FINAL PERFORMANCE REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-151-R

F12AP00888

Endangered and Threatened Species Conservation

Reintroduction of the Louisiana pine snake (*Pituophis ruthveni*) in Texas and Louisiana

Prepared by:

Dr. Craig Rudolph

TEXAS PARKS & WILDLIFE

Carter Smith
Executive Director

Clayton Wolf
Director, Wildlife

5 November 2015

FINAL PERFORMANCE REPORT

STATE: ____Texas_______________ **GRANT NUMBER:** ___TX E-151-R___

GRANT TITLE: Reintroduction of the Louisiana pine snake (*Pituophis ruthveni*) in Texas and Louisiana

REPORTING PERIOD: ____1 September 2012 to 31 August 2015

OBJECTIVE(S): To support reintroduction efforts for the Louisiana Pine Snake in Texas including; capture of additional animals in TX to develop a captive breeding population appropriate for release in TX, continued survey of recently known populations, and analysis of DNA samples to inform management decisions.

Segment Objectives:

Task 1. Shed skin and tissue samples requiring DNA extraction and analysis are collected from all wild caught and captive bred individuals.

Task 2. Surveys in Texas, at the site of the 3 recently existing populations.

Task 3. *Pituophis ruthveni* specimens captured in TX will be incorporated into the TX captive breeding program.

Significant Deviations: None.

Summary Of Progress: See Attachment A, and supplementary materials (Kwiatkowski et al. 2014 draft manuscript to be submitted to Conservation Genetics; Rudolph et al. draft manuscript for submission to scientific journal; Wagner et al. 2014; Rudolph et al. 2012).

Location: Wood, Sabine, Newton, Jasper, and Angelina counties, Texas.

Cost: ___Costs were not available at time of this report.

Prepared by: _Craig Farquhar_____________ **Date:** _5 November 2015_

Approved by: ______________________________ **Date:**__5 November 2015_

C. Craig Farquhar

ATTACHMENT A

REINTRODUCTION OF THE LOUISIANA PINE SNAKE (*PITUOPHIS RUTHVENI)* IN TEXAS AND LOUISIANA

Final Report for Section 6 Funding

Texas Parks and Wildlife Contract 432599

And

U. S. Forest Service Agreement 13-CO-11330124-013

Final Report: 28 October 2015

Principal Investigators:

____

D. Craig Rudolph

____

Daniel Saenz

U. S. D. A., Forest Service

Southern Research Station

706 Hayter Street

Nacogdoches, Texas 75965

Abstract

The Louisiana Pinesnake (*Pituophis ruthveni*) has been declining throughout its historic range, and in Texas, for several decades (Rudolph et al. 2006, In Prep.). The most recent record from Texas was in 2008 (plus a recaptured individual in 2012). Despite the lack of recent records considerable survey effort continues due to the importance of verifying the continued existence of recently extant populations, locating additional extant populations, obtaining specimens for the captive breeding program, and providing additional DNA samples and prey records. During the course of this Section 6 Project (2013-15) a total of 26,043 trap days were accomplished in 6 sites, including the 4 sites represented by the most recent *P. ruthveni* records and two sites represented by older records and inadequately surveyed in the past. Unfortunately, but not unexpectedly, no additional *P. ruthveni* were documented during the course of these trapping surveys. Consequently, no additional DNA samples or prey records were obtained. Data available through 2013 was used to assess the status of *P. ruthveni* throughout the historic range. Existing genetic samples were also analyzed to provide a better understanding of the genetic structure, inbreeding levels, and other genetic characteristics of *P. ruthveni.*

Introduction

Pituophis ruthveni (Louisiana Pinesnake) is one of the rarest snakes in the United States (Conant 1956; Rudolph et al. 2006; Young and Vandeventer 1988). Recent population declines and extirpations have been inferred from available data (Rudolph et al. 2006, In Prep.). The primary causes of these declines are thought to be habitat loss due to changing land use patterns and the reduced suitability of the remaining forested habitat (Rudolph and Burgdorf 1997). Intensive short-rotation pine (*Pinus* spp.) silviculture and widespread fire suppression have reduced the diverse herbaceous vegetation that characterized the original forests by increasing shade (especially early in the rotation), litter buildup in the absence of fire, and herbicide use (Frost 1993). Reduction of the herbaceous vegetation is hypothesized to lead to declines of Baird's Pocket Gopher (*Geomys breviceps*) populations (Rudolph and Burgdorf 1997), the primary prey of *P. ruthveni* (Rudolph et al. 2002, 2012). Declines in pocket gopher abundance are hypothesized to lead to declines of *P. ruthveni* populations (Rudolph and Burgdorf 1997). Wagner et al. (2014) provided a soil suitability model (attached) that clarified the relationship between *P. ruthveni* use and soil characteristics.

Pituophis ruthveni is currently a Candidate Species under the U.S. Endangered Species Act (USFWS 2013a). The historical range included portions of eastern Texas and western Louisiana (Reichling 1995; Sweet and Parker 1991); however, recent surveys suggest a significant decline in overall range in recent decades (Rudolph et al. 2006, In Prep.). In 2008, the U.S. Fish and Wildlife Service (USFWS) delineated seven extant populations, defined as groups with the potential for internal genetic exchange, but genetically isolated from each other (USFWS 2011; Fig. 1). In addition to remnant populations, an ongoing reintroduction effort was initiated on the Catahoula District of the Kisatchie National Forest in 2010.

Beginning in 1992, trapping efforts and substantial field work have been conducted throughout the historical range of *P. ruthveni*. Since that time, efforts and thus record numbers have increased. However, disturbing trends suggest a decline in both overall range and population sizes. The last record in Texas was an individual captured in 2008 with the exception of an individual captured in 2007 and recaptured in 2012. There is a substantial probability that all, or most, populations in Texas are extirpated. Due to the limited historical range, apparent decline in range and numbers, its status as a Candidate Species, and increasing concerns about viability of populations range-wide, additional surveys are warranted within the state.

In addition to increased data on status of *P. ruthveni*, additional specimens from Texas would contribute to ongoing genetic characterization of the species, and bolster the captive breeding program currently in progress. These goals are critical to the recovery of *P. ruthveni* due to the limited number of

DNA samples available range-wide (Kwiatkowski et al. 2010) and the extremely limited number (n = 3) of captive wild-caught animals from Texas. Additional data on the genetic structure of *P. ruthveni*, and a more genetically diverse captive breeding population would greatly improve the probability of population recovery.

Objectives

The objectives of this Section 6 grant were to continue the ongoing surveys of *P. ruthveni* in Texas to provide a better estimate of population status in the state. In addition, any snakes captured would augment the captive breeding program in support of ongoing, and hopefully, expanding reintroduction efforts. This is especially critical due to the limited number of founders in the existing captive population. Captured specimens would also potentially contribute to the increasing knowledge of the diet of *P. ruthveni* through the analysis of any fecal samples obtained from captive individuals.

DNA samples (blood or shed skin) collected from captured animals would be incorporated into an existing library of DNA samples from throughout the historic range to examine questions related to genetic structure of the species. Primary questions to be investigated include 1) Determine the level of genetic structuring among natural populations of Louisiana Snakes, 2) Identify the most likely number of genetic groups or "clusters" in the absence of any geographical information and identify individuals that do not strongly assign to any population, 3) Quantify genetic diversity within and among wild and captive populations of Louisiana Pine Snakes, and 4) Genotype captive snakes to maximize out-crossing potential in the captive population.

Location

Field survey locations were located in 6 counties in eastern Texas (Figure 1). The specific sites are Jarvis Christian University (JU), Wood County; Campbell Timberland Management LLC lands (CAM), Nacogdoches County; James Stutzenburg lands (ST), Tyler County; Angelina National Forest lands (ANF), Angelina and Jasper Counties; Sabine National Forest lands (SNF), Sabine County; and Scrappin' Valley (SV), Newton County. Individual trap locations (Lat./Long.) are available from the authors on request.

Methods

A total of 56 traps were installed and operated during 2013-15, not all operational in any one year. Traps were constructed of treated plywood and hardware cloth following the protocol in Burgdorf et al. (2005). Basically, traps consisted of a 4' X 4' X 16" box with plywood top and bottom and hardware cloth sides. A funnel entrance was installed on each side and a 50' drift fence of 18" hardware cloth extended perpendicular to the side of the trap to guide snakes to the funnel entrance. Traps were provided with a water source (1 gal. chick waterer) and a plastic hide box. This basic design has been the standard trap design for *P. ruthveni* since the early 1990s. Traps were placed to take advantage of any available shade and checked twice per week. All captured vertebrates were removed and released.

Six trapping localities were chosen (Angelina, Nacogdoches, Newton, Sabine, Tyler and Wood Counties) within the historic range of *P. ruthveni* (Figure 1). The specific sites in Angelina, Newton, and Sabine Counties were polygons enclosing multiple *P. ruthveni* records since 1993. The Nacogdoches County site was selected based on suitability of habitat and access in the general vicinity of a 2008 *P. ruthveni* record with only general location data (vicinity of Garrison, TX). The Tyler County site was located near two anecdotal records (unverified) from 2005. The Wood County site was selected in the general vicinity of historical records in the county. This site was included because limited survey efforts had previously been carried out in this northern portion of the historic range.

Data bases containing all information pertaining to the status of *P. ruthveni* (locality records, survey effort) is maintained at the Southern Research Station Lab in Nacogdoches, Texas. The results of current efforts under this research grant through 2013 were used to examine the current status of *P.*

ruthveni (Rudolph et al. In Prep.). These analyses are currently being updated using data available through 2015. The following methodology applies to this document. Acknowledgements and literature cited can also be found in this document (Rudolph et al. In Prep.) which is attached.

A database has been maintained, beginning in 1995, containing all known records of *P. ruthveni* obtained from the published literature, museum specimens, and current research activities by the authors and collaborators. This database currently (through 2013) contains 221 unique (not including recaptures) records, and 24 recaptures. Database records consist of trap and incidental records that were used to delineate occupied range.

Trapping protocol.—Between 1992 and 2013, traps (N = 504) were placed at researcher-selected sites within accessible properties, dispersed as widely as possible throughout the historical range of *P. ruthveni* (Rudolph et al. 2006). Numerous cooperators have been involved in the trapping efforts (see Acknowledgements). The purposes of these trapping efforts have varied over the years to include capture of animals for radio-telemetry studies (Ealy et al. 2004; Himes et al. 2006; Rudolph and Burgdorf 1997; Rudolph et al. 1998), examination of the effects of road mortality on snake populations (Rudolph et al. 1999), and presence/absence surveys (Rudolph et al. 2006).

Traps were operated for variable numbers of years at 31 sites in 10 counties in Texas and 7 parishes in Louisiana (Fig. 2). Traps consisted of 1.2- x 1.2-m plywood and hardware cloth boxes with a funnel entrance on each side and 15.2-m drift fences extending from each entrance (Burgdorf et al. 2005). All traps contained a water source and most traps contained hide boxes for additional cover. The basic trap design underwent minor modification by the various individuals involved in the trapping program, and occasionally major modifications. Minor modifications consisted of changes in mesh size, alteration of funnel entrance diameter to reduce by-catch, and minor structural modifications of the traps themselves. These modifications are unlikely to have had a major impact on trap success. Cooperators made major modifications to trap design in a few instances. One modification consisted of a straight line arrangement instead of one drift fence radiating from each of the four sides of the box trap. Two different configurations were involved with this major modification. One configuration (N = 4) consisted of two box traps connected to each other by 30.5 m of drift fence with one 15.2-m drift fence continuing from each trap. Only those funnel entrances associated with a drift fence were open. These arrays consisted of the same amount of total drift fence (70 m), and the same number of funnel openings (4) as the single trap configuration. A second configuration (N = 2) consisted of three box traps with 53.3 m of drift fence between trap 1 and 2, and 53.3 m of fencing between trap 2 and 3. These arrays consisted of 106.7 m of total drift fence, but used the same number of funnel openings (4) as the single trap configuration. A second major modification consisted of smaller (1.2 x 0.6 m) traps (N = 3) that were partially buried. These traps used funnels that were slightly tilted downward to potentially facilitate snake movement into the box. These major modifications had an unknown effect on trap success and defining a “trap” was not always straightforward. For purposes of this report, we considered all boxes connected by drift fences to be a single trap.

Traps were checked 1–2 times per week and all snake captures were recorded. All *P. ruthveni* were returned to the lab for measuring, sex determination, and collection of DNA and fecal samples. After processing in the lab, *P. ruthveni* were released at their capture site, with the exception of a limited number of animals that were used for radio-telemetry studies or retained for captive breeding. Radio-transmitters and/or passive integrated transponder (PIT) tags were implanted into all released *P. ruthveni* since 1996, with the exception of six animals (5 in Louisiana and 1 in Texas).

Incidental records.—Incidental records include all non-trap records (i.e., road-kills and hand captures). These were obtained from published literature, museum specimens, and current research activities by collaborators. From 1992 to present, an increasingly large group of formal and informal cooperators has been active in the field and alert to the importance of reporting incidental *P. ruthveni* observations.

Occupied range.—We plotted all records obtained from 1992 to 2013, including recaptures, throughout the historical range to update the delineation of occupied habitat and determine temporal trends in known population size. We grouped records into populations, defined as a group of ≥3 records obtained from 1992 to 2013 within 10 km of another record. Thus, we discounted all records that did not include location information (N = 3), single records (N = 3), and groups of two records (N = 2) separated from the nearest adjacent records by >10 km. We constructed minimum convex polygons around these points, including a 1-km buffer, the approximate diameter of a home range (Rudolph, unpublished data) to estimate occupied range. To estimate temporal change in occupied habitat for each population, occupied habitat minimum convex polygons (OHMCPs) were then constructed and plotted for the following time intervals: 1992–1996, 1997–2001, 2002–2007, and 2008–2013.

Model.—To elucidate population trends we used trap capture data from 1992 through 2013. We modeled snake capture rate (unique captures per 1000 trap days) for each population using year as the only predictor variable in a generalized linear model (Littell et al. 2002). Count or rate data generally follow a Poisson distribution. However, numerous factors other than year potentially affect capture rate (e.g., population density) and not including these relevant (but unmeasured) explanatory variables can result in overdispersion of a Poisson model (the variance exceeds the mean; Agresti 2002). Dispersion can also be due to an overabundance of zero responses. Zero captures in a year can reflect either an absence of snakes, or that snakes were present, but not captured. Negative binomial distributions are useful for overdispersed data and both the Poisson and negative binomial distributions can be used in zero-inflated models to include the probability of a zero response in predicted values.

Therefore, we modeled the capture rate distribution from each population with Poisson, negative binomial, zero-inflated Poisson, and zero-inflated negative binomial distributions and included both null models and models with year as a predictor. Models were compared via the Akaike's information criterion for small sample sizes (AIC_c) (Burnham and Anderson 2002). Models with a difference of <4 from the smallest AIC_c value were considered useful (Burnham and Anderson 2002). The Pearson chi-square divided by the number of degrees of freedom was used to evaluate goodness-of-fit (GOF); values close to 1 indicate a good fit (Pedan 2001). From models including year, for each population we plotted actual and predicted capture rates from the model with the lowest AIC_c, along with their 95% confidence intervals, against year to visually inspect the relationships.

In the event *P. ruthveni* were to be captured, they were to be brought to the lab, held for 1-2 weeks, and released near their point of capture. During the period in the lab, length and mass would have been recorded, animals would have had a pit tag implanted, blood and/or shed skins would have been collected for DNA analysis, and any fecal material collected. In the absence of *P. ruthveni* captures during the period of this survey in Texas, prior DNA samples available from Texas and samples from Louisiana were used to examine genetic characteristics of *P. ruthveni* populations. Details of methods and results of genetic investigations are contained in the attached publication (Kwiatkowski et al. 2010) and report (Kwiatkowski et al. 2014). Funds from this grant were used in preparation of the report (attached). Similarly, any fecal samples collected would have been used to add to the existing data base. In the absence of additional samples, Rudolph et al. (2012) is the most recent update on the diet of *P. ruthveni* (attached).

Results

A total of 26,043 trap days at 56 individual trap locations were accumulated in 6 sites in Angelina/Jasper, Nacogdoches, Newton, Sabine, Tyler and Wood Counties within the historic range of *P.*

ruthveni in fiscal years 2013-15. These sites included the three populations (Angelina/Jasper, Newton, and Sabine Counties) as defined by the USFWS (2011) based on multiple records since the mid-1990s, a site in Nacogdoches County with a 2008 record, a site in Tyler County with 2 anecdotal records in 2005, and a site in Wood County near the location of historical *P. ruthveni* records that had not been adequately surveyed previously. These sites were selected as among those most likely to produce additional *P. ruthveni* records. However, no additional *P. ruthveni* records were obtained during this effort (Table 1). The lack of captures contributes to our assessment of the status of *P. ruthveni* in Texas (see Rudolph et al. In Prep.). The lack of captures resulted in no additional prey records being added to the existing data base.

The trap survey data through 2013 was used to prepare a status report that is in the process of being updated using data through 2015 (Rudolph et al. In Prep.). Based on the analysis of data through 2013, the apparent status of *P. ruthveni* has continued to decline since the most recent published account (Rudolph et al. 2006). There have been no records of *P. ruthveni,* with the exception of the 2012 recapture, anywhere in Texas since 2008. *Pituophis ruthveni* were readily trapped at three of the sites reported above as recently as 1995 (Foxhunter's Hill), 2008 (Scrappin' Valley), and 2007 (Angelina National Forest). Despite intensive trapping effort at all three sites since 2007 (Foxhunter's Hill, Angelina National Forest and) 2008 (Scrappin' Valley) no additional *P. ruthveni* have been obtained. Combined with the lack of any additional records (with the exception of the recapture of the male released in 2007) from anywhere within the historic range in Texas since 2007, this suggests that *P. ruthveni* may be extirpated in Texas.

This assessment of the status of *P. ruthveni* in Texas is strengthened by the modeling results using the trap capture data accumulated since 1993. Each of the 3 extant populations for which sufficient data for analysis was available (Foxhunter's Hill, Scrappin' Valley, Angelina National Forest) best fit the model of rapid population decline throughout the trapping period with extirpation in the late 2000s (Foxhunter's

Hill) or mid-2010s (Scrappin' Valley & Angelina National Forest). Figure 2 graphically presents the modeling results, demonstrating that all three populations in Texas approach the zero asymptote by the end of the current decade. The probability of populations reaching a certain zero (ANF and SV) is graphed in Figure 3. Additional detail on the status assessment can be found in the attached document (Rudolph et al. In Prep.).

Despite the lack of *P. ruthveni* captures a total of 1245 snakes of 17 species were captured (Tables 1, 2, 3, 4). These data are available for future analysis as has been the case with previous *P. ruthveni* trap survey data (Steen et al. 2012, 2014).

The lack of *P. ruthveni* captures precluded the addition of DNA samples to the existing data base. However, funding provided by this grant did support analysis of the existing samples. These analyses quantified genetic structure across the historic range of *P. ruthveni,* determined levels of heterozygosity and inbreeding among population segments, and provided information on the genetic situation within the captive breeding population. Details of these results and their implication can be found in the attached report (Kwiatkowski et al. 2014).

Discussion

There is considerable data and analysis suggesting that the Louisiana Pinesnake (*P. ruthveni*) has been in serious decline throughout its historic range in recent decades (Rudolph et al. 2006, In Prep.). Results of trap survey data reported above support this conclusion, especially for Texas. The absence of *P. ruthveni* records in Texas since 2008 (with the exception of a single recapture) raise the possibility that the species is extirpated in the state. Furthermore, even if *P. ruthveni* still exists in Texas, it is extremely unlikely that a viable population remains. These conclusions are strengthened by the fact that survey effort has been substantial since 2008, especially at sites known to have recently supported remnant populations. This conclusion is the primary reason that Texas Parks and Wildlife Department has permitted the transfer of all wild-caught *P. ruthveni* into the captive population. The Texas captive population currently consist of 2 wild-caught females, 4 wild-caught males, and 6 captive-bred offspring as of 2015. Hopefully, these animals will contribute to a future reintroduction of captive bred animals to a suitable site in Texas.

Based on the genetic analyses that examined 16 microsatellite loci several conclusions were made (Kwiatkowski et al. 2014). Genetic structure exists across the historic range of *Pituophis ruthveni*, and the major rivers (Red and Sabine) are barriers to gene flow, especially the larger Red River. Heterozygosity and allelic richness were lower than expected, likely a result of small population size in recent decades. Inbreeding coefficients were above expected values, presumably due to similar reasons. Finally there is evidence of genetic bottlenecks in the recent evolutionary history of *P. ruthveni.* A full discussion of these issues can be found in Kwiatkowski et al. (2014) which is attached.

The impact of these factors on population viability varies widely with magnitude across species. The levels detected in *P. ruthveni* are generally within the range were some species show negative effects and others do not. Consequently, genetic issues should be carefully considered when making decisions concerning the management of this species. The small sample sizes available for genetic analysis also emphasize the importance of continuing to collect samples for DNA analysis at every opportunity. Given the deleterious impacts of loss of heterozygosity, allelic richness, and inbreeding that is well documented in other taxa (i.e. Madsen et al. 1996, 1999, 2011) it is entirely possible that *P. ruthveni* may be detrimentally impacted by genetic issues.

The conservation status of *P. ruthveni* in Texas, and arguably so throughout the historic range, is dire, but certainly not hopeless. A captive population exists that has the potential to produce substantial numbers of offspring to support a major reintroduction effort. The genetic diversity of this captive population and their offspring is limited, but perhaps not to the extent that would preclude the establishment of a viable population. In addition, adding to the genetic diversity of the captive population by obtaining more individuals from the wild, especially from Louisiana, remains a possibility. Implementing genetically informed crosses within the captive population, perhaps including crossing of individuals from across the historic range (which is not currently practiced) can potentially mitigate the effects of small population size in the captive population. However, this would eliminate the genetic structure existing across the historic range (Kwiatkowski et al. 2014). The situation in the captive population has recently improved due to the securing of funding to support the captive-breeding effort, primarily by consolidation of animals in a few zoos, that is anticipated to greatly improve captive breeding success.

Consequently, even if the existing wild populations are not viable, a path forward exists. Several blocks of habitat, primarily on U. S. Forest Service lands, have been restored in recent years to support populations of the federally endangered Red-cockaded Woodpecker (*Picoides borealis*). These provide potential sites for *P. ruthveni* reintroductions that may have the requirements necessary to support viable populations. One of these sites on the Catahoula District of the Kisatchie National Forest in Louisiana is currently the focus of an ongoing reintroduction effort. Ongoing releases of *P. ruthveni* have occurred, and the limited data available to date on survival and growth are encouraging. Animals from the earliest releases are approaching sexual maturity, so evidence of reproduction could be forthcoming at any time.

Acknowledgements

We thank Temple-Inland Inc., Campbell Timberland Management LLC, Jarvis Christian College, National Forests and Grasslands in Texas, Mr Rufus Duncan, and Mr. James Stutzenburg for access to lands under their ownership or management. We also thank Drs. William Godwin, David Wojnowski, and James Childress, Priscilla Lyle, Freddy Vasquez, Howard Williamson, Robert Allen, Jeff Reid, Richard Schaefer, and numerous others for field assistance. We thank Josh Pierce and Nancy Koerth for data management and

analysis throughout this project. We also acknowledge the efforts of Dr. Mathew Kwiatkowski, James Childress, and Josh Pierce for analysis and interpretation of the genetic data.

Literature Cited

Burgdorf, S. J., D. C. Rudolph, R. N. Conner, D. Saenz, and R. R. Schaefer. 2005. A successful trap design for capturing large terrestrial snakes. Herpetological Review 36:421-424.

Conant, R. 1956. A review of two rare pine snakes from the Gulf Coastal Plain. American Museum Novitaties 1781:1-31.

Frost, C. C. 1993. Four centuries of changing landscape patterns in thr: longleaf pine: ecosystem. Proc. Tall Timhers Fire Ecology Conf. 18: 17-44.

Kwiatkowski, Matthew A.; Somers, Christopher M.; Poulin, Ray G.; Rudolph, D. Craig; Martino, Jessica; Tuberville, Tracey D.; Hagen, Chris; Lance, Stacey L. 2010. Development and characterization of 16 microsatellite markers for the Louisiana pine snake, Pituophis ruthveni, and two congeners of conservation concern. Conservation Genetics Resources 2(1): 163-166.

Kwiatkowski, Matthew A., D. Craig Rudolph, and Josh B. Pierce. 2014. Conservation Genetics of Louisiana Pine Snakes, *Pituophis ruthveni*. Final Report to the Louisiana Department of Wildlife and Fisheries.

Madsen, T. J. and B. Ujvari. 2011. The potential demise of a population of adders (*Vipera* berus) in Smygehuk, Sweden. Herpetological Conservation and Biology 6(1):72−74.

Madsen, T., B. Stille, and R. Shine. 1996. Inbreeding depression in an isolated population of Adders *Vipera berus*. Biological Conservation 75:113–118.

Madsen, T., R. Shine, M. Olsson, and H. Wittzell. 1999. Restoration of an inbred Adder population. Nature 402:34–35.

Reichling, S.B. 1995. The taxonomic status of the Louisiana pine snake *(Pituophis melanoleucus ruthveni)* and its relevance to the evolutionary species concept. Journal of' Herpetology 29: 186-198.

Rudolph, D.C. and S.J. Burgdorf. 1997. Timber rattlesnakes and Louisiana pine snakes of the west Gulf Coastal Plain: hypotheses of decline. Texas Journal of Science 49: 11 l-l 22.

Rudolph, D. C., S. J. Burgdorf, R. R. Schaefer, R.N.Conner, and R. W. Maxey. 2006. Status of the Louisiana pine snake, *Pituophis ruthveni.* Southeastern Naturalist 5;463-472.

Rudolph, D. C., S. J. Burgdorf, R. N. Conner, C. S. Collins, D. Saenz, F. F. Schaefer, T. Trees, C. M. Duran, M. Ealy, and J. G. Himes. 2002. Prey handling and diet of Louisiana Pine Snakes (*Pituophis ruthveni*) and black pine snakes (*P. melanoleucus lodingi*), with comparison to other selected colubrid snakes. Herpetological Natural History 9:57-62.

Rudolph, D. C., Melder, C. A., Pierce, J. B., Schaefer, R. R., Gregory, B. 2012. Diet of the Louisiana pine snake (Pituophis ruthveni). Herpetological Review 43(2):243-245.

Rudolph, D. C., J. B. Pierce, and N. E. Koerth. Precipitous Decline of the Louisiana Pine Snake: Can Extinction Be Averted? In Preparation.

Steen, D. A., C. J. W. McClure, J. C. Brock, D.C. Rudolph, J. B. Pierce, and others. 2012. Landscape level influences on terrestrial snake occupancy within the southeastern United States. Ecological Applications 22:1084-1097.

Steen, D. Q., C. J. W. McClure , W. B. Sutton ,D. C. Rudolph, J. B. Pierce , J. R. Lee, L. L. Smith, B. B. Gregory, D. L. Baxley, D. J. Stevenson, and C. Guyer. 2014. Copperheads are common when kingsnakes are not: relationships between the abundance of a predator and one of their prey. Herpetologica 70:69-76.

Sweet, S.S. and W.S. Parker. 1991. *Pituophis melanoleu*cus. Catalog of American Amphibians and Reptiles 474: l-8.

U.S. Fish and Wildlife Service (USFWS). 2011. Species assessment and listing priority assignment form for the Louisiana Pine Snake (*Pituophis ruthveni*). 32 pp.

Wagner, R. O.; Pierce, J. B.; Rudolph, D. C.; Schaefer, R. R.; Hightower, D. A. 2014. Modeling Louisiana pine snake (Pituophis ruthveni) habitat use in relation to soils. Southeastern Naturalist 13 (Special Issue 5):146-158.

Young, R.A. and T.L. Vandeventer. 1988. Recent observations on the Louisiana pine snake, *Pituophis melanolerrcus ruthvmi,* Stull. Bulletin of the Chicago Herpetological Society 23:203-207.

Significant Deviations

This Section 6 grant was initially conceived as a bi-state Section 6 with a similar proposal developed for the state of Louisiana. Both grants were awarded. However, a difference of opinion concerning the contracting of the Louisiana Section 6 led to a severing of the two grants. We removed our names from the Louisiana Section 6 grant, and proceeded with the Texas Section 6 grant as documented in this report. The Louisiana Section 6 grant proceeded independently. The Louisiana grant produced multiple captures of *P. ruthveni*, and the resulting data (fecal samples, DNA samples, collection records) were included in status reviews, genetic analyses, and other manuscripts.

Figure 1. Location of trap survey sites for Louisiana Pinesnakes (*Pituophis ruthveni*) in eastern Texas during 2013-15. Counties not included in historic range omitted.

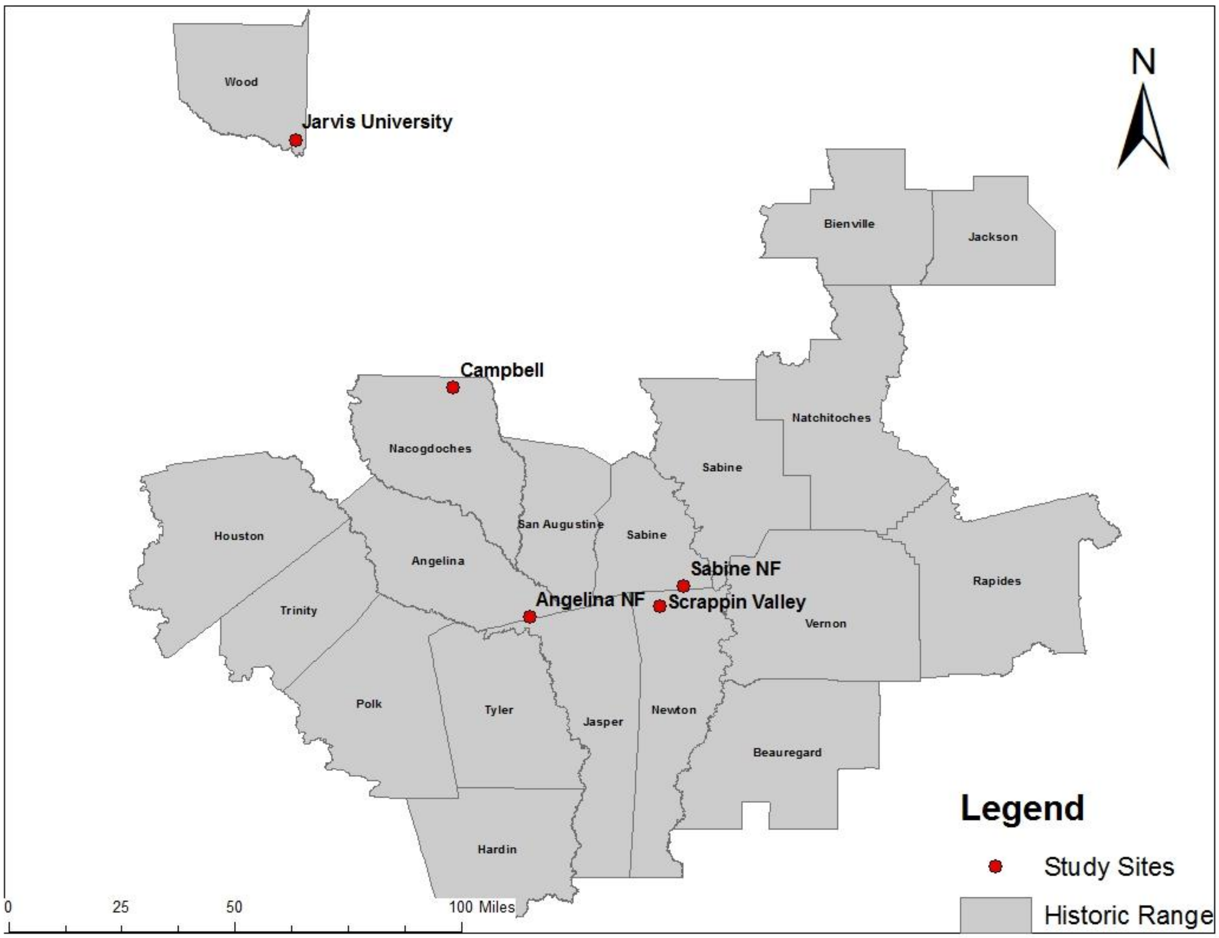

Figure 2. Louisiana Pine Snake (*Pituophis ruthveni*) trap success data fitted to a zero-inflated negative binomial model in a count regression procedure to model the effects of year on the unique capture rate of snakes for populations in the Angelina National Forest (ANF), Scrappin' Valley (SV), and Foxhunter's Hill (FHH), Texas

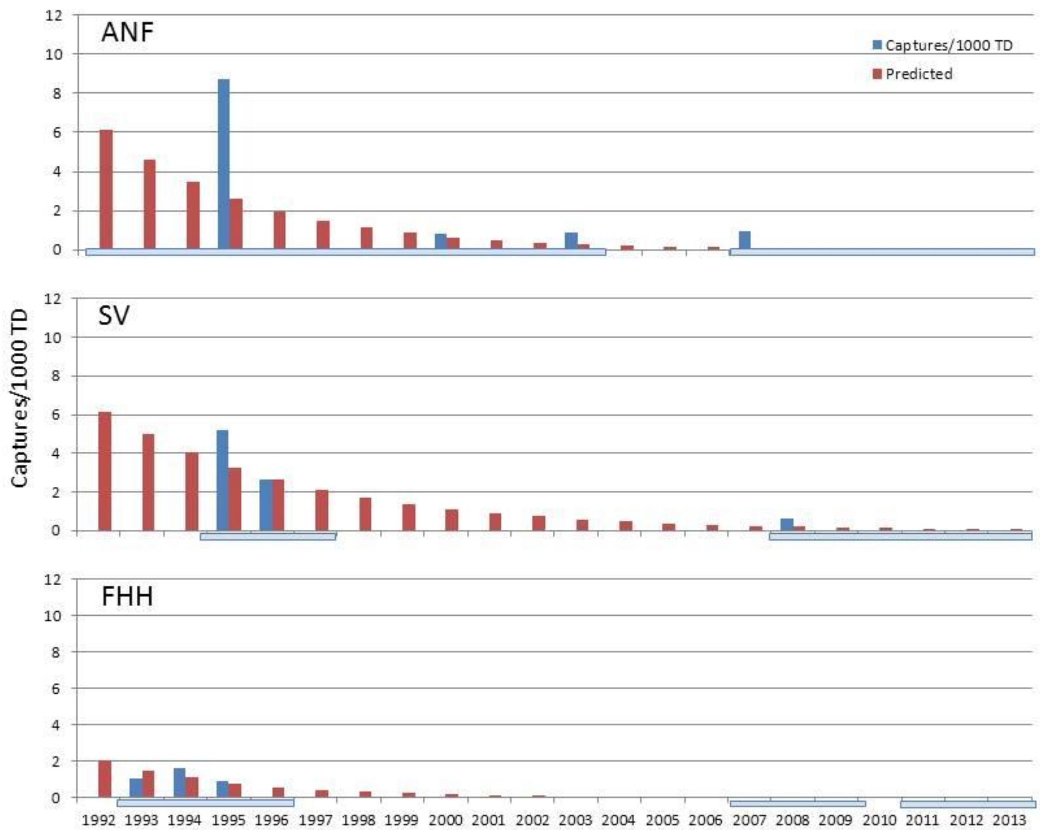

Figure 3. Estimated probability of each year being a certain zero for populations of Louisiana Pine Snakes (*Pituophis ruthveni*) in the Angelina National Forest (ANF), and Scrappin' Valley (SV) Texas.

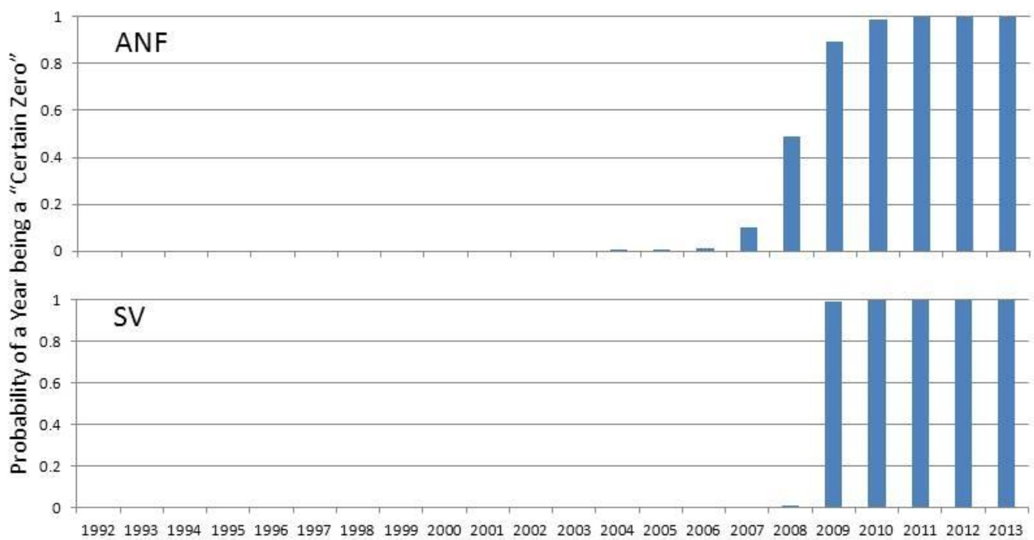

Table 1. Location, number of trap days, number of *Pituophis ruthveni,* and total number of snakes captured during fiscal years 2013-2015 in eastern Texas.

FISCALYEAR	LOCATION	# TRAP DAYS	# *PITUOPHIS*	# SNAKES
2013	SABINE N. F. SABINE COUNTY	620	0	60
	ANGELINA N.F. ANG,/JASPER CO.	3663	0	217
	SCRAPPIN' VALLEY NEWTON COUNTY	2015	0	76
	JARVIS UNIV. WOOD COUNTY	698*	0	29
	STUTZENBURG PROPERTY TYLER COUNTY	94	0	5
2014	SABINE N. F. SABINE COUNTY	788	0	26
	ANGELINA N. F. ANGELINA/JASPER CO.	4692	0	188
	SCRAPPIN' VALLEY NEWTON COUNTY	2379	0	76
	JARVIS UNIV. WOOD COUNTY	953	0	No data
	STUTZENBURG PROPERTY	41	0	3

	TYLER COUNTY			
	GARRISON, TX NACOGDOCHES COUNTY	715	0	131
2015	SABINE N. F. SABINE COUNTY	788	0	13
	ANGELINA N. F. ANGELINA/JASPER CO.	4853	0	165
	SCRAPPIN' VALLEY NEWTON COUNTY	2707	0	130
	JARVIS UNIV. WOOD COUNTY	576	0	8
	GARRISON, TX NACOGDOCHES COUNTY	1061	0	118
TOTAL 2013-2015		26,043	0	1245

*Mis-reported as 2995 trap days in 2013 Interim Report.

Table 2. Snake species and number of individuals captured in fiscal year 2013 by site.

2013	**Site**					
Snake Species	**ANF**	**FHH**	**JU**	**ST**	**SV**	**Total**
Agkistrodon contortrix	53	15	5	0	6	79
Agkistrodon piscivorus	0	0	4	1	8	13
Cemophora coccinea	4	0	0	0	0	4
Coluber constrictor	6	4	0	0	6	16
Coluber flagellum	134	36	14	4	39	227
Crotalus horridus	0	0	0	0	2	2
Heterodon platirhinos	1	4	0	0	1	6
Lampropeltis calligaster	0	0	0	0	1	1
Lampropeltis getula	0	0	0	0	1	1
Micrurus tener	4	0	0	0	0	4
Nerodia fasciata	1	1	0	0	0	2
Nerodia rhombifer	0	0	0	0	1	1
Pantherophis obsoletus	1	0	6	0	5	12
Pantherophis slowinskii	13	0	0	0	6	19
Thamnophis proximus	0	0	1	0	0	1
Total	**217**	**60**	**29**	**5**	**76**	**387**

Table 3. Snake species and number of individuals captured in fiscal year 2014 by site.

2014			**Site**			
Snake Species	**ANF**	**CAM**	**FHH**	**ST**	**SV**	**Total**
Agkistrodon contortrix	54	25	2	1	4	86
Agkistrodon piscivorus	0	2	0	2	6	10
Arizona elegans	0	1	0	0		1
Cemophora coccinea	2	0	0	0	1	3
Coluber constrictor	8	7	1	0	4	20
Coluber flagellum	106	78	18	0	41	243
Crotalus horridus	0	0	0	0	1	1
Heterodon platirhinos	1	2	1	0	0	4
Lampropeltis calligaster	0	2	0	0	0	2
Micrurus tener	1	1	0	0	2	4
Nerodia erythrogaster	0	1	0	0	0	1
Pantherophis obsoletus	3	12	1	0	4	20
Pantherophis slowinskii	10	0	2	0	13	25
Thamnophis proximus	2	0	0	0	0	2
Unknown snake (skeleton)	1	0	1	0	0	2
Total	**188**	**131**	**26**	**3**	**76**	**424**

Table 4. Snake species and number of individuals captured in fiscal year 2015 by site.

2015			**Site**			
Snake Species	**ANF**	**CAM**	**FHH**	**JU**	**SV**	**Total**
Agkistrodon contortrix	46	14	2	0	14	76
Agkistrodon piscivorus	2	3	0	0	6	11
Arizona elegans	0	1	0	0	0	1
Cemophora coccinea	1	0	0	0	7	8
Coluber constrictor	4	5	0	0	9	18
Coluber flagellum	90	63	9	3	63	228
Crotalus horridus	0	0	0	0	3	3
Heterodon platirhinos	1	3	0	0	5	9
Lampropeltis calligaster	1	2	0	0	3	6
Lampropeltis getula	1	1	0	1	3	6
Micrurus tener	0	2	0	0	5	7
Nerodia erythrogaster	0	0	0	0	1	1
Pantherophis obsoletus	2	22	0	1	3	28
Pantherophis slowinskii	15	0	2	0	7	24
Thamnophis proximus	2	1	0	0	1	4
Unknown Snake	0	0	0	3	0	3
Unknown snake (skeleton)	0	1	0	0	0	1
Total	**165**	**118**	**13**	**8**	**130**	**434**