CHAPTER 27

Reintroduction of the Louisiana Pinesnake (*Pituophis ruthveni*) to the longleaf pine ecosystem

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Abstract—The rare and declining Louisiana Pinesnake (*Pituophis ruthveni*) is highly vulnerable to extinction in the wild. Fortunately, insightful members of the zoo community established a captive population of this species in the 1980s. Since 2010, this breeding population has been used as a source of individuals for release into restored habitat with intent to establish a viable population. Initial results are encouraging, including seemingly adequate individual growth rates and the documentation of in situ reproduction. Essential elements of a reintroduction program include a committed and effective stakeholder group, a well-managed captive breeding population, sufficient genetic diversity, restored habitat to serve as reintroduction sites, and adequate funding. Recovery of this characteristic species of the fire-maintained pine forests of the West Gulf Coastal Plain of the United States will likely involve establishing several viable, reintroduced populations.

Keywords—captive breeding; habitat management; head-start; population monitoring; prescribed fire; repatriation; reproduction; structured decision making

Introduction

The longleaf pine ecosystem is one of the most threatened ecosystems in the United States as approximately 98% has been lost since the arrival of Europeans (Bridges and Orzell 1989). This ecosystem is located within the North American Coastal Plain, a global diversity hotspot, which harbors a high diversity of plants and animals, many of which are found nowhere else (Noss *et al.* 2015). The Louisiana Pinesnake (*Pituophis ruthveni*), once considered a subspecies of the more widely distributed Eastern Pinesnake (*Pituophis melanoleucus*; Stull 1929, 1940), is a rare species that is restricted to West-central Louisiana and eastern Texas and is a denizen of the threatened longleaf pine ecosystem. The Louisiana Pinesnake was elevated to full species status in 1995 based on morphometric analyses (Reichling 1995). This taxonomic change was supported by subsequent genetic analyses (Pyron and Burbrink 2009, Rodriguez-Robles and Jesus-Escobar 2000). Prior to the initiation of standardized trapping protocols, the species was rarely reported and concerns about its conservation status were raised as early as the 1950s (Conant 1956) and maintained over the next 40 years (Young and Vandeventer 1988, Reichling 1995, Rudolph and Burgdorf 1997).

Surveys to assess the status of the Louisiana Pinesnake were initiated in 1992 by personnel at U.S. Department of Agriculture, Forest Service (USFS), Southern Research Station (SRS) in Nacogdoches, Texas, USA (Rudolph and Burgdorf 1997). These surveys continue today with the collaboration of various agencies and individuals from research and management. Little was known about the ecology and status of the Louisiana Pinesnake when surveys were initiated. From the first specimens in 1927 through 1992, only 92 records (*i.e.*, roadkills, captures, museum specimens, and verified observations) have been documented (JBP, *pers. obs.*). Knowledge regarding the biology of the Louisiana Pinesnake is limited to data associated with these specimens and information obtained from captive individuals in zoos.

Ecology of the Louisiana Pinesnake

The Louisiana Pinesnake is mainly found in fire-maintained, forested habitats (primarily longleaf pine, *Pinus palustris*) in western Louisiana and eastern Texas. Records also exist in recent decades from sites that have been modified, including non-forested habitats (JBP, *pers. obs.*). Typical habitat consists of open pine forests with a sparse, woody midstory and abundant herbaceous vegetation (Rudolph and Burgdorf 1997) and sandy, well-drained soils (Wagner *et al.* 2014). However, these suitable soils are highly fragmented within the historical range (Wagner *et al.* 2014; Fig. 1). Sandy, well-drained soils with abundant herbaceous vegetation also support populations of Baird's Pocket Gophers (*Geomys breviceps*), the primary prey of the Louisiana Pinesnake, comprising approximately 85% of prey biomass (Rudolph *et al.* 2002, 2012). In addition, the Louisiana Pinesnake uses pocket gopher burrows as subsurface retreats (Ealy *et al.* 2004) and hibernation sites (Rudolph *et al.* 2007), as well as for escape from threats, including fire (Rudolph *et al.* 1998).

Information on breeding and reproduction is limited to data obtained from captive animals (Foster 2023). Louisiana Pinesnakes produce remarkably small clutches, large eggs, and consequently large hatchlings compared to other members of the genus (Reichling 1988, 1990, Reichling *et al.* 2016). Rudolph and Burgdorf (1997) hypothesized that this was an evo-

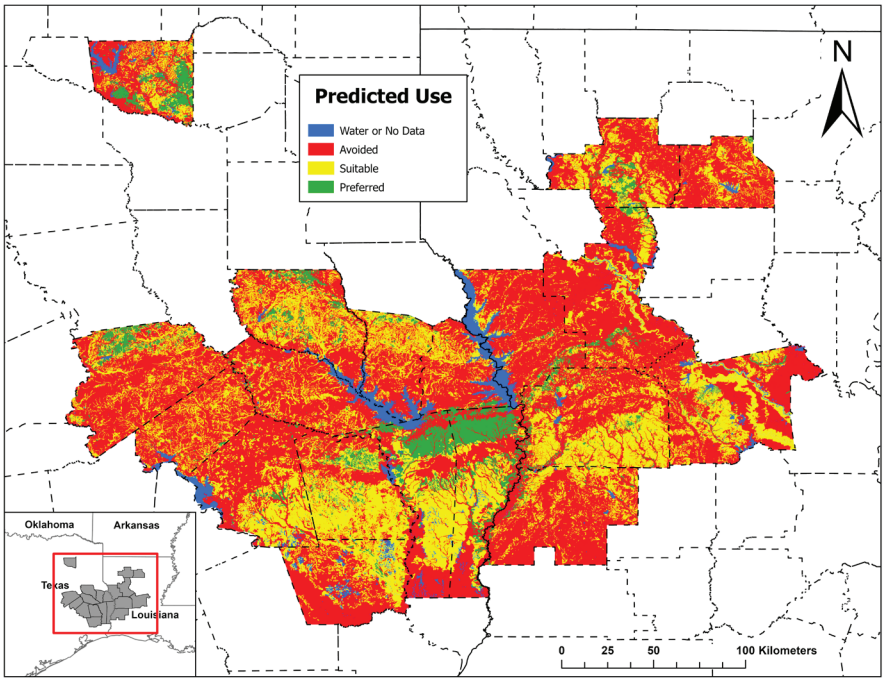

Fig. 1. Habitat suitability index for the Louisiana Pinesnake (*Pituophis ruthveni*) based on soils. Adapted from Wagner *et al.* (2014).

lutionary consequence of the dependence on pocket gophers, allowing hatchlings to rapidly attain a size capable of preying on and consuming large prey. To date, natural oviposition sites have not been located.

Threats—Loss of the longleaf pine ecosystem is due to changes in landcover to urbanization, agriculture, intensive silviculture, and road construction (Rudolph and Burgdorf 1997). This fire-maintained ecosystem historically burned frequently (several times per decade; Frost 1993). Frequent fire encouraged an abundant herbaceous ground cover and prevented encroachment of woody mid-story (Bridges and Orzell 1989, Frost 1993). The loss of this well-defined herbaceous understory, and associated pocket gopher populations, are hypothesized to be a major factor in Louisiana Pinesnake declines (Rudolph and Burgdorf 1997).

Additional threats, some related to habitat loss and alteration of the fire regime, include habitat fragmentation, vehicle mortality (Rudolph *et al.* 1999), collection for the pet trade, snake fungal disease (Lorch *et al.* 2016), and deployment of erosion control blankets (Barton and Kinkead 2005, Ebert *et al.* 2019, Stuart *et al.* 2001). In addition, genetic and demographic viability decrease with increasing habitat fragmentation and population declines (Madsen *et al.* 1996, 1999). Studies on population genetics (Kwiatkowski *et al.* 2010, 2015), captive breeding (JBP, *pers. obs.*), and failure of populations to respond to habitat restoration suggest that issues typical of small populations (*e.g.*, inbreeding depression and genetic drift) may be occurring.

Population status and trends—In 1982, the U.S. Fish and Wildlife Service (USFWS) designated the Louisiana Pinesnake as a candidate species (USFWS 1982). In 2000, the USFWS was petitioned to list the species as Endangered and to designate critical habitat (Boykin 2000, USFWS 2016). The USFWS proposed to list the Louisiana Pinesnake as "Threatened" in 2016 (USFWS 2016) and officially listed it as Threatened two years later (USFWS 2018). In 2022, USFWS proposed designation of critical habitat (USFWS 2022) but has yet to be finalized. For a historical review of previous federal actions see USFWS (2016).

In 2010, the USFWS delineated the historical range, which included 12 counties and 7 parishes (USFWS 2010; Fig. 2). The agency also delineated seven blocks of habitat thought to be occupied at that time (USFWS 2010; Fig. 2). The Louisiana Pinesnake is currently thought to occur in only three isolated blocks of habitat (excluding the reintroduction site) in Louisiana. Other habitat blocks known to have been occupied in the last quarter century have not produced occurrence records for 10 years or more, including all sites in Texas (JBP, *pers. obs.*).

Because of its rarity, sufficient data are lacking to inform the current status of the Louisiana Pinesnake. However, given the issues addressed above, it is reasonable to conclude that populations inhabiting the few remaining blocks of occupied habitat are extremely small, isolated, and likely declining. The species is considered endangered throughout its entire range by the IUCN Red List (Hammerson 2007) and may be extirpated in Texas.

Zoo contributions

Concerns about perceived population declines convinced individuals associated with zoos (*i.e.*, Terry Vandeventer, Jackson Mississippi Zoo and Steven Reichling, Memphis Zoo) to initiate captive breeding of the Louisiana Pinesnake in 1988. In 2003, the Memphis Zoo held a meeting of stakeholders and interested parties and was the precursor of annual meetings that continue today. These Louisiana Pinesnake Stakeholders Consortium (LPSC) annual meetings have been the primary drivers of continued conservation efforts involving the Louisiana Pinesnake. Active participants include the USFWS, USFS Southern Research Station, Kisatchie National Forest, and National Forests and Grasslands in Texas, Ft. Johnson and the Joint Readiness Training Center (Department of Defense), Texas Parks and Wildlife Department and Louisiana Department of Wildlife and Fisheries (LDWF), zoos including Memphis Zoo, Ellen Trout Zoo, Audubon Zoo, and Fort Worth Zoo, Stephen F. Austin State University and Texas A&M University, Louisiana and Texas Forestry Associations, Texas A&M Forest Service, and private landowners, as well as individual citizens

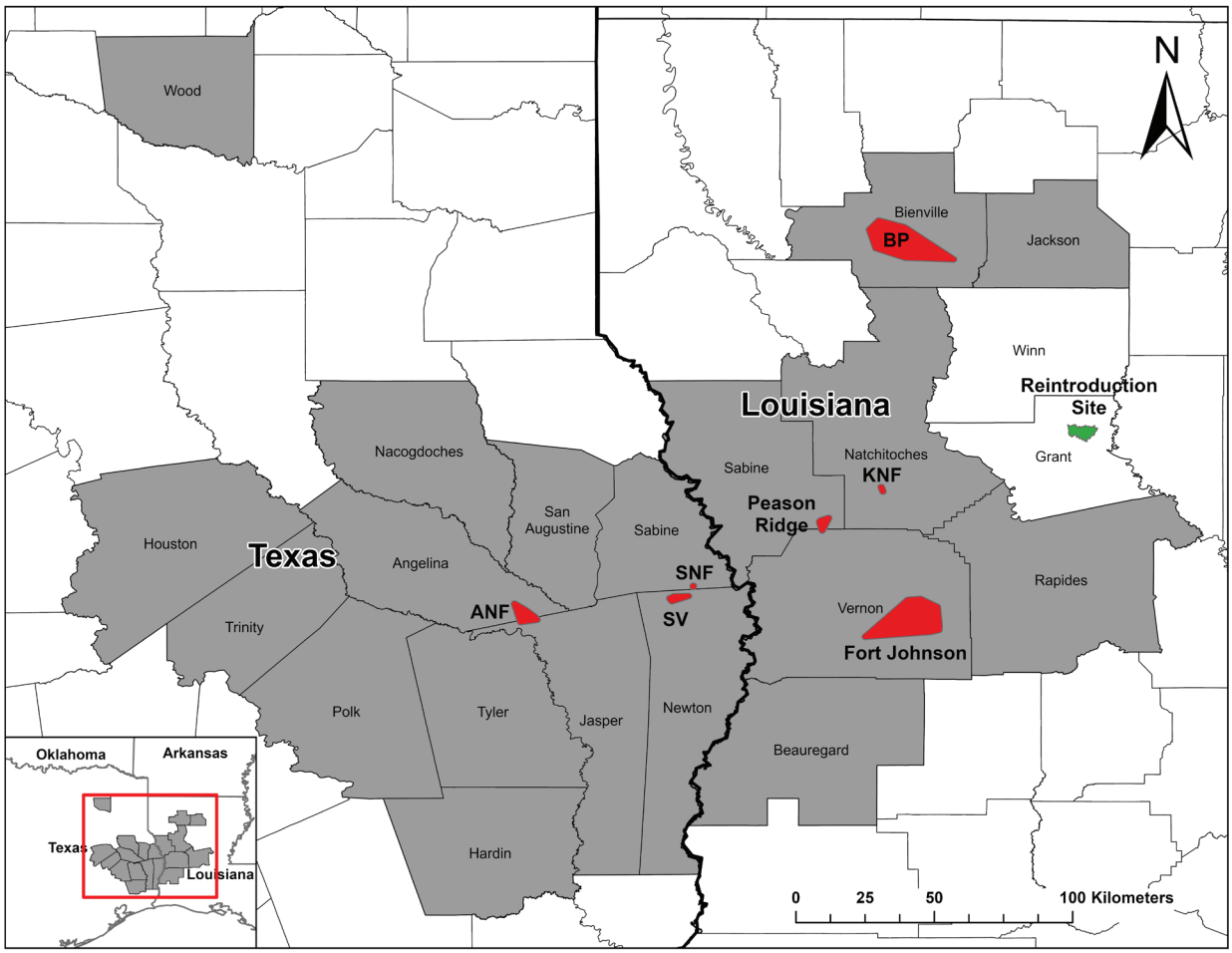

Fig. 2. Seven populations (red polygons) of the Louisiana Pinesnake (*Pituophis ruthveni*) delineated by the U.S. Fish and Wildlife Service (2008); ANF: Angelina National Forest; BP: private land in Bienville Parish, Louisiana; Ft. Johnson, Department of Defense; KNF: Kisatchie District of the Kisatchie National Forest; Peason Ridge, Department of Defense; SNF: Sabine National Forest; SV: private land in Newton County, Texas. The SNF population is currently considered extirpated by the USFWS (2014). Counties and parishes with known records are shaded gray. Map produced by Josh B. Pierce.

that demonstrate interest or support.

Consortium members foresaw that a captive assurance colony would become increasingly valuable for conserving the species and only more difficult to establish in ensuing decades. The initial captive breeding founders (n = 14) were collected from 1984 to 1990 and were the genesis of the zoo breeding program (Reichling 1992). These snakes were obtained from individuals responding to posters offering a cash reward for unharmed, live specimens. Posters were distributed uniformly throughout the historical range in Louisiana, but all snakes acquired ultimately were found in Bienville Parish. Seven snakes were added from Bienville Parish in subsequent years. Ultimately, the number of founders of the captive population from north of the Red River in Louisiana consisted of 21 individuals from Bienville Parish. South of the Red River (*i.e.*, Texas and central Louisiana), a very limited number of additional founders (n = 9) have been obtained since 2000.

In 1985, the Ellen Trout Zoo achieved the first successful captive breeding with a wild-caught pair (Henley 1985); annual reproduction at multiple zoos soon followed. The initial focus for the founding population was to achieve sufficient reproduction to provide a surplus of one or two pairs for distribution to additional zoos each year, thus expanding program participation and helping secure the species' persistence. Space limitations were not a factor, and any breeding that avoided sibling or sib/parent crosses was encouraged. Due to varying husbandry methods, annual population growth rates in captivity varied greatly during 1984-2000 (0.93-1.80; Reichling and Lynch 2001).

Pedigree records for the growing captive population were tracked carefully and updated annually (Foster 2023). With continued population growth and demographic stability, along with the precarious status of wild populations, S. Reichling established a Species Survival Plan® (SSP) in 2001. The SSP formalized management practices as dictated by the Association of Zoos and Aquariums (Reichling *et al.* 2022) and was the first one for a reptile native to the United States.

As the program expanded, both in terms of facilities and snakes, population growth was controlled to remain within the carrying capacity (n = 150 individuals) of participating zoos. To stay below this population size, yet maintain founder representation into new generations, many potential reproductive

pairs were not bred in favor of higher priority pairings (utilizing those from underrepresented lines or individuals approaching reproductive senescence). Therefore, actual growth rates (1.076-1.105) were below the potential during this period of expansion (Reichling and Marti 2013, Reichling *et al*. 2016).

Coincident with the establishment of the SSP, S. Reichling decided to manage lineages from Bienville and Vernon Parishes, Louisiana and Texas as three discrete populations. Up until that time, these populations had been kept separate due to convenience, as most Texas and Vernon Parish snakes resided in only two facilities. In contrast to the Bienville population that had a founder base of 18 individuals in 2001 (10.31 founder genome equivalents; population mean kinship = 0.0485), the Vernon and Texas populations had very small founder bases at that time (2 and 4, respectively; Reichling and Lynch 2001).

By 2016, the population reached its largest institutional participation, with 99 snakes distributed among 22 zoos (Reichling *et al*. 2016). Thus, each zoo held small groups of 2–4 individuals and followed breeding recommendations according to the standard model for SSPs, which was designed to maintain a level census and a pyramidal age structure, avoid inbreeding, and capture and maintain founding genetic diversity (Foster 2017). Maximizing participation by wide distribution of individuals among many zoos is a strategy that provided protection against catastrophic events such as disease and fire. However, this diversification restricted ready availability of multiple potential pairings and potentially hampered maximum annual reproductive recruitment. Participating zoos followed this management model, designed for the long-term maintenance of an assurance colony but inadequate to support an effective reintroduction program, until 2016. At that time, as preparations for consolidation of the zoo holding facilities were being made and based on genetic analyses of these putative demes (Kwiatkowski *et al*. 2015), the USFWS made the decision to combine the Vernon and Texas snakes into one population (8 potential founder genome equivalents; population mean kinship 0.1899) while retaining the discreteness of the larger Bienville population. In 2022, the Bienville Parish lineage was combined with the Vernon Parish/Texas lineages and redefined as a single population, both in terms of reproduction and as the source of snakes for release anywhere selected within the historical range. The USFWS made this decision after discussions with some of the members of the LPSC, due to the apparent extinction of the species in Texas, the limited number of release sites, and the low numbers of founders that started each of the three original managed populations. For example, as stated in the 2019 SSP Breeding and Transfer Plan (Reichling *et al*. 2019), the southern population (Texas and Vernon Parish lineages) was descended from only nine founders and average kinship was around the half-sibling level (Reichling *et al*. 2022). Because the captive breeding population was redistributed from 22 holding facilities to four zoo breeding centers, which diverged from SSP organizational structure, the program ceased to be managed according to SSP criteria in 2023.

The reintroduction program

Goals—Currently, there is a general understanding of the basic ecology, status and population trends, and threats to remaining populations of the Louisiana Pinesnake. This information, combined with the existing captive population, supported a reintroduction program initiated in 2009 with the goal of establishing a viable population on restored habitat on the Kisatchie National Forest, Grant Parish, Louisiana. Although there are no historical records from this area, we are considering this effort a reintroduction instead of an introduction. We assumed the Louisiana Pinesnake occurred in this area historically because (1) the paucity of historical records failed to accurately delineate the historical range; (2) there are species' occurrence records from adjacent parishes; and (3) there are suitable soils at the reintroduction site that are contiguous with adjacent parishes. Ultimately, success in the recovery of the Louisiana Pinesnake will likely involve the establishment of multiple viable populations through reintroductions.

Site selection—Members of the LPSC initiated the reintroduction program in 2009 with a search for a suitable reintroduction site and instructions to the > 20 zoos that then held portions of the captive population to prepare to breed animals for release in 2010. With the notable exception of Bienville Parish, Louisiana, occupied habitat and recent records were concentrated on or near public ownerships. Given the extant conditions and fewer hurdles involved on public lands, members of the LPSC made the decision to evaluate sites on public lands as potential reintroduction sites. The LPSC also focused on sites that were currently unoccupied to avoid any issues involving genetic structure or disease. In addition, the largest subset of the captive population was descended from stock derived from Bienville Parish, north of the Red River, whose wide bottomlands are potentially a significant barrier to gene flow (Kwiatkowski *et al*. 2015). Thus, given our desire to maintain any potential genetic structure that might exist across the historical range, we restricted our search to locations northeast of the Red River.

Our anecdotal knowledge narrowed the search to five proposed reintroduction sites on the Kisatchie National Forest. We attempted to choose areas that best represented current and future Louisiana Pinesnake habitat. Each of the five sites were evaluated for total area (hectares), percent in USFS ownership, percent in Red-cockaded Woodpecker Habitat Management Area (HMA), percent in suitable soils, percent of unsuitable habitat, and road density.

After site visits and GIS analysis of the above criteria, we narrowed the choices to two potential sites located on the Catahoula District of the Kisatchie National Forest. To determine the final site, we evaluated habitat characteristics at a finer scale: midstory height and density, herbaceous cover, hardwood basal area (BA), pine BA, and pocket gopher mound presence. Habitat restoration on the chosen site, primarily driven by the needs of the federally-endangered Red-cockaded Woodpecker (*Leuconotopicus borealis*), was well advanced with a vigorous prescribed fire regime in place. Suitable soils and pocket gophers were widespread, and roads were infrequent. The chosen reintroduction site included 3,328 ha of USFS ownership. Not all of this habitat was suitable, but adjacent potentially suitable habitat could accommodate population expansion.

Table 1. Number of head-started, neonate, male, and female Louisiana Pinesnakes released on the Catahoula District of the Kisatchie National Forest, Louisiana.

	Age		Sex	
Release year	Head-starts	Neonates	Males	Females
2010	0	11	6	5
2011	8	7	5	10
2012	3	0	3	0
2013	14	1	15	0
2014	1	0	1	0
2015	0	15	7	8
2016	17	6	10	13
2017	5	3	5	3
2018	3	4	2	5
2019	25	0	11	14
2020	55	0	19	36
2021	52	0	27	25
2022	61	0	35	26
2023	117	0	55	62
Totals	361	47	201	207

Release and monitoring protocols and preliminary results—Releases began on the Catahoula District of the Kisatchie National Forest in Louisiana in 2010, at which time there was limited information on the relative merits of releasing neonates versus head-started snakes. Consequently, the LPSC decided to release both age classes in an attempt to evaluate the efficacy of the two approaches. The basic protocol was to release 50% unfed neonates (balanced as best possible by sex and clutch) as soon as practical after the post-natal shed. The remaining 50% were head-started for approximately 9 months (from the July -September hatching until the next April/May), and then released. Head-started animals were fed *ad libitum* and not brumated to achieve maximum growth. Snakes were released following this protocol from 2010 to 2018. However, in their stage-based population model, Waddle and Glorioso (2018) determined overall annual survival of head-started individuals (63.7%) was almost double the survival of neonates (33.0%). As a result, only head-started individuals have been released since 2019. This also reduced the logistical burden on zoos by maintaining single age release groups. Annual releases occurred from 2010 to 2023 resulting in a total of 408 captive-bred snakes released. Prior to release, snakes were weighed, measured, inserted with a passive integrated transponder (PIT) tag, and sex determined. A post-natal shed was collected for future DNA analysis (*i.e.*, to determine parentage and identify unknown individuals).

The LPSC believed it was important to monitor the reintroduction population from the beginning of releases to potentially identify any problem that may arise from the monitoring techniques and to track the progress of the reintroduced population. Automated PIT tag recorders (APTR; Biomark HPR Plus) were the primary monitoring technique, supplemented by box trapping (Burgdorf *et al.* 2005). Initially, deep cycle 12 V batteries were the single power source for the APTRs; however, they required exchanging approximately every two weeks for re-charging. This methodology was quite labor intensive, thus the design was recently improved by implementing solar panels to power the external batteries. These recorders were installed throughout the area where snakes were released. The antennas were buried about 8 cm under the soil at a convergence of drift fences. If a snake with a PIT tag traveled over an antenna, its location, date, and time were recorded by the APTR. Data were collected from the recorders approximately every 3 weeks. Box trapping was also used which allowed the ability to weigh, measure, determine body condition, palpate females for eggs, and obtain fecal and blood samples. Traps (121.9 cm X 121.9 cm; Burgdorf *et al.* 2005) included water sources and hide boxes and were checked weekly. Important information that could be obtained through monitoring includes disease status, lack of activity, gravidity, and abnormal behavior.

Of the 408 snakes released prior to February 2024 (Table 1), 57 have been detected following the winter after release. We detected 5 individuals at least 6 years after release and 2 individuals 10 years after release (JBP, *pers. obs.*). These detections provided a minimum level of survival since there are likely surviving snakes that have gone undetected. Using data from the reintroduction effort, annual survival rates have been estimated for hatchlings (25.4%), 1-year olds (47.7%), and 2-year olds (70.8%; Waddle and Glorioso 2018). Fifty-three unique individuals have been captured to date; mean growth rate based on snout-vent length (SVL) of 48 snakes was estimated to be 1.8 cm/month (SD = 1.7), which is higher than past estimates for adult Louisiana Pinesnakes (0.20 cm/month SVL [Himes *et al.* 2002] and 0.19 cm/month SVL [Pierce *et al.* 2014]). This higher growth rate likely can be attributed to a longer length of monitoring and the comparison of multiple age classes (often juveniles and neonate to adult). The growth rate of 4 female juveniles (1.3 cm/month SVL (Himes *et al.* 2002) was more similar to our overall growth rate. In addition to the encouraging survival and growth, the snakes captured appeared to be healthy.

Reproduction has recently been documented in this reintroduced population. In 2016, an untagged adult was captured, and genetic analysis revealed that it was the offspring of a captive-bred female released in 2011 (Hinkson *et al.* 2020). The identity of the male parent was ambiguous. Subsequently, in October 2019, three untagged neonates were captured. An additional untagged snake, presumed to be from one of the 2019 clutches based on size and location, was captured in 2021. Since the reintroduction site was presumed to have been unoccupied prior to the releases of captive snakes, it is highly probable that these individuals are the offspring of captive-bred releases. Genetic analysis has not yet been performed on these four snakes; however, paternity analyses to confirm this hypothesis are pending.

Obstacles and solutions

The numbers problem—Arguably, the primary obstacle involved in the reintroduction effort was the limited number of animals in the captive breeding program maintained in the various zoos. Initially (2009), this was approximately 65 individuals, nearly all descended from founders captured in Bienville Parish. The captive population was essentially at carrying capacity, which limited the number of animals available for release each year. During the initial 5 years of the reintroduction program, 45 animals were released. This was below initial expectations and presumably insufficient in terms of establishing a functional population at the release site. Modeling of life history parameters suggested that, based on the current number of releases, few reproductively mature individuals would exist at the reintroduction site at any point in the future (JBP, *pers. obs.*).

In addition to the limited population size, the captive population exhibited low productivity of potential release animals. Potential factors involved in low reproductive output included, but were probably not limited to, inefficiencies due to population dispersal across many zoos, low percentage of females laying eggs (<20%), low percentage of eggs hatching (<40%), and variation in husbandry skills among facilities. By way of comparison, hatching success of *Pituophis melanoleucus* under similar environmental conditions as our study ranged from 87 to 97% for eggs brought into a laboratory immediately after oviposition (Burger *et al*. 1987) and 100% for gravid females collected from the wild that laid in a laboratory (Gutzke and Packard 1987).

The LPSC participated in a structured decision making (SDM) workshop organized by the USFWS in 2014. The workshop assisted the consortium in reaching a unanimous decision to consolidate the Louisiana Pinesnake population into fewer zoos to potentially reduce husbandry inefficiencies. This consolidation would also potentially address, or at least allow identification of, factors involved in low reproductive success. Consolidation of the captive population into four zoos was committed to in early 2016 and essentially achieved in time for the 2017 breeding season. As a result, the hoped-for improvements in reproductive success appeared to have happened. In 2017, hatching success increased from the 2014-2015 average (29.0%) by approximately 26%. Additionally, due to an increase in the number of breeding females, 117 offspring were released in 2023 compared to an average of 22 for the previous 13 years (JBP, *pers. obs.*). Approximately 170 snakes are expected to be released in 2024. Presumably, producing a sufficient number of snakes for a successful reintroduction program at one site is no longer our major issue.

Genetic issues—In the past, husbandry issues may have contributed to low captive reproductive success and improvements in these metrics post-consolidation of zoo holding facilities suggest that was at least partially the case. However, and despite being now managed as a single population, the founder representation of zoo snakes is still small at 30 (Reichling *et al*. 2022). Analysis of polymorphic microsatellite loci from wild and captive populations of the Louisiana Pinesnake revealed only modest genetic structure (pairwise F_{ST} (a measure of variability among populations) values for wild-caught samples ranged from 0.026 to 0.06) for three *a priori* populations delineated by major rivers, potential barriers to gene flow (Kwiatkowski *et al*. 2010, 2015). Anticipation of this result was the basis for the initial decision to avoid crossing animals from north and south of the Red River in the captive population.

Observed heterozygosity was consistently lower than expected, and heterozygosity in wild-caught samples was consistently lower than in captive samples. For wild populations, F_{IS} (inbreeding coefficient) values were consistently higher (0.12–0.17) than they were for captive populations (-0.19–0.07), which suggests moderate levels of inbreeding in wild snakes. The lower values in captive snakes are likely due to founders originating from different regions. However, given the low number of founders, along with current captive populations having very few individuals that originated from southern Louisiana and Texas, inbreeding could become a problem in the captive population. In addition, using an infinite alleles model, there is evidence of bottlenecks for all populations (Kwiatkowski *et al*. 2015). These results, combined with reproductive metrics observed in the captive population and the apparent failure of wild populations to respond to habitat restoration, suggest that genetic viability of the wild and captive populations is a concern. The consolidation of the captive population into fewer zoos was specifically designed to facilitate appropriate pairings among captives and increase the number of captive breeders to mitigate issues related to low genetic diversity.

Although it would be possible to increase the captive founder base by bringing wild snakes into the breeding program, doing so could negatively impact the demographics and genetic diversity of the tenuous native populations. As an alternative, assisted reproductive technologies have been developed for the Louisiana Pinesnake (Sandfoss *et al*. 2021, 2022, Oliveri *et al*. 2022) and continue to be refined. Breeding facilities have begun collecting and cryopreserving semen from every male Louisiana Pinesnake found in box traps, resulting in a growing gene bank for the species. Roberts *et al*. (2024) used freshly collected semen to achieve the first successful reproduction of the Louisiana Pinesnake by artificial insemination. Subsequently, Sandfoss *et al*. (*pers. comm.*) accomplished another breakthrough with the successful reproduction of Louisiana Pinesnakes—a first for any reptile—by artificial insemination using cryopreserved semen.

Decision making by stakeholders—From its inception, the LPSC was envisioned as a group of interested stakeholders committed to addressing the conservation problems confronting the Louisiana Pinesnake. The group was open to all with an interest in the species, and from its initiation was a very diverse group of representatives from agencies, academia, management, and landowners. The LPSC was, and remains, a very informal group and has never had any official organization, bylaws, or other mechanisms regulating its activities. Initially, this structure was adequate with the zoo community, researchers, and managers each moving forward with the obvious needs of the species. As the conservation efforts transitioned into a formal reintroduction program, the need for coordination to avoid working at cross-purposes greatly increased. It became apparent that the LPSC did not have a mechanism to address decision making, and this deficiency was becoming increasingly serious. Subgroups developed that made independent decisions relevant to their areas of expertise and in-

terests, but most members were only participating in the annual meetings. Meetings often ended without a definitive plan for the coming year.

Our 2014 workshop used a decision-analytic process, known as structured decision making (see O'Donnell and Grant, Case Study 13, this volume), to arrive at the key decision to consolidate captive snakes into fewer zoos. This was a major accomplishment that resulted in a plan to increase the number of pinesnakes available for release. However, participation by only a few individuals, lack of resources to repeat the process when additional decisions were required, and the inherent complexity of the SDM process have precluded follow-up. The basic problem of lack of formal organization remains, complicating the process of making and implementing decisions. This is perhaps the most fundamental issue confronting recovery efforts for the Louisiana Pinesnake, and resolving this issue is an ongoing challenge. The formation in 2017 of a Louisiana Pinesnake Species Survival Plan Steering Committee (SSPSC) to specifically address issues involved in the captive-breeding program, a body which continues to function after captive population management diverged from SSP structure in 2023, was a step forward.

Funding—To date, funding for the conservation of the Louisiana Pinesnake has generally been adequate given the needs of the species. Research activities have been funded by a diverse combination of allocated budgets of the USFWS, USFS Southern Research Station and Kisatchie National Forest, state wildlife agencies, the Department of Defense, and universities, supplemented by Section 6 grants, grants from state agencies, and private landowners.

Consolidation of the captive population into four zoos and a major increase in carrying capacity in those zoos emerged as a major challenge. A funding stream sufficient to provide space to house animals and provide for personnel with a dedicated responsibility for husbandry was lacking. This was provided through the USFS Stewardship Authority, basically retained timber sale receipts, to participate in projects beneficial to the management of National Forests in partnership with third parties (in this case, zoos). This cleared a major obstacle and, given the improvements in breeding success in the first year after consolidation, represented a potentially critical accomplishment in the conservation of the Louisiana Pinesnake. Challenges remain, especially in maintaining a funding stream for the captive-breeding program, but the physical consolidation and improvements in breeding success are encouraging.

Improvements in captive breeding, resulting in greater numbers of animals for release, will allow for acceleration of releases at the current site and initiation of releases at additional sites. However, if monitoring is required or desired at future reintroduction sites, additional resources will be needed (*e.g.*, personnel and funding for equipment). The USFWS has developed a Draft Recovery Plan for the Louisiana Pinesnake that will help guide managers through monitoring requirements (USFWS 2023a).

The future

Additional reintroduction sites—The four zoos currently participating in Louisiana Pinesnake reproduction (*i.e.*, Memphis Zoo, Ellen Trout Zoo, Fort Worth Zoo, and Audubon Zoo) are nearing their respective snake holding capacity and must manage their annual recruitment. Since annual reproductive output has reached over 150 individuals, members of the SSP-SC believe there are enough snakes to begin releases at a second reintroduction site. Currently, the USFWS does not intend to stop releases at the current release site; however, there are plans to release a portion of annual recruitment from the zoos at the next identified release site. A population viability analysis (PVA) would help determine how to allocate individuals across multiple reintroduction sites.

In 2021, USFWS established a cooperative agreement with USFS Southern Research Station to evaluate the habitat of potential Louisiana Pinesnake reintroduction sites. The SSP-SC recommended six sites for further evaluation as a potential second reintroduction site (*i.e.*, Winn District, Kisatchie District, Catahoula District (a potential second site), and Vernon and Evangeline Units of the Calcasieu District of the Kisatchie National Forest in Louisiana and the Angelina National Forest in Texas). The USFS SRS used a similar methodology to that used in the 2010 analysis except we also considered areas already occupied by Louisiana Pinesnakes. The preservation of genetic structure was no longer considered since it was decided to interbreed Louisiana Pinesnakes across captive populations in 2022. Additionally, concerns of disease introduction were alleviated due to stringent health screening initiated by zoos in 2022 (USFWS 2023b). Last, we only considered blocks of suitable habitat ≥2,900 ha, which represented the estimated minimum area needed for a Louisiana Pinesnake population (USFWS 2022). Based on the results from the selection process, and after discussion with the SSPSC, in 2023 USFWS chose the Vernon Unit of the Calcasieu District of the Kisatchie National Forest in Louisiana as the next potential reintroduction site (Pierce *et al.* 2023).

Habitat management—Habitat management for the remnant, naturally occurring populations and those at the reintroduction site is similar. The fire-maintained habitat occupied by the Louisiana Pinesnake requires active management. Because of political and legal decisions in the 1980s and 1990s, public land management to support recovery of the endangered Red-cockaded Woodpecker changed dramatically across the southeastern United States (Conner *et al.* 2001). On the West Gulf Coastal Plain, this resulted in major improvements in the suitability of habitat for the Louisiana Pinesnake, primarily due to intensification of the prescribed fire regime and targeted timber thinnings. Additional habitat restoration is required outside the Habitat Management Areas designated for Red-cockaded Woodpeckers, especially in areas potentially suitable for the Louisiana Pinesnake. To date, habitat management has been the responsibility of the land-management agencies involved, primarily the USFS and Department of Defense. On private lands, limited habitat management suitable for the Louisiana Pinesnake has occurred, often assisted by cooperative agreements with state wildlife agencies.

Using prescribed fire to restore and maintain suitable habitat is inherently difficult. Issues such as smoke management, ad-

equate personnel, and safety can result in sub-optimal timing, intensity, and frequency of burns. As one consequence, the habitat on the current release site is not exhibiting characteristics associated with suitable Louisiana Pinesnake habitat. Woody vegetation is dominant over most of the area resulting in sub-optimal habitat for both gophers and the Louisiana Pinesnake. Current burning practices are not reducing the rootstock of woody species resulting in very rapid regrowth following burns. Consequently, the overall habitat quality at the release site has declined to the extent that it might not be considered a suitable site for reintroduction if it was being evaluated today (JBP, *pers. obs*.).

Not surprisingly, habitat management for the Louisiana Pinesnake can be broadly compatible with overall ecosystem management using fire and timber removal to maintain conditions that reasonably mimic the historical condition. This has been demonstrated by management for the federally endangered Red-cockaded Woodpecker (Conner *et al*. 2001) on this same landscape. Ideally, future efforts to restore Louisiana Pinesnake populations will further ecosystem restoration on suitable remnants of the fire-maintained pine forests of the West Gulf Coastal Plain.

Recognizing success

Ultimately, the recovery of the Louisiana Pinesnake depends on success at two levels, the captive breeding program and the establishment of viable populations within the historical range. Determining the success of these two elements is not a trivial task.

Evaluating the captive-breeding program is the easier of the two because the required data are more accessible. The captive-breeding program has already been successful at producing sufficient hatchlings to support the reintroduction effort at multiple sites, close to 200 individuals per year. However, the population of hatchlings produced must have genetic diversity sufficient to allow establishment of a viable population. The 2017 consolidation of captive snakes into four zoos was a major step towards achieving these goals.

Confirming the establishment of viable populations at the reintroduction sites is much more difficult (Germano and Bishop 2009). Members of the SSPSC have recognized interim goals of adequate survival, individual growth rates, and reproduction in the field (*sensu* Walls, Chapter 2, this volume). The ongoing surveys and establishment of a DNA library provide data to address these topics. Compared to data available on growth and survival of other species in the genus *Pituophis*, data from the reintroduced population are encouraging and reproduction has been documented. The ultimate recognition of a viable population is much more difficult and will not be determined until years in the future. It will likely require a PVA based on data collected from the reintroduced populations in the future.

Lessons learned

A number of lessons have been learned to date. First, it is critical to have a large and diverse group of committed individuals with expertise in captive breeding, habitat restoration, genetics, and population biology. This group of participants requires coordination, decision-making procedures, and effective leadership, in addition to constant communication. A reasonably consistent funding stream is also required. Finally, it would be beneficial for group members to have at least a general concept of how success, in this case preventing extinction in the wild, can be accomplished on the landscape available. Maintaining such a group of committed individuals is an important step towards recovery of the Louisiana Pinesnake.

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