) marked individuals were yellow-phase and 15 (25%) were black-phase (p = 0.0011) with five individuals of unknown or unrecorded color phase. Sex was unavailable for most individuals at MNOR.

Of the 146 marked individuals at MBER >1-year old, 48 were females and 98 were males (1:2.04, p < 0.0001). Of marked individuals of known sex ≤1-year old, 15 were females and 16 were males (1:1.07, p = 0.86). No individuals ≤1-year old were recaptured. Researchers captured 19, 49, 23, and 88 individuals at MBER3, MBER1, MBER2, and MBER4 of which 1, 5, 1, and 18 individuals were neonates or ≤1-year olds, respectively. Researchers captured 83 (47%) yellow-phase individuals and 91 (51%) black-phase individuals (1:1.10, p = 0.54) and 73 (50%) and 72 (49%) black- and yellow-phase >1-year olds (1:0.99, p = 0.93), respectively. However, these ratios differed among subpopulations (the following ratios refer to individuals >1-year old). Researchers captured four females and 14 males in MBER3 (1:3.5, P = 0.0184), 15 females and 25 males in MBER1 (1:1.67, p = 0.1138), 11 females and 11 males in MBER2, and 18 females and 48 males in MBER4 (1:2.67, p = 0.0002). Researchers captured 16 yellow-phase and three black-phase individuals in MBER3 (1:0.19, p = 0.0029), 28 yellow-phase and 18 black-phase individuals in MBER1 (1:0.64, p < 0.0001), 19 yellow-phase and four black-phase individuals in

MBER2 (1:0.21, $p = 0.0018$), and 20 yellow-phase and 66 black-phase individuals in MBER4 (1:3.30, $p < 0.0001$).

At the southern population of CHAR, 53 adults, 10 juveniles, 81 neonates, and three first-year individuals (i.e., born the previous fall) were marked. Of marked individuals of known sex, 28 were females and 34 were males (1:1.21, $p = 0.45$, $n = 85$ of unknown sex), and of adults of known sex, 27 were females and 25 were males (1:0.93, $p = 0.78$, $n = 1$ of unknown sex). Sex was unknown for all but two neonates and first-year individuals. Fifty-two marked individuals were yellow morphs (35%) 17 were black morphs (12%, 1:0.33, $p < 0.0001$). Color phase was unknown or unrecorded for 78 individuals (53%).

Body Size and Condition

We used data from VRUT2 ($n = 112$), MBER ($n = 125$), MHAD1 ($n = 32$), and CHAR ($n = 60$) to compare body sizes for adults and juveniles. A regression of log(weight) against log(SVL) was highly significant ($p < 0.0001$) with $R^2 = 0.87$ (Fig. 15). For SVL, the models with an additive and interactive effect of population and sex had >0.99 of the model weight (Table 11). Males had longer SVL in all populations (Table 12) and females in the two Massachusetts populations had lower SVL than individuals in VRUT2 and CHAR (Fig. 16). For BCI, the population model had the highest support followed by the model with an additive effect of population and sex ($\Delta AIC_c = 2.07$) and these two models together had 0.93 of the model weight (Table 11). Body condition did not differ by sex but was highest for CHAR and MBER and lowest for VRUT2 (Fig. 16).

We used data from VRUT2 ($n = 24$), MBER ($n = 25$), and CHAR ($n = 81$) to compare body sizes for neonates among populations. Neonate log(weight) was significantly associated with log(SVL) but the R^2 value was low ($p = 0.0023$, $R^2 = 0.18$, $n = 50$). The models with population differences in SVL and weight had 100% of the model weight. Neonate SVL and weight were lowest for VRUT2 while neonate SVL was highest for MBER and neonate was highest for MBER and CHAR (Table 13, Fig. 16).

Shedding Rates

At VRUT2, we estimated a mean shedding rate of 1.9 sheds per year (SE 0.53, range = 1–3 sheds) per active season from 13 adult individuals (mean SVL = 103.0 cm, range = 91.4–116.0

cm). Only two individuals shed once in the activity season and one individual shed three times between May 2012 and May 2013. Another individual shed five times in five activity season.

At the southern population of CHAR, we used shedding information from six adults in 1998 (five females and one male) and 17 adults in 1999 (11 females and six males) to estimate shedding rates. We estimated a shedding rate of 1.33 and 1.41 sheds per activity season in 1998 and 1999, respectively, and an overall shedding rate of 1.39 (SE 0.10) sheds per activity season.

Reproduction Frequency

The smallest gravid female observed at VRUT1 had SVL = 91.4 cm. Only two gravid females were observed at VRUT1 with seven and nine embryos. A total of nine gravid females were observed at VRUT2 with a mean SVL of 99.0 cm (SE 1.94). The smallest gravid female was 92.7 cm SVL and of females ≥92.7 cm SVL at VRUT2 that were assessed for reproductive condition, five of 21 (24%) were gravid with a median of seven follicles (range 2–12 follicles) in 2011 and three of 11 (27%) were gravid in 2012 with a median of two follicles (range 1–3 follicles). The ratios of gravid:non-gravid females for 2011 and 2012 were 1:3.20 and 1:2.67, respectively. Only six females ≥91.4 cm SVL were assessed for reproductive condition in 2013 and one was gravid (16%, 1:5.00) with six follicles. The overall ratio of gravid to non-gravid females at VRUT2 was 1:3.22 (9:29) and the overall mean number of follicles was 5.2 (SE 1.18 SE, IQR 2–7). We had insufficient recaptures to estimate reproduction frequency directly. We recorded at least four litters at VRUT1 and at least nine litters at VRUT2 from 2011–2015 with a mean of 5.00 (SE 0.71) and 5.67 (SE 0.73) neonates per litter, respectively.

We used data from 13 females and a total of 47 female-years to estimate the frequency of reproduction at NH. We excluded data from two individuals (#6 and #38) that were initially marked as sub-adults because we were uncertain if these individuals were reproductively mature. We observed a mean of 4.3 females (SE 0.41, range 3–7 females) per year, of which a mean of 1.10 (SE 0.46, range = 0–4 gravid females) were gravid per year. We observed four gravid females in 2011 and 2014 out of a total of four and seven females, respectively. The mean annual proportion of gravid females was 24.4% (SE 9.50%, range 0–100%). The overall ratio of gravid:non-gravid females across years was 1:2.92 suggesting that females on average reproduce every two to three years. Three females (# 41, 42, and 44) were gravid in two different years with two years between reproductions for each individual. All three individuals were gravid in both 2011 and 2014. A fourth female (#36) was gravid on three occasions: 2008, 2011, and 2014. Another female (#5) was gravid once in four years (2009) and two others (#37 and #43) were gravid once in three years (2010 and 2013, respectively). One female (#16) was not gravid in three years (2008–2010). Using the four females observed gravid in multiple years we estimate a 3 year reproductive cycle following Brown (2016). However, if we assume that the two females not observed gravid over three years (#5 and 16) had 4 year reproductive cycles the mean reproductive cycle would increase to 3.29 years (SE 0.18).

A total of five gravid females were observed at MBER with a mean SVL of 88.75 cm (SE 2.63). The smallest gravid female observed at MBER had SVL = 85.0 cm. Of females with SVL ≥85 cm at MBER, researchers observed two gravid and one non-gravid females in 2009, no gravid and three non-gravid females in 2010, one gravid and 13 non-gravid females in 2011, two gravid and three non-gravid females in 2012, and no gravid and three non-gravid females in 2013. In total, we had 28 female-year observations of which five (18%) were gravid for an overall ratio of 1:4.6. Of three radio-tracked females who were found to be gravid, one (PIT # 4938594B63) was radio-tracked from 2009–2012 and observed gravid in 2009 and 2012, one (PIT # 4A05177228) was radio-tracked from 2009–2011 and was observed gravid in 2009, and one (PIT # 4963183B74) was radio tracked from 2009–2012 and observed gravid in 2009. If we assume that the second individual was gravid in 2012 and the third individual was gravid in 2013 then the observed reproduction cycle would be 3.33 years (SE 0.33).

Eleven different females were recorded as gravid in all three years of the study at the southern population of CHAR. The smallest gravid female was 94 cm and mean SVL of the smallest 10 was 99.7 cm (0.99). Because we lacked SVL for all adult females and because the only adult female we observed with SVL <94 cm and whose reproductive status was not uncertain was 92 cm we considered all adult females when calculating the proportion of gravid females. Reproductive status was recorded for 14, 13, and 13 adult females (SVL ≥94 cm) in each year of which 8 (57%), 4 (31%), and 9 (69%) were recorded as gravid, respectively, resulting in ratios of gravid:non-gravid females of 1:0.75, 1:2.25, and 1:0.56, respectively. We estimated the overall study-wide proportion of gravid females as 53% with a ratio of 1:0.90. Of females whose reproductive condition was assessed in two consecutive years (*n* = 7), none were gravid in each year. Reproductive condition was assessed in three consecutive years for three females and two were gravid in one year while the third was not gravid in any year.

Reproductive condition was assessed in 1998 and 2000 for two females and both were gravid in 1998 and 2000 suggesting a two year reproductive cycle. However, if we assume that the female not observed gravid in three years had a 4 year cycle and the female observed gravid in 1998 but not gravid in 1999 and 2000 had a 3 year cycle then the mean reproductive cycle would be 2.75 years (SE 0.48). The identity of the mother was recorded for eight litters (five in 1998, two in 1999, and one in 2000) and the number of neonates per litter ranged from 5–10 (mean = 7.75, SE 0.59, mode = 7).

Counts of Gravid Females

Counts of gravid females were obtained annually from 1991–2017 at the southern population of CHAR. There was a clear distinction between years with low numbers of gravid females ($n = 16$, mean = 5.88, SE 0.64, range = 0–9) and high numbers of gravid females ($n = 11$, mean = 16.09, SE 0.49, range13–19, Fig. 17). These means were significantly different ($p < 0.0001$). High intervals lasted a median of 1 years (range 1–3) while low intervals lasted a median of 2.5 years (range 1–4, Wilcoxon signed rank test $p = 0.0999$).

None of the correlation structures improved model AIC_c using all years and there was no evidence of a trend effect of year across all years ($\beta = -0.018$, SE 0.14, $p = 0.90$). When just low years were considered, including the autoregressive moving average correlation structure with one parameter improved model fit by 0.13 AIC_c units but there was still no significant effect of year ($\beta = 0.067$, SE 0.14, $p = 0.63$). When just high years were considered, including the autoregressive moving average correlation structure with two parameters improved model fit by 2.39 AIC_c units but there was still no significant effect of year ($\beta = 0.013$, SE 0.03, $p = 0.69$).

Movement Patterns

For VRUT2, median total distance moved and maximum displacement was 10.23 km (mean = 9.97 km, SE 1.04, range = 1.54–20.34 km) and 3.42 km (mean = 3.18 km, SE 0.27, range = 1.25–5.72 km), respectively. Median total distance moved for males ($n = 17$) and non-gravid females ($n = 3$) was 11.22 km (range = 4.91–20.34 km) and 3.49 km (range = 2.40–9.37 km, Wilcoxon sign-rank test $p = 0.0123$), respectively. Median maximum displacement for males and non-gravid females was 3.52 km (range = 1.45–5.72 km) and 1.55 km (range = 1.52–3.77 km, Wilcoxon sign-rank test $p = 0.1789$), respectively. The relationship between the log of total distance moved and SVL while controlling for number of locations was not significant (β = 0.009, SE 0.008, $p = 0.304$) but the relationship between maximum displacement distance and SVL was significant (β = 0.013, SE 0.008, $p = 0.173$, respectively). However, when the observation for a male with SVL = 116 cm and total distance moved = 4.91 km was removed, the relationship between the log of total distance moved and SVL was significantly positive (β = 0.014, SE 0.006, $p = 0.0373$). Males at MBER also had significantly greater displacement distances than non-gravid females (Stengle 2018).

Population Growth Rate and Abundance

Vermont

We estimated population growth rate (λ) for VRUT2 using data from 94 PIT-tagged individuals >1-year old. There was equivocal support between models with constant population growth rate and models with different population growth rates between sexes (Table 14). Model-averaged estimates of λ were 0.51 (95% CI = 0.36–0.74) for females and 0.59 (0.48–0.79) for males. We estimated abundance from the spring of 2011 through the spring of 2013 for VRUT2 using 88 PIT-tagged individuals >1-year old. The model-averaged estimate of f_0 for VRUT2 using full-likelihood models was 130.03 (95% CI = 109.23–884.34) for an estimated abundance of 218.03. The model-averaged estimate of abundance using Huggins closed-population models was 223.00 (95% CI = 159.33–343.54).

Massachusetts

We estimated population growth rate (λ), size, and abundance using 125 PIT-tagged individuals >1-year old. Models with constant population growth rate had greater support than models with different population growth rates between sexes ($\Delta AIC_c \geq 2.31$, Table 14). Model-averaged estimates of λ were 0.95 (95% CI = 0.66–1.36) for females and 0.96 (0.68–1.36) for males. The model-averaged estimate of f_0 for MBER using full-likelihood models was 500.57 (95% CI = 216.14–1,159.29) for an estimated abundance of 625.27. The model-averaged estimate of abundance using Huggins closed-population models was 414.73 (95% CI = 294.30–620.81).

Connecticut

We were unable to estimate abundance or population growth rate using open-population JS models for CHAR. Closed population models including ≤1-year olds contained inestimable parameters so we only used data from 29 PIT-tagged individuals >1-year old. The model-averaged estimate of f_0 using full-likelihood models was 50.66 (95% CI = 0.04–62,270.93) for an estimated abundance of 79.66. The model-averaged estimate of abundance using Huggins closed-population models was 377.52 (SE 6,443.21) although estimated abundance from the best supported model (AIC_c w = 0.56) was 54.09 (95% CI = 38.85–92.91).

Population Viability Analyses

VRUT2

Across three initial population sizes and both sets of 2–4 year old survival estimates, probability of extinction for VRUT2 was 100% and occurred in <40 years (Table 15). When adult and juvenile survival were equal, the population showed positive growth (mean r >0) and a probability of extinction ≤ 0.02 when survival was >0.88 with the exception of the highest survival values we considered when probability of extinction was 0.15 (Table 16).

NH

Using the survival estimates from models with no temporal effects, probability extinction for NH was 100% for all three initial population sizes regardless of the presence or severity of inbreeding depression (Table 15). When juvenile survival was held constant (0.577) and adult survival was varied ($N_{initial}$ = 30), probability of extinction was ≤1.5% when adult survival was ≥0.963 but increased to ≥22.8% when survival was ≤0.938 (Table 17). When juvenile survival was held constant at 0.844 (approximately the black-phase juvenile survival reported by Brown et al. (2017), probability of extinction was ≤1.6% when adult survival was ≥0.888 and increased to ≥13.7% when survival was ≤0.864 (Table 17). When adult survival was held constant (0.764) and juvenile survival was varied ($N_{initial}$ = 30), probability of extinction was ≥94% across all values of juvenile survival (Table 17). Probability of extinction did not fall below 5% until adult survival was increased to 0.839 and juvenile survival was 0.978 (Table 17).

MBER

When juvenile and adult survival were equal the population exhibited positive growth and 0% probability of extinction (Table 15).

CHAR

Across three initial population sizes and using empirical survival estimates, probability extinction for CHAR was ≥57% and median time till extinction was 44–88 years in all scenarios (Table 15). Probability of extinction was highest with 12 lethal equivalents (71–87%) followed by 6.29 lethal equivalents (64–83%) and no inbreeding depression (57–67%). When adult survival was varied and survival for all other age classes held constant, probability of population extinction was ≥34.8% in all scenarios (Table 18). When juvenile survival was varied and survival for all other age classes held constant, probability of population extinction was ≥4.7% (Table 18).

Discussion

Survival

Our estimates of annual apparent survival from five populations of timber rattlesnakes in four New England states varied among populations (median estimates = 0.74–0.94 not including NH) yet was consistent with survival estimates reported for other north-temperate communally-denning rattlesnakes (Table 19). While survival estimates for NH showed considerable variation, by 2016 these estimates were among the highest we observed and were >0.80 for adults for approximately the last third of the study. Brown et al. (2007) reported estimates of annual apparent survival estimates of 0.82 and 0.96 for yellow- and black-phase adult (≥5 years old) timber rattlesnakes, respectively, in a metapopulation in the Adirondack Mountains of eastern New York. While our annual apparent survival estimates for PIT-tagged individuals at MBER (0.87) and PIT-tagged males at VRUT2 (0.86) were within this range, survival estimates were lower at other populations (Table 10), although our broad 95% CI intervals suggest caution when interpreting differences among populations. Nevertheless, we are unsure of the mechanisms responsible for the variation in survival among these populations. Our two populations with lowest survival include a single isolated population bordered by a paved road (VRUT2) and two subpopulations divided by a paved road (CHAR). Although we did not observe any mortalities along the road at VRUT2 during our study and all radio-tracked individuals moved away from the road, nine road mortalities have been recorded on this road from 2001–2013 (M. McHugh, personal communication). Additionally, multiple private properties are utilized by rattlesnakes from VRUT2 during the summer and road mortalities have been reported along paved roads in these areas. Despite the presence of a “nuisance” rattlesnake removal program since 2010, it is possible that some rattlesnakes are still intentionally killed on these properties. In contrast, MBER is a metapopulation with four major subpopulations occurring over many square kilometers of relatively undisturbed forest. However, the New York metapopulation is also paralleled and bisected by a paved road where road mortalities have been observed (Aldridge & Brown 1995). Clark et al. (2010) found that this road had caused a significant reduction in gene flow between subpopulations within 7–8 timber rattlesnake generations. A more comprehensive understanding of the role of anthropogenically-induced mortality on timber rattlesnake survival requires a more formal comparison of road densities and anthropogenic development among our populations. However, these factors seem unlikely to explain the lower female survival or male-biased sex ratio we observed at VRUT2 because males and, to a lesser extent, non-gravid females generally move more extensively than gravid females (Reinert & Zappalorti 1988b; Stengle 2018; Waldron et al. 2006, this study). While MNOR is completely surrounded by roads and urban development and had relatively low survival, none of our mortalities were of confirmed anthropogenic origin. The low survival rate of this population likely reflects small sample sizes ($n = 13$) and short study duration although multiple road-killed individuals have been observed at MNOR over the past several years (A.G.S., personal observation). Other factors that could explain variation in survival rates among populations include anthropogenic disturbance and prey availability or quality (Jenkins et al. 2017; Jenkins & Peterson 2008), climate, or elevation, although additional data are required to test these hypotheses.

Low initial survival at NH is apparently due to the outbreak of SFD observed in 2006 and 2007 (Clark et al. 2011). Four of our six confirmed or probable mortalities occurred in the spring or early summer of 2006 and 2007 which was also likely responsible for initially low summer survival. An additional 17 individuals were marked (not radio-tracked) in 2006 but never again seen and an additional three individuals were never seen after 2007. Many of these individuals exhibited signs of SFD or were observed basking near the hibernaculum late into the fall. While timber rattlesnakes may commonly be observed emerging in the spring with skin lesions these lesions are often lost after the first shed (Clark et al. 2011; A.G.S., personal observation). However, the summers of 2005 and 2006 were unusually wet summers and this likely facilitated the outbreak of this disease. While summer survival at NH increased over the course of the study and reached the highest rates observed in our study, winter survival was remained comparatively consistent and was lower than summer survival after approximately the middle of the study. We suspect this pattern was driven by the fact that several individuals throughout the study did not emerge during the spring. While SFD has been reported from many snake taxa and been associated with other mortality events (Lorch et al. 2016), information on the status and severity of SFD among timber rattlesnakes in New England is still emerging. In contrast to NH, we observed no severe lesions or mortalities directly attributable to SFD among timber rattlesnakes at MBER (A.G.S., unpublished data). In nine radio-tracked individuals at MBER that displayed symptoms of SFD, symptoms largely healed over time and generally improved following shedding. The presence of SFD at VRUT1 and VRUT2 is confirmed and one individual at VRUT2 was observed with severe degenerative scale rot and later died in captivity although tests were inconclusive for SFD (D.B., unpublished data). We strongly recommend that managers record and monitor the presence of SFD within timber rattlesnake populations, particularly small, isolated populations with reduced genetic diversity or signs of inbreeding depression.

With the exception of VRUT2, we found very little support for sex-specific differences in survival, a trend consistent with results from other north-temperate viperids (Brown et al. 2007; Diller & Wallace 2002; Flatt et al. 1997; Forsman & Lindell 1997; Jenkins et al. 2017; Jones et al. 2012). Females at VRUT2 had lower survival than males and this pattern was present in both radio-tracked and PIT-tagged individuals, although broad CI suggest that these differences be interpreted with caution. Studies of three different gartersnake species (*Thamnophis* spp.) found that females generally had higher survival than males (Lind et al. 2005; Shine et al. 2001; Stanford & King 2004) although Bronikowski and Arnold (1999) found that western terrestrial gartersnake survival rates at two montane populations in California did not differ between males and females. Weatherhead et al. (2012) reported no differences in mortality rates between male and female black ratsnakes (*Pantherophis alleghaniensis* formerly *Elaphe obsoleta*, Crother 2017) in Ontario while Sperry and Weatherhead (2009) (Sperry & Weatherhead 2009) found that female Texas ratsnakes *(Pantherophis obsoletus* formerly *Elaphe obsoleta*, Crother 2017) had lower survival than males despite more extensive movements by males due to mate-searching. These authors hypothesized that the longer activity season (potentially year round) in Texas increased the costs of reproduction for females, specifically by basking more in the mid-summer through fall than males. In contrast, they hypothesized that shorter activity seasons at northern latitudes imposed behavioral and foraging constraints on both sexes such that the costs of reproduction were approximately equal between males and females.

Increased movements by male timber rattlesnakes associated with mate-searching (Reinert & Zappalorti 1988b; Stengle 2018; Waldron et al. 2006, this study) could put males at greater mortality risk than females. While males at VRUT2 did move more extensively than non-gravid females (albeit with small sample sizes), males had higher survival than females. Furthermore, radio-tracked individuals at MBER showed the same sex-specific differences in movement (Stengle 2018) yet had higher survival and no differences in survival between sexes. In contrast, the energetic costs of reproduction combined with behavioral thermoregulation of gravid females could potentially result in equal mortality risks between sexes or even increase female mortality. Olson et al. (2015) found lower female survivorship in radio-tracked adult timber rattlesnakes in south-central Indiana but only following years of low prey abundance. The need to acquire resources for reproduction may expose females to greater mortality risks and/or magnify impacts of low-prey abundance (Forsman & Lindell 1997; Sperry & Weatherhead 2008). However, additional data are required to evaluate either of these hypotheses at VRUT2.

Our results suggest that surgical implantation of radio transmitters and associated radio-tracking does not negatively impact timber rattlesnake survival in our study populations. Model support was weak for differences in survival between PIT-tagged and radio-tracked individuals and radio-tracked individuals at VRUT2 exhibited slightly higher survivorship. It is possible that higher survival rates of radio-tracked individuals at VRUT2 are an artifact of a small sample size monitored for a short duration. These results are encouraging given the imperiled status of timber rattlesnakes in our study populations and the importance of spatially-explicit information on movement patterns and space use. Other studies have also found little or no differences in snake survival rates between radio-tracked and non-radio tracked individuals (Hyslop et al. 2012). However, we note that our results do not rule out the possibility of negative impacts due to capture, handling, and surgery. Transmitter surgeries may still induce clinical reactions (Lentini et al. 2011; Sperry et al. 2009). Weatherhead & Blouin-Demers (2004) found that surgically implanted transmitters negatively impacted black ratsnake weight gain and relative clutch mass but not SVL. They were unable to rigorously compare survivorship. These responses may prove lethal for individuals with low body condition (e.g., post-partum females) or other stressors. We pooled individuals receiving internal and external radio transmitters in our analyses which may have influenced our results because individuals receiving external transmitters are not exposed to the stressors of surgery. However, external transmitters were only used in Vermont (*n* = 6), where survivorship was higher for radio-tracked individuals, and NH (*n* = 3). Moreover, with the exception of one NH individual that was monitored for 264 days (including one winter), individuals with external transmitters were monitored ≤64 days. We therefore think it unlikely that pooling individuals with internal and external transmitters influenced our survival estimates.

The negative relationship between survival and body size for VRUT2 was unexpected given that other snake studies have found that survivorship increases with body size (Blouin-Demers et al. 2002; Hansen et al. 2015; Hyslop et al. 2012) or is higher in adults compared to juveniles (Altwegg et al. 2005; Brown et al. 2007; Diller & Wallace 2002; Jenkins et al. 2017). In contrast, Forsman (1993) found that survival was greatest for individuals of intermediate SVL in European adders (*Vipera berus*) and Brown & Weatherhead (1999) found a similar pattern for female northern watersnakes (*Nerodia sipedon sipedon*). However, the wide 95% CI around our survival estimates across SVL suggest that this relationship may be an artifact of small sample sizes. Moreover, this relationship could be driven by both of our confirmed mortalities at VRUT2 occurring with above-average length individuals (103.5 cm and 121.1 cm vs. mean SVL = 102.4).

We were unable to estimate age-specific survivorship for all populations except CHAR due to our inability to accurately age individuals and a complete absence of recaptures of ≤1-year olds. While we found differences in survival between adults and sub-adults at NH we are unsure of the exact ages these two groups represent. However, our analysis found no support for differences in survival among age classes at CHAR. This was surprising given that other rattlesnake studies have reported differences in age-specific survivorship (Brown et al. 2007; Jenkins et al. 2017). In particular, neonate survival is invariably lower than adult survival in previously studied rattlesnake populations as well as snake species in general (e.g., Bronikowski & Arnold 1999; but see Pike et al. 2008; Stanford & King 2004). However, Brown et al. (2007)

estimated first year survival rates of 0.53 and 0.77 for yellow- and black-phase timber rattlesnakes, respectively, which approaches our estimated annual neonate survival rates at CHAR (0.63–0.78). Jenkins et al. (2017) estimated first year survival rates of 0.29–0.47 for Great Basin rattlesnakes in southeast Idaho. Using simulation studies and assuming stable populations, Pike et al. (2008) estimated a mean juvenile annual survival rate of approximately 0.45 in viviparous squamates and found that juvenile survival was positively correlated with adult survival. It is therefore possible that neonate and juvenile survival at the Hartford County population is relatively high. However, we note that the 95% CI around our model-averaged survival estimates for neonates and juveniles nearly spanned zero to one throughout our study due to our low numbers of recaptures. We therefore suggest that additional data are required to accurately estimate age-specific survivorship at CHAR.

With the exception of NH, we found little evidence of differences in summer and winter survival. Estimates of seasonal survival rates are rare in snake mark-recapture studies because low recapture rates typically necessitate pooling captures into annual capture occasions. However, Sperry et al. (2010) found that black ratsnake survivorship in populations from Texas, Illinois, and Ontario, Canada, was highest during the winter in all populations and Olson et al. (2015) reported higher winter survivorship for timber rattlesnakes in south-central Indiana. Similarly, studies of four snake species, including Great Basin rattlesnakes, at a multi-species communal hibernaculum in Utah suggested that summer mortality was higher than winter mortality for four snake species (Brown & Parker 1984; Parker & Brown 1974, 1980). A range-wide analysis of eastern massasauga (*Sistrurus catenatus*) survival from 13 different locations found that winter survival was generally higher than activity season survival (mean = 0.89 and 0.77, respectively, Jones et al. 2012). These results are consistent with most of our observed mortalities occurring during the activity season rather than during the winter (i.e., through individuals not emerging during the spring). Altwegg et al. (2005) found that winter severity negatively affected juvenile survival of European asps (*Vipera aspis*) but had no effect on adult survival. Our patterns of age- and season-specific survival rates from NH partially contradict this pattern of higher winter survival. Although summer survival was initially lower than winter survival for both sub-adults and adults, this was due to the outbreak of SFD in 2006 and 2007 and multiple confirmed mortalities during the summers of 2006 and 2007. Moreover, sub-adult winter survival was relatively low throughout the study. By the end of the study, winter survival was lower than summer survival for both age classes. We are unsure of the mechanisms behind this pattern since outbreaks of SFD were largely confined to 2006 and 2007 but hypothesize that the younger sub-adults may be more prone to winter mortality (e.g., Altwegg et al. 2005).

It is important to emphasize that each of our studies lasted a relatively short duration compared to the potential lifespan of timber rattlesnakes. Brown (2008; 2016) estimated that timber rattlesnakes in eastern New York may live over 40 years. Our results therefore represent relatively brief "snapshots" of timber rattlesnake survival and demographic metrics which may partially explain the lack of strong temporal variation in survivorship. It is unclear how our results may change over longer time spans or under different environmental or climatic conditions. Other studies have reported annual variation in snake survival rates which could be due to temporal variation in environmental conditions or prey abundance (Olson et al. 2015; Sperry & Weatherhead 2008). Forsman & Lindell (1997) found that annual variation in survival for European adders on a Swedish island varied from 0.62–0.99 and that this variation was best explained by changes in vole prey densities. On a second island population, annual survival rates also varied from 0.62–99 but these values were not related to temporal changes in prey densities. Baron et al. (2010) also found marked variation in annual survivorship (approximately 0.00–0.75) among five different annual cohorts meadow vipers (*Vipera ursinii ursinii*). It is therefore important to use caution when inferring patterns of survivorship (and other demographic metrics) over broad temporal scales from short duration studies.

Recapture Rates

Recapture rates were generally low (<0.20) across populations (with the exception of NH) consistent with other studies of communally-denning rattlesnakes in north-temperate climates (Brown et al. 2007; Jenkins et al. 2017). Recapture rates were highest at single populations while conducting relatively high-intensity mark-recapture combined with radio telemetry (i.e., NH, CHAR, and VRUT2 from 2011–2012). Recapture rates at NH exceeded 0.50 in nine of 21 recapture occasions. The low recapture rates at MBER likely reflect the larger spatial extent of the population and presence of multiple subpopulations which correspondingly limited field effort while the decline in recapture rates in VRUT2 is directly attributable to a decline in field effort. Other studies have also found a positive association between field effort and recapture rate in snakes (Lind et al. 2005; Maritz & Alexander 2012; Prival & Schroff 2012) but see Flatt et al. (1997). Jenkins et al. (2017) compared *p* among three communal hibernacula for Great Basin rattlesnakes and *p* was regularly >0.30 at their smallest hibernacula which was also encircled by a drift fence with funnel traps during multiple years. Diller and Wallace (2002) also used partial drift fences and funnel traps and achieved high recapture rates (0.42–0.85) for northern Pacific rattlesnakes (*Crotalus oreganus oreganus*). Recapture rates for twin-spotted rattlesnakes at two small (2.30–3.0 ha) denning complexes ranged from 0.19–0.58 (mean = 0.31, Prival & Schroff 2012). These results suggest that high levels of field effort and/or additional capture methods (e.g., drift fences and funnel traps) are needed to obtain even moderate recapture rates. Because the precision of survival and abundance estimates are directly related to recapture probability, the consistently low recapture rates in our studies have implications for the utility of monitoring population status using mark-recapture based estimates of survival and abundance.

The response of recapture rate to our covariates varied among populations. At CHAR, recapture rates were highest for adults which may reflect their larger body size (Koons et al. 2009). Recapture rate was positively associated with increasing SVL at VRUT2 but negatively associated with SVL at MBER. Diller and Wallace (2002) estimate higher recapture rates for neonates (0.85) and adults (0.63) than juveniles (1–2 year olds, 0.42) northern Pacific rattlesnakes while both Brown et al. (2007) and Jenkins et al. (2017) found little or no differences in recapture rates among age classes for timber and Great-Basin rattlesnakes, respectively. In contrast, Davis et al. (2015) found higher recapture rates for juvenile ridge-nosed rattlesnakes (*Crotalus willardi obscurus*) than for adults (0.39 vs. 0.21, respectively). There was no evidence of sex-specific differences in recapture rates in any population consistent with other results from communally-denning north-temperate rattlesnakes (Brown et al. 2007; Diller & Wallace 2002; Jenkins et al. 2017). The fact that most individuals were captured near dens during the spring and fall may have negated any effects of inter-sex behavioral differences (e.g., male mate-searching) on recapture rates. Recapture rates were also very similar for PIT-tagged individuals prior and subsequent to radio-tracking for VRUT2 and MBER. While recapture rates were lower for individuals after radio tracking at CHAR these differences were relatively small. This suggests that surgeries and telemetry monitoring did not have a long-term negative impact on individual behavior (c.f., Brown 2008). However, our use of approximately 3 month capture occasions may have prevented us from detecting short-term behavioral responses. For example, Breininger et al. (2012) found the probability of encountering radio-tracked eastern indigo snakes (*Drymarchon couperi*) was approximately 0.15 lower the week following transmitter surgery. Our small sample sizes of individuals recaptured following radio tracking may also have limited our ability to detect differences.

Population Demographics

Sex Ratio

We observed differences among populations in the demographic metrics we examined (Table 10) although we caution that our small sample sizes limit our ability to make meaningful comparisons among populations. Nevertheless, we make note of several inter-population differences in multiple metrics. For example, sex ratios were significantly male-biased at VRUT2 and MBER but approximately equal at NH, MHAD1, and CHAR. Jenkins et al. (2009) reported different sex ratios in field captures of Great Basin rattlesnakes at three populations; only one population was significantly male-biased. McGowan & Madison (2008) observed a significant male-biased sex ratio based on adult timber rattlesnake field captures at one population in southeastern New York but equal sex ratios at population 30 km away. Brown (1992) reported approximately equal sex ratios of timber rattlesnakes in northeastern New York across all age classes and Brown et al. (2007) reported equal sex ratios at birth in the same population. Other snake studies have reported equal sex ratios (Godley et al. 2017; Kapfer et al. 2008) based on field captures, including multiple rattlesnake studies (Davis et al. 2015; May et al. 1996; Reinert et al. 2011; Wastell & Mackessy 2016). In contrast, other snake studies have reported male-biased sex ratios (Linehan et al. 2010; Stevenson et al. 2009) or increasingly male-biased sex ratios with age (Weatherhead et al. 1995) based on field captures. Luiselli et al. (2011) reported equal sex ratios for dice snakes (*Natrix tessellata*) based on JS model estimates although the ratio varied from year-to-year. Unequal sex ratios may be artifacts of unequal capture probabilities between males and females due, for example, to increased male-mate searching or greater exposure by reproductive females during gestation (McKnight & Ligon 2017; Parker & Plummer 1987). Alternatively, unequal sex ratios may reflect differences in sex-specific survivorship (e.g., Sealy 2002) therefore making it important to determine the extent to which unequal sex ratios are sampling artifacts. Our survival analyses showed no evidence of sex-specific differences in recapture rates. However, only VRUT2 showed evidence of differences in survivorship between males and females with lower female survivorship, a trend consistent with a male-biased sex ratio. It is unclear why the sex ratio at MBER would be male-biased given the lack of evidence for sex-specific recapture probabilities and survival. Snake sex ratios are generally equal at birth (Brown et al. 2007; Diller & Wallace 2002; Parker & Plummer 1987). One possible explanation is sex-specific biases in initial capture probabilities such that females had a lower probability of being included in our sample but those females that were included in our sample had the same probability of recapture as males. Gravid females were poorly represented in our sample at MBER and field work did not focus on gestation sites so the male-biased sex ratio could reflect an underrepresentation of gravid females. We encourage additional research to evaluate this hypothesis, in particular the use of model-based estimates of abundance to control for sex-specific variation in capture probability (Luiselli et al. 2011; McKnight & Ligon 2017).

Reproductive Biology

We also observed differences in reproduction frequency among populations although our small sample sizes again limit the strength of our comparisons. Additionally, growing evidence suggests that reproduction frequency is best estimated using direct observations of females over multiple years because the proportion of gravid females can vary widely among years and even a ratio of gravid:non-gravid females taken over multiple years may incorrectly estimate reproduction frequency (Brown 1991; Farrell et al. 2008; Macartney & Gregory 1988). Despite small samples, our limited evidence suggests that females at VRUT2, NH, and MBER reproduce approximately every three years. This is consistent with timber rattlesnakes in eastern New York (Brown 1991, 2016) and West Virginia (Martin 2002), Great Basin rattlesnakes in eastern Idaho (Jenkins et al. 2009), and northern Pacific rattlesnakes in British Columbia, Canada (Macartney & Gregory 1988). However, reproduction frequency of timber rattlesnakes ranged from 2–7 years with a modal frequency of 5 years in West Virginia (Martin 2002). In contrast, we observed two females at CHAR reproducing twice in three years although another female did not reproduce during three years of observation. While our small sample size makes it impossible to determine if these patterns are characteristic of timber rattlesnakes at CHAR, current research suggests that biennial reproduction is uncommon in northeastern timber rattlesnakes. However, Diller & Wallace (2002) reported biennial reproduction among rattlesnakes at a low elevation

(700 m) site in western Idaho and even documented short-term annual reproduction in three females. Reproduction frequency in timber rattlesnakes appears to decrease with decreasing latitude (Martin et al. 2008). Biennial reproduction has been reported for rattlesnakes in the southern U.S. (Farrell et al. 2008; Schuett et al. 2011). The presence of biennial reproduction in CHAR would be consistent with this population having the southernmost latitude and longest activity season of our populations.

However, we highlight two important caveats when considering our results pertaining to reproduction frequency. First, as previously noted with timber rattlesnakes, reproduction frequency can vary widely within a population and over time making it difficult to generalize with small sample sizes from short duration studies. Second, we are unsure how researchers at CHAR determined reproductive status. Additionally, these researchers note that proportions of gravid females may be biased high because gravid females at gestation sites were easier to detect (G. Hammerson, personal communication). However, we only used observations where reproductive status was not marked as uncertain and direct observations from radio-tracked females still provides evidence of biennial reproduction. Nevertheless, we recommend additional long-term data on individual females to better understand reproduction frequency at this site. Alternatively, multi-state mark-recapture models can be used to estimate the probability of a female transitioning from a non-gravid to gravid state (Nichols et al. 1994). This approach was used by Altwegg et al. (2005) to estimate annual probabilities of reproduction for female European adders of 0.26–0.40.

We were unable to obtain robust estimates of fecundity from any population. While estimated litter size from VRUT2 determined via palpation is consistent with (although slightly lower) than observed litter size for timber rattlesnakes in the Adirondack Mountains of eastern New York (Brown 2016), Brown also found that infertile oocytes and/or stillborns may make up a non-trivial proportion of some litters (as much as 100% in one litter). We observed multiple litters at VRUT2 and CHAR with mean litter size at CHAR being very similar to mean litter size reported by Brown (2016). However, Brown’s (2016) reported litter sizes were from females brought into captivity for birthing. Observations of wild born litters may fail to detect all neonates from a litter and/or incorrectly specify litter membership when multiple litters are observed in close proximity.

The cyclical pattern of gravid females observed at the northern subpopulation in CHAR is consistent with similar patterns observed for timber rattlesnakes in the Adirondack Mountains of eastern New York (Brown 2008; Brown 2016) and West Virginia and Virginia (W.H. Martin, personal communication). Cycles in West Virginia and Virginia appear correlated with acorn productivity with peaks in the number of gravid females occurring approximately two years after a peak acorn year (W. H. Martin, personal communication). Cyclical patterns of reproduction also noted in pygmy rattlesnakes in Florida (Farrell et al. 2008). Diller & Wallace (2002) found a strong correlation between the proportion of reproductive female northern Pacific rattlesnakes and prey abundance. However, we lack data to test the hypothesis that cyclical patterns of gravid females at CHAR are due to prey abundance.

Body Size and Condition

Males at all our study sites were significantly longer and heavier than females, a pattern consistent the male-biased sexual size dimorphism observed for timber rattlesnakes (Brown 1993). While male SVL was generally consistent among populations, female SVL was noticeably lower at MHAD1 and MBER compared to VRUT2 and CHAR. However, BCI was greatest for MBER and CHAR and lowest for VRUT2. Similarly, neonate SVL and weight was lowest for VRTU2 and highest for MBER and CHAR, respectively. Inter-population differences in body size and condition have been reported in other snake studies (e.g., Jenkins et al. 2009) yet understanding the mechanisms responsible for such differences is comparatively more difficult and beyond the scope of this current study. Jenkins et al. (Jenkins & Peterson 2008; Jenkins et al. 2009) demonstrated that differences in reproduction frequency, body size, and condition among three populations of Great Basin rattlesnakes was driven by differences in disturbance levels and prey abundance. Lomas et al. (2015) found that body size and condition in northern Pacific rattlesnakes decreased with increasing levels of within-home range human disturbance. We note that in our northernmost population, VRUT2, adult body condition was lowest and neonate body size was lowest. While we are unable to test tentative hypotheses explaining the variation in body size and condition among our populations, we note that activity season was shortest at VRUT2. Ultimately, additional research is needed to elucidate the mechanisms responsible for geographic variation in body size and condition of timber rattlesnakes in New England and their relevance for population conservation.

Population Viability of Timber Rattlesnakes in New England

Our population viability analyses suggests that the VRUT2, NH, and CHAR populations have a high probability of going extinct in the next 100 years under the parameter values we considered. In contrast, MBER and a metapopulation in the Adirondacks of eastern New York did not go extinct in any of our simulations under the parameter values we considered. However, we emphasize that inferences regarding probability of extinction are dependent upon our parameter values and simulation assumptions (discussed below). For late-maturing species with low reproductive output, population viability is generally most sensitive to changes in survival (Oli & Dobson 2003). Our sensitivity analyses were consistent with this trend. Gregory (2009) found that adult and juvenile survival had much stronger effect on population growth for northern populations of common gartersnake (*Thamnophis sirtalis*) and northern Pacific rattlesnake than did age at maturity, reproductive cycle length, and litter size. Hyslop et al. (2012) found a similarly strong effect of adult survival and weak effect of reproductive parameters on eastern indigo snake population growth rate. In a comparison of two closely-related and ecologically-similar elapids, Webb et al. (2002) found that adult survival had a greater impact on population growth rate on the species with slower life history traits (e.g., late-maturing, high adult survival, long generation length) compared to the species with faster life history traits. For species with long life histories, even minor reductions in survival could lead to population declines (Tack et al. 2017). Our results suggest that reducing anthropogenically-induced mortality on adult timber rattlesnakes is important for maintaining viable populations. Such mortality can come from poaching or road mortality and may disproportionately affect adults because of their higher movement potential and greater conspicuousness.

Our estimates of survival and other demographic parameters for VRTU2 are consistent with its high probability of extinction. Specifically, VRUT2 exhibited the lowest survival rates, a male-biased sex ratio, low body condition, low neonate body size, and a negative model-based estimate of population growth rate. While we are unsure of the mechanisms responsible for the differences between VRUT2 and other populations we examined, the lower body size and condition of rattlesnakes at VRUT2 may suggest that factors other than anthropogenically-induced mortality are responsible. The relatively low population viability of NH and CHAR is also consistent with the relatively low survival estimates from these populations. While estimates of fecundity and reproduction frequency at CHAR were the highest amongst the New England populations we examined (albeit with small sample sizes), our sensitivity analyses suggest that high reproductive output for a long-lived species may not be sufficient to overcome relatively low survival. However, it is important to note that the long-term data set of gravid female counts from the second (northern) subpopulation in CHAR showed no evidence in the decline of gravid females over 27 years. It is possible that including this second subpopulation in the PVA would have resulted in increased population viability although we lacked data on inter-population dispersal. However, increasing carrying capacity at CHAR from 800 to 1,600 did not strongly reduce the probability of extinction nor did varying initial population size from 80–400. Whiting et al. (2008) found that asymptotic estimates of finite population growth rate (λ) from deterministic matrix models showed declining populations of Concho watersnakes (*Nerodia paucimaculata*) while model-based estimates of λ (Pradel 1996) showed a stable population, albeit with large 95% CI. This example illustrates the importance of using multiple lines of evidence to evaluate population persistence and not make management decisions solely on the basis of PVA.

While we did not rigorously evaluate the influence of initial population size of population persistence, we did use three different values of initial population size for each population and results were virtually identical across these three different values. While small populations are more susceptible to inbreeding depression, increasing the number of lethal equivalents in our simulations from zero to 6.29 to 12 had very little impact on our results. Nevertheless, we suggest that estimates of population size are still important for accurately evaluating population persistence. This is particularly true for very small populations where stochastic events (e.g., unusual weather events, disease outbreaks) can have a strong impact on population demographics. Knowing if population size falls below some threshold can be used to evaluate the need for particular management actions, such as population augmentation (i.e., the release of individuals into areas where residents are still present, Kingsbury & Attum 2009). Headstarting neonate timber rattlesnakes, defined here as raising captive-born offspring of wild-caught parents in captivity prior to release, is a potential management tool for population augmentation that has been used with other snake species although the success of previous efforts has varied (Kingsbury & Attum 2009). For example, King & Stanford (2006) found that headstarted neonate plains gartersnakes in northern Illinois successfully reproduced as adults and survival was higher for larger headstarted individuals. In contrast, Harvey et al. (2014) found that 27 headstarted eastern massasaugas in southern Ontario failed to survive their first winter despite being released in mid-summer after. However, Connor et al. (2003) found that eight of nine headstarted timber rattlesnakes in eastern Texas survived their first year and three individuals were alive and had entered the breeding population five years after release. Headstarting neonates have the advantage of not permanently removing individuals from the wild and increasing survival during the first one to few years of life when survival is generally lowest for snakes. Headstarting requires considering multiple factors related to captive birthing and the release of captive-born individuals including, but not limited to, time until release, captive conditions, timing of release, and conditions of the release site (Harvey et al. 2014; King et al. 2004; King & Stanford 2006; Kingsbury & Attum 2009; Roe et al. 2010; Roe et al. 2015; Sacerdote-Velat et al. 2014). We provide a more detailed discussion of these considerations in the Management Options of Chapter 3.

Many authors have stressed the limitations of PVA for identifying minimum population sizes or absolute extinction probabilities, instead emphasizing their utility to describe relative differences and identify factors influencing declines (Beissinger & Westphal 1998; Coulson et al. 2001; Reed et al. 2002) but see Brook et al. (2000). We therefore highlight several limitations to our PVA and again emphasize caution when interpreting our results. First, small sample sizes prevented us from accurately estimating many population-specific demographic parameters. While the research of Brown and colleagues on timber rattlesnakes in the Adirondack Mountains of eastern New York (Aldridge & Brown 1995; Brown 1991; Brown 2008; Brown 2016; Brown et al. 2007) provides accurate estimates of many parameters we are ultimately unsure of how representative these estimates are of our populations. The difficulty in obtaining population demographic parameters for snakes is widely noted (Parker & Plummer 1987; Shine & Bonnet 2009). However, many studies have noted marked spatio-temporal differences in snake demographic parameters, even within populations. For example, Brown (2016) found that patterns of reproduction varied between two timber rattlesnake subpopulations separated by 9.2 km with significant differences in peak reproduction years, frequency of reproduction, proportion of gravid females, and female body condition. Jenkins et al. (2017; 2009) found marked differences in population demographic parameters, including survival, fecundity, and population growth rate, among three Great Basin rattlesnake populations ≤42 km apart. The transferability of demographic parameters may be more problematic with small isolated populations where the effects of genetic drift and inbreeding depression may affect demographic traits.

We were also unable to separately estimate the process variance of our survival estimates which may have overestimated annual variation in survival within the PVA. Similarly, our data were collected over relatively short time periods which may underestimate the degree of temporal variability in demographic parameters which may have important implications for long-term population persistence. Reed et al. (2003) found that using data from short-term studies in PVA consistently underestimated extinction risk. We also did not incorporate catastrophes in our simulation which could result in marked and sudden changes in demographic traits. This again may be important for small populations, particularly those suffering from inbreeding depression, because factors such as extreme climatic events or disease outbreaks may have a large impact on population demographics (e.g., Clark et al. 2011). Our simulations of populations with multiple subpopulations (MBER, CHAR, and New York) did not incorporate migration among subpopulations due to a lack of empirical estimates of age- and sex-specific migration rates. Finally, we assumed that parameter values would remain constant throughout the simulation period. This ignores potential effects of future disturbances or environmental changes such as climate change habitat loss, or increased anthropogenic mortality. We therefore suggest that our PVA results be used to prioritize populations and identify critical demographic parameters (e.g., adult survival) for management or recovery efforts. Specifically, we do not intend that our estimates of potential population stability in MBER be used as justification to forgo management or monitoring efforts at this population. Rather, if the population is stable it is likely due in part to ongoing outreach, management, and conservation efforts. Further, our analysis did not evaluate whether one or more of the subpopulations may be at risk of local extirpation. Similarly, we do not wish to imply that populations with high probabilities of extinction should not be managed or otherwise restored. Indeed, the observed increase in timber rattlesnake survival at NH over the course of our study indicates that increasing critical demographic parameters is possible with small, isolated populations.

Recommendations for Future Research

We make three general recommendations for prioritizing future research to better evaluate the population status of timber rattlesnakes in New England. The first is to obtain age-specific survival estimates. Our survival estimates for VRUT2 and MBER apply to individuals >1-year old whereas we found no strong evidence for differences in survival among neonates, juveniles, and adults at CHAR. While the majority of individuals at VRUT2 and MBER were adults it may therefore be reasonable to assume that our estimated survival rates are representative of adults. However, as previously noted, survival in snakes can vary markedly among age classes. We also suggest that researchers formally estimate the age of all marked snakes at each capture occasion to facilitate the estimation of age-specific survival rates. An alternative approach may be to estimate stage-specific (i.e., juvenile, adult) transition and survival probabilities using multi-state models (e.g., Hyslop et al. 2012) if such classes are formally defined and all individuals can be assigned to an age class at each capture. This negates the need to age individuals at each capture and can be incorporated into PVA using stage-based stochastic matrix models. However, multi-state models require relatively large amounts of data to estimate additional parameters and therefore require sufficient recaptures of individuals across stages. The second priority is to accurately estimate fecundity and the frequency of reproduction while the third priority is to accurately estimate population size. Addressing each of these priorities ideally requires long-term mark-recapture data sets although it may be possible to obtain accurate estimates of fecundity through counts of neonates at gestation sites. Mark-recapture data may also allow the estimation of reproduction frequency using multi-state models (Altwegg et al. 2005; Nichols et al. 1994). An additional benefit to long-term mark-recapture data is the potential to estimate population growth rates, the latter of which may be particularly helpful to corroborate the results of PVA (e.g., Whiting et al. 2008). However, given the challenges of low recapture rates observed in this study, obtaining sufficient mark-recapture data will require a considerable financial and logistical investment. Moreover, managers must carefully consider how well their data meet the assumptions of open and closed population models before estimating population size.

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Table 1. Model-selection results evaluating effects on timber rattlesnake recapture probability (*p*) from the VRUT2 population. In each model, apparent survival (Φ) was held constant using an effect of Capture (PIT-tagged or radio-tracked), SVL, and a linear trend effect of time (Time). We fixed *p* = 1 for radio-tracked individuals. The number of parameters is given by *K* and *w* is the QAIC$_c$ model weight. We report models with *w* ≥0.001.

Model	*K*	*Deviance*	*QAIC$_c$*	*ΔQAIC$_c$*	*w*
p(Capture+SVL+Year)	11	266.51	289.95	0.00	0.4307
p(PostTrans1+SVL+Year)	12	266.51	292.22	2.27	0.1385
p(Capture+SVL+time)	14	262.96	293.29	3.34	0.0813
p(Capture+Sex*SVL+Year)	13	265.39	293.4	3.45	0.0769
p(PostTrans2+SVL+Year)	13	266.10	294.11	4.16	0.0538
p(Capture+Sex+SVL+time)	15	262.40	295.08	5.13	0.0332
p(PostTrans1+SVL+time)	15	262.82	295.50	5.55	0.0269
p(PostTrans1+Sex*SVL+Year)	14	265.39	295.72	5.76	0.0241
p(Capture+Time*SVL+Season)	10	274.85	296.05	6.09	0.0205
p(Capture+Sex*SVL+time)	16	261.79	296.85	6.89	0.0137
p(Capture+Year*SVL)	16	262.00	297.06	7.10	0.0123
p(Capture+Year)	10	276.04	297.24	7.28	0.0113
p(PostTrans1+Sex+SVL+time)	16	262.38	297.44	7.49	0.0102
p(PostTrans2+SVL+time)	16	262.42	297.47	7.52	0.0100
p(PostTrans2+Sex*SVL+Year)	15	265.37	298.05	8.10	0.0075
p(PostTrans1+Time*SVL+Season)	11	274.85	298.29	8.34	0.0067
p(PostTrans1+Year*SVL)	17	261.42	298.88	8.93	0.0050
p(PostTrans1+Sex*SVL+time)	17	261.74	299.20	9.24	0.0042
p(Capture+Sex+Year)	11	275.97	299.41	9.46	0.0038
p(PostTrans1+Year)	11	276.03	299.47	9.52	0.0037
p(Capture+Time*SVL+Sex)	9	280.56	299.53	9.58	0.0036
p(PostTrans1+Time*SVL)	9	280.78	299.75	9.80	0.0032
p(PostTrans2+Sex+SVL+time)	17	262.41	299.87	9.91	0.0030
p(PostTrans2+Time*SVL+Season)	12	274.20	299.92	9.96	0.0030
p(Capture+time)	13	272.94	300.95	11.00	0.0018
p(PostTrans2+Time*SVL)	10	280.14	301.33	11.38	0.0015
p(PostTrans1+Sex+Year)	12	275.96	301.68	11.72	0.0012
p(PostTrans1+Time*SVL+Sex)	10	280.53	301.72	11.77	0.0012
p(PostTrans2+Sex*SVL+time)	18	261.85	301.73	11.78	0.0012
p(PostTrans2+Year)	12	276.39	302.10	12.15	0.0010

Table 2. Model-selection results evaluating effects on timber rattlesnake apparent survival (Φ) and recapture probability (*p*) from the VRUT2 population. Terms for *p* were those with $\Delta QAIC_c \leq 4$ from the analysis of *p*. We fixed *p* = 1 for radio-tracked individuals. The number of parameters is given by *K* and *w* is the $QAIC_c$ model weight. We report models with *w* ≥0.01.

Model	*K*	*Deviance*	$QAIC_c$	$\Delta QAIC_c$	*w*
Phi(Capture)p(Capture+SVL+Year)	9	268.26	287.23	0.00	0.05
Phi(Time*SVL+Sex)p(Capture+SVL+Year)	12	261.67	287.39	0.16	0.04
Phi(Study*SVL+Sex)p(Capture+SVL+Year)	12	262.56	288.28	1.05	0.03
Phi(Study)p(Capture+SVL+Year)	9	269.47	288.44	1.21	0.02
Phi(SVL+Time)p(Capture+SVL+Year)	10	267.39	288.58	1.36	0.02
Phi(Capture+Sex)p(Capture+SVL+Year)	10	267.39	288.59	1.36	0.02
Phi(Study+SVL)p(Capture+SVL+Year)	10	267.49	288.68	1.45	0.02
Phi(Sex*Study+SVL)p(Capture+SVL+Year)	12	263.19	288.90	1.68	0.02
Phi(Study*SVL+Sex+Capture)p(Capture+SVL+Year)	13	261.02	289.03	1.80	0.02
Phi(Time)p(Capture+SVL+Year)	9	270.17	289.14	1.91	0.02
Phi(Study*SVL)p(Capture+SVL+Year)	11	265.76	289.20	1.98	0.02
Phi(Capture)p(p_Group+SVL+Year)	10	268.13	289.32	2.09	0.02
Phi(Sex*SVL+Time)p(Capture+SVL+Year)	12	263.61	289.32	2.10	0.02
Phi(Time*SVL+Sex)p(Capture+Sex*SVL+Year)	14	258.99	289.33	2.10	0.02
Phi(Study*SVL+Capture)p(Capture+SVL+Year)	12	263.61	289.33	2.10	0.02
Phi(Sex*Time+SVL)p(Capture+SVL+Year)	12	263.66	289.38	2.15	0.02
Phi(Time*SVL+Sex)p(p_Group+SVL+Year)	13	261.44	289.45	2.22	0.02

Phi(Sex*SVL+Study)p(Capture+SVL+Year)	12	263.80	289.51	2.28	0.01
Phi(Sex*SVL+Capture)p(Capture+SVL+Year)	12	263.85	289.57	2.34	0.01
Phi(Time*SVL+Sex+Capture)p(Capture+SVL+Year)	13	261.58	289.59	2.36	0.01
Phi(Sex+Study)p(Capture+SVL+Year)	10	268.63	289.83	2.60	0.01
Phi(SVL*Time)p(Capture+SVL+Year)	11	266.45	289.89	2.66	0.01
Phi(Study*SVL+Sex)p(p_Group+SVL+Year)	13	262.03	290.04	2.81	0.01
Phi(Sex+SVL)p(Capture+SVL+Year)	10	269.12	290.32	3.09	0.01
Phi(Capture)p(Capture+Sex*SVL+Year)	11	266.91	290.35	3.12	0.01
Phi(Capture)p(Capture+SVL+time)	12	264.70	290.41	3.19	0.01
Phi(Sex*Study+SVL+Capture)p(Capture+SVL+Year)	13	262.41	290.42	3.19	0.01
Phi(SVL)p(Capture+SVL+Year)	9	271.45	290.42	3.19	0.01

Table 3. Model-selection results evaluating effects on timber rattlesnake dead recovery probability (*r*) from NH. In each model, apparent survival (*S*) was held constant using the term *S*(Captivity+Age*Time+Age*Season+Transmitter) and recapture probability (*p*) was held constant using the term *p*(Fixed_p+Age*Season*Captivity+Post_Trans). Within each model we included the time-varying individual covariates (TVIC) Fixed_p and Fixed_r to numerically force $p = r = 1$ when an individual was radio-tracked or in captivity and Captive to fix $S = 1$ when an individual was in captivity. We fixed $F = 1$ in all models. The number of parameters is given by *K* and *w* is the AIC$_c$ model weight.

Model	*K*	*Deviance*	*AIC$_c$*	*ΔAIC$_c$*	*w*
r(.)	30	539.12	605.60	0.00	0.3540
r(Time)	31	537.93	606.87	1.27	0.1871
r(SubAdult)	31	538.06	607.00	1.40	0.1756
r(Season)	31	539.12	608.05	2.46	0.1037
r(SubAdult:Season)	32	537.01	608.42	2.82	0.0864
r(SubAdult+Time)	32	537.70	609.11	3.51	0.0612
r(SubAdult+Time:Adult+Time:SubAdult	33	536.50	610.40	4.80	0.0320

Table 4. Model-selection results evaluating effects on timber rattlesnake recapture probability (*p*) from NH. In each model, apparent survival (*S*) was held constant using the term S(Captivity+Age*Time+Age*Season+Transmitter) and recovery probability (*r*) was modeled using terms with $\Delta AIC_c \leq 4$ from the analysis of *r*. Within each model we included the time-varying individual covariates (TVIC) Fixed_p and Fixed_r to numerically force *p* = *r* = 1 when an individual was radio-tracked or in captivity and Captive to fix *S* = 1 when an individual was in captivity. We fixed *F* = 1 in all models. The number of parameters is given by *K* and *w* is the AIC_c model weight. We report models with *w* >0.01.

Model	*K*	*Deviance*	AIC_c	ΔAIC_c	*w*
p(Year)r(.)	22	553.09	600.52	0.00	0.0678
p(AgeClass+Year)r(.)	23	551.16	600.92	0.39	0.0557
p(Year)r(Time)	23	551.66	601.41	0.89	0.0435
p(Year)r(SubAdult)	23	551.71	601.47	0.94	0.0423
p(AgeClass+Year)r(Time)	24	549.80	601.90	1.37	0.0341
p(AgeClass+Year)r(SubAdult)	24	549.89	601.99	1.46	0.0326
p(Captivity+Year)r(.)	23	552.25	602.01	1.48	0.0323
p(AgeClass+p_t+Year)r(.)	24	549.95	602.04	1.52	0.0318
p(p_t+Year)r(.)	23	552.40	602.16	1.63	0.0299
p(Year)r(SubAdult:Season)	24	550.54	602.64	2.11	0.0236
p(Year)r(Season)	23	553.07	602.82	2.30	0.0215
p(Season+Year)r(.)	23	553.09	602.85	2.32	0.0212
p(Captivity+Year)r(Time)	24	550.81	602.90	2.38	0.0206
p(Captivity+Year)r(SubAdult)	24	550.81	602.91	2.38	0.0206
p(AgeClass+p_t+Year)r(Time)	25	548.60	603.05	2.53	0.0192
p(p_t+Year)r(Time)	24	550.97	603.06	2.54	0.0190
p(p_t+Year)r(SubAdult)	24	551.08	603.18	2.65	0.0180
p(AgeClass+p_t+Year)r(SubAdult)	25	548.78	603.23	2.71	0.0175
p(AgeClass+Season+Year)r(.)	24	551.15	603.25	2.72	0.0174
p(AgeClass+Year)r(Season)	24	551.15	603.25	2.73	0.0173
p(Year)r(SubAdult+Time)	24	551.28	603.38	2.85	0.0163
p(AgeClass+Year)r(SubAdult:Season)	25	548.98	603.43	2.90	0.0159
p(Season+Year)r(Time)	24	551.65	603.75	3.22	0.0135
p(Season+Year)r(SubAdult)	24	551.71	603.81	3.28	0.0131
p(AgeClass+Year)r(SubAdult+Time)	25	549.48	603.93	3.40	0.0124
p(AgeClass+Captivity+Season+Year)r(.)	25	549.50	603.95	3.42	0.0122
p(Captivity+Year)r(SubAdult:Season)	25	549.56	604.02	3.49	0.0118
p(AgeClass+Season+Year)r(Time)	25	549.80	604.25	3.72	0.0105
p(Captivity+Year)r(Season)	24	552.22	604.32	3.79	0.0102

p(AgeClass+Season+Year)r(SubAdult)	25	549.88	604.34	3.81	0.0101
p(Captivity+Season+Year)r(.)	24	552.25	604.34	3.82	0.0100
p(Season*AgeClass+Year)r(.)	25	549.90	604.35	3.83	0.0100

Table 5. Model-selection results evaluating effects on timber rattlesnake apparent survival (*S*) from NH using a post-hoc analysis including the term *S*(Captive+Age*Season+Age*Time). In each model, recapture probability (*p*) and recovery probability (*r*) were modeled using terms with $\Delta AIC_c \leq 4$ from the analysis of *p*. Within each model we included the time-varying individual covariates (TVIC) Fixed_p and Fixed_r to numerically force *p* = *r* = 1 when an individual was radio-tracked or in captivity and Captive to fix *S* = 1 when an individual was in captivity. We fixed *F* = 1 in all models. The number of parameters is given by *K* and *w* is the AIC_c model weight. We report models with *w* >0.01.

Model	*K*	*Deviance*	*AIC_c*	*ΔAIC_c*	*w*
S(Season*Time)p(Captivity+Year)r(.)	20	552.61	595.44	0.00	0.1635
S(Season*Time)p(Year)r(.)	19	555.77	596.32	0.87	0.1056
S(Season*Time)p(Year)r(SubAdult)	20	553.94	596.76	1.32	0.0845
S(Season*Time)p(AgeClass+Year)r(.)	20	555.03	597.86	2.42	0.0488
S(Season*Time)p(Year)r(Time)	20	555.36	598.19	2.74	0.0415
S(AgeClass*Season+AgeClass*Time)p(Year)r(.)	21	553.10	598.22	2.77	0.0408
S(Season*Time)p(AgeClass+Year)r(SubAdult)	21	553.22	598.34	2.90	0.0384
S(Season*Time)p(p_t+Year)r(.)	20	555.62	598.45	3.01	0.0363
S(AgeClass*Season+AgeClass*Time) p(AgeClass+Year)r(.)	22	551.18	598.61	3.16	0.0336
S(AgeClass*Season+AgeClass*Time)p(Year)r(Time)	22	551.67	599.10	3.66	0.0263
S(AgeClass*Season+AgeClass*Time) p(Year)r(SubAdult)	22	551.72	599.15	3.71	0.0256
S(AgeClass*Season+Time)p(Year)r(.)	20	556.43	599.25	3.81	0.0243
S(AgeClass*Season+AgeClass*Time) p(AgeClass+Year)r(Time)	23	549.81	599.56	4.12	0.0209
S(AgeClass*Season+AgeClass*Time) p(AgeClass+Year)r(SubAdult)	23	549.90	599.65	4.21	0.0199
S(AgeClass*Season+AgeClass*Time) p(Captivity+Year)r(.)	22	552.28	599.71	4.26	0.0194
S(Season*Time)p(AgeClass+Year)r(Time)	21	554.62	599.74	4.30	0.0190
S(AgeClass*Season+AgeClass*Time) p(AgeClass+p_t+Year)r(.)	23	550.07	599.82	4.38	0.0183
S(AgeClass*Season+AgeClass*Time) p(p_t+Year)r(.)	22	552.42	599.85	4.41	0.0181
S(AgeClass*Season+Time)p(Year)r(Time)	21	554.74	599.87	4.42	0.0179
S(AgeClass*Season+Time)p(Year)r(SubAdult)	21	554.74	599.87	4.42	0.0179
S(AgeClass*Season+Time)p(AgeClass+Year)r(.)	21	554.80	599.92	4.48	0.0174

S(Season*Time)p(AgeClass+p_t+Year)r(.)	21	554.99	600.11	4.67	0.0159
S(AgeClass*Season+Time)p(Captivity+Year)r(.)	21	555.20	600.33	4.88	0.0142
S(AgeClass*Season+Time)p(AgeClass+Year)r(Time)	22	553.17	600.60	5.16	0.0124
S(AgeClass*Season+Time)p(AgeClass+Year) r(SubAdult)	22	553.22	600.65	5.20	0.0121

Table 6. Model-selection results evaluating effects on timber rattlesnake recapture probability (*p*) from MBER. In each model, apparent survival (Φ) was held constant using a linear trend effect of time (Time), snout-vent length (SVL), and Transmitter and we fixed *p* = 1 for radio-tracked individuals. The number of parameters is given by *K* and *w* is the AIC$_c$ model weight. We report models with *w* ≥0.01.

Model	*K*	*Deviance*	*AIC$_c$*	*ΔAIC$_c$*	*w*
p(Capture+SVL*Time)	8	247.02	263.61	0.00	0.1246
p(Capture+SVL*Time+Den)	11	240.66	263.75	0.14	0.1162
p(Capture+Time)	6	252.85	265.19	1.58	0.0564
p(p_Group+SVL*Time)	9	246.83	265.57	1.96	0.0467
p(Capture+SVL*Time+Den+Sex)	12	240.40	265.69	2.08	0.0439
p(p_Group+SVL*Time+Den)	12	240.65	265.94	2.34	0.0387
p(Capture+Den)	8	249.75	266.34	2.73	0.0318
p(Capture+Den+Time)	9	247.74	266.48	2.87	0.0296
p(Capture+SVL+Time)	7	252.35	266.81	3.20	0.0251
p(p_Group+Time)	7	252.69	267.14	3.53	0.0213
p(Capture+Sex+Time)	7	252.76	267.22	3.61	0.0205
p(Capture)	5	257.25	267.50	3.89	0.0178
p(Capture+Den+SVL)	9	248.93	267.67	4.06	0.0164
p(Capture+Season*SVL+Den)	11	244.65	267.74	4.13	0.0158
p(Capture+Season*SVL+Den+Season)	11	244.65	267.74	4.13	0.0158
p(Capture+Den+Season)	9	249.10	267.84	4.23	0.0150
p(Capture+Sex*Time)	8	251.25	267.84	4.23	0.0150
p(p_Group+SVL*Time+Den+Sex)	13	240.39	267.91	4.30	0.0145
p(Capture+Den+Study)	9	249.20	267.94	4.33	0.0143
p(Den+p_Group)	9	249.50	268.24	4.63	0.0123
p(Capture+Den+Season+Time)	10	247.43	268.34	4.73	0.0117
p(Capture+Sex+SVL+Time)	8	251.88	268.47	4.86	0.0110
p(Capture+Den+Sex)	9	249.75	268.48	4.88	0.0109
p(Den+p_Group+Time)	10	247.70	268.60	4.99	0.0103
p(Capture+Den+Sex+Time)	10	247.73	268.63	5.03	0.0101
p(Capture+SVL*Time)	8	247.02	263.61	0.00	0.1246

Table 7. Model-selection results evaluating effects on timber rattlesnake apparent survival (Φ) and recapture probability (*p*) from MBER. Terms for *p* were those with ΔAIC$_c$ ≤4 from the analysis of *p*. We fixed *p* = 1 for radio-tracked individuals. The number of parameters is given by *K* and *w* is the AIC$_c$ model weight. We report models with *w* ≥0.01.

Model	*K*	*Deviance*	*AIC$_c$*	*ΔAIC$_c$*	*w*
Phi(.)p(Capture+SVL*Time+Den)	8	241.36	257.94	0.00	0.0406
Phi(.)p(Capture+SVL*Time)	5	247.80	258.04	0.10	0.0386
Phi(Season)p(Capture+SVL*Time+Den)	9	240.33	259.07	1.12	0.0231
Phi(Season)p(Capture+SVL*Time)	6	246.79	259.13	1.18	0.0224
Phi(SVL)p(Capture+SVL*Time+Den)	9	240.79	259.53	1.58	0.0184
Phi(.)p(Capture+Time)	3	178.54	259.55	1.61	0.0182
Phi(SVL)p(Capture+SVL*Time)	6	247.29	259.63	1.69	0.0175
Phi(.)p(Capture+SVL*Time+Den+Sex)	9	241.08	259.82	1.88	0.0159
Phi(Time)p(Capture+SVL*Time+Den)	9	241.25	259.99	2.04	0.0146
Phi(Sex)p(Capture+SVL*Time)	6	247.69	260.03	2.09	0.0143
Phi(.)p(p_Group+SVL*Time)	6	247.69	260.03	2.09	0.0143
Phi(Transmitter)p(Capture+SVL*Time)	6	247.71	260.05	2.11	0.0141
Phi(Time)p(Capture+SVL*Time)	6	247.71	260.05	2.11	0.0141
Phi(Sex)p(Capture+SVL*Time+Den)	9	241.32	260.05	2.11	0.0141
Phi(.)p(p_Group+SVL*Time+Den)	9	241.35	260.09	2.15	0.0139
Phi(Transmitter)p(Capture+SVL*Time+Den)	9	241.36	260.09	2.15	0.0139
Phi(.)p(Capture+Den+Time)	6	173.10	260.36	2.42	0.0121
Phi(Season+SVL)p(Capture+SVL*Time+Den)	10	239.65	260.56	2.61	0.0110
Phi(Season)p(Capture+Time)	4	177.50	260.58	2.63	0.0109
Phi(Season+SVL)p(Capture+SVL*Time)	7	246.18	260.64	2.69	0.0106

Table 8. Model-selection results evaluating effects on timber rattlesnake recapture probability (*p*) from CHAR. In each model, apparent survival (Φ) was held constant using a time- and age-dependent model structure (Φ(Adult:Year + Neonate:Time + Season)) and we fixed *p* = 1 for radio-tracked individuals. The number of parameters is given by *K* and *w* is the $QAIC_c$ model weight. We report models with *w* ≥0.01.

Model	*K*	*Q Dev-iance*	*$QAIC_c$*	*Δ $QAIC_c$*	*w*
p(Age3+Capture)	9	54.84	154.25	0.00	0.0284
p(Adult+Capture+Year2)	9	55.13	154.54	0.29	0.0246
p(Capture+Time:Adult+Time:neo_age_p2)	9	55.25	154.67	0.42	0.0231
p(PostTrans+Time:Adult:Season)	10	53.03	154.68	0.43	0.0230
p(Capture+Time:Adult:Season)	9	55.27	154.68	0.43	0.0229
p(Capture+neo_ageDep_p+Time:Adult)	9	55.27	154.68	0.43	0.0229
p(Capture+juv_ageDep_p+neo_ageDep_p)	9	55.27	154.68	0.44	0.0229
p(Capture+neo_ageDep_p+Time:Adult:Season)	10	53.05	154.69	0.44	0.0228
p(Capture+neo_age_p2:Time+Time:Adult:Season)	10	53.05	154.70	0.45	0.0227
p(Capture+Time:Adult)	8	57.54	154.74	0.49	0.0222
p(Capture+Year2:Adult)	9	55.34	154.75	0.50	0.0221
p(Capture+Season+Time:Adult)	9	55.50	154.91	0.66	0.0204
p(Capture+neo_ageDep_p+Season+Time:Adult)	10	53.29	154.93	0.69	0.0202
p(neo_age_p2:Time+PostTrans+Time:Adult:Season)	11	51.10	155.00	0.75	0.0195
p(neo_ageDep_p+PostTrans+Time:Adult:Season)	11	51.11	155.02	0.77	0.0194
p(Capture+Season+Year2:Adult)	10	53.43	155.07	0.82	0.0188
p(Capture+neo_age_p2+Time:Adult)	9	55.74	155.15	0.90	0.0181
p(Capture+neo_age_p2+Time:Adult:Season)	10	53.54	155.18	0.93	0.0178
p(Age3+Capture+Time)	10	53.65	155.29	1.04	0.0169
p(Age3+PostTrans)	10	53.83	155.47	1.22	0.0154
p(neo_age_p2+PostTrans+Time:Adult:Season)	11	51.58	155.48	1.23	0.0154
p(neo_ageDep_p+PostTrans+Time:Adult)	10	53.90	155.55	1.30	0.0149
p(Adult+PostTrans+Year2)	10	53.92	155.56	1.31	0.0147
p(PostTrans+Time:Adult+Time:neo_age_p2)	10	53.98	155.63	1.38	0.0143
p(neo_ageDep_p+PostTrans+Season+Time:Adult)	11	51.78	155.68	1.43	0.0139
p(PostTrans+Season+Time:Adult)	10	54.06	155.70	1.45	0.0137
p(PostTrans+Time:Adult)	9	56.30	155.71	1.46	0.0137
p(Capture+Season+Time:Adult+Time:neo_age_p2)	10	54.19	155.84	1.59	0.0128
p(Age3+Capture+Season+Time)	11	51.97	155.87	1.62	0.0126
p(juv_ageDep_p+neo_ageDep_p+PostTrans)	10	54.26	155.91	1.66	0.0124
p(PostTrans+Year2:Adult)	10	54.26	155.91	1.66	0.0124

p(Capture+Time:Adult+Time:neo_ageDep_p)	9	56.54	155.95	1.70	0.0121
p(Age3+PostTrans+Time)	11	52.05	155.95	1.70	0.0121
p(Capture+neo_ageDep_p:Time+ Time:Adult:Season)	10	54.34	155.98	1.73	0.0120
p(Capture+Season+Time:Adult+ Time:neo_ageDep_p)	10	54.43	156.08	1.83	0.0114
p(neo_age_p2+PostTrans+Time:Adult)	10	54.47	156.11	1.86	0.0112
p(PostTrans+Season+Year2:Adult)	11	52.21	156.12	1.87	0.0112
p(neo_ageDep_p:Time+PostTrans+ Time:Adult:Season)	11	52.32	156.22	1.97	0.0106
p(Capture+neo_age_p2+Season+Time:Adult)	10	54.61	156.25	2.00	0.0104
p(Adult+Capture+neo_ageDep_p+Season+Time)	11	52.37	156.28	2.03	0.0103

Table 9. Model-selection results evaluating effects on timber rattlesnake apparent survival (Φ) and recapture probability (*p*) from CHAR. Terms for *p* were those with $\Delta QAIC_c \leq 2$ from the analysis of *p*. We fixed *p* = 1 for radio-tracked individuals. The number of parameters is given by *K* and *w* is the AIC_c model weight. We report models with *w* ≥0.025.

Model	*K*	*QDeviance*	*$QAIC_c$*	*$\Delta QAIC_c$*	*w*
Phi(1)p(Age3+Capture)	4	57.17	145.78	0.00	0.0052
Phi(1)p(Adult+Capture+Year2)	4	57.22	145.83	0.05	0.0051
Phi(1)p(Capture+juv_ageDep_p+neo_ageDep_p)	4	57.42	146.04	0.25	0.0046
Phi(Capture)p(Age3+Capture)	5	55.45	146.17	0.39	0.0043
Phi(1)p(Capture+Year2:Adult)	4	57.58	146.19	0.41	0.0042
Phi(1)p(Age3+Capture+Time)	5	55.70	146.42	0.64	0.0038
Phi(Capture) p(Capture+juv_ageDep_p+neo_ageDep_p)	5	55.88	146.61	0.82	0.0034
Phi(Capture)p(Adult+Capture+Year2)	5	55.88	146.61	0.83	0.0034
Phi(1)p(Adult+PostTrans+Year2)	5	55.93	146.66	0.88	0.0033
Phi(Capture)p(Capture+Year2:Adult)	5	55.94	146.67	0.89	0.0033
Phi(1)p(Capture+Time:Adult)	3	60.26	146.78	1.00	0.0031
Phi(Season)p(Age3+Capture)	5	56.09	146.81	1.03	0.0031
Phi(neo_age_Phi)p(Capture+Time:Adult)	4	58.21	146.82	1.04	0.0031
Phi(1)p(Capture+Season+Year2:Adult)	5	56.10	146.83	1.05	0.0031
Phi(1)p(Age3+PostTrans+Time)	6	54.01	146.87	1.09	0.0030
Phi(1)p(Age3+PostTrans)	5	56.17	146.90	1.12	0.0030
Phi(Season)p(Adult+Capture+Year2)	5	56.34	147.06	1.28	0.0027
Phi(1)p(Capture+Time:Adult+Time:neo_age_p2)	4	58.48	147.10	1.31	0.0027
Phi(Adult:Time)p(Adult+Capture+Year2)	5	56.37	147.10	1.31	0.0027
Phi(Season) p(Capture+juv_ageDep_p+neo_ageDep_p)	5	56.42	147.14	1.36	0.0026
Phi(1)p(juv_ageDep_p+neo_ageDep_p+PostTrans)	5	56.42	147.15	1.37	0.0026
Phi(1)p(PostTrans+Year2:Adult)	5	56.45	147.18	1.40	0.0026
Phi(1)p(Capture+neo_ageDep_p+Time:Adult)	4	58.64	147.26	1.48	0.0025
Phi(Adult:Time)p(PostTrans+Time:Adult:Season)	6	54.41	147.27	1.48	0.0025
Phi(Adult:Time)p(Age3+Capture)	5	56.54	147.27	1.49	0.0025

Table 10. Comparison of activity season lengths and start and end times among northeastern timber rattlesnake populations. The 95th range is the difference between the 97.5th and 2.5th quantiles of the date of each observation (following Brown 2008) and the 98th range is the difference between the 99th and 1st quantile. Only captures made without the assistance of radio telemetry were included. Correlations between activity season metrics and latitude were conducted using Spearman's rank correlations and we report Spearman's rho (ρ) and *p* values. Data from New York were taken were taken from Brown (2008). Populations are ranked in order of decreasing latitude.

95th range

Population	*Length*	*Start Date*	*End Date*
VRUT2	161 days	30 April	8 October
New York	159 days	29 April	5 October
NH	159 days	2 May	8 October
MHAD1	191 days	17 April	26 October
MBER	181 days	16 April	14 October
CHAR	191 days	21 April	29 October
	$\rho = -0.72$, $P = 0.14$	$\rho = 0.66$, $P = 0.18$	$\rho = -0.77$, $P = 0.10$

98th range

Population	*Length*	*Start Date*	*End Date*
VRUT2	175 days	18 April	10 October
NH	181 days	30 April	28 October
MHAD1	191 days	17 April	26 October
MBER	186 days	15 April	18 October
CHAR	198 days	19 April	4 November
	$\rho = -0.90$, $P = 0.08$	$\rho = 0.20$, $P = 0.78$	$\rho = -0.60$, $P = 0.35$

Table 11. Model selection tables comparing snout-vent length and body condition index among populations of timber rattlesnakes in New England. The number of parameters is given by *K* and *w* is the AIC_c model weight.

Snout-vent Length					
	K	*Deviance*	AIC_c	ΔAIC_c	w
Population*Sex	9	-213.80	-195.29	0.00	0.6588
Population+Sex	6	-206.20	-193.97	1.32	0.3405
Sex	3	-187.76	-181.69	13.59	0.0007
Population	5	-165.64	-155.47	39.81	0.0000
Null	2	-146.60	-142.57	52.72	0.0000

Body Condition Index					
	K	*Deviance*	AIC_c	ΔAIC_c	w
Population	5	-94.66	-84.47	0.00	0.6898
Population+Sex	6	-94.66	-82.40	2.07	0.2445
Population*Sex	9	-98.34	-79.77	4.70	0.0657
Null	2	-29.62	-25.58	58.89	0.0000
Sex	3	-29.62	-23.55	60.92	0.0000

Table 12. Comparison of snout-vent length (SVL) and body weight between males and females for populations of timber rattlesnakes in New England. Values presented are means or medians (SE), the inter-quartile range, and number of individuals (*n*). We tested for differences in means using t-tests and medians using Wilcoxon signed rank tests. All individuals were >1-year old unless otherwise noted. Note that some individuals were not included in the analyses of SVL and body condition among populations because of missing SVL or weight values.

	SVL (cm)		*Weight (g)*	
	Females	*Males*	*Females*	*Males*
VRUT1	91.4*	101.1*	501 ± 88.15	803 ± 63.07
	87.4–91.9	100.1–102.95	448–601	700–860
	$n = 6$	$n = 6$, $p = 0.07$	$n = 6$	$n = 5$, $p = 0.02$
VRUT2	95.0 (1.92)	105.4 (1.93)	702 g (43.3)	936 g (42.8)
	92.7–101.6	96.0–116.8	518–831	700–1,217
	$n = 45$	$n = 73$, $p < 0.001$	$n = 43$	$n = 73$, $p < 0.001$
VRUT2 (≤1-year old)	28.1 (0.44)	29.0 (1.75)	24 (1.01)	31 (4.72)
	27.1–28.9	27.2–29.1	23–27	25–31
	$n = 10$	$n = 10$, $p = 0.63$	$n = 10$	$n = 8$, $p = 0.23$
MHAD1	82.5 cm (3.00)	99.6 cm (5.05)	522 g (68)	976 g (133)
	70.0–93.5	90.3–113.6	263–756	485–1,396
	$n = 27$	$n = 18$, $p = 0.007$	$n = 20$	$n = 15$, $p = 0.006$
MBER	85.4 cm (1.92)	101.6 cm (1.87)	651 g (40.79)	1,131 g (58.14)
	81.2–94.0	91.5–114.5	442–803	750–1,508
	$n = 45$	$n = 95$, $p < 0.001$	$n = 43$	$n = 87$, $p < 0.001$
MBER (≤1-year old)	34.85 cm (1.56)	33.1 cm (1.35)	28*	29*
	30.25–37.50	32.0–33.0	26–39	29–33
	$n = 15$	$n = 15$, $p = 0.41$	$n = 8$	$n = 10$, $p = 0.54$
CHAR	100.3 (1.26)	116.9 (2.42)	914 g (48)	1,653 g (108)
	97.1–105.7	112.0–124.5	702–1,060	1,394–2,064
	$n = 27$	$n = 25$, $p < 0.001$	$n = 27$	$n = 25$, $p < 0.001$

* Median

Table 13a. Summaries of demographic metrics for select New England populations of timber rattlesnakes. Means (SE) are reported unless otherwise specified. Values for New York are taken from Brown (1991; 2016) and Brown et al. (2007). All survival estimates are medians of model-averaged point estimates across all sampling intervals unless otherwise noted. Note that some values are not reported in the text.

	VRUT2	*New York*	*NH*
Years studied	7	25–36	10
Elevation (m)	200	150–390	100–200
Number of sub-populations	1	9[b]	1
Activity season length (days)	161 (156–173)	159	159 (156–176)
Total abundance (>1-year olds)[c]	218.03 (197.23–972.34)	NA	NA
Annual non-neonate survival	0.77 (PIT-females)	0.82 (yellow-phase)	0.21–0.94 (adults-summer)[d]
	0.86 (PIT-males)	0.96 (black-phase)	0.68–0.83 (adults-winter)[d]
	0.85 (radio-females)		0.40–0.97 (sub-adults-summer)[d]
	0.94 (radio-males)		0.62–0.51 (sub-adults-winter)[d]
Body condition index (> 1 year olds)	-0.13 (-0.17–-0.09)	NA	NA
Neonate snout-vent length (SVL, cm)	28.2 (0.27, n = 19)	NA	NA
Neonate weight (g)	25 (0.73, n = 19)	23.16 (0.22)[e]	NA
Sex ratio (F:M, >1-year olds)	1:1.60 (p = 0.0126)	NA	1:1.10 (p = 0.66)
Color phase ratio (Y:B, >1-year olds)	1:11.33 (p < 0.0001)	1:0.84 (p = 0.04)[f]	0:1.00
SVL of the 10 longest females (cm)	106.9 (1.09)	112g	NA
SVL of the 10 smallest gravid females (cm)	99.0 (1.94, n = 9)	83[h]	NA
Number of marked snakes	151	588, 4,025[i]	100
Number of >1-year old marked snakes	123	NA	98

Number of radio-tracked snakes	28	0	34
Number of recaptures (non-telemetry)	7 (6%)	114 (19%), NR[j]	33 (50%)
Overall ratio of gravid:non-gravid females[k]	1:3.22 (9:29)	1:0.9 (219, 208)	1:2.62 (13:34)
Observed mean reproductive cycle length (years)	NA	4.2 (*n* = 162 snakes, 247 cycles)	3 (*n* = 4 snakes, 5 cycles)
Observed range of reproductive cycle length (years)	NA	2–7 (mode = 3)	3
Number of follicles	5.2 (1.18)	NA	NA
Sheds per year	1.9 (0.53, *n* = 13)	1.31[n]	NA
Litter size	5.67 (0.73, *n* = 9)	7.7 (*n* = 31 in lab, 4 in field)	NA

[a] *All values are from the southern subpopulation within CHAR*

[b] *All reproductive metrics are from two subpopulations (Brown 2016)*

[c] *Model-averaged full-likelihood estimates with 95% CI*

[d] *Point estimates from the first and last intervals*

[e] *Standard error derived from values reported in the text*

[f] *Ratio from neonates born in captivity*

[g] *Maximum reported size of a gravid female*

[h] *Smallest size of a post-partum female*

[I] *Numbers marked from each subpopulation*

[j] *Not reported*

[k] *Numbers are female-years*

[l] *Increases to 3.33 if two questionable cycles are included*

[m] *Increases to 2.75 if two questionable cycles are included*

[n] *Females only*

Table 13a. Summaries of demographic metrics for select New England populations of timber rattlesnakes. Means (SE) are reported unless otherwise specified. Values for New York are taken from Brown (1991; 2016) and Brown et al. (2007). All survival estimates are medians of model-averaged point estimates across all sampling intervals unless otherwise noted. Note that some values are not reported in the text.

	MHAD1	*MNOR*	*MBER*	*CHAR*[a]
Years studied	7	2	6	3
Elevation (m)	240	150	655	215
Number of sub-populations	1 (2)	1	4	1 (2) [a]
Activity season length (days)	191 (171–191)	NA	181 (166-185)	191 (185–196)
Total abundance (>1-year olds)[c]	NA	NA	625.27 (341.14-1,284.29)	79.66 (29.04–62,299.93)
Annual non-neonate survival	NA	0.78	0.87	0.77
	NA	NA		
	NA	NA		
	NA	NA		
Body condition index (> 1 year olds)	0.04 (-0.09–0.08)	NA	0.11 (0.06–0.13)	0.01 (0–0.09)
Neonate snout-vent length (SVL, cm)	NA	NA	32.1 (0.74, *n* = 23)	29.5 (0.28, *n* = 28)
Neonate weight (g)	NA	NA	28 (0.91, *n* = 13)	28 (0.44, *n* = 62)
Sex ratio (F:M, >1-year olds)	1:0.74 ($p = 0.31$)	NA	1:2.04 ($p < 0.0001$)	1:0.93 ($p = 0.78$)
Color phase ratio (Y:B, >1-year olds)	NA	NA	1:1.01 ($p = 0.93$)	1:0.33 ($p < 0.0001$)
SVL of the 10 longest females (cm)	98.2 (2.01)	NA	100.2 (1.33)	106.5 (0.53)
SVL of the 10 smallest gravid females (cm)	NA	NA	88.8 (2.63, *n* = 5)	99.4 (0.99)
Number of marked snakes	61	NA	178	147
Number of >1-year old marked snakes	51	24	145	63
Number of radio-tracked snakes	0	13	20	34
Number of recaptures (non-telemetry)	NA	0	15 (9%)	16 (14%)

Overall ratio of gravid:non-gravid females[k]	NA	NA	1:4.6 (5:24)	1:0.90 (21:19)
Observed mean reproductive cycle length (years)	NA	NA	3 (*n* = 1 snake, 1 cycle)[l]	2 (*n* = 2 snakes, 2 cycles)[m]
Observed range of reproductive cycle length (years)	NA	NA	3 years	2 years
Number of follicles	NA	NA	NA	NA
Sheds per year	NA	NA	NA	1.39 (0.10, *n* = 23)
Litter size	2 & 3 (*n* = 2)	NA	NA	7.75 (0.59, *n* = 8)

[a] *All values are from the southern subpopulation within CHAR*

[b] *All reproductive metrics are from two subpopulations (Brown 2016)*

[c] *Model-averaged full-likelihood estimates with 95% CI*

[d] *Point estimates from the first and last intervals*

[e] *Standard error derived from values reported in the text*

[f] *Ratio from neonates born in captivity*

[g] *Maximum reported size of a gravid female*

[h] *Smallest size of a post-partum female*

[I] *Numbers marked from each subpopulation*

[j] *Not reported*

[k] *Numbers are female-years*

[l] *Increases to 3.33 if two questionable cycles are included*

[m] *Increases to 2.75 if two questionable cycles are included*

[n] *Females only*

Table 14. Model-selection results evaluating effects on timber rattlesnake population growth rate (λ) from the MBER and VRUT2 populations using Pradel's (1996) model. We only included PIT-tagged individuals >1-year old. The number of parameters is given by *K* and *w* is the AIC_c model weight. We report models with *w* ≥0.02.

MBER

Model	*K*	*Deviance*	*AIC_c*	*ΔAIC_c*	*w*
Phi(.)p(Year)lambda(1)	8	255.50	722.48	0.00	0.1358
Phi(.)p(Den+Year)lambda(1)	11	249.66	723.56	1.09	0.0789
Phi(Season)p(Year)lambda(1)	9	254.82	724.08	1.60	0.0610
Phi(.)p(Sex+Year)lambda(1)	9	254.97	724.22	1.75	0.0567
Phi(.)p(Year)lambda(Sex)	9	254.99	724.24	1.76	0.0562
Phi(Sex)p(Sex+Year)lambda(1)	10	252.91	724.47	1.99	0.0501
Phi(Sex)p(Year)lambda(1)	9	255.46	724.72	2.24	0.0443
Phi(Time)p(Year)lambda(1)	9	255.49	724.75	2.27	0.0436
Phi(Season)p(Den+Year)lambda(1)	12	248.54	724.82	2.34	0.0421
Phi(Sex)p(Den+Year)lambda(1)	12	249.23	725.52	3.04	0.0297
Phi(.)p(Den+Year)lambda(Sex)	12	249.30	725.59	3.11	0.0287
Phi(Time)p(Den+Year)lambda(1)	12	249.61	725.90	3.42	0.0246
Phi(Season)p(Year)lambda(Sex)	10	254.36	725.93	3.45	0.0242
Phi(Season)p(Sex+Year)lambda(1)	10	254.37	725.93	3.45	0.0241
Phi(Sex)p(Sex+Year)lambda(Sex)	11	252.28	726.19	3.71	0.0213
Phi(.)p(Sex+Year)lambda(Sex)	10	254.69	726.25	3.77	0.0206
Phi(Season+Sex)p(Year)lambda(1)	10	254.74	726.30	3.82	0.0201

VRUT2

Model	*K*	*Deviance*	*AIC_c*	*ΔAIC_c*	*w*
Phi(Study)p(Year)lambda(Sex)	10	86.68	525.39	0.00	0.1835
Phi(Season)p(Year)lambda(1)	10	87.80	526.51	1.11	0.1051
Phi(Study)p(Year)lambda(1)	9	90.39	526.72	1.33	0.0942
Phi(Season)p(Year)lambda(Sex)	11	85.86	526.99	1.60	0.0824
Phi(Sex+Study)p(Year)lambda(Sex)	11	86.16	527.29	1.90	0.0711
Phi(Study)p(Sex+Year)lambda(Sex)	11	86.64	527.77	2.38	0.0559
Phi(Study)p(Sex+Year)lambda(1)	10	90.01	528.72	3.33	0.0347
Phi(Season)p(Sex+Year)lambda(1)	11	87.77	528.90	3.51	0.0317
Phi(Season+Sex)p(Year)lambda(1)	11	87.79	528.92	3.53	0.0314
Phi(Season)p(Sex+Year)lambda(Sex)	12	85.38	528.98	3.58	0.0306
Phi(Sex+Study)p(Year)lambda(1)	10	90.35	529.06	3.67	0.0293
Phi(Season+Sex)p(Year)lambda(Sex)	12	85.57	529.17	3.78	0.0278
Phi(Time)p(Year)lambda(Sex)	10	90.49	529.20	3.81	0.0273
Phi(Sex+Study)p(Sex+Year)lambda(Sex)	12	86.00	529.60	4.21	0.0224

Phi(Sex*Study)p(Year)lambda(Sex)	12	86.16	529.76	4.36	0.0207

Table 15. Mean (SD) of simulated intrinsic rate of population growth, probability of extinction (% simulations going extinct in 100 years), and median number of years to extinction for select populations of timber rattlesnakes in the northeastern United States across 1,000 simulations (r = 0 indicates a stable population). The New York population is from the Adirondack Mountains of eastern New York (Brown 1991, 2008, 2016, Brown et al. 2007). Inbreeding was specified usin 6.29 or 12 lethal equivalents. Scenarios with equal Adult and Juvenile survival used neonate (first year) survival estimates from Brown et al. (2007) and empirically estimated survival estimates for all subsequent ages. Scenarios with unequal Adult and Juvenile survival estimates used estimates from Brown et al. (2007) for second through fourth year individuals except for CHAR were population- and age-specific survival estimates were used. Low, Medium, and High represent initial population sizes (Appendix 1)..

Population	Lethal Equivalents	Survival	Low	Med.	High
VRUT2	6.29	adult = juv.	-0.23 (0.32), 100%, 17 yrs	-0.24 (0.31), 100%, 21 yrs	-0.24 (0.31), 100%, 23 yrs
VRUT2	6.29	adult ≠ juv.	-0.15 (0.26), 100%, 27 yrs	-0.15 (0.25), 100%, 33 yrs	-0.15 (0.24), 100%, 38 yrs
VRUT2	12	adult = juv.			
New York*	6.29	adult ≠ juv.	0.08 (0.11), 0%, 0 yrs	0.08 (0.11), 0%, 0 yrs	0.08 (0.11), 0%, 0 yrs
NH	6.29	adult ≠ juv.	-0.16 (0.40), 100%, 14 yrs	-0.15 (0.36), 100%, 19 yrs	-0.14 (0.31), 100%, 28 yrs
NH	12	adult ≠ juv.	-0.15 (0.38), 100%, 15 yrs	-0.15 (0.35), 100%, 19 yrs	-0.15 (0.30), 100%, 27 yrs
MBER	6.29	adult = juv.	0.05 (0.18), 0%, 0 years	0.05 (0.18), 0%, 0 years	0.05 (0.18), 0%, 0 years
CHAR*	6.29	adult ≠ juv.	-0.04 (0.55), 83%, 51 yrs	-0.03 (0.55), 72%, 65 yrs	-0.03 (0.55), 64%, 82 yrs

**Used empirical estimates for neonate, juvenile, and adult survival in all simulations*

Table 16. Mean (SD) of simulated population growth rate, probability of extinction (% simulations going extinct in 100 years), and median time to extinction (years) for different values of annual survival for the VRUT2 population of timber rattlesnakes. All other parameter values were held constant at their base values unless otherwise noted. Inbreeding was specified as 6.29 lethal equivalents.

Adult Survival (F, M)	*Adult = Juv. Φ* *$N_{initial}$ = 545*	*Adult = Juv. Φ* *$N_{initial}$ = 218*	*Juv. Φ = empirical estimates* *$N_{initial}$ = 218*
0.983, 0.986	0.11 (0.34), 14.8%, 0 yrs	0.11 (0.34), 18.0%, 0 yrs	0.04 (0.26), 10.5%, 0 years
0.949, 0.958	0.09 (0.25), 2.1%, 0 yrs	0.09 (0.25), 2.4%, 0 yrs	0.01 (0.23), 8.8%, 0 years
0.916, 0.930	0.06 (0.22), 1.2%, 0 yrs	0.06 (0.22), 1.4%, 0 yrs	-0.02 (0.24), 31.4%, 0 years
0.882, 0.902	0.03 (0.22), 2.2%, 0 yrs	0.02 (0.22), 4.2%, 0 yrs	-0.04 (0.27), 69.4%, 80 years
0.848, 0.873	-0.01 (0.23), 13.7%, 0 yrs	-0.02 (0.24), 32.3%, 0 yrs	-0.07 (0.28), 96.3%, 53 years
0.814, 0.845	-0.06 (0.25), 71.2%, 81 yrs	-0.06 (0.26), 85.5%, 65 yrs	-0.10 (0.29), 99.9%, 39 years
0.780, 0.817	-0.09 (0.27), 98.2%, 53 yrs	-0.10 (0.28), 100%, 41 yrs	-0.13 (0.3), 100%, 32 years
0.747, 0.789	-0.13 (0.28), 100%, 37 yrs	-0.13 (0.29), 100%, 30 yrs	-0.16 (0.3), 100%, 25 years
0.713, 0.761	-0.17 (0.3), 100%, 28 yrs	-0.18 (0.31), 100%, 22 yrs	-0.19 (0.32), 100%, 22 years
0.678, 0.733	-0.22 (0.31), 100%, 23 yrs	-0.22 (0.32), 100%, 18 yrs	-0.22 (0.32), 100%, 18 years
0.662, 0.719 (base)	-0.23 (0.31), 100%, 22 yrs	-0.23 (0.32), 100%, 18 yrs	-0.22 (0.32), 100%, 18 years

Table 17. Mean (SD) of simulated population growth rate (r), probability of extinction, and median time to extinction (years) for different values of annual survival for the NH population of timber rattlesnakes. All other parameter values were held constant at their base values unless otherwise noted and 12 lethal equivalents were used to denote inbreeding depression.

Adult survival	$N_{initial} = 30$ *(juvenile survival = 0.577)*	$N_{initial} = 30$ *(juvenile survival = 0.844)*	*Juvenile survival*	$N_{initial} = 30$ *(adult survival = 0.764)*	$N_{initial} = 30$ *(adult survival = 0.839)*
98.76	0.06 (0.16), 0.1%, 0 years	0.14 (0.12), 0%, 0 years	97.775	-0.04 (0.21), 94%, 51 years	0.05 (0.12), 3.1%, 0 years
96.28	0.03 (0.18), 1.5%, 0 years	0.12 (0.12), 0%, 0 years	93.325	-0.05 (0.23), 98.2%, 41 years	0.03 (0.14), 11.4%, 0 years
93.8	0 (0.22), 22.8%, 0 years	0.1 (0.12), 0%, 0 years	88.875	-0.06 (0.25), 99.8%, 36 years	0.01 (0.16), 28.6%, 0 years
91.32	-0.03 (0.26), 75.3%, 74 years	0.07 (0.13), 0.1%, 0 years	84.425	-0.07 (0.26), 100%, 31 years	-0.01 (0.19), 51.7%, 97 years
88.84	-0.04 (0.3), 98%, 46 years	0.05 (0.13), 1.6%, 0 years	79.975	-0.08 (0.28), 100%, 26 years	-0.03 (0.22), 78.9%, 67 years
86.36	-0.06 (0.33), 100%, 33 years	0.02 (0.15), 13.7%, 0 years	75.525	-0.09 (0.3), 100%, 23 years	-0.04 (0.24), 95.3%, 51 years
83.88	-0.08 (0.35), 100%, 26 years	-0.01 (0.19), 51.7%, 97 years	71.075	-0.1 (0.32), 100%, 21 years	-0.05 (0.27), 99.4%, 42 years
81.4	-0.1 (0.36), 100%, 21 years	-0.03 (0.22), 88.2%, 56 years	66.625	-0.12 (0.34), 100%, 18 years	-0.06 (0.29), 100%, 35 years
78.92	-0.13 (0.37), 100%, 17 years	-0.05 (0.24), 99.2%, 41 years	62.175	-0.13 (0.36), 100%, 16 years	-0.07 (0.32), 100%, 29 years
76.44	-0.15 (0.38), 100%, 15 years	-0.07 (0.26), 100%, 31 years	57.725	-0.15 (0.38), 100%, 15 years	-0.08 (0.35), 100%, 26 years

Table 18. Mean (SD) of simulated population growth rate (r), probability of extinction, and median time to extinction (years) for different values of annual survival for the CHAR population of timber rattlesnakes. All other parameter values were held constant at their base values unless otherwise noted. Inbreeding is specified using 6.29 or 12 lethal equivalents.

Adult survival (holding neonate and juvenile survival constant)	$N_{initial}$ *= 80, 12 LE*	$N_{initial}$ *= 160, 6.29 LE*	$N_{initial}$ *= 160, 12 LE*	$N_{initial}$ *= 160, 12 LE, K = 1,600*	*Juvenile survival (holding neonate and adult survival constant)*	$N_{initial}$ *= 160, 12 LE*
96.62	0.15 (0.38), 96.7%, 21 years	0.16 (0.38), 96.3%, 21 years	0.15 (0.37), 97.2%, 20 years	0.16(0.38), 97.7%, 21 years	98.89	0.09 (0.34), 4.7%, 0 years
93.24	0.05 (0.5), 42.8%, 0 years	0.06 (0.52), 39.4%, 0 years	0.06 (0.51), 39.6%, 0 years	0.06(0.51), 36.9%, 0 years	96.66	0.06 (0.44), 14.8%, 0 years
89.86	0.01 (0.53), 46.6%, 0 years	0.02 (0.55), 34.8%, 0 years	0.02 (0.54), 40.5%, 0 years	0.02(0.54), 36.4%, 0 years	94.43	0.05 (0.48), 17.8%, 0 years
86.48	-0.02 (0.53), 69.2%, 67 years	-0.01 (0.55), 53%, 96 years	-0.02 (0.54), 59.9%, 82 years	-0.01(0.54), 53.4%, 94 years	92.20	0.03 (0.52), 31.4%, 0 years
83.10	-0.05 (0.53), 87.6%, 46 years	-0.04 (0.55), 75.4%, 61 years	-0.04 (0.54), 79.9%, 60 years	-0.04(0.54), 77.7%, 59 years	89.97	0.02 (0.55), 39.7%, 0 years
79.72	-0.08 (0.54), 97.5%, 32 years	-0.07 (0.55), 92.5%, 45 years	-0.07 (0.54), 93.8%, 42 years	-0.07(0.54), 91.5%, 43 years	87.74	0.02(0.45), 31%, 0 years
76.34	-0.10 (0.54), 99.7%, 26 years	-0.09 (0.56), 98.5%, 34 years	-0.1 (0.55), 99.5%, 34 years	-0.09(0.54), 98.1%, 34 years	85.51	0.00 (0.48), 39.6%, 0 years
72.96	-0.13 (0.55), 100%, 21 years	-0.12 (0.57), 99.9%, 26 years	-0.13 (0.56), 99.8%, 25 years	-0.12(0.56), 99.9%, 25 years	83.28	-0.01 (0.5), 50.2%, 100 years
69.58	-0.15 (0.56), 100%, 18 years	-0.15 (0.57), 100%, 22 years	-0.15 (0.57), 100%, 23 years	-0.15(0.56), 100%, 22 years	81.05	-0.02 (0.52), 60.9%, 81 years
66.20	-0.19 (0.57), 100%, 15 years	-0.18 (0.59), 100%, 19 years	-0.19(0.58), 100%, 18 years	-0.19(0.58), 100%, 18 years	78.82	-0.03 (0.54), 68.3%, 72 years

0.831 (base)	-0.05 (0.53), 87.6%, 46 years		-0.04 (0.54), 79.9%, 60 years	-0.04 (0.54), 77.7%, 59 years	0.777 (base)	-0.21 (0.86), 100%, 16 years

Table 19. Reported estimates of survival (*S*) and recapture probability (*p*) for viperids using mark-recapture or radio telemetry studies. Single values represent reported point estimates while multiple values represent ranges.

Species	*S*	*SE*	*Age/Attribute*	*State (Location)*	*p*	*No. marked*	*Annual Sampling Occasions*	*Reference*
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.84	0.02	Adult	Idaho (CINB)	<0.20	992	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.70	0.06	Juvenile	Idaho (CINB)	<0.20	992	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.29	0.06	Neonate	Idaho (CINB)	<0.20	992	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.75	0.02	Adult	Idaho (CRAB)	<0.20	894	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.70	0.06	Juvenile	Idaho (CRAB)	<0.20	894	12	Jenkins et al. (2017)

Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.46	0.11	Neonate	Idaho (CRAB)	<0.20	894	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.80	0.01	Adult	Idaho (RCAV)	>0.20	755	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.69	0.04	Juvenile	Idaho (RCAV)	>0.20	755	12	Jenkins et al. (2017)
Great basin rattlesnake (*Crotalus oreganus lutosus*)	0.47	0.05	Neonate	Idaho (RCAV)	>0.20	755	12	Jenkins et al. (2017)
Timber rattlesnake (*Crotalus horridus*)	0.53	0.10	Neonate (Y)[a]	New York	0.10-0.20	588	25	Brown et al. (2007)
Timber rattlesnake (*Crotalus horridus*)	0.77	0.20	Neonate (B)[a]	New York	0.10-0.20	588	25	Brown et al. (2007)
Timber rattlesnake (*Crotalus horridus*)	0.84	0.03	2–4 years (Y)[a]	New York	0.10-0.20	588	25	Brown et al. (2007)

Timber rattlesnake (*Crotalus horridus*)	0.99	—	2–4 years (B)[a]	New York	0.10-0.20	588	25	Brown et al. (2007)
Timber rattlesnake (*Crotalus horridus*)	0.82	0.03	≥5 years (Y)[a]	New York	0.10-0.20	588	25	Brown et al. (2007)
Timber rattlesnake (*Crotalus horridus*)	0.96	0.04	≥5 years (B)[a]	New York	0.10-0.20	588	25	Brown et al. (2007)
Timber rattlesnake (*Crotalus horridus*)	0.927–0.983	NA	Active season, adult males	Indiana	NA	47	NA	Olson et al. (2016)
Timber rattlesnake (*Crotalus horridus*)	0.720–0.955	NA	Active season, adult females	Indiana	NA	47	NA	Olson et al. (2016)
Timber rattlesnake (*Crotalus horridus*)	0.97	NA	Winter, adult males	Indiana	NA	47	NA	Olson et al. (2016)
Timber rattlesnake (*Crotalus horridus*)	0.99	NA	Winter, adult females	Indiana	NA	47	NA	Olson et al. (2016)
Northern pacific rattlesnake (*Crotalus oreganus oreganus*)	0.66	0.07	Neonate	Idaho	0.85	306	9	Diller and Wallace (2002)

Northern pacific rattlesnake (*Crotalus oreganus oreganus*)	0.77	0.06	Juvenile	Idaho	0.42	306	9	Diller and Wallace (2002)
Northern pacific rattlesnake (*Crotalus oreganus oreganus*)	0.82	0.03	Adult	Idaho	0.63	306	9	Diller and Wallace (2002)
Twin-spotted rattlesnake (*Crotalus pricei*)	0.71	0.03	Non-neonates	Arizona	0.19–0.58	249	13	Prival et al. (2012)
Eastern massasauga (*Sistrurus catenatus*)	0.77 (0.51–0.95)	NA	Active season both sexes	Range-wide	NA	499	NA	Jones et al. (2012)
Eastern massasauga (*Sistrurus catenatus*)	0.89 (0.69–1.00)	NA	Winter season both sexes	Range-wide	NA	499	NA	Jones et al. (2012)
Eastern massasauga (*Sistrurus catenatus*)	0.44	0.05	Males, first year since marking	Ontario, Canada	0.53 (study-wide)	262	16	Jones et al. (2017)
Eastern massasauga (*Sistrurus catenatus*)	0.74	0.03	Males, >1 year since marking	Ontario, Canada		262	16	Jones et al. (2017)
Eastern massasauga	0.47	0.06	Females, first year since marking	Ontario, Canada	0.43 (study-wide)	262	16	Jones et al. (2017)

(*Sistrurus catenatus*)								
Eastern massasauga (*Sistrurus catenatus*)	0.73	0.03	Females, >1 year since marking	Ontario, Canada		262	16	Jones et al. (2017)
Cottonmouth (*Agkistrodon piscivorus*)	0.79	0.69–0.88	Adults and juveniles, both sexes	Alabama	<0.077–0.168[b]	198	3[c] (9 per year)	Koons et al. (2009)
European viper (*Viper berus*)	0.80–0.90		Good year, both sexes		0.59–0.80	172	6	Forsman (1993)
European viper (*Viper berus*)	0.45–0.70		Bad year, both sexes		0.40–0.74	172	6	Forsman (1993)
Asp viper (*Vipera aspis*)	0.75	0.61–0.85	Mean for both adults of both sexes		0.33 (0.18–0.52)	72	6	Flatt et al. (1997)
Asp viper (*Vipera aspis*)					0.55 (0.35–0.72)	72	6	Flatt et al. (1997)

[a] Yellow (Y) and black (B) color phases

[b] Only maximum values were reported

[c] Three years with nine sampling periods per year

Figure 1. Numbers of new captures (Nm), recaptures (Rc), and radio-tracked individuals (Telemetry) from four populations of timber rattlesnake in New England. PostTrans represents recaptures of individuals after their radio transmitter batteries expired. Asterisks (*) represent number of neonates.

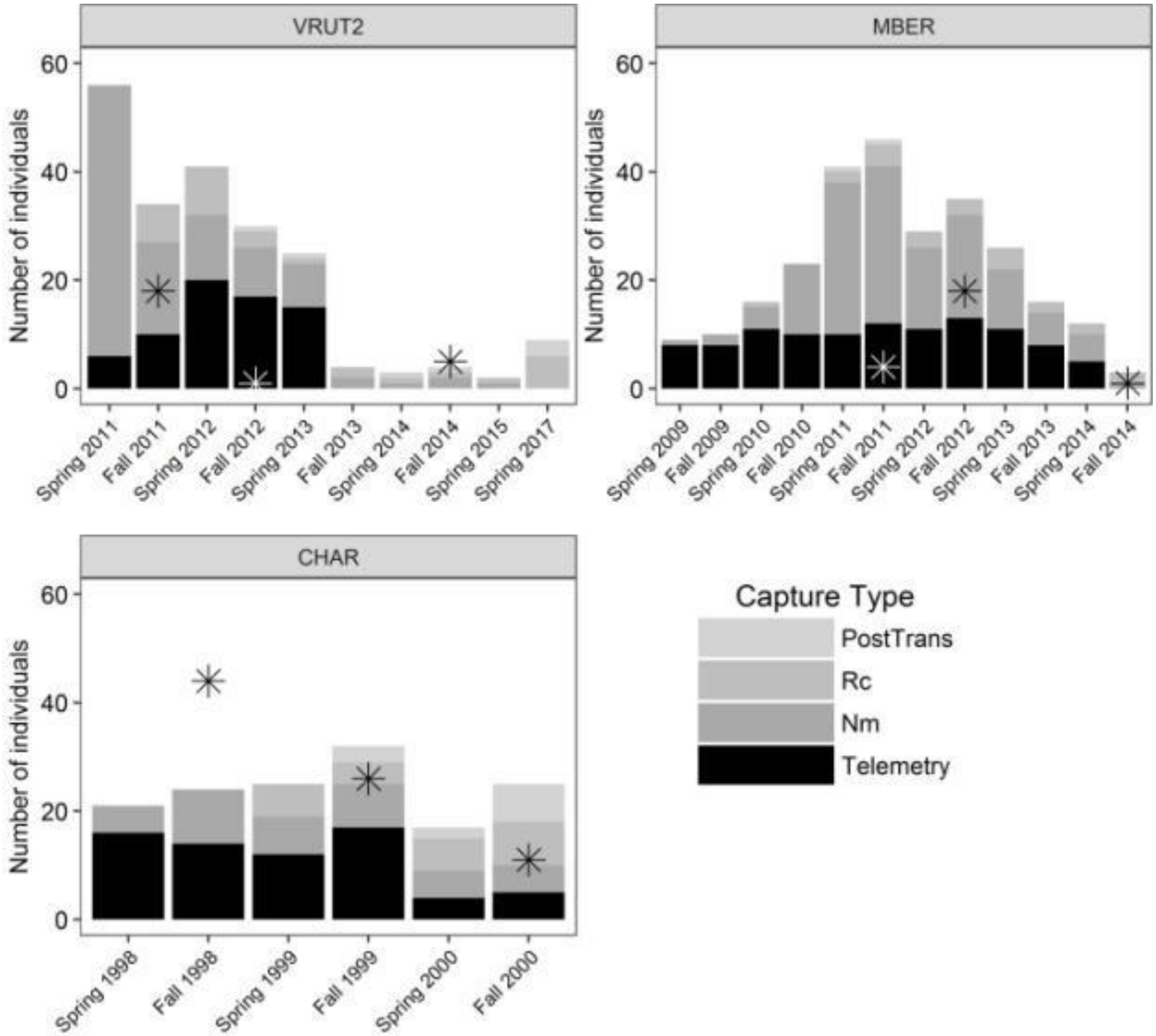


Figure 2. Model-averaged recapture probability (p) and 95% CI for male and female timber rattlesnake from VRUT2 as a function of snout-vent length (SVL) across capture occasions for PIT-tagged individuals prior to or not having received a radio transmitter. Results for PIT-tagged individuals recaptured after radio-tracking are virtually identical and not shown.

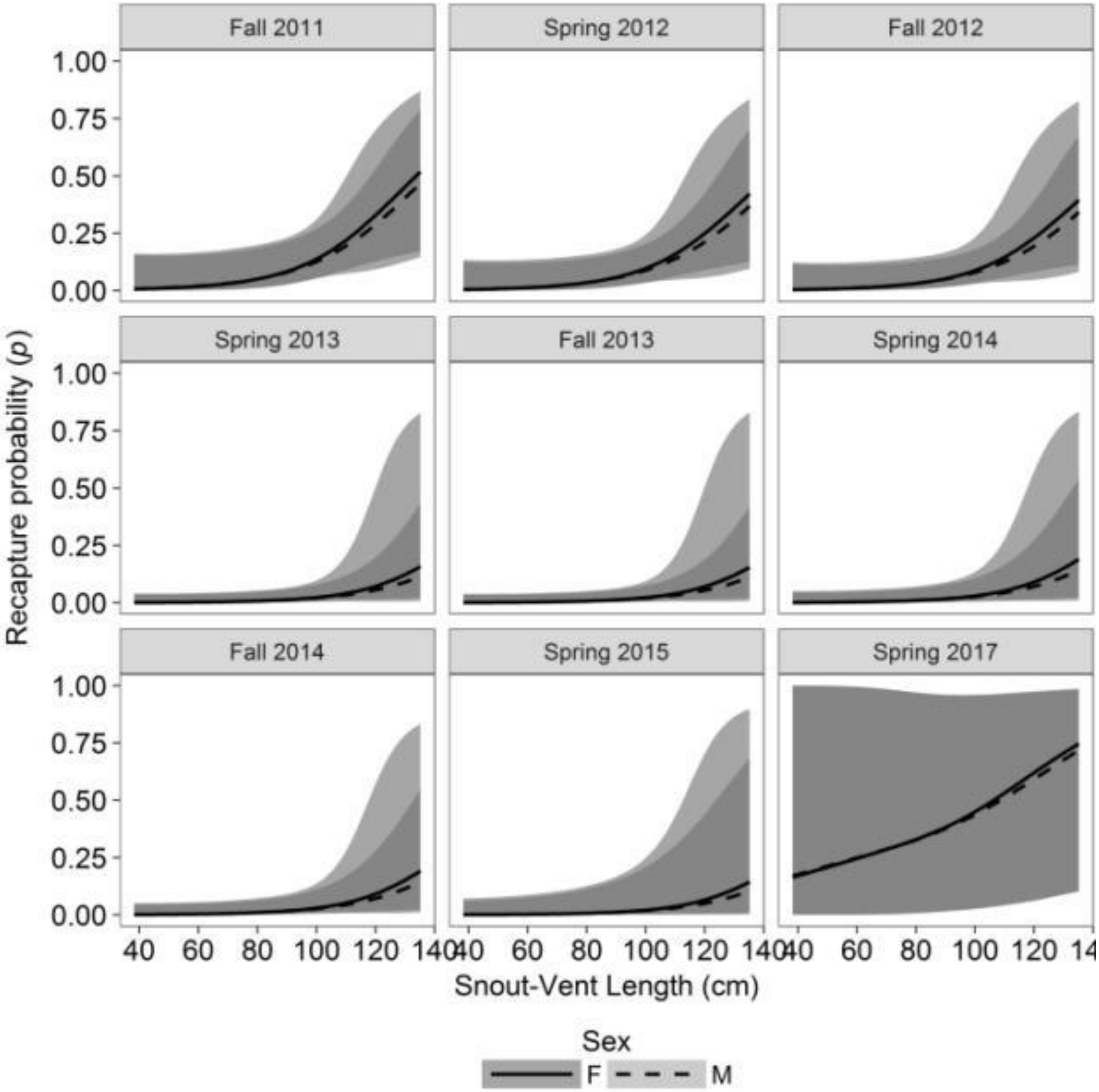


Figure 3.Model-averaged recapture probability (p) and 95% CI for timber rattlesnake from VRUT2 for average-length (SVL = 102.4 cm) male and female PIT-tagged individuals prior to or not having received a radio transmitter.

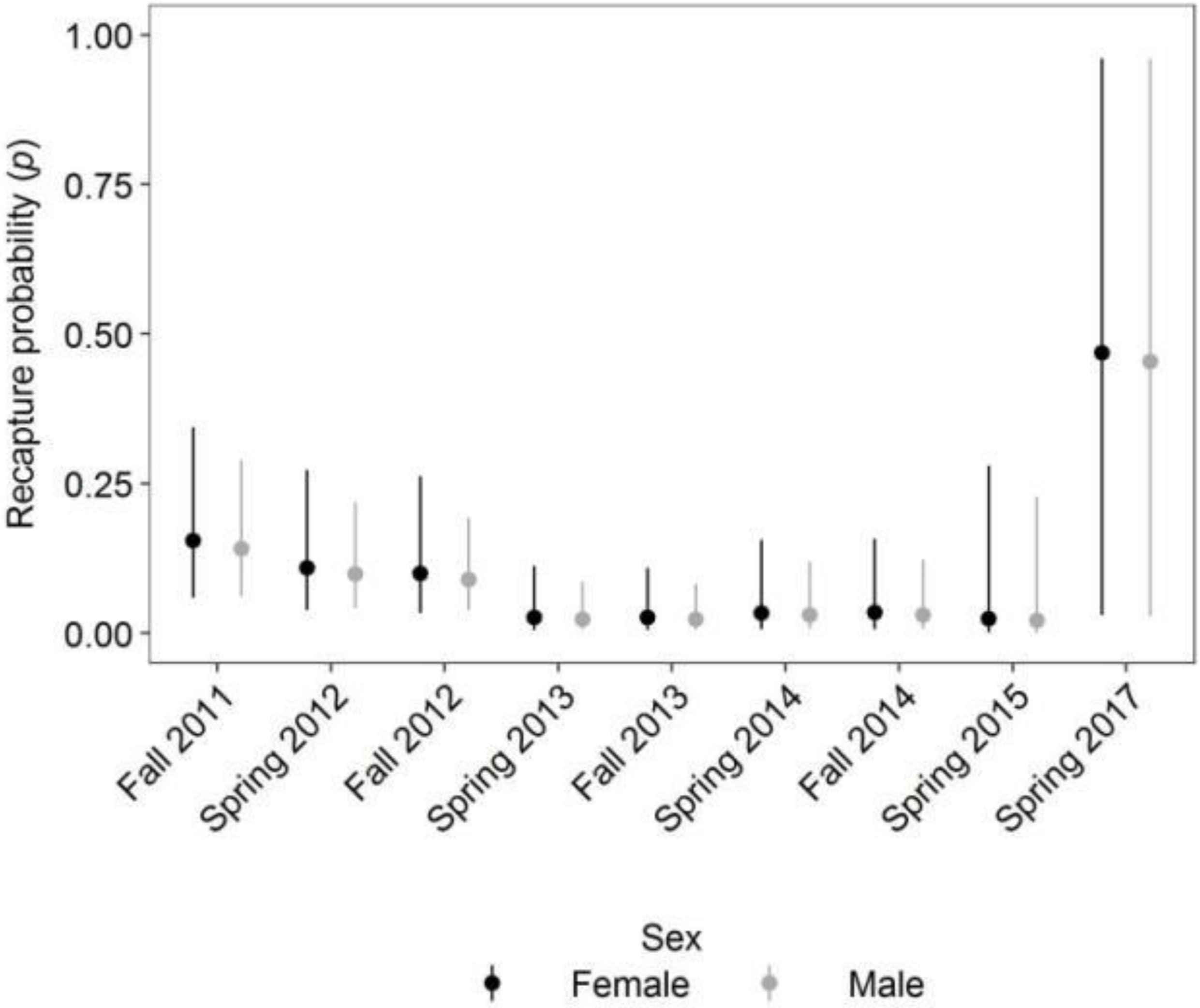


Figure 4. Model-averaged estimates of apparent survival (Φ) and 95% CI across time for timber rattlesnakes from VRUT2 for average-length (SVL = 102.4 cm) male and female PIT-tagged and radio-tracked individuals. Recapture probability (p) was modeled using terms for p with ΔQAIC$_c$ ≤4. We fixed p = 1 for radio-tracked individuals.

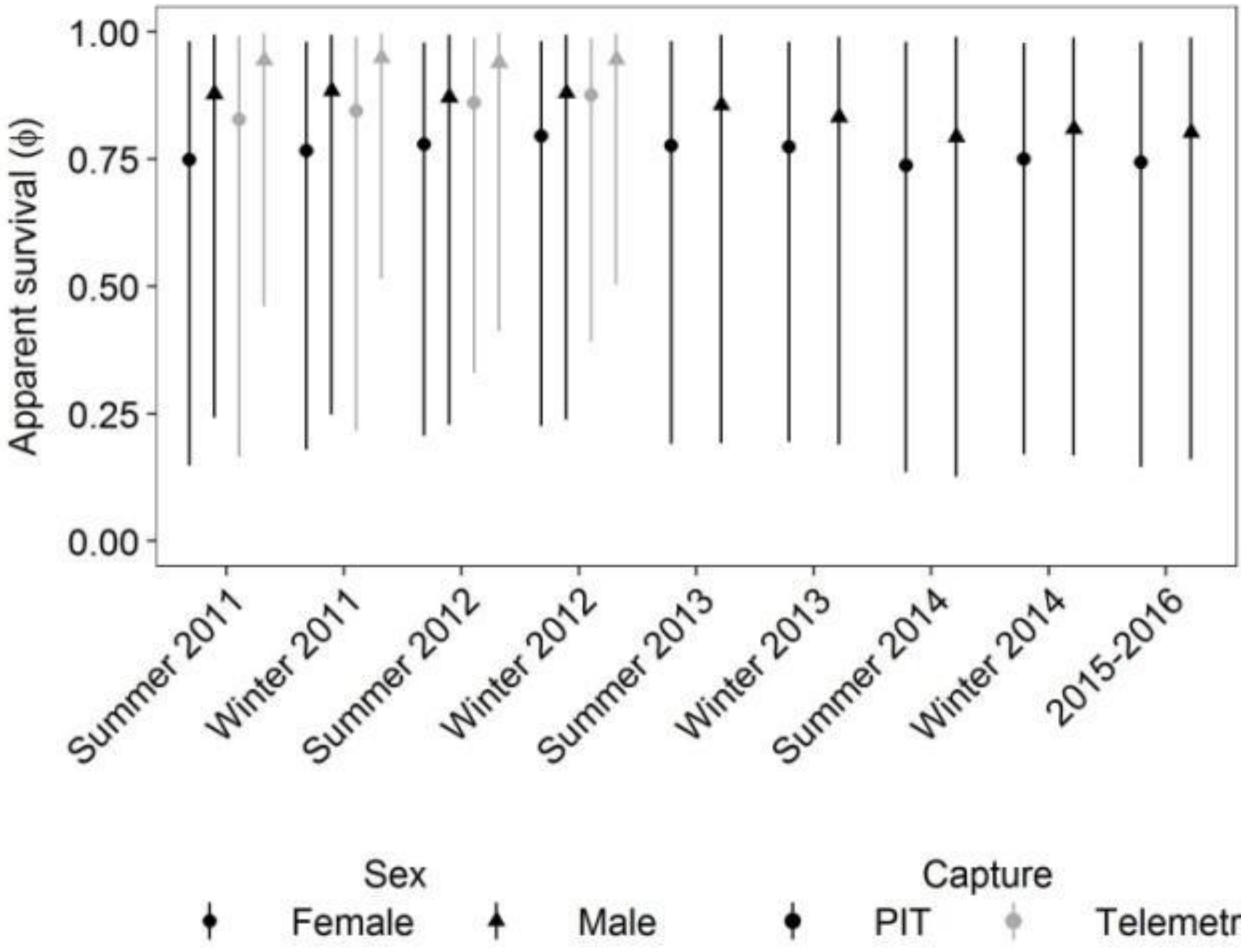


Figure 5. Model-averaged apparent survival (Φ) and 95% confidence intervals as a function of snout-vent length (SVL) for timber rattlesnakes from VRUT2 for PIT-tagged males and females. We fixed p = 1 for radio-tracked individuals.

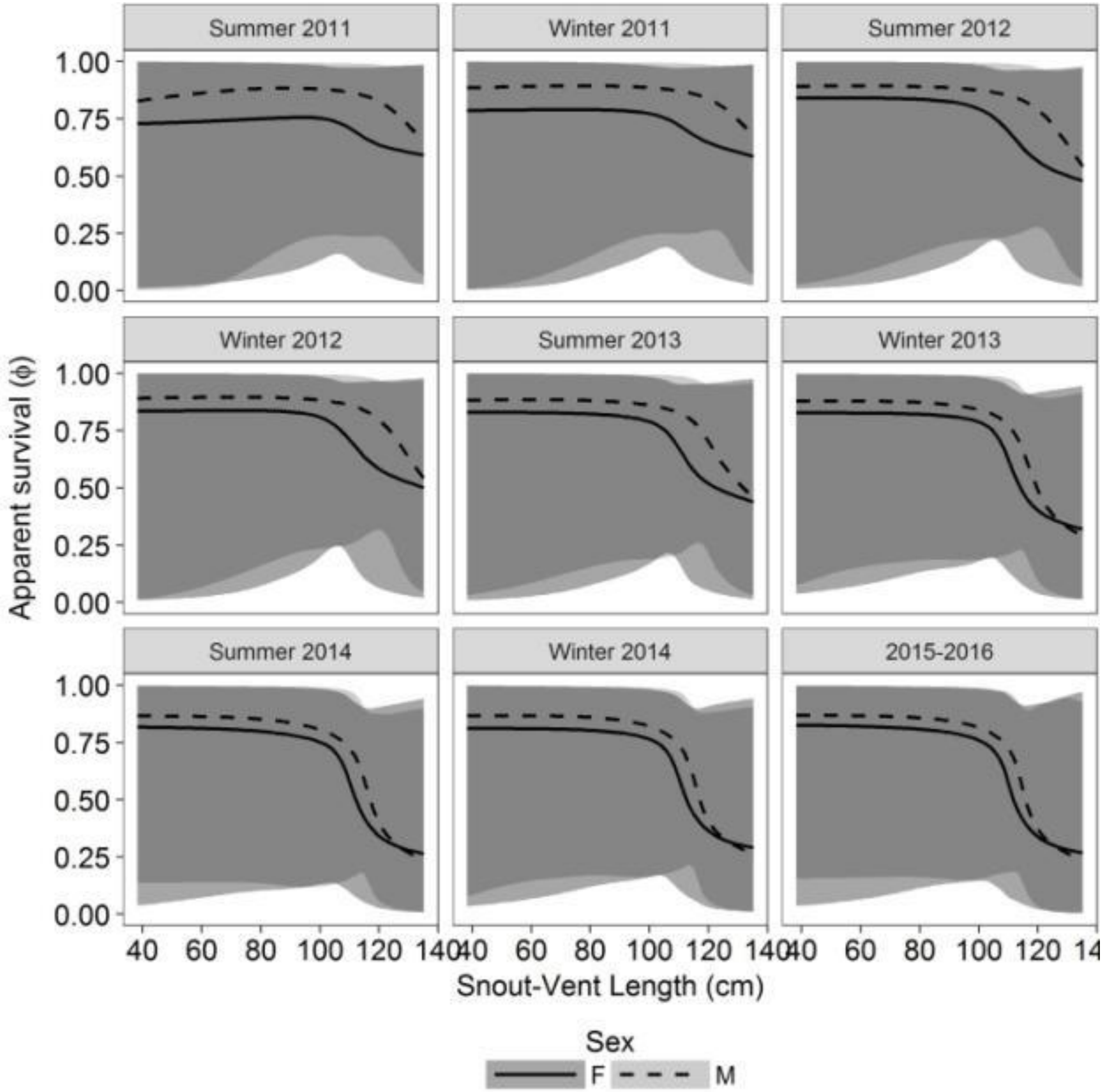


Figure 6. Model-averaged estimates of recapture probability (p) and 95% CI for PIT-tagged adult and sub-adult timber rattlesnakes from NH. Results for other groups are virtually identical and not presented. We included the time-varying individual covariates (TVIC) Fixed_p and Fixed_r to numerically force p = r = 1 when an individual was radio-tracked or in captivity and Captive to fix S = 1 when an individual was in captivity. We fixed F = 1 in all models.

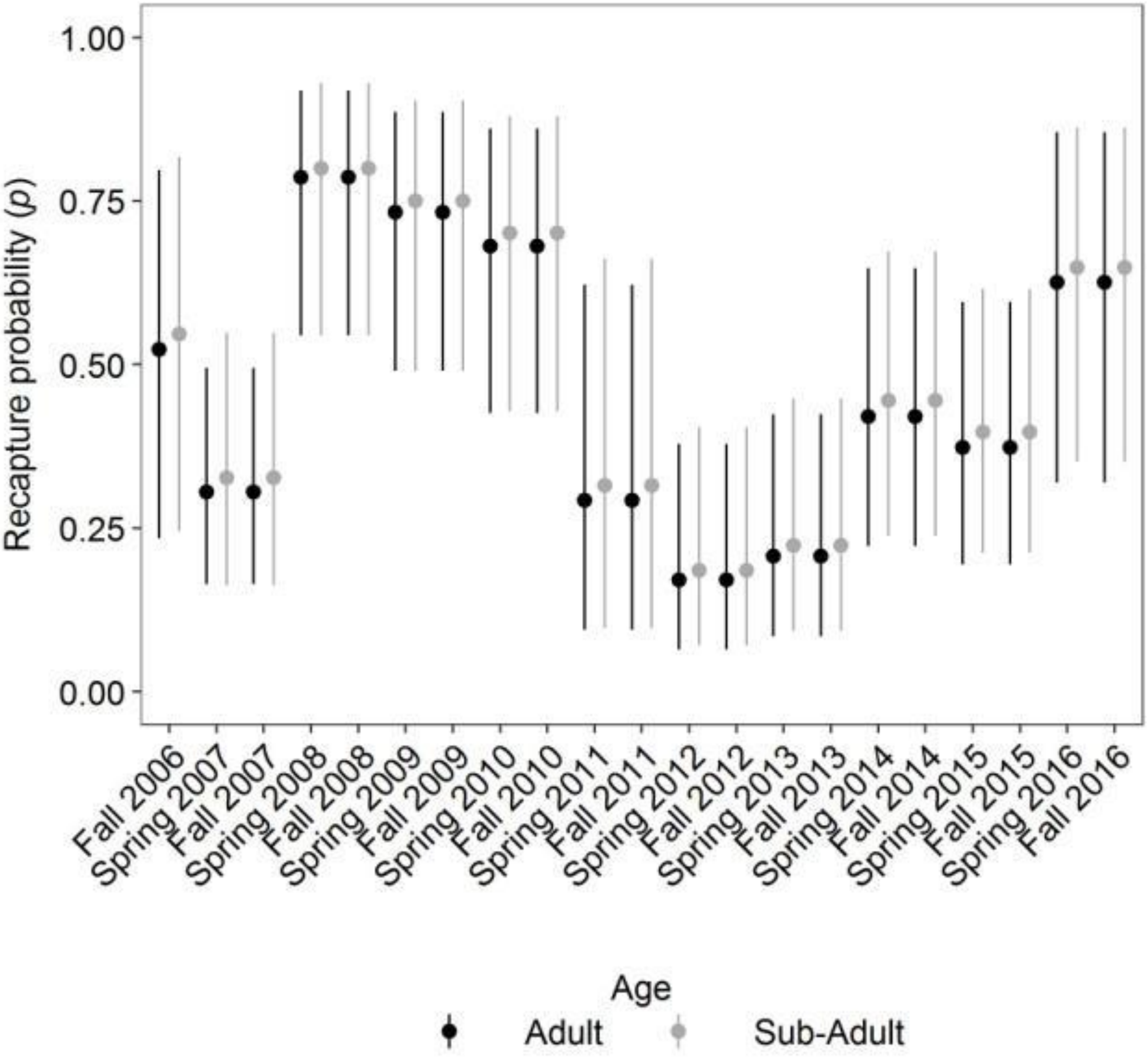


Figure 7. Model-averaged estimates of annual apparent survival (S) and 95% CI for timber rattlesnakes at NH. Recapture probability (p) and recovery probability (r) were modeled using terms with $\Delta AIC_c \leq 4$ from the analysis of p. Within each model we included the time-varying individual covariates (TVIC) Fixed_p and Fixed_r to numerically force p = r = 1 when an individual was radio-tracked or in captivity and Captive to fix S = 1 when an individual was in captivity. We fixed F = 1 in all models.

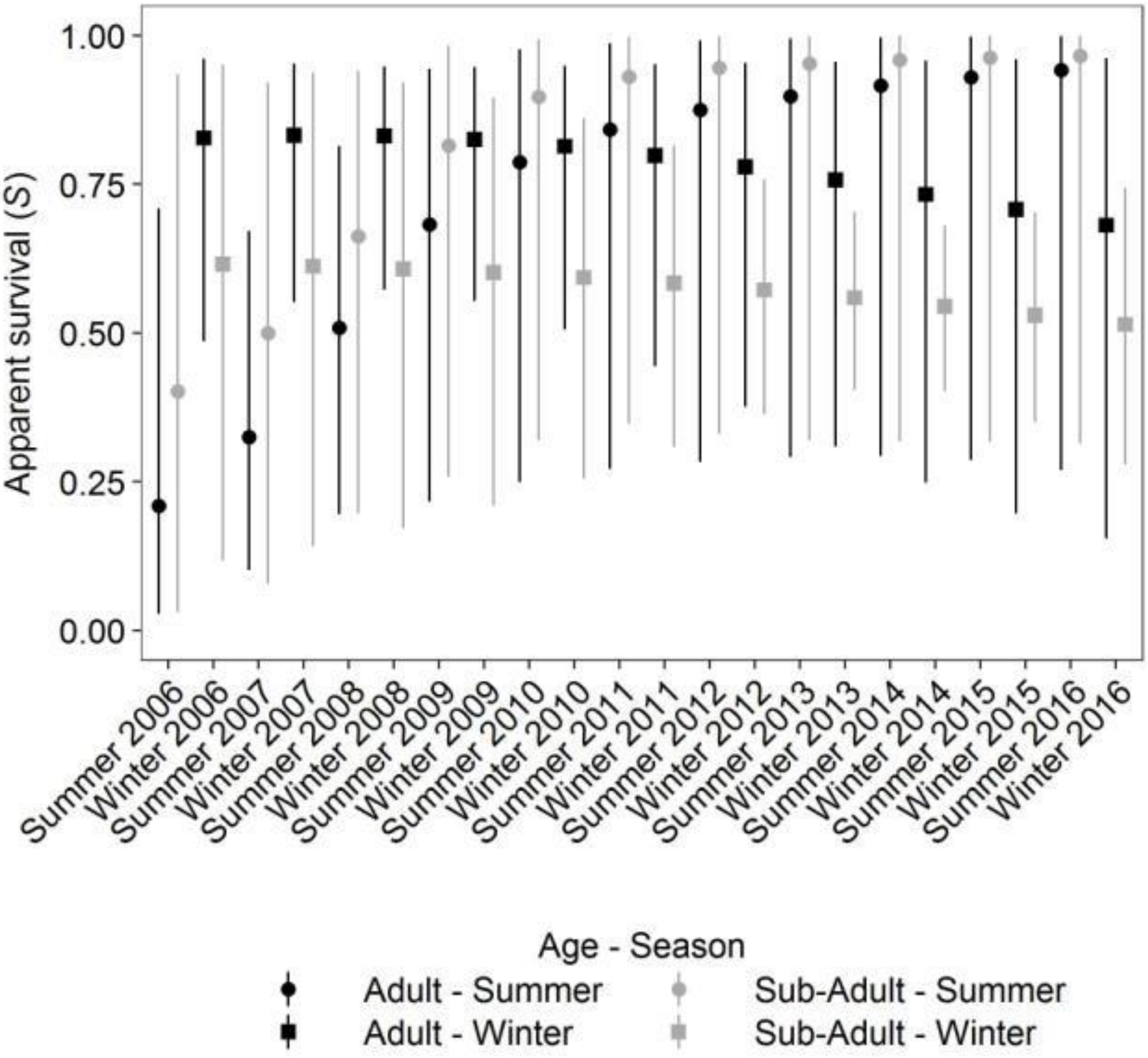


Figure 8. Model-averaged recapture probability (p) and 95% CI for PIT-tagged male timber rattlesnakes from MBER as a function of snout-vent length (SVL) across capture occasions from subpopulations MBER2 and MBER3. Apparent survival (Φ) was held constant in all models using SVL, Transmitter, and a linear trend effect of time (Time). Results are virtually identical for females and other subpopulations.

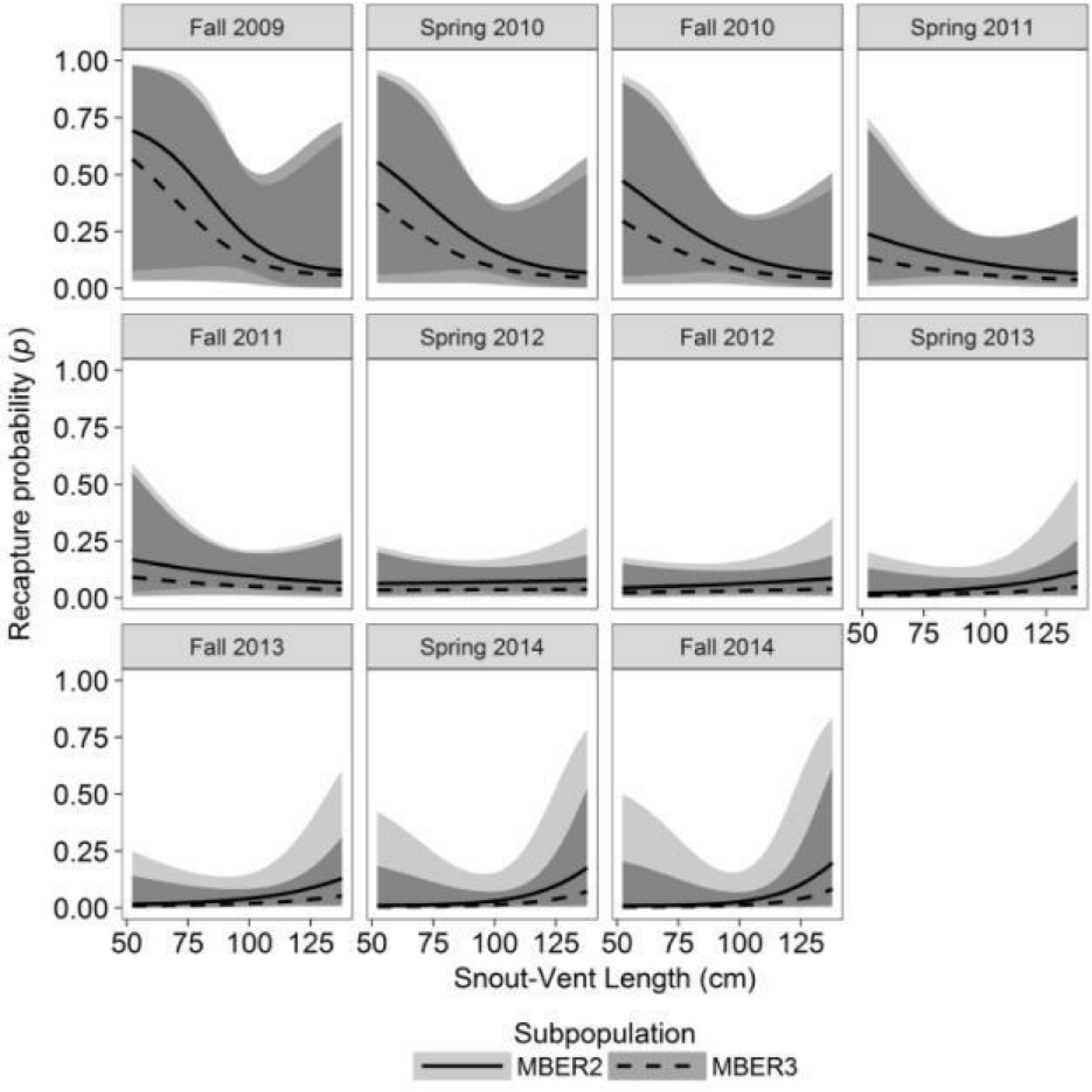


Figure 9. Model-averaged estimates of recapture probability (p) and 95% CI for timber rattlesnakes from MBER across capture occasions for averaged-sized male and female PIT-tagged individuals (SVL = 96.7 cm) from subpopulations MBER2 and MBER3. Apparent survival (Φ) was held constant in all models using a linear trend effect of time (Time) and SVL.

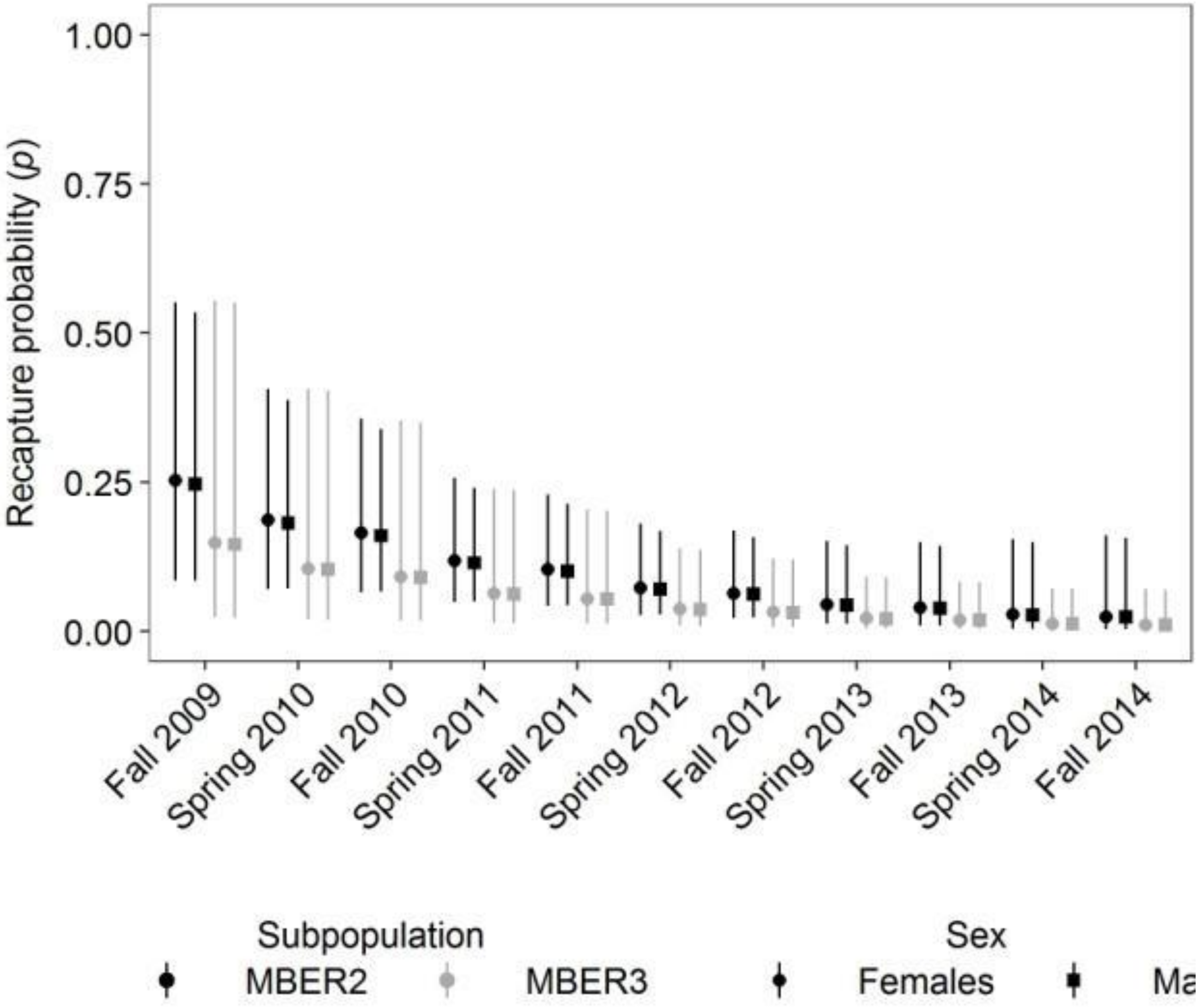


Figure 10. Model-averaged estimates of apparent survival (Φ) and 95% CI for timber rattlesnakes from MBER for an average-length (96.7 cm) individual across sampling occasions. Recapture probability (p) was modeled using terms for p with ΔAIC$_c$ ≤4. We fixed p = 1 for radio-tracked individuals.

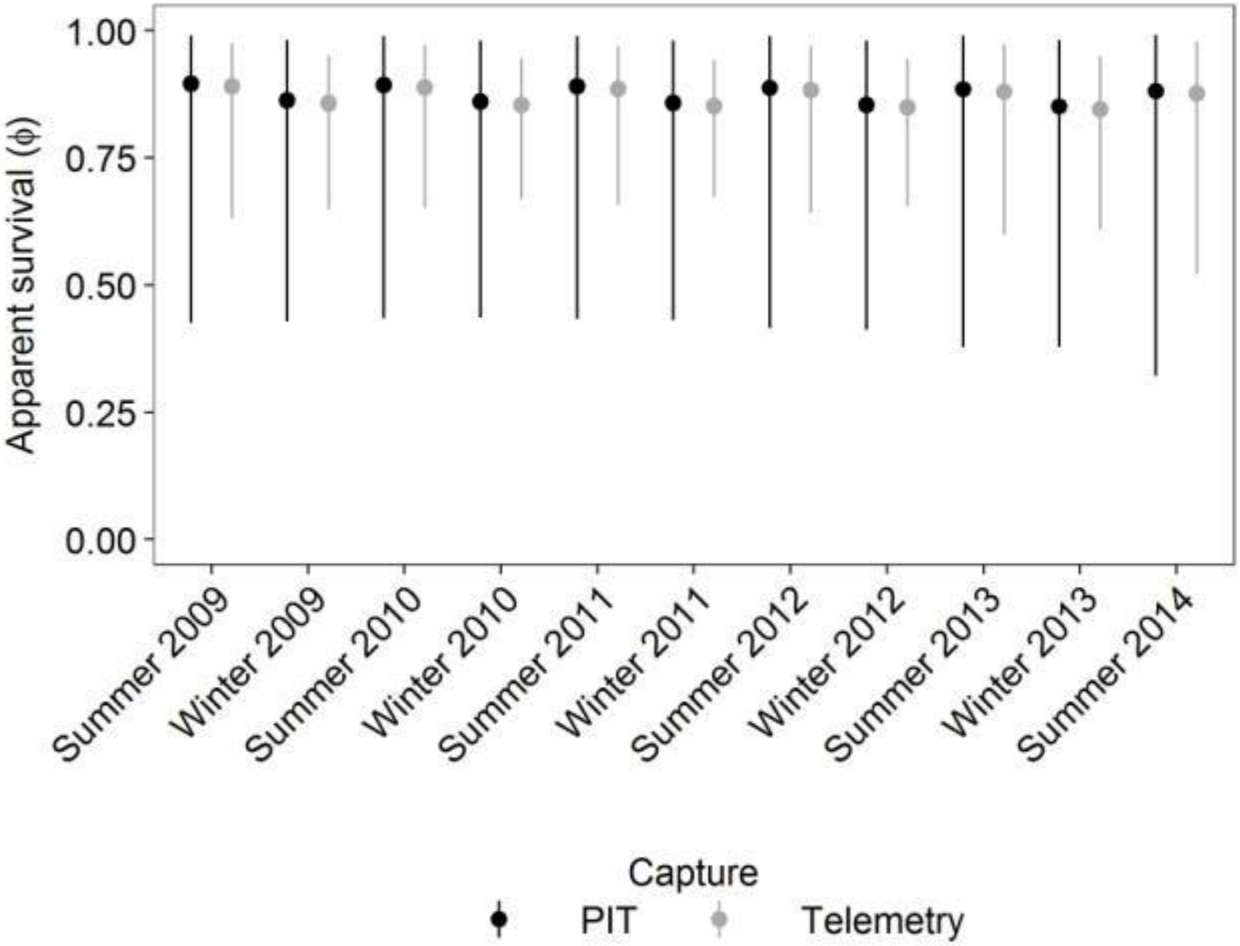


Figure 11. Model-averaged estimates of apparent survival (Φ) and 95% CI for PIT-tagged timber rattlesnakes from MBER as a function of SVL across sampling occasions. Patterns were identical for radio-tracked individuals and are not reported Recapture probability (p) was modeled using terms for p with $\Delta AIC_c \leq 4$. We fixed p = 1 for radio-tracked individuals.

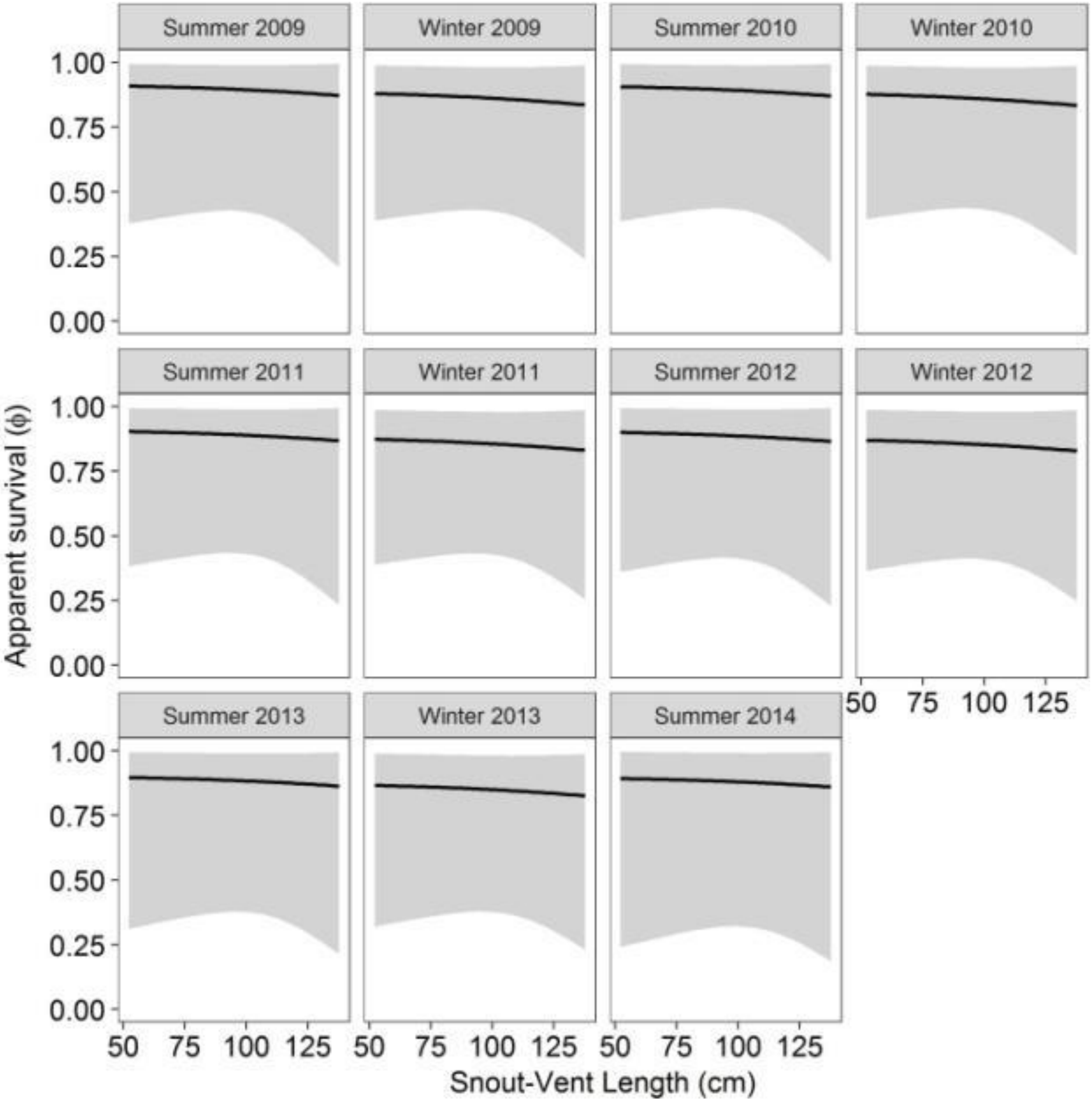


Figure 12. Model-averaged recapture probability (p) and 95% CI for male timber rattlesnakes from CHAR by age class and capture type.

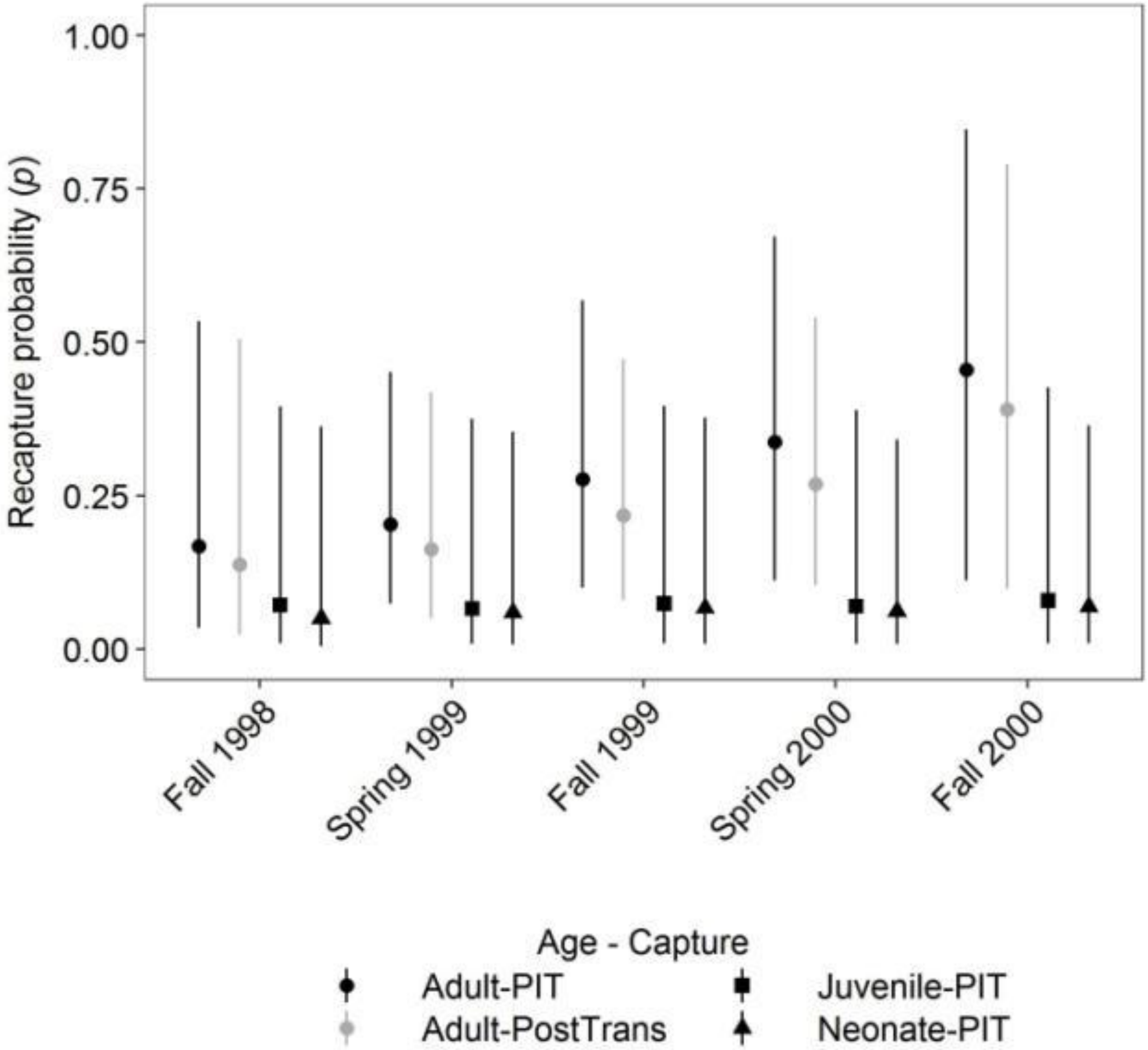


Figure 13. Model-averaged apparent survival (Φ) and 95% confidence intervals for timber rattlesnakes from CHAR by age class and capture type. Recapture probability (p) was modeled using all p terms with $\Delta QAIC_c \leq 2$. We fixed p = 1 for radio-tracked individuals.

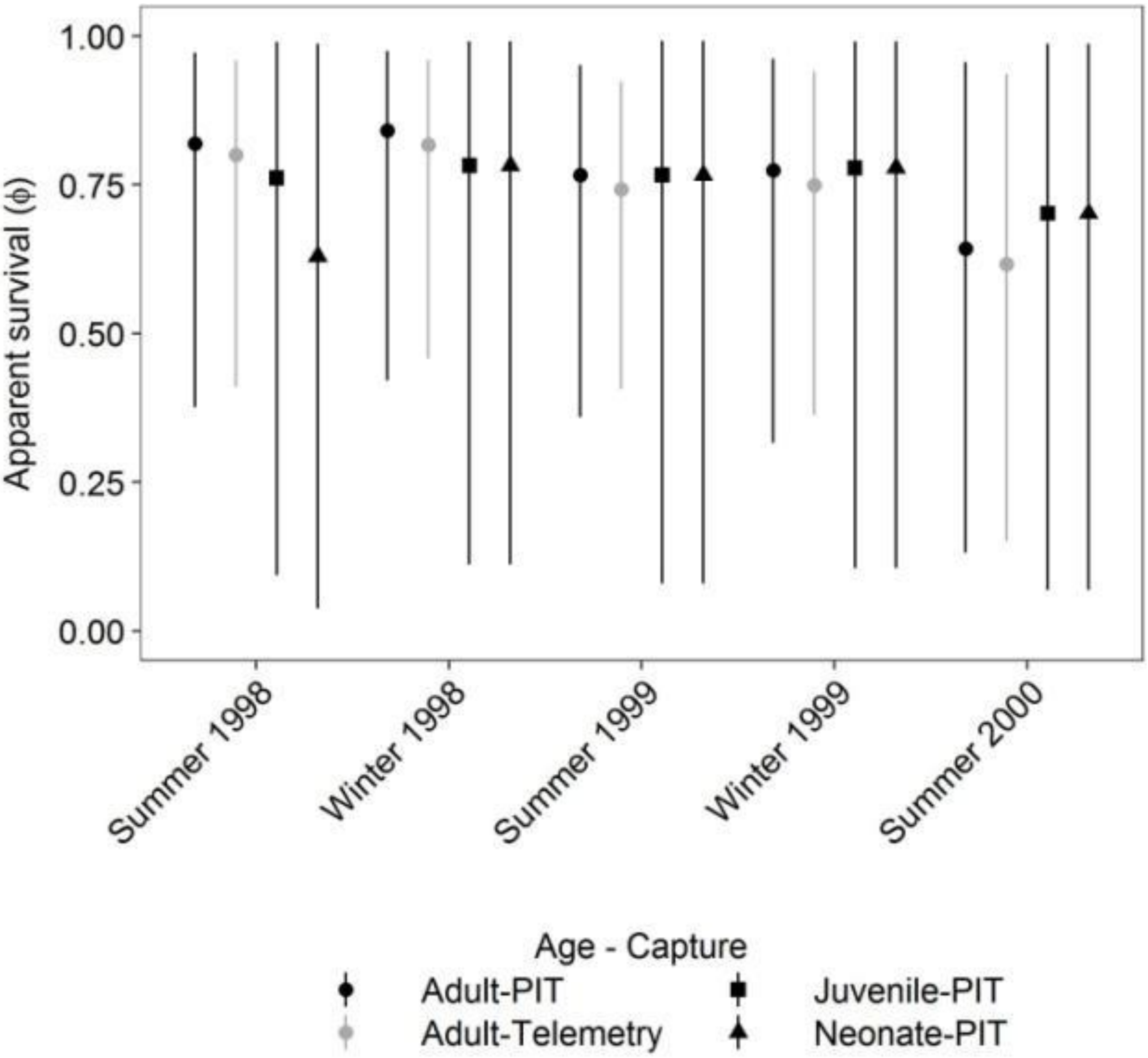


Figure 14. Mean activity season length and egress and ingress date with bootstrapped 95% CI among northeastern populations of timber rattlesnakes. Activity season length was calculated as the difference between upper and lower quantiles of observation dates across all non-radio telemetry observations while egress and ingress dates are the lower and upper quantiles, respectively. Metrics were calculated using the 2.5-97.5th quantiles (black symbols) and 1-99th quantiles (gray symbols). Values for NY (New York) were taken from Brown (2008).

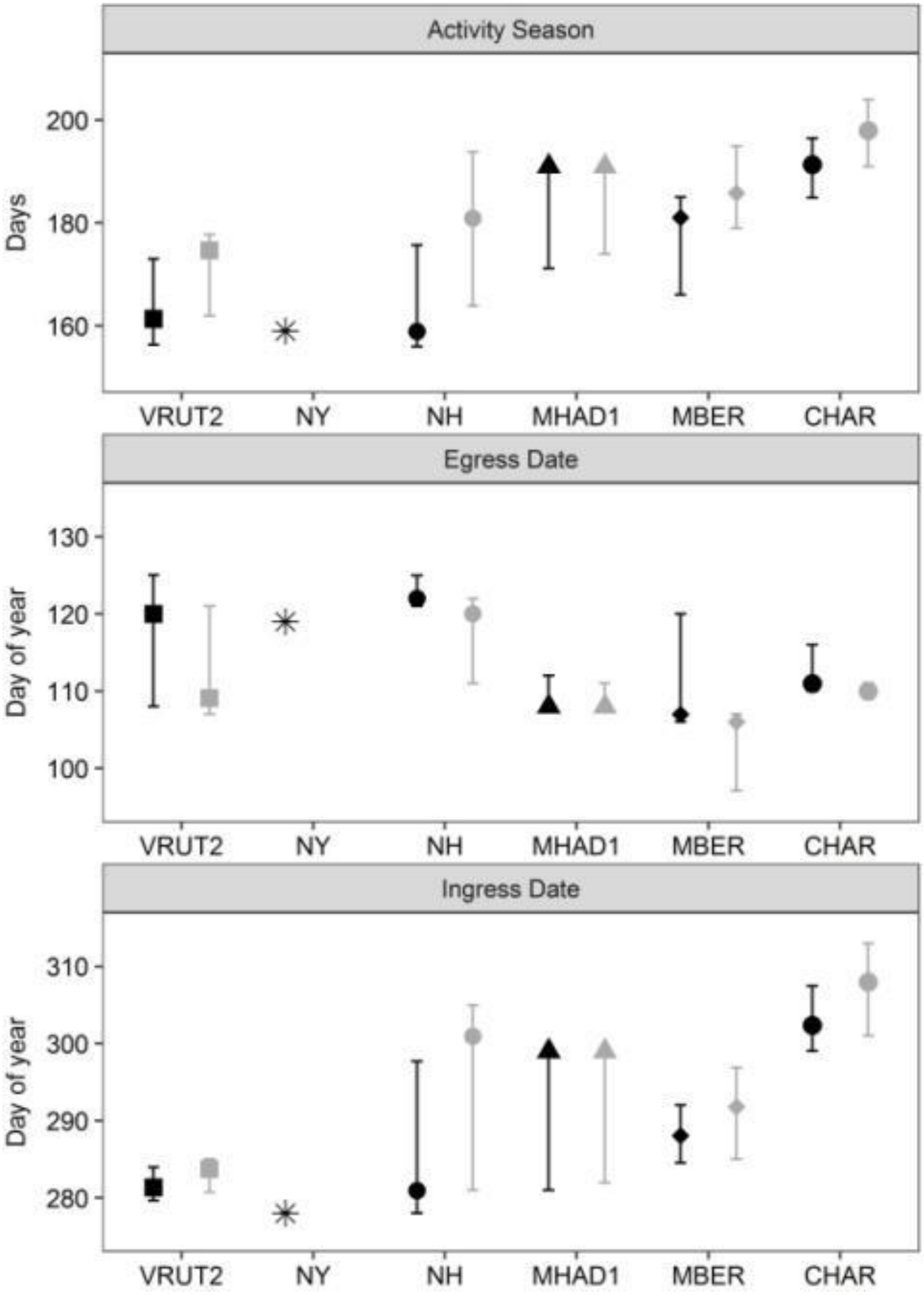


Figure 15. Regression of the log of body weight (g) against the log of snout-vent length (SVL, cm) for timber rattlesnakes >1-year old from four New England populations. The solid black line is the least-squares estimated regression line ($R^2 = 0.87$, $p < 0.0001$).

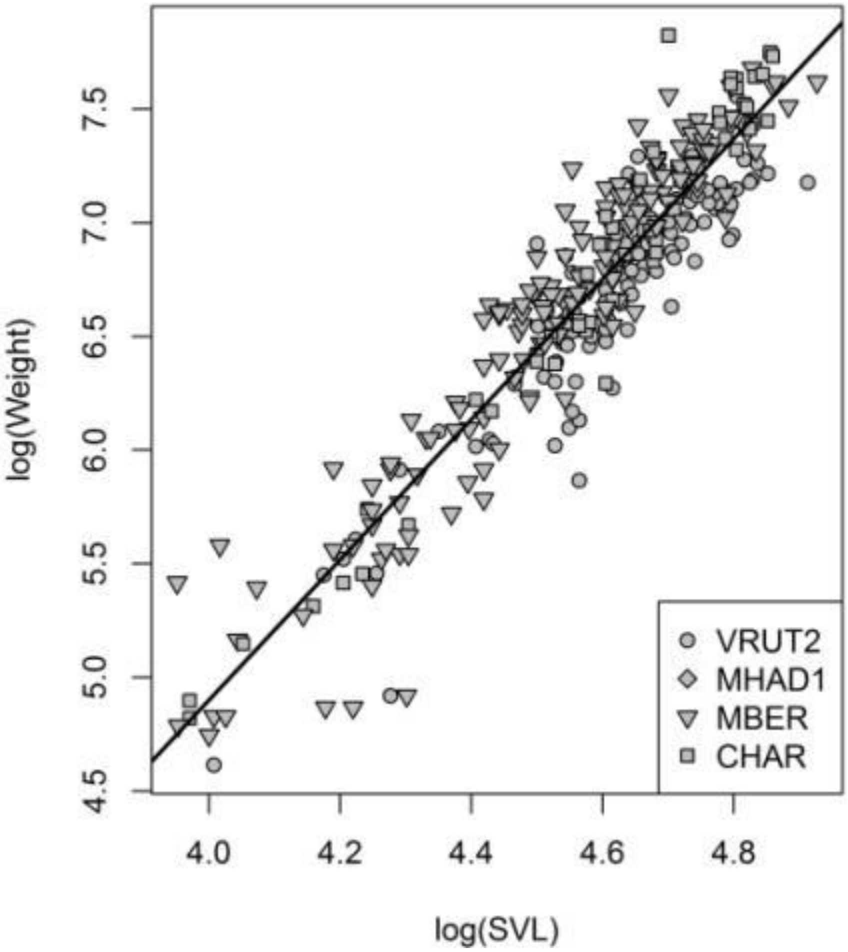


Figure 16. Comparisons of body size metrics by sex and population for timber rattlesnakes in New England. Values for adults and juveniles (>1-year old) are model-averaged predicted values and bootstrapped 95% CI. Values for neonates are observed means and bootstrapped 95% CI. Body condition index was calculated by taking the residuals of a linear regression of log(weight) against log(SVL) using all observations.

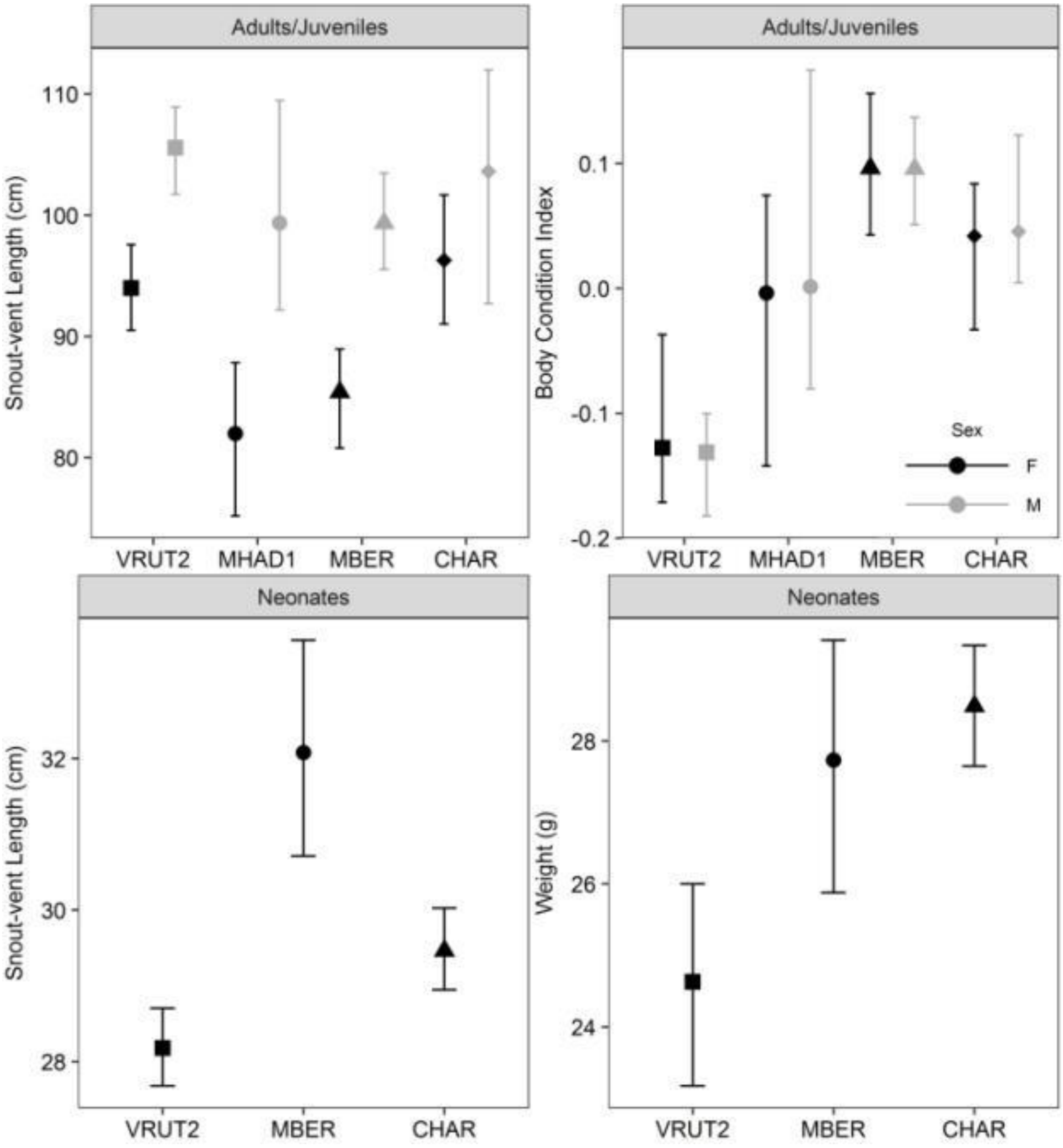
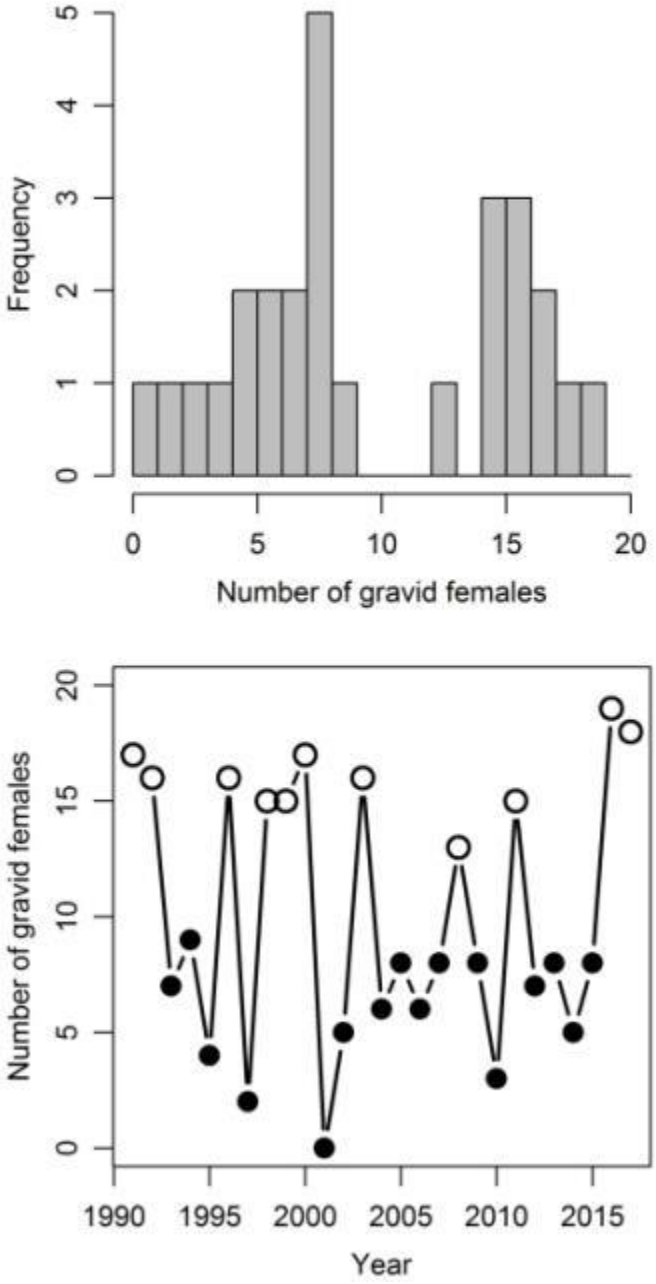


Figure 17. Frequency distribution and time series of annual gravid female counts at the northern population at CHAR from 1991–2017. Open circles indicate "high" years and closed circles indicate "low" years.



Appendices

Appendix 1. Parameter values used in the VORTEX population viability analyses for select timber rattlesnake populations in the northeastern United States. The New York population is from the Adirondack Mountains of eastern New York (Brown 1991, 2008, 2016, Brown et al. 2007). Values are provided for females (F) and males (M) when possible. See text for the values of parameters not listed here.

	VRUT2	New York	NH	MBER	CHAR
Initial population sizes	218, 545, 1,090	255[a], 510, 1275	30, 60, 200	625, 1250, 3125	80, 160, 400
Carrying capacity	2,180	2,550	1,000	6,250	800
Age at first breeding - F[a]	7 years	7 years	7 years	7 years	7 years
Age at first breeding - M[b]	4 years	4 years	4 years	4 years	4 years
Maximum age at first breeding - F[a]	39 years	39 years	39 years	39 years	39 years
Maximum age at first breeding - M	45 years	45 years	45 years	45 years	45 years
Maximum lifespan[a]	45 years	45 years	45 years	45 years	45 years
Maximum litters per year	1	1	1	1	1
Mean offspring per litter	5.67	7.71[a]	7.71[a]	7.71[a]	7.75
SD offspring per litter	2.18	3.15[a]	3.15[a]	3.15[a]	1.67
Maximum offspring per litter[a]	14	14	14	14	14
Percent breeding adults - F	26%[c]	24%[d]	30.95%	33%	39.58%
SD of percent breeding adults - F	5.69%	7.36%[d]	4.07%	4.80%	12.50%
Mortality for Year 1	0.348[e]	0.348[e]	0.348[e]	0.348[e]	0.373
SD of mortality for Year 1	0.1537[e]	0.1537[e]	0.1537[e]	0.1537[e]	0.4169
Mortality for Years 2-4	0.281, 0.338 (M, F)	0.078[e]	0.428	0.143, 0.144 (M, F)	0.223
SD of mortality for Years 2-4	0.1595, 0.1503 (M, F)	0.0836[e]	0.0689	0.123, 0.130 (M, F)	0.2653

Mortality for Years ≥5	0.281, 0.338 (M, F)	0.11[e]	0.247	0.143, 0.144 (M, F)	0.169
SD of mortality for Years ≥5	0.1595, 0.1503 (M, F)	0.0365[e]	0.0498	0.123, 0.130 (M, F)	0.1624

[a] Brown (2016)

[b] Aldridge and Brown (1995)

[c] Observed ratio, all others are derived from observed and/or probable reproductive intervals

[d] Estimated from Figure 6 in Brown (2016)

[e] Brown et al. (2007), mid-point values between yellow- and black-phase values

Chapter 2—Population Genetics and Status of Timber Rattlesnakes (*Crotalus horridus*) in New England with Recommendations for Genetic Monitoring and Management

Abstract

Small, isolated populations are at risk from inbreeding depression which reduces population viability. Genetic rescue (i.e., moving individuals among populations to increase genetic diversity) is a potential management option for restoring genetic connectivity and diversity yet few empirical guidelines exist to inform managers when and how genetic rescue should be implemented. Timber rattlesnakes have declined throughout New England and current populations are largely isolated. In this study, we undertake a comprehensive analysis of the population genetics status of timber rattlesnakes in New England to evaluate the potential need and benefits of genetic rescue. Specifically, we examined the population genetic structure of timber rattlesnakes in the northeastern United States using microsatellite DNA, conducted a literature review to evaluate the potential for developing genetic metric-based thresholds for initiating genetic rescue, and developed a framework of implementing genetic rescue programs. We found significant genetic structure among populations and no evidence of isolation-by-distance suggesting a strong effect of genetic drift. Populations in New Hampshire, Vermont, and 1–2 populations in Massachusetts were most genetically isolated and had lowest genetic diversity. Our literature review found no consistent genetic-based threshold for initiating genetic rescue suggesting instead that phenotypic traits (e.g., abnormal color morphs) may be more reliable indicators of the need for genetic rescue. We therefore recommend a multi-facetted approach incorporating genetic, phenotypic, and demographic data to determine if and when genetic rescue is warranted. Available data strongly suggest that some timber rattlesnake populations in New England will benefit from genetic rescue. We discuss alternative approaches for implementing genetic rescue for timber rattlesnakes in New England. We discuss approaches for identifying timber rattlesnake conservation units in New England but strongly recommend integrating an understanding of historical distribution and connectivity and the potential for local adaptation into any such exercise. While long generation times may make genetic and phenotypic monitoring difficult, we recommend that it accompany any genetic rescue efforts.

Introduction

Small and isolated populations are at increased risk of extirpation due to loss of genetic diversity, inbreeding depression, and random fluctuations in population demographics (Blows & Hoffmann 2005). Inbreeding depression, defined as a decrease in individual fitness that occurs when close relatives reproduce (Keller & Waller 2002; Savage & Zamudio 2011; Spielman et al. 2004), is most likely to occur in small, isolated populations and is associated with reduced population viability (Keller & Waller 2002). Inbred populations are also at greater risk from stochastic events, such as introduction of a new disease pathogen, habitat loss, or climate change. The cumulative effect leads to an extinction vortex where multiple factors interact to produce a positive feedback cycle further reducing population size (Brook et al. 2008). Genetic exchange among subpopulations is required to maintain genetic diversity, minimize inbreeding depression, and maintain the evolutionary potential to respond to changing environments (Slatkin 1987; Wright 1969). Fortunately for species conservation, the rate of gene flow needed for maintaining genetic diversity can be as low as one reproductive migrant per generation in stable populations (Mills & Allendorf 1996; Speith 1974). Peripheral populations are often isolated from each other and may exhibit lower genetic diversity, small effective population sizes, and a stronger signal of genetic drift (Eckert et al. 2008; Safriel et al. 1994). It is important to ensure that peripheral populations maintain adaptive potential (Safriel et al. 1994). In particular, the effects of climate change are often most realized on the margins of species' distributions where populations are often most at-risk due to small population sizes and inbreeding depression.

Because peripheral populations in several taxa were likely connected historically and their genetic pattern would likely have been similar to the populations in the core range, there is debate over how such populations should be managed in contemporary landscapes. One argument has been that isolated and genetically distinct populations should be protected as separate units and treated as separate management units (MU) to avoid the risk of outbreeding depression, defined as a decrease in fitness in offspring from reproduction between genetically divergent individuals (Templeton 1986; Thornhill 1993). This concern is based on the assumption that contemporary population genetic patterns, specifically the degree of genetic divergence among populations, represent genetic adaptations to local environmental condition (e.g., growing season, habitat conditions, etc.). An alternative argument is that, because isolated peripheral populations are more prone to genetic drift, observed population differentiation is more likely due to random genetic drift (Frankham et al. 2012; Vucetich & Waite 2003). Under this argument, treating isolated peripheral populations as separate MU may exacerbate the effects of population isolation and genetic drift, a dilemma referred to as ‘managing drift’ (Coleman et al. 2013). Conservation of imperiled peripheral populations therefore requires an accurate understanding of their population genetic structure and the factors responsible for such structure.

One potential management option in for genetically isolated 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 (Whiteley et al. 2015). Genetic rescue has been implemented for multiple taxa including Florida panther (*Puma concolor*, Johnson et al. 2010), European adder (*Viper berus*, Madsen et al. 1999; Madsen et al. 2004), Greater Prairie Chicken (*Tympanuchus cupido*, Bouzat et al. 2009), and bighorn sheep (*Ova Canadensis*, Miller et al. 2012). Genetic rescue appears to have beneficial fitness effects even when source and recipient populations have elevated levels of inbreeding (Heber et al. 2012). Furthermore, genetically isolated populations may become extinct due solely to the effects of genetic isolation even in the presence of other management tools (e.g., habitat restoration, Coleman et al. 2013).

Despite its potential, genetic rescue has been relatively infrequently implemented compared to the number of taxa for which it might be suitable (Whiteley et al. 2015). This may reflect the costs and uncertainties of genetic rescue. Many issues must be addressed when implementing genetic rescue including the identification of suitable source populations, ensuring translocated individuals survive and contribute genetically to the recipient population, the minimizing the introduction of novel pathogens, and planning follow-up monitoring to determine if genetic rescue has had a positive impact on population fitness. Genetic rescue also carries a risk of promoting outbreeding depression although in their review Whiteley et al. (2015) only found one example of genetic rescue having a negative effect on recipient population fitness. However, there is very little empirical guidance on when and how genetic rescue should be implemented.

Defining the population genetic structure of a species across its range can help guide genetic rescue programs, particularly by identifying small, genetically-isolated populations and defining conservation units below the species level (Moritz 1994; Ryder 1986). One commonly-used within-species conservation unit is the evolutionarily significant unit (ESU) although multiple definitions of ESU exist (Allendorf et al. 2013). For example, Waples (1991) defines ESU 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. In contrast, Mortiz (1994) defines ESU based on reciprocal monophyly in mitochondrial DNA (mtDNA) alleles and significant divergence in nuclear DNA alleles. Similar to Waples (1991) definition, Crandall et al. (2000) used the principle of ecological exchangeability whereby individuals can be moved among populations and fulfill the same ecological role without outbreeding depression. Lack of exchangeability implies different ESU. Evolutionarily significant units are recognized by the Federal Endangered Species Act where they are referred to synonymously as Distinct Population Segments (DPS, U. S. Fish and Wildlife Service 1996). Within a DPS, groups of spatially proximate populations connected by gene flow are often designated as MU to provide a smaller-scale unit of management and conservation. While MU are genetically distinct groupings of populations (Moritz 1994; Palsboll et al. 2007), by definition they do not exhibit differences in local adaptation, in contrast to ESU (Moritz 1994).

Timber rattlesnakes (*Crotalus horridus*) are distributed throughout most of the eastern United States and reach the northern periphery of their range in New England (Brown 1993) (Brown 1993). In all but the southernmost parts of their range, timber rattlesnakes utilize communal den sites and suitable denning habitat may be a limiting resource, particularly in the northern parts of their distribution (Brown 1993, 2008; Martin et al. 2008). Within the core of their range (i.e., approximately Tennessee to Pennsylvania), timber rattlesnake populations tend to have high within-population genetic diversity and generally form metapopulations according to the number and spatial proximity of denning sites (Bushar et al. 2015). Timber rattlesnake populations in the northern periphery of their range generally have low abundances and are more spatially distant, likely reflecting the decreasing availability of suitable denning sites (Brown 1993; Martin et al. 2008). Timber rattlesnakes show very high fidelity to denning and gestation sites and neonates often use their maternal den site (Anderson 2010; Brown 1993; Brown et al. 2007; Cobb et al. 2005; Reinert & Zappalorti 1988a, b). Genetic connectivity among denning sites appears driven by male movements during the late summer breeding season (Clark et al.

2008). As a result, population subdivision is strongly associated with the den sites and different subpopulations tend to correspond to a single den site (Clark et al. 2008).

In the northeastern United States, small and geographically distant timber rattlesnake populations may contain one to few den sites (Martin et al. 2008; Stengle 2018). These populations could be both genetically depauperate and highly genetically divergent. Isolated peripheral populations in the northeast are often widely separated by human modified and fragmented habitats thereby strongly reducing the opportunities for mating between individuals from different dens. These isolated populations are at greater risk of extirpation due to genetic drift and inbreeding depression (Wright et al. 2007) making it critical to evaluate their genetic structure.

In this study, we undertake a comprehensive analysis of the population genetics status of timber rattlesnakes in New England to evaluate the potential need and benefits of genetic rescue. First, we use microsatellite genetics data from timber rattlesnake populations from West Virginia to Vermont to examine within- and among-population genetic diversity and to evaluate the potential risk of future inbreeding. We also conduct a literature review to develop empirical guidelines for evaluating when genetic rescue should be considered and develop a framework of implementing a genetic rescue program for timber rattlesnakes in the northeastern United States. Our specific objective was to identify one or more thresholds based on metrics of genetic diversity and structure which would indicate both when genetic rescue was warranted and when genetic rescue could be considered successful in mitigating inbreeding depression. We then provide a discussion of potential approaches for identifying timber rattlesnake management units within the northeastern United States. Finally, we evaluate different options for the genetic management of timber rattlesnakes in New England.

Methods

Samples and the majority of the population genetic analyses presented here were conducted under the PhD dissertation, Stengle (2018), entitled ‘Habitat Selection, Connectivity, and Population Genetics of a Timber Rattlesnake (*Crotalus horridus*) Metapopulation in Southwestern Massachusetts and New England’.

Sample Collection

We used data from 996 tissue samples from 20 sites collected 2009–2014 including all ten remaining New England populations and several populations in eastern New York, Virginia, and Pennsylvania (Table 1). Populations were abbreviated by the two letter state abbreviation, followed by the first three letters of the county name, and a number to distinguish multiple populations in a county (Table 1). Further locality details are not provided due to concerns of poaching. A 2 mm scale clip from each individual and either stored clips air dried or in 95% ethanol, and stored at <0° C. Shed skins were used opportunistically including those previously collected samples from Massachusetts Wildlife (n = 8) and the Harvard Museum of Comparative Zoology (n = 22). Samples from New York were from the same population studied by Clark et al. (2008). Because our sampling occurred afterwards it is unknown if any individuals were used in both studies. Thirty-four New Hampshire samples used by Clark et. al. (2011) were provided by New Hampshire Fish and Game Department. Within the putative Massachusetts metapopulation (MBER), we sampled 253 individuals across four potential subpopulations with sample sizes of n = 124, 20, 28 and 79, respectively.

Thirteen microsatellite loci were amplified using standard laboratory procedures described in detail in Stengle (2018). GIMLET (Valiere 2002) was used to identify duplicate multi-locus genotypes possibly representing samples from the same individual corroborated by field notes on age, sex, color phase, and location. 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 & Wang 2012). COLONY 1.2 (Wang 2004) was used to estimate the number of litters when multiple neonate sheds were collected from the same communal rookery.

Conformation of Hardy-Weinberg equilibrium proportions in each population by calculating F_{IS} was calculated for each locus 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 & Van Tienderen 2004) was used to estimate observed heterozygosity (H_O), expected heterozygosity (H_E), and mean within-population expected heterozygosity (H_S) per locus and per population. We calculated allelic richness (mean number of alleles scaled to the smallest sample size, $n = 12$), F_{IS}, and F_{ST} using FSTAT 2.9.3.2 (Goudet 2001).

Effective number of breeders (N_b) was estimated using LDNE 1.31 (Waples & 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 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 with a minimum allele frequency cutoff (P_{crit}) of 0.02 to balance bias and precision (Waples & Do 2008). We used the jackknife approach to calculate 95% CI.

Population Genetic Structure

Pairwise F_{ST} and F'_{ST} values were calculated between all populations with ≥12 samples using GENODIVE 2.0b22 and Fisher's method (Meirmans & Van Tienderen 2004). To assess population genetic distinctiveness we calculated mean population-specific F_{ST}, a measure akin to "genetic uniqueness" (Coleman et al. 2013). For each locus, we then plotted the mean population-specific F_{ST} against metrics of genetic distinctiveness including allelic richness, expected heterozygosity, and mean number of alleles. A negative relationship between genetic distinctiveness metrics 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. Migration among populations is unlikely given the geographic distances among populations or separated by major highways which act a barrier to this species (Clark et al. 2010) so we did not apply F_{ST} coalescence analyses. We tested for isolation by distance (IBD) by comparing the relationship between genetic distance (population pairwise F_{ST}) and Euclidian distance using a Mantel test in the package vegan (v. 2.2-1, Oksanen et al. 2015) and R version 3.1.3 (v. 3.1.3, R Core Team 2017). Pairwise F_{ST} was transformed as $F_{ST}/(1-F_{ST})$ (Slatkin 1987) 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 et al. 2010) using populations with ≥12 samples. 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*) we used 100,000 replicates, with 50,000 burn-in cycles, an admixture model, and correlated allele frequencies. Ten runs were performed for *K* = 1–20, 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. We used STRUCTURE HARVESTER (Earl & vonHoldt 2012) to visualize STRUCTURE results and inferred the number of clusters (*K*) based on interpretation of the relationship of estimated log probability of the data (LnP(D)) with *K*. We used the program CLUMPP (Jakobsson & Rosenberg 2007) to achieve permutations of all 10 iterations for each *K*, using the "greedy" algorithm, and the program DISTRUCT version 1.1 (Rosenberg 2004) to create bar plots for each value of *K* showing the probability of cluster membership for each sample.

For the DAPC analysis we used successive *K*-means clusters in the *find.cluster* function with the R package *adegenet* (v.1.4-2, Jombart et al. 2010). We evaluated *K* = 1–30 with ten runs for each *K* and considered the *K* with the lowest Bayesian Information Criterion (BIC) to represent the optimal number of clusters. The *dapc* function in *adegenet* was executed using optimal grouping, retaining the principal components analysis axes that explained >90% of variation in the data.

Radio telemetry data (Stengle 2018) suggest that the nine dens of the largest Massachusetts (MBER) population could be a metapopulation. We therefore conducted an additional STRUCTURE analysis to evaluate the degree of genetic clustering within this population. We evaluated *K* = 1–9 using the same settings as described previously.

To evaluate the sensitivity of our inferred patterns among New England populations, Virginia and Pennsylvania were excluded, and re-tested for IBD and re-ran the DPAC analysis.

Genetic Rescue Literature Review

We reviewed the peer-reviewed literature to find all relevant cases of genetic rescue in wild vertebrate populations. We summarized the geographic distances between donor and recipient population, genetic methods and metrics, and observations of inbreeding depression. Most studies did not disclose precise population locations so we estimated locations based on descriptions provided in each study.

Results

Population Genetics

A mean of 48 samples (range 13–124) were collected from 19 timber rattlesnake populations across the northeastern United States (Table 2). Mean A was 5.71 (range 3.71–10.29), mean A_R was 4.02 (range 1.90–5.75), mean H_E was 0.53 (range 0.24–0.66), and mean F_{IS} was 0.14 (range 0.03–0.22). Pairwise F_{ST} estimates were significant for all population pairs (171 tests, Table 3). Overall F_{ST} was 0.173 (95 % CI 0.141 – 0.205). The relationships between genetic diversity (A, H_E and A_R) and genetic distinctiveness (mean population-specific F_{ST}) were consistently negative: A, R^2 =0.17, p = 0.068; H_E R^2 = 0.72, p < 0.0001; A_R, R^2 = 0.62, p < 0.0001 (Fig. 1). There was no correlation between population pairwise genetic and geographic distances (Mantel 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 (Fig 2). Only one sample (a shed skin collected in 1995) was excluded as a duplicate sample.

The STRUCTURE analysis indicated that K = 7 had the greatest support as indicated by the log-likelihood probability reaching an asymptote after K = 8 with increasing variance after K = 10. With K = 5 NH and MNOR each formed a separate cluster as did all MBER subpopulations (Fig. 4). Both Vermont populations and NWAS clustered together. At K = 5 and K = 6, both MHAD1 and MHAD2 fell into separate clusters. At K = 7 VRUT1, MHAD1, and MHAD2 fell into separate clusters. Other distinct clusters did not appear to have distinct geographic relationships. The two Connecticut populations do not appear to cluster strongly with any of the groups; however they have a relatively low F_{ST} value among them of 0.09.

The estimate of BIC from the DAPC analysis decreased monotonically with increasing K from K = 1–30 preventing us from identifying an empirically value of K (Fig 5). We therefore used K = 7, based on the STRUCTURE results, for further DAPC analyses. Results from DAPC were consistent with those of STRUCTURE. The populations most distinct were the peripheral New England populations, NH, MNOR, MHAD1, VRUT1 and VRUT2 (Fig 6), consistent with STRUCTURE results. In regions where populations were larger, (PA, VA, 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 $F_{ST} = 0.401$).

The STRUCTURE analysis of the putative Massachusetts metapopulation (MABER) indicated the greatest support for $K = 4$ with each subpopulation forming a separate cluster (Fig. 7). Each subpopulation contained multiple individuals with a high probability of assignment to other subpopulations. The two most spatially proximate subpopulations, MBER2 and MBER3, showed the greatest degree of admixture. Estimated pairwise F_{ST} (mean = 0.081, range 0.042–0.123) indicated some genetic differentiation among the four subpopulations, with the least differentiation between the two most geographically proximate subpopulations ($F_{ST} = 0.042$). MBER1 was the most genetically differentiated from other den regions. MBER4, the largest site, was the least genetically differentiated (Table 3). Point estimates of N_b for each subpopulation ranged from 3.7–83.7 (Table 2).

The IBD and DAPC results were similar regardless of whether non-Northeastern populations (i.e., Virginia and Pennsylvania) were included. There was no significant relationship between geographic and genetic distance ($R = 0.020$, $p = 0.474$, Fig. 8). The estimate of BIC from the DAPC analysis decreased monotonically from $K = 1$–30 (results not presented) so we again used $K = 7$ based on the STRUCTURE results. Similar to our results with all populations, peripheral populations (New Hampshire, Vermont, and MHAD1) were the most distinct populations with less evidence of distinction among other populations (Fig. 9, 10).

Genetic Rescue Literature Review

We reviewed 12 studies of nine vertebrate species that employed genetic rescue and reported genetic metrics prior and/or subsequent to genetic rescue (Table 4). There was great variability in species natural history, genetic methods and metrics, sampling methods (i.e. opportunistic samples, museum specimens, active capture, etc.) used. Studies appeared to initiate genetic rescue primarily based on the presence or suspected presence of inbreeding depression through observations of decreased fitness or the presence of unusual phenotypes. In studies where inbreeding depression was detected or presumed present (*n* = 7, Bouzat et al. 2009; Fredrickson et al. 2007; Heber et al. 2012; Hogg et al. 2006; Johnson et al. 2010; Madsen et al. 1999; Madsen et al. 2004; Miller et al. 2012; Westemeier et al. 1998), fitness did improve and unusual phenotypes decreased after genetic rescue was implemented. For example, after genetic rescue the rate of stillbirths decreased in European adders (Madsen et al. 1999; Madsen et al. 2004) and litter size increased for the Mexican wolf (Fredrickson et al. 2007). Of the 12 studies we reviewed, all but one showed an increase in all genetic metrics, including four studies where no inbreeding depression was observed or reported (Olson et al. 2012; Whittaker et al. 2004; Yamamoto et al. 2006). The twelfth study, on Eurasian otters (*Lutra lutra*), showed an increase mtDNA haplotype diversity but decreases in microsatellite-based metrics (Arrendal et al. 2004).

Discussion

Population Genetics of Northeastern Timber Rattlesnakes

We observed a relatively high degree of genetic structure among northeastern timber rattlesnake populations. Previous studies have found that even lightly-used paved roads can result in genetic differentiation between timber rattlesnake populations (Clark et al. 2010). Extant timber rattlesnake populations in the northeast, particularly in New England, are widely separated by landscapes with a diversity of anthropogenic disturbance levels ranging from low-density rural development to multi-lane paved highways and high-density urban development. Patterns of timber rattlesnake genetic structure may, in part, reflect this species' association with specific microhabitats for denning, gestation, and shedding (Brown 1993; Martin et al. 2008). These habitat features are not, and likely were not historically, continuously distributed across the landscape. Among timber rattlesnake subpopulations in New York separated by 1.2–9.9 km, Clark et al. (2008) found no correlation between genetic distance and geographic distance but a significant positive correlation between genetic distance and a cost distance based on the amount of potential basking habitat (i.e., areas with ≥30° slope and SE-to-SW aspects). This suggests that genetic connectivity among populations is limited by the amount of potential basking habitat. Therefore, the extent to which these habitat features occurred across the historical New

England landscape could inform the degree to which timber rattlesnake populations were historically connected. We therefore encourage efforts to empirically model the historical distribution of timber rattlesnakes in New England to better understand historical connectivity. Such an effort is currently underway in Massachusetts using historical observations of timber rattlesnakes and climatic and topographic variables (M.J., personal communication). Our results did not change when the larger, more distant populations in Pennsylvania and Virginia were removed, suggesting including more distant populations in the analyses did not drive this unusual pattern within New York and New England. In contrast, Clark et al. (2003) determined spatial variation in timber rattlesnake mtDNA do not support subspecies classifications a conclusion supported by a later, more extensive mtDNA analysis (Stengle 2018).

The lack of IBD was surprising given that the geographic distances among populations typical exceed known dispersal distances for timber rattlesnakes (e.g., 7 km, Brown 1993). Other studies of snake population and landscape genetics have reported significant IBD at scales from 10–400 kilometers (e.g., DiLeo et al. 2013; King 2009; Klug et al. 2011; Row et al. 2010) including studies of timber rattlesnakes (Bushar et al. 2014; Bushar et al. 2015). Bushar et al. 2014 conducted a study similar to ours across PA and NJ, an area geographically larger than New England, and found significant IBD. The same was found with the black ratsnake (*Pantherophis alleghaniensis* formerly *Elaphe obsoleta*, Carothers et al. 2017), across a similar geographic range as Bushar et al. 2014 (Lougheed et al. 1999). Both of these studies, however, were done in regions where the study species is more abundant than the timber rattlesnake is in New England. While Clark et al. (2008) found no evidence of IBD, the distances between subpopulations was <10 km and they did find a significant positive correlation between genetic distance and cost distance based on potential basking habitat. Anderson (2010) found no evidence of IBD among timber rattlesnake dens <3 km apart.

IBD is theoretically expected under a stepping-stone model of population genetic structure when gene flow and drift are in equilibrium with the relative effects of genetic drift becoming stronger than those of gene flow at increasing distances and vice versa (Hutchison & Templeton 1999). In contrast, a flat relationship between genetic and geographic distance with wide scatter suggests a greater effect of genetic drift than gene flow. Hutchison & Templeton (1999) examined the genetic similarity of collard lizards (*Crotaphytus collaris collaris*) in Ozark Mountains where they occupy isolated rocky glades within a forest matrix and found a flat relationship between genetic and geographic distance with wide scatter. They concluded that recent fire-suppression has reduced gene flow among glades such that genetic drift was the driving factor behind observed patterns of genetic structure. Our observed lack of IBD may therefore suggest disequilibria between gene flow and drift and the high levels of genetic structuring suggest a greater effect of genetic drift than gene flow. Similarly, a region wide study of the eastern massasauga (*Sistrurus catenatus*), a rattlesnake species also exhibiting isolated populations in fragmented areas found strong genetic divergence among several populations (Chiucchi & Gibbs 2010). While the authors did not test for IBD, they concluded that long-term genetic drift was the cause extreme differentiation among populations. However, isolated populations may still show evidence of IBD. Bighorn sheep (*Ovis canadensis*) populations restricted to single mountaintops with no migration between them still showed a strong relationship of IBD and genetic diversity (Forbes & Hogg 1999).

Genetic Rescue Summary

Our literature provides a comprehensive summary of genetic rescue and a detailed description of the genetic metrics used in previous studies. Given the variability in species life histories, genetic markers and metrics, and sampling methods and the relatively small number of studies available, there does not appear to be a genetic-based threshold to determine if genetic rescue is warranted. Rather, we suggest that the presence or suspected presence (e.g., through the observation of unusually phenotypes) may be a more informative metric for evaluating the need for genetic rescue. Our review found that in all populations exhibiting signs of inbreeding depression that overall fitness increased and abnormal phenotypes decreased post-genetic rescue. In all but one study, measures of genetic diversity also increased post genetic rescue. Even though we cannot provide a multi-taxa, genetic-based threshold, metric, our review corroborates previous reviews indicating that genetic rescue is an effective method to increase fitness and genetic diversity across many taxa and geographic regions (Whiteley et al. 2015).

Caution should be used when comparing genetic metrics across the studies we reviewed. Values of genetic diversity using different genetic markers (i.e. microsatellite, mtDNA) cannot be directly compared. Measures of genetic diversity can also vary across taxa so these metrics should not be directly compared, even if the methods used are the same. The number of markers used in each study also varies, which can affect the values of measured metrics. Sampling methods also differed among studies, some collecting only museum or opportunistically-encountered specimens while collected samples systematically. The number of generations pre- and post-genetic rescue also varied across studies. Finally, the natural history and phenology of each species differs greatly. Different species presented here disperse in different patterns and distances, and these patterns may affect the efficacy of the rescue effort. In some studies, individuals were translocated greater distances than would be possible naturally, up to 30 times farther, as is the case with the Prairie Chicken (Bouzat et al. 2009; Westemeier et al. 1998). Habitats also vary to some degree between translocation populations in most of these studies presented here. These differences suggest that attempts to develop a genetic-based threshold for initiating genetic rescue using multi-taxa studies may be uninformative and potentially misleading. However, our review highlights the importance of using phenotypic signs of inbreeding depression to consider implementing genetic rescue. While we also recommend that managers consider the genetic structure and connectivity of their population(s), we suggest that managers not rely solely on genetic metrics but rather recommend a multi-facetted approach incorporating genetic, phenotypic, and demographic data to determine if and when genetic rescue is warranted.

Identification of Timber Rattlesnake Management Units

Many factors influence the designation of units for conservation including genetic, phenotypic, ecological, and geographic distinctiveness, the degree of connectivity with other potential units, and economic, sociological, political, and legal considerations (Crandall et al. 2000; Moritz 1994; Ryder 1986; Waples 1991, 1995). One complicating factor in using contemporary patterns of genetic structure and connectivity to assist in designating conservation units is determining the extent to which these patterns are the result of anthropogenic factors such as habitat loss and fragmentation and human persecution. For example, two populations may have been demographically and genetically connected historically but have since been sufficiently anthropogenically isolated so as to become genetically divergent. In this situation, genetic distinctiveness is due to random genetic drift which could be exacerbated by designating the populations as separate conservation units (Coleman et al. 2013; Frankham et al. 2012; Vucetich

& Waite 2003). Conversely, contemporary genetic isolation may reflect a historical lack of genetic connectivity due to naturally fragmented habitats. Under the latter scenario, a lack of historical connectivity may have resulted in varying degrees of local adaptation making it important to maintain the genetic distinctiveness of the population. Outbreeding depression can be a concern when translocating individuals among locally-adapted populations depending on the degree of local adaptation and the differences in selective pressures among populations (Frankham et al. 2012). However, the potential for outbreeding depression can be difficult to determine *a priori*, particularly in populations showing a high degree of phenotypic plasticity (Edmands 2007). However, outbreeding depression may be more likely when marked differences in fitness related traits correlate to spatial genetic structure and differences in environmental features, such as climate or elevation, and when historical genetic connectivity has been minimal (Frankham et al. 2011). For example, severe outbreeding depression leading to local population extinction was observed when ibex (*Capra ibex*) from Austria were introduced into Czechoslovakia following a local extirpation but then subsequently crossed with Ibex introduced from Turkey and Egypt (Greig 1979). Therefore, an accurate and robust designation of conservation units requires an understanding of both contemporary and historical population distributions, genetic structure and connectivity as well as the factors influencing such patterns. Unfortunately, such data is often difficult or impossible to obtain for many species. Additionally, the critical conservation status of some populations may require conservation action in the absence of sufficient data. In all situations, managers must weigh the relative risks and benefits of different approaches for identifying conservation units within existing legal and political constraints and mandates.

To our knowledge, only Martin et al. (2008) have undertaken a formal designation of timber rattlesnake conservation units. They proposed dividing the entire distribution of timber rattlesnakes into five units which they termed “management units” on the basis of “shared geographical characteristics and on the on the ecological, morphological, and genetic characteristics of the snakes as currently understood or surmised” (pg. 448). The genetic characteristics they used were based on the mtDNA analysis of Clark et al. (2003). Within each management unit, they further identified eco-regional sub-units to account for “strong regional variation […] in life history traits, and in the snake’s population sizes, threats, and conservation status” (pg. 450). They classified New England in the Appalachian/Northeastern Unit and the

New England Uplands sub-unit. However, Martin et al. (2008) do not provide further justification for their definition or delineation of eco-regional sub-units or empirical evidence that timber rattlesnake population demographics are homogenous across New England. While our demographics analysis did not show evidence of large differences in population demographics among the New England population we considered (Chapter 1), there exists marked differentiation in the conservation status of timber rattlesnakes across New England. Moreover, our genetics results show strong intra-regional variation in population genetics structure. We suggest, therefore, that both the existing genetics and demographic data be incorporated into any designation of timber rattlesnake conservation units within New England.

Patterns of contemporary genetic structure indicate a high degree of structure among New England timber rattlesnake populations combined with a lack of IBD. In particular, Massachusetts timber rattlesnake populations in the Berkshire Mountains and Connecticut River Valley showed strong genetic structure within and between these two regions despite close spatial proximity. It is quite possible that such patterns represent the effects of random genetic drift rather than local adaptation or evolutionary distinctiveness (Frankham et al. 2012). Genetic drift changes the allele frequencies of a population in one generation and the magnitude of these changes increase as population size decreases. We therefore suggest that any definition of timber rattlesnake conservation units in New England not solely be based on our genetic clustering analyses as doing so may exacerbate the effects of genetic drift and inbreeding depression.

Alternatively, divergent genetic structure could reflect local adaptation. There are relatively few studies demonstrating a genetic basis to inter-population differences in adaptive phenotypes in snakes. Bronikowski and Arnold (Bronikowski 2000; Bronikowski & Arnold 1999) found evidence of local adaptation in western terrestrial gartersnake (*Thamnophis elegans*) populations separated by 5–15 km. Individuals from lakeshore habitats with high prey availability had faster life histories than individuals from mountain meadows with lower prey availability and common garden laboratory experiments indicated these differences were genetically based (Bronikowski 2000). Dubey et al. (2015) observed correlations between population genetics structure and color morph frequency in asp vipers (*Vipera aspis*) and suggested such differences might be driven by variation in selection pressures. Local adaptation is most probable when local environmental conditions differ in a manner affecting fitness with the degree of local adaptation reflecting the strength of selective pressures (e.g., Hereford 2009).

Local adaptation can be difficult to distinguish from phenotypic plasticity but can be identified through genomic analyses or common garden and reciprocal transplant experiments (Allendorf et al. 2013; Kawecki & Ebert 2004). However, these approaches are costly to implement and the latter may be prohibitive with critically endangered species. The longevity and long times to sexual maturity for timber rattlesnakes as well as their imperiled status in New England make reciprocal transplant experiments logistically challenging.

An alternative approach to evaluate the potential for local adaptation among timber rattlesnake populations in New England might be to correlate inter-population variation in fitness related traits (e.g., adaptive phenotypes, demographic parameters) as a function of potential selection pressures such as elevation or climatic variables. Correlations between variation in traits and selection pressures may suggest that variation in traits is due to local adaption, particularly if the variation in selection pressures has persisted for a relatively long time. For example, Santos et al. (Santos et al. 2014) found that spatial variation in European adder color morphs was correlated with both spatial genetic structure and spatial variation in lithology and climate. However, in some cases inter-population differences in demographic traits may be influenced by local differences in prey availability or recent anthropogenic disturbances (Jenkins et al. 2017; Jenkins & Peterson 2008; Jenkins et al. 2009). Timber rattlesnake body size is known to vary among populations (Chapter 1; W.H. Brown and L. Perrotti, personal communication). However, this may be related to population density, food availability, or anthropogenically-induced variation in life span. Our demographics analyses do not readily suggest that our observed differences in demographic traits are attributable to differences in climate or elevation although we were limited by small sample sizes (Chapter 1). It is also unclear of the extent to which our observed differences in demographic traits are due to phenotypic plasticity or variation in anthropogenic disturbance. Despite its challenges, we encourage additional research into the possibility of local adaptation of New England timber rattlesnakes given its potential to inform the designation of management units and inform genetic rescue efforts.

Given the uncertainties associated with contemporary and historical timber rattlesnake population structure and connectivity in New England and the need to balance the risks of inbreeding and outbreeding depression (Edmands 2007; Frankham et al. 2012; Frankham et al. 2011), we strongly recommend that additional research be conducted prior to formally designating conservation units for timber rattlesnakes in this region. Moreover, concordant results from multiple lines of evidence should be used to identify conservation units (Allendorf et al. 2013). Designating conservation units in the absence of sufficient data may overly restrict potential management options. Political and legal factors (e.g., state and federal mandates for species recovery) may also be considered when designating conservation units. In the absence of conclusive empirical data, a precautionary approach to wildlife conservation is often advised (Edwards-Jones 2006). However, whether or not a particular action is considered precautionary may depend on particular circumstances. For example, for a small, highly inbred population, precautionary management may involve actions more extreme than what might be considered precautionary for a larger, more well-connected population (e.g., translocating between management units or subspecies in the case of severe inbreeding depression, Edmands 2007). Overly precautionary approaches may limit potential management options while insufficient precaution may lead to unintended consequences from which small, imperiled populations may not recover. We encourage the continued collaborative participation of New England timber rattlesnake stakeholders to identify and implement priority research programs that will contribute towards timber rattlesnake conservation in this region.

Management Options

The data provided here can serve as a guideline when determining which populations may benefit from genetic rescue. However, based on the limited extent of existing data and the absence of other data sources, we suggest that it may be premature to develop a comprehensive genetic rescue plan for all timber rattlesnake populations in New England, with the exception of New Hampshire. Both previous work (Clark et al. 2011) and our results suggest that some form of genetic rescue will greatly facilitate the recovery and long-term persistence of this population. The Massachusetts Hampshire County population may also be a priority candidate for genetic rescue based on its strong differentiation from other Massachusetts populations in the Connecticut River Valley. Moreover, given the widespread success of genetic rescue in wildlife conservation (Whiteley et al. 2015), we recommend that other states consider genetic rescue as a potential conservation tool for their respective populations. In this section, we provide a discussion of different approaches for implementing genetic rescue together with their benefits and drawbacks.

Regardless of the management option chosen, we do not recommend translocating adult individuals. There is limited data available on translocating adult timber rattlesnakes, and snake species in general (Kingsbury & Attum 2009). However, Reinert & Rupert (1999) found that translocated adult timber rattlesnakes moved more frequently and greater distances compared to resident snakes. Such increases in movement patterns may increase the risk of natural and anthropogenic mortality, and decrease the amount of time available for foraging and mate-searching. However, Reinert & Rupert (1999) found that surviving translocated adults exhibited movement patterns more similar to resident snakes and stable home ranges after at least one year. Nuisance adult viper species in Malaysia that were relocated also moved more frequently, more sporadically, and longer distances than native snakes (N. Karraker, unpublished data). Similar patterns were observed with translocated tiger snakes (*Notechis scutatus*, Butler et al. 2005) and eastern hog-nosed snakes (*Heterodon platirhinos*, Plummer & Mills 2000). We therefore do not recommend that adults be used for genetic rescue or translocation. However, if circumstances necessitate the use of adults, we strongly recommend that all individuals are regularly monitored using radio telemetry.

Choosing a Source Population

Because genetic rescue involves removing some individuals from the source population, careful consideration should be given to ensure that this does not negatively impact the source population. Potential source populations should, therefore, represent stable and relatively large populations. While demographic analysis was unable to accurately estimate population size, survival analyses for VRUT2, MBER, and CHAR showed increasing, stable, or negligibly decreasing survival (Chapter 1). However, population viability analyses on these three populations suggested that only MBER is stable given our estimated survival rates (Chapter 1). Another option is to trade individuals between populations, so there is no change in population size, assuming that individuals from both populations contribute equally to their respective recipient populations.

The genetic similarities between source and recipient populations should be considered although the implications of any similarities or differences may be difficult to determine. Genetic structure may be due to naturally low rates of gene flow, local adaptation, genetic drift due to anthropogenically-induced isolation, or some combination of the above factors. However, two observations may mitigate concerns about outbreeding depression due to local adaptation: First, in their review of genetic rescue, Whiteley et al. (2015) found only one example of genetic rescue that resulted in a negative fitness response (i.e., outbreeding depression) and in this example the source and recipient population were extremely divergent at the cytochrome oxidase I gene. Second, genetic rescue appears to have beneficial fitness effects even when source and recipient populations have elevated levels of inbreeding (Heber et al. 2012). Because differences in local adaptation are generally maximized where local environmental conditions are very different, selecting source populations from similar environmental conditions may mitigate any effects of outbreeding depression (Edmands 2007; Frankham et al. 2011). Assuming differences in local adaptation where none exist may also unnecessarily exclude some potential source populations from consideration. We acknowledge that political, sociological, and legal constraints regarding moving individuals across state boundaries may constrain the choice of donor populations. Even though all New England populations have tested positive for snake fungal disease (SFD), we recommend not translocating individuals that exhibit signs of an active SFD infection. All individuals brought into captivity for any length of time should have a DNA sample collected for future analyses. We encourage a collaborative approach among states to identify a single, central repository for tissue samples.

Neonates and Headstarting

Headstarting is a technique that can be used with or without genetic rescue. We have concluded based on the demographic risk to populations and individuals, that only neonates and/or headstarted juveniles should be translocated. The removal of neonates and juveniles from a population will have less of an overall population-level effect, particularly since these age classes exhibit higher mortality rates. Headstarting neonates within a population will likely have a minimal chance of causing a negative effect on population size and allows individuals to survive the first one to few years of life with increased body condition. Although individuals translocated in one Massachusetts population successfully adapted to the new population, data are very limited (A.G.S., unpublished data). We therefore recommend that all headstarted and translocated individuals are monitored with radio telemetry to determine if individuals survive, find adequate overwintering sites, forage successfully, and mate with resident individuals. Transmitters can be surgically implanted or attached externally using glue or tape. Surgical implantation allows for the use of larger transmitters and enables tracking for longer periods (up to two years). External attachment is less invasive and smaller transmitters can be used with smaller individuals (e.g., neonates). However, external transmitters are usually lost when an individual sheds and small transmitters have reduced battery life and signal range. We therefore recommend that each state make the decision to use internal or external transmitters according their particular needs and concerns. King et al. (2004) observed greater survival in repatriated 2–3 year old eastern massasaugas released in July compared to individuals released in September. We therefore recommend releasing individuals in the spring or summer rather than the fall to allow individuals an opportunity to adjust to their new surroundings prior to hibernation.

We recommend that managers consider only removing half the individuals from a single litter so as to not entirely remove the genetic signature of the litter from the source population. With captive breeding (see below) half the litter can be returned to the parental population(s). Translocations of headstarts in New England suggest releases during emergence are more successful than during ingress.

Headstarting requires a high capital investment including a facility and staff to raise individuals for 2–3 years, depending on individual growth rates, prior to release. A size of approximately 450g is the ideal size for release. At this size, survivorship is greatly increased (Brown et al. 2007) and the individual is large enough to safely carry a radio. Additionally, releasing larger individuals closer to sexual maturity gives those individuals an opportunity to contribute to the resident population sooner. Rapid growth can be obtained by not allowing captive individuals to brumate over the winter (e.g., King & Stanford 2006). However, Sacerdote-Velat et al. (2014) found that headstarted smooth greensnakes (*Opheodrys vernalis*) that underwent brumation achieved similar pre-release body sizes as individuals that did not undergo brumation due to compensatory growth. In the presence of such compensatory growth, forgoing brumation could be more efficient through a reduction in husbandry requirements. Roe et al. (2015) found that 22-month old headstarted northern watersnakes that were not bromated their first winter but were brumated their second winter had similar behavioral and movement patterns and survival as wild, resident individuals. The extent to which brumation is advantageous for headstarted timber rattlesnakes in north-temperate climates is currently unclear and we encourage additional research on this topic.

Other resources needed include food, caging, heating equipment and access to veterinary care. Staff time and radio telemetry equipment is needed for follow up monitoring. However, it may be possible to forgo radio telemetry monitoring once it has been determined that the translocated individuals are regularly surviving and breeding with resident snakes.

Captive Breeding or Propagation

We refer to captive breeding, or propagation, specifically as the breeding and birthing of individuals in captivity. Individuals are removed from source populations for a set length of time which could include one or more reproductive cycles. Previous captive breeding work suggests that adults should be in captivity for at least two years prior to successful birthing unless the female is already gravid. Adults could be rotated through the program so individuals are not permanently removed from the source population and to increase genetic diversity of captive-born offspring. Another option is to keep females longer, and rotate males more frequently. Female timber rattlesnakes adapted to captivity can reproduce on an annual cycle, instead of every 3–5 years seen with wild females (Brown 1991, 2016). The average wild female in northeast only produces an average of 1–2 litters throughout her entire life span (Brown 2016). Captive breeding therefore offers the advantage of potentially increasing female reproductive output.

Captive breeding could involve breeding individuals from a single population or breeding individuals across multiple populations although we note the previously discussed considerations with regards to the risks of outbreeding depression. Mating individuals across populations could potentially create more genetically diverse offspring. We strongly recommend that conditions in captivity including temperature and photoperiod follow the natural phenology of timber rattlesnake populations in the Northeast.

The costs, facilities, and supplies required for captive breeding are similar to those required for headstarting. We suggest using radio telemetry to track released individuals whenever possible. Again, it may be possible to cease radio telemetry once initial releases are determined to be successful.

Future Genetic and Fitness Monitoring

Future genetic and fitness monitoring poses several challenges, however monitoring population genetics and fitness is necessary for evaluating the necessity and success of any genetic rescue projects undertaken. We suggest that genetic rescue not be undertaken if no post-release monitoring can occur. To determine if the rescue effort was successful an increase in genetic diversity needs to be observed. This also confirms that released snakes are mating with resident snakes. Any data on fitness metrics or phenotypic abnormalities collected prior to genetic rescue should also be collected post-rescue.

The first challenge with monitoring genetic rescue in timber rattlesnakes is that their slow life histories mean that several years of elapsed time will be required to detect changes in genetic metrics or fitness. The late age at first reproduction combined with the difficulty in detecting neonates and juveniles mean that over 10 years may be required before F1 individuals can be sampled (assuming that released individuals are immature). Genetic changes may be detected more quickly if released individuals are sexually mature and immediately breed with resident individuals within one to a few years of being released, if litters can be located and sampled. Given a 30–≥40 year lifespan (W.H. Brown, personal communication) and high percentage of overlapping generations, it could potentially be much longer before a change is observed, especially if a large portion of the population is unmarked.

It is also likely that genetic laboratory techniques will change over the next several years as genomic approaches become more widespread in wildlife conservation. If microsatellite markers become obsolete in the future, all of the DNA samples used here would have to be re-analyzed using the current method (i.e. SNPs). Even if microsatellites were still a viable technique in the future, several to all of the original samples would need to be re-amplified. All future genetic scenarios require adequate financial resources for laboratory work and field technicians which may change as state agency staff and management priorities change over time. Therefore we suggest state agencies develop a protocol for monitoring the effects of genetic rescue before any such effort is undertaken. Even if samples can’t be analyzed immediately, (i.e. funding isn’t available, etc.) samples can be stored for future work.

Radio telemetry may be the most efficient way to confirm the success of genetic rescue because it allows researchers to document courtship and mating behavior and new mothers with their litters. However, if headstarted juveniles were released, it would be several years before they would reach maturity which would require multiple years of telemetry monitoring. While mark-recapture will likely be insufficient to document such behavior, given the low recapture rates in our populations (Chapter 1), it may be possible to observe released gravid females during birthing rookery surveys. In populations with abnormal phenotypes visual surveys may be sufficient to document a decrease in their frequency.

Other Management Recommendations

Although genetic rescue is widely recognized as a tool to increase genetic diversity and fitness, it should not be viewed as the only management for a population. All managers should continue to pursue other management options to protect the population(s). This includes but not limited to: habitat protection and restoration, technical assistance to key landowners, refined regulatory approaches, increasing protection from poachers, and public outreach and education.

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Table 1. Population abbreviations and locations of sampled northeastern timber rattlesnake populations.

Abbreviation	Abbreviation	State	County
CHAR	CTHAR	Connecticut	Hartford
CLIT	CTLIT	Connecticut	Litchfield
MBER	MABER	Massachusetts	Berkshire
MHAD	MAHAD	Massachusetts	Hampden
MHAM	MAHAM	Massachusetts	Hampshire
MNOR	MANOR	Massachusetts	Norfolk
NWAS	NYWAS	New York	Washington
NH	NHNA	New Hampshire	N/A
NORA	NYORA	New York	Orange
NSUL	NYSUL	New York	Sullivan
NULS	NYULS	New York	Ulster
NWAR	NYWAR	New York	Warren
PA	PACEN	Pennsylvania	Multiple
VA	VASHE	Virginia	Multiple – Shenandoah National Park
VRUT	VTRUT	Vermont	Rutland

Table 2. Population genetic parameter estimates for all timber rattlesnake populations sampled. Sample size (*n*), mean number of alleles per locus (*A*), allelic richness (A_R), expected heterozygosity (H_e), observed heterozygosity (H_o), inbreeding coefficient (F_{IS}), genetic divergence (F_{ST}), and effective number of breeders (N_b) with 95% CI.

Population	*N*	*A*	A_R	H_e	H_o	F_{IS}	F_{ST}	N_b
NH	69	4.929	1.896	0.241	0.207	0.14	0.391	2 (1.3-3.1)
MNOR	110	6.643	4.201	0.600	0.524	0.128	0.135	47.5 (36.2-64.2)
MHAD1	55	4.786	3.164	0.438	0.375	0.145	0.200	11 (6.4-17.8)
MHAM	19	4.500	3.723	0.495	0.433	0.125	0.202	2.2 (1.7-2.8)
MHAD2	28	4.571	3.495	0.504	0.456	0.094	0.166	9.2 (5.3-15.5)
CHAR	38	5.643	4.117	0.563	0.495	0.122	0.136	26.8 (16.3-50.7)
CLIT	13	5.429	4.800	0.614	0.532	0.134	0.097	6.4 (3.6-9.5)
MBER1	79	6.071	3.640	0.481	0.414	0.139	0.107	29.5 (21.5-41.5)
MBER2	28	5.428	4.061	0.509	0.418	0.178	0.310	23.7 (14.6-45.9)
MBER4	124	7.429	4.077	0.533	0.477	0.106	0.236	83.7 (59.8-125.5)
MBER3	22	5.428	4.367	0.574	0.459	0.201	0.209	3.7 (2.7-6.3)
VRUT1	39	3.714	3.065	0.499	0.425	0.148	0.221	25.6 (13.7-62.6)
VRUT2	94	4.071	3.190	0.413	0.399	0.034	0.212	107.9 (54.4-406.6)
NWAS	26	5.214	4.014	0.513	0.415	0.192	0.213	18.8 (12.7-30.3)
NWAR	29	6.071	4.656	0.594	0.465	0.217	0.145	893.9 (45.9-INF)
NULS	14	4.357	4.129	0.542	0.461	0.150	0.068	24.5 (9.6-INF)
NORA	19	5.714	4.568	0.607	0.521	0.142	0.153	43 (25.4-108.0)
PA	33	8.143	5.408	0.635	0.534	0.159	0.111	60 (35.8-150.5)
VA	65	10.286	5.750	0.659	0.546	0.170	0.092	184.1 (106.1-539.1)
Mean	33.05	5.879	4.017	0.528	0.4503	0.145	0.175	84.39

Table 3. 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.

	NH	MNOR	MHAD 1	MHAM	MHAD 2	CHAR	CLIT	MBER	VRUT1	VRUT2	NWAS	NWAR	NULS	NORA	PA	VA
NH	0.00	0.30	0.41	0.44	0.43	0.35	0.35	0.28	0.48	0.42	0.41	0.38	0.47	0.37	0.35	0.33
MNOR	0.56	0.00	0.18	0.18	0.17	0.12	0.08	0.10	0.22	0.21	0.16	0.11	0.14	0.10	0.08	0.07
MHAD 1	0.66	0.35	0.00	0.30	0.19	0.18	0.15	0.15	0.23	0.28	0.23	0.15	0.23	0.19	0.18	0.15
MHAM	0.71	0.37	0.53	0.00	0.23	0.17	0.18	0.15	0.28	0.28	0.25	0.14	0.17	0.16	0.13	0.15
MHAD 2	0.71	0.35	0.33	0.42	0.00	0.12	0.13	0.15	0.23	0.25	0.21	0.10	0.15	0.13	0.13	0.11
CHAR	0.63	0.27	0.33	0.35	0.25	0.00	0.09	0.08	0.22	0.22	0.17	0.08	0.10	0.05	0.08	0.09
CLIT	0.62	0.16	0.28	0.37	0.28	0.21	0.00	0.05	0.19	0.13	0.10	0.05	0.07	0.06	0.05	0.03
MBER	0.56	0.22	0.30	0.31	0.30	0.17	0.10	0.00		0.19	0.13	0.06	0.08	0.06	0.05	0.07
VRUT1	0.59	0.42	0.49	0.51	0.47	0.43	0.24	0.38	0.00	0.16	0.15	0.12	0.23	0.22	0.17	0.18
VRUT2	0.70	0.42	0.38	0.47	0.40	0.41	0.33	0.30	0.39	0.00	0.09	0.12	0.21	0.20	0.17	0.16
NWAS	0.68	0.33	0.41	0.47	0.40	0.35	0.19	0.26	0.14	0.26	0.00	0.07	0.05	0.14	0.10	0.11
NWAR	0.65	0.23	0.27	0.25	0.19	0.17	0.07	0.13	0.21	0.21	0.11	0.00	0.05	0.05	0.04	0.06
NULS	0.76	0.27	0.40	0.29	0.27	0.20	0.12	0.15	0.35	0.38	0.27	0.04	0.00	0.07	0.06	0.08
NORA	0.66	0.22	0.37	0.35	0.27	0.11	0.15	0.12	0.39	0.39	0.28	0.09	0.13	0.00	0.04	0.04
PA	0.65	0.16	0.36	0.28	0.28	0.19	0.12	0.11	0.35	0.32	0.21	0.06	0.09	0.09	0.00	0.04
VA	0.57	0.17	0.31	0.34	0.25	0.22	0.05	0.15	0.35	0.37	0.26	0.13	0.17	0.10	0.09	0.00

Table 4a. Results of a literature review of studies employing genetic rescue and the population genetics metrics of recipient populations prior to and following genetic rescue. Species names are given only at species level and not subspecies level because some studies occur with translocation across subspecies. Approximate Distance represents distance between source and recipient populations. For genetic metrics: n = census population size, A = average number of alleles, A_R = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, * = reported means or means estimated from figures in the paper. For Genetic Methods: MHC = Major histocompatibility complex, RFLP = Restriction fragment length polymorphism, mSat = Microsatellites, mtDNA = Mitochondrial DNA.

Species	Population Location	Source Location	Approx. Distance (km)	Metric Measured	Genetic Method	Metric Before	Metric After
European adder *Vipera berus*	Smygehuk, Sweden	Lund, Sweden	50	male n		8*	40*
				H_O	MHC RFLP	mostly homozygote *	increase in heterozygostiy *
Mexican wolf	Arizona	Durango and Chihuahua, Mexico	550	n		7	52
Canis lupus			1200	A		1.55–2.5*	NA
				H_O		0.128–0.457*	NA
				H_E		0.174–0.403*	NA
				F_{IS}		0.18–0.61*	NA
Bighorn sheep	Montana	Alberta, Canada	500	n		~40	58
Ova canadensis				H_O	mSat	0.5	0.67

Prairie Chicken	Illinois	Minnesota	640	n		50	2000
Tympanuchus cupido				A	mSat	3.67	
Florida panther	Florida	Texas	1770	n		30–50	104
Puma concolor				H_O	mSat	0.167	0.244
				H_O			
Eurasian otter	Sweden	Norway	550	n		15	20
Lutra				H_E	mSat	0.694	0.613
				H_O	mSat	0.656	0.642
				A	mSat	4.83	3.67
				haplotype A:B	mtDNA	15:00	11:06
Bighorn sheep	Oregan-Steens MT	Nevada-Santa Rosa Mt	350 km	H_O	mSat	0.42	0.44
Ova canadensis				H_E	mSat	0.41	0.47
				A_R	mSat	2.54	3.16
Bighorn sheep	Oregan Leslie Gulch	Nevada-Santa Rosa Mt	450 km	haplotype A:B:C:D:E	mtDNA	0:0:0:0:13	8:0:1:39
Ova canadensis				H_O	mSat	0.39	0.46
				H_E	mSat	0.43	0.48
				A_R	mSat	2.71	3.34

				haplotyple A:B:C:D:E	mtDNA	0:0:0:0:22	6:0:5:0:37
South Island Robin *Petroica australis*	Allports Island, New Zealand	Motuara Islands, New Zealand	24 km	n		45	50
				A	mSat	2.68	3.79
				A_R	mSat	2.68	3.76
				H_E	mSat	0.462	0.527
				H_O	mSat	0.511	0.554
South Island Robin *Petroica australis*	Motuara Islands, New Zealand	Allports Island, New Zealand	24 km	n	mSat	82	122
				A	mSat	2.96	3.75
				A_R	mSat	2.96	3.7
				H_E	mSat	0.489	0.527
				H_O	mSat	0.492	0.507
White-spotted charr *Salvelinus leucomaenis*	above dam Kame River, Japan	below dam Kame River, Japan	<2km	H_O	mSat	0.28	0.63
				H_E	mSat	0.27	0.52
				A	mSat	2	2.5
				n	mSat	50	48
White-spotted charr *Salvelinus leucomaenis*	above dam Hitozuminai River, Japan	below dam Hitozuminai River, Japan	<2km	H_O	mSat	0.28	0.39
				H_E	mSat	0.24	0.48
				A	mSat	2	2.8
				n	mSat	52	46

Table 4a. Results of a literature review of studies employing genetic rescue and the population genetics metrics of recipient populations prior to and following genetic rescue. Species names are given only at species level and not subspecies level because some studies occur with translocation across subspecies. Approximate Distance represents distance between source and recipient populations. For genetic metrics: *n* = census population size, *A* = average number of alleles, A_R = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, * = reported means or means estimated from figures in the paper. For Genetic Methods: MHC = Major histocompatibility complex, RFLP = Restriction fragment length polymorphism, mSat = Microsatellites, mtDNA = Mitochondrial DNA.

Species	Generation Sampled	Inbreeding Depression?	Phenotypes Observed	Phenotypes Post	Reference	Notes
European adder *Vipera berus*	F1	Yes	increased still births	decreased still births	Madsen et al. 1999; 2004	*values estimated from graph, no formal genetic metric reported *no formal genetic metrics reported, paper only provides a picture of a gel of 7 males prior to rescue and post rescue
Mexican wolf *Canis lupus*	F1	Yes	decreased litter size	increased litter size	Fredrickson et al. 2007	mixing strains was done experimentally at a zoo, and mixed strains had higher fitness then lone strains
			decreased pup survival	increased		*Ranges are reported from the three lineages
			lower litter size	increased		litter size increased from *n* = 3 to *n* =6
Bighorn sheep *Ova canadensis*	F2	Yes	decreased lamb weaning	increased by 2.2x	Hogg 2006	
			fewer lambs sired	increased by 2.6x	Miller 2012	

			shorter lifespan	increased		
Prairie Chicken	F6	Yes	lower fertility	increased	Bouzat 2009	*provide A values for other current populations A = 5.33-5.83, historic A for Illinois was 5.12
Tympanuchus cupido			lower hatching rate	increased	Westemeier 1998	alleles found in IL were also found in other populations, indicates bottle neck occurred after range expansion
Florida panther	F2	Yes	poor sperm quality	NA	Johnson 2010	
Puma concolor			kinked tails 90%	25%		
			cryptorchid 66%	10%		
			lower survivorship	increased by 3x		
			small range	range expansion		
			atrial deptal defects 17%	8%		
			cowlicks 81%	27%		
Eurasian otter	1966-1985:1993-1999	Not observed	N/A	NA	Arrendal 2004	Genetic samples only taken from road-killed individuals
Lutra						Given the length of sampling time, cannot comment of generation time
Bighorn sheep	F1, F2	Not observed	N/A	NA	Olson 2012	released n = 15, females only

Ova canadensis					Whitaker 2004	Was extirpated in Oregon in 1915, *n* = 20 were reintroduced from British Columbia in 1954 Samples collected from hunter kills
Bighorn sheep *Ova canadensis*	F1, F2	Not observed	N/A	NA	Olson 2012 Whitaker 2004	See above notes
South Island Robin *Petroica australis*	F1, F2	Yes	decreased juvenile survival 29% decreased recruitment 59% abnormal sperm 46% immunodeficiency 0.012 inability to find territory 40%	79% survival 95% recruitment 13% abnormal sperm 0.099 immune response 5% could not find territory	Heber 2017	These translocations where across isolated bottlenecked populations Both populations are the result of 5 founders each in 1973 (i.e. they were extirpated) Only females moved (*n* = 31) Immune response-swelling of wing web following immune challenge with phytohaemagglutinin (PHA)
South Island Robin *Petroica australis*	F1, F2	Yes	decreased juvenile survival 29% decreased recruitment 59%	79% survival 95% recruitment	Heber 2017	See above notes

			abnormal sperm 46% immunodeficiency 0.012 inability to find territory 40%	13% abnormal sperm 0.099 immune response 5% couldn't find territory		
White-spotted charr *Salvelinus leucomaenis*	F1	Not observed	N/A	NA	Yamamoto 2006	*n* = 20 females translocated above and below damn were genetically distinct prior, and not distinct post translocation
White-spotted charr *Salvelinus leucomaenis*	F1	Not observed	N/A	NA	Yamamoto 2006	see notes above

Figure 1. Relationship of multiple genetic diversity metrics to genetic distinctiveness (i.e., average population genetic diversity (F_{ST}*)) for northeastern timber rattlesnake populations. Genetic diversity metrics are: (a) mean allelic richness, (b) average number of alleles per locus, and (c) and expected heterozygosity. Solid lines represent least-square regression lines. All populations had >10 samples.*

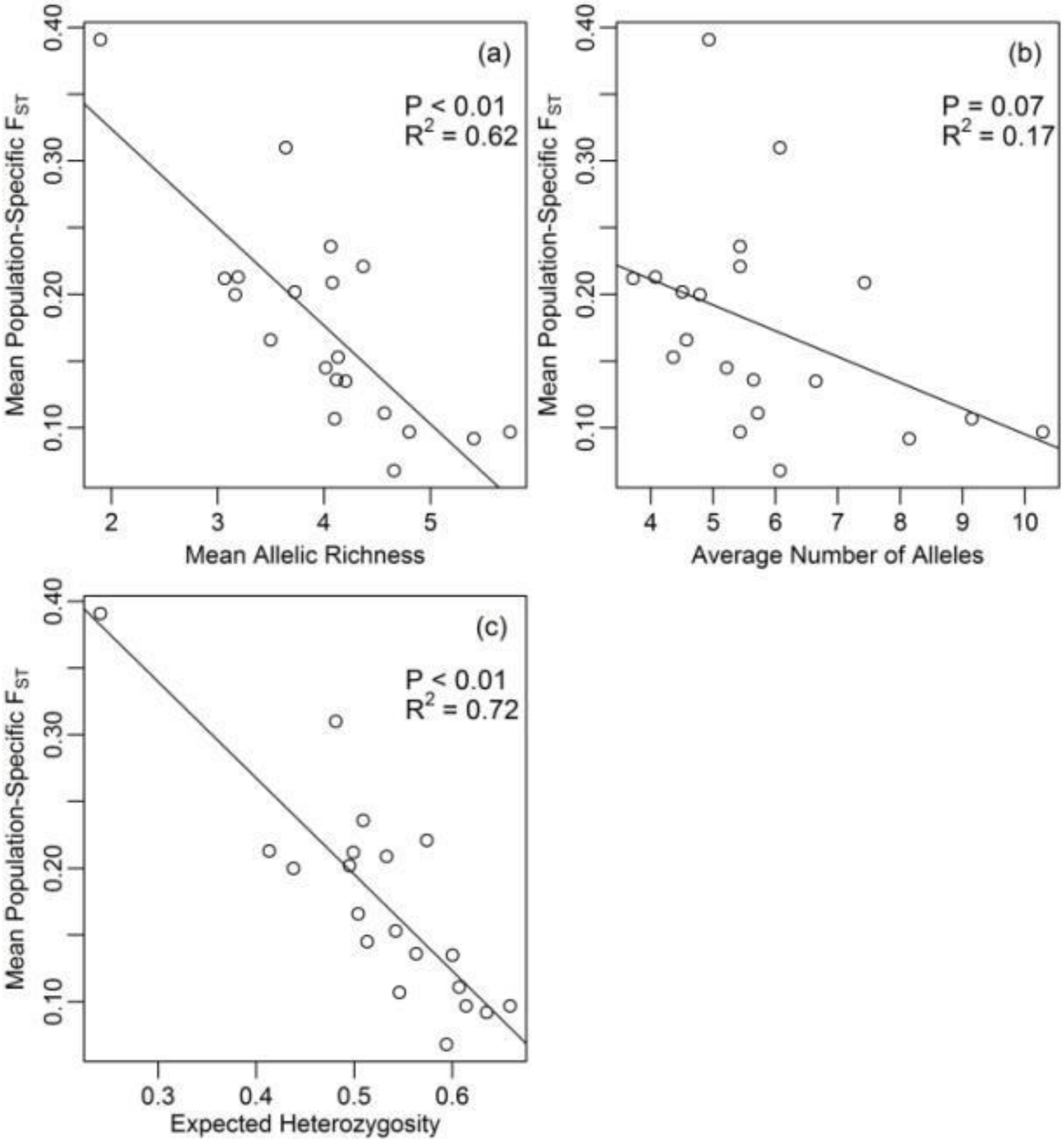


Figure 2. Relationship between genetic distance (F_{ST}/1-F_{ST}) and geographic (i.e., Euclidean) distance for all northeastern timber rattlesnake population pairs (n = 20). Filled circles indicate the New Hampshire population, the most genetically isolated population.

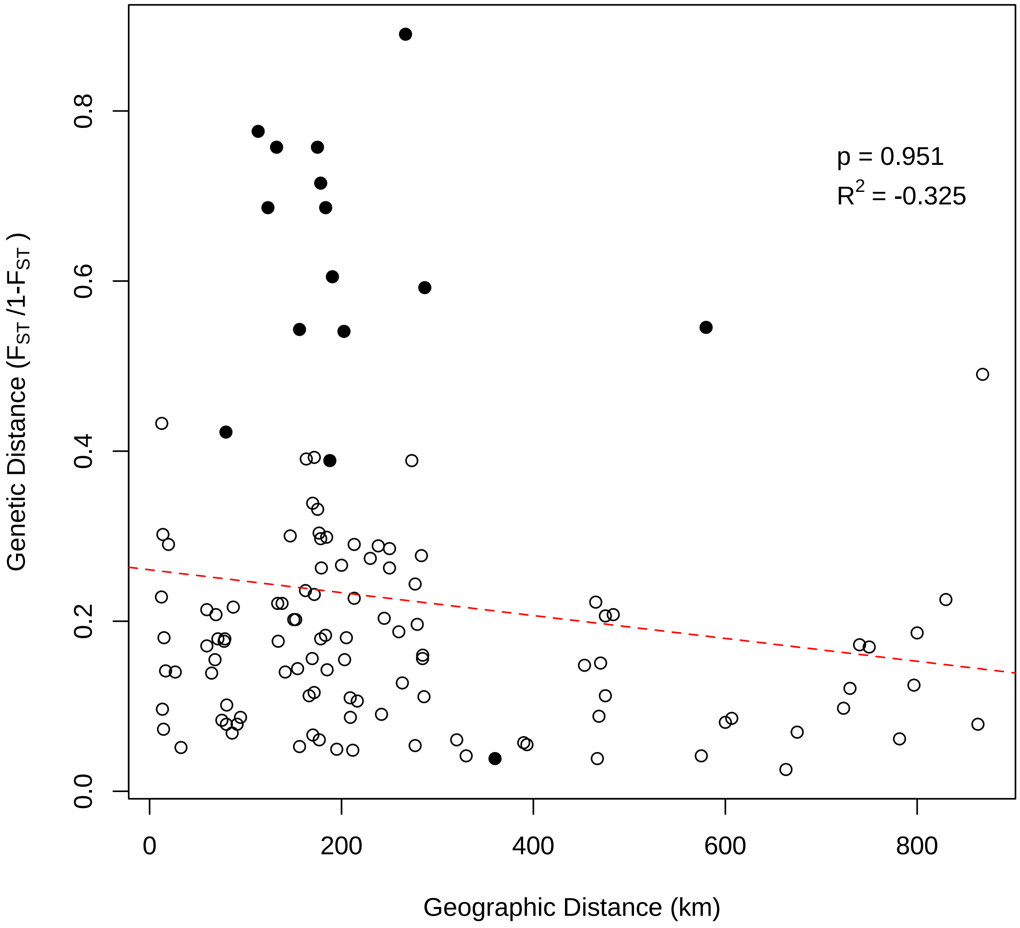


Figure 3. Relationship between log-likelihood probability and number of genetic clusters (K) for 20 timber rattlesnake populations across K = 1–20 using STRUCTURE.

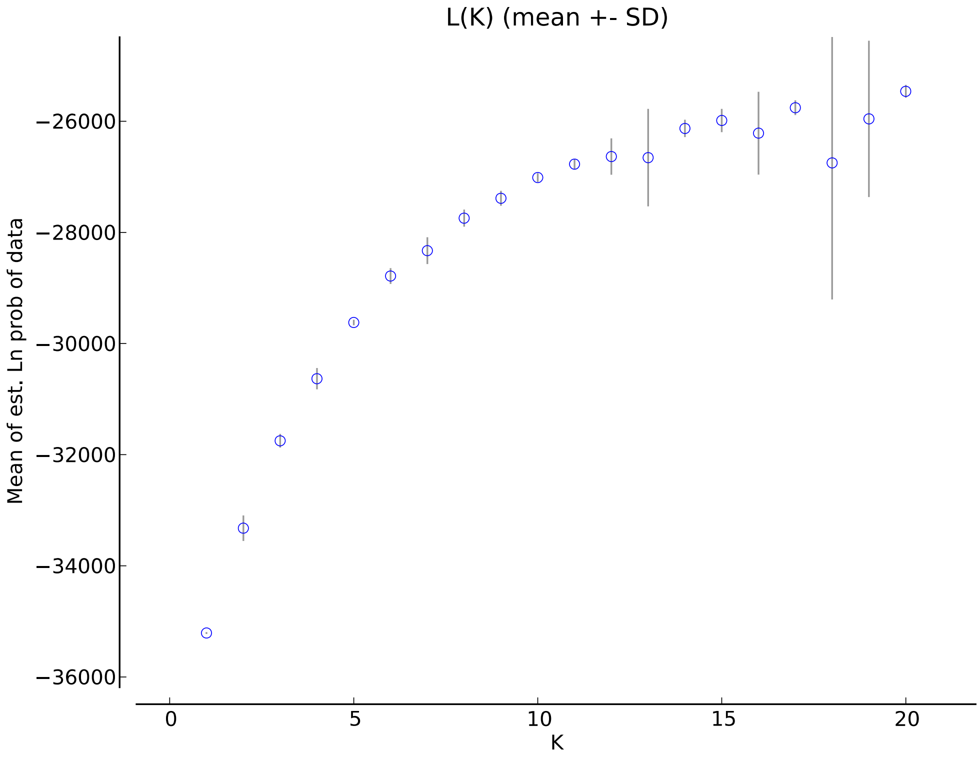


Figure 4. Proportion of the genome (Q) of each individual assigned by STRUCTURE to each cluster (K) across northeastern timber rattlesnake populations (n = 20). Each column corresponds to an individual and horizontal black bar separates sample locations. Each cluster (K) corresponds to a separate color. Results are shown for K = 5, 6, and 7.

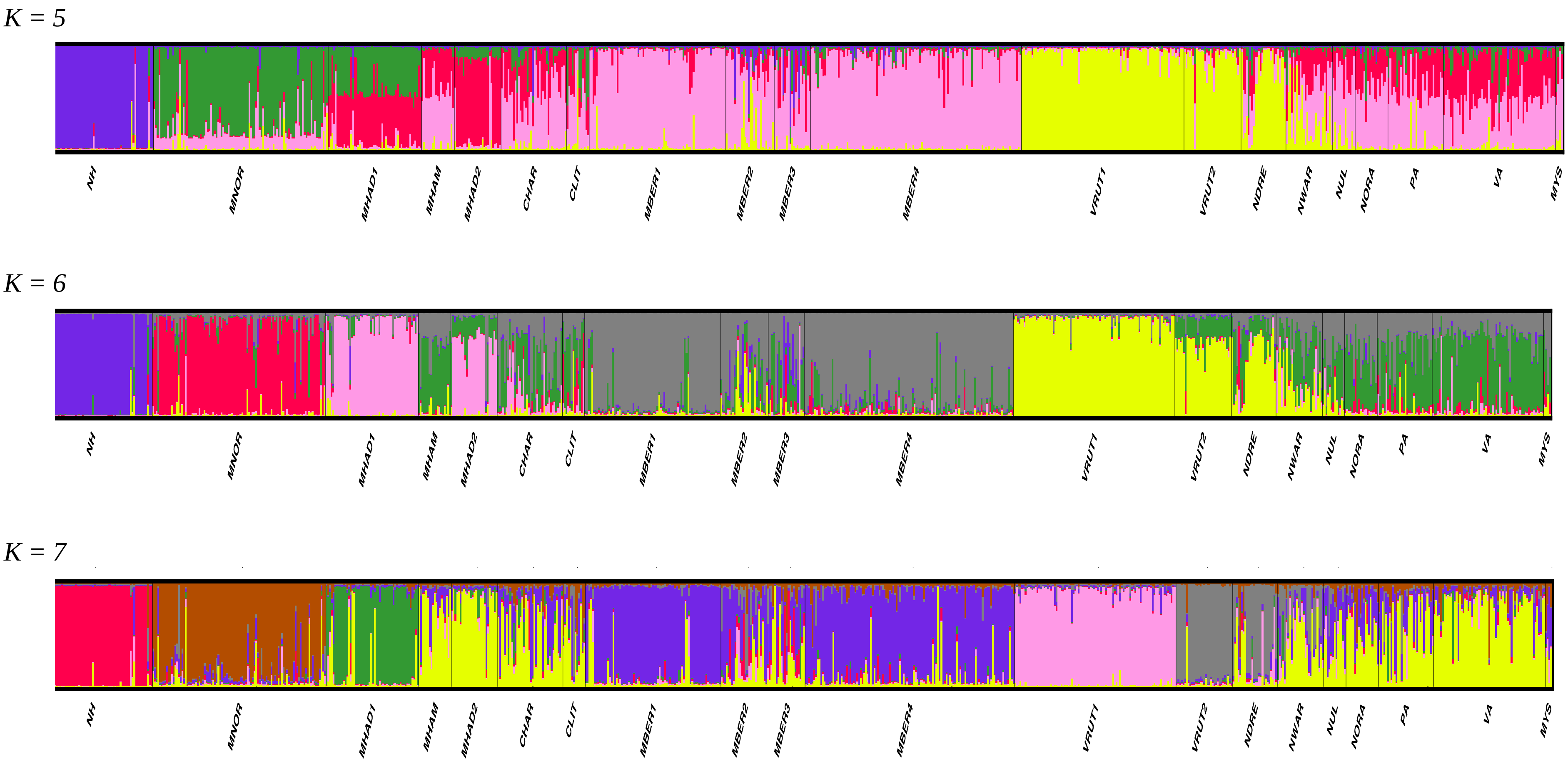


Figure 5. Relationship of Bayesian Information Criterion (BIC) to number of population clusters using discriminant analysis of principal components (DAPC) for K = 1 to 30 for 20 northeastern timber rattlesnake populations.

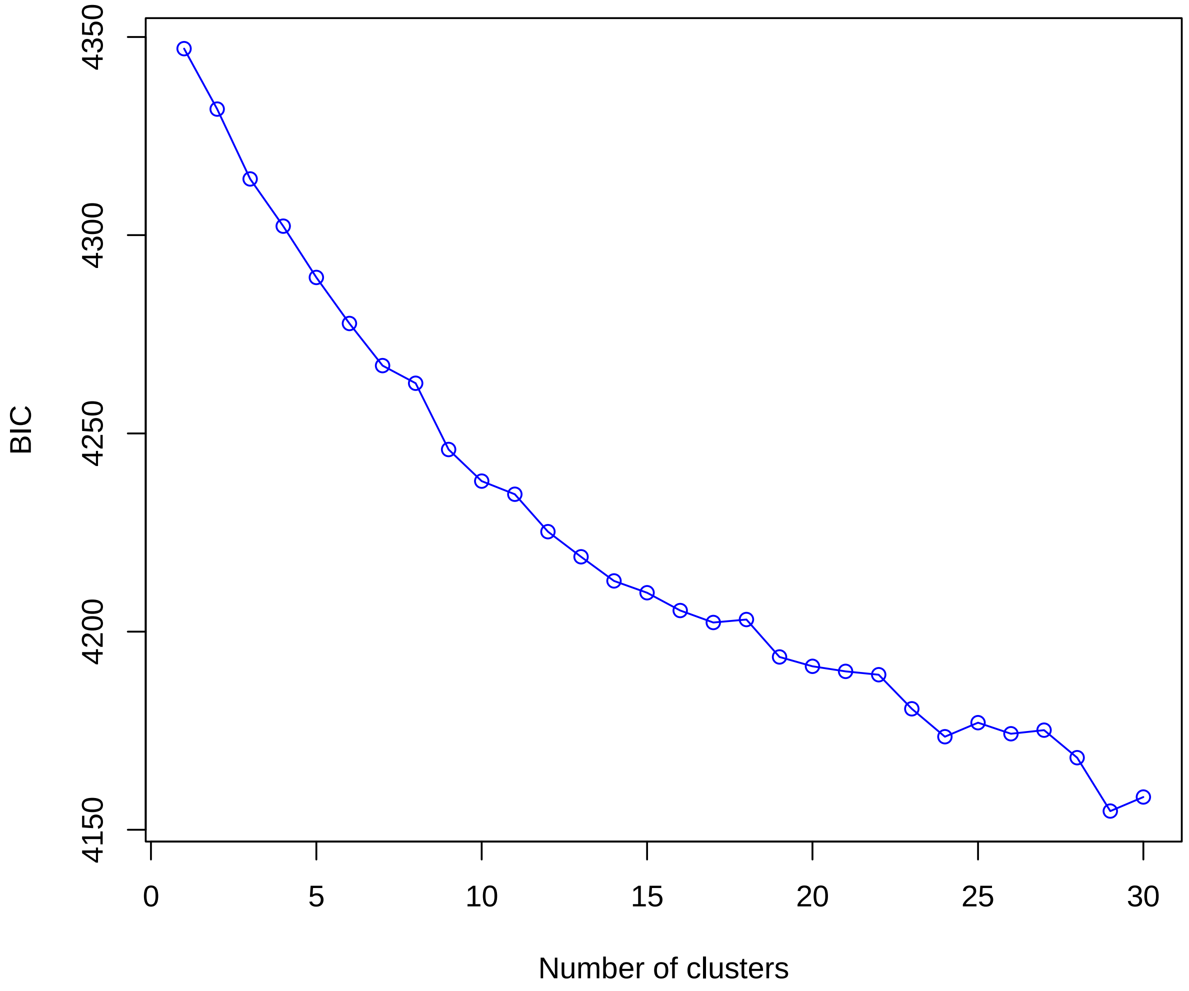


Figure 6. Discriminant analysis of principal components (DAPC) of the first two principal components for population clusters (K) for K = 7 across 20 populations of northeastern timber rattlesnake. Distinct single population clusters are labeled, with all other assigning equally in clusters 5 and 6.

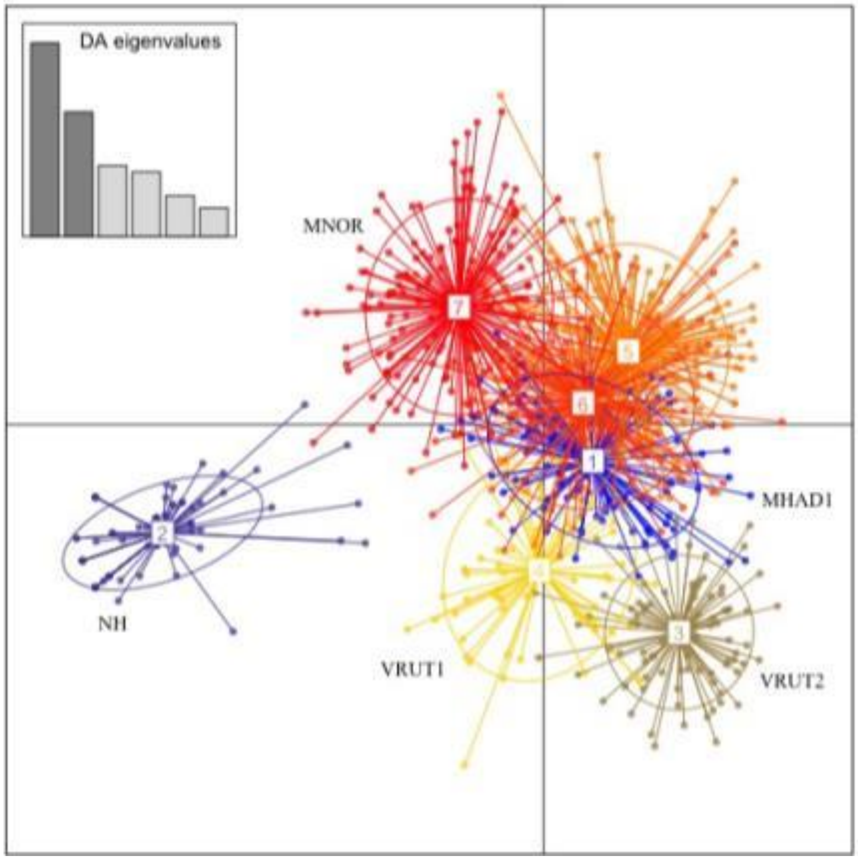
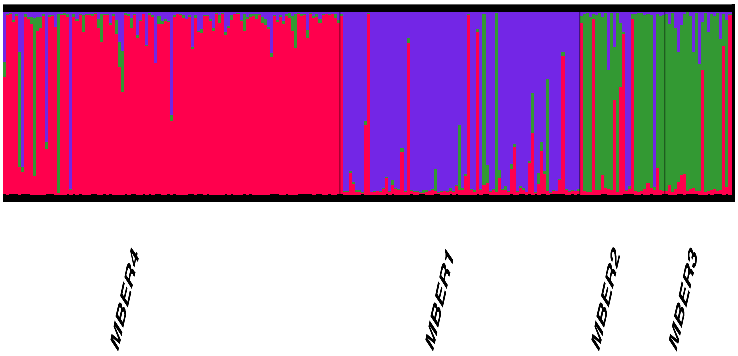
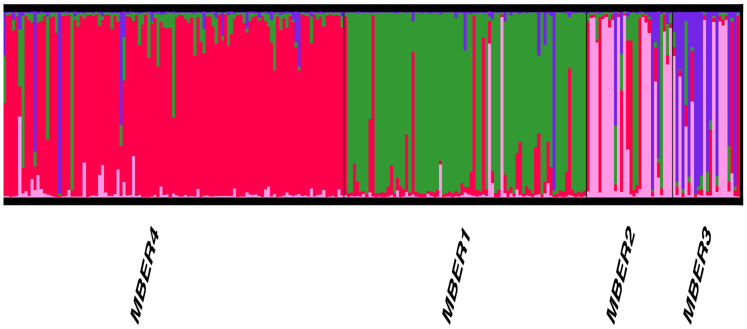


Figure 7. Proportion of the genome (Q) of each individual assigned by STRUCTURE to each cluster (K) among the four putative subpopulations within MBER. Each column corresponds to an individual and horizontal black bar separates sample locations. Each cluster (K) corresponds to a separate color. Results are shown for K = 5, 6, and 7.

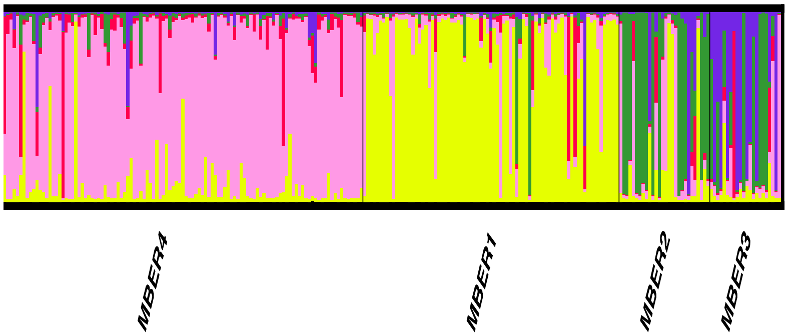


Q

K =3



Q
K = 4



Q
K = 5

Figure 8. Relationship between genetic distance ($F_{ST}/1-F_{ST}$) and geographic (i.e., Euclidean) distance for all northeastern timber rattlesnake population pairs (n = 20) excluding populations from Pennsylvania and Virginia.

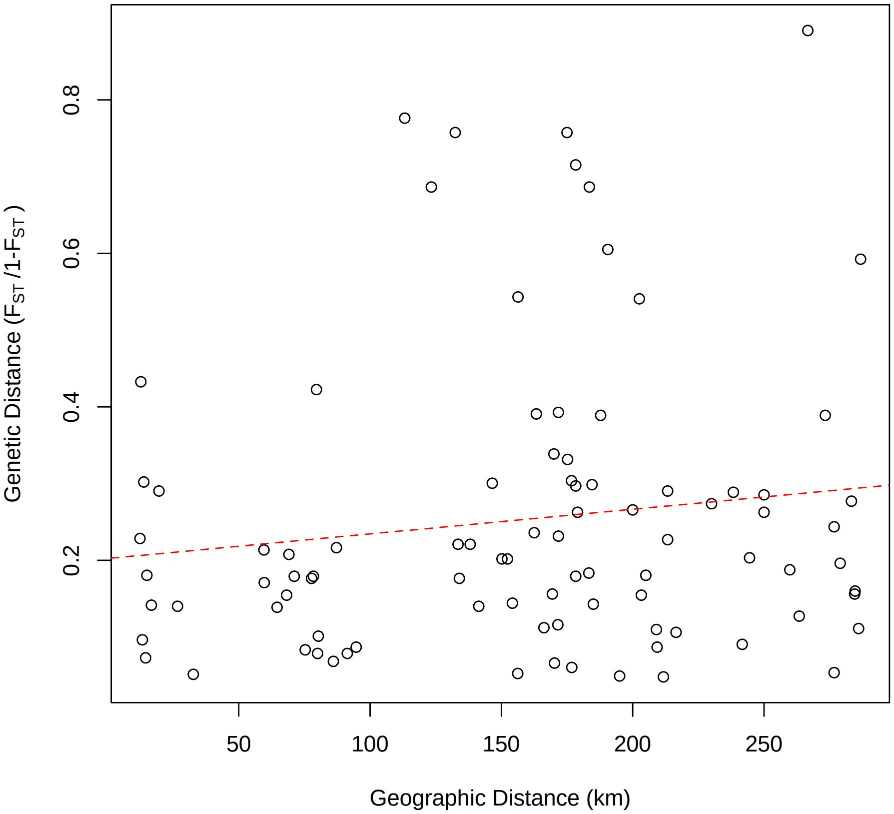


Figure 9. Relationship between genetic distance ($F_{ST}/1-F_{ST}$) and geographic (i.e., Euclidean) distance for all northeastern timber rattlesnake population pairs (n = 20) excluding populations from Pennsylvania, Virginia, and New Hampshire populations.

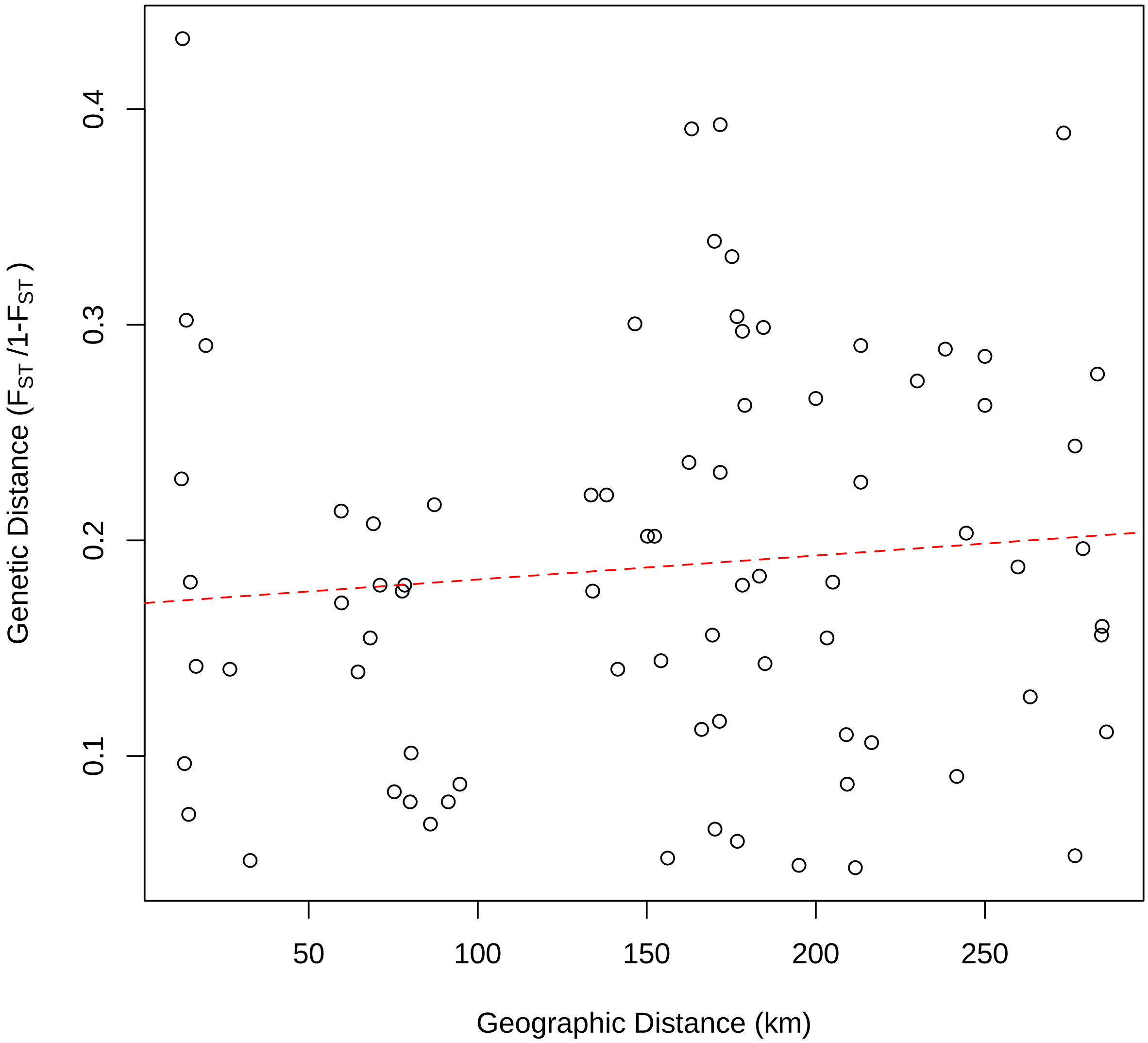
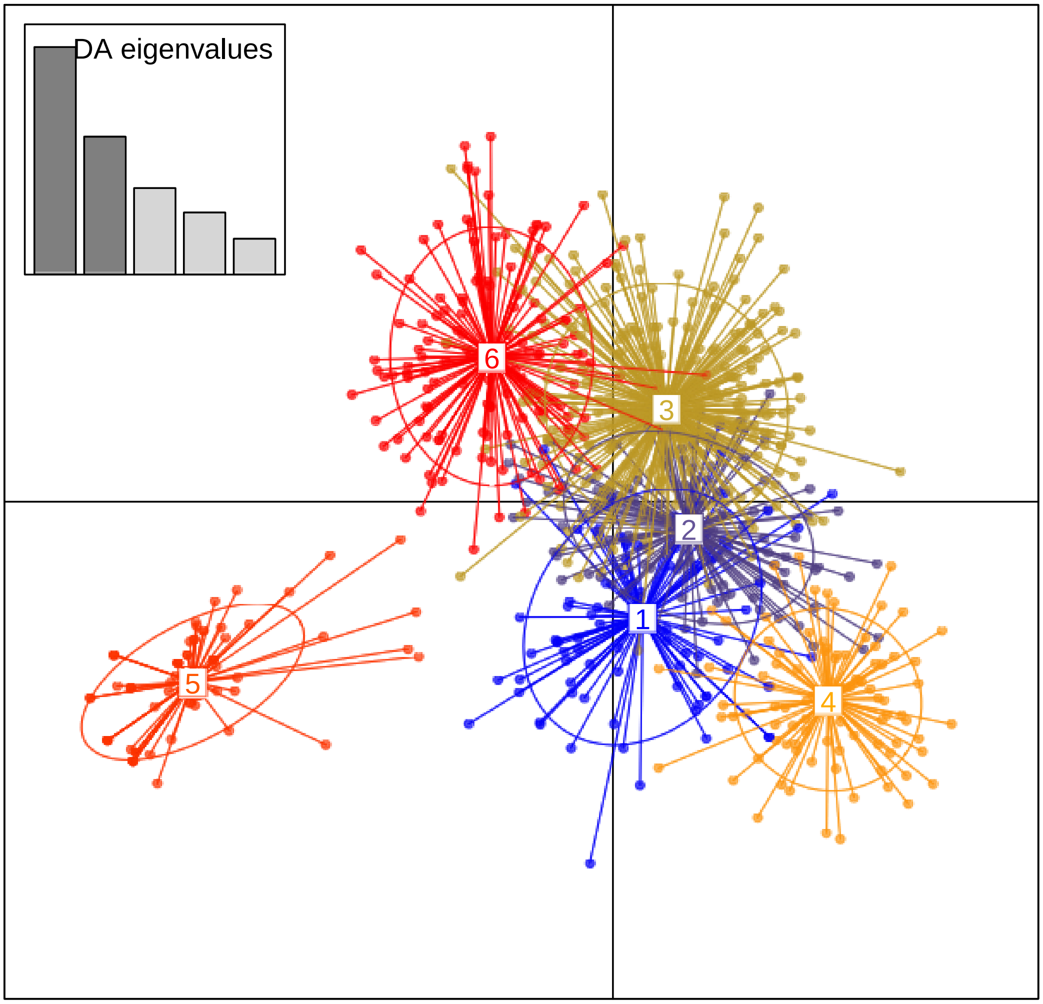


Figure 10. Discriminant analysis of principal components (DAPC) of the first two principal components for population clusters (K) for K = 7 across 20 populations of northeastern timber rattlesnake excluding populations in Pennsylvania and Virginia. Distinct single population clusters are labeled, with all other assigning equally in clusters 5 and 6.



Chapter 3—Long-Term Monitoring Protocols for Timber Rattlesnakes (*Crotalus horridus*) in New England

Abstract

Monitoring programs are essential for evaluating the status of wildlife populations over time. It is important to carefully consider the choice of state variable(s) for monitoring and to determine if a given study design will have sufficient statistical power to detect changes in those state variables over time. Within New England, the timber rattlesnake is a species of great conservation concern yet no region-wide monitoring protocol currently exists. Our goal was to evaluate multiple approaches for developing standardized region-wide monitoring protocols for timber rattlesnakes in New England. In particular, we use simulation studies to evaluate our ability to 1) estimate abundance from counts of unmarked individuals at the dens and 2) estimate occupancy and abundance of individuals at gestation and shedding sites. Our simulations showed that counts of unmarked individuals at the dens do not provide unbiased estimates of abundance. Moreover, statistical power to detect trends in occupancy and abundance at gestation and shedding sites was generally very low at most sampling designs we considered but was greater for abundance than occupancy. Maximizing the number of sites and detection rates provided the greatest increases in statistical power and we recommend pooling data among populations and regions when possible. Because temporary emigration will likely be present at gestation and shedding sites we recommend that managers collect data, at least initially, to estimate the degree of temporary emigration and understand the interpretation of their parameter estimates. We provide a detailed evaluation of the suitability of radio telemetry, mark-recapture, and gestation/shedding site occupancy and abundance for monitoring timber rattlesnakes. We recommend that managers utilize mark-recapture to monitor population status because it permits the estimation of multiple demographic parameters. However, mark-recapture may require a prohibitive level of effort to obtain sufficient recapture rates and mangers must consider the impacts to individual animals. In these situations, we recommend managers not rely primarily on den counts for monitoring but instead focus on monitoring occupancy and abundance of gestation and shedding sites. We provide specific recommendations for site delineation and selection, data collection, and analysis.

Introduction

Monitoring programs are a critical tool for assessing population trends in the face of anthropogenic threats to species and their ecosystems (e.g., Gauthier et al. 2013; Weller 2008). While monitoring programs are often designed to simply detect trends in state variables (e.g., occurrence or abundance), there is a recent emphasis in the integration of *a priori* hypotheses and their associated models into monitoring programs to determine the mechanisms behind temporal changes in these variables (Nichols & Williams 2006; Yoccoz et al. 2001). Identification of such factors can provide natural resource managers with information necessary to develop management actions to reverse or mitigate population declines.

While the development and implementation of wildlife monitoring studies involves numerous challenges, we highlight two in particular. The first is the choice of state variable(s) used to evaluate population trends. While the choice of state variable should ideally reflect specific study objectives, logistical and financial constraints may preclude the use of certain metrics. For example, obtaining estimates of abundance or indices of abundance using count data are often less costly and labor intensive than estimates of population size, abundance, or survival from mark-recapture or radio telemetry methods. A second challenge is designing a sampling design with sufficient statistical power to accurately identify trends in state variables and the factors influencing those trends. While simulations can provide valuable insights into optimal sampling designs and the tradeoffs therein, such efforts are rarely undertaken prior to initiating a monitoring study (Couturier et al. 2013; Sewell et al. 2012; Ward et al. 2017).

Within New England, the timber rattlesnake has undergone substantial declines and is now a species of conservation concern (Brown 1993; Martin et al. 2008). Timber rattlesnakes are extirpated from Maine and Rhode Island, critically imperiled in New Hampshire and Vermont, and imperiled in Massachusetts and Connecticut (Clark et al. 2011; Martin et al. 2008). The effects of habitat fragmentation and human persecution are exacerbated by life history constraints at high latitudes including long times to reproduction, long birthing intervals, and small litter sizes (Brown 1993, 2008; Martin 2002). Monitoring remaining populations is of paramount importance to identify not only future declines but potential recovery as the result of management and conservation efforts. However, there is currently no standardized region-wide monitoring protocol. Furthermore, no studies have evaluated different sampling designs to identify the efficiency or efficacy of those designs. Such efforts are particularly important for uncommon or imperiled species occurring at a limited number of sites which may limit sample sizes and make it difficult or impossible to statistically detect trends (Ward et al. 2017).

It is widely recognized in wildlife ecology that it is generally impossible to detect all individuals within a site and estimates of state variables must account for imperfect detection rates (Mackenzie et al. 2004; Pollock et al. 1990; Williams et al. 2002). The widespread development of hierarchical models and associated statistical software provides researchers with multiple approaches for estimated state variables of interest while accounting for imperfect detection and incorporating covariate effects (Kery & Royle 2016; MacKenzie et al. 2006). In particular, occupancy models (MacKenzie et al. 2003; MacKenzie et al. 2002) and N-mixture abundance models (Royle 2004) have received increased use in studies of snake ecology (e.g., Bauder et al. 2017; Sewell et al. 2012; Steen et al. 2012a; Steen et al. 2012b; Ward et al. 2017) and use presence-absence data and counts, respectively, of unmarked individuals making them attractive for low-impact, long-term monitoring. However, low detection rates may limit their utility for monitoring snake populations in many cases (Steen 2010; Steen et al. 2012a; Ward et al. 2017).

In this chapter, we discuss multiple approaches for developing standardized region-wide monitoring protocols for timber rattlesnakes. While radio telemetry and mark-recapture have received widespread use in timber rattlesnake research and monitoring (Brown 2008; Brown et al. 2007), less attention has been given to the use of presence-absence and count data to provide model-based estimates of occupancy and detection. We therefore conduct a simulation study to evaluate the use of model-based estimates of occupancy and abundance to monitor timber rattlesnake populations. We focus on two aspects of timber rattlesnake ecology that may hold potential for long-term monitoring: communal denning and gestation sites. We first use simulation studies to evaluate these models' ability to provide unbiased estimates of occupancy and abundance and identify optimal sampling designs. We then conclude with recommendations for standardized protocols for data collection and analysis for multiple methodologies including radio telemetry, mark-recapture, counts of unmarked individuals, and presence-absence surveys.

Methods

Description of Study Systems

Communal Denning

Throughout much of their range, including New England, timber rattlesnakes utilize communal hibernacula for overwintering (Brown 1993; Martin et al. 2008). Individuals are readily observed at communal hibernacula during the spring and fall and can easily be counted making abundance of individuals at the denning area a potentially useful state variable for monitoring. However, these systems are open populations which violate the key assumption of population closure in occupancy and abundance models (Kery & Royle 2016) although some models can accommodate temporary emigration (Chandler et al. 2011) or open populations (Dail & Madsen 2011). We therefore used simulation studies to address the following questions: 1) Can N-mixture abundance models provide unbiased estimates of timber rattlesnake abundance using den count data?; 2) What classes of N-mixture models provide unbiased estimates of abundance?; and 3) What sampling designs produce the most unbiased estimates of abundance?

Gestation and Shedding Sites

Gravid female rattlesnakes in many species maintain elevated and constant body temperatures to facilitate embryo development and seek open habitats for access to thermally suitable microhabitats (Brown 1993; Gardner-Santana & Beaupre 2009; Graves & Duvall 1993; Peterson et al. 1993). Within the northeastern United States, such habitats are generally restricted to open, rocky balds and ridges and likely represent a limiting resource, particularly in New England with its forested landscapes and relatively short growing season (Brown 1993; Martin 2002; Martin et al. 2008). As a result, gravid females regularly congregate at gestation sites making them relatively easy to observe and count. Males and non-gravid females may also utilize similar microhabitats for shedding (i.e., ecdysis) during the spring and summer (Brown 1993; Stengle 2018). Timber rattlesnakes in the northeast may predominately shed in June (Aldridge & Brown 1995; Stengle 2018) although shedding may also occur throughout the activity season in different microhabitats (J.M.B & D.B., unpublished data). Because gestation sites are relatively distinct and discrete habitat features, it is possible to delineate these features as sampling sites (e.g., a single rocky bald) and monitor occupancy and abundance. Importantly, the assumption of population closure or temporary emigration at these sites can likely be assumed if surveys are conducted over sufficiently short durations during the appropriate times of year. We therefore used simulation studies to address the following questions: 1) What sampling designs provide the most unbiased and precise estimates of occupancy and abundance?; 2) What sampling designs provide the greatest statistical power to detect declines in occupancy and abundance over time?

Simulation Study Design

Den Count Simulations

We developed a novel approach to simulate counts of rattlesnakes at multiple dens (i.e., hibernacula) during spring egress or fall ingress. Our model simulates the emergence of individuals who then remained at the den for some number of days before leaving the sampling area and becoming unavailable for detection. We loosely based our model on the Poisson-binomial N-mixture model (Royle 2004) by simulating the total number of individuals at each den (N) according to a Poisson distribution with mean = λ and the probability of detecting an individual according to binomial distribution with mean = p. We considered N analogous to an estimate of superpopulation size (Kendall et al. 1997) as it represents the number of individuals that were on the surface in the denning area over the course of the egress/ingress period. We note that this model does not directly estimate N for each den but these values can be derived for each den from the Poisson distribution of N's with mean = λ (Kery & Royle 2016).

After simulating the total number of individuals at each den, we then defined a probability function describing the probability of an individual emerging at any date during the defined survey period. We used a beta distribution to create this function because it can take on a variety of shapes and skews while being constrained between zero and one (Fig. 1A, 1B). We used the function to determine the number of snakes emerging on each date during the survey period. We then defined a residency time which was the number of days an individual would remain at the den and available for detection after emerging. We then calculated the number of individuals available for detection on each day of the survey period for each site. Longer residency times increased the proportion of the population available for detection on any given day (Fig. 1C-1F). Finally, we determined the number of individuals counted at each visit as a binomial draw with trial size equal to the number of individuals available for detection and *p* as the individual detection rate.

We created a full-factorial simulation experiment to assess our ability to reliably estimate *N* using den count data collected under different sampling intensities. We describe our experimental design to represent surveys conducted during spring egress although the design could also represent fall ingress. Because we lacked precise data on model parameter values for our New England populations we select values to span a range of plausible values. We varied the number of sites (10, 30, 100), number of visits per site (3, 6, 9), detection probability (0.25, 0.75, 0.90), residency time (2, 5, and 15 days), and the parameters of the beta distribution (weak peak: a = 1, b = 1.25, strong peak: a = 2, b = 3, Fig 1). We note that existing data suggests a peaked pattern of egress/ingress (Brown 2008, J.M.B. & D.B., unpublished data) analogous to our strong peak scenario but we include the weak peak scenario to test the hypotheses that a longer egress/ingress period would make more individuals available for detection and lead to more accurate estimates of abundance. We evaluated a relatively small number of sites (10 and 30) because the number of potential dens within each New England state may approximate these values. We selected a 60 day emergence period based on observed patterns (e.g., most egress occurs in April and May, Brown 2008) and systematically placed visits across the emergence period. We acknowledge that a more efficient design would be to concentrate visits during periods of peak egress/ingress. However, the peak period may not be known *a priori* and/or may vary from year-to-year. We therefore considered it likely that some visits would occur prior or subsequent to peak emergence. We specified $\lambda = 100$ and created 100 replicate data sets under each parameter combination. All simulation and analyses were conducted using R (R Core Team 2017) unless otherwise noted.

We analyzed our simulated data using three different N-mixture models representing plausible survey protocols for timber rattlesnake den counts. The first was the binomial N-mixture model of Royle (2004). This model estimates abundance using spatially replicated count data and assumes population closure during the sampling period although random emigration/immigration can change the interpretation of λ to the number of individuals ever associated with a site (i.e., the superpopulation, Chandler et al. 2011; Kendall et al. 1997; Kery &

Royle 2016). We fit these models using the *pcount* function in R package *unmarked* (v. 01.12-2, Fiske & Chandler 2011) and refer to them hereafter as the *pcount* model.

The second model was the temporary emigration model of Chandler (2011). This model expands upon the binomial N-mixture model of Royle (2004) by explicitly modeling temporary emigration using an additional parameter, Φ, describing the probability of an individual being present at the survey site. The temporary emigration model requires both primary and secondary sampling periods where population closure is assumed during the secondary visits. To meet this assumption within our simulated data, we envisioned a design wherein a single observer conducted two visits at each site on a single day. We assumed that the number of individuals available for detection would not change within a day. Within our simulation, we conducted two binomial draws at each visit from the number of individuals available for detection. We fit these models using the *gpcount* function in *unmarked* and refer to them hereafter as *gpcount* models. The third model also used the temporary emigration model but the observed data represented multinomial outcomes rather than binomial outcomes. Such data can be obtained using double-observer survey methods (Nichols et al. 2000; Nichols et al. 2009). Two forms of double-observer methods are available. The first assumes observers independently record their observations (i.e., the independent double-observer survey). We thought observer independence would be impractical while surveying timber rattlesnakes because rattlesnakes often rattle in response to the presence of an observer thereby violating the assumption of independence between observers. We therefore simulated data according to a dependent double-observer survey. Under this design, the primary observer records individuals they detect and the secondary observer records all individuals detected by the primary observer as well as any missed by the primary observer. The observation process of these data is modeled using a multinomial distribution which can be fit using the *gmultmix* function in the *unmarked* package. This model also estimates Φ and we constrained p to be equal between observers. We refer to these models as the *gmultmix* models. Examples of our simulation code are available in Appendix 1.

We fit multiple candidate models to each type of model. In the *pcount* models, we either held p constant (i.e., $p(.)$) or modeled p as a linear, categorical, or quadratic function of visit date. For the *gpcount* and *gmultmix* models, we held p constant and either held Φ constant or modeled

Φ as a linear, categorical, or quadratic function of visit date while holding p constant in all cases. We calculated the percent bias of λ and p relative to the true parameter values.

Occupancy Simulations

We simulated occupancy data across a range of scenarios to identify optimal sampling designs for estimating occupancy of timber rattlesnake gestation and shedding sites and changes in occupancy over time. We simulated our data according to a hierarchical model where occupancy (ψ) and detection (p) represent separated Bernoulli processes with probability equal to ψ and p, respectively. Note that in an occupancy framework, p now represents the probability of detecting any individual at a site conditional upon the species being present. We simulated data under three scenarios representing different plausible patterns of occupancy. The first was a constant scenario (Null) where ψ and p were held constant. The second scenario (Trend) included a linear decline in ψ over a time. The final scenario (Trend+Site) combined a linear decline in ψ over time with a two-level categorical covariate (Site) denoting high- and low-occupancy sites. Occupancy in the low-occupancy sites was 67% of ψ for the high-occupancy sites and is designed to represent additional heterogeneity in ψ.

For each true model we simulated data across a range of sites (3, 5, 10, 20, 30, 50), number of visits (2, 4, 6, 8, 10), ψ values (0.25, 0.50, 0.75, 0.95), and p values (0.25, 0.50, 0.75, 0.95). We explored a wider range of sampling scenarios than our den count simulations our occupancy models were quicker to fit. We simulated 5%, 10%, and 20% declines in ψ over 5 and 10 year durations. For a given decline (T) and duration (D), we calculated the change per-year decline in occupancy (i.e., slope, b) such that ψ at the final year (t_D) was equal to $\psi_{1-(}\psi_1*T)$ and simulated site occupancy at each subsequent time period (t_2, …, t_D) with $\psi_1+(b*t-1)$. So if $\psi_1 = 0.80$, $T = 20\%$, $D = 10$, then $b = -0.01778$ and $\psi_2 = 0.7822$ and $\psi_D = 0.64$. For each year, we specified the occupancy status (0 or 1) for each site using a new random binomial draw with $p = \psi_t$. We conducted separate binomial draws for high- and low-occupancy sites. Note that we did not specific the true values of the estimate covariate (i.e., beta estimate) effect of year on the logit scale. We simulated 500 replicate data sets under each scenario.

We fit single-season occupancy models to our data using the *occu* function in the *unmarked* package. We fit a null (i.e., $\psi(.)p(.)$) model for each scenario and a model with a site effect on ψ for the Trend+Site scenario. For the Trend scenarios, we “stacked” each year’s data into a $m*n$ matrix where m = number of sites*duration and n = number of visits (Bauder et al. 2017; Miller et al. 2013). We then fit a model with year (e.g., 1, 2, …, D) as a continuous covariate to estimate the trend, as well as a model with year as a categorical covariate. Examples of our simulation code are available in Appendix 1.

We evaluated the performance of these models using different metrics. We calculated root-mean-squared-error (RMSE) as an overall measure of bias and precision. For the Null scenario, we calculated RMSE between the true and estimated value of ψ. For the Trend and Trend+Site scenarios we calculated RMSE between the true percent decline and the estimated percent decline. We calculated the estimated percent decline as (plogis(β_0) – plogis($\beta_0 + \beta_{Year}*D$))/plogis(β_0) where β_0 = the estimated intercept and β_{Year} = the per year change in ψ on the logit scale. Because we calculated percent bias relative to the intercept, our estimates of percent bias were biased slightly high (approximately 10% depending on the percent decline and duration) relative to the true simulated percent decline. We also compared models fit to data from the Trend and Trend+Site scenarios using AIC adjusted for small sample size (AIC_c) and calculated AIC_c model weights (w) which represent the probability that a given model is the correct model amongst all candidate models for that data set (Burnham & Anderson 2002). Finally, we calculated statistical power for detecting a significant effect of year as the proportion of significant P values for these covariates. We used $\alpha = 0.05$.

Abundance Simulations

We simulated abundance data across a range of scenarios to identify optimal sampling designs for estimating abundance of timber rattlesnakes at gestation and shedding sites and changes in abundance over time. We simulated data using the Poisson-binomial N-mixture model of Royle (2004) by simulating the total number of individuals at each site (N) according to a Poisson distribution with mean = λ and the probability of detecting an individual according to binomial distribution with mean = p.

We simulated abundance data under two scenarios. In the first scenario (Site), sites were divided into two equal groups where λ in one group was twice that of the other group. This scenario was meant to approximate a system with high- and low-quality sites that differ markedly in abundance. The second (Trend) included a linear decline in λ. We again “stacked” the Trend data as described above.

For each true model we simulated data across a range of values for λ (6, 10, 20), sites (3, 5, 10, 20, 30), number of visits (2, 4, 6, 8, 10), and p values (0.25, 0.50, 0.75, 0.95). We simulated 5%, 10%, and 20% declines in λ over 5 and 10 year durations. However, we specified that the decline would not start on year 2 but rather on year 3 (10% decline over 5 years), year 4 (5% over 5 years), year 3 (20% decline over 10 years), year 4 (10% over 10 years), and year 5 (5% over 10 years). As a result, the decline in λ for 10 year durations was not truly a continuous decline across the entire duration and the beta estimate for linear effect of year on λ will indicate a slightly greater rate of decline. Under this design, the Trend model is still the true model even though the function representing that trend is not linear. For a given decline (T) and duration (D), we calculated the change per-year decline in λ (i.e., slope, b) such that λ at the final year (t_D) was equal to $\lambda_{1-(}\ \lambda_1*T)$ and simulated abundance at each subsequent time period ($t_2, \ldots, t_D$) with $\lambda_1+(b*t-1)$. So if $\lambda_1 = \lambda_2 = 10$, $T = 20\%$, $D = 10$ years and the decline starts at year 3, then $b \approx -0.3865$ and $\lambda_3 \approx 9.6135$ and $\lambda_D = 8$. For each year, we specified abundance for each site using a new random Poisson draw with $\lambda = \lambda_t$. We conducted separate Poisson draws for high- and low-abundance sites in the Site scenarios. We simulated 500 replicate data sets under each scenario. Examples of our simulation code are available in Appendix 1.

We fit modeled each data set using the *pcount* function in *unmarked*. We fit a null (i.e., $\lambda(.)$) model as well as a trend model to the Trend data and compared model support using AICc model weights (w). We also calculated the statistical power to detect a significant effect of year as described above using $\alpha = 0.05$. We calculated RMSE for the Site model using the estimated value of λ for both the low- and high-abundance sites and for the Trend model using the estimated amount of decline. We calculated the estimated amount of decline as $(\exp(\beta_0) - \exp(\beta_0 + \beta_{\mathrm{Year}}*D))/\exp(\beta_0)$ where β_0 = the estimated intercept and β_{Year} = the per year change in λ on the log scale. We also report the percent bias in the estimated amount of decline relative to D. Because our simulated decline did not start on year 2 the estimated amount of decline calculated in this manner will overestimate the amount of decline relative to the true amount of decline (i.e., 5%, 10%, or 20%). However, because we used the same “true” values within a given scenario, the bias for a given amount of decline and duration is consistent and RMSE values within a scenario can be directly compared.

Observed Use of Gestation Sites

To help determine the extent to which the assumption of site closure is met with regards to gestation sites, we examined data from radio-tracked gravid females in three New England populations. Specifically, we noted the dates during which they utilized gestation sites and the extent of their movements during these times. We used radio-telemetry data from a population in Vermont (VRUT2), New Hampshire (NH), and western Massachusetts (MABER). Details of study sites and data collection procedures are provided in Chapter 1.

Results

Den Count Simulations

All model types produced biased estimates of λ using simulated den count data regardless of whether *p* or Φ were held constant or allowed to vary by visit date. We illustrate the results from the data simulated using a strong peak in probability of emergence and two day residency time (Fig. 2) and present the results of other simulation scenarios in Appendix 2 (Fig. S1-S6).

Estimates of lambda were predominately biased high and imprecise. While the estimates of λ often spanned the true value of λ, particularly for the *gpcount* and *gmultmix* models where Φ was modeled as a non-linear function of survey date, these estimates varied widely in their precision. For example, while the mean percent bias for λ with the *gpcount* model with a quadratic effect of visit date on Φ with 100 sites, 6 visits, and $p = 0.25$ was -1.57% the percent bias ranged from -76.5–45.5%. We note in particular that under the most optimal survey designs (i.e., the largest number of sites and visits and highest detection rates), estimates of λ were very precise but biased high (Fig. 2).

The *gpcount* and *gmultmix* models generally reliably estimated *p*. We again illustrate the results from the data simulated using a strong peak in probability of emergence and two day residency time (Fig. 3, see also Fig. S4-S6). The most unbiased estimates of *p* were when Φ was modeled either as a constant or as a non-linear function in visit date. At the lowest number of sites (10), precision in *p* appeared most sensitive to the true value of *p* rather than the number of visits. The *pcount* models consistently underestimated *p*.

Occupancy Simulations

Null Scenario

Under the Null scenario, RMSE almost always decreased with increasing numbers of sites (Fig. 4). We suspect that any exceptions to these trends are due to random variation in our simulated data which would like disappear with additional replications. While RMSE generally decreased with increasing numbers of visits, this relationship was more complex. The rate at which RMSE decreased with increasing numbers of visits was generally greatest when ψ and p was ≤ 0.50. For example, when occupancy and detection were low (i.e., $\psi = 0.25$ and $p = 0.25$), increasing the number of visits from two to four with 5 sites resulted in a 14% decrease in RMSE while increasing the number of visits from six to eight reduced RMSE by 15%. Similarly, increasing the number of visits from two to four with 30 sites resulted in a 47% decrease in RMSE while increasing the number of visits from six to eight reduced RMSE by 34%.

In contrast, when ψ or p were ≥ 0.75 increasing the numbers of visits from two to four generally reduced RMSE more than increasing the number of visits beyond four. For example, when $\psi = 0.75$ and $p = 0.25$, increasing the number of surveys from two to four with 5 sites resulted in a 4% decrease in RMSE while increasing the number of surveys from six to eight reduced RMSE by 7%. In contrast, increasing the number of surveys from two to four with 30 sites resulted in a 34% decrease in RMSE while increasing the number of surveys from six to eight reduced RMSE by 6%.

Increasing p reduced RMSE and also reduced the benefits of increasing number of visits on RMSE reduction, particularly at low or moderate ψ. For example, when $\psi = 0.25$ and $p = 0.75$, increasing the number of surveys from two to four with 5 sites resulted in a 47% decrease in RMSE while increasing the number of surveys from six to eight reduced RMSE by 4%. Similarly, increasing the number of surveys from two to four with 30 sites resulted in a 31% decrease in RMSE while increasing the number of surveys from six to eight reduced RMSE by 0.3%.

Variation in the bias and precision of estimated occupancy followed similar patterns as those for RMSE (Fig. 5). Estimates were generally biased with 3–5 sites particularly when ψ and/or p were low (e.g., generally ≤ 0.50). Estimates were most precise with ≥ 20 sites and when both ψ and p were ≥0.50. Similar to RMSE, bias and precision were relatively insensitive to the number of visits.

Trend and Trend+Site Scenarios

Overall patterns were very similar between data simulated under the Trend and the Trend+Site scenarios so we focus on results from the Trend scenario. Under the Trend scenario and the simulation parameters we considered, there was little evidence that we could correctly identify a decline in occupancy. A total of 2,848 out of 2,880 parameter combinations had convergence rates ≥50%. Of the 32 parameter combinations with convergence issues, 27 were with $\psi = 0.95$ and the remainders were with $\psi = 0.75$ and $p = 0.25$.

Of the parameter combinations without convergence issues, 160 (5.6%) had $w \geq 0.50$ for the true Trend model. Of these 160, 24 (15%) were with declines ≤10%, 37 (23%) were with ≤20 sites, and three (1.9%) were with declines ≤10% and ≤20 sites. Most ($n = 85$, 53%) were with 20% declines over 10 years or 20% declines over 5 years ($n = 51$, 32%). The inter-quartile range of the median AIC$_c$ w was 0.26–0.32 for the true Trend model compared to 0.62–0.71 for the null model. At all trend and duration values except the 20% decline, AIC$_c$ w for the true Trend model was relatively invariant with respect to numbers of sites and visits and values of ψ and p (Fig. 6, Fig. S7–S9). At 20% declines, AIC$_c$ w for the true Trend model was greatest with increased numbers of sites, ψ, and, to a lesser extent, p but was comparatively insensitive to number of visits (Fig. 6 and Fig. S7–S9). This trend was most pronounced at moderately-high occupancy ($\psi = 0.75$) and gains in AIC$_c$ w for the true Trend model from increasing numbers of visits were greater. At very high occupancy ($\psi = 0.95$) patterns in model selection were more variable at greater numbers of sites, visits, and degree of decline but otherwise were generally consistent with these trends (Fig. S9).

Statistical power to detect a decline was low with an inter-quartile range of 1.4–7.1% and was ≥80% in 15 scenarios, all including 20% declines and 50 sites. Statistical power was ≥50% in 41 scenarios all including 20% declines with 30 or 50 sites. For these 30-site scenarios, all had $\psi = 0.95$ ($p = 0.25$–0.75) and for the 50-site scenarios, six had $\psi = 0.75$ ($p = 0.75$–0.95) and remainder had 0.95 ($p = 0.25$–0.95). Statistical power showed a similar pattern to AIC$_c$ w in that power was maximized by increasing the number of sites and at higher occupancy and detection while power was relatively invariant to the number of visits (Fig. 7 and Fig. S10–S13).

When ψ = 0.25 and *p* = 0.50 or 0.75, RMSE only showed a clear response to increasing the number of sites. When ψ was ≥0.75 and *p* = 0.25 patterns of RMSE were more variable than when *p* = 0.75 and showed no distinct pattern although increasing the number of sites and visits tended towards lower RMSE (Fig. S14). When ψ was ≥0.75 and *p* = 0.75 increasing the number of sites clearly reduced RMSE and increasing the number of visits up to four or six also reduced RMSE (Fig. 8). The effect of number of visits was strongest when the decline occurred over 10 years compared to 5 years.

Patterns of bias and precision in the estimated percent decline in ψ showed similar patterns as seen with regards to model selection, statistical power, and RMSE. Estimates of the percent decline in ψ were highly imprecise at all but the most severe declines and the largest number of sites (Fig. 9 and Fig. S15–S17). Estimates of the percent decline in ψ where generally biased (Table 1) but the degree of bias was generally an order of magnitude or more less than the precision of the estimates.

These patterns were largely consistent with those observed for the Trend+Site scenario. A total of 2,819 out of 2,880 parameter combinations had convergence rates ≥50%. Of the 61 parameter combinations with convergence issues, 51 where with *p* = 0.25 (ψ = 0.25–0.95) and the remainders were with *p* = 0.50 and two site visits. A total of 35 parameter combinations with convergence issues occurred with two site visits.

Of the parameter combinations without convergence issues, 75 (2.7%) had *w* ≥0.50 for the true Trend+Site model. Of these 75, one (1.3%) had a decline <20% (5%), 12 (16%) were with ≤20 sites, and one had a decline ≤10% and ≤20 sites. Most were with a 20% decline over 10 years (*n* = 61, 81%) or 5 years (*n* = 13, 17%). The model with only a linear trend effect of time (Trend) never had *w* >0.50 and the maximum median *w* was 0.25. The inter-quartile range of the median AICc *w* was 0.11–0.27 for the true Trend+Site model compared to 0.03–0.38 for the null model, 0.02–0.17 for the Trend model, and 0.22–0.48 for the Site model. Statistical power to detect a decline was similarly low with an inter-quartile range of 3.0–8.0%. Maximum power to detect a decline was 78% and was ≥50% in 39 scenarios, all including 20% declines and 30 (*n* = 12, 31%) or 50 (*n* = 27, 69%) sites. All 39 scenarios all had ψ = 0.95 and was *p* = 0.50–0.95 for the 12 30-site scenarios. Patterns in AIC_c *w*, statistical power, and RMSE for the true Trend+Site model with regards to the duration and degree of decline, sampling intensity, and ψ and *p* followed similar patterns as described for the Trend scenario and are not reported.

Abundance Simulations

Site Scenario

Under the Site scenario, only four of 625 parameter combinations had convergence rates < 50% for the *pcount* model, all with $p = 0.95$. Of the remaining 621 combinations, 452 (73%) had $w \geq 0.50$ for the true Site model.

Across all values of lambda and p we considered, RMSE decreased with increasing numbers of sites and increasing p (Fig. 10 and Fig. S18). Surveying 20 or 30 sites resulted in very similar RMSE values. In contrast, the effect of number of visits had less of an effect on RMSE and this effect was generally greatest at four or six visits. For example, RMSE for $\lambda = 4$ and $p = 0.50$, and 10 sites decreased from 1.79 to 1.39 (22%) when going from two to eight visits (20 to 80 surveys) compared to an decrease from 1.56 to 1.40 (10%) when going from ten sites and four visits to 20 sites and two visits (both 40 surveys total).

The relative gains in RMSE reduction by increasing the number of sites and p appeared to vary interactively depending on the true value of λ (Table 2). When $\lambda = 4$ (note that λ represents the expected abundance at low-abundance sites with expected abundance of high-abundance sites equal to $\lambda*2$), increasing the number of sites resulted in the greatest reduction in median RMSE. When $\lambda = 6$ or 10, the greatest gains in RMSE appeared to occur when increasing both p and the number of sites. When $\lambda = 20$, increasing p resulted in greater reductions in median RMSE compared to increasing the number of sites.

Estimates of λ for the low-abundance sites were consistently unbiased across the sampling scenarios we considered (Fig. 11 and Fig. S19). The median absolute value of percent bias in estimated λ for the low-abundance sites was >10% only when p was ≤ 0.25 except for two simulation scenarios with $p = 0.50$ and five sites with two visits. However, the precision of estimated λ varied greatly across sampling scenarios. Precision was greatest at low detection rates and numbers of sites. Similar to our RMSE results, gains in precision appeared less when going from 20 to 30 sites compared to going from three to five or ten sites. Number of visits also had less of an effect on precision than did number of surveys. For example, precision for $\lambda = 4$ and $p = 0.50$, and 10 sites decreased from -60–80% to -43–64% when going from two to eight visits (20 to 80 surveys) compared to an decrease from -51–73% to -48–68% when going from ten sites and four visits to 20 sites and two visits (both 40 surveys total). The absolute values of the 2.5–97.5th quantiles were ≤25% in 51 of 621 parameter combinations including 1, 20, and 30 combinations with $p = 0.50$, 0.75, and 0.95, respectively. Of the 20 combinations for $p = 0.75$, all but two had $\lambda \geq 10$ and/or ≥20 sites. The absolute values of the 2.5–97.5th quantiles were ≤ 50% in 187 of 621 parameter combinations including 4, 34, 65, and 84 combinations with $p = 0.25$, 0.50, 0.75, and 0.95, respectively. Of the combinations for $p \leq 0.50$, all had ≥20 sites.

Trend Scenario

A total of 1,537 out of 2,250 parameter combinations had convergence rates ≥50%. Of the 713 parameter combinations with convergence issues, 128, 222, and 363 where with $p = 0.50$, 0.75, and 0.95, respectively. Of the 363 parameter combinations with $p = 0.95$, the only combinations with insufficient convergence rates had $\lambda = 6$ and ≥5 sites. The 222 parameter combinations with insufficient convergence rates and $p = 0.75$ showed a similar pattern with lower convergence rates when $\lambda > 6$ and number of sites <5. Subsequent analysis showed that trends in model support, RMSE, and statistical power were very erratic with $\lambda = 20$ and $p = 0.75$.

Of the parameter combinations without convergence issues, 361 (23%) had $w \geq 0.50$ for the true Trend model. Of these 361, 3 (0.8%) were with declines ≤10%, 191 (53%) were with ≤20 sites, and 19 (5%) were with 10% declines and 20 sites. Most were with 20% declines over 10 years ($n = 167$, 46%) or 20% declines over five years ($n = 118$, 33%) although 13 (4%) and 60 (17%) were with 10% declines over five and 10 years, respectively. The inter-quartile range of the median AIC_c w was 0.26–0.50 for the true Trend model compared to 0.49–0.74 for the null model.

When the percent decline was <10%, median AIC_c w for the true Trend model never markedly exceeded that of the null model regardless of the number of sites, visits, or the true value of λ and p (Fig. 12 and Fig. S20–S22). When the true decline was 10%, 10 years of simulated data was generally required for the median AIC_c w of the true Trend model to exceed that of the null model. Increasing the number of sites, p, and lambda all increased the median AIC_c w of the true Trend model. In particular, using 20 or 30 sites with a 20% decline clearly identified the Trend model as the best-supported model. Increasing the number of visits had comparatively little impact on AIC_c w.

These patterns mirrored by the statistical power to detect a significant trend (Fig. 13 and Fig. S23–S25). Power only exceeded 50% when the true decline was <20% under parameter combinations where 20 sites were surveyed with $p \geq 0.50$ or those where 30 sites were surveyed. Power was increased with increasing number of sites, p, and λ. Increasing the number of surveys increased power under parameter combinations with ≥10% declines. These patterns were also mirrored by RMSE (Fig. 14 and Fig. S26).

The percent decline in abundance was generally underestimated when the true decline was 5% and overestimated when the true decline was 10% and 20% (Fig. 15 and Fig. S27–S29). However, the magnitude of this bias was relatively low, particularly when compared to the precision of the estimates (Table 3). Precision of the estimated rate of decline vary by up to three orders of magnitude across parameter combinations. Precision showed marked decreases with increasing number of sites, p, and λ. Precision was less affected by number of visits or the true rate or duration of decline.

Observed Use of Gestation Sites

Two gravid females were monitored with radio telemetry in 2012 at VRUT2. The first gravid female was present at a gestation site from 24 Jul–5 Sep 2012 and gave birth between 27 and 29 Aug. During this time she was located on 13 occasions and observed using two gestation rocks approximately 5 m apart. On 7 Sep she was located in the surrounding forest matrix approximately 175 m from her gestation site. The second gravid female was present at a gestation site from 19 Jul–29 Sep and gave birth between 2 and 5 Sep. During this time she was located on 22 occasions and observed using three gestation rocks within approximately 15 m. These two females were approximately 20–35 m apart during gestation.

Three radio-tracked females at MABER were gravid during 2009 and one of these three was also gravid in 2012. The first female was present at a gestation site from 24 Jul–20 Sep 2009 and used four gestation rocks within approximately 10 m. This female was present at a different gestation site from 14 Jul–27 Aug 2012 (approximately 660 m from the 2009 site) although it was also observed at the original gestation site on 3 Sep 2012. The second female was observed using three distinct sets of locations from 16 Jul–13 Sep 2009 where sets were approximately 75–145 m apart and locations within sets were within approximately 20 m. The third female was observed at a gestation used by the previous two females in 2009 from 27 Jul–3 Aug 2009 but was observed approximately 660 m away on 18 and 21 Jul and in the vicinity of the den from 27 Aug until ingress.

One radio-tracked and two PIT-tagged gravid females at NH were observed throughout the activity season. The radio-tracked female was observed using two locations approximately 25 m apart from 16 Jun–8 Aug 2014 during which time it shed (approximately 7 July). It then moved approximately 460 m to a location where it remained from 16–28 Aug and then used approximately six different locations 25–525 m apart before ingress. The first PIT-tagged female was observed 17 times between 2 May and 28 Aug 2009. This female was observed in one location from 4 Jun–10 Jul, moved approximately 86 m on 31 Jul, moved approximately 450 m on 3 Aug, after which it moved approximately 445 m to a cluster of locations within approximately 20 m where it remained from 15–28 Aug. The second PIT-tagged female was observed 18 times between 1 May and 2 Oct 2008. This female was observed at a single location from 22 Aug–2 Oct except for a single observation on 9 Sep approximately 90 m away.

Discussion

Estimating Abundance from Den Counts

Our simulations show that counts of unmarked individuals at communal hibernacula during egress and ingress cannot reliably estimate total abundance of individuals at the hibernacula using N-mixture models. Abundance was consistently overestimated, consistent with previous simulation studies showing that violation of the closure assumption leads to overestimating λ (Kery & Royle 2016). This pattern was consistent across a wide range of sampling designs, detection rates, and patterns of emergence and departure. It was possible to accurately estimate detection probability using a robust sampling design with secondary sampling occasions occurring during periods of population closure. While estimating detection probability may be of interest in some applications (e.g., understanding how detection is influenced by weather or varies among observers) detection probability only represents the probability of detecting an individual that is at the denning area and available for detection, not the probability of an individual remaining within the denning area. While temporary emigration models can estimate the probability of remaining within the dennng area, our simulations showed that even these models cannot reliably estimate total abundance. We therefore recommend that managers not use counts of individuals during ingress and egress to obtain model-based estimates of abundance.

It is possible that the relative differences in abundance estimated from den count data using N-mixture models are consistent across space and time despite the bias of the estimates themselves. If this assumption held true, it may be possible to use these estimates to monitor relative changes in abundance over time. This could be evaluated using simulation studies. However, spatio-temporal differences in emergence patterns and den residency, as well as spatio-temporal variation in detection, would result in spatio-temporal variation in the extent, and possibly direction, of the bias and obscure true variation in abundance.

An alternative to closed-population N-mixture models is the Dail-Madsen model (hereafter the DM model) for open populations (Dail & Madsen 2011). This model generalizes the closed-population N-mixture model by allowing recruitment (births or immigration, γ) and survival (ω) between primary sampling periods. The model requires spatially replicated counts within multiple primary sampling periods although one can also use a robust design with secondary sampling periods during which closure is assumed (Dail & Madsen 2011). We briefly explored the applicability of the DM model using secondary sampling periods (analogous to the *gpcount* data) using a small number of simulated data sets. Under this sampling design, we interpret γ as the number of individuals emerging between visits and ω as the probability of remaining at the den site between visits. We fit our DM models using the *pcountOpen* function in *unmarked* and modeled γ and ω either as constant or with a quadratic effect of visit date. We held λ and *p* constant. We found that the DM model was able to accurately estimate true abundance at each visit. However, the DM model does not provide an estimate analogous to total abundance or superpopulation size and summing estimated abundance across visits consistently underestimated true abundance, likely due to individuals emerging and departing between survey periods without being detected. Therefore, any spatio-temporal variation in emergence patterns or residency times would further bias summed estimated abundance relative to true total abundance.

Gestation/Shedding Site Occupancy and Abundance

Our simulations suggest that under the parameter combinations we considered there is very little ability to statistically detect a decline in gestation/shedding site occupancy or abundance particularly weak declines over short durations. While surveying the maximum number of sites we considered (30–50) provides sufficient statistical power to detect relatively severe declines, the absolute number of potential timber rattlesnake gestation/shedding sites per population, and even per state, may be less than the number of sites we considered. Ward et al. (2017) conducted a similar simulation study regarding occupancy and abundance of a rare, insular population of grass snakes (*Natrix natrix*) in the U.K. Using estimated occupancy (0.44) and detection (0.33) rates they also concluded that a prohibitively large number of sites would be required to detect 15% and 30% declines. Surveying 41–75 sites per year for each year of a six year survey cycle (the approximate effort of current monitoring programs) with moderate to high per-survey sampling effort and eight visits per site per year would only allow detection of 50% declines between cycles. Our results and those of Ward et al. (2017) highlight the challenges of monitoring imperiled species with restricted distributions. A possible remedy to the issue of limited site numbers for timber rattlesnakes in New England is to pool sites across populations or states. This approach will likely increase the spatio-temporal heterogeneity of the data (e.g., population-specific differences in occupancy or detection) which should be evaluated by evaluating additional covariates through formal model-selection approaches. However, increases in heterogeneity may be such that the need to incorporate additional covariates reduces statistical power beyond gains made by increasing the number of sites. The presence of interactive effects among populations/regions may further impede the ability to detect and estimate trends in state variables.

Our results suggest two general guidelines for managers wishing to monitor occupancy and abundance at timber rattlesnake gestation/shedding sites. First, we recommend that managers maximize the number of survey sites as far as resources permit and prioritize surveying additional sites rather than additional site visits. While increasing the number visits generally increased statistical power these gains were usually less than gains from increasing the number of sites, particularly when the number of visits was increased beyond 4–6 visits. We therefore suggest a maximum of 4–6 visits per site where resources permit. While increasing the number of visits did appear more beneficial at higher occupancy rates, our results indicate that increasing the number of sites had a stronger effect. Second, we recommend that managers increase detection rates to the extent possible. This can be accomplished by conducting surveys under optimal conditions (e.g., warm temperatures, low wind speeds), using highly trained observers and randomly rotating observers among sites, and by surveying each site as thoroughly as possible. These guidelines are consistent with those recommended by other researchers monitoring state variables estimated using hierarchical models (Kery et al. 2009; Ward et al. 2017).

Our results suggest that surveying 20–50 sites with at least four visits per site and moderate to high detection rates will allow managers to statistically detect moderate to severe declines. However, we do not wish to suggest that monitoring timber rattlesnake gestation/shedding sites is futile under less intensive sampling designs. While managers should realize that less intensive sampling designs will substantially limit statistical power, a visual assessment of model-based estimates of occupancy and abundance over time may still be informative for evaluating population status. Subtle yet sustained declines in occupancy or abundance in small, imperiled populations may warrant management action, particularly if declines can be corroborated with other demographic data (e.g., declines in survival or fecundity).

Mackenzie and Royle (2005) compared a standard occupancy sampling design to a removal sampling design where a site is no longer surveyed once a species is detected. They concluded that removal designs were more efficient when occupancy was ≥ 0.50, particularly when $p \leq 0.50$. However, they noted that removal designs provide less information for modeling detection which may be important if detection is highly variable. We did not evaluate removal designs in our simulation studies although we suspect that gestation/shedding site occupancy for timber rattlesnakes is likely moderate to high given the limited distribution of appropriate microhabitats and high site fidelity. If estimating occupancy is the sole objective we suggest that managers consider evaluating a removal sampling design. However, a removal design for estimating occupancy does not allow for the concurrent collection of count data to estimate abundance.

We note that a rotational study design may be used when resources do not permit managers to survey all sites annually. We recommend a rotational design wherein non-overlapping subsets of sites are surveyed each year such that each site is surveyed once within a single multi-year cycle. Data within a single cycle are pooled (e.g., by “stacking” the data) and considered to represent a single sampling occasion. Year can still be included in the analysis to account for inter-annual heterogeneity. Such an approach is potentially beneficial for long-lived species which may show little year-to-year variation in state variables. We do not recommend that managers consider each year to represent a single “cycle” because this confounds variation over time with variation among sites. Our guidelines can still be used to inform rotational study designs with the number of sites in our simulations representing the total number of sites surveyed each cycle.

One potential drawback of our simulations is that we did not simulate cyclical patterns in the abundance of gestating females. Such patterns have been noted in many timber rattlesnake populations (Brown 2016, Chapter 1, W.H. Martin, personal communication). However, introducing cyclical patterns would have only increased the signal-to-noise ratio in our data making it even more difficult to statistically detect a trend in abundance. Alternatively, one could consider our use of low- and high-abundance sites somewhat analogous to low- and high-abundance years assuming that all site-years can be classified as low or high abundance. In any case, we recommend that managers statistically control for cyclical variation in the abundance of gestating females through the use of a binary low-high covariate and recognize that such variation may further limit the ability to statistically detect trends.

We also emphasize that our gestation/shedding site simulations were conducted under the assumption of site closure with respect to occupancy or abundance. Our limited sample sizes of radio-tracked gravid females prevented us from rigorously evaluating this assumption. While radio-tracked gravid females were present at gestation sites from approximately mid July through early September some individuals did not consistently use a single site for that entire period, perhaps either moving among gestation sites or to other habitat features. This suggests that gestation sites may be subject to temporary emigration (i.e., individuals entering and leaving the survey site) and violation of the closure assumption. However, we emphasize that the presence or degree of temporary emigration is likely to vary among sites and populations and should be evaluated whenever possible. For example, small sites may be more prone to temporary emigration (Rodda 2012) and the spatial ecology of gravid females may vary within and among populations (Anderson 2010; Gardner-Santana & Beaupre 2009; Reinert & Zappalorti 1988b;

Stengle 2018). Random temporary emigration affects the interpretation occupancy and abundance differently. First, closure may be more readily assumed with regards to occupancy because the occupancy status (occupied vs. unoccupied) may be less likely to change within the survey period than the number of individuals present at the site. If changes in site occupancy are random, probability of site occupancy is then interpreted as probability of site use which may still prove useful for monitoring population status. Second, in the presence of completely random temporary emigration abundance (λ) still represents an unbiased estimate superpopulation size (i.e., the total number of individuals ever associated with a site, Chandler et al. 2011; Kendall et al. 1997; Kery & Royle 2016) and is likely to still prove a useful metric for monitoring timber rattlesnake population status.

We provide two recommendations to researchers with regards to temporary emigration. First, when resources permit, we suggest that researchers collect data that permit estimating the degree of and factors influencing temporary emigration. This can be accomplished by a robust survey design with secondary sampling periods during which site closure is maintained. For example, a single observer could repeatedly survey a site within a short period of time (e.g., a single day) although this risks introducing effects of prior knowledge of detections. This could be avoided by rotating observers among sites such that the same observer does not survey a site multiple times within the same secondary sampling period. Data collected under such a design can be modeled using binomial temporary emigration models in the *gpcount* function in *unmarked* (Fiske & Chandler 2011) although these models are computational demanding, particularly for large sample sizes. An alternative approach is the dependent double observer method (Nichols et al. 2000) which can be modeled using multinomial temporary emigration models in the *gmultmix* function in *unmarked* (Fiske & Chandler 2011). While computational restrictions prevented us from fully evaluating the effects of sampling intensity on the ability of temporary emigration models to detect trends in abundance, preliminary analyses suggest that patterns of bias and precision of parameter estimates with respect to the number of sites and visits observed with the binomial N-mixture model hold true with temporary emigration models (e.g., precision is maximized by increasing the number of sites). However, we found that the multinomial temporary emigration models fit with simulated double observer data were prone to convergence issues particularly when detection probabilities were similar, but not identical, between observers. We recommend additional simulation studies, preferably informed with preliminary estimations of temporary emigration and detection, to fully evaluate the suitability of binomial and multinomial temporary emigration models for estimating timber rattlesnake abundance at gestation/shedding sites. In the meantime, we suggest that managers collect data using a robust design methodology and model those data using the *gpcount* function. Second, we recommend that researchers collect and evaluate covariates hypothesized to affect the probability of being present and available during a survey (i.e., Φ from Chandler et al. 2011). This will not only permit more accurate estimates of λ but can also guide study design to focus surveys during conditions when Φ is maximized. If researchers can design sampling protocols such that Φ is close it may be reasonable to revise the sampling protocol to assume spatial closure.

While some researchers have compared the feasibility and efficacy of monitoring occupancy vs. abundance (Joseph et al. 2008; Ward et al. 2017), monitoring timber rattlesnakes at gestation/shedding sites allows managers to simultaneously collect data to estimate both occupancy and abundance. That both variables responded similarly to changes in sampling intensity and detection probability indicates that guidelines for optimal study design pertain similarly to both variables. However, our results suggest that it may require less survey effort to detect changes in abundance than occupancy. For example, when $p = 0.75$ and $\psi = 0.75$ and a 10% decline over 10 years, median AIC_c *w* for the true trend model only approached 0.50 when 50 sites were surveyed and never exceeded the median AIC_c *w* for the null model. In contrast, when $p = 0.75$ and $\lambda = 10$, median AIC_c *w* for the true trend model exceeded the median AIC_c *w* for the null model with 30 sites and a ≥10% decline. Median AIC_c *w* for the true trend model also exceeded the median AIC_c *w* for the null model with a 20% decline and ≥10 sites. Under these same scenarios, power to detect a decline in occupancy never exceeded 50% but was >50% for abundance with a 10% decline over 10 years with 30 sites or a 20% decline at either duration with ≥20 sites. The precision of the estimated percent decline in abundance was also greater than the precision of the estimated percent decline in occupancy. Additionally, monitoring occupancy may have limited utility if all or most known gestation sites are occupied. Furthermore, changes in occupancy may occur slowly and fail to correspond to actual population declines given the longevity and site fidelity of timber rattlesnakes (Brown 1993; Brown 2016). While the design of our simulations prevented us from directly evaluating bias in the estimated percent decline, our use of the same “true” percent decline within a given scenario (i.e., a given percent decline and duration) ensures that this bias is consistent across different sampling intensities and detection rates. We therefore have no reason to suspect that estimation of trends in abundance will be biased. Furthermore, the precision of the estimated percent decline in ψ and λ increased rapidly by increasing the number of sites. However, natural cyclical changes in gravid female abundance may increase the difficulty in detecting trends in gravid female abundance. Nevertheless, we suggest that managers focus on estimating abundance at timber rattlesnake gestation/shedding sites and, if necessary, prioritize a sampling protocol to estimate temporary emigration to improve estimates of abundance.

In summary, we make the following specific recommendations with regards to study design for monitoring occupancy and abundance of timber rattlesnake gestation/shedding sites.

- Surveys at gestation sites should be conducted from approximately mid July through early September.
- We recommend that managers focus on monitoring abundance.
- The number of sites surveyed per population should be maximized, given logistical and financial constraints, while conducting at least three but no more than six repeat visits per site.
- At least one pilot year should be used to formally estimate the degree of temporary emigration, preferably using a robust survey design. If temporary emigration is minimal or can be substantially reduced through study design, managers may consider adopting a survey design assuming population closure. In either case, managers should clearly recognize the interpretation of λ.
- Surveys should be conducted during conditions optimal for rattlesnake surface activity using trained observers. When logistically feasible observers should be rotated among sites.
- Data should be analyzed annually, pooled across populations and regions where possible, to identify influential covariates and qualitative trends in state variables.
- Managers should recognize and appreciate the statistical limits to their ability to detect trends given their sample sizes.

Evaluation of Different Monitoring Approaches

In this section, we evaluate and provide guidelines for approaches for monitoring timber rattlesnake populations organized by decreasing level of sampling intensity. For each methodology, we provide a brief overview followed by a detailed discussion of implementation. Our two goals with this discussion are to 1) help managers make informed decisions about timber rattlesnake monitoring based on their available resources and the management needs of their particular populations, and 2) coordinate timber rattlesnake monitoring efforts across the New England states through the standardization of monitoring methodologies and data collection. In particular, we provide additional details on study design and data collection for monitoring timber rattlesnake occupancy and abundance at gestation/shedding sites.

We focus our discussion primarily on methodologies that permit managers to account for imperfect detection (or, in the case of radio telemetry, assure perfect detectability). Herpetofauna often have low and variable detection rates which, if not statistically accounted for, have the potential to produce misleading inferences with respect to occupancy and abundance (Mazerolle et al. 2007). Many factors influence herpetofaunal detection rates including weather condition, age and body size, reproductive class, as well as variation due to season, observer, or site (Christy et al. 2010; Durso et al. 2011; Lardner et al. 2015; Spence-Bailey et al. 2010; Waldron et al. 2013; Willson et al. 2011). Some variation in detection rates may be reduced by conducting field surveys using standardized protocols and under optimal conditions for surface activity. However, research on other taxa indicates that this is insufficient to fully account for variation in detection (Hochachka & Fiedler 2008; Kery et al. 2009; Schmidt 2005). While metrics computed from data collected under optimal conditions with standardized methodology may provide accurate indices to state variables (e.g., abundance, Nichols et al. 2009), we concur with other researchers in recommending that the accuracy of indices be thoroughly evaluated prior to their use or that monitoring metrics explicitly account for imperfect detection through model-based approaches (Anderson 2001; MacKenzie & Kendall 2002). Because timber rattlesnake gestation or shedding sites may include a relatively small number of easily observable individuals, particularly gravid females, it may be possible to estimate the number of individuals present through the identification of individuals based on their size, sex, and coloration. However, we still recommend that the burden of proof fall on the researcher to demonstrate that such an approach truly provides a reliable estimate in the presence of imperfect detection.

Radio Telemetry

Radio telemetry has a long tradition in wildlife ecology and conservation and considerations for the design and implementation of radio telemetry studies have been discussed in detail elsewhere for wildlife species in general (Millspaugh & Marzluff 2001; White & Garrott 1990) and snakes in particular (Dorcas & Willson 2009; Plummer & Ferner 2012; Reinert 1992). We therefore provide a brief discussion regarding the applicability of this methodology for monitoring timber rattlesnakes. The primary advantage of radio telemetry for monitoring population status is its ability to provide relatively continuous monitoring of individual demographic parameters including survival, reproductive status, fecundity, shedding rates, health status (e.g., presence of snake fungal disease), as well as detailed and spatially-explicit information on movement patterns and habitat use. However, many of these demographic parameters can be obtained through mark-recapture studies. The high financial and logistical costs of radio telemetry often mean that the gains in the accuracy and precision of demographic parameter estimates are offset by small sample sizes and limited study durations. Radio telemetry is also generally limited to larger individuals (but see Cobb et al. 2005; Howze et al. 2012) and carries the potential for negative impacts associated with transmitter surgeries (e.g., Lentini et al. 2011; Weatherhead & Blouin-Demers 2004). However, radio telemetry is an ideal methodology for obtaining spatially-explicit information on movement patterns and for identifying key habitat features (e.g., new den or gestation sites, movement corridors). We therefore suggest that radio telemetry is more appropriate for relatively short-term research projects compared to long-term monitoring efforts. The specific data collected as part of a radio telemetry study will depend on the particular study goals (e.g., evaluating habitat use) but we recommend that managers record at each telemetry location, at a minimum, the identifier (preferably PIT tag or unique ID number to facilitate comparison with mark-recapture data), date, time, observer, location coordinates (e.g., latitude and longitude or Universal Transverse Mercator (UTM) coordinates with the appropriate datum and UTM zone), whether the individual has shed since its last observation, reproductive status (e.g., is the individual gravid?), health status (e.g., evidence of snake fungal disease, injuries), and a brief description of the presence of prey bolus, behavior, habitat, and fate (i.e., mortality). An example data sheet is provided in Appendix 3. Data recorded at the initial capture of each radio-tracked individual should follow the format for mark-recapture data collection (described below).

Mark-Recapture

Overview

Mark-recapture, also called capture-mark-recapture, refers to the process of uniquely marking and recapturing individuals and is also widely used among snake ecologists (Dorcas & Willson 2009; Plummer & Ferner 2012). Mark-recapture is well suited for monitoring the population status of communally-denning snake species because the spatial concentration of individuals during egress and ingress provides an opportunity to increase the numbers of captures and recaptures. Mark-recapture data can be used to provide model-based estimates of demographic parameters (e.g., survival, abundance) as well as information relating to body size and condition, shedding rates, and reproduction frequency. Mark-recapture is comparatively less expensive and labor intensive than radio-telemetry and has comparatively less of an impact on individual animals, although the effects of capturing and handling individuals must still be considered. However, low recapture rates are common in many snake mark-recapture studies (Brown et al. 2007; Jenkins et al. 2017; Chapter 1) which may limit the utility of this methodology for population monitoring. In this section, we provide guidance for mark-recapture study design and data collection and discuss the use of mark-recapture data to obtain model-based demographic parameters potentially suitable for population monitoring.

Study Design and Data Collection

Arguably the key objective in mark-recapture studies is to maximize recapture rates and the independence among recaptures as recapture probabilities directly influence the precision of state variables (e.g., survival, abundance). Increased recapture rates also increase the accuracy of estimates of parameters such as shedding and growth rates and reproduction frequency. Recapture probability is often associated with field effort (Lind et al. 2005; Maritz & Alexander 2012; Prival & Schroff 2012) but see Flatt et al. (1997). Brown (2008) found that timber rattlesnake captures increased with time per day spent in the field and that the number of snakes captured per day increased with the number of days spent in the field. Therefore, timber rattlesnake mark-recapture studies should strive to maximize field effort during egress and ingress while individuals are spatially concentrated. Some mark-recapture models (e.g., open population abundance models) assume constant study size so field personnel should record the precise location of individual captures to identify captures made at the dens. While individuals could be marked and recaptured at shedding or gestation sites, only a subset of the population will occur at these sites and this may unduly disturb individuals, particularly gravid females. We therefore suggest that managers focus mark-recapture efforts around the dens unless additional information from shedding and gestation sites is required.

We recommend that managers continuing using Passive Integrated Transponder (PIT) tags. We have successfully marked neonates with standard 12 mm tags although smaller 8 mm tags are available (e.g., Biomark and AVID). We strongly encourage the marking of neonates because population-specific estimates of neonate and juvenile survival are lacking from all but one New England timber rattlesnake population (Chapter 1). Additionally, we recommend that researchers record sex, snout-vent length, tail length, and body weight. Reproductive condition of females can be determined by palpating the ventral side and feeling for the presence of follicles and embryos although this method may overestimate fecundity (Brown 2016). Radiographs and ultrasound can also be used to evaluate reproductive status and estimate fecundity (A.G.S., unpublished data) although this may not be feasible for most populations. The presence/number of follicles and embryos should be recorded. Painting the basal rattle segment can be used to estimate shedding rates. Managers should record the number of sheds based on painted rattle segments rather than the number of rattles. An example data sheet is provided in Appendix 4.

Survival

Survival can be estimated from mark-recapture data using the Cormack-Jolly-Seber (CJS) model (Cormack 1964; Jolly 1965; Seber 1965). When radio-telemetry data with confirmed mortalities are available, the CJS model and Burnham's (1993) joint recapture-recovery model or the similar Barker model (Barker 1997, 1999) can also be used to estimate survival. We focus our discussion on the CJS and its applicability to timber rattlesnake survival estimation. The CJS model requires data from at least three capture occasions and estimates apparent survival (Φ) and probability of recapture (*p*). Although apparent survival cannot distinguish between permanent emigration and mortality, the former is unlikely in timber rattlesnakes in New England given their high den fidelity and the distances among populations. The CJS model assumes 1) marks are not lost, overlooked, or misrecorded; 2) no unmodeled heterogeneity in Φ or *p*; 3) independence of individual's fates; 4) any emigration is permanent; and 5) sampling occasions are instantaneous. The first three assumptions are likely to be met with communally denning timber rattlesnakes marked with PIT tags. The assumption of instantaneous sampling occasions means that sampling occasions should be as short as possible relative to the probability of mortality of the interval between occasions. However, when recapture rates are low it may be necessary to aggregate captures to sufficiently increase the number of recaptures within each occasion (e.g., Diller & Wallace 2002; Jenkins et al. 2017). However, the relatively high annual survival of adult rattlesnakes likely mitigates violations of this assumption and CJS models are relatively robust to pooling across capture occasions (Hileman et al. 2015). If data permit, captures can be divided into spring and fall capture occasions (Chapter 1). Data from individuals captured/recaptured during the summer away from the den can be included survival analyses although this risks introducing additional heterogeneity in *p* because factors influencing recapture probability may differ for individuals away from the den (e.g., unmarked individuals are captured through association with radio-tracked individuals). Data from radio-tracked individuals can be included in CJS models if *p* is fixed to one for those individuals.

Many snake studies have used CJS models to estimate survival (e.g., Bronikowski & Arnold 1999; Brown et al. 2007; Diller & Wallace 2002; Hyslop et al. 2012; Jenkins et al. 2017; Lind et al. 2005; Prival & Schroff 2012; Stanford & King 2004), some despite relatively low recapture rates. However, low recapture rates will reduce the precision of parameter estimates and the statistical power to detect differences among groups or changes over time. We therefore recommend that projects monitoring survival conduct power analyses to determine the recapture rates needed to obtain a desired level of precision in survival estimates. While survival is important for evaluating the health and status of a population and an essential parameter in population viability analyses, it may have limited utility as a monitoring metric in isolation because high survival may be offset by low fecundity or vice versa. We therefore recommend that managers not solely rely on survival as a monitoring metric.

Open Population Metrics of Abundance/Population Size

Abundance can be estimated using the open-population Jolly-Seber model (JS, Jolly 1965; Seber 1965) and its variants. The data and model assumptions used in the JS model are identical to those used in the CJS model. Additionally, the JS model assumes that unmarked and marked individuals in the population have identical capture probabilities and that the size of the study area remains constant. Violations of these assumptions will produce biased estimates of abundance, particularly when capture probabilities are low (Carothers 1973; Gilbert 1973; Pollock et al. 1990; Williams et al. 2002). Careful consideration should therefore be given to whether the assumption of equal capture probabilities between unmarked and marked animals is met. For example, if some individuals are more likely to be captured and subsequently recaptured (e.g., larger individuals or basking, gravid females) population estimates will be negatively biased. As a result, radio-tracked individuals cannot be incorporated into JS estimates. This assumption may be problematic with timber rattlesnakes due to a hypothesized "spook" factor (Brown 2008; Brown et al. 2007). Such a response would positively bias abundance estimates. Yet if this response can be assumed to be temporary and of short duration relative to the length of the study it can be accounted for by using a time-since-marking covariate and assuming that individuals captured and marked on a given occasion have the same capture probability as unmarked individuals (Williams et al. 2002). Alternatively, this response could be evaluated using radio telemetry where radio-tracked individuals are captured, handled, and their behavioral response recorded, although their response relative to unmarked individuals would still be unknown.

As with survival estimation, we recommend that efforts monitoring estimated abundance from open population models conduct power analyses to determine sufficient capture probabilities. Using the figures provided in Pollock et al. (1990), achieving the recommended 20% CV in estimated population size with population sizes of 100–200 individuals with 0.75 annual survival over 5, 10, and 20 years would require capture probabilities of approximately 0.40–0.50, 0.30–0.40, and 0.25–0.35, respectively. Recapture probabilities in our studies were generally ≤0.20 (Chapter 1) suggesting that timber rattlesnake mark-recapture studies may have limited statistical power to detect changes in abundance over time. However, it may still be possible to use estimates of abundance to determine if abundance is greater or less than some critical threshold. Alternatively, Pradel’s (1996) variation to the JS model provides an estimate of population growth rate. Whiting et al. (2008) used Pradel’s model to estimate population growth rates for the endangered Concho watersnake (*Nerodia harteri paucimaculata*).

Multiple snake studies have used the JS model or its derivatives to estimate abundance (e.g., Godley et al. 2017; Reinert et al. 2011). King et al. (2006) used closed and open population models, including the JS model, to estimate population size of Lake Erie watersnakes (*Nerodia sipedon insularum*) and found no significant differences among estimators although standard errors were larger around estimates from open populations. However, their sites were individual islands where geographic closure during their survey period is likely. Prival & Schroff (2012) used the POPAN formulation of the JS model (Schwarz & Arnason 1996) to estimate population size of twin-spotted rattlesnakes (*Crotalus pricei*) at communal hibernacula and suggested that a negative behavioral response to capture may have occurred in their populations. While open population estimates of abundance are a potentially valuable monitoring metric for timber rattlesnakes, we recommend that mangers carefully consider the assumption of equal capture probabilities and design their field methodology to minimize heterogeneity in capture probability. We also recommend that managers test for evidence of post-marking effect on capture probability using a time-since-marking covariate in their model selection process. Finally, we recommend that managers interpret population size or growth rate estimates from JS models with some degree of caution.

Closed Population Metrics of Abundance/Population Size

Closed population models have a long tradition in wildlife ecology for estimating abundance. These models assume both demographic (i.e., no births or deaths) and geographic closure (i.e., no immigration or emigration) between one or more sampling occasions (Pollock et al. 1990; Williams et al. 2002). Additionally, closed population models assume no unmodeled heterogeneity in capture probabilities among marked and unmarked animals and that marks are not overlooked or incorrectly recorded. Different closed population models are available to test for heterogeneity in capture rates due to behavioral effects (e.g., “trap happy” or “trap shy” responses) or individual heterogeneity.

While some snake studies have reported abundance estimates from closed population models, it is unclear how well many of these studies met the closure assumption. For example, researchers may pool capture data collected over multiple years (e.g., Kapfer et al. 2008; Reinert et al. 2011). While it may be reasonable to assume population closure of particular groups (e.g., adults with high annual survival) over a period of a few months to one or two years, to our knowledge this assumption has not been rigorously evaluated using field or simulation studies. Wastell & Mackessy (2016) used a closed population model to estimate desert massasauga (*Sistrurus tergeminus,* Crother 2017, formerly *Sistrurus catenatus*) population size with data collected over three years. However, they used an approach suggested by Krebs (2008) to evaluate the closure assumption by regressing the proportion of marked snakes against the number of previously marked snakes at each capture occasion. Their high R^2 value (0.94) suggested that their closure assumption was met.

Given the potential for violations of geographic closure with communally-denning timber rattlesnakes we do not recommend that managers use closed population estimates of abundance as monitoring metrics. However, within-year estimates of closed population abundance may provide reasonable approximations of abundance, although this hypothesis is untested. Specifically, captures made at the dens could be pooled into spring and fall capture occasions. Non-den captures should be excluded because capture probabilities are almost certainly lower once an individual has left the denning area. The effects of recruitment can be minimized by only including adult observations (King et al. 2006) while mortality between sampling occasions can be ignored if mortality rates are equal between marked and unmarked individuals. While this assumption is difficult to test, our research indicates that timber rattlesnake survival rates were very similar between PIT-tagged and radio-tracked individuals (Chapter 1). A similar approach could be used using consecutive fall and spring captures in the same manner. We recommend that managers adopting this approach use formal likelihood based estimation (e.g., using program MARK, White & Burnham 1999). However, closed population models generally requiring marking a large proportion of the population (0.33–0.67, Krebs 1998), which may be difficult given our low recapture rates (Chapter 1). Furthermore, preliminary applications of this approach produced values that were highly correlated with the number of captures suggesting that this approach may be confounded with sampling effort.

Conclusion

Mark-recapture provides managers with an opportunity to estimate multiple demographic parameters with which to monitor timber rattlesnake population status including survival, abundance, reproduction frequency, fecundity, shedding rates, and body size and condition. We therefore recommend that managers consider implementing mark-recapture monitoring efforts when financial and logistical resources permit the long-term investment of a sufficiently high level of field effort to obtain sufficient recapture rates. However, we note two important considerations. First, it may be difficult to support a sufficient level of field effort long-term. What constitutes a sufficient level of effort will vary among populations but we note that concurrent radio telemetry and mark-recapture studies at three New England populations failed to obtain sustained recapture probabilities >0.20 (Chapter 1). Preliminary data combined with formal power analyses can inform whether a sufficient level of effort can be maintained. Second, mark-recapture necessarily involves capturing and handling individuals at least once and this level of disturbance may be undesirable in some situations given the potential for negative behavioral responses (Brown 2008; Brown et al. 2007). However, subsequent “recaptures” can be obtained with minimal disturbance by attaching a PIT-tag scanner to the end of a long pole or holding the scanner with a pair of snake tongs (M.M. & A.G.S., personal observation).

Presence/Counts of Unmarked Individuals at Shedding/Gestation Sites

In this section, we expand upon our simulation study to provide specific guidelines for developing and implementing protocols for monitoring occupancy and abundance at gestation/shedding sites. Because the study design and data collection methodology is similar between these two approaches we suggest a unified approach to site selection and then discuss differences in survey design and data collection. We then conclude with suggestions for monitoring fecundity through observations of unmarked neonates at birthing sites.

Site Definition

The definition and delineation of potential survey sites should reflect the specific objectives (e.g., monitor gestation sites vs. shedding sites) and local conditions. We suggest four principles to consider when defining sites: 1) sites are fixed, discrete units in space, 2) sites should represent a random sample from a larger statistical population of sites or a complete census of known sites, 3) closure should be maintained within sites with regards to the state variable of interest, and 4) variation among sites that influences model parameters is modeled using covariates. Site definitions in occupancy and abundance studies vary widely according to study-specific objectives and field conditions and may include wetlands or catchments (Gould et al. 2012), fixed radius point counts (Chandler et al. 2011), discrete habitat patches (Bauder et al. 2017; Collier et al. 2010), fixed area plots (Haan et al. 2007), transects (Kery et al. 2009), or trapping arrays (Steen et al. 2012b). We suggest that sites be delineated so as to maximize site closure. Beyond that, managers have substantial flexibility in delineating sites according to local conditions. Because timber rattlesnake gestation sites in the northeast are typically patches of open, rocky habitats within a matrix of forest (Brown 1993; Stengle 2018) we suggest that sites be delineated to include a single such patch. These patches may partially or entirely include the den area. Variation in site-level microhabitats within and among populations is permitted (e.g., site size, substrate, canopy cover, etc.) and can be accommodated in the modeling process using site-level covariates. This may be particularly important when pooling sites across populations or regions due to inter-population differences in site characteristics. However, such variation may help elucidate processes influence variation in occupancy/abundance among sites. While there is no theoretical minimum (or maximum) site size, small sites may be more prone to violations of population closure (Rodda 2012).

Site delineation should consider the biological and statistical independence among sites. The former may be reduced if sites are closely spaced such that the same individuals readily move among sites. Complete biological independence may be unobtainable in many populations, particularly if gravid females utilize multiple gestation sites in a single activity season. However, consider two rock balds separated by only 15 m of forest habitat. It may be preferable in this case to consider them as one site and include the proportion of open rock or rocky substrate as a site-level covariate. Statistical independence refers to the independence of model residuals and is violated in the presence of residual spatial autocorrelation which occurs when model covariates do not completely explain spatial variation in the response variable (Beale et al. 2010; Dormann et al. 2007; Legendre 1993). Residual spatial autocorrelation can be minimized by maintaining sufficient spacing among sites although this may be unfeasible with regards to timber rattlesnake gestation/shedding sites. Accounting for residual spatial autocorrelation is not straightforward in hierarchical models although it can be accomplished using spatial autocovariates (Augustin et al. 1996; Bauder et al. 2017; Moore & Swihart 2005) or through Bayesian modeling approaches (Royle & Dorazio 2008).

We see three possible approaches for identifying the statistical population of sites for a given timber rattlesnake population (note that a statistical population may or may not be synonymous with a biological population). The first is to restrict the statistical population to known sites. Surveyed sites could then represent a probabilistic sample or complete census of known sites. Such an approach may prove problematic for estimating occupancy using model-based approaches if the naïve occupancy rate approaches 100%. Under this approach, statistical inferences can only be made to known sites. Managers must also recognize that known sites may only represent an unknown and potentially misrepresentative proportion of all sites within a biological population.

The second approach is to identify all potential gestation or shedding sites (i.e., suitable habitat patches) available to the biological population using remotely sensed or aerial imagery. However, it may not be clear what potential sites are indeed available to a population. Availability could be defined using observed individual movement distances, particularly for gravid females. For example, two radio-tracked gravid females in Vermont moved 1.25 and 1.41 km from their den (J.M.B. & D.B., unpublished data) while gravid females in western Massachusetts moved up to 3.5 km from their den (A.G.S., unpublished data). While this approach may yield a greater number of potential sites from which to sample, it may overestimate the area potentially available to individuals from that den and devote a substantial portion of potentially limited field resources to surveying unoccupied sites. Furthermore, it may not be possible to comprehensively identify all potential sites, particularly shedding sites which may occur in multiple habitat types (J.M.B. & D.B., unpublished data). In this case, statistical inferences should clearly be limited to only those potential sites that managers identify. However, surveying unoccupied sites may be important for determining if such sites become occupied in the future.

A third approach is a hybrid approach wherein new sites are added as they are detected. While models can accommodate the addition of new sites, this risks confounding temporal changes in state variables with spatial changes due to the addition of new sites. For example, if new sites have consistently lower abundance than previously known sites estimated abundance may decline as new sites are added to the analyses. This confounding may be minimized if differences in state variables between old and new sites can be explained by factors incorporated into the model as covariates (e.g., canopy cover, elevation, substrate, numbers of gestation rocks, etc.). The magnitude of confounding will likely depend on the differences in state variables between old and new sites and the number of sites in each group. While we recognize that most managers will need to add new sites to their monitoring program, we encourage managers to identify as many potential sites as possible prior to initiating monitoring.

Our results suggest that gestation site closure is maximized by conducting surveys between mid July and early September although we note that the degree of closure within this window may vary among populations. Timber rattlesnakes in New England appear to complete their first shed in June (Aldridge & Brown 1995; Stengle 2018, J.M.B. & D. Blodget, unpublished data) suggesting that shedding site surveys be conducted during this time. However, we note that temporary emigration is likely to be present and should be considered during study design and in the interpretation of parameter estimates. We strongly encourage pilot seasons to quantify the degree of temporary emigration, particularly for shedding sites

Given the potential complexities in defining and delineating sites, particularly when sites are to be pooled for inter-population analyses, we strongly recommend a collaborative approach to defining and delineating potential timber rattlesnake gestation and shedding sites across the northeastern United States. While a collaborative approach does not mean that site definitions must be identical among populations, it is likely to minimize systematic biases and inter-population heterogeneity in the data which may affect inter-population comparisons. We also encourage managers to consider the first one to few years of a monitoring effort as pilot years where new sites are identified and added to the population. Inter-population differences in site delineations may also be problematic for inter-population analyses of site occupancy. For example, suppose sites at one population are delineated using only known gestation/shedding sites but at another population sites are delineated by identifying all south-facing rock exposures within 1.5 km of the den. The second population may include a greater proportion of unoccupied sites than the first population.

Covariate Selection and Measurement

We discuss a non-exhaustive suite of potential site- and survey-level covariates. The former can be used to model both state variables and detection while the latter can be used to model detection. The same covariate can be used in a single model to model both the state variable and detection. Prior to data collection, we strongly recommend that researchers identify factors hypothesized to influence states variables and detection and collect covariates reflecting those factors. In particular, factors hypothesized to explain temporal variation in state variables (e.g., declines) should be collected so that the mechanisms influencing such variation can be inferred.

Potential site-level covariates for state variables include the spatial area of site (i.e., size), the number of potential or actual gestation/shedding rocks, or site-average canopy cover. Site-level covariates can also include covariates measured beyond the scale of the site such as amount of surrounding potential basking habitat (Clark et al. 2008), distance to nearest den, or road density within varying radii buffers. The aforementioned site-level covariates measured at the level of the site can also serve as covariates for detection. In particular, the number, spatial arrangement, and characteristics of gestating/shedding rocks may be influential. Characteristics of individual rocks (e.g., size, proportion of underside visible) can be used to calculate site-wide averages.

While we strongly recommend conducting surveys during optimal weather conditions for surface activity to minimize weather-induced heterogeneity in detection and maximize detection rates, observers should record weather variables at each survey with which to model detection. These can include shaded air temperature approximately 2 cm and 2 m above ground level, estimated cloud cover, wind speed and direction, and the presence of precipitation. Shaded air temperature can be obtained by using a notebook or hand to shade a quick-reading thermometer. Cloud cover and wind speed could be recorded as categorical (e.g., low, medium, high) or ordinal (e.g., on a scale from 1 to 5) variables. Date, time of day, and observer initials should be recorded.

Data Collection and Organization

We recommend observers record data using data sheets to standardize data collection and organization (Appendix 5). Even if abundance will be not estimated we recommend observers record the number of individuals because occupancy can easily be ascertained from count data (e.g., >0 individuals detected). While it is possible to estimate occupancy/abundance for different age/sex/reproductive classes this depends on the accuracy with which such classes can be identified. Nevertheless, data on age/sex/reproductive class may still be informative. Data should be maintained in electronic data bases or spreadsheets. An example Excel spreadsheet with dropdown boxes for data entry will be provided to managers as requested.

An alternative to visual surveys of gestation/shedding sites is the use of time-lapse cameras. Time-lapse cameras are widely used to estimate species occupancy and may allow managers to monitor more sites more frequently than would be possible with field observers (O'Connell et al. 2011). However, time-lapse cameras have received relatively little use for monitoring snake occupancy or abundance (Fisher 2012). Monitoring occupancy using time-lapse cameras involves activating one or more cameras at a site for some time period (e.g., a single day) which then represents a single visit. Species presence or non-detection is then assessed from video footage. Multiple "visits" are then conducted in the same manner as visits by field observers. Similarly, abundance could be monitored by counting the number of individuals observed in video footage although care must be taken to avoid double counting individuals, particularly when using multiple cameras. Cameras should be positioned so as to maximize site coverage but individuals under rocks may be missed. It is therefore conceivable that detection rates using time-lapse cameras could be lower than detection rates using field observers (e.g., because field observers can look under cover objects). Additionally, the field effort needed to position and move cameras, change batteries, and download SD cards may offset gains from reduced use of field observers. We therefore encourage that any use of time-lapse cameras be implemented concurrently with field surveys to compare the two approaches and assess the long-term suitability of monitoring with time-lapse cameras.

Data Analysis

We recommend that managers analyze their data after every survey season using single-season models to: 1) identify non-influential covariates that may not need to be collected in the future, 2) identify additional covariates to collect, and 3) obtain preliminary estimates of model parameters and their precision. We recommend an information-theoretic model selection approach where models are formulated to represent different competing hypotheses and compared using AIC (potentially adjusted for small sample size and/or overdispersion, Anderson

2008; Burnham & Anderson 2002). AIC model weights are then used to compare the relative support for each model/hypothesis and identify important/unimportant covariates. Model-averaging can be used to obtain estimates of state variables and detection across models. We recommend these analyses be conducted in R using the *unmarked* package (Fiske & Chandler 2011) to facilitate repeatability among states and over time. We provide R script illustrating the analysis of a simulated data set, including model-selection and model-averaging as an example (Appendix 6). We also recommend conducting regional analyses using data pooled among populations after the first one to few survey seasons to evaluate the feasibility of pooled analyses and identify additional covariates needed to account for inter-regional heterogeneity in the data.

When multiple years of data become available, at least two approaches are available for estimating changes in state variables over time. The first is the approach used in our simulation studies where data from multiple years are "stacked" and year included as a covariate in the model. If year is a continuous covariate one can formally test for a linear trend in the state variable over time. We recommend that year be included as either a factor or continuous covariate in all models regardless of its support or significance to account for year-to-year variation in the data. For example, the null model in a multi-year analysis would still contain an effect of year (Bauder et al. 2017).

The second approach uses dynamic population models to formally estimate changes in state variables over time. These include the multi-season occupancy model (MacKenzie et al. 2003) and the Dail-Madsen model (Dail & Madsen 2011). These models estimate occupancy/abundance for the first occasion, extinction/emigration, and colonization/recruitment, respectively, and derive occupancy/abundance at subsequent occasions using the population dynamic parameters. Population dynamic parameters can be modeled as a function of covariates. While these models can provide insights into the mechanisms responsible for temporal variation in occupancy/abundance, the presence of additional parameters may make these models difficult to fit with limited data sets. Moreover, because the timber rattlesnake is long-lived and shows relatively high fidelity to denning, shedding, and gestation sites, parameters such as site extinction and colonization may not be relevant or meaningful over relatively short durations.

Conclusions

We recognize that many options are available for monitoring the status of timber rattlesnake populations and that no single approach is best in all situations. We therefore recommend that managers consider the ecology and conservation status of each population, their available financial and logistical resources, potential impacts to individual animals, and the type and quality of information provided by each methodology. We also strongly recommend that managers implement methodologies that can estimate state variables for monitoring while formerly accounting for imperfect detection. We suggest that radio telemetry is most appropriate for addressing specific research questions requiring detailed information on individual movements and habitat use, population-specific estimates of space use, or precise information on the presence, cause, and timing of mortality. We recommend that managers implement monitoring protocols using mark-recapture and/or gestation/shedding site occupancy and abundance. We suggest, all things being equal, that mark-recapture is preferable to monitoring gestation/shedding site occupancy and abundance because mark-recapture allows managers to estimate a suite of demographic parameters and can target all ages and reproductive classes which together may provide a more comprehensive assessment of population status. However, mark-recapture may require a prohibitive level of field effort to obtain accurate parameter estimates. Importantly, the potential risks of repeated capture and handling of individuals must be weighed against the benefits of the data obtained. In these cases, we recommend that managers monitor populations using gestation/shedding site occupancy and abundance rather than counts of unmarked individuals at dens. We recommend that managers maximize the number of sites to increase statistical power to detect trends in state variables but recognize that substantial limits to statistical power may occur. Because temporary emigration will likely be present, we recommend that managers, at least initially, adopt a robust sampling design to formally estimate the degree of temporary emigration using binomial temporary emigration models and recognize the proper interpretation of parameter estimates in the presence of temporary emigration. Pooling analyses among populations and regions may substantially increase the statistical power for detecting trends in occupancy and abundance and we encourage a collaborative approach to delineating sites and collecting and analyzing data.

Table 1. Median, 2.5th, and 97.5th quantiles of the percent bias in the estimated percent decline of occupancy (ψ) across different true rates of decline and values of detection rate (p). The true percent decline is always negative so positive biases indicate that the estimated rate was smaller (i.e., less negative) than the true rate. Because the estimated percent decline in occupancy was estimated using the estimated intercept (see text for details), the estimated percent decline will be biased relative to the true decline. Data were simulated using a single-season occupancy model.

Median % bias in estimated decline	*2.5th to 97.5th quantile % bias in estimated decline*	*Estimated decline*	*True decline*	*True p*	*Sites*	*Duration*	*Visits*	*True ψ*
-19.99%	-1,157.05–487.44%	-4.00%	-5.0%	0.25	30	10 years	4	10
8.62%	-415.71–311.62%	-5.43%	-5.0%	0.75	30	10 years	4	10
-13.97%	-399.51–233.16%	-8.60%	-10.0%	0.25	30	10 years	4	10
7.46%	-201.08–165.52%	-10.75%	-10.0%	0.75	30	10 years	4	10
6.02%	-143.59–120.06%	-21.20%	-20.0%	0.25	30	10 years	4	10
9.78%	-85.67–83.19%	-21.95%	-20.0%	0.75	30	10 years	4	10

Table 2. The percent reduction in median root-mean-squared-error (RMSE) in estimated abundance for each value of true abundance (λ) across different scenarios of increasing detection rate (*p*) and/or the number of sites visited. Data were simulated using a Poisson-binomial N-mixture abundance model with sites divided into low-abundance (expected abundance = λ) and high-abundance (expected abundance = λ*2) sites.

	$\lambda = 4$	$\lambda = 6$	$\lambda = 10$	$\lambda = 20$
Increasing *p* from 0.10 to 0.75	25%	64%	68%	75%
Increasing no. sites from 3 to 30	63%	61%	59%	57%
Increasing *p* from 0.10 to 0.75 with 5 sites	44%	53%	62%	71%
Increasing no. sites from 3 to 30 with *p* = 0.25	64%	60%	54%	54%
Increasing *p* from 0.10 to 0.75 with 20 sites	55%	65%	73%	82%
Increasing no. sites from 3 to 30 with *p* = 0.75	69%	70%	74%	74%

Table 3. Median, 2.5th, and 97.5th quantiles of the percent bias in the estimated percent decline of abundance (λ) across different true rates of decline and values of detection rate (*p*). The true percent decline is always negative so positive biases indicate that the estimated rate was smaller (i.e., less negative) than the true rate. Because declines in abundance did not start at year 2 and was estimated using the estimated intercept (see text for details), the estimated percent decline will be biased relative to the true decline Data were simulated using a Poisson-binomial N-mixture abundance model with sites divided into low-abundance (expected abundance = λ) and high-abundance (expected abundance = λ*2) sites.

Median % bias in estimated decline	*2.5th to 97.5th quantile % bias in estimated decline*	*Estimated decline*	*True decline*	*True p*	*Sites*	*Duration*	*Visits*	*True λ*
19.03%	286.72%–-210.46%	-4.0%	-5.0%	0.25	20	10 years	4	10
20.77%	118.20%–-61.48%	-4.0%	-5.0%	0.75	20	10 years	4	10
-33.72%	88.88%–-145.56%	-13.4%	-10.0%	0.25	20	10 years	4	10
-33.77%	2.68%–-69.93%	-13.3%	-10.0%	0.75	20	10 years	4	10
-12.57%	41.05%–-62.26%	-22.5%	-20.0%	0.25	20	10 years	4	10
-13.42%	4.26%–-28.37%	-22.7%	-20.0%	0.75	20	10 years	4	10

Figure 1. Examples of the beta distributions used to define probability of emergence (A and B) and the resulting number of individuals available for detection (C–F) from den count simulations. The parameters of the beta distribution are a and b. For this particular simulation, we used 30 sites, six visits per site, a detection rate (p) of 0.75, and a true λ of 100.

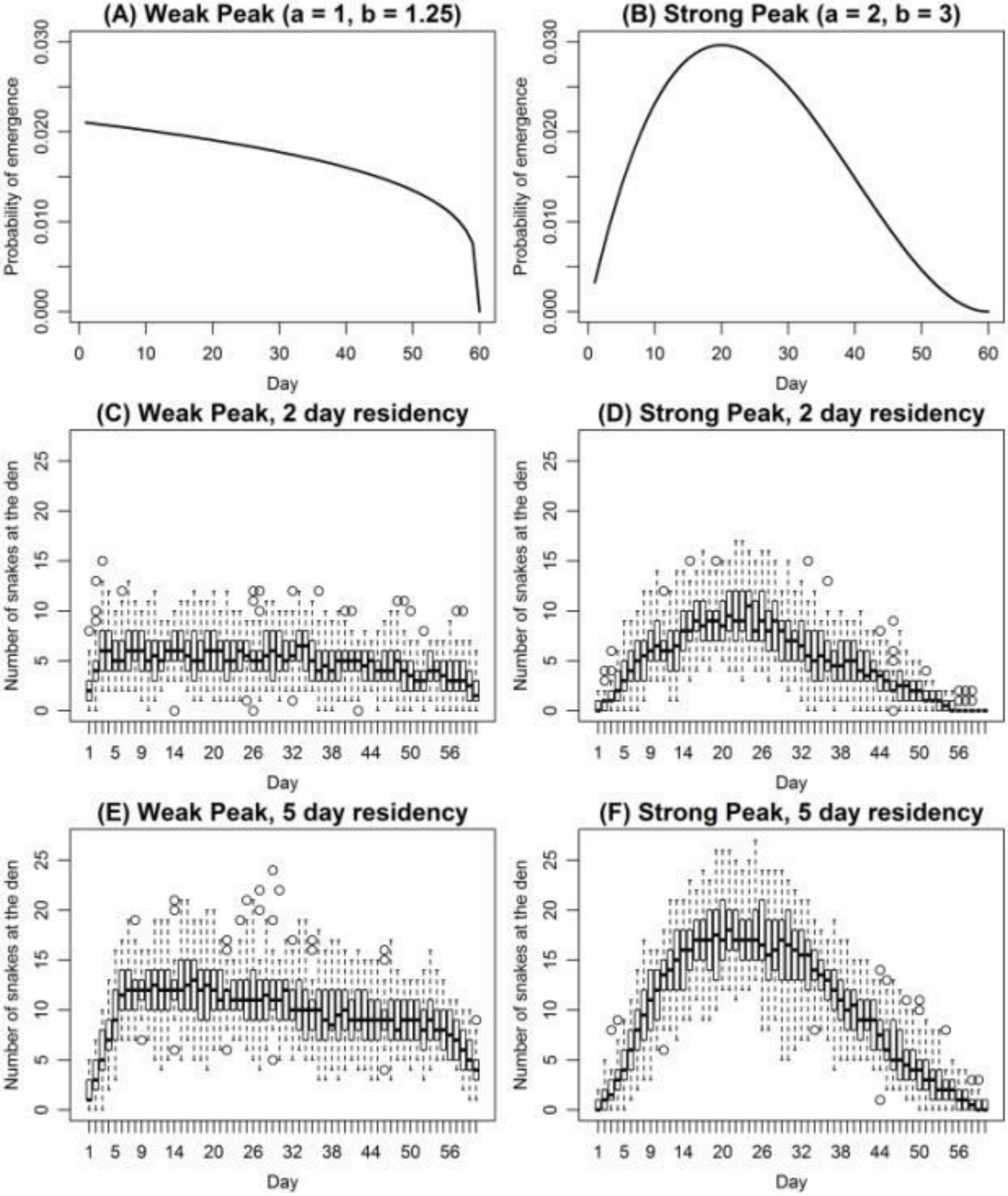


Figure 2. Percent bias in λ from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the strong peak beta distribution and a two day residency period.

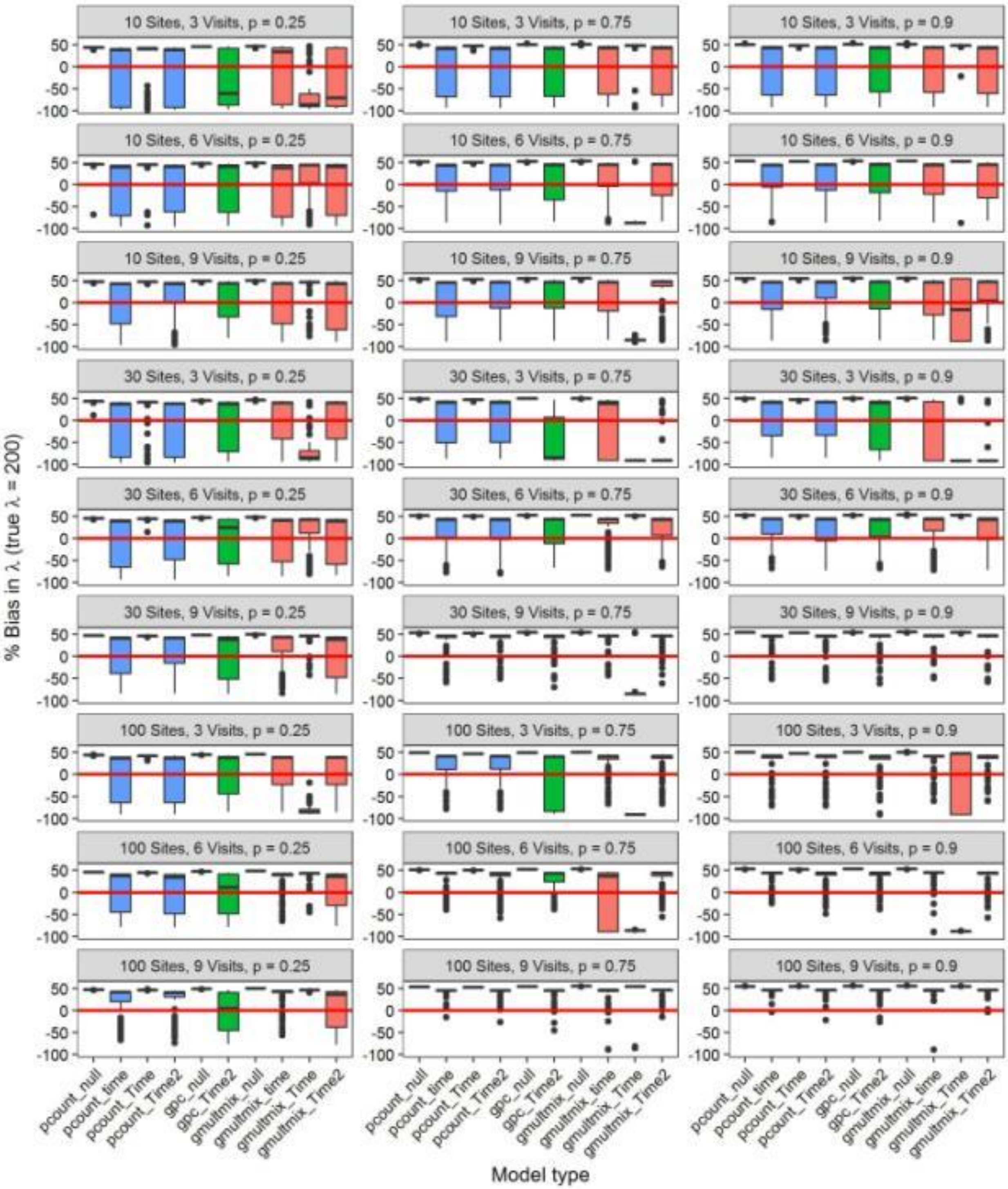


Figure 3. Percent bias in p from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the strong peak beta distribution and a two day residency period. Estimated p is shown for the first, middle, and last visit for each model and scenario.

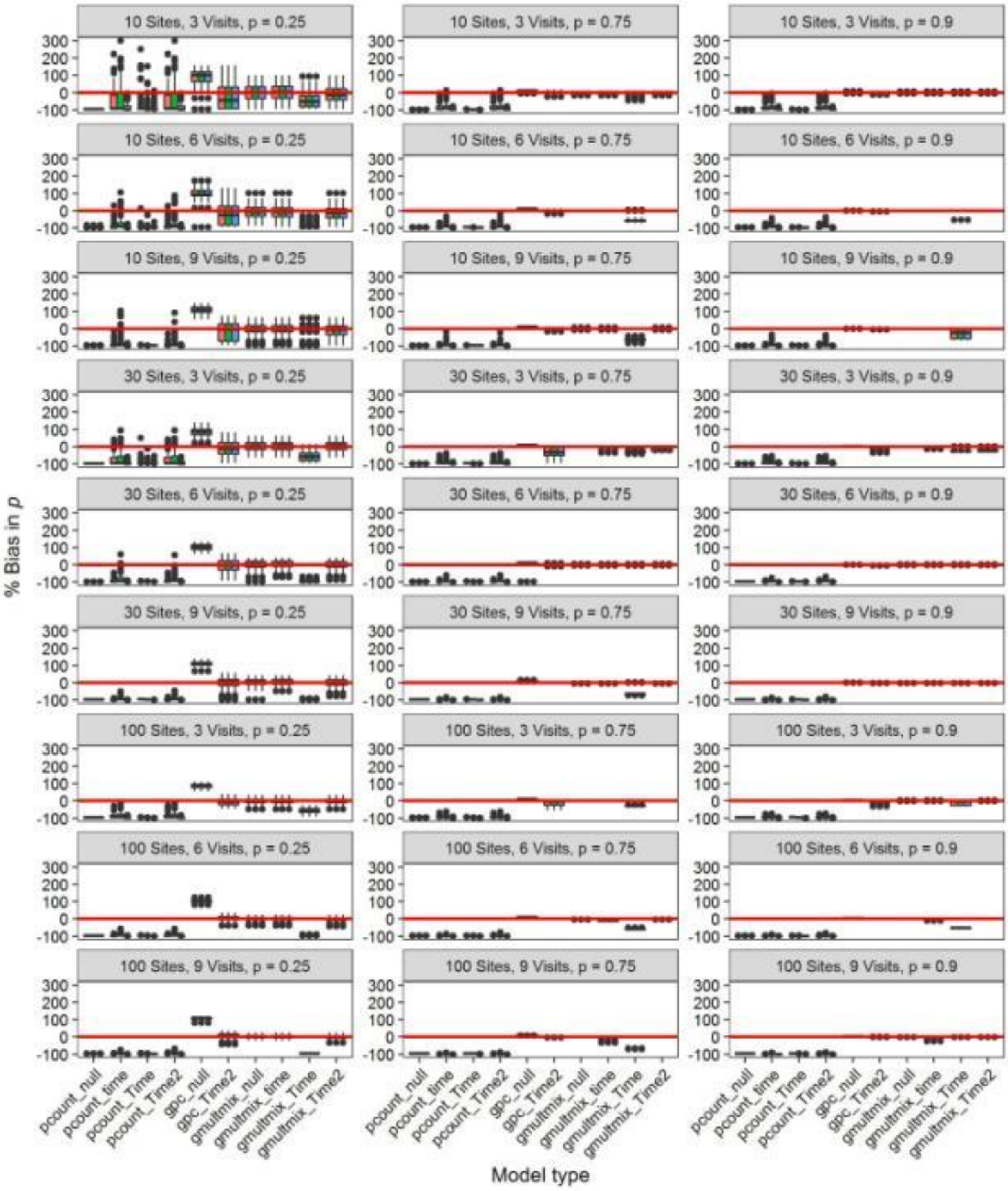


Figure 4. Root mean squared error for estimating occupancy (psi, ψ) under a constant (ψ(.)) model for different values of detection (p) and numbers of sites and visits.

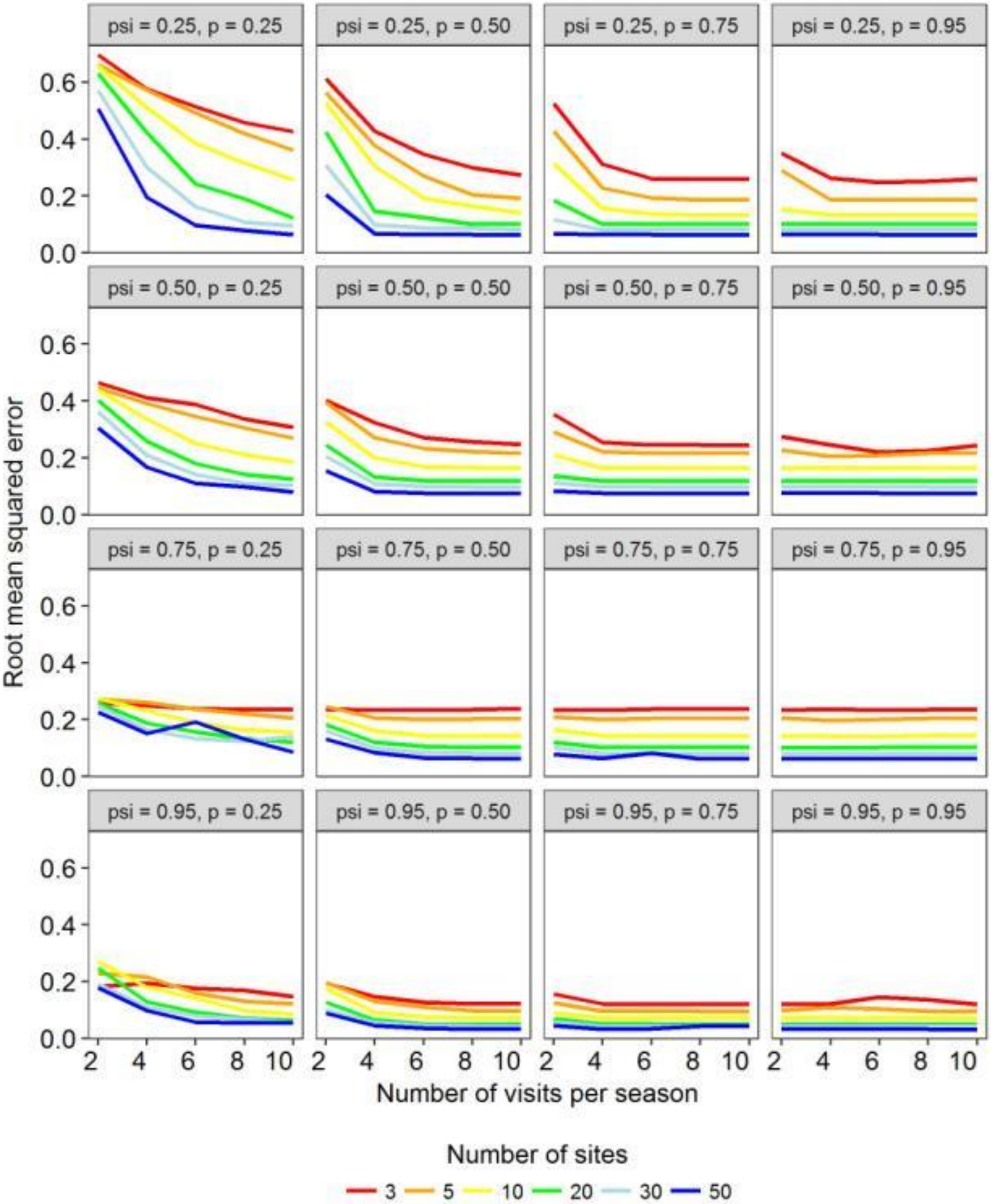


*Figure 5. Percent bias (((truth-estimate)/truth)*100) in estimated occupancy (ψ) under a constant (ψ(.)) model for different values of detection (p) and numbers of sites and visits. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero.*

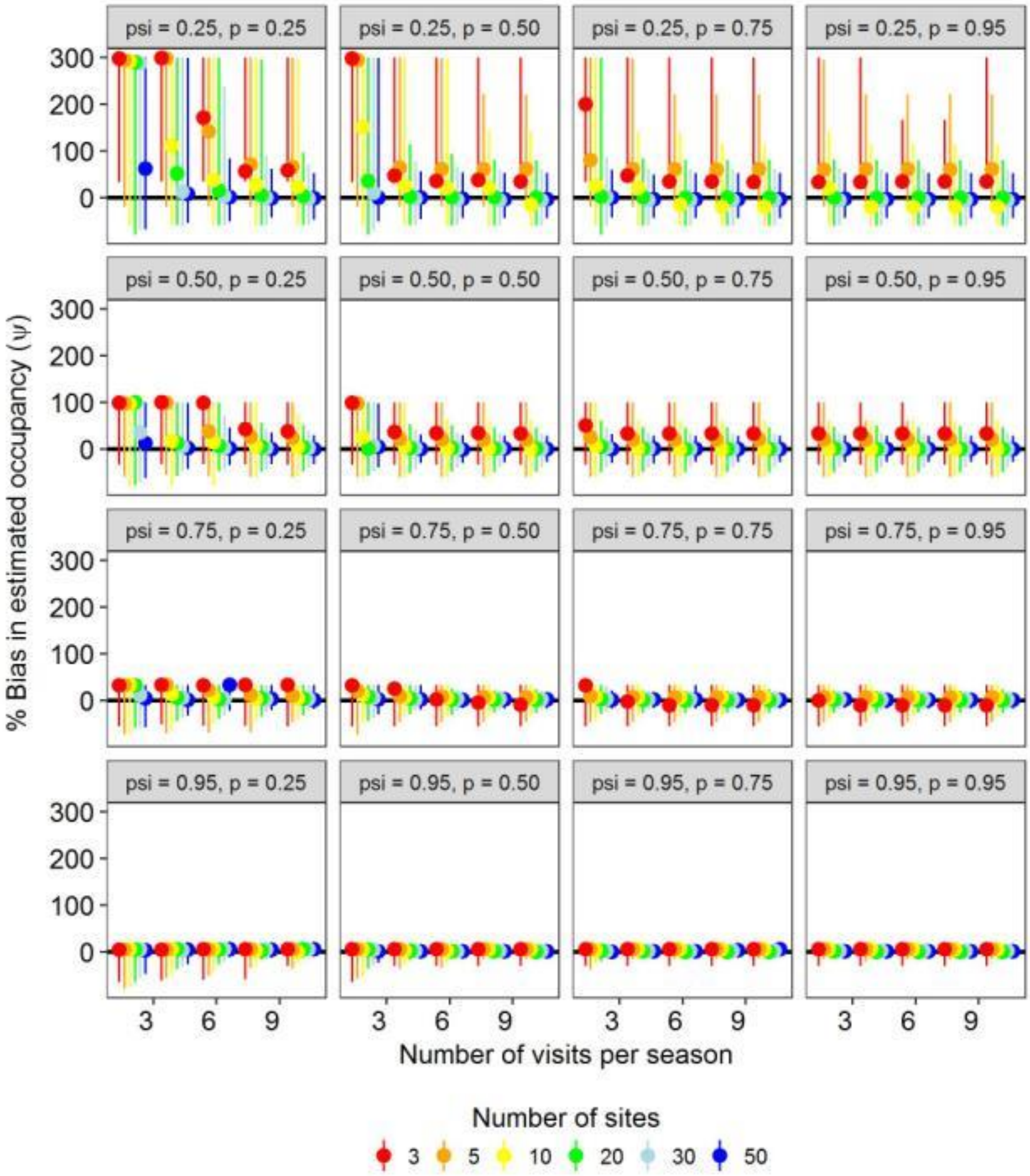


Figure 6. Variation in AICc model weight (w) for data simulated with a linear decline in occupancy (psi, ψ) across different rates and durations of decline and sampling intensities when initial ψ = 0.75 and p = 0.75. Points represent median w and error bars represent the inter-quartile range. The TREND model represents the true model.

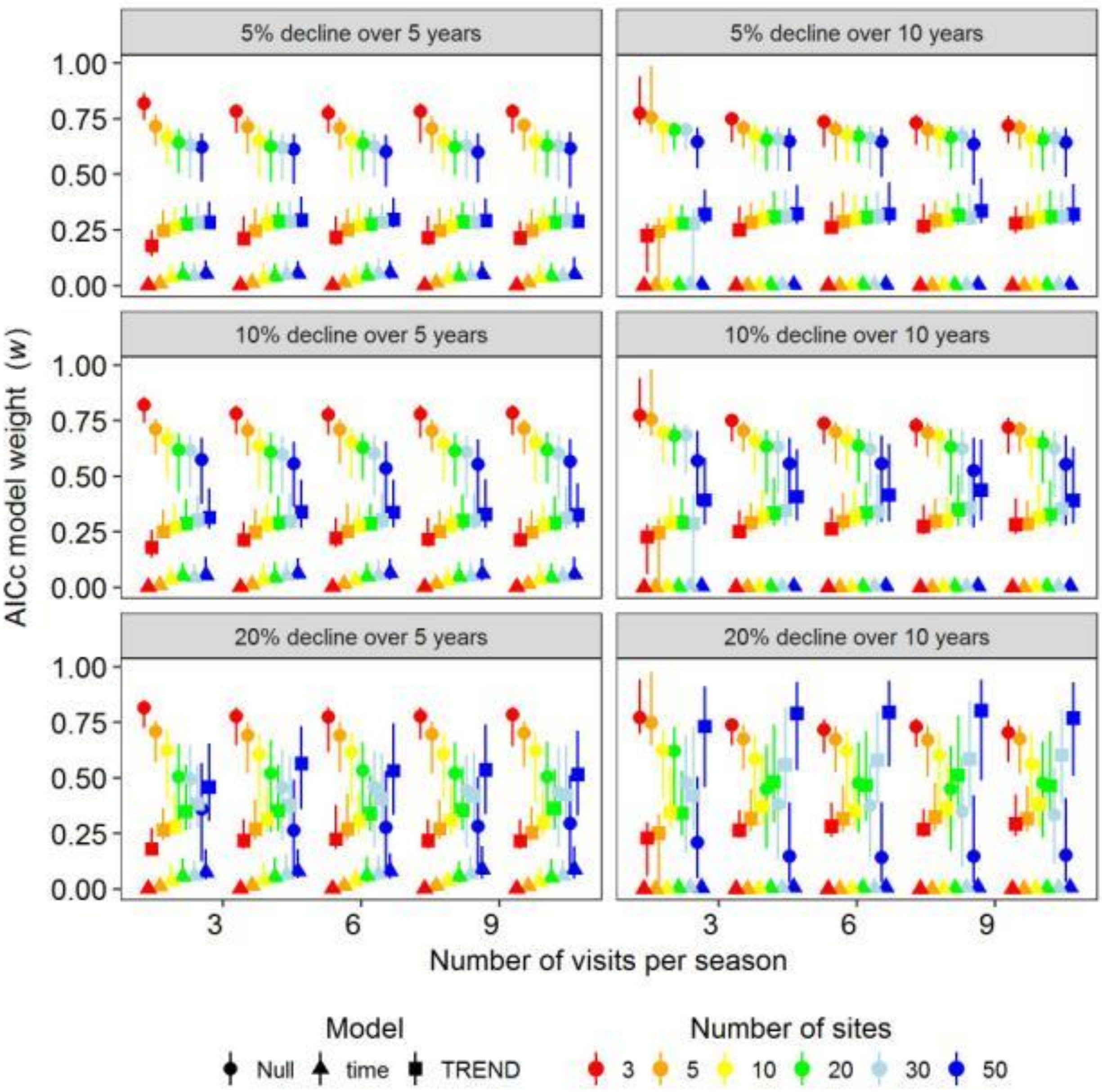


Figure 7. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on occupancy (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.75 and p = 0.75.

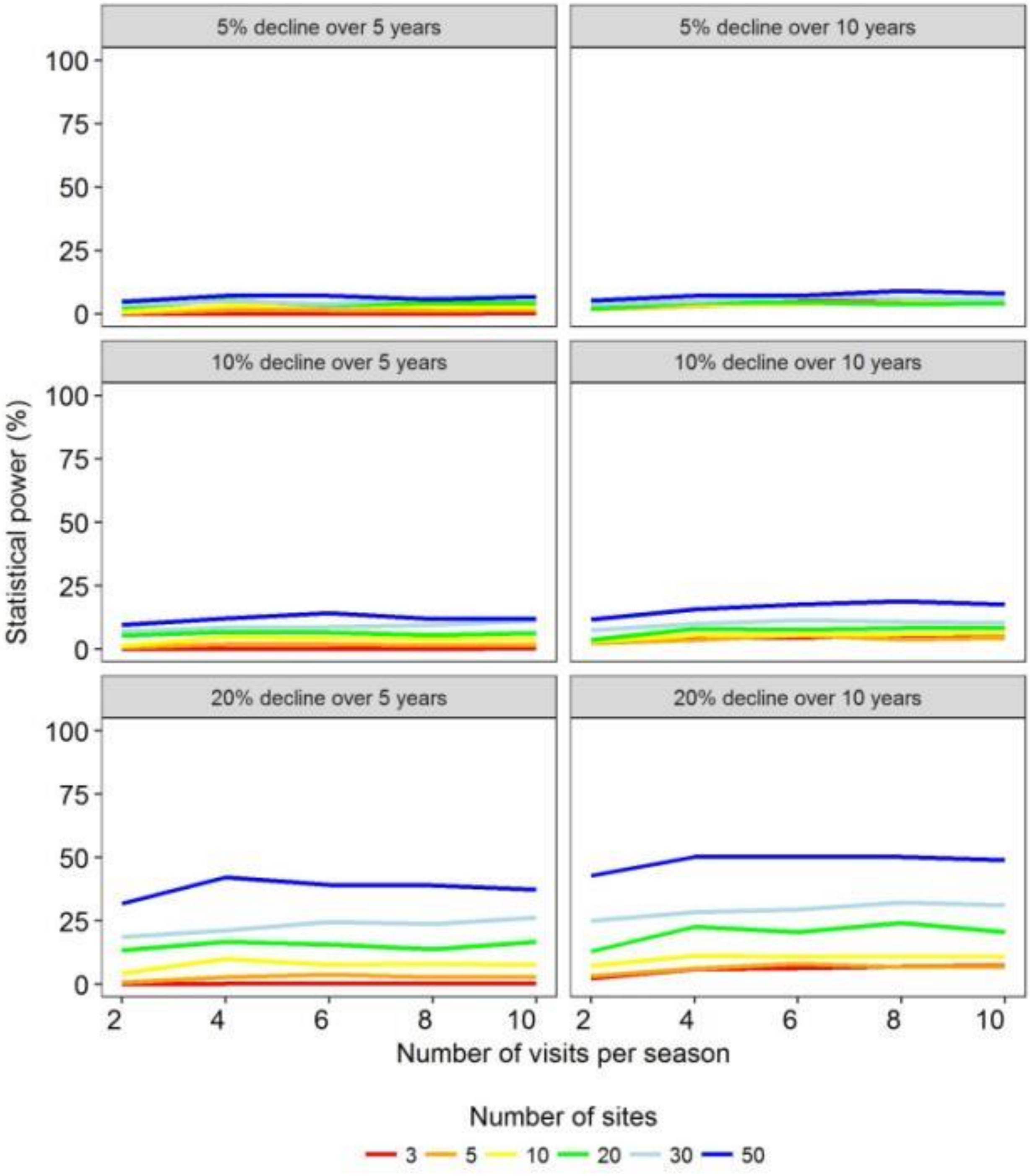


Figure 8. Root mean squared error for the estimated linear trend in occupancy across different rates and durations of decline and sampling intensities when initial ψ = 0.75 and p = 0.75.

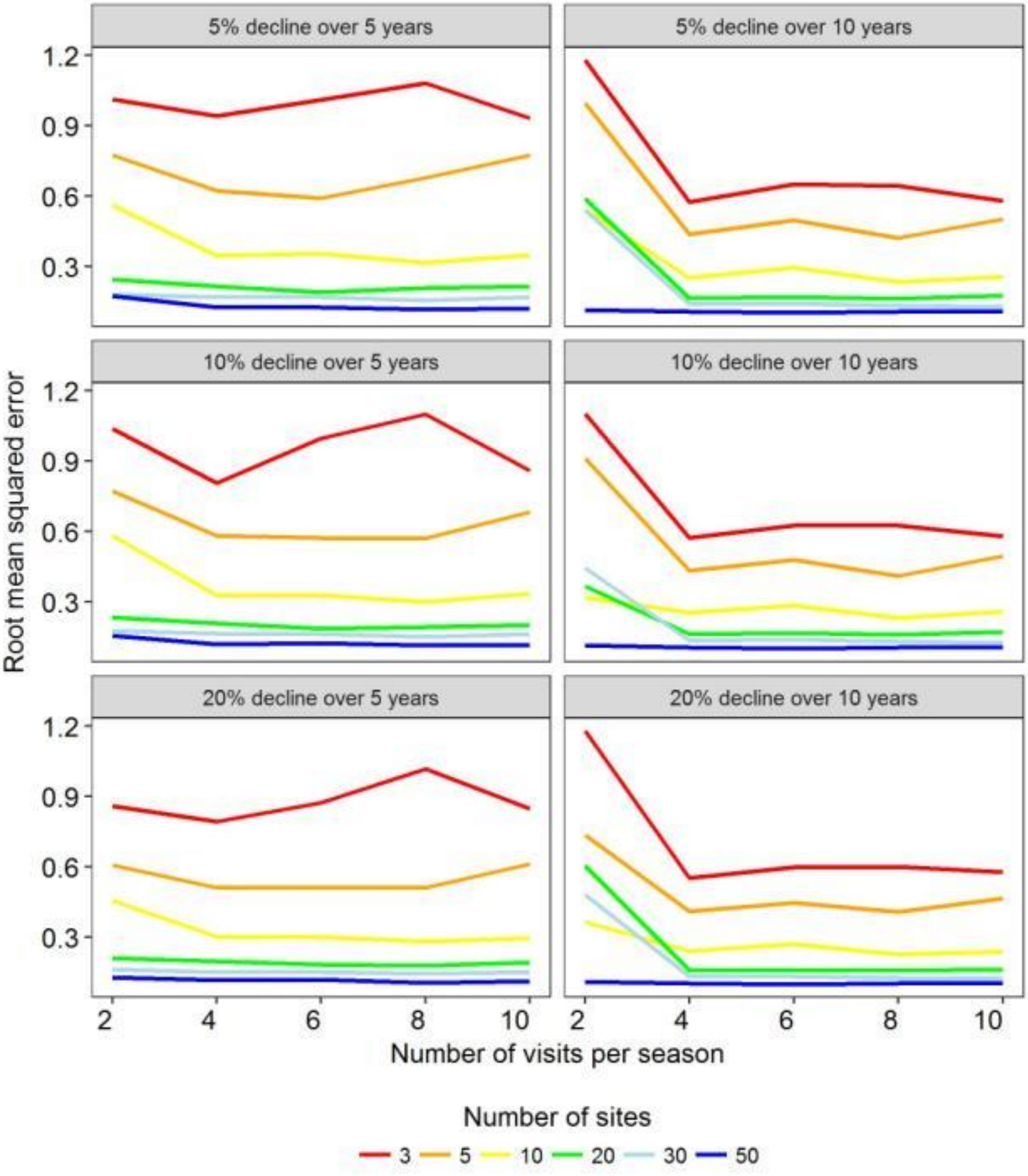


*Figure 9. Percent bias (((truth-estimate)/truth)*100) in the estimated percent decline in occupancy (ψ) under different sampling intensities and rates and durations of declines when initial $\psi = 0.75$ and $p = 0.75$. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5^{th} and 97.5^{th} quantiles. Missing values have 2.5^{th} quantiles <-1,000 The dark horizontal line represents zero.*

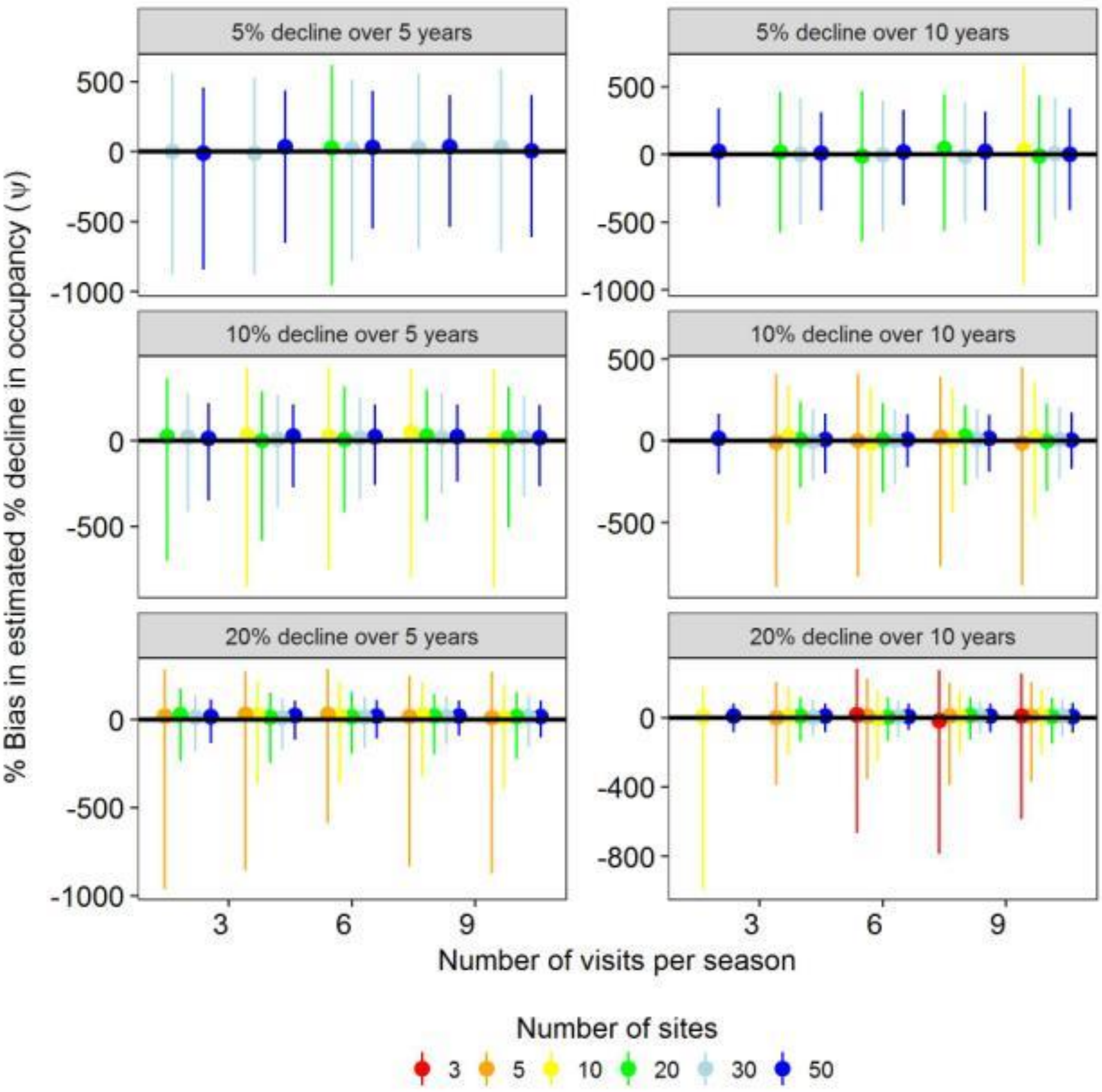


Figure 10. Root mean squared error for estimated abundance (λ) under different sampling intensities and true values of λ and detection probability (p). Data were fit using the pcount model.

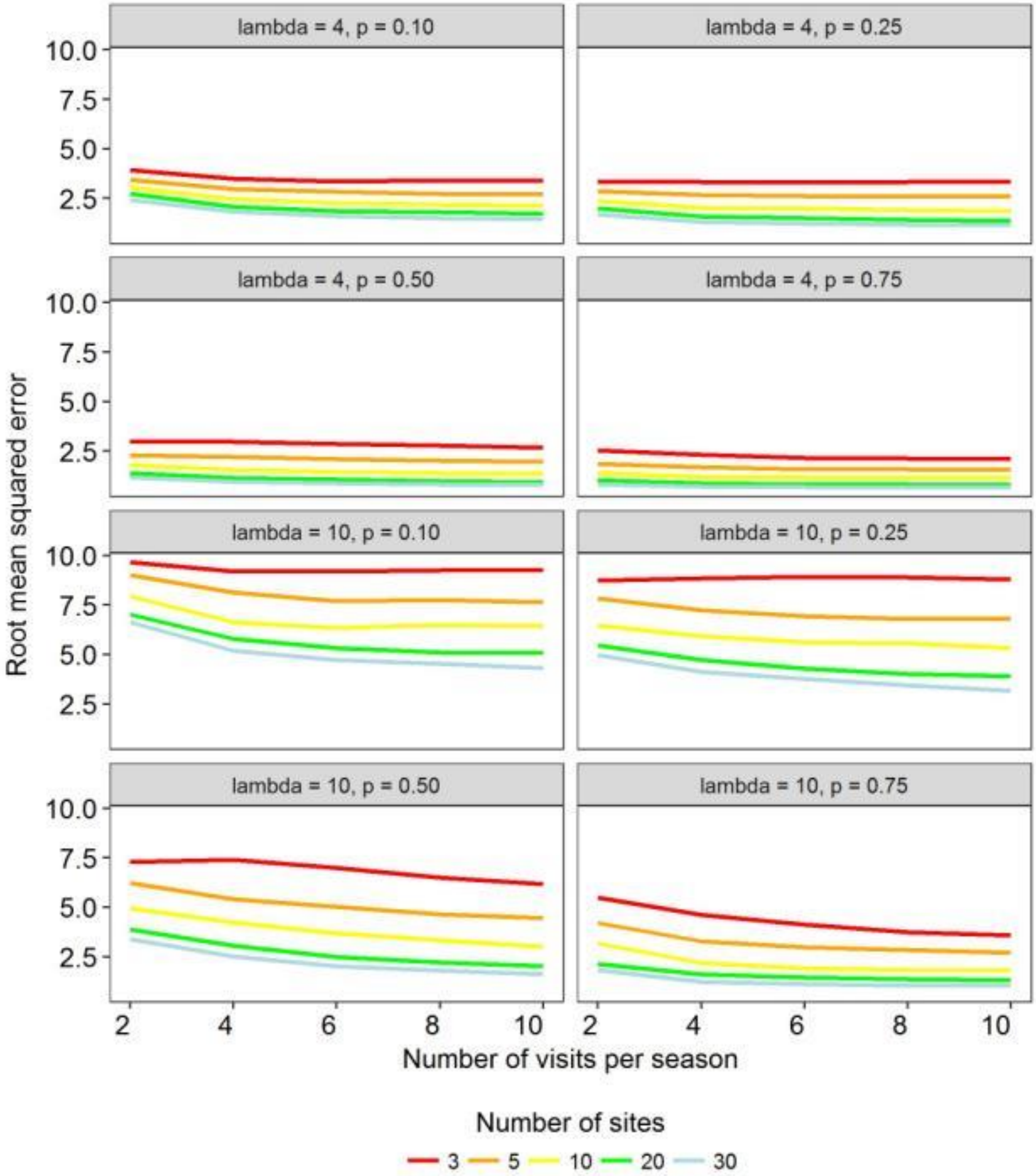


*Figure 11. Percent bias (((truth-estimate)/truth)*100) in estimated abundance (λ) under different sampling intensities and true values of λ and detection probability (p). Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero. Data were fit using the pcount model.*

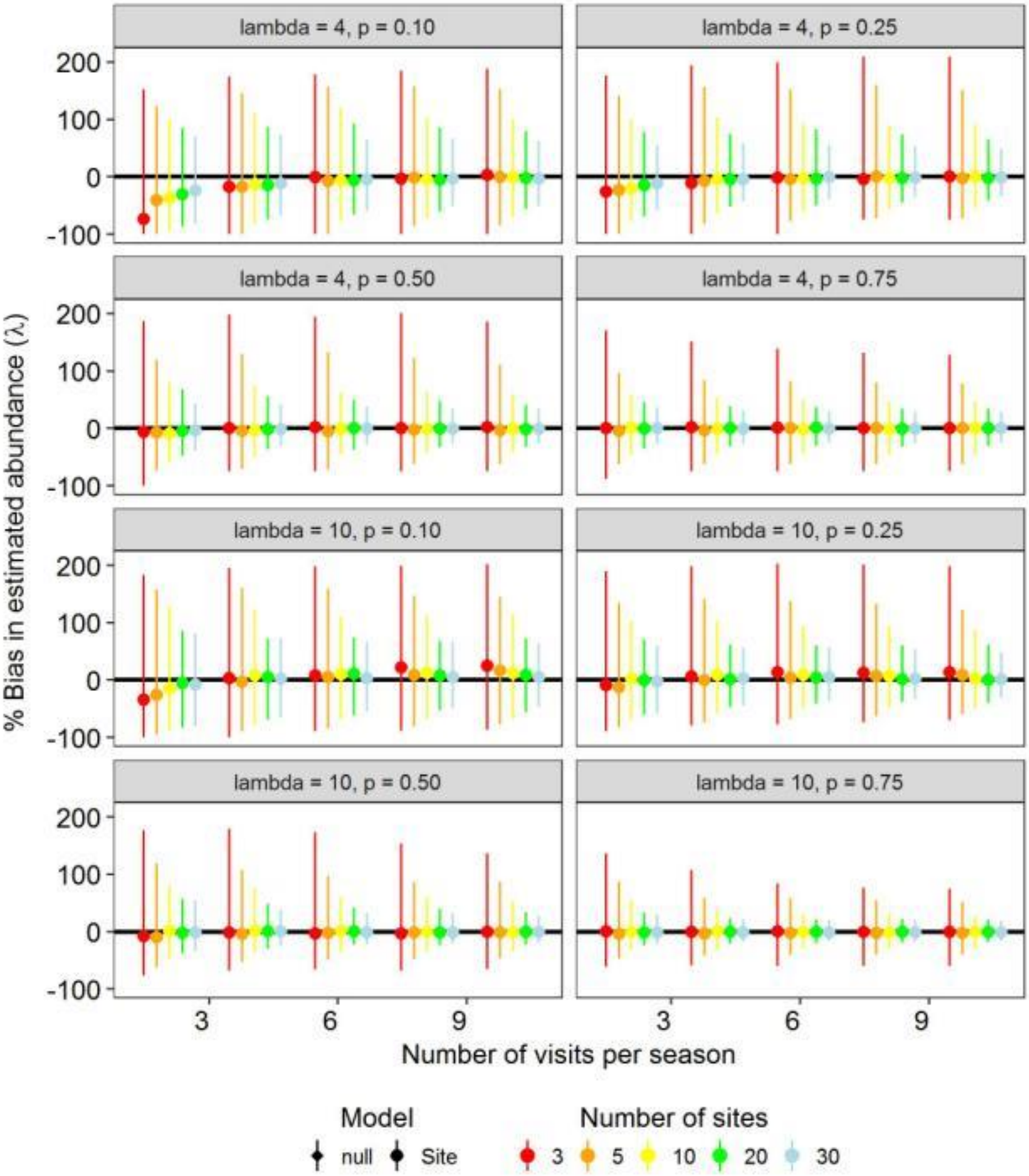


Figure 12. Variation in AICc model weight (w) for data simulated with a linear decline in abundance (lambda, λ) across different rates and durations of decline and sampling intensities when initial λ = 10 and p = 0.25. Points represent median w and error bars represent the inter-quartile range. The TREND model represents the true model.

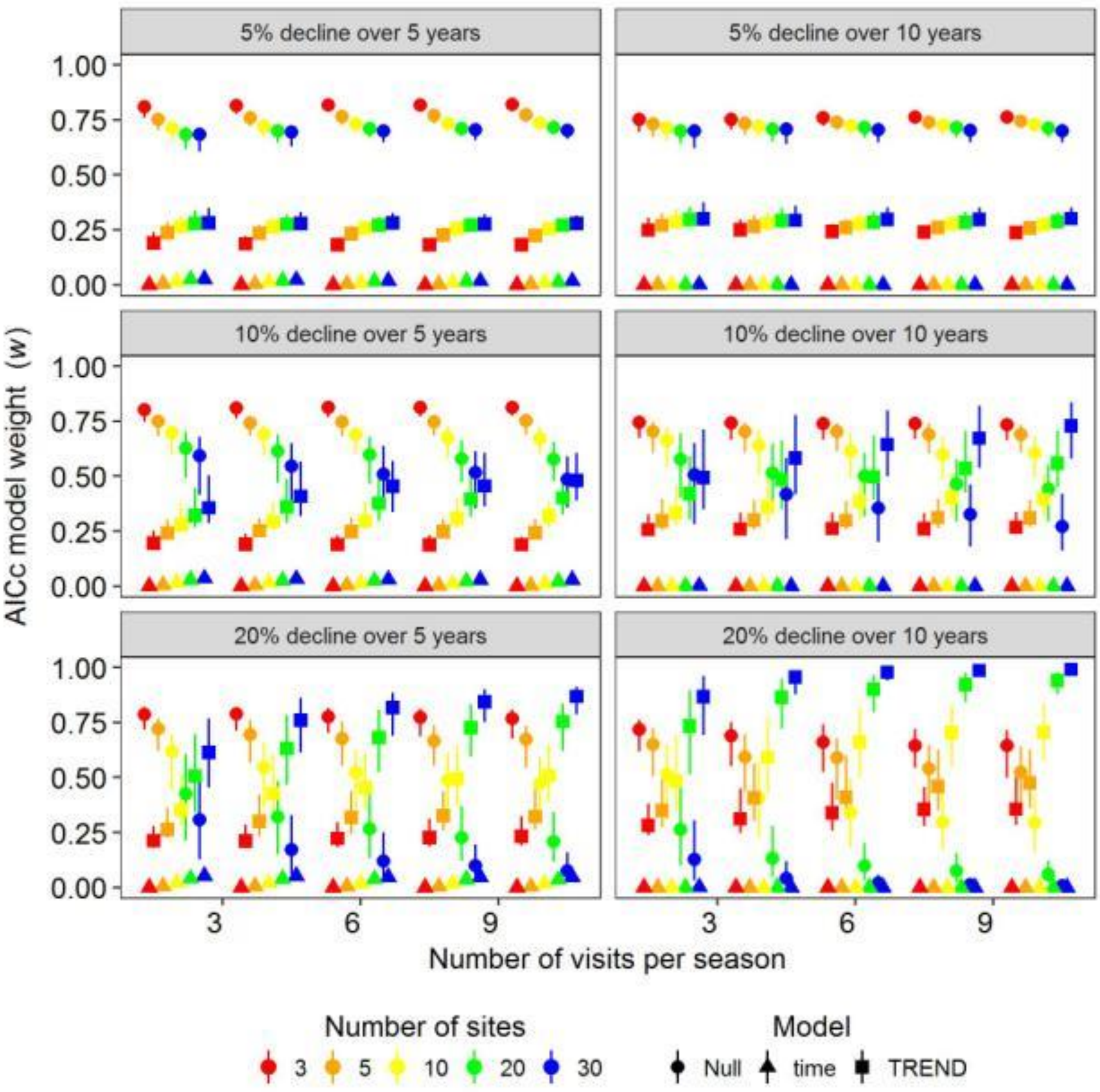


Figure 13. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on abundance (λ~Year) across different rates and durations of decline and sampling intensities when initial λ = 10 and p = 0.25.

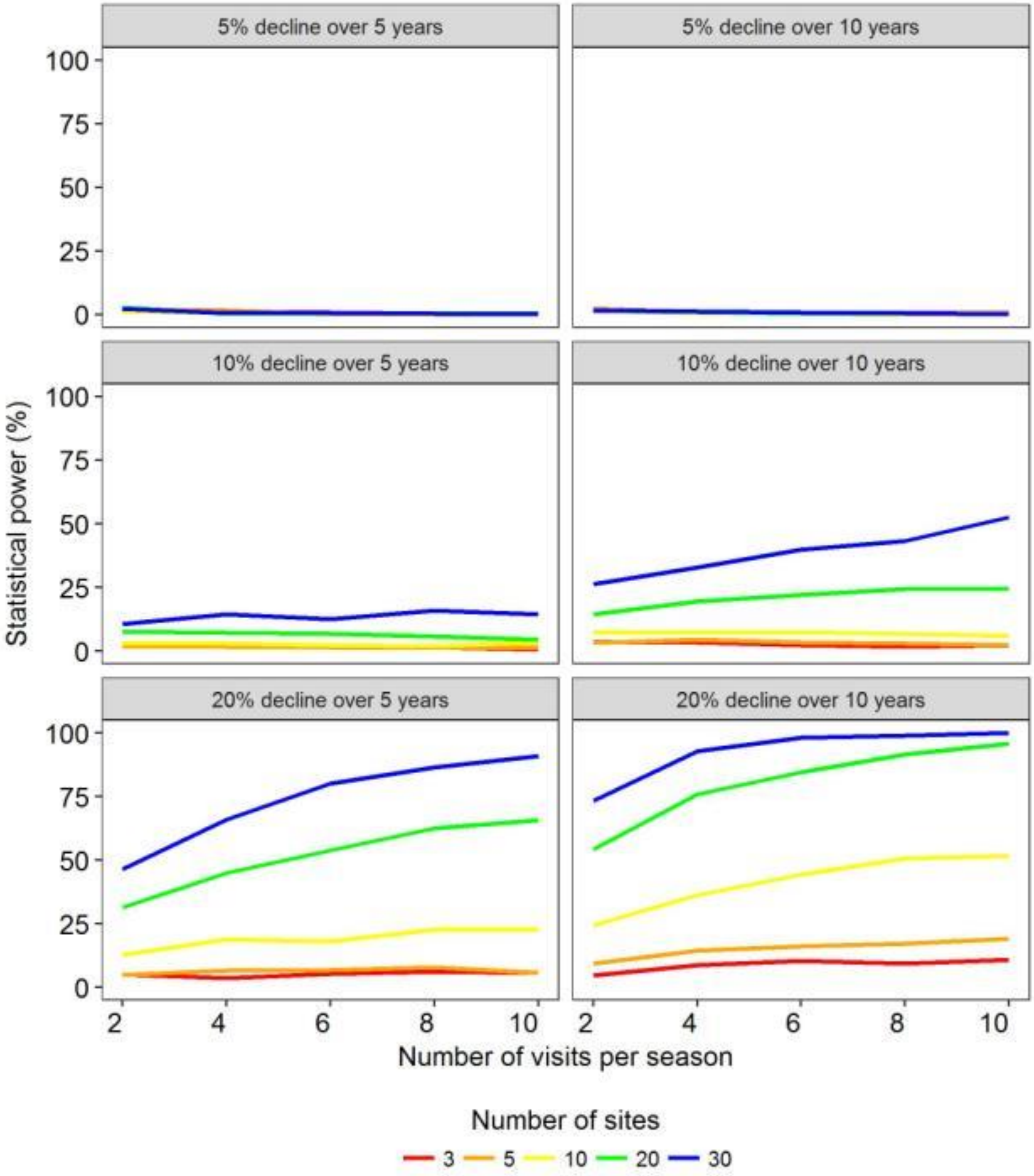


Figure 14. Root mean squared error for the estimated linear trend in abundance across different rates and durations of decline and sampling intensities when initial λ = 10 and p = 0.25.

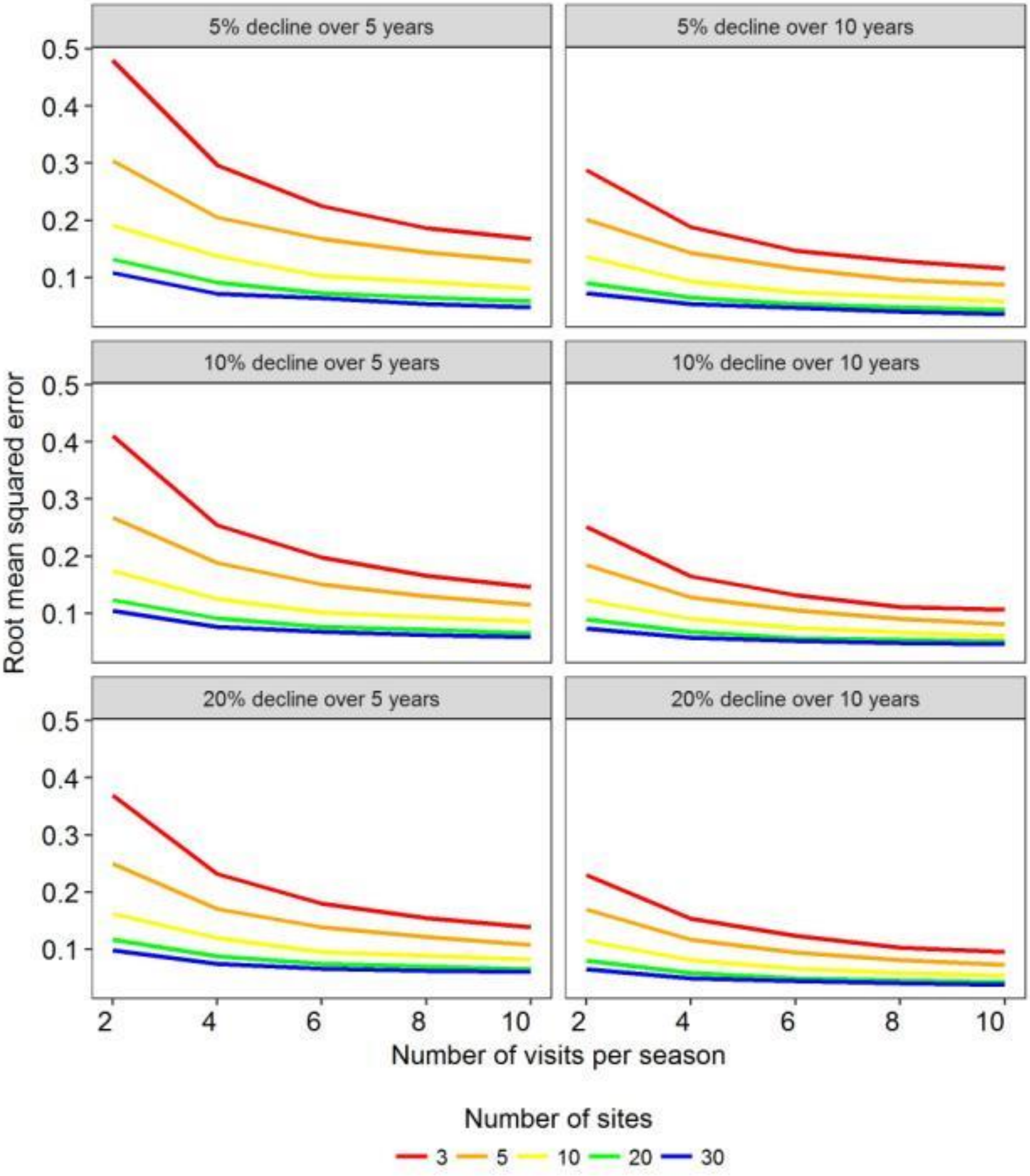
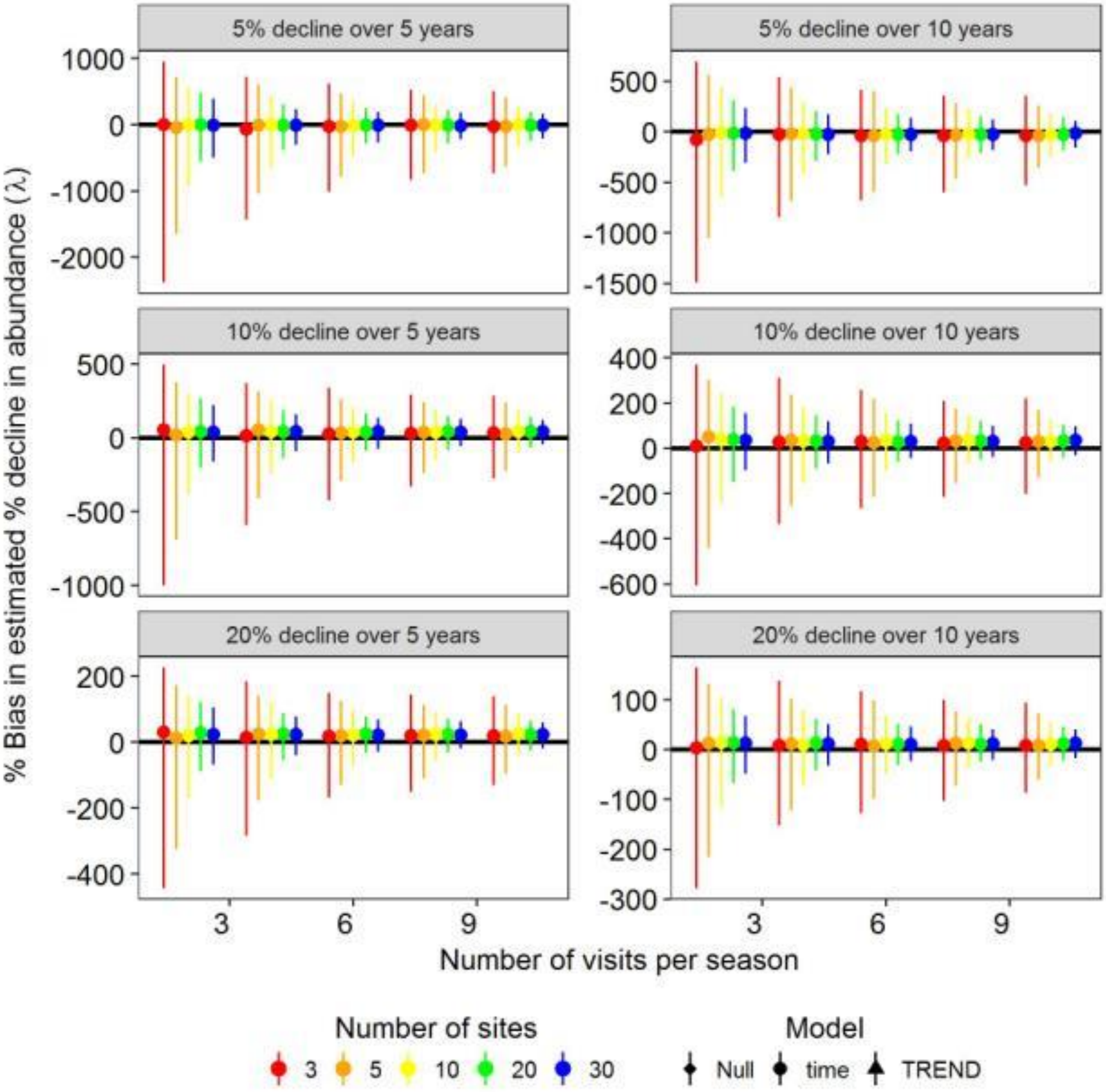


*Figure 15. Percent bias (((truth-estimate)/truth)*100) in the estimated percent decline in abundance (λ) under different sampling intensities and rates and durations of declines when initial λ = 10 and p = 0.25. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero. Data were fit using the pcount model.*



Appendices

Appendix 1

Example of R code used to conduct the den count, occupancy, and abundance simulations

```
# R code for simulating den count data and occupancy/abundance data at gestation/sheddin sites

# Load packages -------------------
library(unmarked)
library(AICcmodavg)
library(ggplot2)

# Den count data ---------

# Source function to simulate observations according to a dependent double-observer design
depDoubPiFun <- function(p) {
  M <- nrow(p)
  pi <- matrix(NA, M, 2)
  pi[,1] <- p[,1]
  pi[,2] <- p[,2]*(1-p[,1])
  return(pi)
}

# Specify parameter values
nSites <- 30
lambda <- 200 # Superpopulation size
nVisits <- 6
p <- 0.75
Season <- 60 # Length of the ingress/egress season in days
nReps <- 2 # Number of secondary surveys within a primary period where population
# is assumed (2 here represents two site visits per day where each site is
# assumed to be closed within a day)
pA <- 0.75 # Detection probability for the primary observer
pB <- 0.75 # Detection probability for the secondary observer
residency <- 2 # Number of days a rattlesnake remains in the survey area
a <- 2 # Parameter for the beta distribution
b <- 3 # Parameter for the beta distribution

# Determine the abundance at each site
print(lambda)
Ni <- rpois(nSites, lambda)
print(summary(Ni))

# Determine the dates of each visit
surveys <- round(seq((Season/nVisits/2),Season,by=Season/nVisits))
```

```
# Determine the number of snakes available at each visit
N_true  <- matrix(Ni,nSites,Season,byrow=F)

# Create a matrix for "time" as a survey-level covariate
time <- matrix(rep(surveys, each=nSites), nSites, nVisits)
time <- (time - (mean(time)))/sd(time)

# Create the distribution of emergence probabilities
x <- (1:Season)/Season
pr_emerg <- dbeta(x, a, b) / sum(dbeta(x, a, b))

plot(pr_emerg, main=paste0("a = ",a,", b = ",b))
abline(v=surveys, lwd=2, lty=3, col="red")

# Simulate the number of rattlesnakes available for detection on each day
N_avail <- N_true
for(j in 1:length(Ni)){
  Nj <- as.vector(rmultinom(1,Ni[j],pr_emerg))
  Nj_avail <- as.vector(filter(Nj,rep(1,residency+1),sides=1))
  Nj_avail[which(is.na(Nj_avail))] <- cumsum(Nj[which(is.na(Nj_avail))])
  N_avail[j,] <- Nj_avail
}

# Boxplot of number of rattlesnakes availabe for detection on each day across sites
boxplot(N_avail, main=paste0("a = ",a,", b = ",b,", residency = ",
                             residency,", lambda = ",lambda))
abline(v=surveys, lwd=2, lty=3, col="red")

N_surveys <- N_avail[,surveys]

# Create umf for pcount
Y_pcount <- matrix(rbinom(length(N_surveys), N_surveys,p), nSites, nVisits)
umf_pcount <- unmarkedFramePCount(y=Y_pcount, obsCovs = list(time=time))

# Create umf for gpcount
Y_gpcount <- N_surveys[,rep(1:ncol(N_surveys), each=nReps)]
Y_gpcount[,] <- NA
for(i in 1:nrow(Y_gpcount)) {
  survey <- 1
  for(j in 1:nVisits) {
    Y_gpcount[i,survey:(survey+1)] <- rbinom(nReps, N_surveys[i,j], p)
    survey <- survey + 2
  }
}
obs_time <- time[,rep(1:ncol(time),each=nReps)]
```

```
umf_gpcount <- unmarkedFrameGPC(y=Y_gpcount,
                                numPrimary=nVisits,
                                yearlySiteCovs = list(time=time),
                                obsCovs = list(obs_time=obs_time))

# Create umf for gmultmix with dependent double observer sampling
obs_time <- time[,rep(1:ncol(time),each=nReps)]
Y_gmultmix <- N_surveys[,rep(1:ncol(N_surveys), each=nReps)]
cp <- c(pA, pB * (1 - pA))
cp[3] <- 1 - sum(cp)
for(i in 1:nrow(Y_gmultmix)) {
  survey <- 1
  for(j in 1:nVisits) {
    Y_gmultmix[i,survey:(survey+1)] <- c(rmultinom(1,
                                         Y_gmultmix[i,survey],
                                         cp)[1:2])
    survey <- survey + 2
  }
}
observer <- matrix(c("A", "B"), nSites, nVisits*2, byrow=TRUE)
obsToY <- matrix(1, 2, 2)
obsToY <- kronecker(diag(nVisits), obsToY)
umf_gmultmix <- unmarkedFrameGMM(y = Y_gmultmix,
                                 obsCovs = list(observer=observer, obs_time=obs_time),
                                 yearlySiteCovs = list(time=time),
                                 numPrimary=nVisits, obsToY=obsToY,
                                 piFun="depDoubPiFun")

# Fit pcount models -----------
pcount_models <- list("null"=pcount(~1 # Detection
                                    ~1, # Abundance,
                                    data=umf_pcount, K=lambda*1.75, mixture="P",
                                    control=list(trace=TRUE, REPORT=1)),
                      "Time"=pcount(~time ~1, data=umf_pcount, K=lambda*1.75, mixture="P",
                                    control=list(trace=TRUE, REPORT=1)),
                      "time"=pcount(~as.factor(time) ~1, data=umf_pcount, K=lambda*1.75,
                                    mixture="P",
                                    control=list(trace=TRUE, REPORT=1)),
                      "Time_2"=pcount(~time+I(time^2) ~1, data=umf_pcount, K=lambda*1.75,
mixture="P",
                                      control=list(trace=TRUE, REPORT=1)))
aictab(pcount_models)

# Estimate abundance
exp(coef(pcount_models$null, type="state"))
exp(coef(pcount_models$Time, type="state"))
```

```
exp(coef(pcount_models$time, type="state"))
exp(coef(pcount_models$Time_2, type="state"))
lambda

# Fit gpcount models -------------------
# Note, these models take considerably longer than pcount models to fit

gpcount_models <- list("null"=gpcount(~1, # Abundance
                        ~1, # Availability/temporary emigration
                        ~1, # Detection
                        data=umf_gpcount, K=lambda*1.75, mixture="P",
                       control=list(trace=TRUE, REPORT=1)),
              "Time"=gpcount(~1, ~time, ~1 data=umf_gpcount, K=lambda*1.75, mixture="P",
                       control=list(trace=TRUE, REPORT=1)),
              "time"=gpcount(~1, ~as.factor(time), ~1, data=umf_gpcount, K=lambda*1.75,
                       mixture="P",
                       control=list(trace=TRUE, REPORT=1)),
              "Time_2"=gpcount(~1, ~time+I(time^2), ~1, data=umf_gpcount, K=lambda*1.75,
mixture="P",
                        control=list(trace=TRUE, REPORT=1)))
aictab(gpcount_models)

# Estimate abundance
exp(coef(gpcount_models$null,type="lambda"))
exp(coef(gpcount_models$Time,type="lambda"))
exp(coef(gpcount_models$time,type="lambda"))
exp(coef(gpcount_models$Time_2,type="lambda"))
lambda

# Fit the gmultmix models -----------------
# Note, these models take longer to fit than pcount models but not as long as gpcount models!

gmultmix_models <- list("null"=gmultmix(~1, # Abundance
                        ~1, # Availability/temporary emigration
                        ~1, # Detection
                        data=umf_gmultmix, K=lambda*1.75, mixture="P",
                        control=list(trace=TRUE, REPORT=1)),
               "Time"=gmultmix(~1, ~time, ~1, data=umf_gmultmix, K=lambda*1.75,
                         mixture="P",control=list(trace=TRUE, REPORT=1)),
               "time"=gmultmix(~1, ~as.factor(time), ~1, data=umf_gmultmix, K=lambda*1.75,
                        mixture="P",
                        control=list(trace=TRUE, REPORT=1)),
               "Time_2"=gmultmix(~1, ~time+I(time^2), ~1, data=umf_gmultmix,
K=lambda*1.75, mixture="P",
                          control=list(trace=TRUE, REPORT=1))
               )
```

```
aictab(gmultmix_models)

# Estimate abundance
exp(coef(gmultmix_models$null,type="lambda"))
exp(coef(gmultmix_models$Time,type="lambda"))
exp(coef(gmultmix_models$time,type="lambda"))
exp(coef(gmultmix_models$Time_2,type="lambda"))
lambda

# Occupancy data -----------------

# Specify true parameter values, number of sites and visits -----------

# Use a large number of sites (>>1000) to obtain estimates close to the true parameter values
nSites <- 5000
nVisits <- 4
psi <- 0.75
psi_2 <- 0.50 # occupancy of the low-occupancy sites
p <- 0.95
trend <- -0.20
duration <- 10

# Simulate data for NULL model --------

psi_null_true <- matrix(NA, nrow = nSites, ncol = nVisits)
psi_null <- rbinom(n = nSites, size = 1, prob = psi)
for(j in 1:nVisits){
  psi_null_true[,j] <- rbinom(n = nSites, size = 1, prob = psi_null*p)
}

umf_null <- unmarkedFrameOccu(y=psi_null_true)

null <- occu(~1  # Detection
             ~1, # Occupancy
             umf_null)

# Estimated occupancy
plogis(coef(null, type="state"))
psi

# Estimated detection
plogis(coef(null, type="det"))
p

# Simulate data for SITE model --------
```

```
psi_Site_true <- matrix(NA, nrow = nSites, ncol = nVisits)
psi_Site_high <- rbinom(n = floor(nSites/2), size = 1, prob = psi)
psi_Site_low <- rbinom(n = ceiling(nSites/2), size = 1, prob = psi_2)
psi_Site <- c(psi_Site_high,psi_Site_low)
Site_Covs <- data.frame(Site=c(rep("A",floor(nSites/2)),rep("B",ceiling(nSites/2))))
for(j in 1:nVisits){
  psi_Site_true[,j] <- rbinom(n = nSites, size = 1, prob = psi_Site*p)
}

umf_psiSite <- unmarkedFrameOccu(y=psi_Site_true, siteCovs = Site_Covs)

site_mods <- list("null_site" = occu(~1 ~1, umf_psiSite),
                  "site_site" = occu(~1 ~Site, umf_psiSite))

aictab(site_mods)

# Estimated occupancy of high-occupancy sites
plogis(coef(site_mods$site_site)["psi(Int)"])
psi

# Estimated occupancy of low-occupancy sites
plogis(coef(site_mods$site_site)["psi(Int)"]+coef(site_mods$site_site)["psi(SiteB)"])
psi_2

# Estimated detection
plogis(coef(site_mods$site_site, type="det"))
p

# Simulate data for the TREND model ---------

psi_TREND_true <- matrix(NA, ncol = nVisits, nrow = nSites*duration)
psi_time <- matrix(NA, nrow = nSites, ncol = duration)
psi_time[,1] <- rbinom(n = nSites, size = 1, prob = psi)
print(psi)
for(j in 1:(duration-1)){
  trend_slope <- (psi*trend)/(duration-1)
  psi_j <- (psi + ((j)*trend_slope))
  print(psi_j)
  psi_time[,j+1] <- rbinom(n = nSites, size = 1, prob = psi_j)
}
i.row <- 1
for(i in 1:duration){
  for(j in 1:nVisits){
    psi_TREND_true[i.row:(i.row+nSites-1),j] <- rbinom(n = nSites,
                                                     size = 1,
                                                     prob = psi_time[,i]*p)
```

```
  }
 i.row <- i.row + nSites
}

Site_Covs_TREND <- data.frame(Year=rep(1:duration,each=nSites))
umf_psi_TREND <- unmarkedFrameOccu(y=psi_TREND_true,
                              siteCovs = Site_Covs_TREND)

trend_mods <- list("null_trend" = occu(~1 ~1, umf_psi_TREND),
              "Year_trend" = occu(~1 ~Year, umf_psi_TREND),
              "year_trend" = occu(~1 ~as.factor(Year), umf_psi_TREND))

aictab(trend_mods)

# Estimated occupancy at time = 0
plogis(coef(trend_mods$Year_trend)["psi(Int)"])
psi

# Estimated percent decline in occupancy. Note that this will be biased because
# percent change is calculated relative to the intercept rather than year 1.
beta0 <- coef(trend_mods$Year_trend)["psi(Int)"]
beta1 <- coef(trend_mods$Year_trend)["psi(Year)"]
as.numeric((plogis(beta0)-(plogis(beta0+(beta1*duration))))/plogis(beta0))*-1
trend

# Estimated detection
plogis(coef(trend_mods$Year_trend, type="det"))
p

# Extract p-value for the continuous effect of Year for power analysis
as.numeric(summary(trend_mods$Year_trend)$state["Year","P(>|z|)"])

# Simulate data for the TREND+SITE model ---------

psi_TREND_Site_true <- matrix(NA, ncol = nVisits, nrow = nSites*duration)
psi_Site_time <- matrix(NA, nrow = nSites, ncol = duration)
psi_Site_time[1:(floor(nSites/2)),1] <- rbinom(n = length(1:(floor(nSites/2))),
                                      size = 1, prob = psi)
psi_Site_time[((floor(nSites/2))+1):nSites,1] <- rbinom(n = length(((floor(nSites/2))+1):nSites),
                                            size = 1, prob = psi_2)
Site_TREND_Covs <- data.frame(Site=c(rep("A",length(1:(floor(nSites/2)))),
                              rep("B",length(((floor(nSites/2))+1):nSites))),
                         Year=c(rep(1:duration,each=nSites)))
print(psi)
# Simulates the occupancy status of each site based on the decline in occupancy over time
# This code prints the occupancy for high and low sites for year 2 through year (duration)
```

```
for(j in 1:(duration-1)){

  trend_slope1 <- (psi*trend)/(duration-1)
  trend_slope2 <- (psi_2*trend)/(duration-1)
  psi_j <- (psi + (j)*trend_slope1)
  psi_2_j <- (psi_2 + (j)*trend_slope2)

  cat("Year ",j+1,", psi =",psi_j,"\n")
  cat("Year ",j+1,", psi_2 =",psi_2_j,"\n")

  psi_Site_time[1:(floor(nSites/2)),j+1] <- rbinom(n = length(1:(floor(nSites/2))),
                                                   size = 1, prob = psi_j)
  psi_Site_time[((floor(nSites/2))+1):nSites,j+1] <- rbinom(n =
length(((floor(nSites/2))+1):nSites),
                                                            size = 1, prob = psi_2_j)

}

i.row <- 1
for(i in 1:duration){
  for(j in 1:nVisits){
    psi_TREND_Site_true[i.row:(i.row+nSites-1),j] <- rbinom(n = nSites,
                                                            size = 1,
                                                            prob = psi_Site_time[,i]*p)
  }
  i.row <- i.row + nSites
}

Site_TREND_Covs$year_factor <- as.factor(Site_TREND_Covs$Year)

umf_psi_TREND_Site <- unmarkedFrameOccu(y=psi_TREND_Site_true,
                                        siteCovs = Site_TREND_Covs)

trend_site_mods <- list("null_trend_site" = occu(~1 ~1, umf_psi_TREND_Site),
                        "Year_trend_site" = occu(~1 ~Year, umf_psi_TREND_Site),
                        "year_trend_site" = occu(~1 ~as.factor(Year), umf_psi_TREND_Site),
                        "site_trend_site" = occu(~1 ~Site, umf_psi_TREND_Site),
                        "site_year_trend_site" = occu(~1 ~Site+as.factor(Year), umf_psi_TREND_Site),
                        "site_Year_trend_site" = occu(~1 ~Site+Year, umf_psi_TREND_Site))
aictab(trend_site_mods)

# Estimated occupancy at year 1 at high-occupancy site
plogis(coef(trend_site_mods$site_Year_trend_site)["psi(Int)"])
psi

# Estimated occupancy at year 1 at low-occupancy site
```

```
plogis(coef(trend_site_mods$site_Year_trend_site)["psi(Int)"]+
        coef(trend_site_mods$site_Year_trend_site)["psi(SiteB)"])
psi_2

# Estimated percent change in occupancy at high-occupancysite
beta0 <- coef(trend_site_mods$site_Year_trend_site)["psi(Int)"]
beta1 <- coef(trend_site_mods$site_Year_trend_site)["psi(Year)"]
as.numeric(((plogis(beta0))-(plogis(beta0+(beta1*duration))))/(plogis(beta0)))*-1
trend

# Estimated detection
plogis(coef(trend_site_mods$site_Year_trend_site, type="det"))
p

# Extract p-value for the continuous effect of Year for power analysis
as.numeric(summary(trend_site_mods$site_Year_trend_site)$state["Year","P(>|z|)"])

# Abundance -----------------------

# Specify true parameter values, number of sites and visits ----------

nSites <- 5000
nVisits <- 8
lambda <- 10
p <- 0.95
lambda_2 <- 5
trend <- -0.10
duration <- 10

# Determine the abundance at each site at time t = 1 -----

Ni <- rpois(nSites, lambda)

# Set K based on lambda
max_K <- ifelse(lambda<10,50,100)

# Simulate data for the SITE model -------------------

N_1 <- rpois(floor(nSites/2), lambda)
N_2 <- rpois(ceiling(nSites/2), lambda_2)
Ni <- c(N_1,N_2)
site_Covs <- data.frame("Site"=c(rep("Low",length(N_1)),rep("High",length(N_2))))
site_Covs$Site <- factor(site_Covs$Site,levels=c("Low","High"))

N_true  <- matrix(Ni,nSites,nVisits,byrow=F)
```

```
Y_pcount <- matrix(rbinom(nSites*nVisits, N_true, p), nSites, nVisits)
umf_pcount <- unmarkedFramePCount(y=Y_pcount,
                                  siteCovs=site_Covs)

site_models <- list("null"=pcount(~1  # Detection
                                  ~1, # Abundance
                                  data=umf_pcount, K=max_K),
                    "site"=pcount(~1 ~Site, data=umf_pcount, K=max_K)
)
aictab(site_models)

# Estimate lambda at the high-abundance sites
exp(coef(site_models$site, type="state"))[1]
lambda

# Estimate lambda at the low-abundance sites
exp(coef(site_models$site)[1]+coef(site_models$site)[2])
lambda_2

# Estimate detection
plogis(coef(site_models$site, type="det"))
p

# Simulate data for the TREND model ----------

N_TREND_true <- matrix(NA, ncol = nVisits, nrow = nSites*duration)
N_time <- matrix(NA, nrow = nSites, ncol = duration)
N_time[,1] <- Ni
print(N_time)
for(j in 1:(duration-1)){
  trend_slope <- (Ni*trend)/(duration-1)
  N_j <- (Ni + ((j)*trend_slope))
  print(N_j)
  N_time[,j+1] <- round(N_j,0)
}
i.row <- 1
for(i in 1:duration){
  for(j in 1:nVisits){
    N_TREND_true[i.row:(i.row+nSites-1),j] <- rbinom(nSites,
                                              size = N_time[,i],
                                              prob = p)
  }
  i.row <- i.row + nSites
}

Site_Covs_TREND <- data.frame(Year=rep(1:duration,each=nSites))
```

```
umf_N_TREND <- unmarkedFramePCount(y=N_TREND_true,
                          siteCovs=Site_Covs_TREND)

trend_models <- list("null"=pcount(~1 ~1,
                          data=umf_N_TREND, K=max_K),
                "trend"=pcount(~1 ~Year, data=umf_N_TREND, K=max_K),
                "year"=pcount(~1 ~as.factor(Year), data=umf_N_TREND, K=max_K)
                )
aictab(trend_models)

# Estimate lambda at time = 0
exp(coef(trend_models$trend, type="state"))[1]

# Estimate percent change in lambda
# Note that this will be biased low (i.e., more extreme) because the simulation code
# does not start the decline in lambda until after year 2 and percent change is calculated
# relative to the intercept rather than year 1.
beta0 <- (coef(trend_models$trend)["lam(Int)"])
beta1 <- (coef(trend_models$trend)["lam(Year)"])
as.numeric((exp(beta0)-(exp(beta0+(beta1*duration))))/exp(beta0))*-1
trend

# Estimate detection
plogis(coef(trend_models$trend, type="det"))
p

# Extract p-value for the continuous effect of year for power analysis
summary(trend_models$trend)$state["Year","P(>|z|)"]
```

Appendix 2

Additional graphical results of simulation studies evaluating the use of den counts to estimate timber rattlesnake abundance and presence-absence and count data to estimate occupancy and abundance at timber rattlesnake gestation/shedding sites.

Figure S1. Percent bias in λ from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the strong peak beta distribution and a five day residency period.

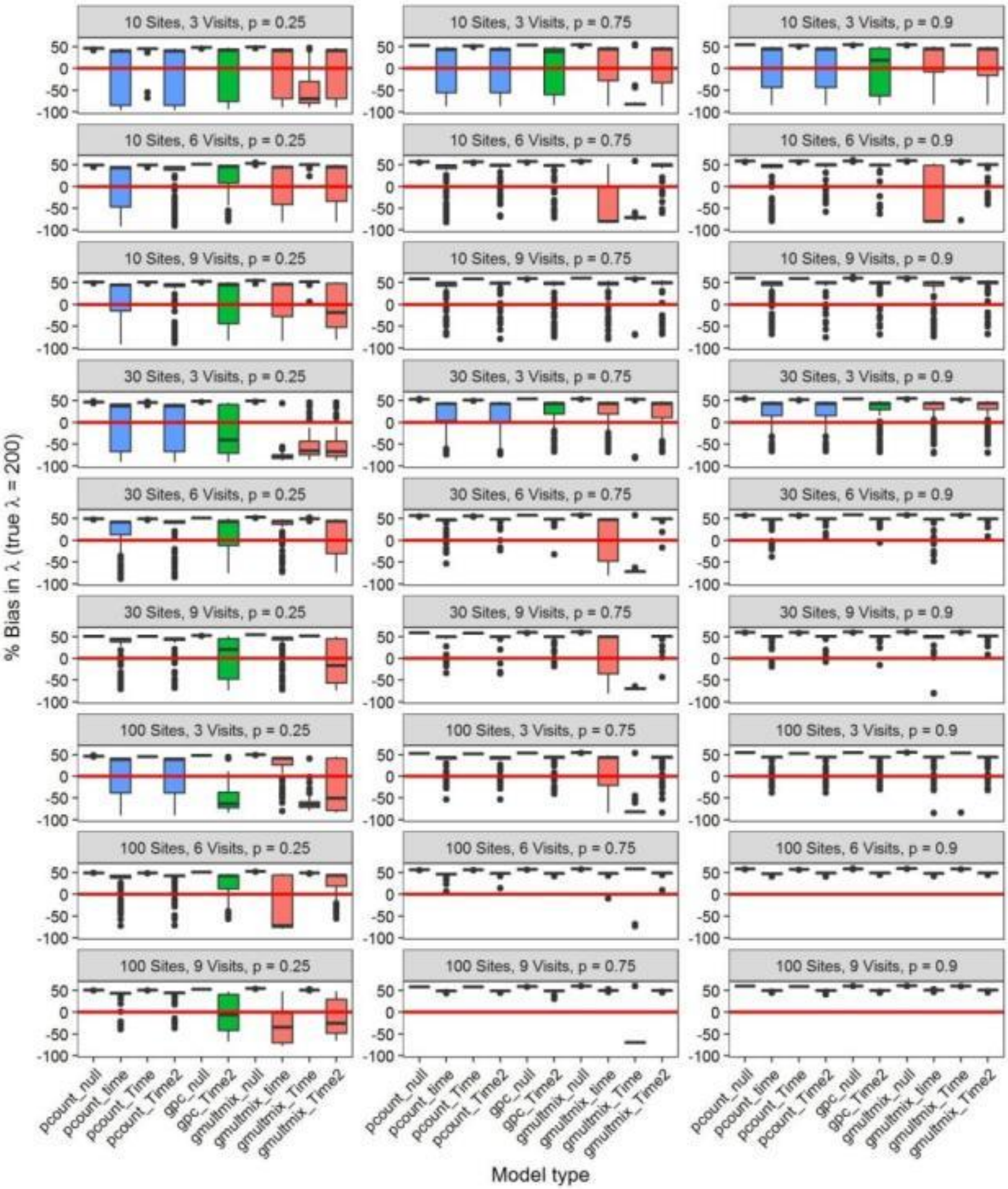


Figure S2. Percent bias in λ from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the weak peak beta distribution and a two day residency period.

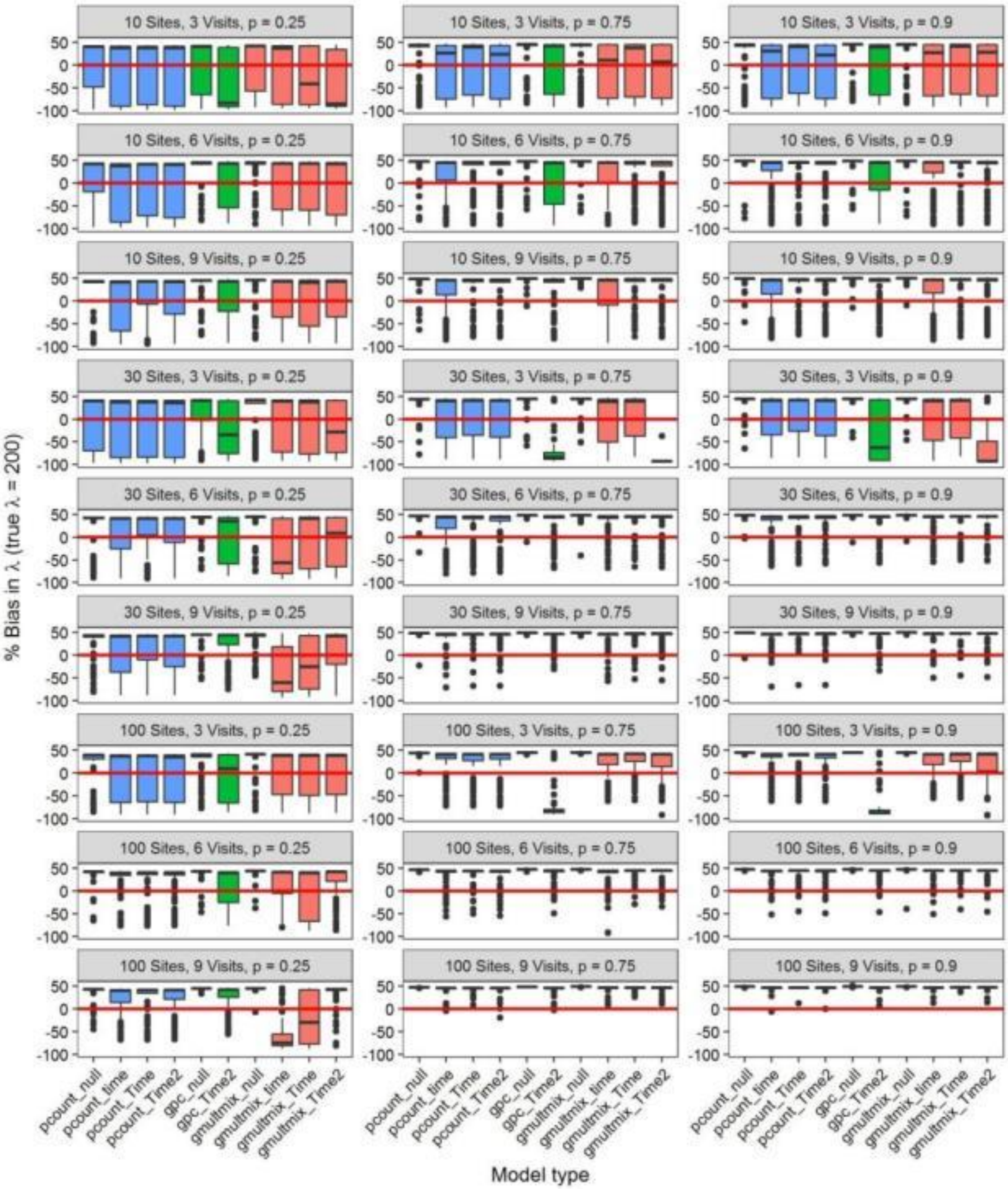


Figure S3. Percent bias in λ from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the weak peak beta distribution and a five day residency period.

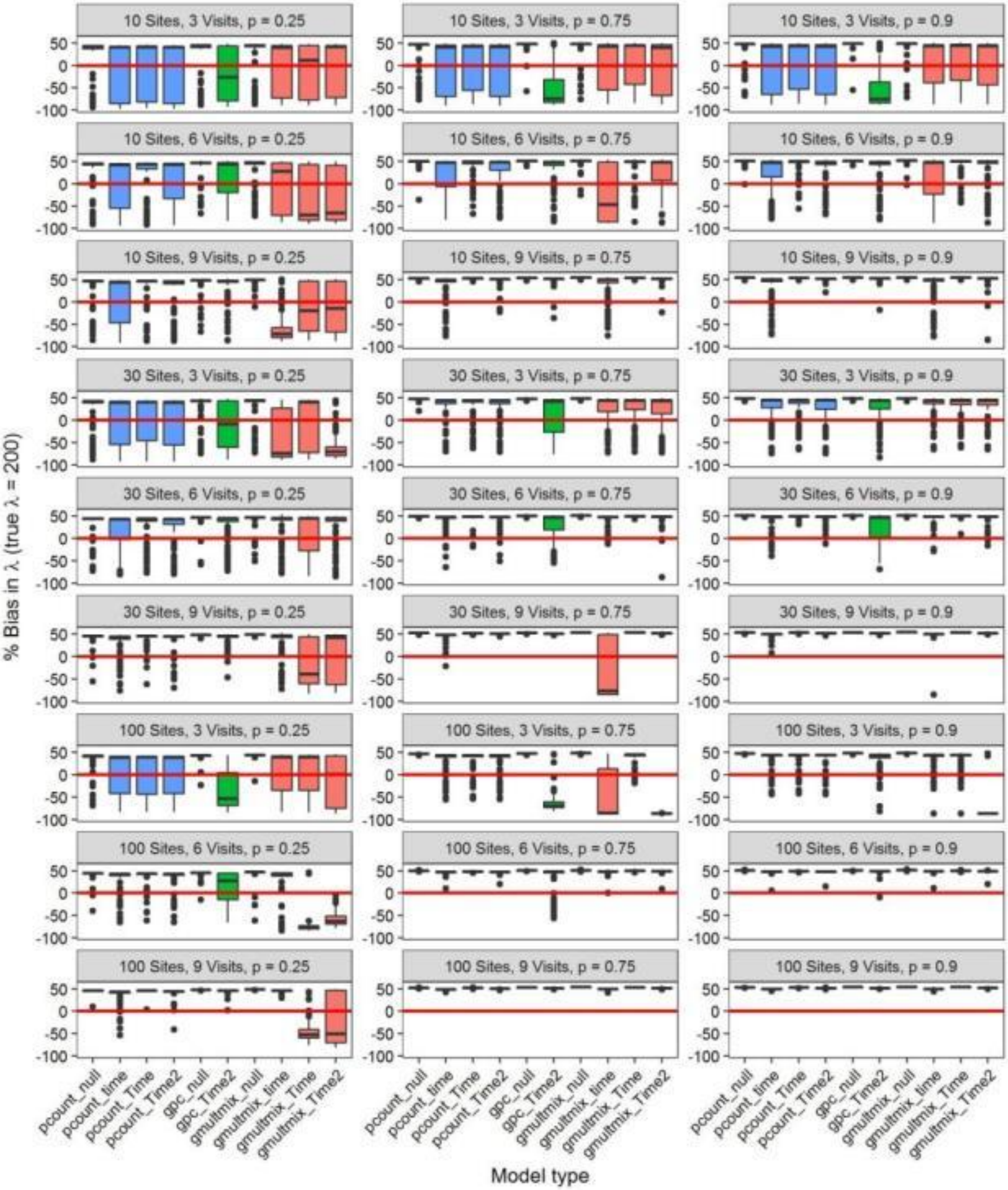


Figure S4. Percent bias in p from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the strong peak beta distribution and a five day residency period. Estimated p is shown for the first, middle, and last visit for each model and scenario.

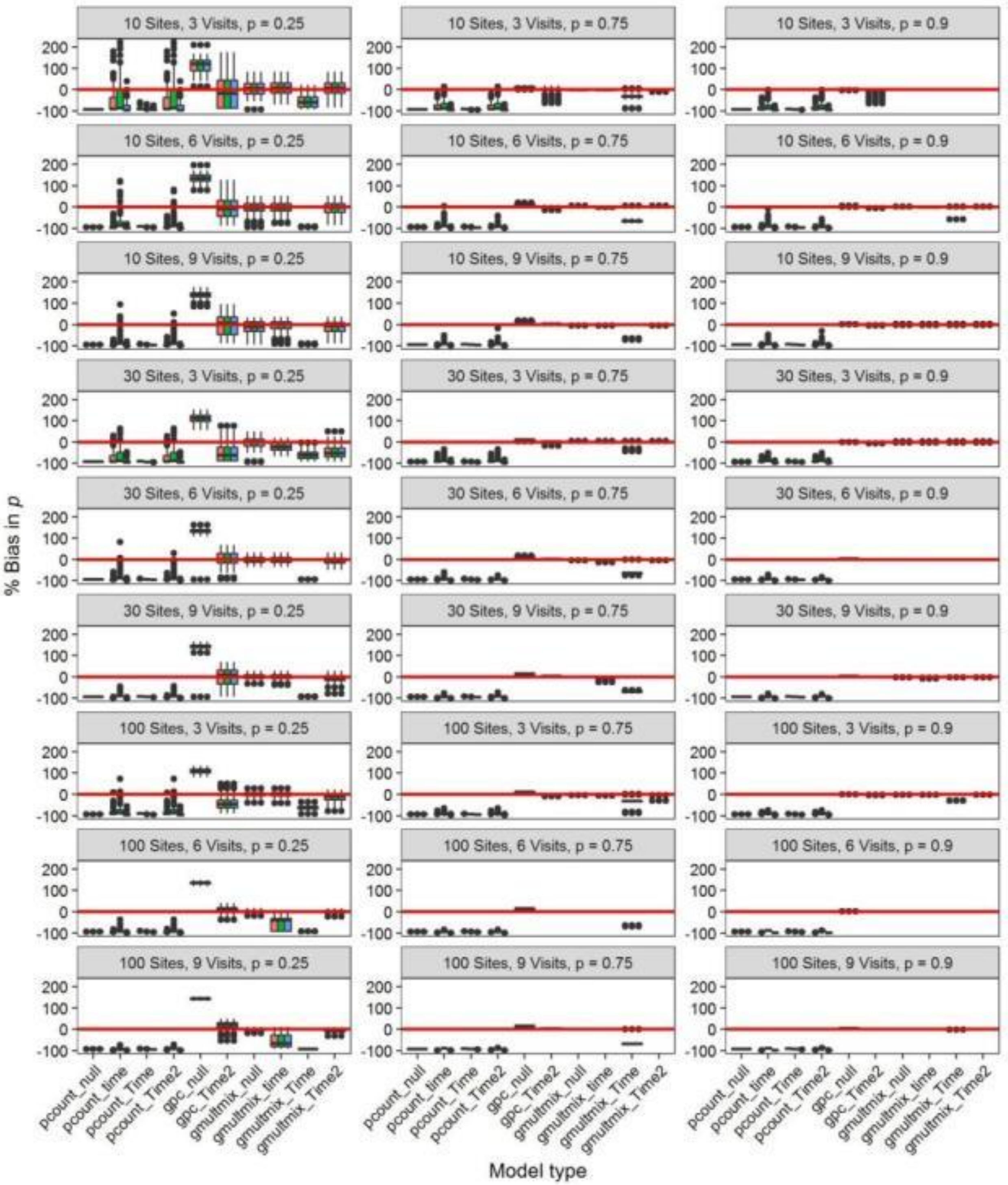


Figure S5. Percent bias in p from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the weak peak beta distribution and a two day residency period. Estimated p is shown for the first, middle, and last visit for each model and scenario.

Figure S6. Percent bias in p from simulated den count data by number of sites, number of visits, and detection rate (p). Data were simulated using the weak peak beta distribution and a five day residency period. Estimated p is shown for the first, middle, and last visit for each model and scenario.

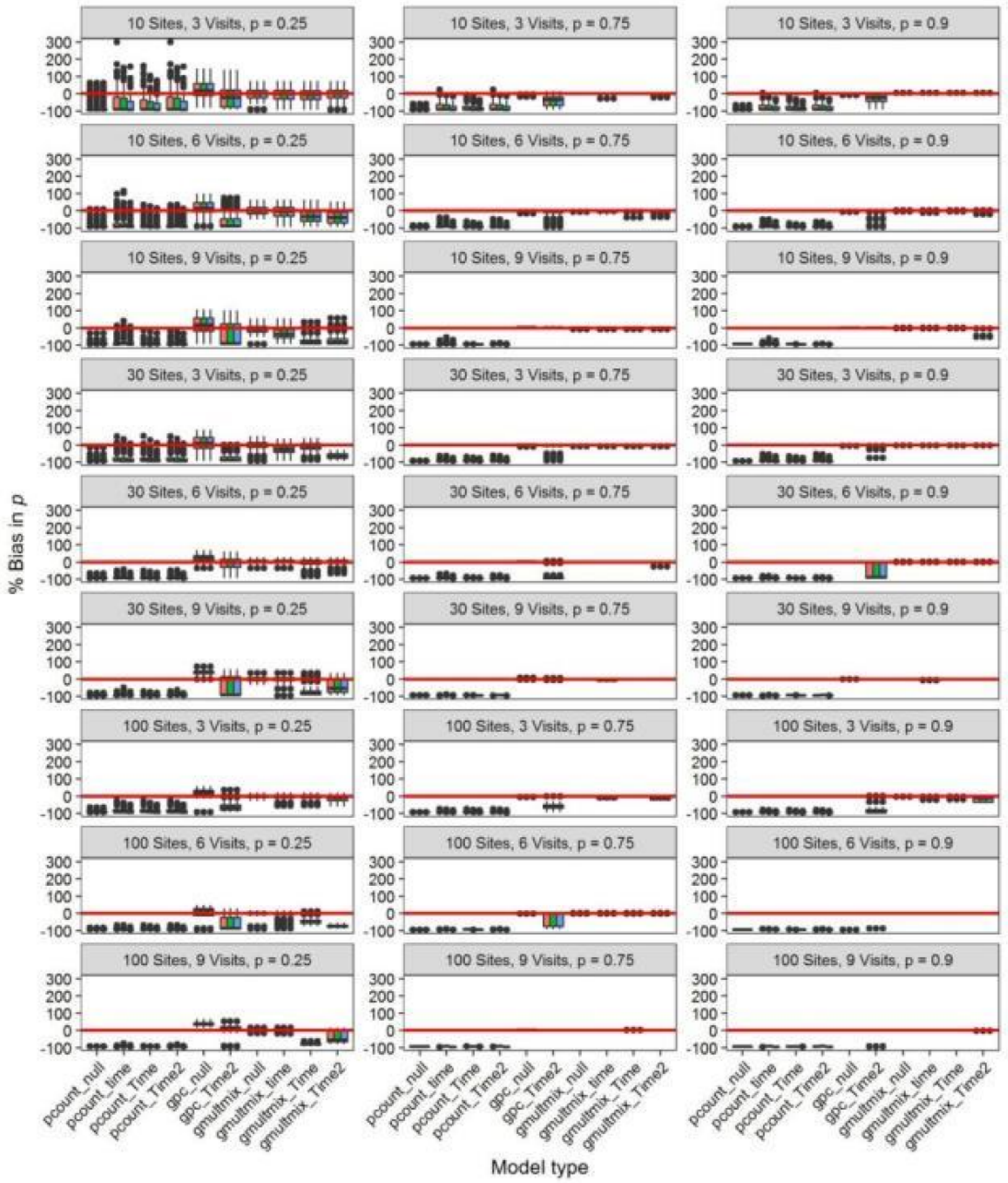


Figure S7. Variation in AICc model weight (w) for data simulated with a linear decline in occupancy (ψ) over time (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.75 and p = 0.25. The TREND model represents the true model.

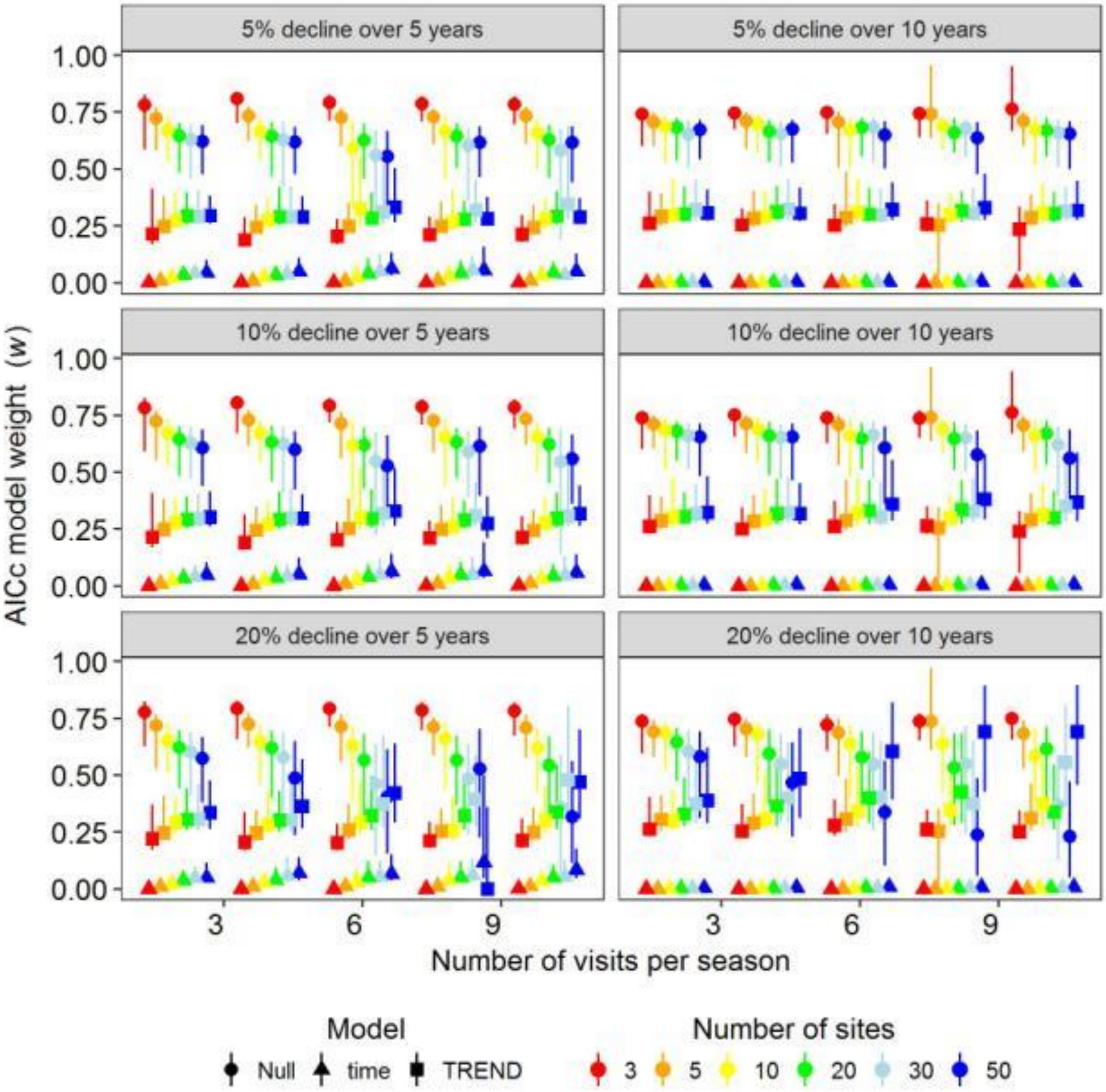


Figure S8. Variation in AICc model weight (w) for data simulated with a linear decline in occupancy (ψ) over time (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.25 and p = 0.50. The TREND model represents the true model.

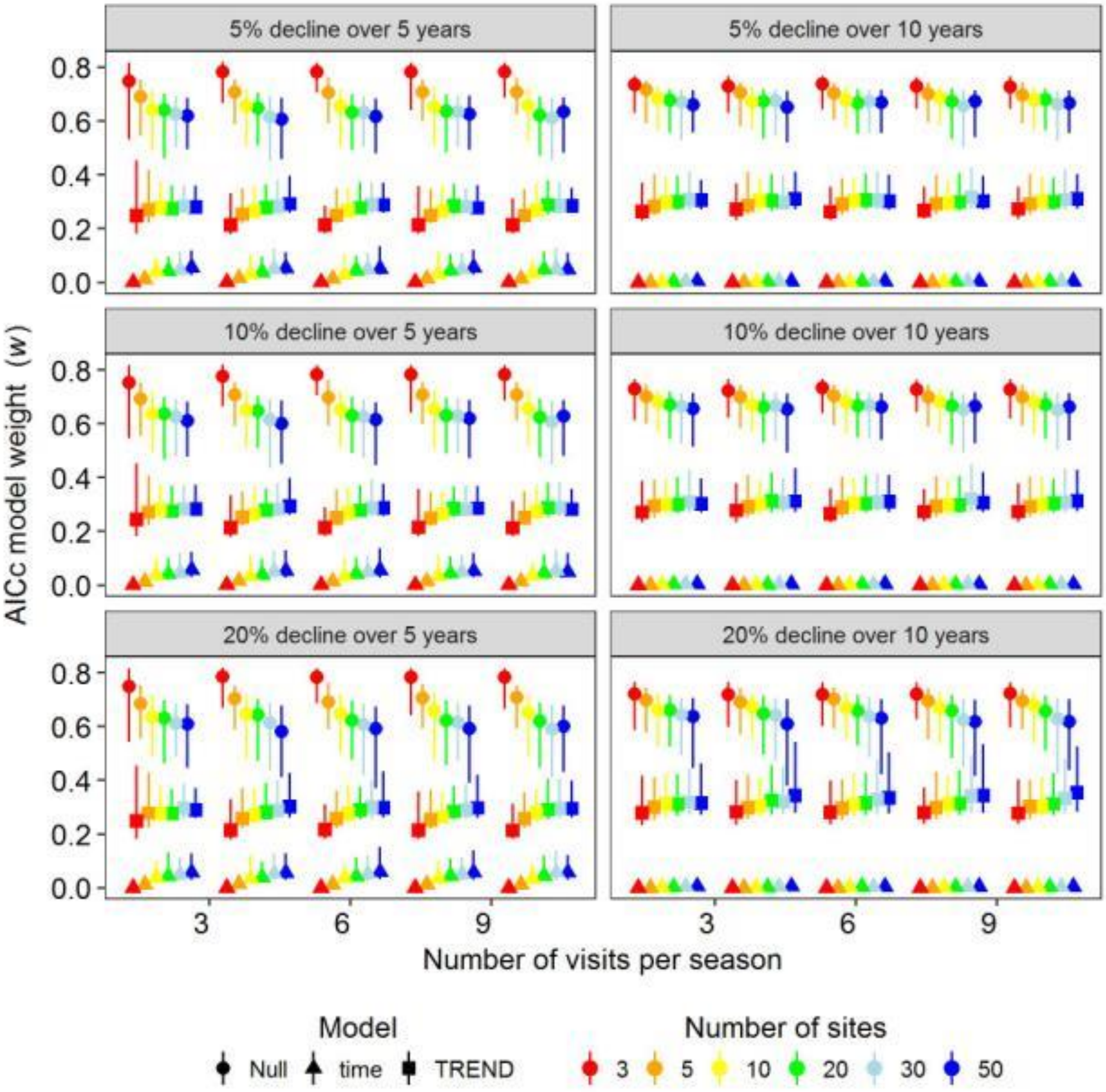


Figure S9. Variation in AICc model weight (w) for data simulated with a linear decline in occupancy (ψ) over time (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.95 and p = 0.75. The TREND model represents the true model.

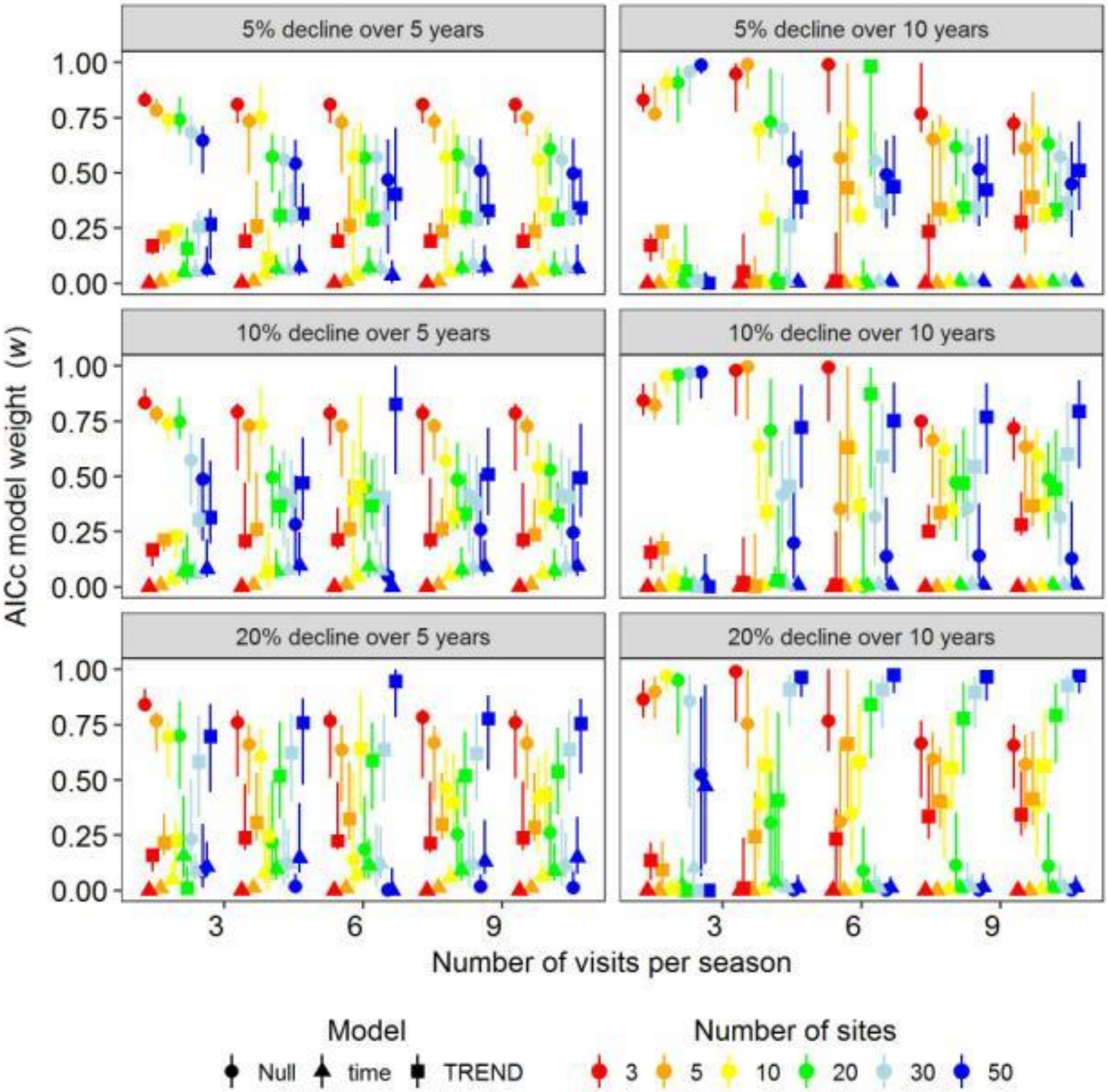


Figure S10. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on occupancy (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.75 and p = 0.25.

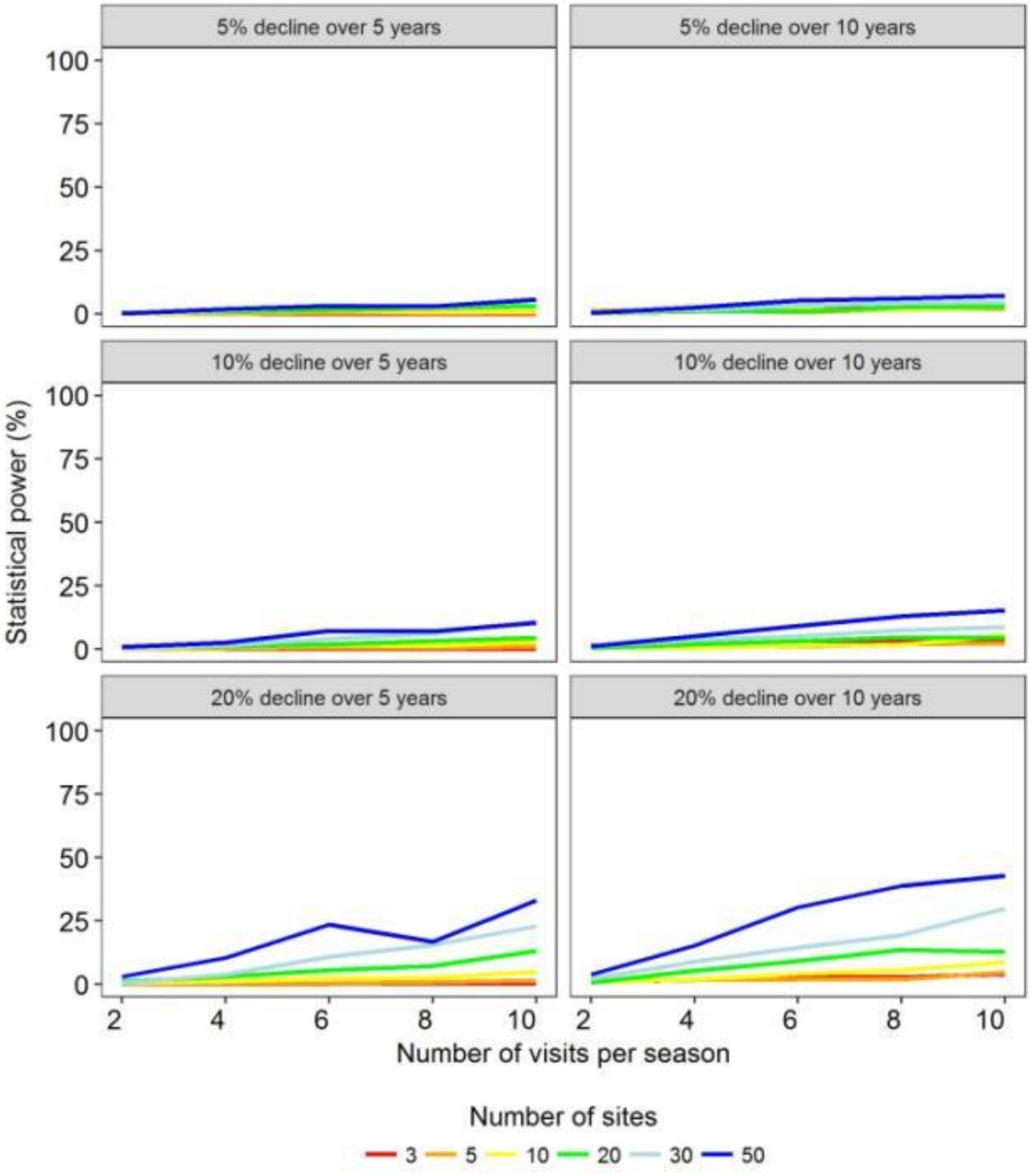


Figure S11. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on occupancy (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.25 and p = 0.50.

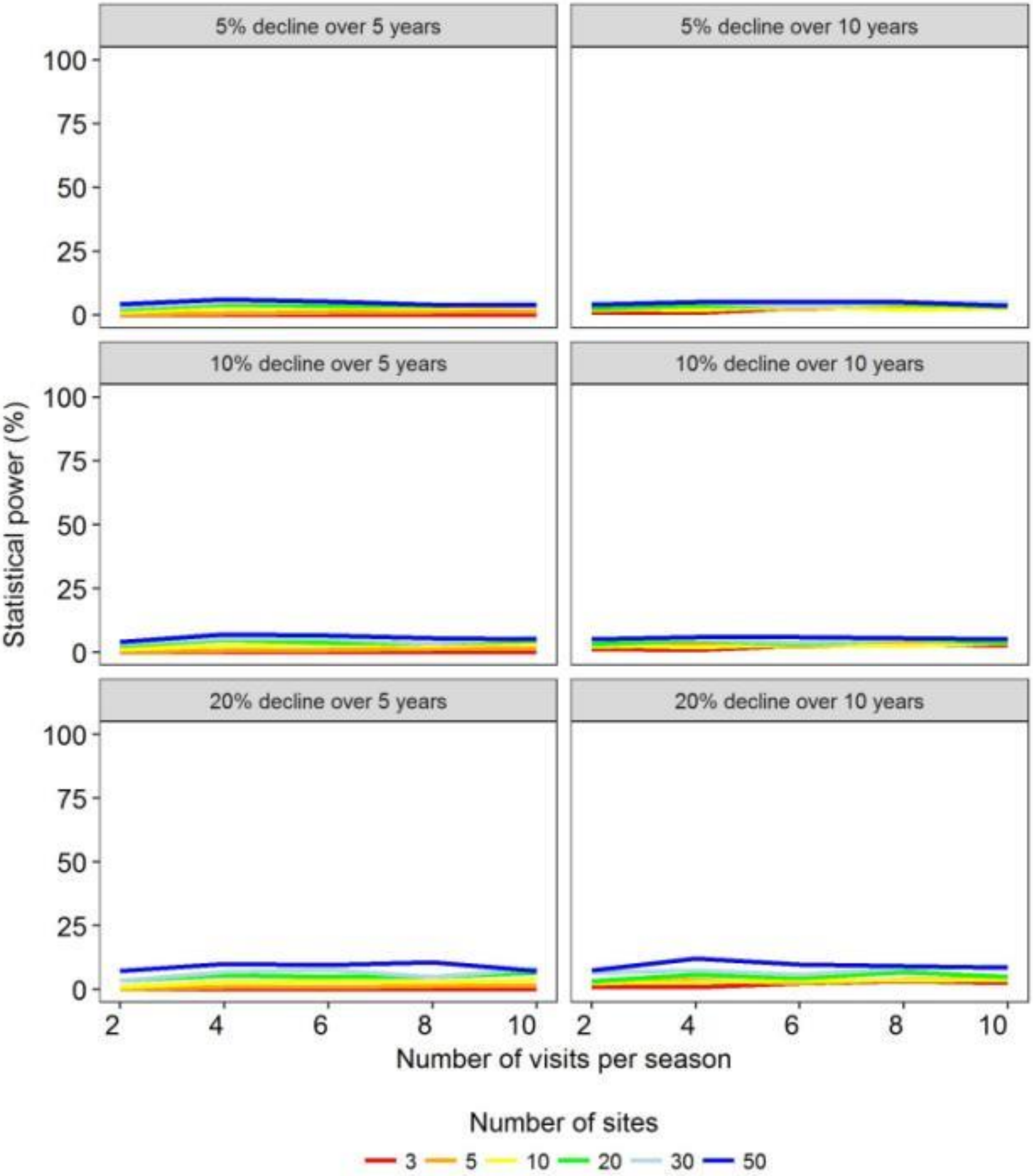


Figure S12. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on occupancy (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.95 and p = 0.25.

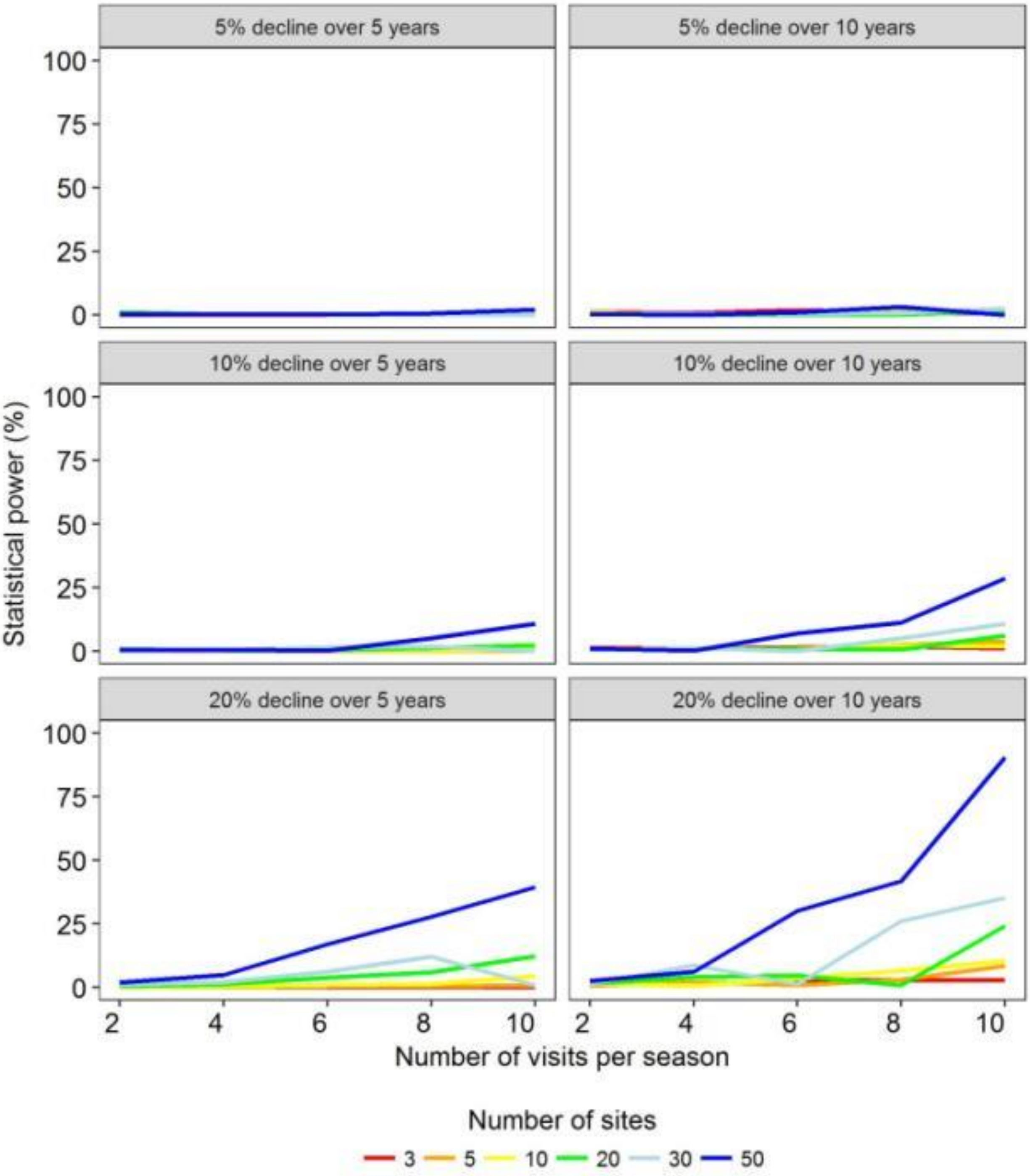


Figure S13. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on occupancy (ψ~Year) across different rates and durations of decline and sampling intensities when initial ψ = 0.95 and p = 0.75.

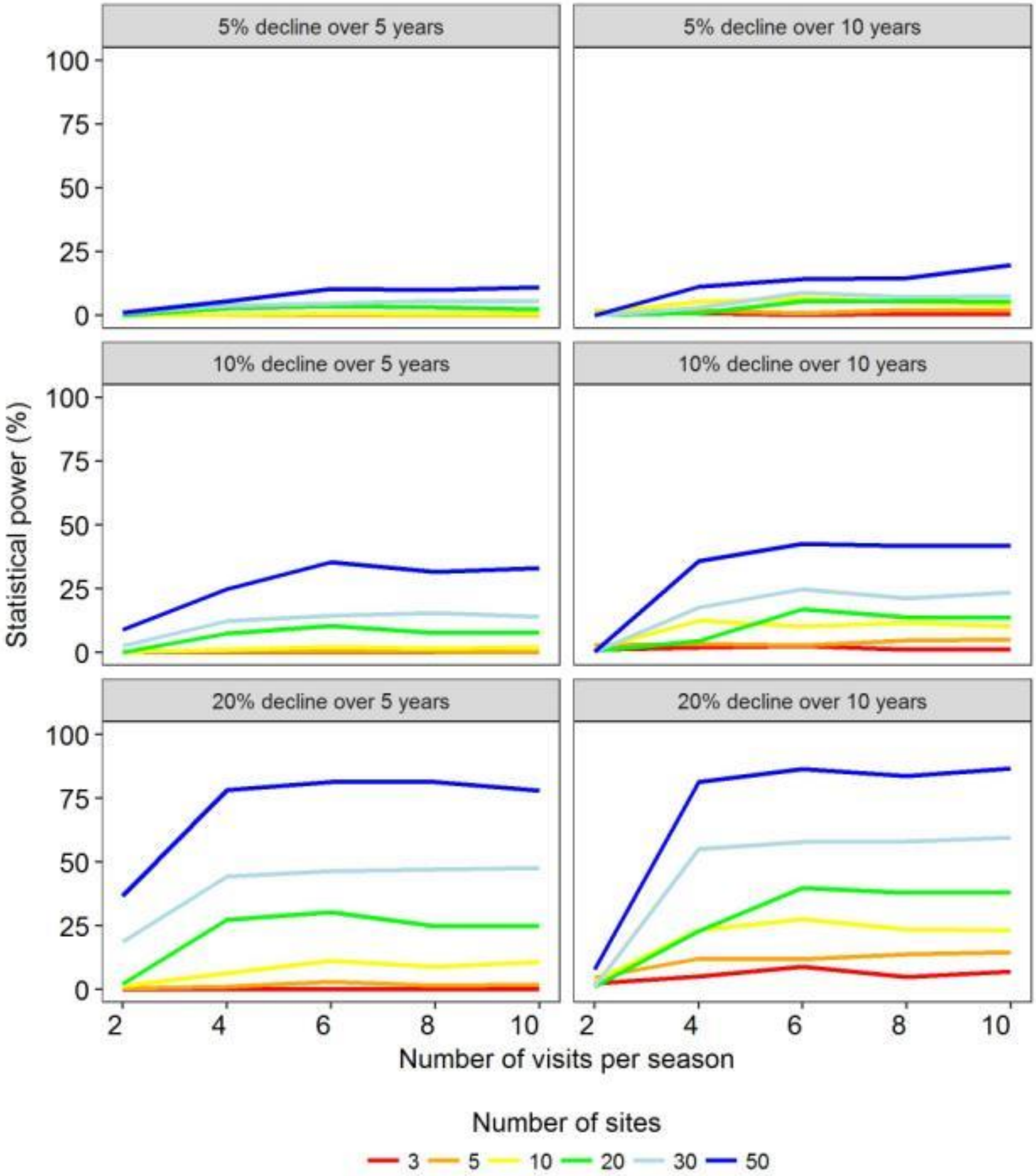


Figure S14. Root mean squared error for the estimated linear trend in occupancy across different rates and durations of decline and sampling intensities when initial $\psi = 0.75$ and $p = 0.25$.

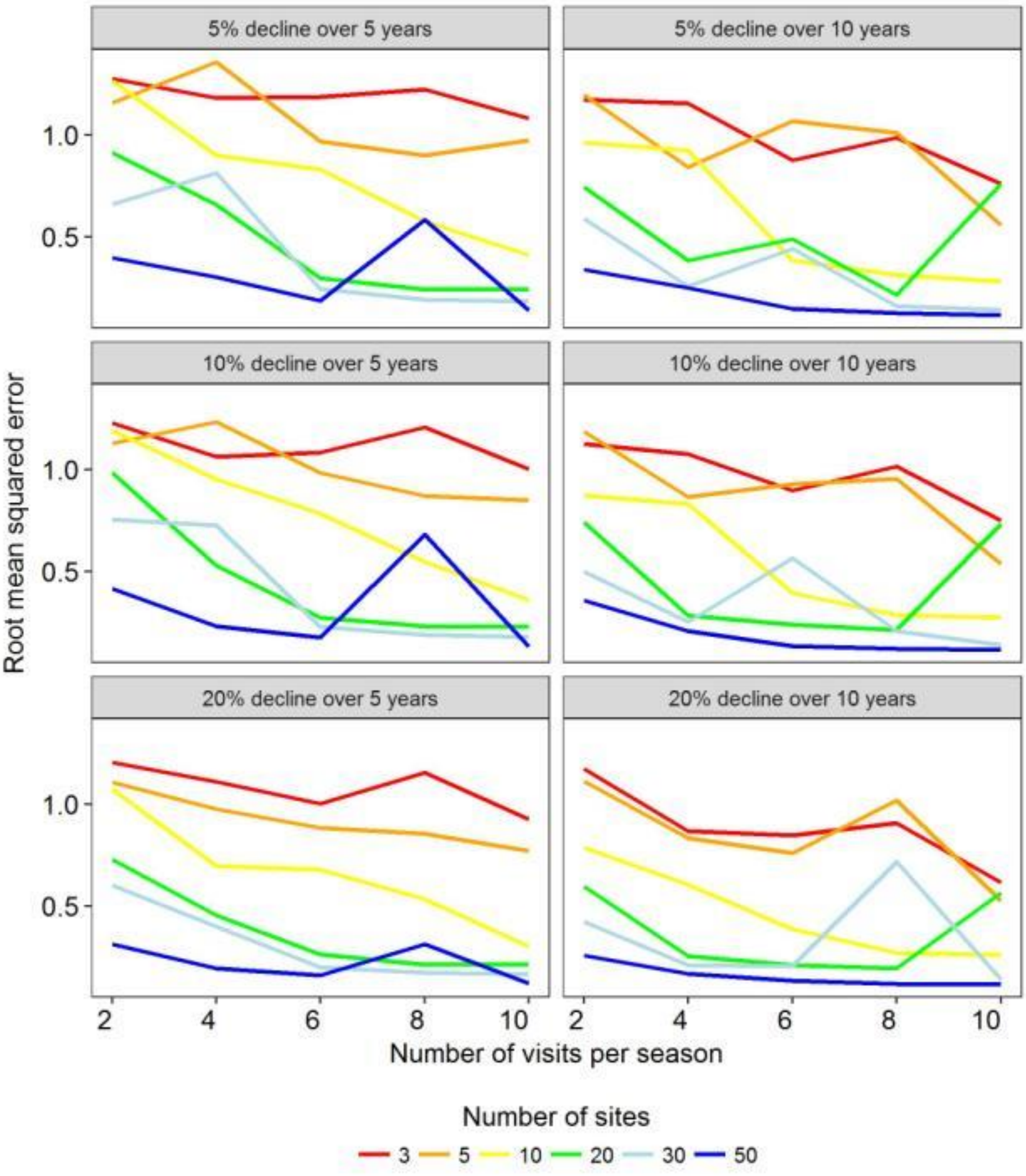


*Figure S15. Percent bias (((truth-estimate)/truth)*100) in the estimated rate of decline in occupancy (ψ) under different sampling intensities and rates and durations of declines when initial ψ = 0.75 and p = 0.25. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. Missing values have 2.5th quantiles <-5,000. The dark horizontal line represents zero. Data were fit using the pcount model.*

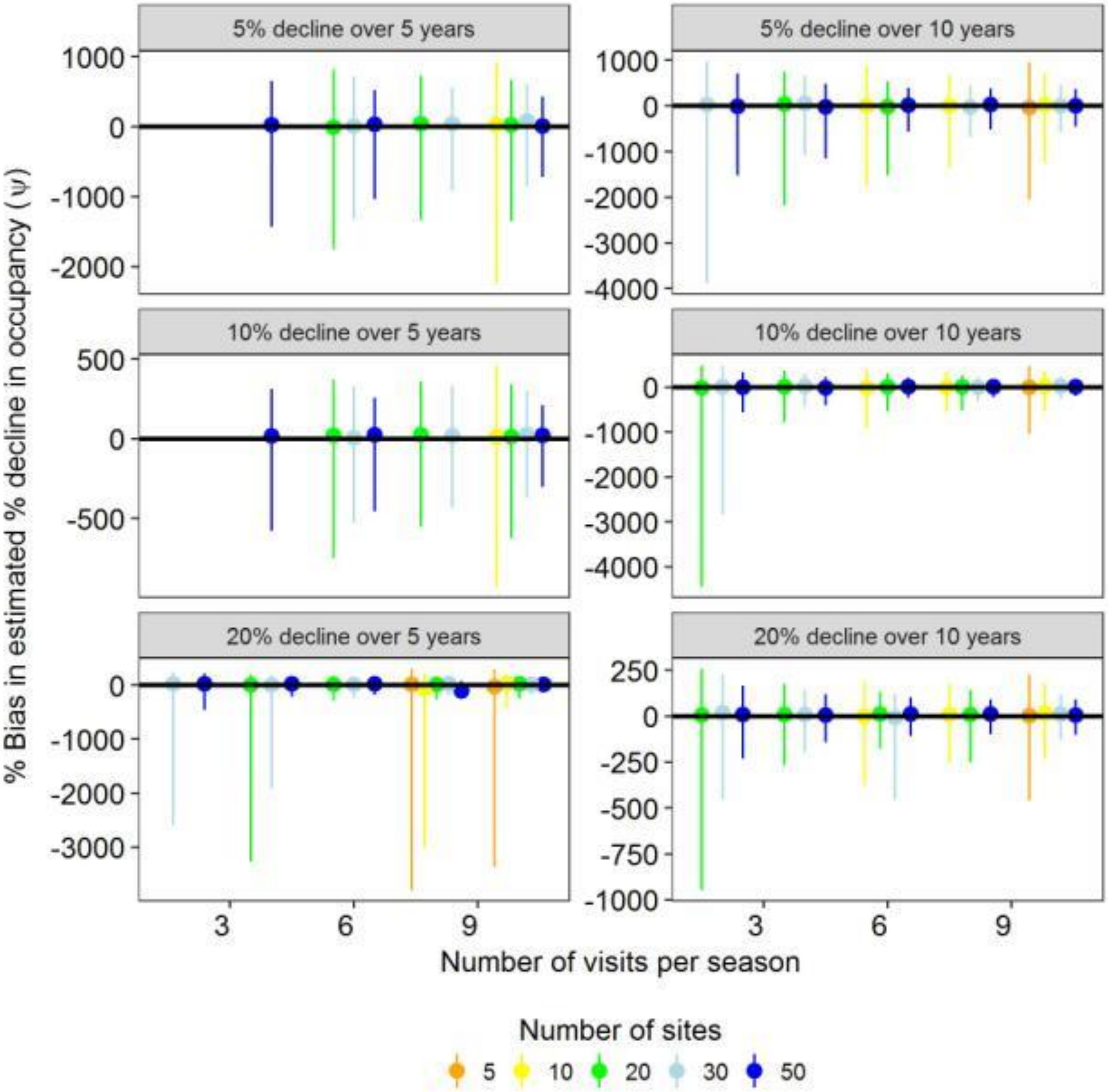


*Figure S16. Percent bias (((truth-estimate)/truth)*100) in the estimated rate of decline in occupancy (ψ) under different sampling intensities and rates and durations of declines when initial ψ = 0.25 and p = 0.50. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. Missing values have 2.5th quantiles <-5,000. The dark horizontal line represents zero. Data were fit using the pcount model.*

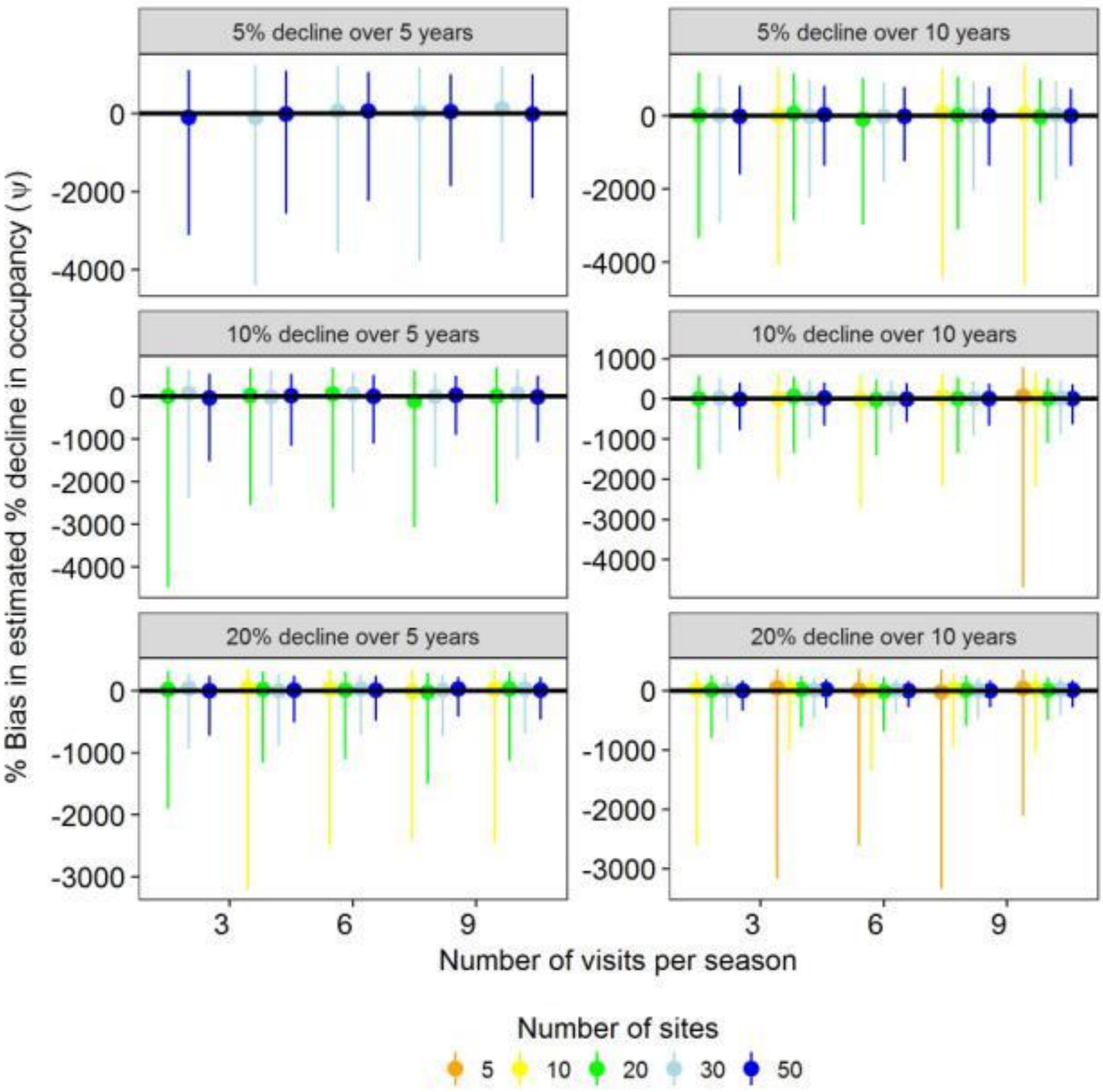


*Figure S17. Percent bias (((truth-estimate)/truth)*100) in the estimated rate of decline in occupancy (ψ) under different sampling intensities and rates and durations of declines when initial $\psi = 0.95$ and $p = 0.75$. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5^{th} and 97.5^{th} quantiles. Missing values have 2.5^{th} quantiles <-1,000. The dark horizontal line represents zero. Data were fit using the pcount model.*

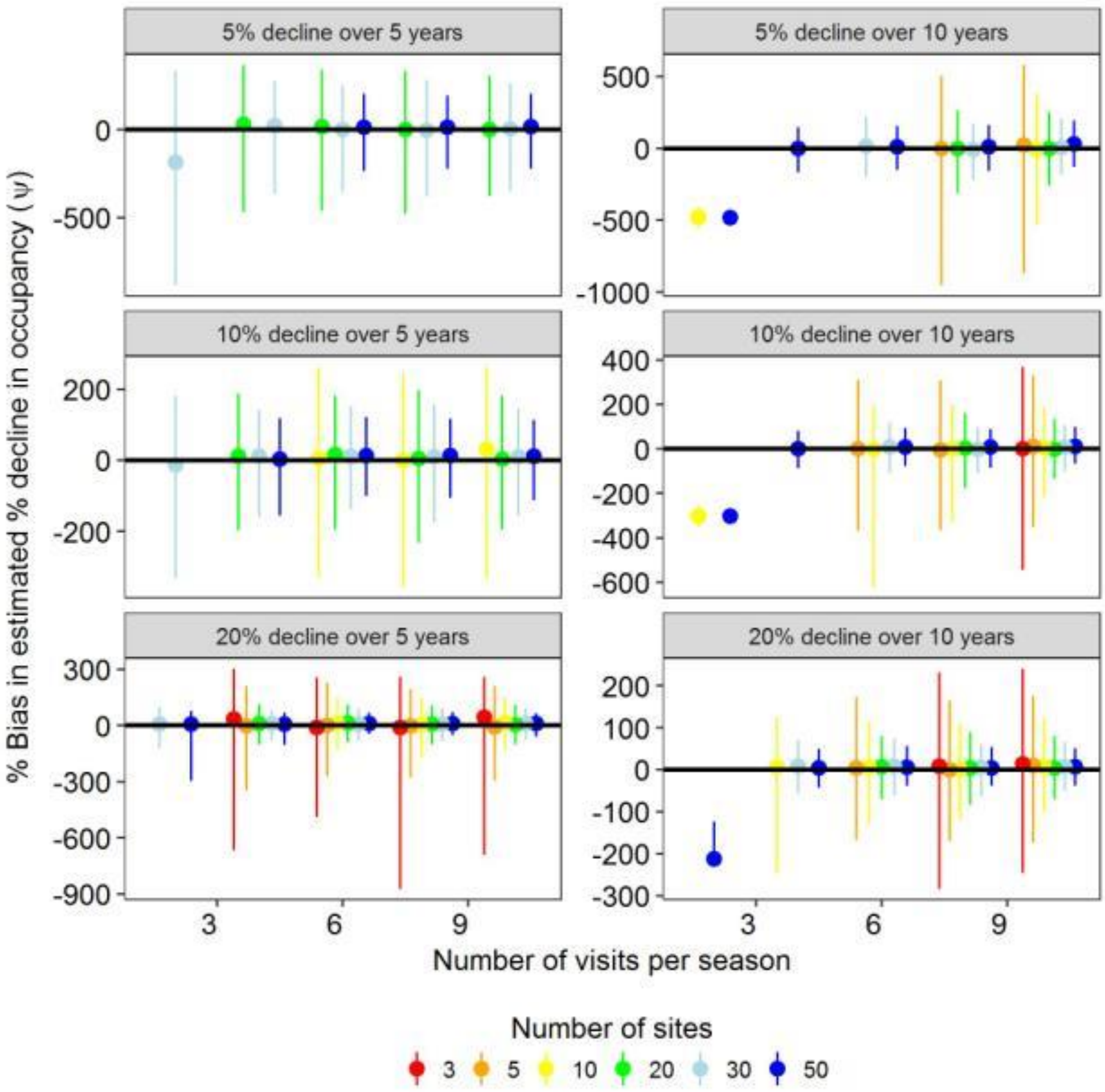


Figure S18. Root mean squared error for estimated abundance (λ) under different sampling intensities and true values of λ and detection probability (p). Data were fit using the pcount model.

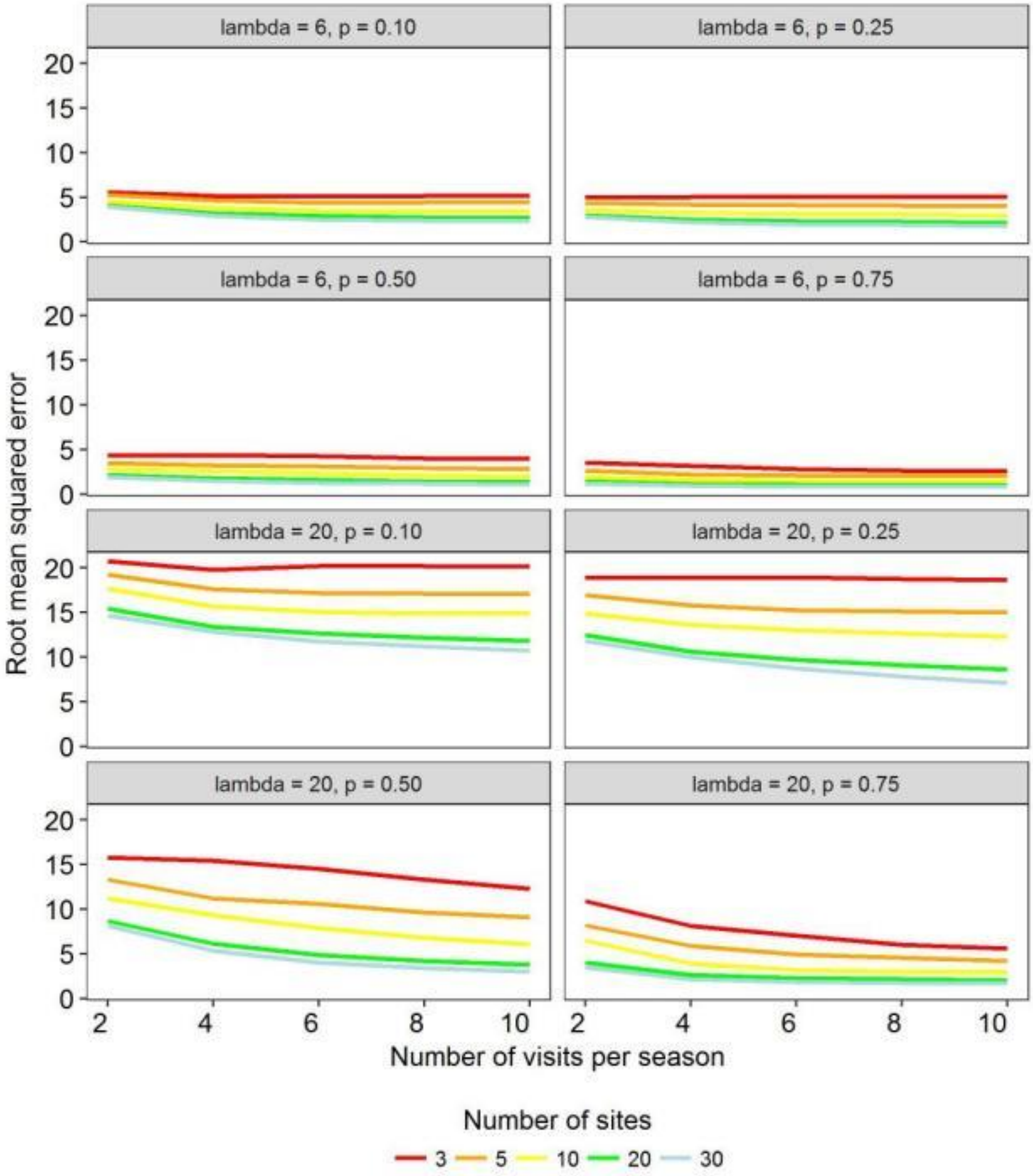


*Figure S19. Percent bias (((truth-estimate)/truth)*100) in estimated abundance (λ) under different sampling intensities and true values of λ and detection probability (p). Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero. Data were fit using the pcount model.*

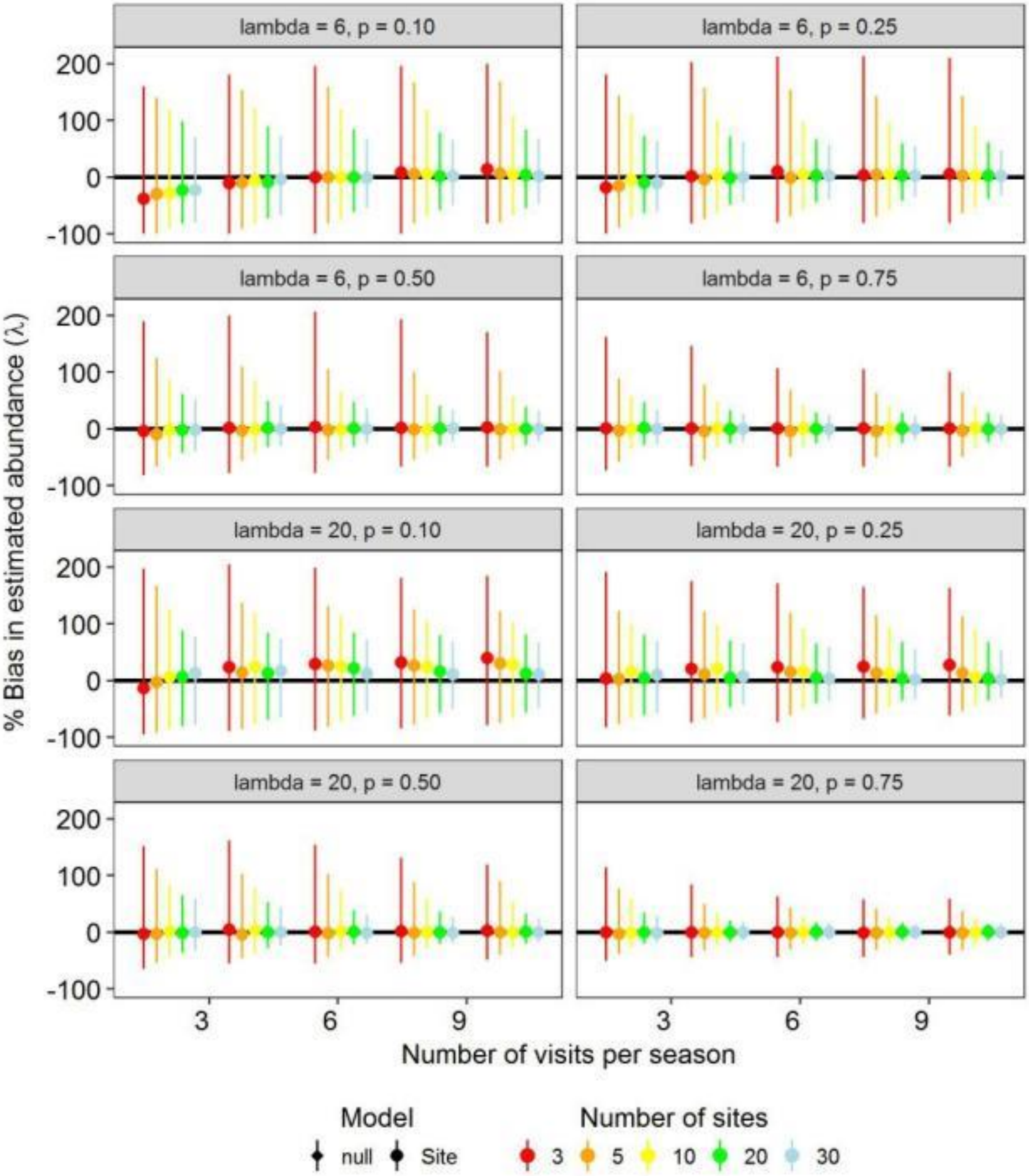


Figure S20. Variation in AICc model weight (w) for data simulated with a linear decline in abundance (lambda, λ) across different rates and durations of decline and sampling intensities when initial λ = 10 and p = 0.75. Points represent median w and error bars represent the inter-quartile range. The TREND model represents the true model.

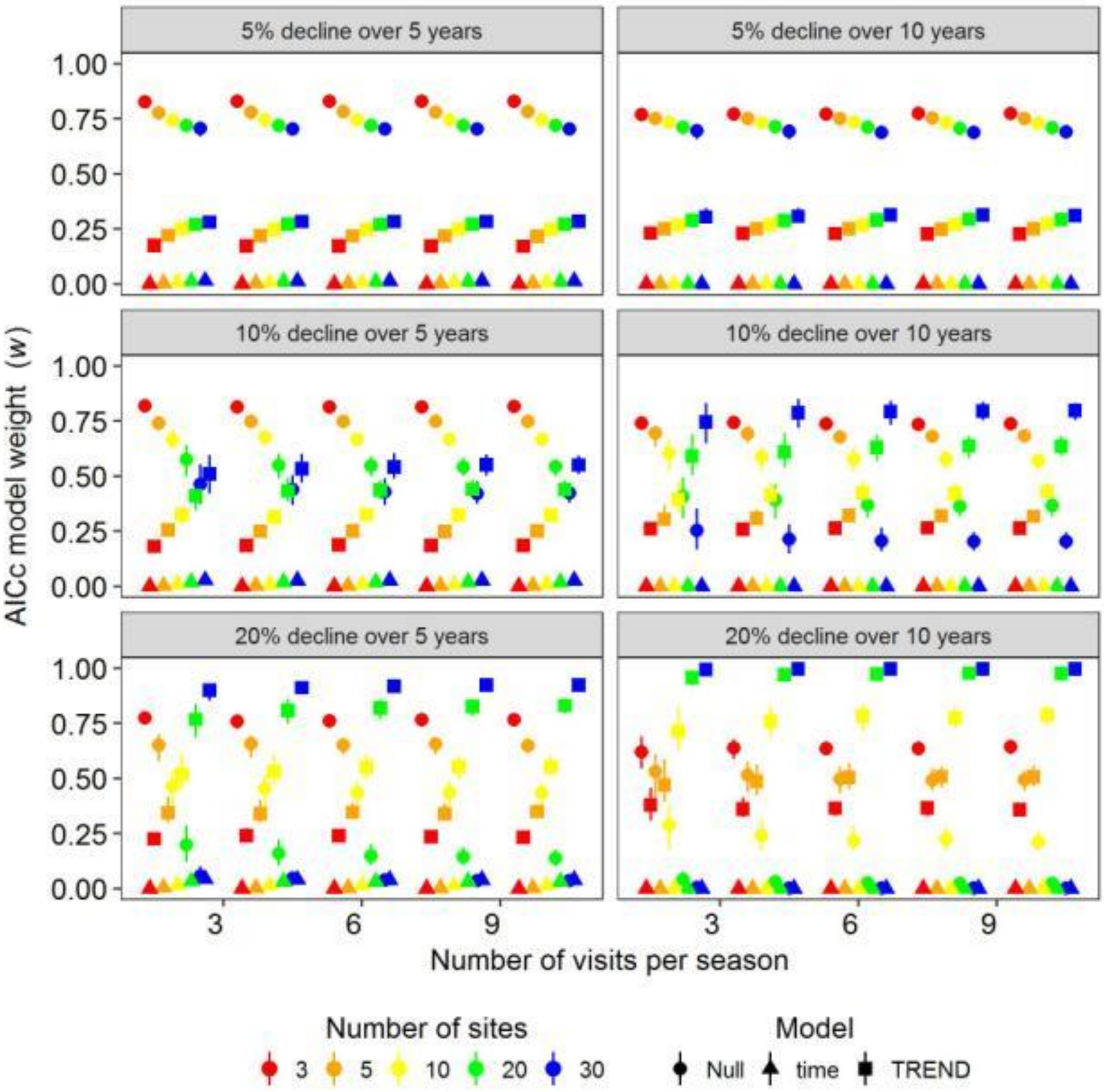


Figure S21. Variation in AICc model weight (w) for data simulated with a linear decline in abundance (lambda, λ) across different rates and durations of decline and sampling intensities when initial λ = 6 and p = 0.25. Points represent median w and error bars represent the inter-quartile range. The TREND model represents the true model.

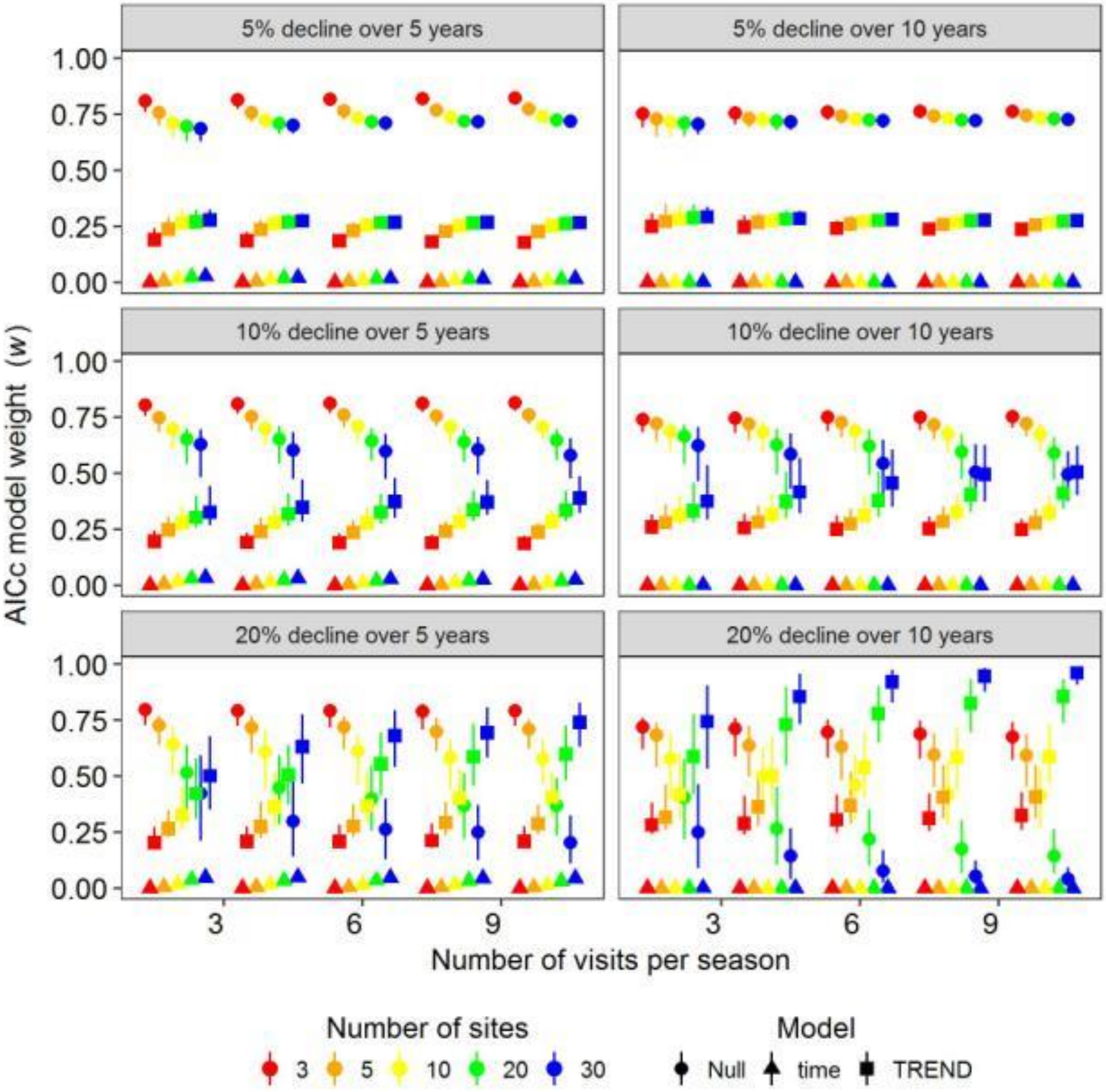


Figure S22. Variation in AICc model weight (w) for data simulated with a linear decline in abundance (lambda, λ) across different rates and durations of decline and sampling intensities when initial λ = 6 and p = 0.75. Points represent median w and error bars represent the inter-quartile range. The TREND model represents the true model.

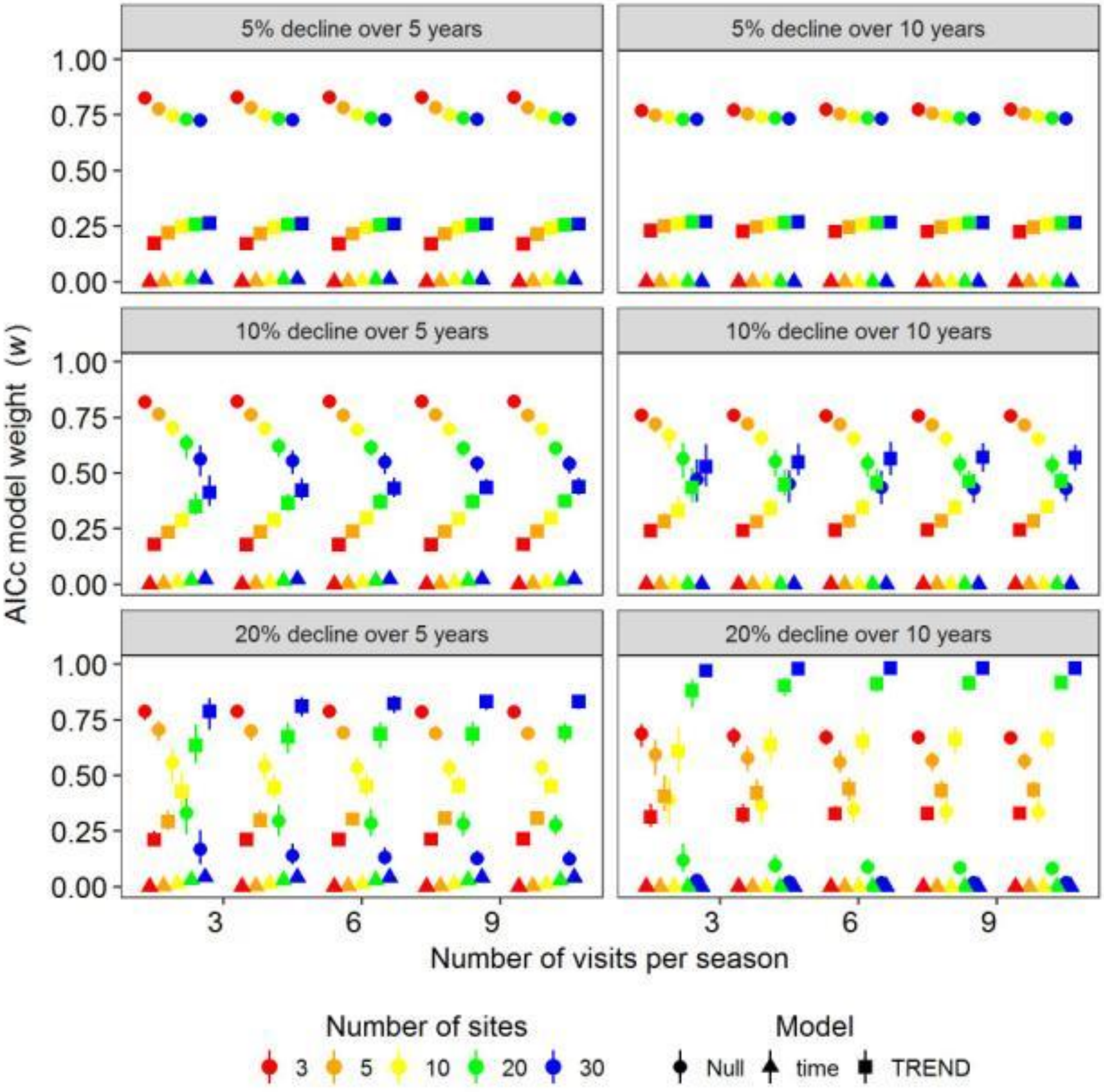


Figure S23. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on abundance (λ~Year) across different rates and durations of decline and sampling intensities when initial λ = 10 and p = 0.75.

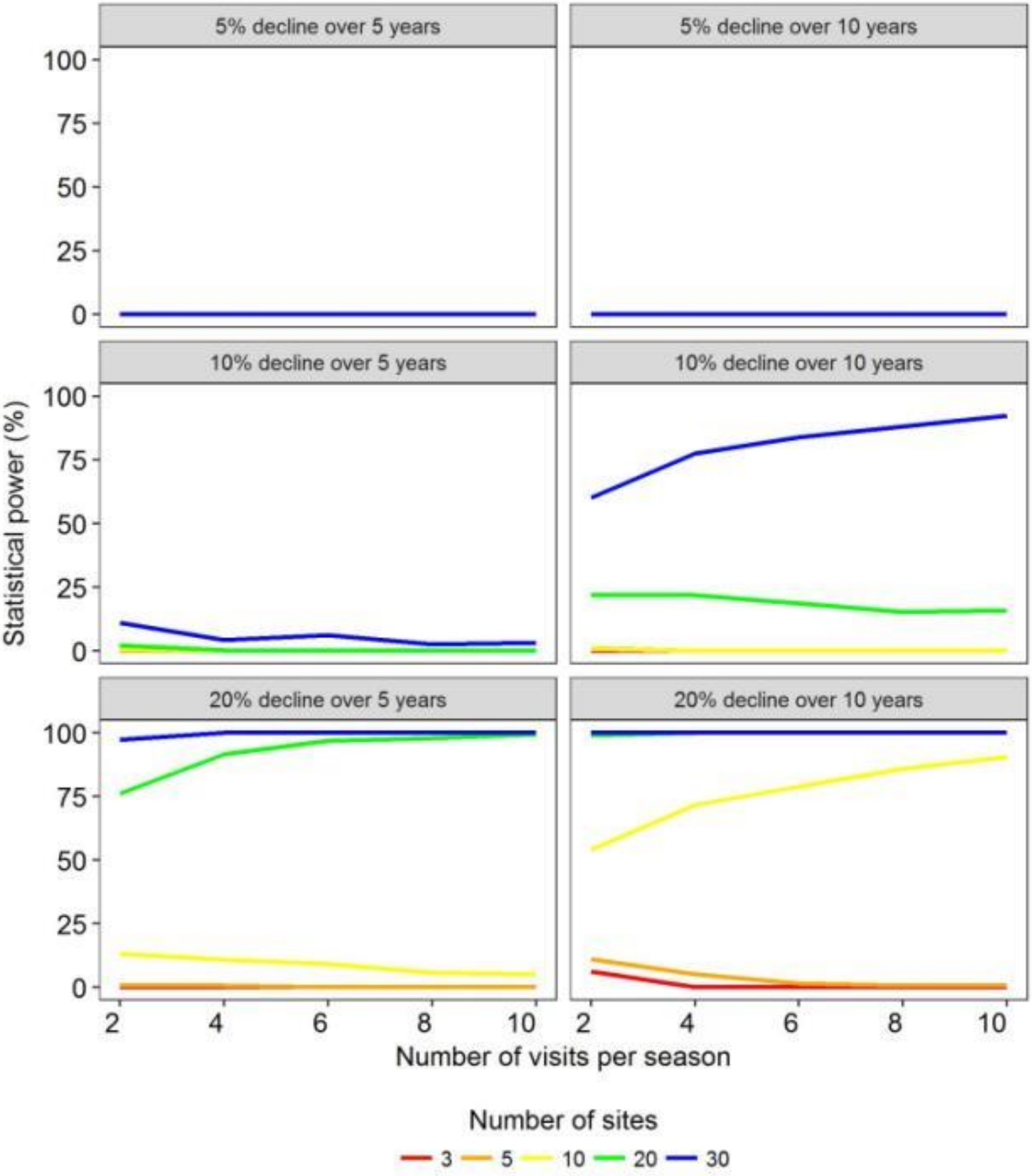


Figure S24. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on abundance (λ~Year) across different rates and durations of decline and sampling intensities when initial λ = 6 and p = 0.25.

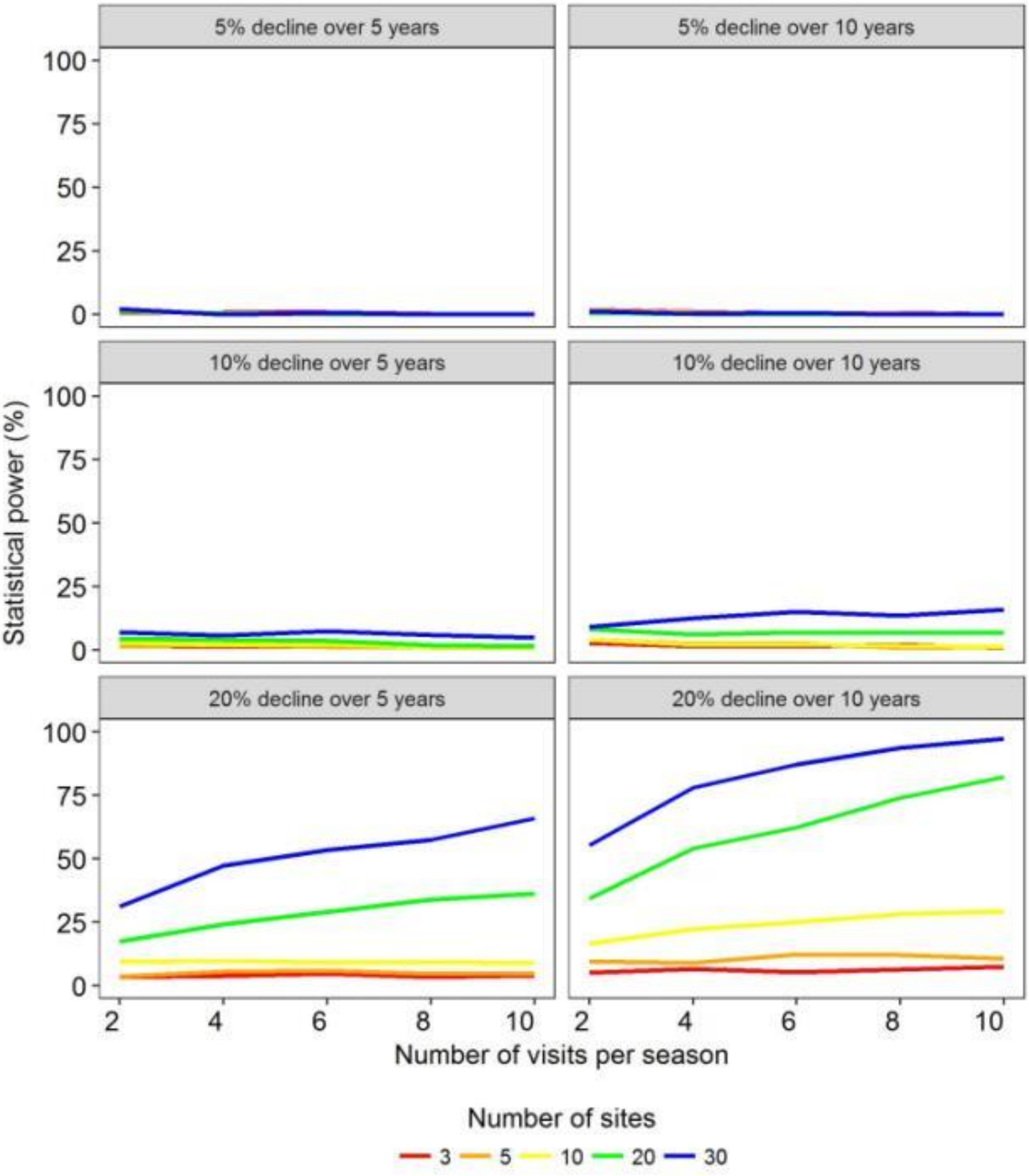


Figure S25. Statistical power to detect a significant (α = 0.05) effect of Year as a continuous, linear covariate on abundance (λ~Year) across different rates and durations of decline and sampling intensities when initial λ = 6 and p = 0.75.

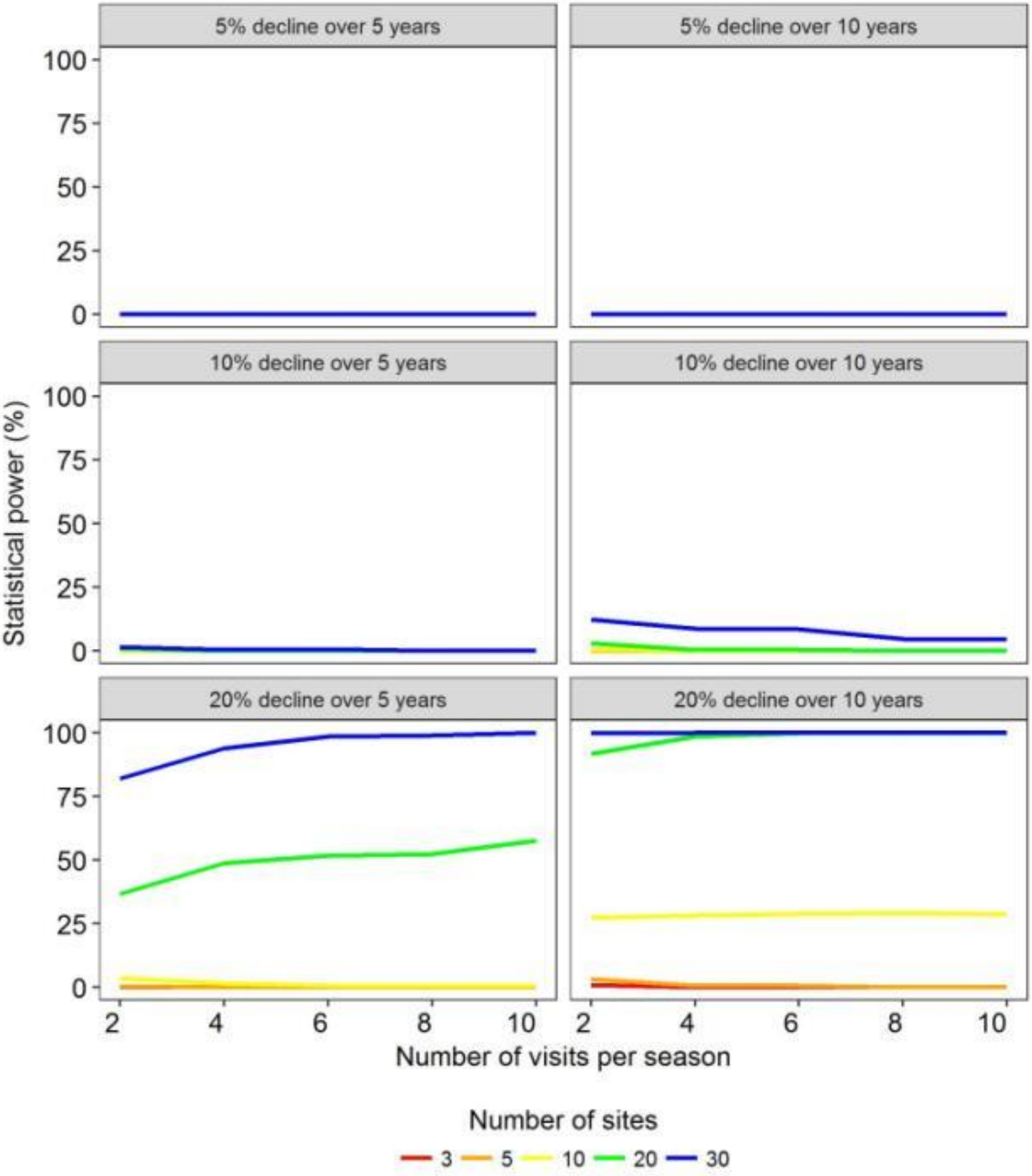


Figure S26. Root mean squared error for the estimated linear trend in abundance across different rates and durations of decline and sampling intensities when initial λ = 10 and p = 0.75.

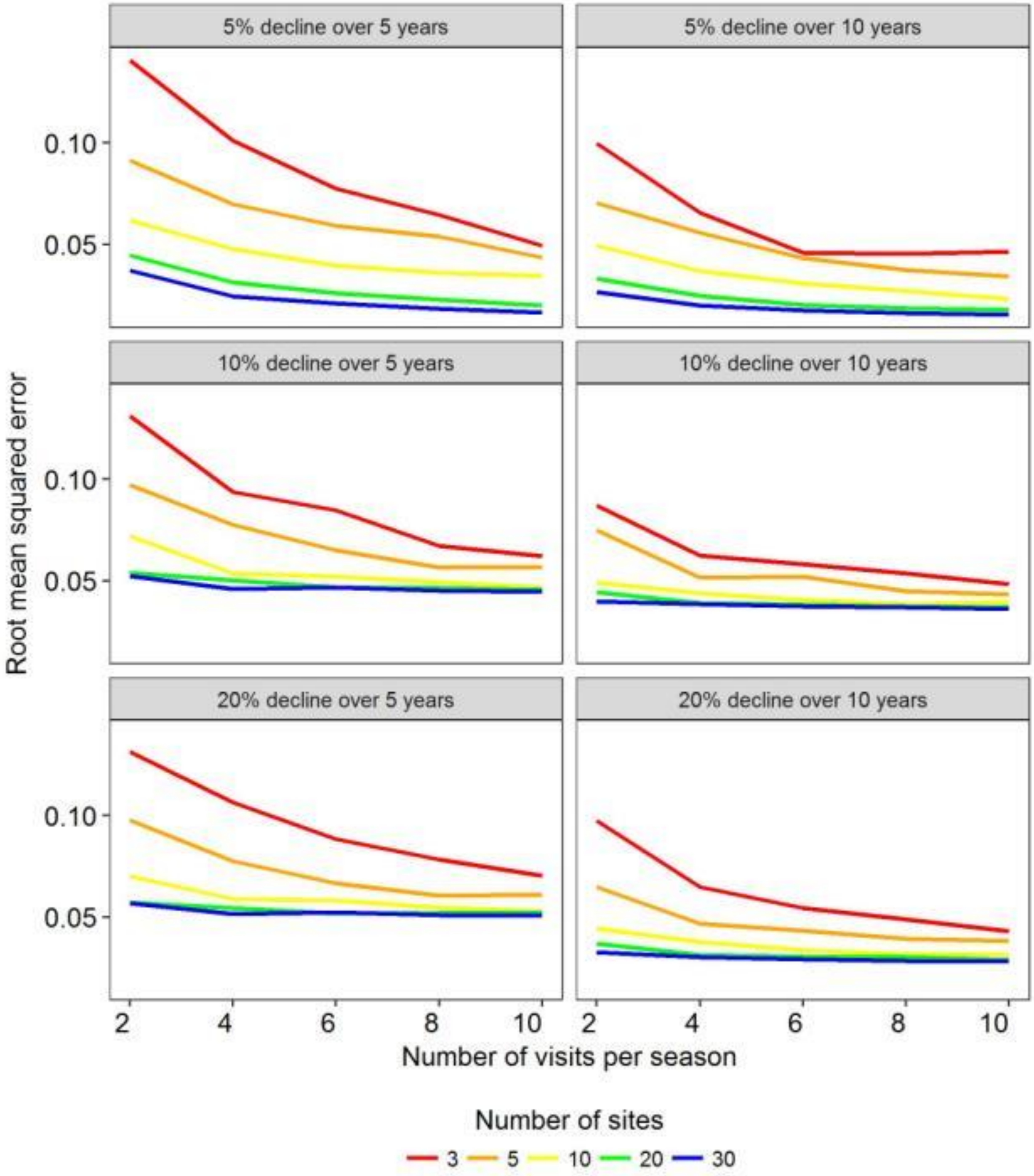


*Figure S27. Percent bias (((truth-estimate)/truth)*100) in the estimated rate of decline in abundance (λ) under different sampling intensities and rates and durations of declines when initial λ = 10 and p = 0.75. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero. Data were fit using the pcount model.*

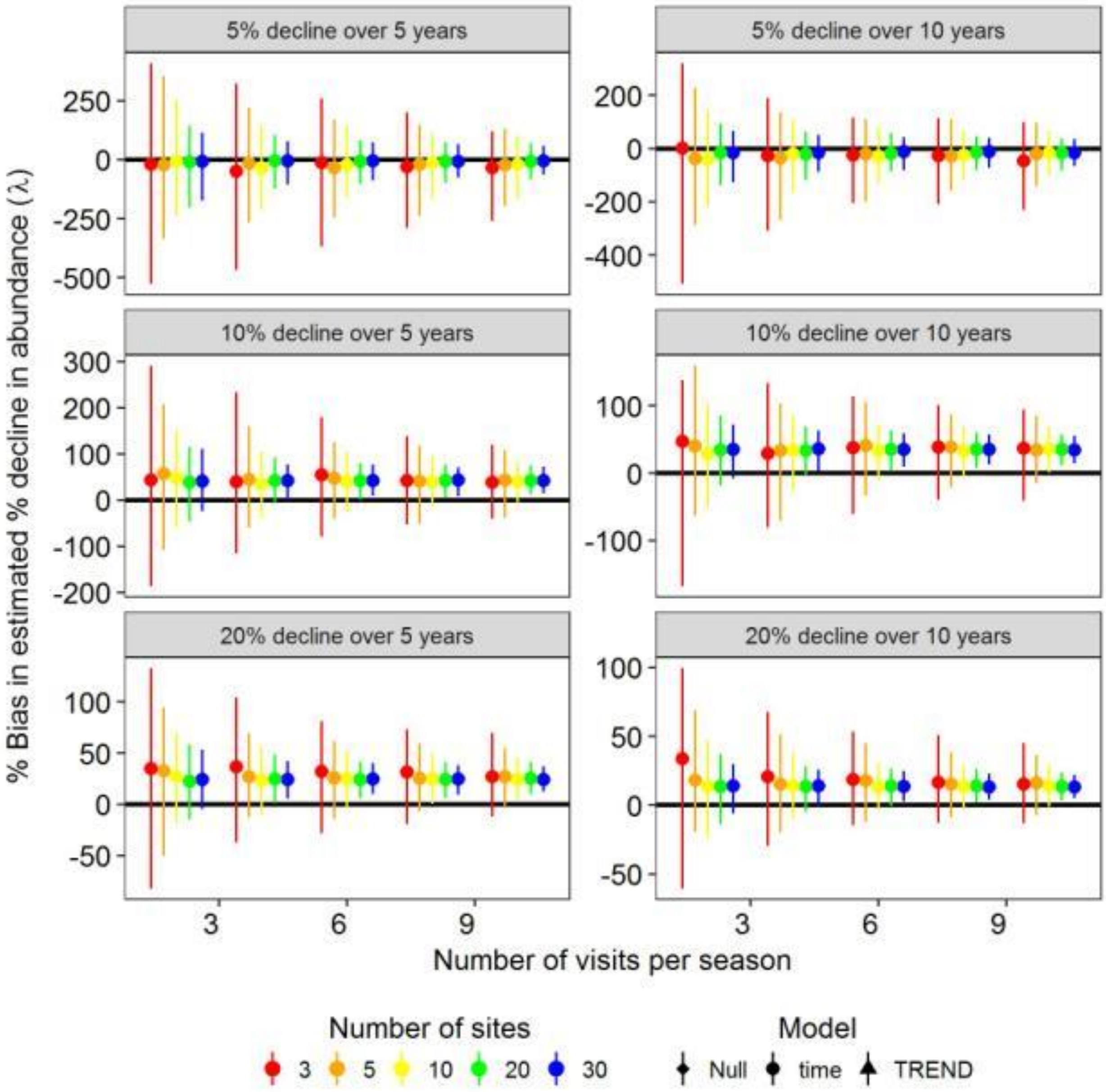


*Figure S28. Percent bias (((truth-estimate)/truth)*100) in the estimated rate of decline in abundance (λ) under different sampling intensities and rates and durations of declines when initial λ = 6 and p = 0.25. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero. Data were fit using the pcount model.*

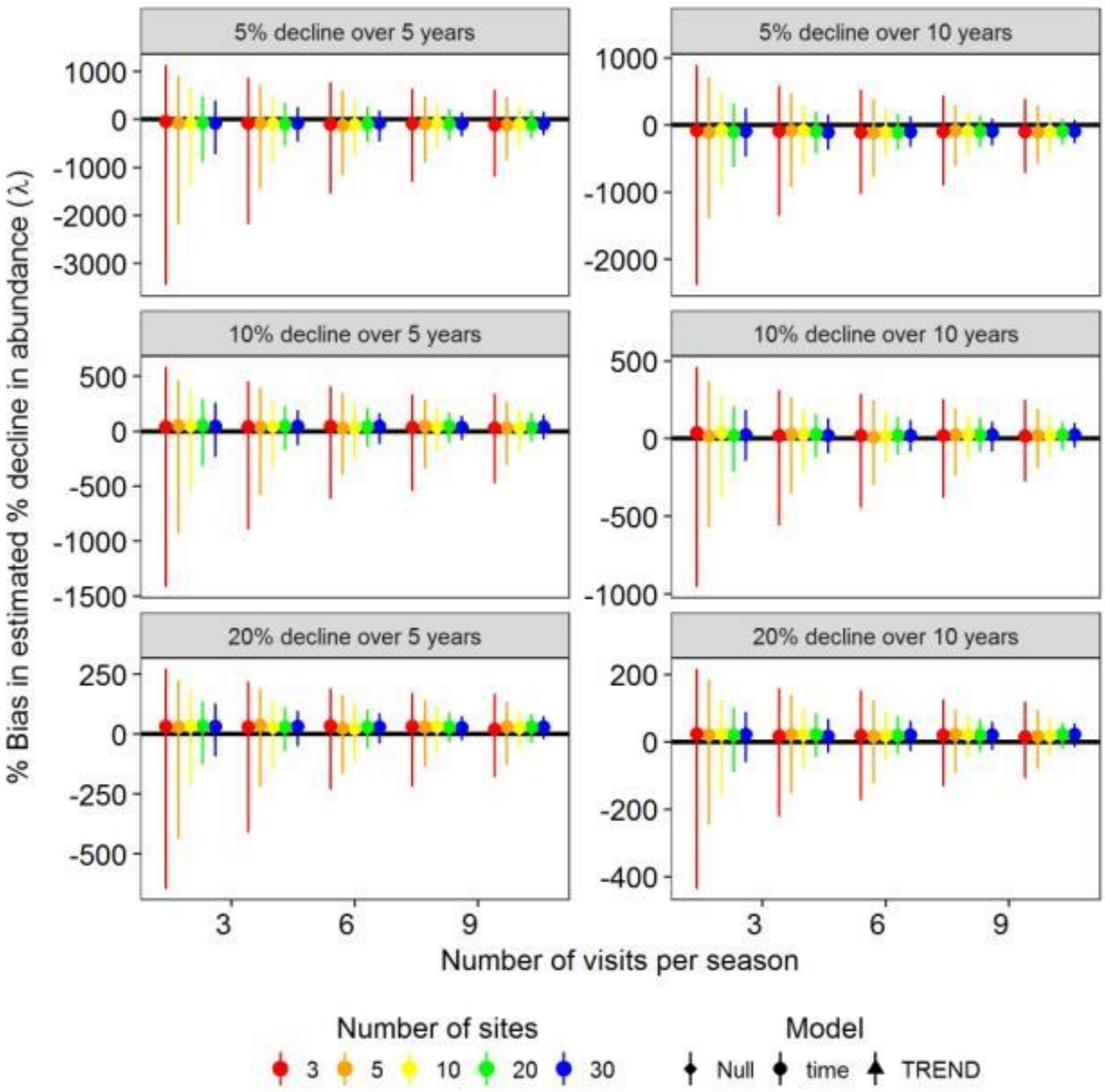
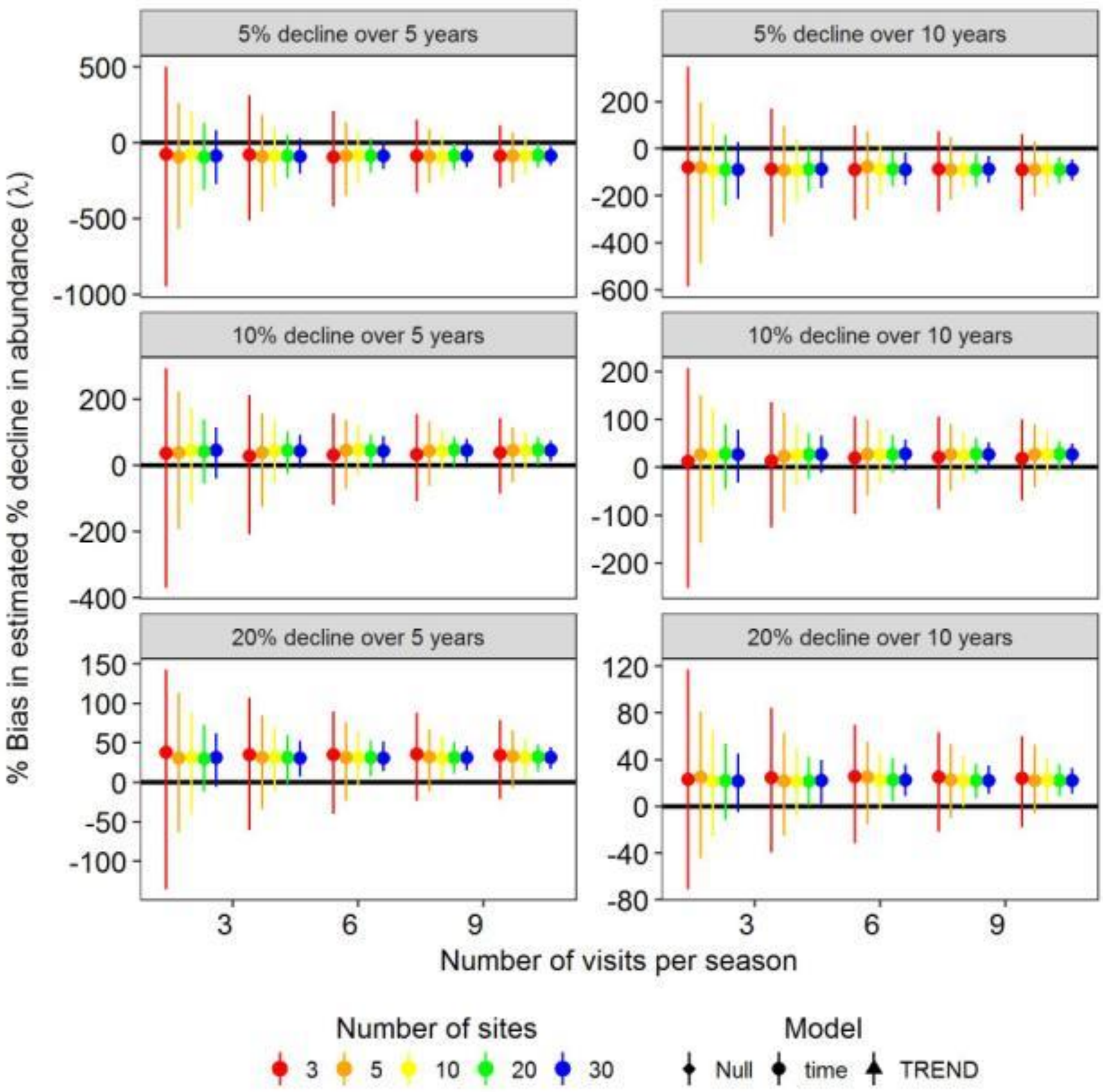


*Figure S29. Percent bias (((truth-estimate)/truth)*100) in the estimated rate of decline in abundance (λ) under different sampling intensities and rates and durations of declines when initial λ = 6 and p = 0.75. Points are the median estimate across 500 simulated data sets and error bars represent the 2.5th and 97.5th quantiles. The dark horizontal line represents zero. Data were fit using the pcount model.*



Appendix 3—Example of radio telemetry data sheet

Date____________Study Site ____Observers_____________________

Coordinate Type (e.g., lat/long, UTM)_______________________Datum/UTM Zone________________________

Snake ID	Date	Time	Coordinates (X)	Coordinates (Y)	New Shed	Reproductive Status	Health Status	Comments (Prey bolus, Behavior, Habitat, Fate)

Additional Comments____

____

____

Appendix 4—Example of mark-recapture data sheet (can also be used to record individual-level data for individuals seen during gestation/shedding site surveys)

Date___________**Study Site** _____________________________________**Observers**__________________

Date	Snake ID (PIT tag #)	Coordinates (X)/Captured at Den?	Coordinates (Y)	Sex	Snout-vent length (cm)	Tail length (cm)	Weight (g)	Reproductive (Y/N)/ # of follicles	Shed since last capture?	# of rattles	Comments (health status, prey bolus, behavior, preparing to shed, etc.)

Additional Comments____

____

Appendix 5—Example of gestation/shedding site occupancy/abundance data sheet

Survey Site: ___________________________________ Survey #:__________ Use this space to record
Start/stop times of breaks:

Date: _______________________________ Observer: ________________________

Start Time:__________ End Time: _________ Time Spent Surveying:__________

Weather Conditions:

Time	Air Temp (2 cm)	Air Temp (2m)	Cloud Cover (nearest 10%)	Wind Speed (1–5)	Wind Direction	Precip. (P/A)	Other

Total Number of Timber Rattlesnakes Observed:____________________________________

Total Number of Gravid Female Timber Rattlesnakes Observed:_______________________

Total Number of Shedding Timber Rattlesnakes Observed:___________________________

Total Number of Neonate Timber Rattlesnakes:_____________________________________

Additional Comments____

____

Appendix 6—Example of R code illustrating occupancy and abundance analyses using model-selection and model-averaging

```r
# Example code for simulating and analyzing occupancy and abundance data using hierarchical
models

# Load packages -------------------
library(unmarked)
library(AICcmodavg)
library(ggplot2)
# The package MuMIn can also be used for model-selection and model-averaging but contains
# several duplicate functions with AICcmodavg

# Use the help pages for additional details.
# As an example,

?unmarkedFrameOccu
?occu
?aictab
?modavgPred
?unmarkedMultFrame
?colext

# The unmarked package also contains several very useful vignettes. To access, click on the
# "Index" link at the bottom of any unmarked help page and then click on the
# "User guides, package vignettes and other documentation" link at the top.
# Also see the unmarked Google group for answers to specific questions.

# Occupancy data -----------------

# Specify true parameter values, number of sites and visits -----------

# Simulate data collected over multiple years with high- and low-occupancy sites and a linear
# trend effect of year on occupancy with constant detection. This is to illustrate how to "stack"
# data to test for a trend using a single-season occupancy model.

# Specify number sites, visits, occupancy and detection rates, number of years, and rate of
decline
# Use a large number (>1000) to verify that the model can return the parameter estimates but
note that
# models take longer to fit as the number of sites increases.

nSites <- 5000
nVisits <- 4
psi <- 0.75
psi_2 <- 0.50 # occupancy of the low-occupancy sites
```

```
p <- 0.95
trend <- -0.20
duration <- 10

# Simulate data ---------

psi_TREND_Site_true <- matrix(NA, ncol = nVisits, nrow = nSites*duration)
psi_Site_time <- matrix(NA, nrow = nSites, ncol = duration)
psi_Site_time[1:(floor(nSites/2)),1] <- rbinom(n = length(1:(floor(nSites/2))),
                                   size = 1, prob = psi)
psi_Site_time[((floor(nSites/2))+1):nSites,1] <- rbinom(n = length(((floor(nSites/2))+1):nSites),
                                          size = 1, prob = psi_2)
Site_TREND_Covs <- data.frame(Site=c(rep("A",length(1:(floor(nSites/2)))),
                             rep("B",length(((floor(nSites/2))+1):nSites))),
                        Year=c(rep(1:duration,each=nSites)))
print(psi)
# Simulates the occupancy status of each site based on the decline in occupancy over time
# This code prints the occupancy for high and low sites for year 2 through year (duration)
for(j in 1:(duration-1)){

  trend_slope1 <- (psi*trend)/(duration-1)
  trend_slope2 <- (psi_2*trend)/(duration-1)
  psi_j <- (psi + (j)*trend_slope1)
  psi_2_j <- (psi_2 + (j)*trend_slope2)

  cat("Year ",j+1,", psi =",psi_j,"\n")
  cat("Year ",j+1,", psi_2 =",psi_2_j,"\n")

  psi_Site_time[1:(floor(nSites/2)),j+1] <- rbinom(n = length(1:(floor(nSites/2))),
                                     size = 1, prob = psi_j)
  psi_Site_time[((floor(nSites/2))+1):nSites,j+1] <- rbinom(n =
length(((floor(nSites/2))+1):nSites),
                                            size = 1, prob = psi_2_j)

}

# Simulates the observed status of each site-year under imperfect detection
i.row <- 1
for(i in 1:duration){
  for(j in 1:nVisits){
    psi_TREND_Site_true[i.row:(i.row+nSites-1),j] <- rbinom(n = nSites,
                                            size = 1,
                                            prob = psi_Site_time[,i]*p)
  }
  i.row <- i.row + nSites
}
```

```
# Fit first year of data using a single-season model --------

year1 <- psi_TREND_Site_true[1:nSites,]
umf_year1 <- unmarkedFrameOccu(y=year1,
                               siteCovs = Site_TREND_Covs[Site_TREND_Covs$Year==1,])

# Conduct model-selection and model-averaging -------

models_year1 <- list("null"=occu(~1 ~1, data=umf_year1),
              "site"=occu(~1 ~Site, data=umf_year1))

model_table <- aictab(models_year1)
model_table

# Calculate model-averaged predicted occupancy for high and low sites

pred_data <- data.frame(Site=c("A","B"))
mod_avg_pred_psi <- modavgPred(models_year1, parm.type="psi",
                               newdata = pred_data)
mod_avg_pred_p <- modavgPred(models_year1, parm.type="detect",
                             newdata = pred_data)
pred_data$Psi <- mod_avg_pred_psi$mod.avg.pred
pred_data$Psi_l <- mod_avg_pred_psi$lower.CL
pred_data$Psi_u <- mod_avg_pred_psi$upper.CL
pred_data$p <- mod_avg_pred_p$mod.avg.pred
pred_data$p_l <- mod_avg_pred_p$lower.CL
pred_data$p_u <- mod_avg_pred_p$upper.CL
pred_data
psi
psi_2

# Fit a single-season model to each year's data ---------
# Model-average and plot estimated occupancy for each year.
# Note, this code should be made into a function to save space but is shown here
# for illustrative purposes.
# Inferences regarding trends can be derived using the 95% CI of the model-averaged
# predicted values for each year.

ss_results <- data.frame(year=rep(seq(1,duration),each=2),
                        Site=rep(c("High","Low")), psi=NA, lcl=NA, ucl=NA)

i_row  <- 1
for(i in 1:duration){
  year_i <- psi_TREND_Site_true[i_row:(i_row+nSites-1),]
  umf_psi_year_i <- unmarkedFrameOccu(y = year_i,
```

```
                              siteCovs = Site_TREND_Covs[Site_TREND_Covs$Year==i,])
  models_year_i <- list("null"=occu(~1 ~1, data=umf_psi_year_i),
                  "site"=occu(~1 ~Site, data=umf_psi_year_i))
  mod_avg_pred_year_i <- modavgPred(models_year_i, parm.type="psi",
                          newdata = data.frame(Site=c("A","B")))
  ss_results[which(ss_results$year==i),"psi"] <- mod_avg_pred_year_i$mod.avg.pred
  ss_results[which(ss_results$year==i),"lcl"] <- mod_avg_pred_year_i$lower.CL
  ss_results[which(ss_results$year==i),"ucl"] <- mod_avg_pred_year_i$upper.CL
  i_row <- i_row + nSites
}

# Plot data using ggplot2, can also be done using the plot() function

g1 <- ggplot(data=ss_results,aes(x=as.factor(year),ymin=lcl,ymax=ucl,y=psi))+
  geom_pointrange(aes(colour=Site),size=0.45,position = position_dodge(width=0.85)) +
  geom_point(aes(colour=Site),size=0.6,position = position_dodge(width=0.85)) +
  geom_hline(yintercept = c(psi,psi_2))+
  scale_colour_manual(values=c("black","darkgrey"),
                 name="Site")+
  theme_bw(base_size = 14)+
  guides(colour=guide_legend(title.position='top',nrow=1,title.hjust=0.5))+
  scale_y_continuous(limits = c(0, 1),
                 name=expression(paste("Occupancy (", psi,")")))+
  scale_x_discrete(name="Year")+
  theme(plot.margin=unit(c(0.5,0.5,0.5,2),"mm"),
      legend.key.width = unit(2,"cm"),
      legend.background = element_rect(fill="white",
                              colour="white", size=0.25,
                              linetype=1),
      panel.grid.major = element_blank(),
      panel.grid.minor = element_blank(),
      panel.background = element_blank(),
      legend.position="bottom",legend.direction="horizontal",
      axis.text=element_text(size=14, colour="black"),
      axis.title=element_text(size=14,colour="black"),
      plot.title=element_text(size=14,face='plain'),
      legend.text = element_text(colour="black", size = 14, face = "plain"),
      legend.title = element_text(colour="black", size=14, face="plain")
  )
g1

ggsave(g1,filename="Model_averaged_occupancy_by_year.jpeg",
     width=6.5,height=5.5,units='in',dpi=300)

# Create unmarkedFrameOccu object using the stacked data ------
# This is how the simulated data were analyzed for the power analysis.
```

```
# This approach could be used to formally test for a trend effect of year.
# However, note that model support in this example is generally low for the model
# incorporating year-to-year variation in psi (i.e., year as a factor)
# so the model-averaged estimates of psi
# will generally follow a linear trend (or no trend depending on the weight of the null
# model). It may therefore be preferable to only use this approach to test for the presence
# of a trend effect and then calculate model-averaged estimates of occupancy for each year
# using single-season models as described above or multi-season occupancy models (see
example below).

# Create another column for Year as a factor, will make model-averaging easier
Site_TREND_Covs$year_factor <- as.factor(Site_TREND_Covs$Year)

umf_psi_TREND_Site <- unmarkedFrameOccu(y=psi_TREND_Site_true,
                                        siteCovs = Site_TREND_Covs)

# Fit candidate models and store in a list object -------------

models <- list("null"=occu(~1  # Detection
                           ~1, # Occupancy
                           data=umf_psi_TREND_Site),
               "site"=occu(~1 ~Site, data=umf_psi_TREND_Site),
               "trend"=occu(~1 ~Year, data=umf_psi_TREND_Site),
               "year"=occu(~1 ~year_factor, data=umf_psi_TREND_Site),
               "trend_site"=occu(~1 ~Year+Site, data=umf_psi_TREND_Site))

# Model-selection and model-averaging ----------------------

# Compare models using AICc

model_table <- aictab(models)
model_table

# Create model-averaged predicted occupancy for each site-year

pred_data <- data.frame(Site=rep(c("A","B"),each=duration),
                        Year=rep(seq(1,duration),2),
                        year_factor=as.factor(rep(seq(1,duration),2)))

mod_avg_pred_psi <- modavgPred(models, parm.type="psi",
                               newdata = pred_data)
mod_avg_pred_p <- modavgPred(models, parm.type="detect",
                             newdata = pred_data)
pred_data$Psi <- mod_avg_pred_psi$mod.avg.pred
pred_data$Psi_l <- mod_avg_pred_psi$lower.CL
pred_data$Psi_u <- mod_avg_pred_psi$upper.CL
```

```
pred_data

# Plot data using ggplot2 -------

g2 <- ggplot(data=pred_data,aes(x=year_factor,ymin=Psi_l,ymax=Psi_u,y=Psi))+
  geom_pointrange(aes(colour=Site),size=0.45,position = position_dodge(width=0.85)) +
  geom_point(aes(colour=Site),size=0.6,position = position_dodge(width=0.85)) +
  scale_colour_manual(values=c("black","darkgrey"),
                      name="Site")+
  theme_bw(base_size = 14)+
  guides(colour=guide_legend(title.position='top',nrow=1,title.hjust=0.5))+
  scale_y_continuous(limits = c(0, 1),
                     name=expression(paste("Occupancy (", psi,")")))+
  scale_x_discrete(name="Year")+
  theme(plot.margin=unit(c(0.5,0.5,0.5,2),"mm"),
        legend.key.width = unit(2,"cm"),
        legend.background = element_rect(fill="white",
                                         colour="white", size=0.25,
                                         linetype=1),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        legend.position="bottom",legend.direction="horizontal",
        axis.text=element_text(size=14, colour="black"),
        axis.title=element_text(size=14,colour="black"),
        plot.title=element_text(size=14,face='plain'),
        legend.text = element_text(colour="black", size = 14, face = "plain"),
        legend.title = element_text(colour="black", size=14, face="plain")
  )
g2

ggsave(g2,filename="Model_averaged_occupancy_by_year_stacked_data.jpeg",
       width=6.5,height=5.5,units='in',dpi=300)

# A note on testing for model goodness-of-fit (GOF) in single-season occupancy models ------

# In any formal analysis, one must always test for model GOF and estimate c-hat.
# If c-hat > 1 you can adjust the models' standard errors and AIC as illustrated below.
# c-hat should be estimated using the global (i.e., most heavily parameterized model).
# See Burnham and Anderson (2002), Mackenzie et al. (2006), and Kery and Royle (2016)
# for additional details.
# Note that model parameter estimates are not biased in the presence of overdispersion
# but estimated standard errors will be too small.

# Note tha nsim should be >1000 in any formal analysis but increasing nsim greatly
# increases run time.
```

```
GOF <- mb.gof.test(models$trend_site,nsim=50)
GOF

aictab(models,c.hat=GOF$c.hat.est)

modavgPred(models, parm.type="psi",
          newdata = pred_data, c.hat=GOF$c.hat.est)

# Reformat for analyzing using a multi-season occupancy model ----------

mult_seas <- matrix(NA, nrow = nSites, ncol = nVisits*duration)
i_col <- 1
i_row <- 1
for(i in 1:duration){
  mult_seas[,i_col:(i_col+nVisits-1)] <- psi_TREND_Site_true[i_row:(i_row+nSites-1),]
  i_col <- i_col + nVisits
  i_row <- i_row + nSites
}

years <- data.frame(matrix(rep(1:duration, each=nSites), nSites, duration))
colnames(years) <- "Year"
site_covariates <- data.frame(Site=Site_TREND_Covs[1:nSites,"Site"])
ummf_psi_TREND_Site <- unmarkedMultFrame(y=mult_seas,
                                    siteCovs = site_covariates,
                                    yearlySiteCovs = years,
                                    numPrimary = duration)

# Fit candidate models and store in a list object ----------------

models <- list("null"=colext(~1, # First-year occupancy
                          ~1, # Colonization
                          ~1, # Extinction
                          ~1, # Detection
                          data = ummf_psi_TREND_Site),
              "site"=colext(~Site, ~1, ~1, ~1, data = ummf_psi_TREND_Site))

# Compare models using AICc ---------------

model_table <- aictab(models)
model_table

# Calculate occupancy for each year -------------------

# Because the multi-season model does not directly estimate occupancy for each year,
# these estimates must be derived using the estimates of initial (year 1) occupancy,
```

```
# extinction, and colonization. This code provides an example of how to calculate these values
# but there is currently no straightforward way (to my knowledge) to calculate CI for
# these derived estimates or model-average derived estimates.
# Note that increasing the value of B will increase run time particularly for complex models.
# For "final" analyses, I recommend B > 1000.
# This code is rather complex but see the vignette in the unmarked package for a more detailed
# discussion of using the colext function.

# Also note that this example does not allow calculating derived estimates for different
# treatment levels (e.g., different sites in this example). See the unmarked Google group
# for discussions on how this might be done.

m1 <- nonparboot(models$null, B = 10)
ms_results <- data.frame(year=rep(seq(1,duration)),
                         psi=smoothed(m1)[2,],
                         SE=m1@smoothed.mean.bsse[2,])

# Plot data using ggplot2

g3 <- ggplot(data=ms_results,aes(x=as.factor(year),y=psi))+
  geom_point(size=2,position = position_dodge(width=0.85)) +
  theme_bw(base_size = 14)+
  guides(colour=guide_legend(title.position='top',nrow=1,title.hjust=0.5))+
  scale_y_continuous(limits = c(0, 1),
                     name=expression(paste("Occupancy (", psi,")")))+
  scale_x_discrete(name="Year")+
  theme(plot.margin=unit(c(0.5,0.5,0.5,2),"mm"),
        legend.key.width = unit(2,"cm"),
        legend.background = element_rect(fill="white",
                                         colour="white", size=0.25,
                                         linetype=1),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        legend.position="bottom",legend.direction="horizontal",
        axis.text=element_text(size=14, colour="black"),
        axis.title=element_text(size=14,colour="black"),
        plot.title=element_text(size=14,face='plain'),
        legend.text = element_text(colour="black", size = 14, face = "plain"),
        legend.title = element_text(colour="black", size=14, face="plain")
  )
g3

ggsave(g3,filename="Derived_occupancy_by_year.jpeg",
       width=6.5,height=5.5,units='in',dpi=300)
```

```
# Abundance -----------------------

# pcount model (assumes no temporary emigration) -----------------
# This example also simulates data where abundance differs between "high" and "low"
abundance
# sites and where abundance declines.

# Specify true parameter values, number of sites and visits ----------

nSites <- 100
nVisits <- 8
lambda <- 4
p <- 0.33
lambda_2 <- 2
trend <- -0.10
duration <- 10

# Simulate data --------------------------

N_TREND_Site_true <- matrix(NA, ncol = nVisits, nrow = nSites*duration)

Ni_high <- rpois(length(1:(floor(nSites/2))), lambda) # True abundance at "high" sites
Ni_low <- rpois(length(((floor(nSites/2))+1):nSites), lambda_2) # True abundance at "low" sites
Ni <- c(Ni_high,Ni_low)

print(summary(Ni[1:(nSites/2)]))

N_TREND_Site_true[1:nSites,] <- Ni
j_row <- nSites+1
for(j in 1:(duration-1)){
  trend_slope <- (Ni*trend)/(duration-1)
  print("trend_slope")
  print(summary(trend_slope[1:(nSites/2)]))
  N_j <- round((Ni + ((j)*trend_slope)),0)
  print("N_j")
  print(summary(N_j[1:(nSites/2)]))
  N_TREND_Site_true[j_row:(j_row+nSites-1),] <- N_j
  j_row <- j_row + nSites
}
N_TREND_Site_obs <- N_TREND_Site_true
i.row <- 1
for(i in 1:duration){
  for(j in 1:nVisits){
    N_TREND_Site_obs[i.row:(i.row+nSites-1),j] <- rbinom(nSites,
                                                         size = N_TREND_Site_true[i.row:(i.row+nSites-1),
                                                                                  j],
```

```
                                        prob = p)
  }
  i.row <- i.row + nSites
}

# Create "stacked" unmarkedFramePCount object ----------

site_covariates <- data.frame(Site=c(rep("A",length(1:(floor(nSites/2)))),
                                  rep("B",length(((floor(nSites/2))+1):nSites))),
                              Year=c(rep(1:duration,each=nSites)),
                              year_factor=as.factor(c(rep(1:duration,each=nSites))))
# Note, if you wish to analyze year as a categorical covariate (i.e., a factor or character)
# you can specify Year as a factor within your model. We include it as a factor here to make
# model-averaging easier.

# Use one matrix per observation-level covariate (e.g., mean survey temperature, date, etc.)
# Must be the same dimension as the matrix containing the observed counts (i.e.,
N_TREND_Site_obs)
obs_covariates <- matrix(as.character(1:nVisits), nSites*duration, nVisits, byrow=TRUE)
# Note that this covariate will have no support because detection was simulated using a
# constant p, not a visit-specific p. It is used purely for illustrative purposes.

umf_N_TREND_Site <- unmarkedFramePCount(y=N_TREND_Site_obs,
                                    siteCovs = site_covariates,
                                    obsCovs=list(visit=obs_covariates))

# Extract and analyze the first year of data as an example -------------

year1 <- umf_N_TREND_Site[1:nSites,]

# Conduct model-selection and model-averaging -------
# K should be set larger than the true value of lambda but models take longer to run
# the more K is increased.

models_year1 <- list("null"=pcount(~1  # Detection
                                  ~1, # Abundance
                                  data=year1, K=lambda*10),
                     "p_visit"=pcount(~visit ~1, data=year1, K=lambda*10),
                     "site"=pcount(~1 ~Site, data=year1, K=lambda*10))

model_table <- aictab(models_year1)
model_table

# Calculate model-averaged predicted occupancy for high and low sites

pred_data <- data.frame(Site=c("A","B"))
```

```
mod_avg_pred_lambda <- modavgPred(models_year1, parm.type="lambda",
                          newdata = pred_data)
pred_data$lambda <- mod_avg_pred_lambda$mod.avg.pred
pred_data$lambda_l <- mod_avg_pred_lambda$lower.CL
pred_data$lambda_u <- mod_avg_pred_lambda$upper.CL
pred_data
lambda
lambda_2

pred_data_p <- data.frame(visit=as.character(seq(1,nVisits)))
mod_avg_pred_p <- modavgPred(models_year1, parm.type="detect",
                        newdata = pred_data_p)
pred_data_p$p <- mod_avg_pred_p$mod.avg.pred
pred_data_p$p_l <- mod_avg_pred_p$lower.CL
pred_data_p$p_u <- mod_avg_pred_p$upper.CL
pred_data_p
p

# A note on the "mixture" argument within pcount ------
# The mixture argument specifies the functional form of Ni (site-specific abundance). The
# default value ("P") specifies a Poisson mixture which is likely appropriate in most situations.
# However, options are available to use a negative binomial mixture to account for
overdispersion or a
# zero-inflated Poisson mixture to account for excess zeros (relative to what would be expected
# under a Poisson distribution). See Kery & Royle (2016) for additional details.
# While our data were simulated under a Poisson mixture we illustrate a situation where a
# zero-inflated Poisson might be appropriate by setting lambda equal to a small number.

# Return to "Specify true parameter values, number of sites and visits" and set
# lambda = <4 and p relatively low to ensure some zeros are recorded on some visits.
# Then re-simulate the data and fit the following models.

low_models <- list("P"=pcount(~1 ~1, data=year1, K=lambda*10, mixture="P"),
                "NB"=pcount(~1 ~1, data=year1, K=lambda*10, mixture="NB"),
                "ZIP"=pcount(~1 ~1, data=year1, K=lambda*10, mixture="ZIP"))
aictab(low_models)

# Depending on your parameter values the ZIP model may have more support. Parameter
# interpretation for the NB and ZIP mixture models is generally the same as with
# the Poisson mixture model except there will be an extra parameter to account for the
# overdispersion and zero-inflation, respectively.

summary(low_models$ZIP)
summary(low_models$NB)

# Analyze each year seperately ----------------
```

```
pcount_results <- data.frame(year=rep(seq(1,duration),each=2),
                      Site=rep(c("High","Low")), lambda=NA, lcl=NA, ucl=NA)

i_row  <- 1
for(i in 1:duration){
  year_i <- umf_N_TREND_Site[i_row:(i_row+nSites-1),]
  models_year_i <- list("null"=pcount(~1 ~1, data=year_i, K=lambda*5),
                      "site"=pcount(~1 ~Site, data=year_i, K=lambda*5))
  mod_avg_pred_year_i <- modavgPred(models_year_i, parm.type="lambda",
                                newdata = data.frame(Site=c("A","B")))
  pcount_results[which(pcount_results$year==i),"lambda"] <-
mod_avg_pred_year_i$mod.avg.pred
  pcount_results[which(pcount_results$year==i),"lcl"] <- mod_avg_pred_year_i$lower.CL
  pcount_results[which(pcount_results$year==i),"ucl"] <- mod_avg_pred_year_i$upper.CL
  i_row <- i_row + nSites
}

g1 <- ggplot(data=pcount_results,aes(x=as.factor(year),ymin=lcl,ymax=ucl,y=lambda))+
  geom_pointrange(aes(colour=Site),size=0.45,position = position_dodge(width=0.85)) +
  geom_point(aes(colour=Site),size=0.6,position = position_dodge(width=0.85)) +
  scale_colour_manual(values=c("black","darkgrey"),
                    name="Site")+
  theme_bw(base_size = 14)+
  guides(colour=guide_legend(title.position='top',nrow=1,title.hjust=0.5))+
  scale_y_continuous(limits = c(0, 12),
                   name=expression(paste("Abundance (", lambda,")")))+
  scale_x_discrete(name="Year")+
  theme(plot.margin=unit(c(0.5,0.5,0.5,2),"mm"),
        legend.key.width = unit(2,"cm"),
        legend.background = element_rect(fill="white",
                                    colour="white", size=0.25,
                                    linetype=1),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        legend.position="bottom",legend.direction="horizontal",
        axis.text=element_text(size=14, colour="black"),
        axis.title=element_text(size=14,colour="black"),
        plot.title=element_text(size=14,face='plain'),
        legend.text = element_text(colour="black", size = 14, face = "plain"),
        legend.title = element_text(colour="black", size=14, face="plain")
  )
g1

ggsave(g1,filename="Model_averaged_lambda_by_year.jpeg",
```

```
      width=6.5,height=5.5,units='in',dpi=300)

# Analyze each year "stacked" to test for a trend effect -----------------

# Fit candidate models and store in a list object ------------

models <- list("null"=pcount(~1  # Detection
                         ~1, # Abundance
                         data=umf_N_TREND_Site, K=lambda*5),
             "site"=pcount(~1 ~Site, data=umf_N_TREND_Site, K=lambda*5),
             "trend"=pcount(~1 ~Year, data=umf_N_TREND_Site, K=lambda*5),
             "year"=pcount(~1 ~year_factor, data=umf_N_TREND_Site, K=lambda*5),
             "trend_site"=pcount(~1 ~Year+Site, data=umf_N_TREND_Site, K=lambda*5))

# Model-selection and model-averaging ----------------------

# Compare models using AICc

model_table <- aictab(models)
model_table

# Create model-averaged predicted occupancy for each site-year

pred_data <- data.frame(Site=rep(c("A","B"),each=duration),
                        Year=rep(seq(1,duration),2),
                        year_factor=as.factor(rep(seq(1,duration),2)))

mod_avg_pred_lambda <- modavgPred(models, parm.type="lambda",
                               newdata = pred_data)
pred_data$lambda <- mod_avg_pred_lambda$mod.avg.pred
pred_data$lambda_l <- mod_avg_pred_lambda$lower.CL
pred_data$lambda_u <- mod_avg_pred_lambda$upper.CL
pred_data

(mod_avg_pred_p <- modavgPred(models, parm.type="detect",
                             newdata = pred_data))

# Plot data using ggplot2 -------

g2 <- ggplot(data=pred_data,aes(x=year_factor,ymin=lambda_l,ymax=lambda_u,y=lambda))+
  geom_pointrange(aes(colour=Site),size=0.45,position = position_dodge(width=0.85)) +
  geom_point(aes(colour=Site),size=0.6,position = position_dodge(width=0.85)) +
  scale_colour_manual(values=c("black","darkgrey"),
                      name="Site")+
  theme_bw(base_size = 14)+
  guides(colour=guide_legend(title.position='top',nrow=1,title.hjust=0.5))+
```

```
  scale_y_continuous(name=expression(paste("Abundance (", lambda,")")))+
  scale_x_discrete(name="Year")+
  theme(plot.margin=unit(c(0.5,0.5,0.5,2),"mm"),
        legend.key.width = unit(2,"cm"),
        legend.background = element_rect(fill="white",
                                         colour="white", size=0.25,
                                         linetype=1),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        legend.position="bottom",legend.direction="horizontal",
        axis.text=element_text(size=14, colour="black"),
        axis.title=element_text(size=14,colour="black"),
        plot.title=element_text(size=14,face='plain'),
        legend.text = element_text(colour="black", size = 14, face = "plain"),
        legend.title = element_text(colour="black", size=14, face="plain")
  )
g2

ggsave(g2,filename="Model_averaged_occupancy_by_year_stacked_data.jpeg",
       width=6.5,height=5.5,units='in',dpi=300)

# Testing for model goodness-of-fit (GOF) ------

# Note that nsim should be >1000 in any formal analysis but increasing nsim greatly
# increases run time.

GOF <- Nmix.gof.test(models$trend_site,nsim=25)
GOF

aictab(models,c.hat=GOF$c.hat.est)

modavgPred(models, parm.type="lambda",
           newdata = pred_data, c.hat=GOF$c.hat.est)


# gpcount model (allows for temporary emigration) ------------------
# Note that these models take a long time to fit and the length of time increases
# with the number of sites.

# Simulate a single season (i.e., year) of data ------

# Specify true parameter values, number of sites and visits ----------

nSites <- 30
lambda <- 5
```

```
lambda_2 <- 3
nVisits <- 6
p <- 0.75
nReps <- 2 # Number of secondary surveys within a primary period where population
# is assumed (2 here represents two site visits per day where each site is
# assumed to be closed within a day)
phi <- 0.80

# Simulate data -------

N_1 <- rpois(floor(nSites/2), lambda)
N_2 <- rpois(ceiling(nSites/2), lambda_2)
Ni <- c(N_1,N_2)
site_Covs <- data.frame("Site"=c(rep("Low",length(N_1)),rep("High",length(N_2))))
site_Covs$Site <- factor(site_Covs$Site,levels=c("Low","High"))
N_true  <- matrix(Ni,nSites,nVisits,byrow=F)

N_avail <- N_true
for(i in 1:nVisits) {
  N_avail[,i] <- rbinom(nSites, Ni, phi) # population available during vist j
}
Y_gpcount <- N_avail[,rep(1:ncol(N_avail), each=nReps)]
Y_gpcount[,] <- NA
for(i in 1:nrow(Y_gpcount)) {
  survey <- 1
  for(j in 1:nVisits) {
    Y_gpcount[i,survey:(survey+1)] <- rbinom(nReps, N_avail[i,j], p)
    survey <- survey + 2
  }
}

# Create a matrix for "time" as a continuous survey-level covariate
time <- matrix(seq(1,nVisits), nSites, nVisits, byrow=T)
# z-score standardize time to have mean = 0 and SD = 1
# This is generally recommended with continuous covariates to help model convergence
# but you have to remember to back-transform the predicted values.
time <- (time - (mean(time)))/sd(time)

# Note that time is specified per primary period, not per survey. As a result, it can
# only be used to model temporary emigration (phi) not detection. To use time to model
detection,
# a time needs to be specified for every survey, as shown here.

obs_time <- time[,rep(1:ncol(time),each=nReps)]

# Now create two "random" primary period-level and survey-level covarites just to illustrate
```

```
# including multiple covariates.

time_2 <- matrix(rnorm(nVisits,0,1), nSites, nVisits, byrow=T)
obs_time_2 <- matrix(rnorm(ncol(obs_time),0,1), nrow(obs_time), ncol(obs_time), byrow=T)

# Create umf for gpcount
umf_gpcount <- unmarkedFrameGPC(y=Y_gpcount,
                        siteCovs = site_Covs,
                        numPrimary=nVisits,
                        yearlySiteCovs = list(time=time, time_rand=time_2),
                        obsCovs = list(obs_time=obs_time, obs_rand=obs_time_2))

# Conduct model-selection and model-averaging -------
# K now is the maximum possible superpopulation size so should be larger for high rates of
# temporary emigration (i.e., low phi).
# Mixture can be a Poisson or negative binomial but not zero-inflated Poisson.

gpc_models <- list("null"=gpcount(~1, # Abundance
                           ~1, # Temporary emigration/availability
                           ~1, # Detection
                           data=umf_gpcount, K=lambda*10,
                          control=list(trace=TRUE, REPORT=1)),
                "random_p"=gpcount(~1, ~1, ~obs_time+obs_rand, data=umf_gpcount,
                            K=lambda*10,control=list(trace=TRUE, REPORT=1)),
                "random_phi"=gpcount(~1, ~time, ~1, data=umf_gpcount,
                            K=lambda*10,control=list(trace=TRUE, REPORT=1)),
                "site"=gpcount(~Site, ~1, ~1, data=umf_gpcount, K=lambda*10,
                          control=list(trace=TRUE, REPORT=1)))

model_table <- aictab(gpc_models)
model_table

# Calculate model-averaged predicted abundance, availability, and detection

pred_data <- data.frame(Site=c("A","B"))
mod_avg_pred_lambda <- modavgPred(gpc_models, parm.type="lambda",
                          newdata = pred_data)
pred_data$lambda <- mod_avg_pred_lambda$mod.avg.pred
pred_data$lambda_l <- mod_avg_pred_lambda$lower.CL
pred_data$lambda_u <- mod_avg_pred_lambda$upper.CL
pred_data
lambda
lambda_2

# One advantage of z-score standardizing continuous covariates is that you can easily predict
# at a mean value by using zero (although you may then want to back-transform this for the
```

```
# purpose of plotting the results on the original scale, for example).
pred_data_phi <- data.frame(time=as.vector(unique(time)))
mod_avg_pred_phi <- modavgPred(gpc_models, parm.type="phi",
                        newdata = pred_data_phi)
pred_data_phi$phi <- mod_avg_pred_phi$mod.avg.pred
pred_data_phi$phi_l <- mod_avg_pred_phi$lower.CL
pred_data_phi$phi_u <- mod_avg_pred_phi$upper.CL
pred_data_phi
phi
# Note that because phi was not modeled according to any covariates that the predicted values
# are all relatively close to the true value of phi

pred_data_p <- data.frame(obs_time=as.vector(unique(time)),
                       obs_rand=as.vector(unique(obs_time_2)))
mod_avg_pred_p <- modavgPred(gpc_models, parm.type="detect",
                        newdata = pred_data_p)
pred_data_p$p <- mod_avg_pred_p$mod.avg.pred
pred_data_p$p_l <- mod_avg_pred_p$lower.CL
pred_data_p$p_u <- mod_avg_pred_p$upper.CL
pred_data_p
p




# gmultmix model (dependent double observer method with temporary emigration) ----------------
--
# Note that these models do not take as long to fit as gpcount models but still take longer
# than pcount models.

# Simulate a single season (i.e., year) of data ------

# Specify true parameter values, number of sites and visits ----------

nSites <- 500
lambda <- 10
lambda_2 <- 5
nVisits <- 6
phi <- 0.80
pA <- 0.75 # Detection for the primary observer
pB <- 0.95 # Detection for the secondary observer

# Source this function
depDoubPiFun <- function(p) {
  M <- nrow(p)
  pi <- matrix(NA, M, 2)
```

```
  pi[,1] <- p[,1]
  pi[,2] <- p[,2]*(1-p[,1])
  return(pi)
}

# Simulate data ----------

N_1 <- rpois(floor(nSites/2), lambda)
N_2 <- rpois(ceiling(nSites/2), lambda_2)
Ni <- c(N_1,N_2)
N_true  <- matrix(Ni,nSites,nVisits,byrow=F)
N_avail <- N_true
for(i in 1:nVisits) {
  N_avail[,i] <- rbinom(nSites, Ni, phi) # population available during vist j
}

Y_gmultmix <- N_avail[,rep(1:ncol(N_avail), each=2)]
cp <- c(pA, pB * (1 - pA))
cp[3] <- 1 - sum(cp)
for(i in 1:nrow(Y_gmultmix)) {
  survey <- 1
  for(j in 1:nVisits) {
    Y_gmultmix[i,survey:(survey+1)] <- c(rmultinom(1,
                                          Y_gmultmix[i,survey],
                                          cp)[1:2])
    survey <- survey + 2
  }
}

# Specify covariates
site_Covs <- data.frame("Site"=c(rep("Low",length(N_1)),rep("High",length(N_2))))
site_Covs$Site <- factor(site_Covs$Site,levels=c("Low","High"))
time <- matrix(seq(1,nVisits), nSites, nVisits, byrow=T)
time <- (time - (mean(time)))/sd(time)
obs_time <- time[,rep(1:ncol(time),each=2)]

# Specify covariates to identify primary and secondary observers and create the
# "obsToY" object (see the ?unmarkedFrameGMM for more details)
observer <- matrix(c("A", "B"), nSites, nVisits*2, byrow=TRUE)
obsToY <- matrix(1, 2, 2)
obsToY <- kronecker(diag(nVisits), obsToY)

# Create umf object
umf_gmultmix <- unmarkedFrameGMM(y = Y_gmultmix,
                                 siteCovs = site_Covs,
                                 obsCovs = list(observer=observer, obs_time=obs_time),
```

```
                              yearlySiteCovs = list(time=time),
                              numPrimary=nVisits, obsToY=obsToY,
                              piFun="depDoubPiFun")

# Conduct model-selection and model-averaging -------
# K is simply identified as the upper bound of emigration (see ?gmultmix) but should still be
# large relative to lambda.
# Mixture can be a Poisson or negative binomial but not zero-inflated Poisson.

# Note that double observer models often fail to converge. In my limited experience with
# simulated data and gmultmix models, these models perform best when pA=pB and
performance decreases
# Be sure to check to make sure you are averaging across models that converge.
# Alternatively, you can use the "starts" argument to provide starting values which my allow
# a model to converge. Starting values must be on the link scale for their respective parameters.

gmultmix_models <- list("null"=gmultmix(~1, # Abundance
                               ~1, # Temporary emigration/availability
                               ~1, # Detection
                               data=umf_gmultmix, K=lambda*10,
                               control=list(trace=TRUE, REPORT=1)),
                      "time_phi"=gmultmix(~1, ~time, ~1, data=umf_gmultmix,
                                    K=lambda*20,control=list(trace=TRUE, REPORT=1)),
                      "obs_p"=gmultmix(~1, ~1, ~observer, data=umf_gmultmix,
                                  K=lambda*20,control=list(trace=TRUE, REPORT=1)),
                      "site"=gmultmix(~Site, ~1, ~1, data=umf_gmultmix,
                                    K=lambda*20,control=list(trace=TRUE, REPORT=1)),
                      "site_obs_p"=gmultmix(~Site, ~1, ~observer, data=umf_gmultmix,
K=lambda*10,
                               control=list(trace=TRUE, REPORT=1),
                               starts=c(log(lambda),(log(lambda_2)-log(lambda)),qlogis(phi),
                                    qlogis(pA),(qlogis(pB)-qlogis(pA))))
                      )

model_table <- aictab(gmultmix_models)
model_table

summary(gmultmix_models$site_obs_p)
coef(gmultmix_models$site_obs_p)

# Calculate model-averaged predicted abundance, availability, and detection
# It is usually best to exclude models that did not converge

non_convg <- NULL
for(i in 1:length(gmultmix_models)){
  if(NaN %in% sqrt(diag(vcov(gmultmix_models[[i]])))) non_convg[i] <- i
```

```
}

if(!is.null(non_convg)) gmultmix_models <- gmultmix_models[which(is.na(non_convg))]

pred_data <- data.frame(Site=c("A","B"))
mod_avg_pred_lambda <- modavgPred(gmultmix_models, parm.type="lambda",
                                  newdata = pred_data)
pred_data$lambda <- mod_avg_pred_lambda$mod.avg.pred
pred_data$lambda_l <- mod_avg_pred_lambda$lower.CL
pred_data$lambda_u <- mod_avg_pred_lambda$upper.CL
pred_data
lambda
lambda_2

pred_data_phi <- data.frame(time=as.vector(unique(time)))
mod_avg_pred_phi <- modavgPred(gmultmix_models,
                               parm.type="phi",
                               newdata = pred_data_phi)
pred_data_phi$phi <- mod_avg_pred_phi$mod.avg.pred
pred_data_phi$phi_l <- mod_avg_pred_phi$lower.CL
pred_data_phi$phi_u <- mod_avg_pred_phi$upper.CL
pred_data_phi
phi

pred_data_p <- data.frame(observer=c("A","B"))
mod_avg_pred_p <- modavgPred(gmultmix_models,
                             parm.type="detect",
                             newdata = pred_data_p)
pred_data_p$p <- mod_avg_pred_p$mod.avg.pred
pred_data_p$p_l <- mod_avg_pred_p$lower.CL
pred_data_p$p_u <- mod_avg_pred_p$upper.CL
pred_data_p
c(pA,pB)
```

Conclusions

In this report, we address several components of timber rattlesnake conservation in New England which together represent a substantial contribution towards the management and recovery of this species within the northeastern United States. Our results largely corroborate the imperiled status of timber rattlesnakes in New England. While data limitations prevented us from evaluating the status of most New England timber rattlesnake populations, our results suggest that timber rattlesnakes in one Vermont, one New Hampshire, and one Connecticut population may be declining although timber rattlesnakes in a western Massachusetts metapopulation do not appear to be declining under current conditions. However, we acknowledge the limitations of our data and recommend caution when interpreting the results of our population viability analyses. Given the widespread, and often fine-scale, spatial variation in snake demographic parameters, we suggest that additional data be collected from these and other populations if evaluating population status is a management priority. Age-specific survival estimates and estimates of reproduction frequency, fecundity, and population size from individual populations would be of great value for more rigorous population viability analyses. However, we note that collecting such data will require a substantial commitment of time and resources. Our population viability analyses indicate that management actions designed to reduce anthropogenic impacts on adult survival may have the greatest impact on improving population persistence. Therefore, we suggest that mangers consider implementing management actions intended to reduce adult mortality which could include the creation of road-crossing culverts, implementing removal programs for “nuisance” rattlesnakes, or increased education and outreach. Population augmentation may also be warranted for small populations or those experiencing sudden, rapid population declines.

Our genetics results were consistent with the isolated nature of timber rattlesnakes in New England and indicated substantial genetic differentiation among populations and relatively low population connectivity. These results strongly suggest that genetic rescue be included as a potential management option in timber rattlesnake conservation planning. While our results may indicate that some populations are stronger candidates for genetic rescue than others, we note that uncertainty regarding factors contributing towards contemporary patterns of genetic structure and a lack of understanding of historical patterns of timber rattlesnake distribution and genetic connectivity complicate efforts to identify source populations. It is likely that other factors (e.g., costs, logistical constraints, socio-political factors) will require a case-by-case application of genetic rescue. We therefore strongly encourage a collaborative approach via a New England Inter-Agency Timber Rattlesnake Conservation Group to implementing genetic rescue and a comprehensive evaluation of the costs and benefits of different management actions, which will likely vary from population to population. While we found insufficient information from our literature review to develop genetics-based recommendations for when and how genetic rescue should be implemented, we note that other studies have used phenotypic traits to guide successful genetic rescue efforts. We therefore recommend a multi-facetted approach to implementing genetic rescue incorporating genetic, phenotypic, and demographic data. Furthermore, we suggest that genetic rescue for timber rattlesnakes in New England represents an important learning opportunity and encourage managers to adopt an adaptive management approach to implementing genetic rescue.

Our results emphasize the need for region-wide monitoring efforts to evaluate population trends. Additionally, these results indicate that timber rattlesnake monitoring efforts should not focus primarily on counts of unmarked individuals at dens during egress and ingress but should instead focus on gestation and shedding sites and/or mark-recapture approaches. While mark-recapture data can directly or indirectly provide estimates of many demographic parameters needed to evaluate population persistence, the high level of effort needed for mark-recapture studies combined with frequent low recapture rates limits the widespread applicability of mark-recapture. The potential for behavioral impacts to individuals as a result of repeated capture and handling may also restrict the use of mark-recapture. Unfortunately, our results also suggest that the number of gestation and shedding sites in some states may be too low to statistically detect trends in model-based estimates of occupancy and abundance. Pooling sites across states and testing for regional differences in parameter estimates through formal model-selection approaches represents a potential solution. We discuss a standardized gestation/shedding site monitoring protocol and encourage inter-state collaboration to identify potential sites and coordinate data management and analysis. Because of the potential for temporary emigration at gestation/shedding sites, we recommend that managers, at least initially, adopt a study design that will permit the estimation of and evaluation of factors influencing temporary emigration. The collaborative effort involved in this project represents a key step towards the creation of a

New England Inter-Agency Rattlesnake Conservation Group which will be critical for successfully implementing this monitoring protocol. Ongoing efforts towards implementation include capacity building efforts to purchase field supplies and equipment to initiate monitoring work in the spring of 2018 and the compilation of a spatially-referenced database of potential monitoring sites for select populations. This group will adopt an adaptive management approach where monitoring data will regularly be evaluated to identify potential changes in management actions or priorities.

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