FINAL REPORT

2016

ENDANGERED SPECIES RESEARCH PROJECTS FOR THE LOUISIANA PINE SNAKE (RFP NO. 212D)

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EXECUTIVE SUMMARY

1) **Literature Review** – We prepared a species account for the Catalogue of American Amphibians and Reptiles (pg. 4). This document is the first ever exhaustive review of all literature known about the Louisiana Pinesnake (LPS, *Pituophis ruthveni*) to date, and it represents the best synopsis of our current understanding of the species. The document is in the second round of revisions with the editors of the Catalogue.

2) **Map of localities and populations** – We prepared a map of all known localities of LPS for the species account (pg. 16). Current populations in Texas are only known from two localities, one in Northern Newton County and one on the border of Angelina and Jasper Counties. The most recent LPS capture from either of these populations is 2012.

3) **Assessment of habitat needs and availability** – We combined a 30-year change detection analysis of pine forest timber harvest with an LPS habitat model based on soils. A moving window analysis of this new LPS habitat model identified all remnant habitat patches meeting minimum movement and home range requirements for the species (Map 5 pg. 23). This model helped identify Texas private land owners with suitable habitat on their property for surveys in 2016. It also identified a single candidate reintroduction site in Texas on National Forest land for juvenile snakes produced from the existing captive breeding program.

4) **Assessment of population structure and abundance** – Recent results from genetics studies showed only modest genetic structure among three historically identified populations in Texas and northern and southern Louisiana. The observed low measures of population differentiation alleviated concerns about combining the Texas and southern Louisiana captive breeding populations into one "Southern" population. This Southern population and the Northern Louisiana captive population now represent two distinct captive populations based on geography. Crosses among individuals from all populations should be considered for captive breeding and reintroduction programs, especially given the low likelihood of wild captures

from the Texas populations and the resistance to bringing more wild snakes into captivity from southern Louisiana populations (pg. 29). We surveyed 7 sites across the historical distribution of LPS in Texas using modified camera trapping methods. A total of 8,388,078 images were taken from April to October 2016. No LPS were observed.

5) **Recommendations on species management and analysis of captive breeding** – The lack of captures in this study and other recent studies of LPS in Texas is discouraging; however it is possible that some individuals still exist in the state especially in the areas where they have most recently been captured. Our recommendations for species management have two parts. First, we need to continue to manage the habitat, which means frequent fires and thinning of pine stands to create natural pine savannah habitat with an open herbaceous understory for both current populations and future reintroductions. Second, we need to support the ongoing captive breeding program. This includes the identification of a Texas reintroduction site (southern Angelina National Forest) and the management of that site specifically for LPS. This program will likely be the best way to ensure that a population (or populations) of LPS remain in Texas in perpetuity (pg. 35).

CONTENTS

SECTION I – Literature Review

REPTILIA: SQUAMATA: COLUBRIDAE ***Pituophis ruthveni***

Catalogue of American Amphibians and Reptiles.

Adams, C.S., J.B. Pierce, D.C. Rudolph, W.A. Ryberg and T.J. Hibbitts. 2015. *Pituophis ruthveni*.

Pituophis ruthveni Stull Louisiana Pinesnake

Pituophis melanoleucus ruthveni Stull 1929:1. Type-locality, "Long Leaf, Rapides Parish, Louisiana, USA." Holotype, USNM 76278, adult male, collected by W. D. Harris on 24 March 1927.

Pituophis catenifer ruthveni Fugler 1955

Pituophis ruthveni Conant 1956:28.

CONTENT. No subspecies are recognized.

DEFINITION. The Louisiana Pinesnake (*Pituophis ruthveni*) is a large, heavy-bodied snake with adults reaching 121.9-152.4 cm in length (Werler and Dixon 2000). Male snakes reach slightly larger sizes than females (Himes et al. 2002). Neonates of *P. ruthveni* are, on average, the largest neonates of any North American snake, reaching 55 cm or larger (Reichling 1988 and Reichling 1990). Four prefrontal scales are present on the forecrown, a distinguishing trait of snakes in the genus *Pituophis*. They possess an enlarged rostral scale that is typically longer than it is wide, and an undivided anal plate. The dorsal scales are keeled above the seventh scale row and arranged in 27 to 33 rows at midbody (Wright and Wright 1957). *Pituophis ruthveni* has a distinct pattern consisting of 28 to 42 brown blotches that are conspicuously different at opposite ends of the body (Conant and Collins 1998). Near the head and neck, these blotches are typically a darker brown, and tend to join and intermingle to form a continuous band of darker streaks over a yellowish-beige ground color. Blotches towards the posterior of the body can be a lighter reddish-brown or dark, are widely spaced, and well-defined against a yellow ground color. Dark spots and splotches cover the crown, and in some specimens an indistinct dark bar crosses the head between the eyes. The venter is marked with small, irregular black splotches (Stull 1929). Juvenile coloration does not vary significantly from that of adults.

DIAGNOSIS. The key diagnostic feature that distinguishes *P. ruthveni* from other Pinesnakes (*Pituophis melanoleucus*) is the presence of an enlarged rostral scale in *P. ruthveni*. The rostral scale is raised slightly above the surrounding scales. The conspicuous pattern and coloration of the mid-dorsal markings of *P. ruthveni* is another distinct diagnostic feature that distinguishes this species from *P. melanoleucus* and the associated subspecies (Stull 1929). Mid-dorsal markings in *P. melanoleucus* are fewer in number than in *P. ruthveni,* ranging from 23-30 (Reichling 2008). *Pituophis ruthveni* and the Northern Pinesnake (*P. m. melanoleucus*) both exhibit a pattern in which blotches along the mid-dorsal line will intermingle into darker streaks towards the head and neck, and become more defined posteriorly. These markings in *P. m.*

melanoleucus are distinctly darker than the brown to reddish-brown markings of *P. ruthveni*, and appear as a black coloration over a white ground color. The Florida Pinesnake (*P. m. mugitus*) is characterized as a rusty-brown snake with an indistinct pattern. The darker dorsal markings are vaguely visible. The ventral pattern is typically immaculate compared to the ventral markings seen in specimens of *P. ruthveni*. The Black Pinesnake (*P. m. lodingi*) is distinguished by a plain (or nearly plain) black or dark brown coloration, on both the dorsum and venter. The Bullsnake (*P. catenifer sayi*) is another closely related species that possesses an enlarged rostral scale; however, they can be distinguished by a pattern of 41 or more black, brown, or reddish-brown dorsal blotches (Werler and Dixon 2000). The markings are more defined, and in strongest contrast with the yellow ground color, at both the posterior and anterior ends of the body. A dark band usually extends from each eye to the end of the mouthline. The dorsal scales are strongly keeled and usually in 33 scale rows at midbody. A light band surrounded by darker borders between the eyes on the crown can be present.

PHYLOGENETIC RELATIONSHIPS. Three recent phylogenetic studies of the genus *Pituophis* identify 3 currently recognized species: *P. melanoleucus* (Pinesnakes), *P. catenifer* (Gopher Snakes and Bullsnakes), and *P. ruthveni* (Louisiana Pinesnake) (Rodriguez-Robles and De Jesus-Escobar 1999, 2000, Pyron and Burbrink 2009, Krysko et al. 2014). Notably, two of these studies recognize *P. ruthveni* as a separate species despite the fact that it is nested within a clade of *P. c. sayi* based on Parsimony, Maximum Likelihood (ML), and Bayesian Inference (BI) analyses of the mitochondrial DNA (mtDNA) ND4 region (Rodriguez-Robles and De Jesus-Escobar 1999, 2000, Collins and Taggart 2008, Collins 2010, Krysko et al. 2014). The third study identifies *P. ruthveni* as sister to *P. catenifer* using ML and BI analyses of both nuclear and mtDNA sequences (Pyron and Burbrink 2009). Because of these genetic similarities, evidence supporting the designation of *P. ruthveni* as a separate species comes from the geographic isolation of its populations from other species (Smith and Kennedy 1951, Conant 1956, Thomas et al. 1976, Fitch 2006) and a combination of morphometric characters distinguishing *P. ruthveni* from close relatives (Thomas et al. 1976, Knight 1986, Collins 1991, Reichling 1995). Thus, *P. ruthveni* is recognized as a species under the evolutionary species concept (Reichling 1995). A clear limitation of the three phylogenetic studies described above is the use of only one or two genes to draw inferences among taxa. Additional research including more genes might provide genetic data that further supports *P. ruthveni* as a full species.

PUBLISHED DESCRIPTIONS. Descriptions are given in Stull (1929, 1932, 1940), Smith and Kennedy (1951), Conant (1956), Wright and Wright (1957), Thomas et al. (1976), Tennant (1984), Dixon (1987), Dundee and Rossman (1989), Sweet and Parker (1990), and Conant and Collins (1991). These descriptions discuss *P. ruthveni* as a subspecies of *P. melanoleucus* or as a distinct species. A modern taxonomic review of *P. ruthveni* was given by Reichling (1995). Other published descriptions of *P. ruthveni* are given in Conant and Collins (1998), Tennant (1998), Dixon (2000), Werler and Dixon (2000), Dixon and Werler (2005), Tennant (2006), Dixon (2013), and Wallach et al. (2014).

ILLUSTRATIONS. Color photographs and illustrations of *P. ruthveni* are found in Tennant (1984), Dundee and Rossman (1989), Conant and Collins (1991), Conant and Collins (1998), Tennant (1998), Tennant and Bartlett (2000), Werler and Dixon (2000), Bartlett and Bartlett (2005), Dixon and Werler (2005), Tennant (2006), Reichling (2008), Dixon (2013), Krysko et al.

(2014), Mehrtens (1987). Black and white photographs and illustrations of *P. ruthveni* are found in Cagle (1952), Smith and Kennedy (1951), Conant (1956), and Wright and Wright (1957).

DISTRIBUTION. *Pituophis ruthveni* is an inhabitant of the Longleaf Pine savannahs west of the Mississippi River in Louisiana and eastern Texas. It is known from 8 parishes in Louisiana (Dundee and Rossman 1989) and 12 counties in Texas (Dixon 2013); however, in the last 15 years (2000-2015) it has only been found in 5 Louisiana parishes (Bienville, Natchitoches, Rapides, Sabine, and Vernon) and 4 Texas counties (Angelina, Jasper, Nacogdoches, and Newton). Records from Caldwell, Montgomery and Walker Counties of Texas, and Calcasieu and Jefferson Davis Parishes of Louisiana have been considered erroneous or are questioned (specimens examined in Thomas et al. 1976 and this paper).

FOSSIL RECORD. None known.

PERTINENT LITERATURE. **geographic distribution** (Ashton 1976, Brown 1950, Conant 1956, Conzelmann 2003, Ernst and Barbour 1989, Fugler 1955, Kroll and Hicks 1975, Krysko et al. 2014, Mitchell and Tinkle 1960, Parks and Cory 1936, Parks 1942, Rakowitz et al. 1983, Raun and Gehlbach 1972, Rossi 1992, Smith and Kennedy 1951, Stull 1929, Thomas et al. 1976, Vandeventer and Young 1989, Wilks 1962, Williams and Cordes 1996, Young and Vandeventer 1988) **natural history** (Ealy et al. 2004, Ernst and Ernst 2003, Fritz 1993, Greene 1997, 1999, Himes et al. 2002, 2006, Keiser and Wilson 1969, 1979, Mattison 1995, Pierce et al 2014a, 2014b, 2016, Reichling 2008, Rudolph et al. 2002, 2007, 2012, Scott 1996, Wrobel 2004) **systematics and taxonomy** (Collins 1991, Fitch 2006, Frank and Ramus 1995, Klauber 1941, Krysko et al. 2014, Kwiatkowski et al. 2010, Pyron and Burbrink 2009, Reichling 1995, Smith and Kennedy 1951, Stull 1932, 1940, **field techniques** (Burgdorf et al. 2005, Rudolph et al. 1998) **conservation** (Alvarez 2014, Ashton et al. 2007, Frank and Ramus 1994, Dodd 1987, Greene 1997, 1999, King 2009, Köhler 2004, Louisiana Office of the State Register 2014, Rudolph and Burgdorf 1997, Rudolph et al. 1999, 2006, Siegal and Collins 1993, Skubowius 2004, Texas Parks and Wildlife Department 1978, Texas Parks and Wildlife Department 1993, U. S. Fish and Wildlife Service 2011, 2013a, 2013b) **habitat modeling** (Wagner et al. 2014)

ETYMOLOGY. The genus *Pituophis* is derived from the Greek word *pitys,* which means pine, and *ophios,* which means serpent. The species name *ruthveni* is a patronym honoring Alexander Grant Ruthven, an American herpetologist and former president of the University of Michigan.

COMMENT. *Pituophis ruthveni* is arguably the rarest large North American snake. Less than 100 snakes were known before 1993 when efforts were made to trap for this species to try to learn about their status. As of December 2016 the number of snakes known is less than 250. The longleaf pine habitat, which *P. ruthveni* prefer, was mostly logged by the 1930's relegating populations of *P. ruthveni* to remnant forest patches. Additionally, fire suppression reduced available habitats even where forest remnants remained. At present only 7 small and isolated populations occur between Texas and Louisiana (including the reintroduced population in Louisiana) with no snakes observed in the 2 Texas populations since 2012. Texas populations are listed as endangered by the Texas Parks and Wildlife Department and Lousiana prohibits the collections of this species from the wild. *Pituophis ruthveni* is currently a candidate for federal listing.

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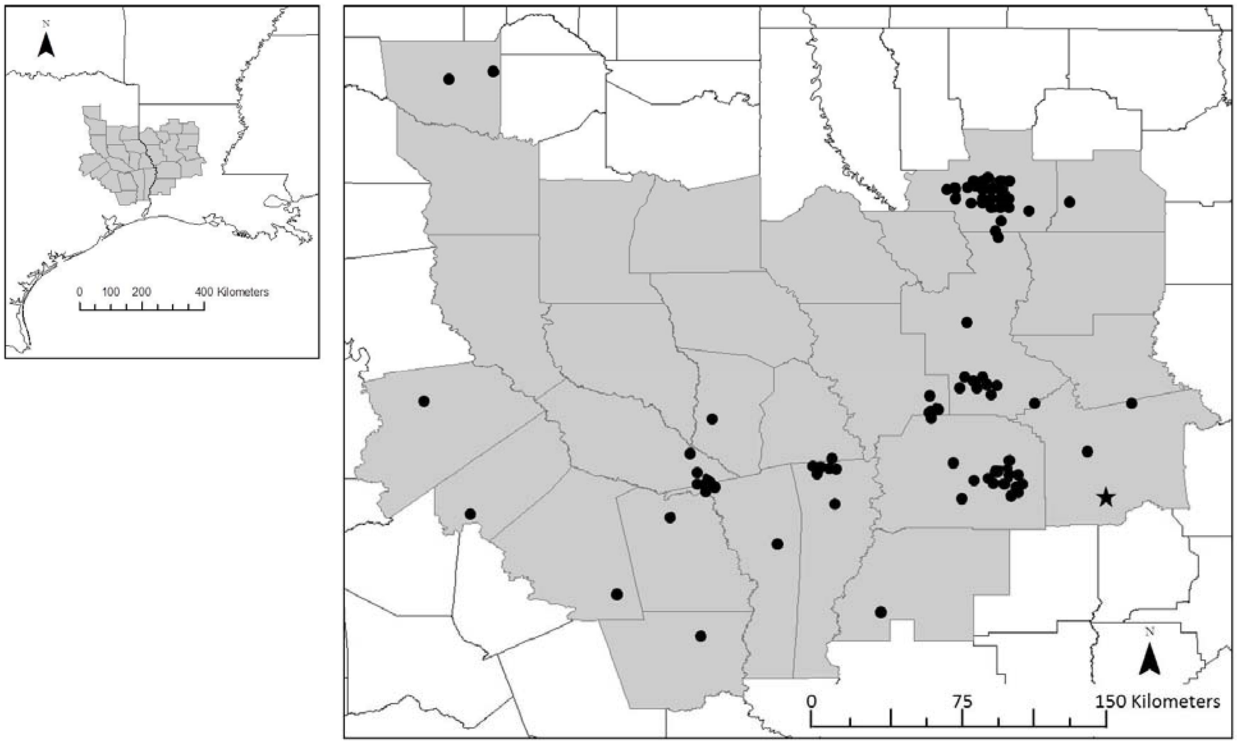
FIGURE 1. A male from Vernon Parish, Louisiana. Photo by Toby J. Hibbitts.

FIGURE 2. A male from Jasper County, Texas. Photo by Toby J. Hibbitts.

SECTION II – Map of localities and populations



MAP 1. Black dots indicate historical records, and the star marks the type locality for *Pituophis ruthveni*. The shaded counties and parishes contain potential habitat for *Pituophis ruthveni* but do not demarcate a distribution for the species.

SECTION III – Assessment of habitat needs and availability

Introduction

The Louisiana Pinesnake (LPS) is an inhabitant of the Longleaf Pine savannahs with limited occurrences in other pine forests, west of the Mississippi River in Louisiana and eastern Texas. It is known from 8 parishes in Louisiana (Dundee and Rossman 1989) and 12 counties in Texas (Dixon 2013); however, in the last 15 years (2000-2015) it has only been found in 5 Louisiana parishes (Bienville, Natchitoches, Rapides, Sabine, and Vernon) and 4 Texas counties (Angelina, Jasper, Nacogdoches, and Newton)(J.B. Pierce unpubl. data). This lack of recent survey success suggests that this species has been extirpated from much of its historical range (Rudolph et al. 2006, Rudolph et al. 2016). Currently, the U.S. Fish and Wildlife Service (USFWS) recognizes 7 extant populations that occupy small, fragmented habitats on federal and private lands (USFWS 2014). The rarity of the LPS has limited research on its ecology, making it difficult to develop landscape-scale habitat models for the species, which are essential for management and conservation.

Previous research suggests that LPS habitat suitability is influenced by the presence of sandy, well-drained soils and LPS prey, the Baird's Pocket Gopher (*Geomys breviceps*)(Rudolph and Burgdorf 1997; Rudolph et al. 1998, 2002). Based on the published descriptions of Baird's Pocket Gopher soil preferences (Davis et al. 1938), Wagner et al. (2014) used edaphic factors (i.e., increasing sand content and decreasing soil saturation) to model potentially suitable habitat for LPS, and then used independently derived telemetry data for this species to validate their results. Their model demonstrated that the distribution of LPS on the landscape is strongly influenced by edaphic factors related to soil permeability and depth to ground water, which are unlikely to be changed at a landscape scale by human activities. They concluded that many areas with suitable soils remain available to support the species throughout its historical range, but that perceived suitable vegetation communities required to support the species on those same sites are lacking due to short-rotation silviculture practices. Perceived suitable vegetative communities for LPS include pine overstory with a sparse midstory and a well-developed herbaceous understory (Himes et al. 2006, Rudolph and Burgdorf 1997). Short-rotation timber harvest practices do not allow such vegetative communities to develop at any part of the harvest rotation, and as a result, these practices are hypothesized causes of decline in this species.

Given the perceived sensitivity of LPS to short-rotation timber harvest, in this study we build on the existing soil-based habitat model by adding a range-wide analysis of timber harvest (Fig. 3). We combined a 30-year change detection analysis of pine forest timber harvest with the LPS habitat model based on soil characteristics. The new harvest habitat model identifies existing mature pine stands, highlighting areas with potential to support LPS. The potential suitability of these remnant mature pine stands for supporting LPS was ranked by patch size according to minimum movement and home range requirements for the species (Himes et al. 2006). This harvest habitat model was used to help identify private land owners with potentially suitable LPS habitat on their property for surveys in 2016. It also identified candidate reintroduction sites in Texas and Louisiana on National Forest land for juvenile snakes produced from the existing captive breeding program.

Methods

Study Area

The study area contained 17 eastern Texas counties and 11 west central Louisiana parishes, including the 14 counties and 7 parishes considered in the soil-based habitat model (Wagner et al. 2014). All verified LPS records were included within the study area (Rudolph et al. 2016), including a recently reintroduced population (USFWS 2016).

Change Detection Analysis

For the change detection analysis, we acquired pre-processed Landsat 5 TM, 7 ETM+, and 8 OLI 30-meter imagery and normalized difference vegetation index (NDVI; i.e., an indicator of photosynthetic activity) products through the United States Geological Survey (USGS) Earth Resources Observation and Science (EROS) Center Science Processing Architecture (ESPA) On Demand Interface website (https://espa.cr.usgs.gov). Using an interactive supervised classification in ArcMap 10 (Esri 2014), we identified existing pine from leaf-off Landsat imagery acquired during winter of 2014-2015 (Map 2). This provided a baseline land cover map from which we eliminated areas where forest was not constantly present over time, using change detection analysis.

We performed an image differencing change detection analysis on Landsat-derived NDVI scenes collected in late summer or early fall from 1985 to 2015 (Lyon et al. 1998, Coppin et al. 2004). We identified areas of vegetation loss from 1985 to 2015 in 5-year intervals (e.g., loss from 2010 to 2015, loss from 2005 to 2010, etc.) by subtracting temporally consecutive NDVI rasters for each time step (e.g., $NDVI_{2010}$ - $NDVI_{2005}$). Significant decreases in NDVI indicated a loss of vegetation. Targeting scenes in this timeframe provided the best opportunity to correctly classify loss of vegetation (Map 3). NDVI, especially for evergreen forest-type land covers, remains generally stable during this time of year, meaning an observed decrease in NDVI during a particular time step is likely associated with loss of vegetation (e.g., due to clear-cut or harvest), and not due to naturally-occurring seasonal variation in photosynthetic activity.

We confirmed appropriate difference thresholds chosen to represent vegetation loss by comparing vegetation enhancing false-color image composites (e.g., shortwave infrared, near infrared, and red band combination) between each time step. Areas identified as vegetation loss from any time period were then eliminated from the existing pine classification. This provided a raster layer of oldest existing pines in the landscape (i.e., areas expected to have continuous pine existence from 1985 to 2015). Our pine model was further refined by extracting coincident areas identified in the Wagner et al. (2014) suitability model (Map 4).

Potential Suitability Rankings

To produce our final suitability model, we used a mean home range size of 33 hectares (range 6.5-107.6 ha; Himes et al. 2006) to estimate LPS abundance for each patch size class. This assumes no overlap of home ranges, which we know occurs to varying degrees in wild populations. The classes were color coded as follows: red polygons represent suitable habitats large enough for 1-3 snakes (30-99 ha), orange polygons are big enough for 4-15 snakes (100-499 ha), yellow polygons are big enough for 16-30 snakes (500-999 ha), and green polygons are large enough for more than 30 snakes (≥1,000 ha) (Map 5).

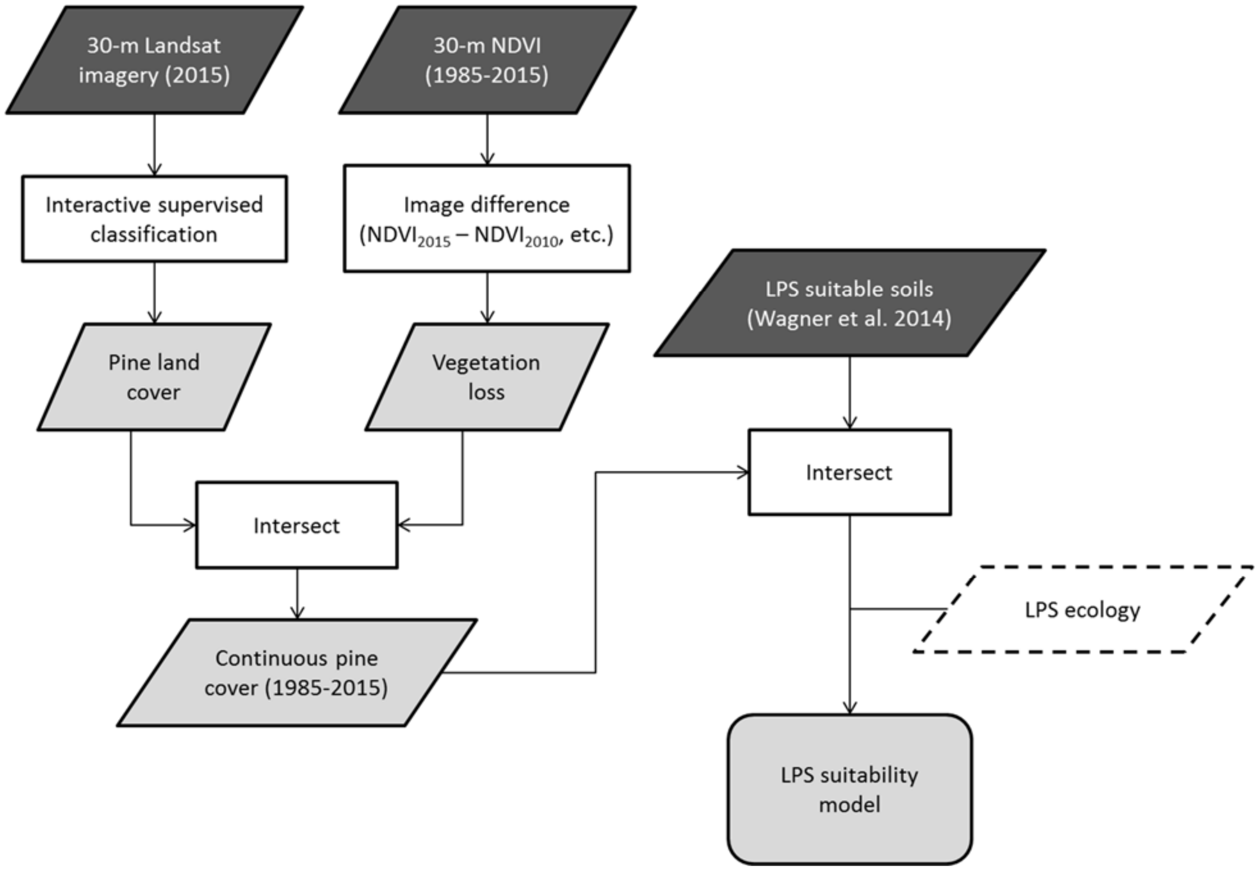
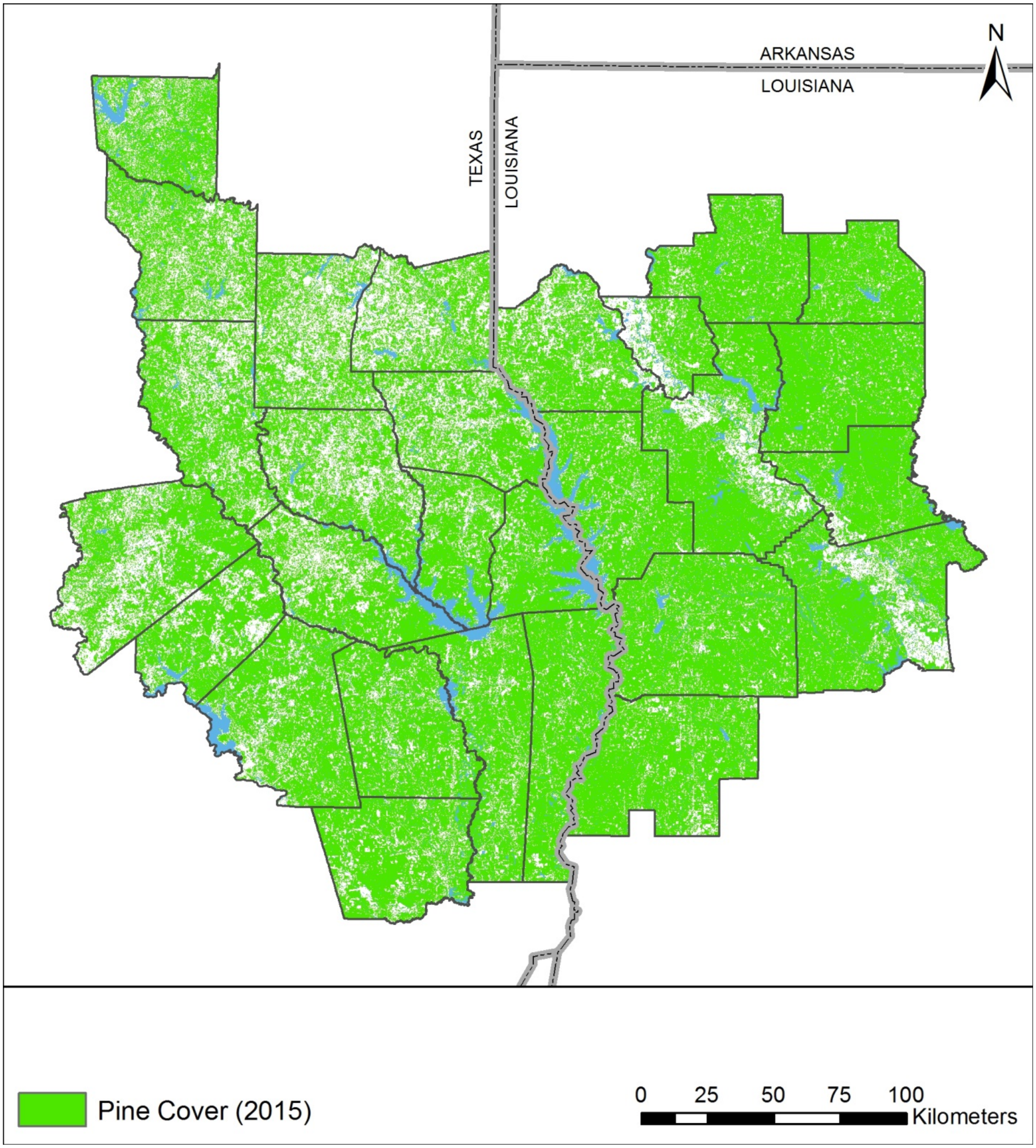
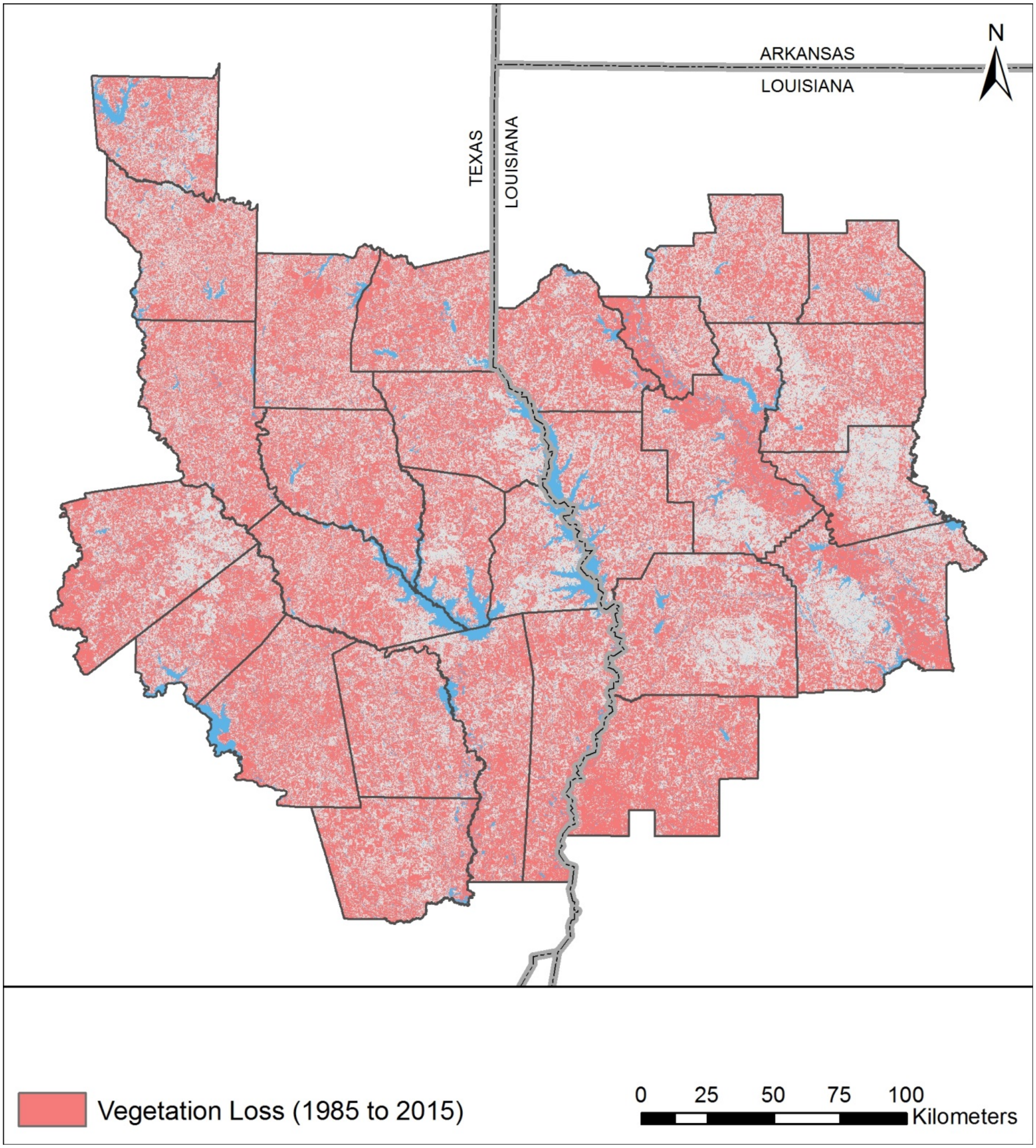


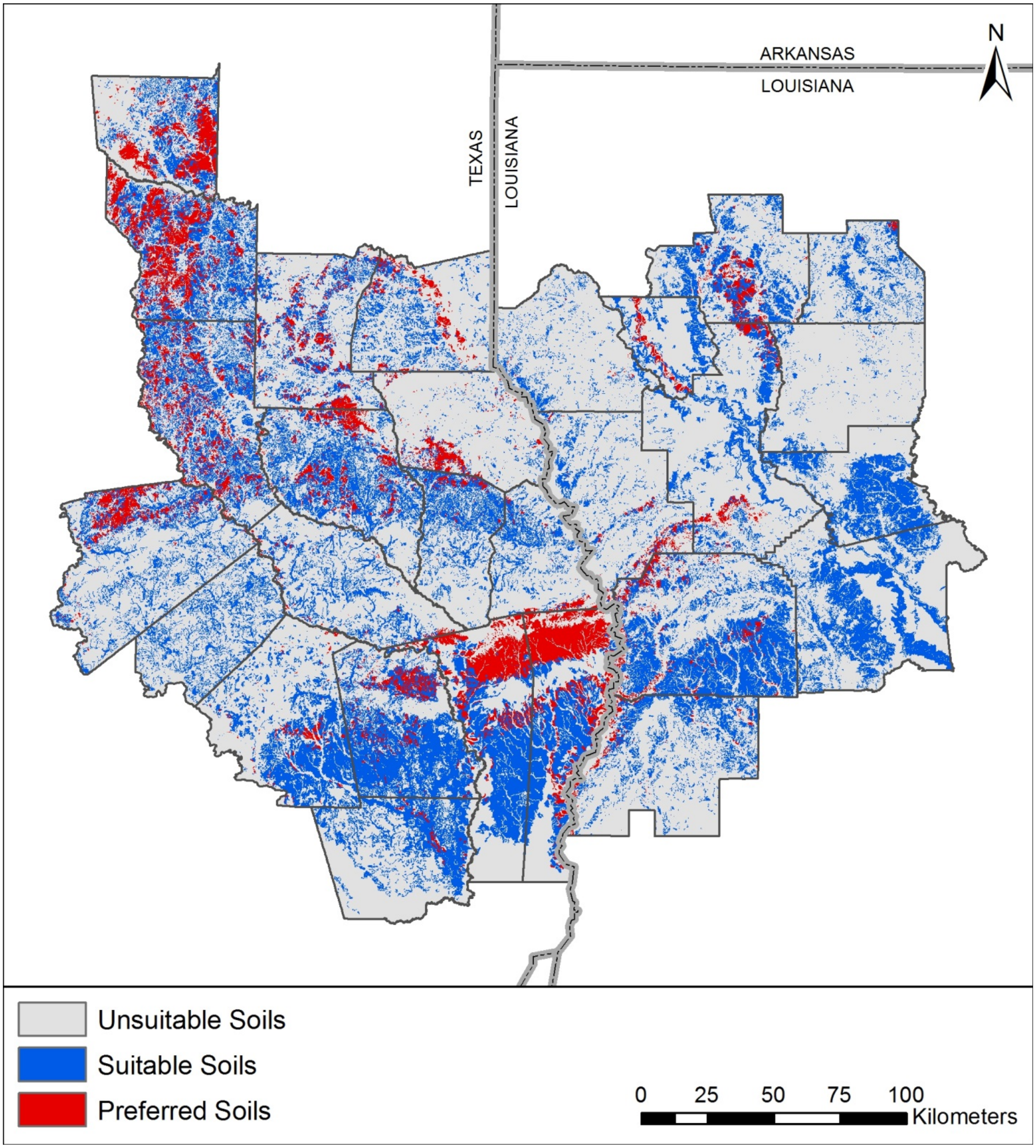
FIGURE 3. Flowchart showing methods used to generate a map of potentially suitable LPS habitat.



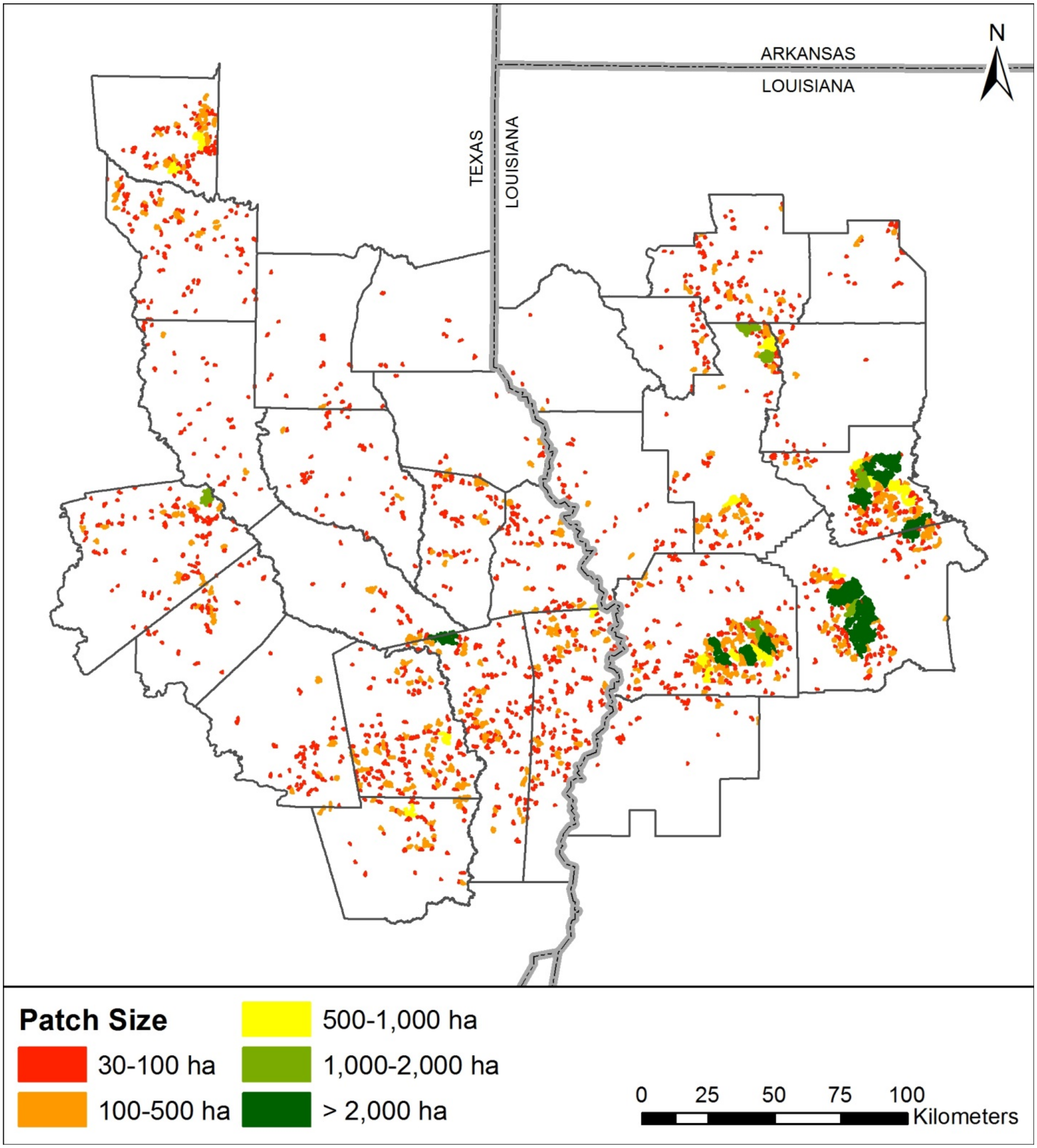
MAP 2. Pine tree cover.



MAP 3. Perceived loss of vegetation through change detection analysis.



MAP 4. Suitable soil model following methods from Wagner et al. (2014).



MAP 5. Model of potentially suitable Louisiana Pinesnake habitat classifying remnant mature pine stands by total patch size (area).

Results

The final LPS potentially suitable habitat model identified all remnant habitat patches that have not been harvested by clear-cutting for more than 30 years and that also meet minimum home range requirements for the species (Map 5). A total of 1,652 patches comprising 180,050 hectares of potential habitat were identified throughout the study area (Table 1). Patch size ranged from 30 to 9,807 hectares, but only 16 patches were greater than 1,000 hectares (i.e., coded green) and considered large enough to support more than 30 snakes. Together, these 16 patches contain ~25% of the total modeled habitat area for the species.

Two of these patches were located in Texas approximately 95 km from one another. The western-most patch of potential LPS habitat was located in Davy Crockett National Forest (Houston County), and the other patch was located in Angelina National Forest (Angelina and Jasper Counties). These two large patches (>1,000 ha) were surrounded by smaller patches (< 500 ha) and relatively isolated (>30 km) from other moderately sized patches (500-999 ha).

The remaining 14 patches located in Louisiana were arranged in four clusters. In Vernon Parish, the cluster was comprised of four large patches (>1,000 ha) spread across the Calcasieu District of Kisatchie National Forest and Fort Polk, the Army's Joint Readiness Training Center. In Rapides Parish, three large patches were clustered in the Calcasieu District of Kisatchie National Forest. Five large patches were clustered in the Catahoula District of Kisatchie National Forest, Grant Parish, and a pair of large patches was located in the Winn District of Kisatchie National Forest, Natchitoches Parish. Each of these clusters also included 1-5 moderately sized patches (500-999 ha).

TABLE 1. Summary stats for habitat model. All area measures are in hectares (ha).

Suitability Class	Number of Patches	Patch Size Range	Mean Patch Area	Total Area	Proportion of Total Area
Red	1,321	30-99	51	67,630	37.6%
Orange	296	100-492	180	53,292	29.6%
Yellow	19	527-948	712	13,523	7.5%
Light Green	6	1,159-1,697	1,377	8,262	4.6%
Dark Green	10	2,006-9,807	3,734	37,343	20.7%
Total	1,652			180,050	100%

Discussion

Our model demonstrates that 9% (180,050 ha) of the potential habitat for LPS identified using edaphic factors described by Wagner et al. (2006) contains pine forest that has not been clearcut for 30 years (1985-2015). When considering only remnant patches estimated to be large enough to support more than 30 snakes (i.e., green patches, n = 16), the amount of potentially suitable habitat shrinks to 2% (45,605 ha), and all of it is contained within U.S. Forest Service (USFS) and U.S. Army Federal lands. This observation makes sense given that as early as the 1980's, the USFS and Army implemented forest restoration and management plans in National Forests and on Fort Polk to restore and maintain open canopy pine forest for the Red-cockaded woodpecker (*Picoides borealis*), which has similar habitat requirements to LPS. In 2003, these conservation actions were formally extended to LPS through a candidate conservation agreement (CCA) with USFS, DOD, Texas Parks and Wildlife Department (TPWD), and Louisiana Department of Wildlife and Fisheries (LDWF) to maintain fire-climax, park-like, open canopy pine forest conditions typical of LPS habitat (USFWS 2003).

In contrast, 99% of the total number of remnant patches (n = 1,636) identified in this study are considered too small to support viable LPS populations (i.e., a few hundred hectares or less; Rudolph et al. 2006, Reichling et al. 2008, Rudolph et al. 2016). This pattern of habitat loss and fragmentation was driven almost entirely by silviculture practices on private lands for fiber production, which has resulted in further degradation of remnant habitat patches for LPS through fire suppression. Because historical fiber production goals favored faster growing pine tree species for shorter harvest rotations, longleaf pine was replaced with less fire-tolerant (i.e., across all life stages) overstory pine species such as loblolly or slash pine. Forest conversion to less fire adapted species made it more difficult to use prescribed fire to manage LPS habitat over multiple rotations (Rudolph 2000, Rudolph and Burgdorf 1997). The large number of small sized remnant patches observed in this study exacerbates problems associated with prescribed fire management by increasing legal liability and expense of liability insurance, straining limited funds and personnel, and intensifying smoke management issues (USFWS 2016). These added constraints on prescribed fire culminate in extended fire intervals and reductions in burn area per fire event, which fail to provide adequate fire intensity or frequency to suppress mid- and understory growth and allow the growth of herbaceous vegetation necessary to support viable pocket gopher, and by extension, LPS populations (Rudolph et al. 2006, Rudolph et al. 2016).

All together, these observations indicate that the loss and degradation of LPS habitat from silviculture practices on private lands remains a current threat to the species despite a reduction in timber harvest from historical levels (USFWS 2016). Reversing this trend will require the reestablishment of more appropriate overstory species (e.g., longleaf pine) and fire regimes on private lands, which can only be achieved through further engagement with private landowners open to conservation strategies like the purchase of conservation easements (Duran 2010, Wagner et al. 2014).

To address these limitations to LPS conservation on private lands, future conservation plans (e.g. HCP, Safe Harbor) should target areas immediately adjacent to the habitat patches estimated to be large enough for LPS populations in this study (i.e., green patches). By focusing conservation and restoration objectives in these areas, existing isolated patches like those found in Texas can increase in size, even if incrementally, and existing clusters of patches like those identified in Louisiana can be reconnected. To insure that these areas are maintained in perpetuity regardless of changes in ownership, language can be included in the conservation plan to bind conditions of the agreement to the land itself. Another conservation option is the outright purchase of private lands adjacent to patches identified in this study. Many of these candidate sites are already considered “inholdings” within the purchase boundary of the National Forests containing remnant habitat patches large enough for LPS populations.

In addition to recognizing candidate areas for LPS habitat restoration and conservation agreements, our model also identifies candidate sites for future LPS reintroductions and surveys. Of the four clusters of patches observed in Louisiana, two coincide with extant populations of

LPS, one of which is a recently reintroduced population. The other two clusters contain historical LPS localities but are now considered extirpated by USFWS. According to our model, those two clusters of patches represent viable candidate sites for future LPS reintroduction efforts. In Texas, one of the two patches identified coincides with an extant LPS population (southern Angelina National Forest), although snakes have not been detected there since 2012. This site is managed for LPS and is the largest patch of habitat remaining in Texas although it is bisected by a heavily traveled state highway. The patch in the Davy Crockett National Forest would need considerable habitat management before it could be considered a viable candidate site for future LPS reintroduction efforts. Finally, the results of this study pinpoint locations of potentially suitable habitat for future surveys on private and federal lands that may not have been adequately surveyed for LPS occurrence.

Four LPS populations in addition to the three mentioned above, persist in areas that were not completely identified as potentially suitable habitat. Portions of the areas containing those extant populations were identified, but the entire area occupied by LPS populations was not included. For example, the Scrappin Valley LPS population in northern Newton County Texas, the Bienville population in Bienville Parish Louisiana, and the Peason Ridge population in Vernon and Sabine Parishes, Louisiana occupy habitats that were not completely identified using our modeling approach. These observations highlight one shortcoming of our approach to modeling clearcutting in pine forest habitat. Portions of the areas occupied by those four extant populations contain open pine savannahs that were not included in the pine forest Landsat imagery due to the low density of individual pine trees. Future habitat modeling efforts should try to incorporate additional spatial data capable of identifying open pine savannah habitats. Alternatively, the locations of most well-managed Longleaf Pine Savannah habitats are already well known and could be incorporated into future versions of this model. Another caveat is that our model does not identify whether the appropriate herbaceous ground cover is present within the forest patches identified as potentially suitable. We know from past research that this is another key habitat factor that allows for the presence of pocket gophers and in turn for potential populations of LPS.

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SECTION IV – Assessment of population structure and abundance

Population Structure

Historically, three geographic populations separated by two rivers were recognized for LPS. Texas populations were thought to be separated from Louisiana populations by the Sabine River, and Louisiana populations were split north and south of the Red River. Recent genetic analyses using microsatellites evaluated this hypothesized population structure (Kwiatkowski et al. 2010, Kwiatkowski et al. 2014). Results from these studies indicated only modest genetic structure among these predicted populations. Indeed, when considering both genetic clustering results and measures of population differentiation due to genetic structure (F_{st}), the Texas and southern Louisiana groups were difficult to distinguish from one another. The northern Louisiana group contained some alleles that were extremely rare in the other two groups, which indicated the Red River was likely a more effective barrier to gene flow than the Sabine.

These genetic results on population structure have implications for future conservation efforts. Given the low measures of population differentiation in LPS, concerns about maintaining three distinct captive populations based on geography should be somewhat alleviated. This was especially true for captive snakes from southern Louisiana and Texas given their small captive population size (see Section V) and was the impetus for the decision to combine the two captive populations. Although the closeness of the Texas and southern Louisiana snakes makes those populations ideal for conservation strategies involving captive breeding and reintroduction, all of the LPS populations are considered closely related. Thus, even crosses of individuals between northern Louisiana and the other two populations should be considered, especially given the low likelihood of wild captures from Texas. The current prohibition on bringing additional animals into captivity from the southern Louisiana populations is a further consideration.

Population Abundance

Data from trapping surveys conducted since the mid-1990s suggest that all LPS populations are in decline (Rudolph et al. 2006, Rudolph et al. 2016). In addition, 4 of the 7 LPS populations recognized by the USFWS in 2008 are either considered extirpated or are approaching the service's criteria for extirpation. In an effort to locate additional occupied habitat, we used the harvest habitat model (Section III) to identify 7 survey sites in areas deemed most likely to support LPS populations (Map 6).

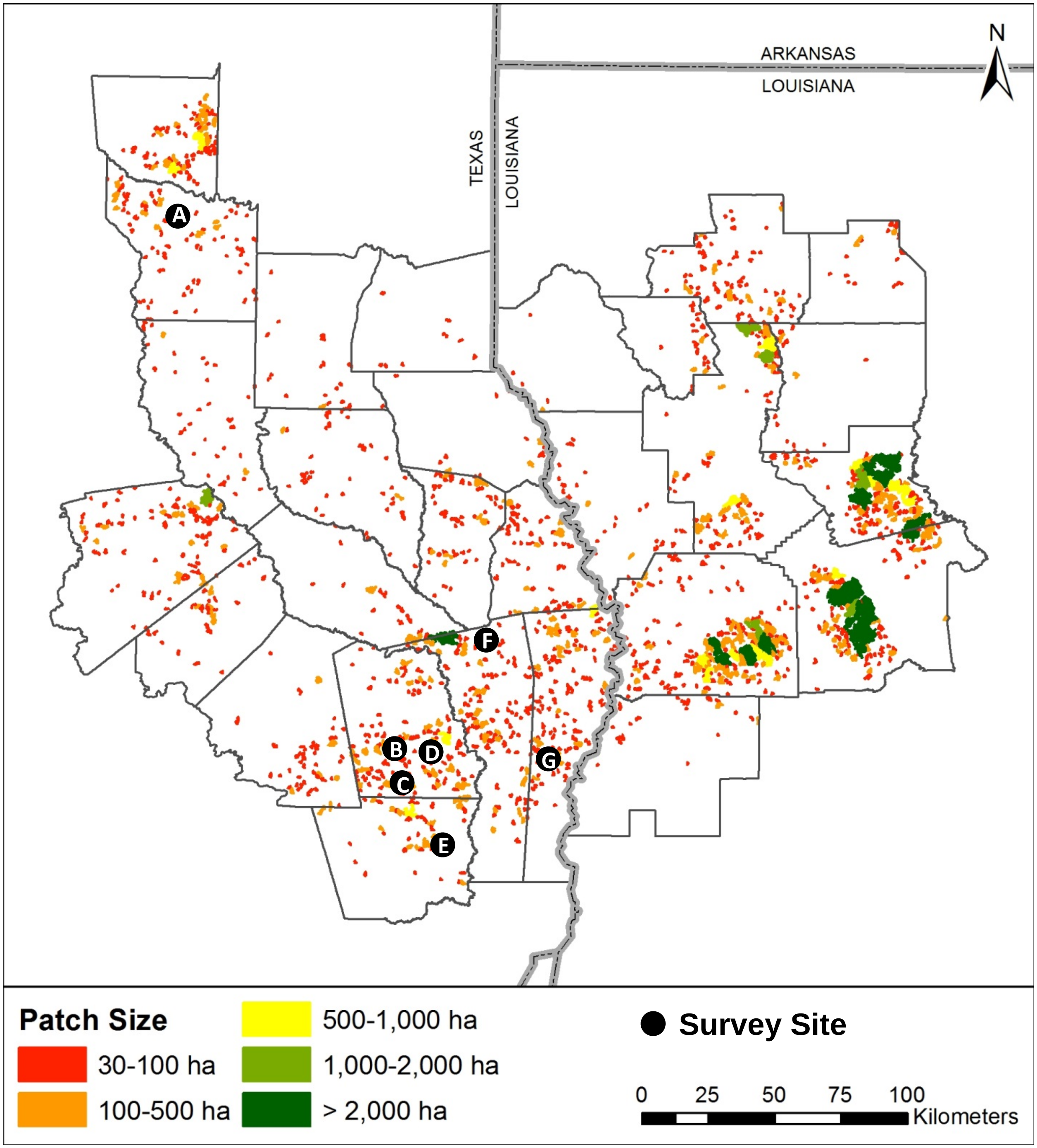
At each site, we applied the same drift fence sampling design used to monitor LPS populations since the mid-1990s with one exception. We replaced the central box trap with a RECONYX PC800™ game camera mounted facing the ground (Fig. 4). All other aspects of the original sampling design were retained. The drift fences were constructed of 6.4 mm mesh hardware cloth, approximately 15 m in length and 61 cm in height (Burgdorf et al. 2005; Rudolph et al. 2006). Four drift fences per array were buried 10 cm in depth in a "+" configuration with a 1 square meter opening at the center (Burgdorf et al. 2005; Rudolph et al. 2006). The camera was mounted on a conduit pole approximately 2 meters above the ground with a flexible Gorillapod™ camera tripod, so that the camera's field of view included the end of each drift fence at the target area in the center of the array (approximately 1 square meter).

Each of the survey sites contained 4 camera trap arrays except one, which contained only two (n = 26 camera arrays total). The cameras were programmed to take an image every 30 seconds, with the assumption that large snakes, such as pinesnakes, exhibiting normal behavior would likely move slowly across the target area and thus be "captured" in an image. Each image was date and time stamped. The cameras were operational from the hours of 0545 to 2200, and they were deployed from March to early October 2016 (Table 2). All images were stored on Verbatim Premium 32 gigabyte SD cards, which were replaced approximately every 24 days along with 12 Energizer Lithium Ion batteries. During each replacement visit, the camera's target area was also raked clean of debris. Images were processed using the Reconyx MapView Professional program. Species, time of detection, and number of consecutive images in which an observation occurred were recorded for each observation. Approximately 1 to 1.5 person hours were needed to analyze 10,000 images.

Across all 26 camera arrays, 8,388,078 images resulted in 523 observations of 18 snake species (Table 3). This equals one snake for every 16,038 images or about one snake every 8.4 camera days (Table 2). No LPS were detected. For comparison, 58 box trap arrays sampling LPS habitats in different locations in Texas over the same time period in 2016, yielded a total of 513 captures of 15 snake species. This equaled 11,919 trap-days with a capture rate of one snake for every 24.2 trap-days. No LPS were captured in box trap arrays either. These data illustrate that the camera trap arrays were much more efficient at detecting snakes and also detected a similar number of snake species as compared to the box trap arrays. For example, the most common snake species observed using either method was the Coachwhip. The two methods also share observations of many other common species such as the Copperhead, Cornsnake, Eastern Hognose, Racer, Texas Coral Snake, Texas Rat Snake, and Western Ribbon Snake.

The major differences observed between the two methods were intentional and expected. First, to match the known diurnal activity pattern of the LPS, the camera trap arrays were operational in the early morning, daylight hours, and early evening hours, but not at night. As a result, the camera traps detected fewer individuals of nocturnal species (e.g., Copperheads) than the box traps, which were open at night also. Second, the camera trap arrays can detect many species regardless of size, whereas the box traps are specifically designed to capture larger snakes. For this reason, the camera trap arrays detected many more small or skinny snake species (e.g., Rough Earth Snake, Rough Green Snake, Texas Brown Snake) capable of escaping the box traps. Nevertheless, it is possible that the camera traps failed to detect all small snakes, because the mesh gaps in the fencing material might not direct the smallest snakes across the target area of the camera.

Species detections using camera traps occurred over the entire daily sampling range, 05:45-22:00. Most detections (n=303, ~58%) were from single images, or 2 consecutive images (n=92, ~18%). Only 24% (n=128) of detections were from 3 or more consecutive images. This means that ¾ of individuals detected were moving through the field of view of the camera in less than 1.5 minutes. Some species (e.g. Coachwhips) potentially moved through in less than 30 seconds, suggesting that longer time intervals between images should be used with caution if applying this camera trapping technology to future LPS survey and monitoring efforts. For example, if we were to have used a one minute photograph interval we would have missed approximately 150 snake detections.



MAP 6. Map showing camera trapping sites A-G in proximity to modeled potentially suitable LPS habitat patches.

FIGURE 4. An example of our camera trap design deployed in longleaf pine habitat.

TABLE 2. Trap effort for 26 camera traps deployed across 7 sites in Texas from March to early October 2016. Location labels correspond to Map 6 locations.

Location	County	Trap Number	Trap-days	LPS Observed	Snake Observations	Trap-days per Snake
A	Smith	4	703	0	65	10.8
B	Tyler	4	572	0	57	10.0
C	Tyler	4	655	0	89	7.4
D	Tyler	2	376	0	19	19.8
E	Hardin	4	689	0	117	5.9
F	Jasper	4	653	0	69	9.5
G	Newton	4	704	0	102	6.9
Total		26	4,352	0	518	8.4

TABLE 3. Total detections by species from 8,388,078 images taken using 26 time-lapse triggered RECONYX PC800™ camera traps deployed across 7 sites in Texas (Map 6) from March to early October 2016. To compare sampling methods, capture results from 58 box traps sampling LPS habitat over the same time interval are also reported.

Common Name	Scientific Name	Box Trap Captures	Camera Trap Detections per Species		Consecutive Detections		
			N	Time Range	Once	Twice	>2
Broad-banded Water Snake	*Nerodia fasciata*	0	2	20:16-20:19	0	1	1
Coachwhip	*Masticophis flagellum*	255	195	7:54-19:44	153	26	16
Copperhead	*Agkistrodon contortrix*	104	14	6:38-20:30	7	2	5
Cornsnake	*Pantherophis slowinskii*	32	7	6:45-21:46	1	2	4
Cottonmouth	*Agkistrodon piscivorus*	5	4	8:33-19:21	1	0	3
Eastern Hognose	*Heterodon platirhinos*	14	12	7:59-18:27	4	3	5
Red-bellied Snake	*Storeria occipitomaculata*	0	1	19:21-19:22	0	0	1
Glossy Snake	*Arizona elegans*	1	0				
Kingsnake	*Lampropeltis getula*	2	3	10:15-17:04	2	1	0
Mud Snake	*Farancia abacura*	1	0				
Prairie King Snake	*Lampropeltis calligaster*	1	11	7:47-18:06	4	3	4
Racer	*Coluber constrictor*	21	54	8:41-18:16	28	7	19
Ring-necked Snake	*Diadophis punctatus*	0	2	8:46-18:26	0	0	2
Rough Earth Snake	*Virginia striatula*	0	3	11:58-16:41	0	1	2
Rough Green Snake	*Opheodrys aestivus*	0	4	11:01-18:20	1	0	3
Scarlet Snake	*Cemophora coccinea*	5	0				
Texas Brown Snake	*Storeria dekayi*	0	7	5:54-20:27	1	1	5
Texas Coral Snake	*Micrurus tener*	11	22	6:58-21:46	9	7	6
Texas Rat Snake	*Pantherophis obsoletus*	54	98	6:02-20:51	49	23	26
Timber Rattlesnake	*Crotalus horridus*	2	0				
Western Ribbon Snake	*Thamnophis proximus*	3	77	5:49-20:59	41	13	23
Yellow-bellied Water Snake	*Nerodia erythrogaster*	0	2	16:43-20:21	1	0	1
Unknown	Unknown	2	5	12:30-20:47	1	2	2
TOTAL		513	523	5:49-21:46	303	92	128

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SECTION V – Recommendations on species management and analysis of captive breeding

The LPS preys heavily on *Geomys breviceps* (pocket gophers), and uses pocket gopher burrow systems for subsurface retreats, including hibernacula and escape from fire (Rudolph and Burgdorf 1997; Rudolph et al. 1998, 2002; Young and Vandeventer 1988). Pocket gophers are closely associated with a well-developed herbaceous ground cover of grasses and forbs (Ealy et al. 2004, Himes et al. 2006, Rudolph and Burgdorf 1997, Rudolph et al. 2002). Well-drained soils and frequent fires are necessary to support the herbaceous vegetation required by pocket gophers. Longleaf pine forests are tolerant of frequent fires and grow well in deep sandy soils. Hence, the LPS was frequently found in longleaf pine savannahs (Conant 1956, Himes et al. 2006, Reichling 1995, Young and Vandeventer 1988), although other pine overstory forests are sometimes occupied under proper herbaceous cover conditions. The once extensive longleaf pine ecosystem of the southeastern United States is one of the most threatened ecosystems in the United States (Bridges and Orzell 1989, Conner et al. 2001, Frost 1993). Indeed, less than 5% of the original extent of the longleaf pine ecosystem survives, and much that remains is extensively altered by changes in fire regimes, silviculture, and land use (Frost 1993).

Most of the longleaf pine ecosystem that occurred on the West Gulf Coastal Plain has been converted to other land uses including urbanization, agriculture, and intensive silviculture. These land uses appear to be incompatible with the survival of LPS populations. The less intensive silvicultural practices of the past, specifically longer rotations and use of prescribed fire, were apparently more compatible with the existence of LPS populations. However, the development and increasing implementation of more intensive silvicultural practices is eliminating much of the remaining suitable habitat on private lands. These practices include clearcutting, intensive mechanical site preparation, planting of pine species other than longleaf, short rotations, fertilization, and use of herbicides instead of prescribed fire for control of competition. The substitution of herbicides for prescribed fire likely has an important impact on LPS. Silvicultural managers use herbicides to control herbaceous as well as woody vegetation, both of which compete with the pine crop. The absence of fire allows the continuous buildup of a thick duff layer further suppressing the herbaceous layer. The ultimate result is a highly altered forest with a minimal herbaceous component, conditions apparently unsuitable for pocket gophers or LPS (Reichling 1995, Rudolph and Burgdorf 1997).

Most critical is the restoration of a prescribed-fire regime sufficient to prevent the encroachment of a dense hardwood midstory and recovery of a vigorous herbaceous community. Economic considerations may preclude improvement on private lands, with the limited exception of small areas specifically managed for LPS and other species adapted to fire-maintained pine ecosystems. However, even on public lands, numerous obstacles exist, especially in implementing an adequate prescribed-fire regime. Managers need to resolve issues relating to liability, smoke management, air-quality standards, and agency regulations to effectively use fire as a management tool to support viable populations of LPS and overall biodiversity in the long term. The potential for restoration on public lands is considerably greater than on private lands. Management of national forest lands and military installations within the range of LPS currently include prescribed fire as a management tool. Increased use of prescribed fire is planned, driven primarily by the management needs of *Picoides borealis* (Red-cockaded Woodpecker), a federally listed endangered species. Habitat management appropriate for *Picoides borealis* in many cases is also appropriate for LPS, and numerous additional species adapted to fire-maintained pine ecosystems, many of which are of conservation concern (Bridges and Orzell 1989, Conner et al. 2001).

The Association of Zoos and Aquariums (AZA) have continuously maintained LPS in captivity since 1972. The first breeding occurred in 1984 and the zoo population has grown slowly since then. The AZA together with the LPS working group have begun an effort to consolidate all captive LPS into four zoos. This consolidation was funded, and began in 2016. The Memphis Zoo, Audubon Zoo, Fort Worth Zoo, and Ellen Trout Zoo were chosen to house the captive breeding program for LPS. Additionally, Texas and southern Louisiana captive populations have now been combined into one “Southern” population. Currently, three Southern population females are reproducing. They have produced 18 offspring in the last 5 years, 8 males, 5 females, and 5 of an unknown sex. The combination of the two southern captive population increases the number of individuals and founders which in turn increases the chances for growth over time. Also, additional founders may be added to this captive population from extant populations south of the Red River in Louisiana (with Louisiana and land owner permission). Without additional founders from the wild, the southern population will likely need the addition of some Bienville Parish snakes to increase genetic diversity and overall captive population numbers.

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