SURVEY PROTOCOL, TRAPPING METHODOLOGY, HANDLING, AND MONITORING OF THE BLACK PINESNAKE (*PITUOPHIS MELANOLEUCUS LODINGI*) IN MISSISSIPPI AND ALABAMA

March 2017

Photo: J. Lee, TNC

PURPOSE

This document describes protocols meant to document presence/absence of black pinesnakes, as well as approved handling and tracking methodologies. Survey protocols were developed using the best scientific information available, and will be updated as new information becomes available. The two types of surveys detailed in this document include:

- **Visual Survey (Transects)** – visually searching for black pinesnakes (or their sheds) via multiple pedestrian transects through suitable habitat during the snake's active season, for the purpose of documenting presence/absence of the species. This type of monitoring does not involve take of black pinesnakes, and therefore does not require a permit. However, the U.S. Fish and Wildlife Service (Service) would appreciate a report detailing the results of these surveys to help better inform our knowledge about the distribution of black pinesnakes.

- **Trapping** – utilizing drift fences and box traps to capture black pinesnakes, for documenting presence/absence, as well as for more informative population evaluation, mark-recapture studies, and radio-telemetry studies. As this monitoring involves potential take of black pinesnakes (in the form of harassment), it requires appropriate state and federal permits prior to the activity.

VISUAL SURVEY PROTOCOL

The purpose of this section is to provide a tool to improve the review of permit applications and proposed land clearing activities for potential effects on the federally-threatened black pinesnake in accordance with the Endangered Species Act of 1973 (ESA), as amended (16 U.S.C. 1531 et seq.).

The tool is a visual encounter survey protocol (Protocol) that is to be conducted by project proponents or their designees for documenting the presence/absence of black pinesnakes at a proposed project site. The results of the Protocol will be used by Federal and non-Federal entities in evaluating permit applications and proposed activities for compliance with the ESA, and the Service encourages the use of the Protocol in situations where habitats that may support black pinesnakes will be impacted by development activities. For projects that encompass vast acreage such as military installations, large restoration projects, and new pipeline and transportation projects, we recommend contacting our office in advance to discuss the best approach for implementation of this Protocol in order to achieve the intended purpose and objective.

This Protocol explains visual encounter survey methodologies that include pedestrian transect surveys and inspection of above-ground refugia. A black pinesnake survey conducted according to this Protocol is an attempt to document presence/absence of the species within the affected area(s) of a proposed site, providing project planners an early opportunity to develop minimization and/or compensation measures and consult with the Service. This Protocol provides a method for surveying black pinesnakes in a manner which does not include handling, trapping, or potentially injuring snakes (therefore no take and no Federal permit required), and includes visually searching for black pinesnakes and their sheds via multiple pedestrian transects through suitable habitat during the snake's active season.

We offer this Protocol as a measure to help us obtain necessary information to make informed regulatory decisions relative to the recovery and management of this federally-threatened species. It is the intent of the Service to recommend this protocol until such time that better survey methodologies are available for project proponents.

Previously Reported Snake Transect Survey Methodologies

The current U.S. Fish and Wildlife Service (Service) technique for eastern indigo snakes (*Drymarchon couperi*) involves visual encounter surveys between October 1st and April 30th, which is the time of year when snakes are less active and expected to be in or near their winter refugia (Service 2011). The survey protocol includes transect surveys and inspection of above-ground and underground refugia. A minimum of five (5) survey days is required, unless an indigo snake is detected prior to the fifth survey day. A survey day is the amount of time required to review the entire survey area once. Surveys are done between 0900-1600 h when snakes are more likely to emerge from their refugia. Above-ground refugia to be inspected include ground litter, trash piles, abandoned structures, rock piles, stumps, plywood, and sheets of metal. Underground refugia include gopher tortoise burrows and stump holes.

A visual search is recommended for the eastern massasauga rattlesnake (*Sistrurus catenatus catenatus*) by qualified personnel only (Casper *et al*. 2001). Surveys are done when temperature is between 50-80 degrees Fahrenheit, preferably during the morning and evening. A minimum accumulation of forty (40) person hours distributed over a standard (April-October) field season is recommended before any evaluations are made regarding the presence/absence of this species. The majority of these hours should be expended in two time windows reflecting presumed maximum activity levels of the massasauga rattlesnake: 1) spring emergence, and 2) mid- to late summer basking and birthing period.

A visual search is also recommended to document presence/absence for the timber rattlesnake (*Crotalus horridus*) by the state of Pennsylvania (Pennsylvania Fish & Boat Commission 2010). Visual surveys should be conducted April 15th through September 15th when the ground temperature is above 75° Fahrenheit, air temperature is above 65° Fahrenheit, when it's not raining, and should target areas that have high potential to support rattlesnakes like talus slopes, boulder fields, and rocky outcrops. Each survey area must be visited four (4) times on separate dates.

Another source investigated the survey effort needed to assume with 95% probability that a site was unoccupied by three species of terrestrial snakes (Kéry 2002). The study found that, based on varying probability of detection, between 12 and 26 independent survey visits were necessary to document that a species was absent from a site, with the more cryptic species requiring more visits to ascertain absence. This analysis also involved considerations for seasonality of surveys as well as time of day and weather. Instead of basing the survey criteria on number of hours, the methodology was based on independent survey visits performed within the boundary of suitable habitat on the tract.

Black Pinesnake Transect Survey Protocol

Visual encounter surveys will be used to ascertain presence or absence of black pinesnakes, and should include examination of above-ground refugia located along transect lines. Above-ground refugia includes ground litter, trash piles, brush piles, abandoned structures, stumps, and cover objects (plywood or sheets of metal); and should be inspected in a non-destructive manner (i.e., attempting to return any potential refugia to its original condition if it needs to be disturbed during inspection). Based primarily on the study by Kéry (2002), but limited by specific information on the probability of detection of black pinesnakes, we estimate that a minimum of 12 independent survey visits are necessary to establish presence/absence of the species. While this number of independent surveys represents the low end of the range determined by Kéry, it is more than twice the number of visits recommended in the other three snake survey protocols discussed previously. Each independent visit must include a survey of all potential habitat within the site, with only one full survey allowable each day, and no more than three separate visits to a site within the same week. Potential black pinesnake habitat is characterized as upland (non-wetland) pine-dominated forest with at least some herbaceous groundcover and some sunlight reaching the forest floor. If there is either a completely closed overstory (canopy) or a dense, impenetrable midstory or understory to the extent that no light is reaching the forest floor, then that is not considered suitable black pinesnake habitat.

While this survey protocol does not require the examination of underground refugia with camera systems, areas immediately surrounding pine stump holes and gopher tortoise (*Gopherus polyphemus*) burrows should be given thorough examination as these are potential refugia for black pinesnakes. Keep in mind that if gopher tortoise burrows are to be examined with a camera system, a permit must first be obtained from the Service and either the Mississippi Department of Wildlife, Fisheries, and Parks; or from the Alabama Department of Conservation and Natural Resources (with the exception of Clarke County, Alabama where the black pinesnake is listed under the ESA but the gopher tortoise is not).

The recommended approach is to systematically search the entire survey area by walking parallel transects spaced appropriately for the habitat conditions (*i.e.*, the length may be consistent or vary with the shape of the site, but the width should allow a reasonable level of ground visibility). Transect widths will be determined based on habitat conditions of the survey site. Transects in habitat such as natural sandhill or open-canopied longleaf forest should be spaced no more than 33 feet apart. In thicker habitat with a heavier understory, transects may need to be spaced as close as 10 feet apart to ensure complete coverage. Surveyors should look for black pinesnakes moving or resting on the surface, noting any signs of shed skins. Surveys can be conducted by one or multiple surveyors depending on the size of the survey area, but must be performed by qualified personnel able to effectively investigate the area (including potential above-ground refugia) and able to correctly identify snake species and snake sheds. Transect edges should be marked with flagging to make sure there are no gaps between transects.

Timing for transect surveys should coincide with increased black pinesnake activity (see Fig. 1); therefore, surveys must be performed between March 15th and September 30th. Surveys should be performed between 0900 and 1800 h, when temperatures are between 60º and 90º F, and should not occur on overcast days (cloud cover > 90%). If a black pinesnake is located at any point during the survey visits, then the surveyor may discontinue the survey for the remaining days since presence has been established. If no pinesnakes are documented after the site is surveyed 12 times, then an assumption can be made that, for the purposes of Service consultations and effects' determinations, the species is absent from the site.

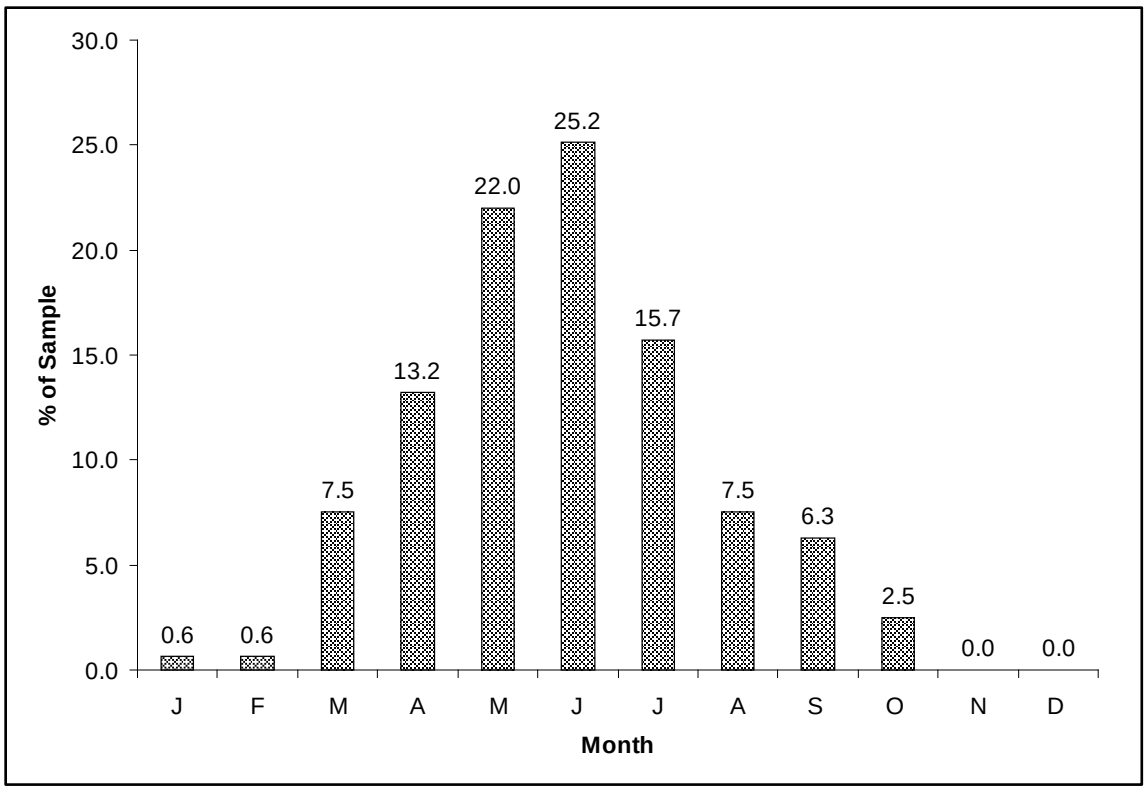

Figure 1. Seasonal activity pattern of black pinesnakes in Mississippi (from Yager *et al*. 2005 and references therein). N = 161. "Percent of sample" refers to the relative number of black pinesnakes captures or records from each month of the year.

Reporting

The surveyor(s) will complete a survey report, including names of surveyors, survey dates and times, and weather/habitat conditions. A map will accompany the survey report, with the delineations of the surveyed area and estimated locations of transect lines. A list of all observed snakes will also be included, along with corresponding coordinates and, to the greatest extent possible, photographs to confirm identification and for voucher purposes. The results of the presence/absence survey will be considered valid for two (2) years from the date of completion, unless the habitat has been significantly modified. Transect surveys can also record gopher tortoise burrows at the same time since most projects containing black pinesnake habitat also likely contain suitable gopher tortoise habitat. Surveyors should always carry a camera to photograph any snake sightings, and use a GPS unit to document snake locations.

TRAPPING PROTOCOL (Activities from here down require a Federal permit)

This protocol describes utilizing drift fences and box traps to capture black pinesnakes, for population evaluation, mark-recapture studies, and/or radio-telemetry studies. Trap arrays should be similar in design to those described by Burgdorf *et al*. (2005) and Lee (2009). They should consist of a drift fence (*i.e*., sedimentation fence; height = 3 ft, length = 295 ft) buried to a depth of 4 inches, with box traps (height and width = 2 ft, length = 4 ft) constructed of hardware cloth (¼-inch mesh) and plywood. Box traps, equipped with a rectangular funnel entrance

(height = 3 in, length and width = 1 ft) and one-way funnel door, should be placed at each end, and on alternating sides, of the drift fences (see Fig. 2). Holes cut in the sedimentation fence will allow animals to access the traps on alternating sides. Drift fences should be placed along or near ridges through potential upland pine habitat. Potential habitat characteristics and trapping timeframe should be the same as the transect survey protocol. All traps must be monitored daily, and during the hotter times of the year (*i.e.*, late spring through early fall), each trap must contain a dish of water to prevent dehydration or overheating of captured animals. While this methodology is primarily to evaluate or monitor populations of black pinesnakes, it may also be used as a tool to document presence/absence of the species. If the latter is the only objective, trapping may cease once a black pinesnake is captured; if no pinesnakes are captured after appropriately-located trap arrays have been continuously open for one full active season (March through September), then an assumption can be made that, for the purposes of Service consultations and effects' determinations, the species is absent from the site.

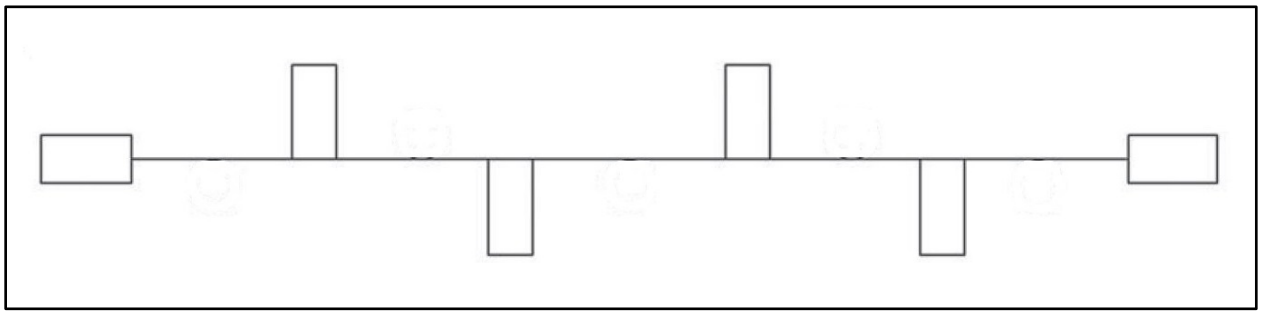

Figure 2. Drift fence design for black pinesnake trapping, including a 295 ft-long drift fence with box traps on either end and on alternating sides (see Lee 2009).

Handling, Marking, and Tagging

All personnel/field technicians must be included on current required permits for handling state- or federally-protected animals. Any snakes captured either by hand or in box traps should be individually measured (snout-to-vent length, total length, and mass), sexed, and marked via scale clipping (Brown and Parker 1976) and/or Passive Integrated Transponder (PIT) tagging prior to release.

It is possible that disease may be transmitted within and among black pinesnake populations during handling if appropriate precautions are not taken. Snake Fungal Disease (SFD) is an emerging disease in certain populations of wild snakes in the eastern and midwestern United States, and laboratory analyses have demonstrated that the fungus *Ophidiomyces ophiodiicola* is consistently associated with SFD (U.S. Geological Survey's National Wildlife Health Center (NWHC); www.nwhc.usgs.gov/disease_information/other_diseases/snake_fungal_disease.jsp). Although SFD has not been documented in black pinesnakes, it has been documented in Florida pinesnakes (*P.m. mugitus*) in Georgia (M. Moore, unpubl. data), as well as in a timber rattlesnake (*Crotalus horridus*) in Copiah County, Mississippi (N. Kennedy, unpubl. data), which is near the range of the black pinesnake.

The most consistent clinical signs of SFD include scabs or crusty scales, subcutaneous nodules, premature separation of the outermost layer of the skin from the underlying skin (or abnormal

molting), white opaque cloudiness of the eyes (not associated with molting), or localized thickening or crusting of the skin. Skin ulcers/lesions, swelling of the face, and nodules in the deeper tissues of the head have also been documented (NWHC). Clinical signs of SFD and disease severity may vary by snake species. Snakes showing severe signs of SFD shall be brought to the attention of State Agency biologists and a wildlife veterinarian; in the case of a federally-listed snake, USFWS shall also be contacted.

Examine any captured/roadkill snakes (all species) for signs of SFD; roadkill showing clinical signs should be refrigerated and sent to the Southeastern Cooperative Wildlife Disease Study (SCWDS) for fungal analysis. For sampling live snakes, the following instructions are taken from the "Snake Fungal Disease Sampling Protocol for SCWDS Submitters". Live snakes showing signs of SFD should be swabbed using sterile technique if lesions are small and the snake is otherwise healthy. Swabs should be similar to those for bacterial culture; they should come in their own sealed container that can be closed after the sample is taken. Cotton-tipped swabs can also be used and the samples stored in sterile whirlpack bags before sending them to SCWDS for diagnostics. No media should be used on the swabs. Lesions on the head and affected scales should be targeted, and if more than one site is targeted, that should be indicated on the sample (trying to maximize the area swabbed and making sure to swab any visible lesions).

As long as the animal of origin can be determined, snake sheds can also be submitted for fungal analysis. Sheds should be placed in sealed plastic bags, with the suspected lesion area circled on the bag, if possible. Scale clips of lesions may be used for testing as well, and an ideal size is approximately 0.5 x 0.5 cm or larger. The method of storing samples prior to shipment depends mostly on when shipment will take place. If the samples are to be sent the next day, keeping the samples in a refrigerator or cooler with ice is appropriate. If the samples will be sent two days later or longer, they should be frozen to at least -20 degrees Celsius, keeping in mind freezing or cooling samples for extended time can affect the test results.

All samples should be packaged in at least two layers of watertight seals. The samples should be packaged with re-freezable ice packs such as blue ice (not regular wet ice); also included in the package should be some absorbent material such as newspaper or paper towels. The contents should be placed in a regular plastic cooler or in a Styrofoam cooler inside a cardboard box, and shipped overnight delivery. Ensure that the diagnostician on duty at SCWDS is notified prior to the package being sent and the proper submission form has been completed.

Southeastern Cooperative Wildlife Disease Study
589 D.W. Brooks Drive
Wildlife Health Building
College of Veterinary Medicine
The University of Georgia
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During field work, all equipment used (hooks, tongs, snake bags, PIT tag injectors, tubes, probes, scale clipping scissors) should be disinfected before moving between sites. Disinfection protocol

recommended for *O. ophiodiicola* includes a minimum 2-minute exposure to at least 3% bleach solution (fresh bleach solution should be made weekly). Cover boards and traps should not be moved between sites unless thoroughly disinfected as well. Additionally, all snakes not being immediately returned to the field must be held in clean, secure, well-ventilated individual containers or bags, in areas without cold temperatures or overheating.

If pinesnakes are to be used for radio-telemetry, transmitter implantation procedures should follow those similar to Reinert and Cundall (1982), with some possible procedural modifications (see Hardy and Greene, 1999, 2000), by trained veterinarians and under sterile conditions. Additional surgical implantation procedures can be referenced from the "Eastern indigo snake (*Drymarchon couperi*) capturing, handling, blood and tissue sampling, marking, PIT tag implantation, and surgical protocol" (Service 2016). All Institutional Animal Care and Use Committee guidelines must be followed, if applicable. Transmitters must weigh less than 3% of each animal's body mass, and all snakes must be in good body condition prior to surgery. Prior to release after surgery, ensure that a minimum of 10 days of thermoregulatory temperatures (70 to 80º F (21 to 27º C) are predicted to ensure proper healing, post-surgery, in the wild. If these conditions are not likely post-surgery, do not implant radio transmitters.

As another alternative for identification and tracking, PIT tags may be subcutaneously injected mid-body into black pinesnakes using sterile syringes and manufacturer's guidelines. When injecting tags, keep the needle parallel to the snake's body; do not force the needle into the muscle tissue or between ribs. Post-injection, sterilize the site using rubbing alcohol and use tissue glue to seal and protect the injection site. Scan the tag to ensure it is reading correctly.

Natural History and Identification of the Black Pinesnake

Description of the Species

Pinesnakes are large, non-venomous, oviparous (egg-laying) constricting snakes with keeled scales and disproportionately small heads (Conant and Collins 1998). Their snouts are pointed and they are good burrowers. Black pinesnakes are distinguished from other pinesnakes by being dark brown to black both on the upper and lower surfaces of their bodies. There is considerable individual variation in adult coloration (Vandeventer and Young 1989), and some adults have russet-brown snouts. They may also have white scales on their throat and ventral surface (Conant and Collins 1998). In addition, there may also be a vague pattern of blotches on the end of the body approaching the tail. Adult black pinesnakes range from 48 to 76 inches long (Conant and Collins 1998; Mount 1975). Young black pinesnakes often have a blotched pattern, typical of other pinesnakes, which darkens with age. The species' defensive posture when disturbed is particularly interesting; when threatened, it throws itself into a coil, vibrates its tail rapidly, strikes repeatedly, and utters a series of loud hisses (Ernest and Barbour 1989). There are three recognized subspecies of *P. melanoleucus* distributed across the eastern United States (Crother 2012; Rodriguez-Robles and De Jesus-Escobar 2000): the northern pinesnake (*P. m. melanoleucus*); black pinesnake (*P. m. lodingi*); and Florida pinesnake (*P. m. mugitus*); however the black pinesnake is distinguished from the other two by being almost completely dark brown to black on both the upper and lower surfaces of their bodies.

Similar Species

Within the black pinesnake's range, the southern black racer (*Coluber constrictor*) is the primary species with an overlapping geographic range that may be mistaken for the black pinesnake. Although only recently re-introduced back to Alabama, and not currently known to occur in Mississippi, the eastern indigo snake (*D. couperi*) could be mistaken for a black pinesnake as well. Others may include the eastern hognose snake (*Heterodon platirhinos*), the eastern coachwhip (*Masticophis flagellum flagellum*), and to a lesser extent, various aquatic snakes (*e.g*., *Nerodia* and *Agkistrodon* spp.); however, by far the most common species for which this species may be mistaken is the black racer (see Figures 1 and 2 for photographs of black pinesnakes and similar snake species). The black racer's scales are smooth and dull black, whereas a black pinesnake has keeled scales that may appear more reflective. The black racer is a much more slender snake than the black pinesnake, and often the chin and throat of the racer are creamy white in color. Young racers are strongly patterned with a mid-dorsal row of dark gray, brown or reddish brown blotches on a gray or bluish gray ground color. Distinguishing features between black pinesnakes and similar species include scale keeling, head shape (pointed snout), overall size, and head/throat coloration and patterning.

Figure 1. Photos of black pinesnakes. Photo credits: USFWS, J. Lee (TNC), and T. Dickinson (MS Army National Guard).

Figures 2a & b. Similar species to the black pinesnake. Photos in 2a are black racers; Photos in 2b are (clockwise from top left): Eastern hognose snake; Eastern coachwhip; and Eastern indigo snake (with insert magnifying the typical head coloration). Photo credits: USFWS, J. Lee (TNC), T. Dickinson (MS Army National Guard), and D. Stevenson (Orianne Society).

Life History

Black pinesnakes are active during the day but only rarely at night. As evidenced by their pointed snout and enlarged rostral scale (the scale at the tip of their snout), they are accomplished burrowers capable of tunneling in loose soil, potentially for digging nests or excavating rodents for food (Ernst and Barbour 1989). In addition to rodents, wild black pinesnakes have been reported to eat nestling rabbits and quail (Vandeventer and Young 1989). During field studies of black pinesnakes in Mississippi, hispid cotton rats (*Sigmodon hispidus*) and cotton mice (*Peromyscus gossypinus*) were the most frequently trapped small mammals within black pinesnake home ranges (Duran and Givens 2001; Baxley 2007). These results suggest that these two species of mammals represent essential components of the snake's diet (Duran and Givens 2001).

Very little information on black pinesnake breeding and egg-laying is available from the wild. Lyman *et al*. (2007) described the time frame of mid-May through mid-June as the period when black pinesnakes breed on Camp Shelby, and Lee (2007) described copulatory behavior in a pair of black pinesnakes in late September. Mating activities may take place in or at the entrance to armadillo burrows, stump holes, or inactive gopher tortoise burrows (J. Lee, pers. comm). Based on dates when hatchling black pinesnakes have been captured, the potential nesting and egg deposition period of gravid females extends from the last week in June to the last week of August (Lyman *et al*. 2009). In 2009, a natural nest with a clutch of 6 recently hatched black pinesnake eggs was found at Camp Shelby (Lee *et al*. 2011) at the terminus of a juvenile gopher tortoise burrow. Since there is only one documented natural black pinesnake nest, it is unknown whether the species exhibits nest site fidelity; however, nest site fidelity has been described for other *Pituophis* species.

Specific information about black pinesnake underground refugia was documented during a study conducted by Rudolph *et al*. (2007) which involved excavating five sites used by the species for significant periods of time from early December through late March. The pinesnakes occurred singly at shallow depths (mean of 9.8 in; maximum of 13.8 in) in chambers formed by the decay and burning of pine stumps and roots (Rudolph *et al*. 2007). The refugia were not excavated by the snakes beyond minimal enlargement of the preexisting chambers. These sites are not considered true hibernacula because black pinesnakes move above ground on warm days throughout all months of the year (Baxley 2007; Rudolph *et al*. 2007).

Duran and Givens (2001) estimated the average size of black pinesnake home ranges (Minimum Convex Polygons) on Camp Shelby, Mississippi, to be 117.4 acres (ac) using data obtained during their radio-telemetry study. Observations made during this study also provided some evidence of territoriality in the black pinesnake. A more recent study conducted on Camp Shelby provided home range estimates from 135 to 385 ac (Lee 2014). Additional studies from the De Soto National Forest and other areas of Mississippi have documented somewhat higher MCP home range estimates, from 225 to 979 ac (Baxley and Qualls 2009).

Habitat

Black pinesnakes are endemic to the upland longleaf pine forests that once covered the southeastern United States. Optimal habitat for these snakes consists of sandy, well-drained soils with an open-canopied overstory of longleaf pine, a reduced shrub layer, and a dense herbaceous ground cover (Duran 1998a). Duran (1998b) conducted a radio-telemetry study of the black pinesnake that provided data on habitat use. Snakes in this study were usually located on well-drained, sandy-loam soils on hilltops, ridges, and toward the tops of slopes in areas dominated by longleaf pine. They were rarely found in riparian areas, hardwood forests, or closed canopy conditions. From radio-telemetry studies, it has been shown that black pinesnakes spend between 53 – 70% of their time below ground (Baxley and Qualls 2009; Duran 1998b; Yager *et al*. 2005). These locations were usually in the trunks or root channels of rotting pine stumps.

During two additional radio-telemetry studies, individual pinesnakes were observed using riparian areas, hardwood forests, and pine plantations periodically, but the majority of their time was still spent in upland longleaf pine habitat. This indicates a tolerance for some degree of patchiness and habitat heterogeneity; but in these studies they repeatedly returned to core areas in the longleaf pine uplands and used the same pine stump and associated rotted-out root system from year to year indicating considerable site fidelity (Yager, *et al*. 2006; Baxley 2007). Several radio-tracked juvenile snakes were observed using mole or other small mammal burrows rather than the bigger stump holes used by adult snakes (Lyman *et al*. 2007).

Snakes may show some seasonal movement trends of emerging from overwintering sites in February, moving to an active area from March until September, and then moving back to their overwintering areas (Yager, *et al*. 2006). The various areas utilized throughout the year may not have significantly different habitat characteristics, but these movement patterns support the need for black pinesnakes to have access to larger, unfragmented tracts of habitat to accommodate fairly large home ranges while minimizing interactions with humans.

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