Captive Propagation of the Threatened Eastern Indigo Snake for Reintroduction into Alabama

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Introduction

The historic range of the eastern indigo snake (*Drymarchon couperi*) spans south Alabama, Georgia, and Florida, encompassing longleaf pine and sandhill habitat (Godwin 2004; Moler 1992; Mount 1975; Stevenson et al. 2008); an ecotype now recognized as one of the most imperiled in the southeastern United States (Frost et al. 1986; Noss 1989; Stout and Marion 1993). Specifically within these habitats, adult indigo snakes utilize gopher tortoise burrows as nest sites, winter dens, and refugia (Stevenson et al. 2003). In both Alabama and Georgia the gopher tortoise has experienced both population declines and local extirpations due to habitat degradation (Aresco and Guyer 2004; Birkhead and Tuberville 2008). Gopher tortoise habitat loss and declines have possibly led to declines of the eastern indigo snake. In 1978 the eastern indigo snake was listed as Threatened by the U.S. Fish and Wildlife Service and a recovery plan was approved in 1982 (USFWS

1982). Reasons given for the listing included declines due to habitat degradation, fragmentation, and loss, over-collection, road fatalities, and gassing of gopher tortoise burrows (Hyslop 2007, Speake 1993).

In 1976 a captive propagation program for the purpose of indigo snake recovery was established under the direction of Dr. Dan Speake of the Alabama Cooperative Wildlife Research Unit at Auburn University; this program continued through 1987. The purpose of the program was to supplement existing populations in the Southeast that were low in eastern indigo snake numbers or restock areas that experienced extirpation. Adult snakes used for captive breeding were obtained from zoos, U.S. Fish and Wildlife Service confiscations, and by wild capture in Florida and Georgia. These snakes formed the nucleus of the captive breeding population. Offspring of this population were used in restocking and reintroduction efforts, but in some cases adults were also released to the wild. Over the period of 10 years the project housed approximately 168 adult indigo snakes, captively bred and produced approximately 300 young, and released snakes in 20 locations, nine in Alabama (Speake et al. 1987, unpublished data) (Table 1). Between 1978 and 1986, 285 juveniles and 34 adults, for a total of 318 snakes, were released at the Alabama locations (unpublished data). Of the nine Alabama release sites, two were outside the historic range of the eastern indigo snake, although during the Pleistocene the eastern indigo snake ranged further north into the Black Belt of Dallas County, as did the gopher tortoise (Dobie and Leary 1996).

In recent years the Alabama Department of Conservation and Natural Resources (ADCNR) has conducted multiple searches of the Alabama release locations (Clay 2006; 2007) with no eastern indigo snakes or evidence of snakes being found at any release site.

Sightings of presumed eastern indigo snakes are occasionally reported, and a number have been from credible sources (Hart 2002); but, to date none of the sightings have been confirmed.

Table 1. A listing of the sites on which Dr. Dan Speake attempted to establish populations of the eastern indigo snake through a captive breeding and release program.

County	Site	Individuals Released	Historic Range
Autauga	Autauga County Community Hunting Area	15 hatchlings; fall 1986	No
Baldwin	Perdido River Hunting Club	2 juveniles & 25 hatchlings; 1978 & 1979	Yes
Baldwin	Gulf State Park Preservation Area	2 juveniles & 35 hatchlings; 1978 & 1979	Yes
Bullock	Swift Plantation, Private Hunting Preserve	1 adult & 37 hatchlings; 1980, 1981, & 1982	No
Covington	Blue Springs WMA, Conecuh National Forest	50 hatchlings; 1981	Yes
Covington-Escambia	Solon Dixon Forestry Education Center	33 adults, 10 juveniles, & 2 hatchlings; 1979 to 1986	Yes
Escambia	T.R. Miller Hunting Area	3 juveniles & 39 hatchlings; 1983	Yes
Mobile	J.L. Hunting Club	50 hatchlings; 1982	Yes
Washington	Annie Jordan Trust Property	14 juveniles; 1986	Yes

Conservation Concerns

Because few places, particularly outside the Florida peninsula, currently support viable populations of the eastern indigo snake (Gunzburger and Aresco 2007), reintroduction of these snakes to areas that once harbored them is a reasonable conservation tool. Although such projects have well-known pitfalls (Dodd and Seigel 1991), no other conservation tool is as likely to place this species on a trajectory to achieve eventual delisting. The previous effort at reintroduction likely failed because

individuals were distributed at low densities, across too many sites, and by hard releases of juvenile snakes into inappropriate habitat (Speake et al.1987). Despite this discouraging outcome, a reintroduction project focused on a single site should be tried before conservation biologists eliminate reintroduction as a management option for this species. The Alabama Comprehensive Wildlife Conservation Strategy (CWCS) identified the threatened eastern indigo snake as a Priority 1 (Highest Conservation Concern) species and recommended intensive surveys of the Alabama reintroduction sites, which are listed in Hart (2002). Surveys (Clay 2006, 2007) have been completed with no sightings or other evidence of the eastern indigo snake at any reintroduction site. While promising Alabama sightings occurred in Mobile and Washington counties (Hart 2002), without substantiation the conclusion that the species has essentially been extirpated from Alabama is unavoidable. Therefore, it appears that successful reintroduction and recovery of this species in Alabama will require an intensive propagation program (Speake 1993). Preliminary data have been generated that show that a restocking effort for this species will take over 10 years and require approximately 300 individuals for a productive outcome (R. Seigel, unpublished data).

If reintroduction is to be attempted at a single site, then this site must be of adequately large acreage with diverse habitat patches as these are large snakes with correspondingly large home ranges that on an annual basis inhabit a suite of habitats. Annual home ranges of adult indigo snakes range from 77 – 126 ha for females and 481 – 538 ha for males (Hyslop 2007). Even juvenile snakes require large home ranges (4.1 – 5.4 ha; Smith 1987). Adult snakes exhibit changes in seasonal preference for habitats in winter favoring xeric upland pine and sandhill habitats containing gopher tortoise

burrows and lowland, wetter areas during summer months (Hyslop 2007). Hatchlings and yearlings occupy pineland, cypress ponds, fields, and open ponds (Smith 1987). Thus, reintroduction of this species will be a challenge because it will require a large area within a carefully managed landscape.

The Conecuh National Forest Restoration Efforts

The Conecuh National Forest is in the process of a 30-year plan to restore the landscape to a pre-settlement native ecosystem composition and structure. This plan includes a managed fire regime, thinning stands, and replacing tree species with longleaf pine where appropriate. These restoration efforts encompass studies such as herpetofaunal surveys of the national forest. Habitats throughout the national forest were evaluated and found adequate for reintroduction or restocking efforts of those species rare or recently extirpated from the area. Additionally, the Conecuh National Forest has healthy populations for the majority of the herpetofauna observed and trapping efforts report no non-native species (Guyer et al. 2007).

Study Objectives

1. Establish a breeding facility in which to rear captive eastern indigo snakes for re-establishment in Alabama.

2. Develop protocol for the successful housing, breeding, and rearing of eastern indigo snakes.

3. Identify sites appropriate for the release of captive-raised snakes.

4. Develop guidelines regarding the number of young snakes to be released and the number of multiple releases needed for the establishment of new populations.

5. Develop guidelines for monitoring of released snakes in years subsequent to the release.

Results

1. Establish a breeding facility in which to rear captive eastern indigo snakes for its re-establishment in Alabama.

A housing and rearing area was designated at Auburn University for eastern indigo snakes in the Physiology Building on the main campus. Space within the building was specifically configured to house wild-caught adults, the incubation of eggs, and rearing of young snakes. The breeding of adults and rearing of young is expected to continue at Auburn for a three-year period (see section #2 below).

In May 2008 Project Orianne Ltd., The Indigo Snake Initiative, a privately funded conservation program, came into existence. One of the goals of Project Orianne is to establish a breeding facility and once this breeding facility is operational the Auburn University facility will be closed. Until that time gravid females will be collected and used as the main source for production of juvenile indigo snakes for release into the Conecuh National Forest. After this three-year period, all eastern indigo snake breeding and rearing will be conducted at the Project Orianne base of operations and Project Orianne has agreed to supply Auburn University with juvenile and young indigo snakes for release into the Conecuh National Forest.

2. **Develop protocol for the successful housing, breeding, and rearing of eastern indigo snakes**.

Gravid Females

Initially a breeding colony of eastern indigo snakes was to be established utilizing wild caught male and female snakes from Georgia. Projections of young to be produced were based on the number of adult snakes that the state of Georgia would allow to be collected, the number of females that could be feasibly housed, and a decline of fertility in females held in captivity over multiple years. Due to permitting issues no eastern indigo snakes could be collected and held in captivity the first year.

During the second year of the project (2008) four gravid females were collected in Georgia and housed at Auburn University where they were maintained until eggs were laid. One female died prior to oviposition. The number one cause of death of female indigo snakes in captivity is egg binding, which is often a result of capture stress; this was not her cause of death. Necropsy results indicated that she died of a chronic severe colitis that was present at time of capture. She exhibited minimal clinical signs, and this condition was non-responsive to the therapies given during her period in captivity. At the time of death she was gravid, and in a late stage of her cycle, with 13 well-developed, normal eggs. For the remaining three females, once eggs were deposited they were released at the site of capture following resumption of feeding and a health assessment by the project veterinarian, Dr. Elizabeth M. Rush (Table 2).

Table 2. Date of capture, location, mass, and number of eggs laid by the eastern indigo snakes captured in Georgia.

Snake ID	Capture Date	Capture and Release Location	Mass (g)	Date of Oviposition	Number of eggs laid	Release Date	Release Location
1	29 January 2008	Ft. Stewart, Evans Co., GA	1714.6 (16 Feb)	6 May 2008	10	21 May 2008	same as capture
2	29 January 2008	Ft. Stewart, Bryan Co., GA	2459.8 (16 Feb)	13 May 2008	11	21 May 2008	same as capture
3*	1 February 2008	Sansavilla WMA, Wayne Co., GA	1815.3 (16 Feb)	NA	NA	NA	NA
4	5 March 2008	Private Property, Telfair Co., GA	1698.8 (5 Mar)	15 May 2008	7	14 July 2008	same as capture

*Died in captivity.

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Continued discussions with Georgia DNR staff, USFWS personnel, and the creation of Project Orianne prompted a reconsideration of establishing a breeding colony of eastern indigo snakes at Auburn University. With Project Orianne constructing a dedicated breeding facility and with their agreement to provide young snakes for future releases the decision to forgo a breeding colony at Auburn was made, but until Project Orianne's breeding facility is fully operational gravid female eastern indigo snakes will continue to be brought to Auburn for production of progeny. The collection of gravid females forgoes the need to house snakes, both male and female, year-round thus saving on husbandry costs and having cage space available for gravid females, space which otherwise would be devoted to males.

Adult eastern indigo snakes captured during winter months often exhibit a skin condition with sores and pustules. These female snakes were typical in that regard. To

assist with sheds, and aid in prevention of blistering (with pustual complications), snakes were treated topically with 0.9% sodium chloride irrigation, applied directly with paper towel. This was followed with either a 1% silver sulfadiazine cream or chlorhexidine antiseptic ointment, as prescribed by the project veterinarian. Medications were administered daily or every other day until the condition improved.

Eggs and Hatchlings

Twenty-eight eggs were laid in three clutches; one female died in captivity prior to egg deposition. All clutches were laid in early to mid-May and hatched in mid- to late-August (Table 3), with 26 of the eggs being viable; temperatures were held at 23°-25.5°C (74°-78°F) during the course of incubation. While the sample size is very small the observed trend is that as female size increases fecundity increases and egg inviability decreases.

Table 3. Clutch data for the initial captive cohort of eastern indigo snakes. All eggs were weighed on 15 July 2008.

Clutch ID	No. of eggs	Date of oviposition	Mean incubation temperature (C°)	Hatching date	Number hatched	Number failing to hatch	Mean mass (g)	Mean length (mm)	Mean diameter (mm)
1	10	6 May 2008	24.7	13 - 17 August 2008	10	0	72.4	64.30	46.36
2	11	13 May 2008	24.8	18 - 21 August 2008	9	2	73.7	61.50	46.55
3	7	15 May 2008	24.6	20 - 23 August 2008	6	1	79.2	77.75	41.47

Average time from hatching to first (natal) shed was 10.2 days. Growth of the snakes has been steady with an approximate 30% increase in length over a two month period (Table 4). Snakes have been offered food on a weekly basis and, as indicated by the growth pattern, most are healthy feeders. Since these individuals will be released into the wild they are being offered prey types for which they will encounter upon release. When necessary, the diet is being supplemented with frozen thawed lab mice. Food items offered thus far include crickets, fish, frogs and toads, lizards, snakes, and mice. Of the prey items offered, crickets, green tree frogs, and skinks have been refused by all snakes.

Table 4. Mean growth measurements of each clutch for mass and snout-vent length (SVL). Clutch 1 was from snake #1, clutch 2 from snake #2 and clutch 3 from snake #4. Period I was 26 August 2008, and Period II was 13 November 2008.

Clutch ID	Mean no. of days from hatch to natal shed	Mean mass (g) Period I	Mean mass (g) Period II	Mean SVL (cm) Period I	Mean SVL (cm) Period II
1	9.8	39.6	45.2	40.0	45.7
2	10.5	40.3	44.2	37.0	46.4
3	10.4	41.2	49.2	35.1	45.6

3. **Identify sites appropriate for the release of captive raised snakes.**

We have selected a primary release site in the Conecuh National Forest (Figure 1) that will serve as the first of potentially several release locations in southern Alabama for the eastern indigo snake. The Conecuh National Forest possesses several characteristics that make it preferred as the initial release site: 1) location within the historic range of the eastern indigo snake; 2) size; 3) habitat heterogeneity; 4) presence of a substantial gopher

tortoise population; 5) management directed toward restoration of a longleaf pine ecosystem; and 6) adjacency to additional lands in public ownership that share similar characteristics. While not a criterion, Conecuh National Forest is near the last reported locality for the eastern indigo snake in Alabama (Neill 1954).

The Conecuh National Forest encompasses approximately 84,000 acres (ca. 34,000 hectares) in Covington and Escambia counties and abuts the Blackwater River State Forest of Okaloosa and Santa Rosa counties, Florida. The Blackwater River State Forest encompasses an additional 206,000 acres (ca, 83,000 hectares) bringing the combined total of state and federal lands in the region to ca. 290,000 acres (ca. 117,000 hectares).

Adult and juvenile eastern indigo snakes utilize a variety of habitats on an annual basis, ranging from sandhill uplands in the fall and winter to forested bottomlands during the warmer months. The heterogeneity of habitat types within the Conecuh National Forest includes those favorable to the eastern indigo snake. Soils across the selected release site range from (upland to lowland) Troup loamy sand (0 to 5% slopes), Bonifay loamy fine sand (5 to 10% slopes), and Muckalee, Bibb, and Osier soils (0 to 2% slopes, frequently flooded) (Cotton 1989) (Figures 2 and 3). In other words, the soils of the site provide a gradient of upland well-drained sandy soils to mucky saturated soils. Thus, the site has the foundation to support the range of biotic and physical features favored by the eastern indigo snake including the presence of gopher tortoises and vegetation characteristic of longleaf uplands and, riparian belts surrounding wetlands (Figure 4).

Figure 1. ArcMap sub-meter georeferenced outline of the enclosures within the Blue Spring Wildlife Management Area on Conecuh National Forest. Nearby landmarks and gopher tortoise burrows are also shown.

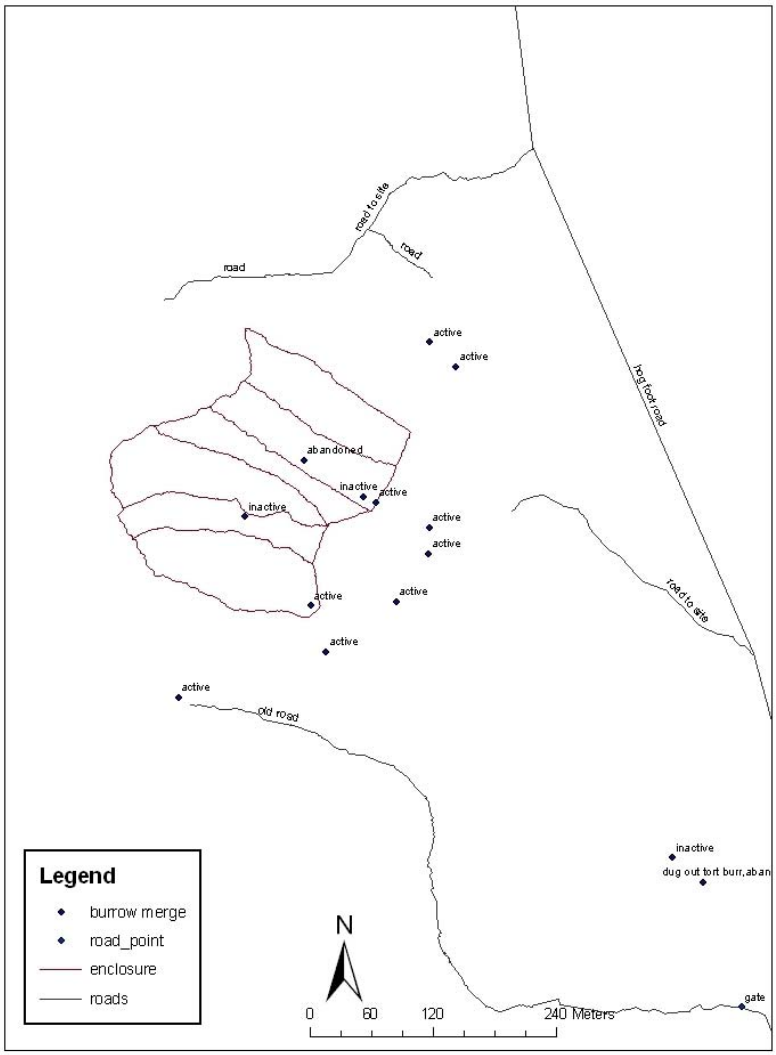


Figure 2. Orientation of the soft-release enclosures as constructed on the topography of the release site in Conecuh National Forest.

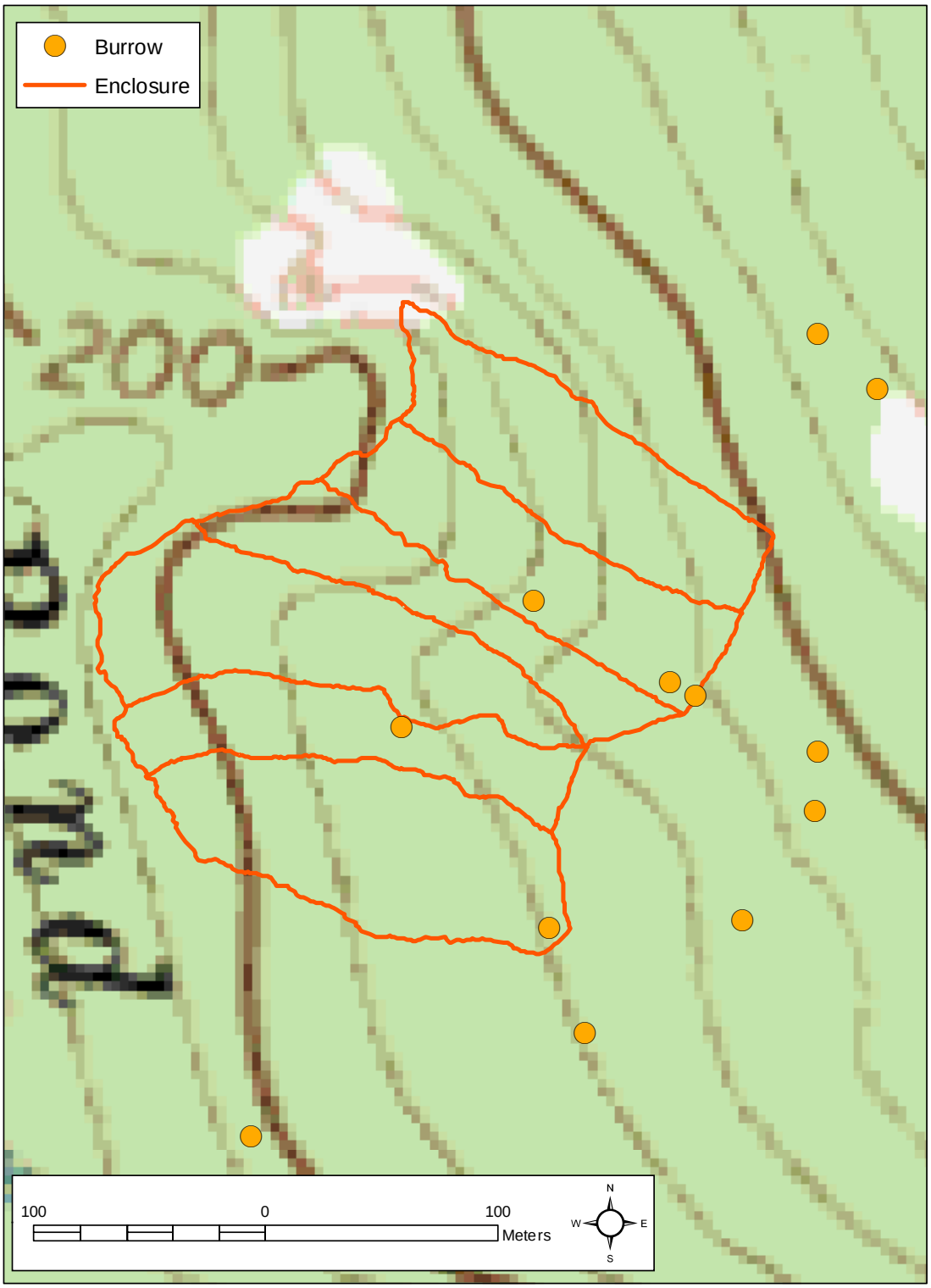


Figure 3. Map illustrating soil type diversity and distribution in the region of Conecuh National Forest where the soft-release enclosures have been constructed.

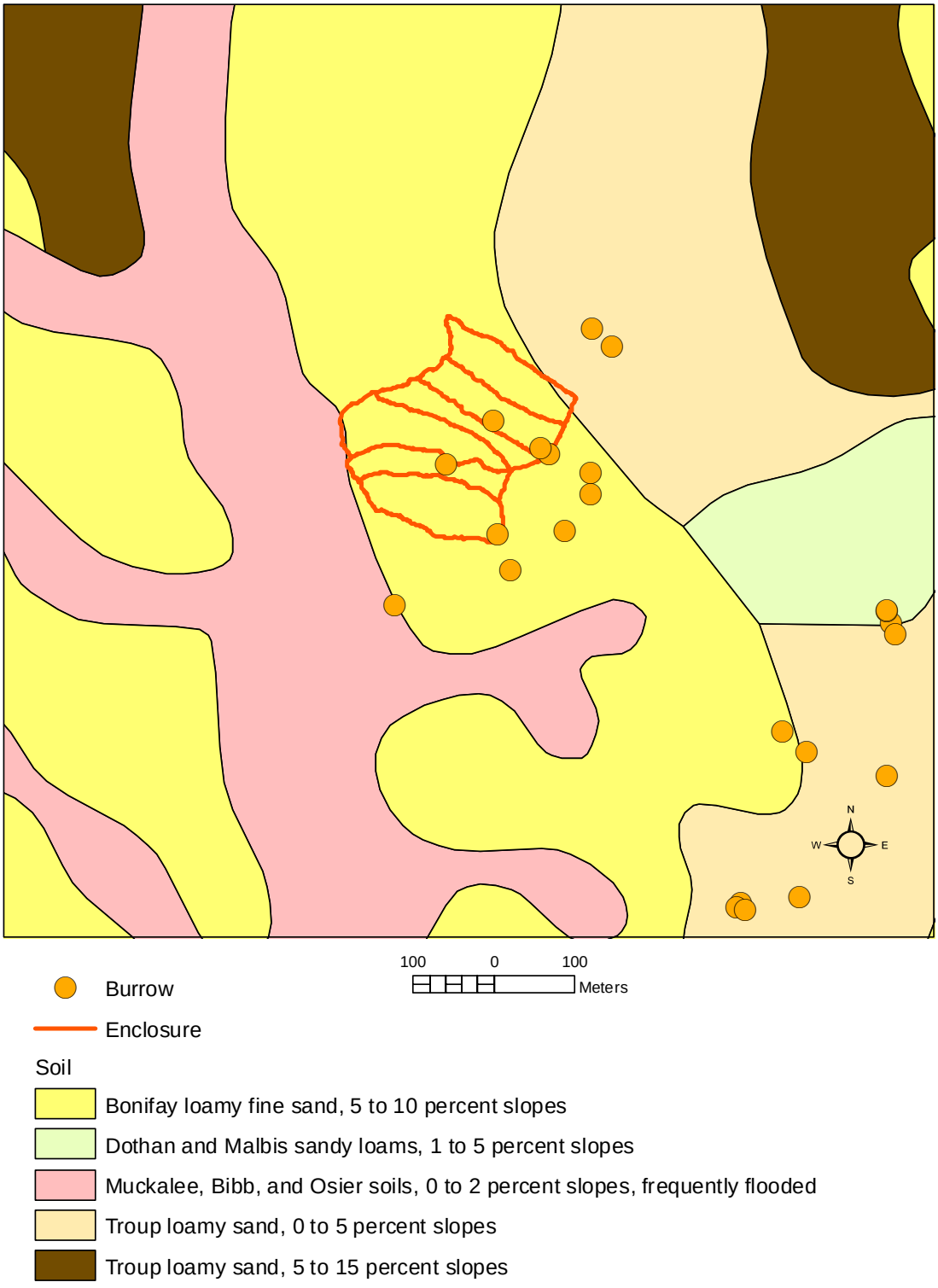
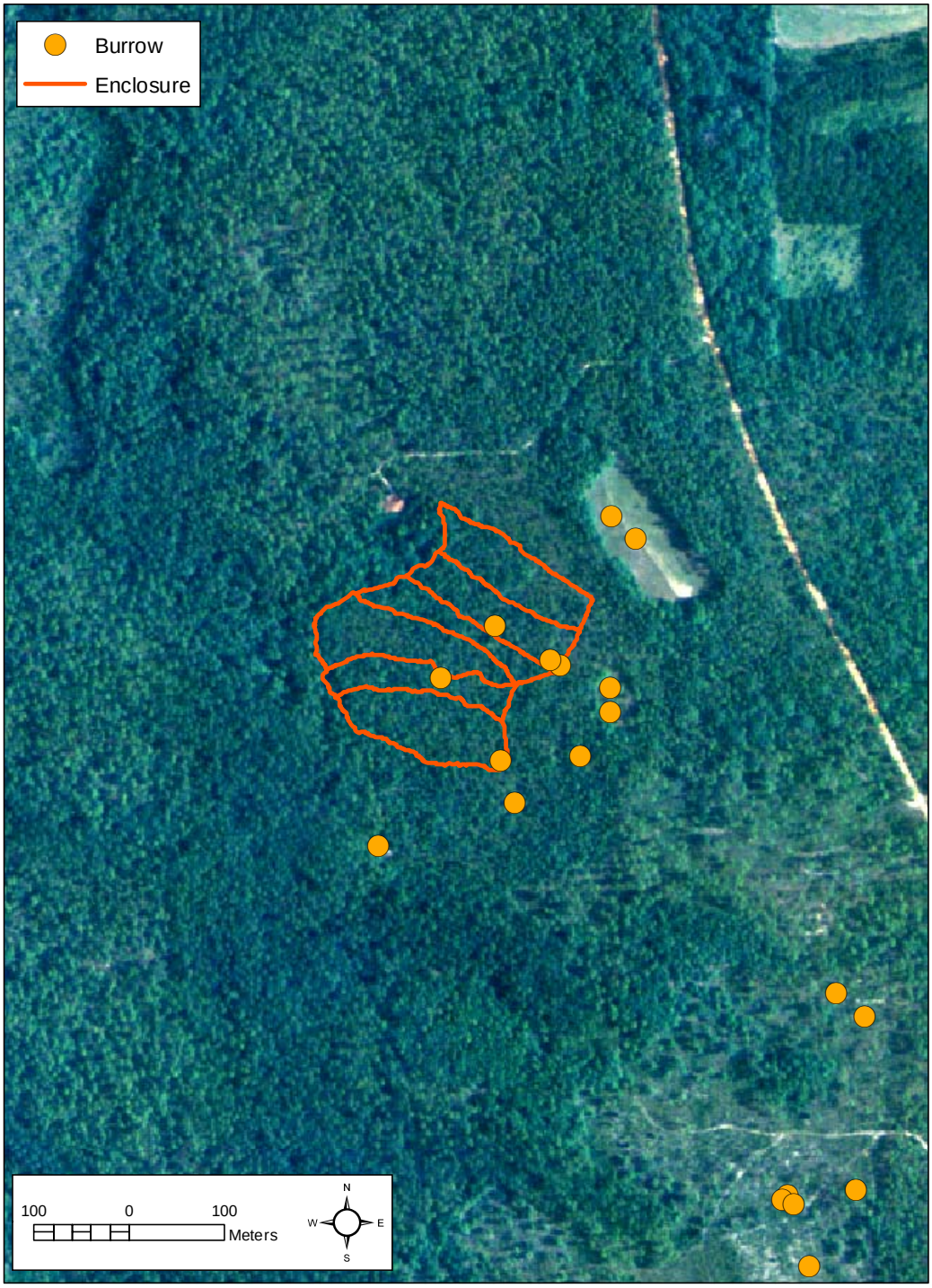


Figure 4. DOQQ aerial and soft-release enclosures overlay. This figure illustrates the vegetation contiguity and diversity in the vicinity of the release site. Riparian and upland pine/mixed pine-hardwood forest cover may be distinguished based on coloration; hardwood dominated riparian vegetation has a darker coloration.



4. **Develop guidelines regarding the number of young snakes to be released and the number of multiple releases needed for the establishment of new populations.**

Building on a model developed by Dr. Richard Siegel, data were generated to estimate the restocking effort that will be required over the next 10 years to release 300 individuals at the study site. Along with an annual influx of yearlings, additional snakes should be considered for release in years 3 – 10 to compensate for mortality of released individuals (Smith, 1987). For example, if the first two years of releases involve 30 snakes, the third years' release and thereafter should probably be increased to 60 snakes because mortality of young snakes is expected to be high. Smith (1987) radio-tracked a group of 19 captive-bred and released snakes and found that nine of the 19 (47%) succumbed to predation. Mammalian predators accounted for loss of 4 individuals, avian predators for three, one died from unknown causes, and cannibalism accounted for one. Snakes in Smith's (1987) study were hard-released; the proposed soft-release technique is intended to provide an increased level of protection for the snakes during their first year in the wild that may lower mortality rates.

Population Growth Projections

The sole purpose of captive husbandry is to facilitate the production of a sufficient number of young snakes so that a viable population of the eastern indigo snake may be established. But what is the time line for this and how many snakes may be required? To examine these questions a projection of population growth has been calculated base on a series of assumptions. As the project proceeds and data are gathered these projections will be refined.

Assumptions:

1) Hatchling to yearling snake survivorship is 61.8% (Speake and Smtih 1987), i.e. within the first year following release approximately 40% of the young snakes will have succumbed to some type of mortality.

2) Post-yearling survivorship is 89% (Speake and Smith 1987). By the time the snakes have reached two years of age they will have acclimated to their environment, experienced predator avoidance, captured prey items, and will be larger in size.

3) Sex ratio of hatchlings is 1:1.

4) Maturity for both female and male snakes is reached in four years. This point of maturity is more important for the female demographic than the male.

5) Average female reproductive output is 9.4 eggs/clutch/year with 80.7% egg viability (Speake and Smith 1987); thus, on average, a clutch produces 7.6 hatchlings.

For the first year of this project the total number of young snakes from three females was 25. During the second year, if we are able to maximize our collection with six gravid females, and average egg production is 10/clutch with an average viability of 10/clutch then the number of snakes available for the second year of release (2010) will be 60. A total of 60 hatchling snakes may be reasonably attained and thus the calculations for the remaining eight years of husbandry produced snakes is based on this figure. During the first year of release mortality is expected to be high, around 40% but after the first year the rate is expected to drop to about 10% and survivorship calculations are based on these numbers. Snakes will reach maturity by year four and females from the first release should then begin contributing young to the population at that time. By year eight offspring produced from this first reproductive cycle in the wild will be maturing and reproducing, this is represented by the row entitled “Population contribution by "wild" FF.”

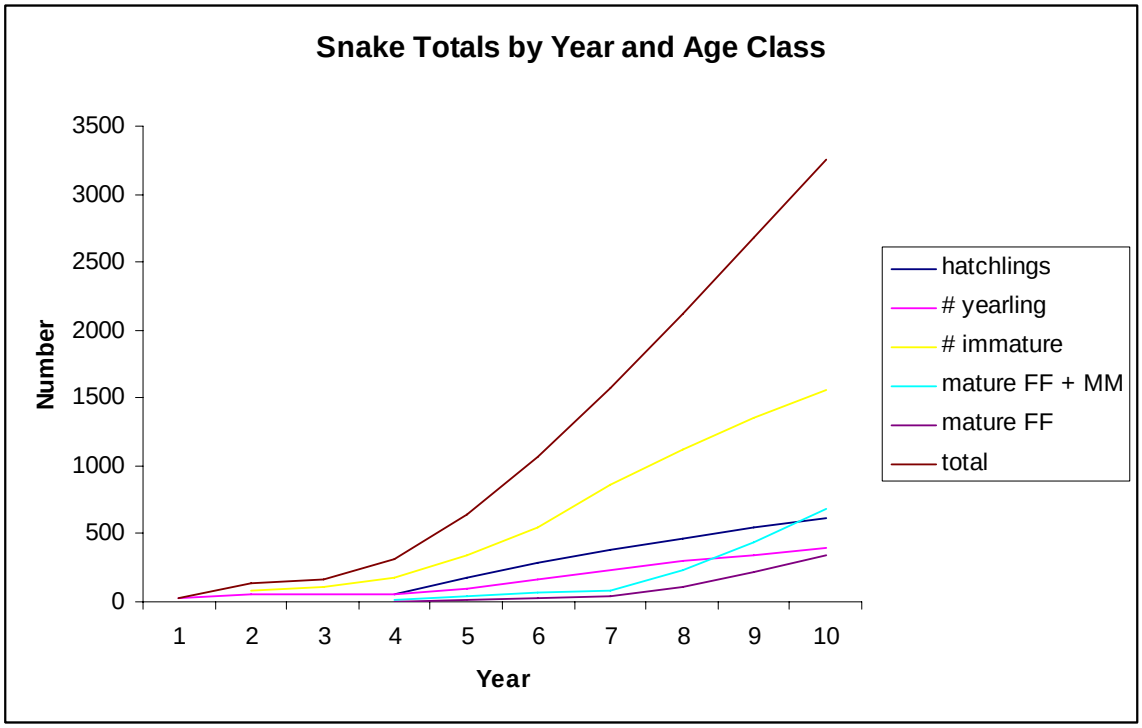
Significant population growth begins around year six and climbs steadily thereafter. In year six 32 mature females will be present and by year 10 the number will be 342 and the population, by this time, is expected to be self-sustaining (Table 3 and Figure 4). These numbers serve as a model and starting point upon which modifications can be made. Calculations are based on a static increase in the population and a static mortality rate. In reality these rates will change from year to year as determined by the myriad of ecological factors under which these snakes will be required to live. Stochastic weather events, fluctuating prey base, shifting predation dynamics, variable indigo snake densities, occupation of optimum and marginal habitats, disease and parasitism, and other aspects of the ecology and natural history of indigo snakes will affect population growth and expansion.

Table 3. Population growth predictions for the eastern indigo snake reintroduction on Conecuh National Forest based on an initial release of 25 snakes with an annual input of 60 snakes thereafter.

Indigo snake population projections beginning with Spring 2009 (year 1)										
year	1	2	3	4	5	6	7	8	9	10
Husbandry produced snakes										
# yearling	25	60	60	60	60	60	60	60	60	60
# immature		15.5	37.1	37.1	37.1	37.1	37.1	37.1	37.1	37.1
			13.8	33.0	33.0	33.0	33.0	33.0	33.0	33.0
bold = cohort maturity				**12.2**	**29.4**	**29.4**	**29.4**	**29.4**	**29.4**	**29.4**
					10.9	**26.1**	**26.1**	**26.1**	**26.1**	**26.1**
						9.7	**23.3**	**23.3**	**23.3**	**23.3**
							8.6	**20.7**	**20.7**	**20.7**
								7.7	**18.4**	**18.4**
									6.8	**16.4**
										6.1
#/year by age and class										
# immature	25	75.5	110.8	130.1	130.1	130.1	130.1	130.1	130.1	130.1
# mature FF + MM				**12.2**	**40.3**	**65.2**	**87.4**	**107.2**	**124.7**	**140.4**
# mature FF				**6.1**	**20.1**	**32.6**	**43.7**	**53.6**	**62.4**	**70.2**
Population contribution by "wild" FF										
reproductive input* (hatchlings)				53.2	175.1	283.6	380.2	466.1	542.6	610.7
# yearlings					32.9	108.2	175.3	235.0	288.1	335.3
# immature						29.3	96.3	156.0	209.1	256.4
							85.7	138.8	186.1	228.2
bold = cohort maturity								**123.6**	**165.6**	**203.1**
									147.4	**180.7**
										160.9
# immature				53.2	208.0	421.2	737.6	996.0	1226.0	1430.6
# mature FF + MM								**123.6**	**313.1**	**544.7**
# mature FF								**61.8**	**156.5**	**272.3**
snake totals by year	1	2	3	4	5	6	7	8	9	10
hatchlings				53.2	175.1	283.6	380.2	466.1	542.6	610.7
# yearling	25	60.0	60.0	60.0	92.9	168.2	235.3	295.0	348.1	395.3
# immature		75.5	110.8	183.3	338.1	551.2	867.6	1126.1	1356.0	1560.7
mature FF + MM				**12.2**	**40.3**	**65.2**	**87.4**	**230.7**	**437.8**	**685.1**
Mature FF				**6.1**	**20.1**	**32.6**	**43.7**	**115.4**	**218.9**	**342.5**
total	25	135.5	170.8	308.8	646.4	1068.3	1570.5	2117.9	2684.6	3251.9

*****Reproductive input = 9.4 eggs/clutch/female/year with 80.7% egg viability

Figure 4. Population growth curve using the results presented in Table 3.



5. **Develop guidelines for monitoring of released snakes in years subsequent to the release.**

These guidelines will be developed as data are collected and results are generated from using the experimental enclosures in the Conecuh National Forest at the first release site. Telemetry data will be gathered using a subset of released juvenile indigo snakes beginning in spring 2009. The survivability of the subset of snakes released into the enclosures will be compared to the subset of snakes released into nearby unenclosed areas of the release site. Using these data, decisions on subsequent releases will be made.

Future

This study may best be viewed as Phase I of a larger endeavor extending past geographical, governmental, and organizational boundaries. Phase II is underway which is being funded by a second State Wildlife Grant, with a generous cash match from Project Orianne. Phase II will see the implementation of the efforts of Phase I with the first snake releases scheduled for Spring 2009. This comprehensive endeavor of collecting gravid snakes, captive rearing of young, and their subsequent release is a collaborative effort bringing together the Alabama Department of Conservation and Natural Resources, Auburn University, Project Orianne, the U.S. Forest Service, and the U.S. Fish and Wildlife Service. During Phase II three snake releases are planned, one during each spring of the project period (2009-2011). These snakes will be the progeny of wild caught gravid females brought into the lab for oviposition following the techniques utilized during Phase I. From year-to-year as the releases progress and

information on released snakes are accumulated monitoring guidelines (item #5 above) will be developed and revised.

Once Phase II is completed snakes will be needed for an estimated six more years in order to meet the projected snake numbers for the proposed 10-year release period. Project Orianne is planning on establishing a breeding facility and has agreed to provide young snakes for the Conecuh National Forest reintroduction needs, post-Phase II.

Nine terrestrial snake species, including the eastern indigo snake, were assigned High (2) or Highest (1) conservation priority during the symposium held on Alabama Wildlife (Mirarchi 2004). The eastern indigo snake is a reptile of Highest Conservation Concern (Priority 1). The other species are the southern hognose (1), black pine snake (1), Florida pine snake (2), northern pine snake (2), prairie kingsnake (2), eastern kingsnake (2), speckled kingsnake (2), and eastern diamondback rattlesnake (2). Protocol, methodologies, and techniques developed during this project are expected to have transference and utility in the conservation of these species. Modifications and adjustments will undoubtedly be needed on a species by species basis, but in general, where conservation needs dictate population supplementation or reintroduction the framework will be available.

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