FINAL PERFORMANCE REPORT
South Carolina ProjectT-26-T, Segment 1

South Carolina Endangered Species Program
South Carolina Department of Natural Resources
October 1, 2007 – September 30, 2011

Project 1: South Carolina Reptile and Amphibian Conservation Planning

Job 1. Gopher Tortoise Habitat and Population Management.

Objective 1. Develop a population management plan for the Gopher Tortoise at the Tillman Sand Ridge.

Accomplishments:
Staff and partners completed development of A Conservation Strategy for the Gopher Tortoise in South Carolina. This document addresses the conservation needs of the specie in South Carolina with a focus on SCDNR properties that support populations of the Gopher Tortoise. The document was appended to the FY10 Interim Performance Report.

Significant Deviations: None

Objective 2. Develop a plan to re-stock (translocate) tortoises from privately owned property, on the ridge, to the newly acquired Goethe tract of the Tillman Sandridge Heritage preserve.

Accomplishments:
Based on the success of our translocation and re-stocking project at the Aiken Gopher Tortoise Heritage Preserve we will use these same protocols at the Goethe Tract. The details of this project are documented in the previous interim reports and summarized below for the Final Performance Report. The protocols for Gopher Tortoise translocation are also documented in Tuberville et al, 2005, Translocation as a conservation tool: Site fidelity and movement of repatriated Gopher Tortoises, (*Gopherus polyphemus*) (see Appendix 1-1). There is no time frame for this re-stocking project due both to budgetary issues and the availability of tortoises for re-stocking. This is addressed in the Gopher Tortoise Conservation Plan submitted with last year's Interim Performance Report.

Significant Deviations: None

Objective 3. Develop a plan to recover and enhance the Gopher Tortoise population at Aiken Gopher Tortoise Heritage Preserve in Aiken County to include the re-stocking of tortoises from the surrounding habitat and from other sites in South Carolina.

Accomplishments:
From October 1, 2009 to September 30, 2010, thirteen tortoises from Pen 1 were radio-tracked at the Aiken Gopher Tortoise Natural Heritage Preserve (AGTHP). Tortoises were tracked weekly during March-October, bi-monthly in November and December, and monthly in January and February. During that time period, all individuals made movements, with some moving greater distances than

others. Additionally, thirteen new burrows were created outside of the original penned area by 6 of the tortoises. All but one tortoise (F3) remained within the property boundaries of the AGTHP. Between August 25 and September 22, female #3 covered a distance of 3.9 linear km, before she was found >2 km north of Pen 1 on the bank of the Edisto River. At this point female #3 was transported back to her longtime burrow within the penned area and considered a failed attempt for tortoise relocation. The tortoises tracked to date have all been waif (unknown origin) animals that were maintained in Pen 1. Currently, there are 6 tortoises within the footprint of the original penned area (Pen 1) and 11 tortoises are within 400 meters. All 13 tortoises are within 750 m of the original penned area. Additional radios have been purchased by SCDNR for use on tortoises in Pen 3 (SC origin). These tortoises will be released during the 2011 field season.

Figure 1. Radio tracking translocated gopher tortoises at the AGTHP in 2010 (left). All locations are recorded with GPS units to facilitate creation of home ranges in Arc GIS (right).

Predator Issues

Domesticated and/or feral dogs are becoming a problem for the tortoises on the AGTHP.. Although no tortoise mortality has been documented on the preserve as a result of interactions, canines are responsible for damaging over 75% of the burrows from the pen 1 tortoises, and destroying at least 2 radio transmitters. The radios that were destroyed had obvious puncture holes and in one case the radio was completely removed from the tortoise's shell where it was anchored with epoxy and wire. The problem is not only the costs of replacing transmitters ($600 in this case), but also affects the health of the tortoises. The individuals harassed by canines had teeth marks on their shell where the dogs had clearly gnawed on them. Although most adult tortoises may be able to withstand this harassment, juveniles and hatchlings, which are smaller and have thinner shells, would not be able to withstand multiple bites and gnawing to their shells. In addition, as a result of their smaller size, juveniles may be more likely to be injured, eaten or carried off the preserve. Clearly, there are human neighbor issues when dealing with trespassing domesticated and/or feral dogs on a large, public piece of land, however these are problems that need to be understood and addressed for the future of the tortoise population.

Figure 2. One of the radio-tracked tortoises with damage to its radio from dog bites, but the radio was still attached and the tortoise seemed fine (left). A radio recovered by the burrow of tortoise #100 after dogs ripped the radio off of the tortoise and ruined the radio; the tortoise is OK (right).

Figure 3. Fresh dog diggings at one of the AGTHP tortoise burrows (left). A dog captured on hidden wildlife camera investigating one of the tortoise burrows at the AGTHP, July 2010 (right).

Reproduction

On August 25, 2010, we excavated burrow aprons on the AGTHP for nests. We found a total of 6 clutches of eggs. Four clutches were from previous years that had not hatched but 2 clutches were from this year, one from the Pen 1 population (waifs) and one from Pen 2 (Native AGTHP tortoises). The clutch from Pen 2 contained 6 eggs and yielded 4 viable hatchlings. These four hatchlings represent the first known recruitment by the native AGTHP tortoises since the onset of this project. The clutch from Pen 1 contained 9 eggs of which 7 hatchlings were emerging from the nest when we discovered it . Eggs and hatchlings were brought to the SREL where hatchlings were measured, weighed, marked and had blood sample taken for future genetic work. Eleven hatchlings were transported back from the SREL to AGTHP to become part of the population there.

Figure 4. Excavating tortoise aprons and discovering nests on the AGTHP in 2010 (left). Hatchling gopher tortoises being readied for release back at the AGTHP (right).

In addition to the hatchlings on the preserve, 9 hatchlings were obtained from a long-term captive pair of tortoises from the Town of Hilda, SC (courtesy of Ms. Betty Everett). Thus, a total of 20 hatchling tortoises were added to the AGTHP population in 2010. Two dog pens were constructed at the Preserve to provide protection for the 20 hatchlings; one was built inside Pen 4 and one inside Pen 2. Twenty artificial starter burrows were constructed in each dog pen for the hatchlings which were randomly placed in the two pens and released on 27 October 2010.

Figure 5. We built a second dog pen for hatchlings in 2010. A total of 20 hatchlings are being head-started in the two dog pens in 2010-11. Multiple starter burrows were made in each dog pen for the hatchlings. Burrows were made by driving a 3 ft long (2.0 inch diameter) PVC pipe into the ground at a 30 degree angle with a post pounder.

Translocations of Adult and Juvenile Tortoises

In addition to the hatchlings obtained from Hilda, SC, three new tortoises were added to the preserve this year: One juvenile tortoise (approximately 6 years of age) from Florida was provided by the South Carolina Department of Natural Resources and two adult male tortoises were acquired from the Georgia Department of Natural Resources. Due to its small size, the juvenile was released with the hatchlings in the dog pen located in Pen 2, while the adult males were released in an unoccupied pen on the preserve. All individuals were measured, weighed, marked and had blood taken before release.

+Figure 7. Two old male tortoises acquired from GA DNR during 2010 for the AGTHP project.

Radio-tracking and Site Fidelity of Release Group 1

During 2011, 13 released gopher tortoises were radio-tracked bi-weekly to determine their site fidelity, movements, and home range sizes on the Aiken Gopher Tortoise Natural Heritage Preserve (AGTHP). These Group 1 tortoises were all of waif origin and had been maintained in a 2.5 acre pen on the AGTHP for 2 years prior to their release in 2009. Thus, these tortoises have been radio-tracked for 2 years post-release. All 13 tortoises were alive at the end of the 2011 activity season and all have remained on the AGTHP property. All radios are due for replacement in 2012. A manuscript reporting on the site fidelity and survival of this translocated group of waif tortoises is in draft and will be submitted to a journal for publication in 2012.

Penning of Groups 2, 3, and 4.

Tortoises in Group 2 represent animals whose origin was the AGTHP or the immediate surrounding area of Aiken County. These animals have also been penned (similar to Group 1 above) and will be released and radio-tracked in spring 2012.
Group 3 animals are from multiple South Carolina sites; all from private property. They will also be released and radio-tracked in Spring 2012.

Groups 4 and 5 consist of unknown waif animals that have been obtained in 2010 and 2011. Five additional animals were added to Group 4 in 2011. These animals will remained penned in 2012, but will be released in 2013.

Reproduction

Researchers searched for gopher tortoise nests in the aprons of female tortoises from Groups 1, 2, and 3 in September of 2011. Two nests were found, both from Group 1 females. One of those clutches did not hatch (0 of 4 eggs). However, 7 of 9 eggs hatched from the second clutch. Those seven hatchlings are been head-started and over-wintered at the Savannah River Ecology Laboratory and will be released onto the AGTHP in late spring 2012. A paper reporting nest guarding by a gopher tortoise from the AGTHP is in press (Grosse et al, Chelonian Conservation and Biology). (Appendix 1-2)

Agreement with Florida Fish and Wildlife Conservation Commission (FFWCC) for Waif Tortoises

Researchers Tuberville and Buhlmann reported on gopher tortoise translocation and head-starting methodologies at the annual Gopher Tortoise Council (GTC) meeting, held 15-16 October 2011 in Orlando, Florida. Following, a conference call was held in November 2011 among Florida FWCC personnel (Deborah Burr, Joan Berish), SCDNR (Brett Moule), and SREL (Tuberville and Buhlmann). Florida and South Carolina are drawing up an MOU that will allow for the transport to SC of waif tortoises of Florida origin that are randomly given to Florida biologists as rescues. These animals will become designated for the AGTHP reintroduction and augmentation project. It is expected that this agreement will near approval at the end to 2011 or early in 2012. Currently it is our understanding that there are 7 hatchlings/yearlings and 13 other tortoises of mixed ages available.

In summary, we continue to build the gopher tortoises at the AGTHP with both new animals and natural reproduction by the current stock. Habitat management commitments by SCDNR continue with additional acreage prescribe burned in 2011 and herbicide treatments scheduled for 2012. Additional animals have and are being made available to us from cooperating state agencies (Georgia DNR, Florida FWCC). Thus, we are strongly encouraged that the project continues to move towards the goal of a viable, self-sustaining gopher tortoise population at the Aiken Gopher Tortoise Heritage Preserve.

Significant Deviations: None

Objective 4. Determine the population structure, size, density and distribution, mortality, survivorship, and reproductive potential for tortoises on the Tillman Sand Ridge. Determine the limiting factors to population growth for this meta-population and analyze population genetics to determine paternity and kinship at this site. Determine activity and movement patterns, home range and habitat use, for the Gopher Tortoise's population at this site. This is a continuation and expansion of an existing SWG project.

Accomplishments:

Home Range & Burrow Use

Methods

In 2003, 8 adult tortoises (4 males, 4 females) at TSR were fitted with radio-transmitters and radio-tracked each activity season during 2003 – 2005, unless otherwise noted. In 2006, radio-transmitters were replaced on 3 males and 3 females tracked in previous years at TSR, and radios were also placed on additional 2 males and 5 females not previously tracked. A total of 5 males and 8 females were tracked at TSR during the activity seasons of 2006-2008. Thus, individuals included in the radio-telemetry study were tracked 3-6 years. At PSA, 4 males and 4 females were fitted with transmitters and radio-tracked from 2003-2005. In 2006, radio-transmitters were replaced on 2 males and 3 females, and radios were placed on an additional 4 males and 3 females not previously tracked at PSA. A total of 12 tortoises (6 males, 6 females) were radio-tracked at PSA from 2006-2008 unless otherwise noted.

Gopher Tortoises were radio-tracked 2-3 times per week during the activity season and periodically during the winter when surface activity and movement is uncommon. Locations were recorded using a Global Positioning System with sub-meter accuracy and mapped using a Geographic Information System (GIS). Minimum convex polygons (MCP) were calculated for each individual for each year tracked, except when fewer than three unique locations (i.e., burrows) were documented. Calculations were made in ArcView 3.3 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) using the Animal Movement Extension (Hooge and Eichenlaub 1997).

Results and Discussion

There was much variation among individuals in their home range size, which also varied from year to year for a single individual. However, several general patterns emerged. First, at both sites, males had significantly larger home ranges (typically <2 ha) than females (typically <1 ha), as has been observed in previous studies. Surprisingly, tortoises at TSR had larger home ranges than tortoises from PSA. The mean male home range (averaged over the 6 year study) at TSR was 11.389 ha compared to 2.691 ha at PSA; mean female home range at TSR was 1.741 ha compared to 0.249 ha at PSA. Previous studies have suggested that home range size will vary as a function of habitat quality, with tortoises in poorer quality habitat having to travel greater distances to presumably meet their nutritional needs or to interact with other individuals in the population (citations). However, Eubanks et al. radio-tracked 102 Gopher Tortoises in high-quality old growth longleaf pine forest and observed home ranges much larger than previously reported. Their explanation for this pattern was that tortoises in other studies, even though in presumably suitable habitat, may have been artificially confined to a small area due to small patch size and the poor quality of surrounding habitat. Our observation of smaller home ranges at PSA, which consists of poor quality, fragmented habitat, compared to TSR, which consists of high quality contiguous habitat, is in keeping with their explanation. Finally, although most individuals in most years conformed to the general patterns above, the one notable exception was TSR male #24 in 2005, which had a home range size of 13.381 ha. Presumably, this male made more frequent, longer distance movements in order to increase his encounter rate and thus mating opportunities with females. In 2005, male #24 used 9 burrows compared to 2 burrows used in 2003 and 3 burrows used in 2004 (Table 1-1). Overall burrow use patterns mirrored the general trends observed with home ranges. Female tortoises at TSR used an average of 8.0 burrows per year, males used 13.4 burrows per year; PSA females used an average of 5.3 burrows per year, males 8.6 burrows per year.

Habitat Selection

Methods

Vegetation data were collected at both randomly-selected plots (hereafter, called “random plots”) and at selected tortoise burrows at TSR and PSA during September-October 2004. Trees were sampled using the point-quarter method. The following data were recorded for the nearest live tree (>2.5 cm dbh) in each quadrant: distance (m), diameter at breast height (dbh in cm), and tree type (pine, oak, other hardwood). In addition, percent canopy cover was estimated in each quadrant using a spherical crown densiometer and averaged over the four quadrants. Percent shrub cover was estimated in 2m x 2m plots using the Braun-Blanquet cover score scale (absent, <5%, 5-25%, 25-50%, 50-75%, 75-

100%). Wiregrass, other grass, legumes, other forbs were similarly quantified in 1m x 1m herbaceous plots. Herbaceous plots were nested within shrub plots.

Potential locations of random plots were generated in ArcView 3.3 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) using the RANDOM POINTS Extension. Random plots were a minimum of 50 m from each other and at least 10 m from a road or tortoise burrow. The target sampling intensity was 1 random plot per 1-2 ha of study area (i.e., the forest stand or stands in which tortoises were radio-tracked). Burrows were selected from the list of burrows known to be used on at least 3 occasions by the animals radio-tracked based on 2003 and 2004 telemetry data. Burrow plots were placed 1 m behind the burrow entrance to minimize potential impacts (e.g., soil disturbance, feeding) that tortoises might have on the vegetation surrounding their burrows (Boglioli et al. 2000).

Vegetation data were analyzed by comparing mean ranks among sites using Kruskal-Wallis and by comparing mean ranks between random plots and burrows using Wilcoxon rank sum test. Prior to analyses, cover class scores were converted to percentages using the midpoint of the corresponding cover class. Alpha was set at 0.05 for all statistical procedures.

These data and analyses were conducted as part of a collaborative study that included two additional study sites with differing management prescriptions for Gopher Tortoises. Detailed results can be found in the Tuberville et al. 2009 report which is included as an appendix at the end of this report. Only the main findings and trends regarding TSR and PSA are presented here.

Results

- Percent canopy cover at TSR was significantly lower (38.4%) than at PSA, which had canopy cover value of approx. 55%. At PSA, tortoises selected burrow sites with significantly lower canopy cover than the average available.
- Tree density was significantly higher at PSA than TSR and tortoises at PSA selected burrow sites with significantly lower tree density than the average available.
- Compared to TSR, PSA had significantly less herbaceous vegetation, including wiregrass and legumes.
- Percent cover of wiregrass at tortoise-selected burrows was similar to the amount available in the surrounding habitat. However, lugume cover was significantly higher at tortoises selected burrows when compared to the surrounding available habitat.
- Vegetation at tortoise-selected burrows was similar between TSR and PSA despite the available habitat being markedly different between the two sites.

Discussion

Gopher Tortoises generally selected burrow locations with 30-50% canopy cover, approximately 20% shrub cover, and lower basal area (total, pine, and oak) than in surrounding available habitat. Although it can be an important food item when other forage is not available (such as in the early growing season; Garner and Landers 1981), wiregrass abundance at burrows varied with management prescription but generally reflected its availability in the surrounding habitat. Under management prescriptions in which availability was limited, legumes—also considered to be an important food item (Garner and Landers 1981)—were significantly more abundant at burrow sites than in surrounding available habitat.

Habitat selection may have been limited by the range of some habitat variables available for them to choose from (e.g., total herbaceous cover, tree density, etc). In addition, we could not control for—or even understand—the potential legacy effects of previous land use and land management history. However, the fact that tortoises consistently selected sites with certain structural features (especially canopy measures) at these two study sites with vastly different habitat quality, suggests that specific management targets for tortoises can be developed.

Isotopic Analysis of Diet

Wiregrass (*Aristida stricta / A. beyrichiana*) was historically a dominant species in the long-leaf pine forests of the southeastern U.S. and is suspected to have played a key role in maintaining the ecological community by facilitating the spread of lightening strike fires. The species may also be an important structural component, providing cover for secretive, cryptic species resident to the longleaf community, and can also serve as a major food item for herbivores. However, due to intensive soil disturbance associated with agriculture and timber production, wiregass has been eliminated or severely reduced in many stands of longleaf pine.

Gopher Tortoises are generalist herbivores, and can consume a wide array of low-growing, herbaceous plants and shrubs. Previous studies have noted the abundance of wiregrass in the Gopher Tortoise diet and a few have even suggested that wiregrass may be a preferred forage species. Other researchers have suggested that wiregrass is *not preferred* but will be consumed when other species are not available or are in low supply, in order to keep gut contents moving. Indeed, wiregrass may not have a high nutritive value but may instead function as a digestive aid. Thus, there appear to be discrepancies among wildlife managers regarding the importance of wiregrass to Gopher Tortoises.

We investigated the diet of Gopher Tortoises in marginal (PSA) and optimal (TSR) habitats by characterizing the isotopic signatures of tortoise fecal pellets and comparing them to the isotopic composition of available food types (wiregrass [narrowleaf grass or NLG], broadleaf grasses [BLG], and herbs [H]). Wiregrass is a C4 plant, whereas most herbs and many of the broadleaf grasses are C3, suggesting that wiregrass can potentially be discriminated isotopically from many of the other available forage species. Our primary goal was to evaluate the relative proportion of the diet that wiregrass comprises in diets of Gopher Tortoises occupying optimal habitat, which presumably supports a high diversity of potential forage plants, versus marginal habitats, where potential food items and their diversity may be limited.

Methods

Tortoise fecal samples were collected from TSR & PSA in 2007 and 2008 (as well as a small number of samples from AGTHP and Grays in 2007) by searching aprons and areas around occupied burrows. All samples were placed in plastic storage bags and labeled with site, date, closest burrow and tortoise ID, if known. All samples were immediately frozen. Potential food plants were collected from both random sites and from burrow sites where fecal samples were collected. Six sampling sites were randomly generated for each study site using GIS. At these random points, we created sampling quadrats by circumscribing a 2.5 m diameter circle, for an approximate plot size of 20 m^2. Within each random plot, we collected small samples of all potential tortoise food plants, including narrow-leaf grasses, broadleaf grasses, and herbaceous forbs. Above-ground portions of plants were placed in plastic storage bags, labeled with study site, sample site, date and sample type. All samples were immediately frozen. Finally, we also collected potential food plants at each of the burrows where a tortoise fecal sample was collected using methods described for the randomly placed sampling plots.

Fecal samples and plant materials were submitted to the Savannah River Ecology Laboratory's (UGA) Isotope Laboratory and analyzed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$.

Results

In 2007, fecal samples were collected from 10 burrows at PSA and 7 burrows at TSR (Table X). In 2008, fecal samples were collected from 14 burrows at PSA and 6 burrows at TSR (Table X). In addition, 52 plant samples were collected in 2007 and 39 in 2008 from those same burrows, for a total of 128 fecal and plant samples. The results of the 2007 isotopic analysis are graphically depicted in Figure X and the combined 2007 and 2008 results are shown in Figure X.

The different plant groups sampled (BLG, NLG, and herbs) had fairly distinct $^{13}C/^{12}C$ ratio values despite a wide range in values for the broadleaf grasses, as illustrated by the separation among the groups along the horizontal axis in Figures X and X. The fecal samples also varied widely in their $^{13}C/^{12}C$ ratio values but did not overlap at all with the narrowleaf grasses. This pattern could be explained by a diverse diet comprised of numerous herbaceous plants and broadleaf grasses (e.g., *Poaceae, Andropogon*) but containing little wiregrass. However, these results should be interpreted cautiously, as noted in the discussion that follows. The $^{15}N/^{14}N$ values could not be distinguished among the plant groups, although the fecal samples had slightly higher values than the potential diet components, as would be expected between consumers and the foods they consume due to isotopic enrichment. Sample sizes were too small to evaluate differences in isotopic signatures of fecal or plant samples between the two primary study sites.

Discussion

Based solely on inspection of the isotopic values from our samples, one might conclude that Gopher Tortoises at both TSR and PSA consume a wide variety of plant species but preferentially consume broadleaf grass and herbaceous species and avoid wiregrass, even at PSA where wiregrass is one of the few forage species available. However, one of the major hindrances to interpreting our isotopic results is uncertainty regarding how well fecal samples reflect dietary choice. Fecal pellets are comprised of the items that are consumed and passed through a tortoise's digestive tract without being digested and/or assimilated. The number species or proportion of items that are completely assimilated and thus go undetected in fecal samples cannot be determined. In addition, digestion rates likely vary among plant species such that consumed items are not represented equally in fecal pellets. Although preliminary field and laboratory studies of mammalian herbivores suggest that fecal analysis may provide a reasonable representation of their diet (Botha and Stock 2005, and references therein), similar work has not been performed with any reptile species. Without corresponding foraging data or from experimental feeding trials (Romanek et al. 2000) it is difficult to evaluate the management significance of our isotope data. In addition, samples from the different years were collected over a broad time period during which both diet and plant quality may vary, introducing additional variability and uncertainty into our results.

However, if fecal composition does reasonably reflect dietary composition, then several our data would provide supporting evidence that Gopher Tortoises at our study sites: a) most likely have diverse diets comprised of numerous taxa; b) may consume less wiregrass relative to herbaceous species and/or broadleaf grasses; and c) and potentially consume less wiregrass than expected based on its availability in the habitat. Previous studies have documented a large number of taxa in diet of Gopher Tortoises based on fecal analysis and foraging observations (70 taxa, Birkhead et al. 2005; 68 taxa, MacDonald and Mushinsky 1988). MacDonald and Mushinsky (1988) found that fecal pellets provided a good indication of overall diet based on a comparison of species documented during foraging observations and represented in their feces, although relative abundance in diet was not assessed. Broadleaf grasses are one of the most commonly documented taxa and are consumed throughout the year, but some taxa such as forbs may be underrepresented in feces due to digestion of identifiable plant parts, making it difficult to quantify preference or avoidance of particular taxa (Birkhead et al. 2005).

Information regarding the importance of wiregrass in the diet of Gopher Tortoises is still conflicting. MacDonald and Mushinsky (1988) noted the prevalence wiregrass in adult fecal samples and determined that wiregrass was consumed by adults in the same relative proportion as its availability. In a previous study at TSR (one of our study sites), Wright (1982) proposed that the bulk of tortoises' diet was comprised of wiregrass. However, Garner and Landers (1981) observed that little wiregrass was consumed by tortoises in southwest Georgia when other forbs were available. In a study of juvenile foraging ecology, Mushinsky et al. (2003) discovered that although wiregrass was the single most frequently encountered plant taxa along juvenile foraging routes it was the only common taxa that was consistently avoided by juveniles during foraging observations. We know from our habitat analyses that wiregrass abundance at Gopher Tortoises at both study sites generally reflects its abundance in the habitat, although wiregrass is much more abundant at TSR than PSA (>30% cover vs. ≤5% cover; Tuberville et al. 2007). Further study is required to elucidate the relative importance of wiregrass in the diet of Gopher Tortoises at our study sites in South Carolina and what effects habitat quality and wiregrass abundance might have on their ecology.

Population biology

Methods

At both study sites, live wire traps were placed at the entrances of active burrows and shaded with burlap or natural vegetation to prevent overheating of tortoises. Traps were checked at least once or twice daily, depending on maximum air temperatures. Inn addition, tortoises were captured incidentally by hand or when found crossing roads. Captured individuals were permanently marked by filing or drilling a unique combination of marginal scutes. Mature individuals were sexed based on plastral concavity and length of gular scute. On initial and each subsequent capture, carapace length (CL) was measured to nearest mm and weight was recorded to the nearest 1 g. Whole blood was collected from all captured tortoises, mixed with lysis buffer and stored at -80 C for subsequent genetic analysis using five species-specific microsatellite markers (Tuberville et al. 2011).

Results: Reproduction

Clutch sizes based on either xradiography or excavation of field-collected nests, which were obtained for seven PSA nests and 20 TSR nests. The smallest gravid female documented through xradiography at PSA was 295 mm CL and at TSR was 277 mm CL, although sizes of females were not known for field-collected nests. Mean clutch size at PSA was 5.85 eggs (range: 2-9 eggs) and was 5.55 eggs at TSR (range: 2-9 eggs). From these clutches, a total of 23 hatchlings were released at PSA and 41 hatchlings at TSR, not including hatchlings that escaped protective nest cages.

Results: Demography

Both populations were skewed towards larger, presumably older adults with few immatures captured (Figure X).

Results: Genetics

Genotypes for adults were provided in Table X and will be used for subsequent parentage analysis of hatchlings collected as part of this study.

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Publications and presentation based on research

<u>Publications</u>

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- Grosse, A.M., K.A. Buhlmann, B.B. Harris, B.A. DeGregorio, B.M. Moule, R.V. Horan III, and T.D. Tuberville. *In press*. Nest guarding in the Gopher Tortoise (*Gopherus polyphemus*). Chelonian Conservation and Biology.
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- Clostio, R.W. 2010. Use of environmental variables to infer gene flow and population structure in Gopher Tortoise (*Gopherus polyphemus*) and predict the seroprevalence of an emerging infectious disease. Dissertation. University of New Orleans, LA. (samples from TSR included in genetic analysis)
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McCoy, H. Mushinsky, T.W. Hentges, and A. Schrey. A mitochondrial DNA reassessment of the phylogeography of the Gopher Tortoise. Submitted to: Journal of Fish and Wildlife Management, 17 Oct 2011.

Presentations

- Tuberville, T.D., S.H. Bennett, K.A. Buhlmann, J.P. Nestor, J.W. Gibbons, H.E. Balbach and R.R. Sharitz. Effects of forestry practices for red-cockaded woodpeckers on space use by Gopher Tortoises (*Gopherus polyphemus*). Joint Meetings of Herpetologists, New Orleans, LA. 2006. (poster)
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Significant Deviations: None

Job 2. Diamondback Rattlesnake Population Management

Objective 1. Develop a management plan for the Longleaf Pine Reptile guild embers, using the eastern diamondback rattlesnake as a model for the guild.

Accomplishments:

The IUCN Viper Specialist Group and The Orianne Society are spearheading the development of an EDB conservation action plain. The goal of the project is to review the EDB’s status and to use the best available information to develop conservation strategies for the species. We are currently collaborating with these efforts, and thus the development of a management plan for South Carolina EDB populations has been incorporated into the range-wide effort. Data derived from the seventeen year study on the Eastern Diamondback Rattlesnake, conducted in coastal South Carolina and funded in part by State Wildlife Grant money will be used in the development of this regional conservation plan.

Significant Deviations: None

Objective 2. Determine the feasibility of managing rattlesnake populations by translocating eastern diamondback rattlesnakes to sites with appropriate habitat within the historic range of the species. Develop a model for eastern diamondback rattlesnake demography, to include population size, survivorship, mortality, growth patterns, age classes and sex ratio.

Accomplishments

The eastern diamondback rattlesnake (*Crotalus adamanteus*; EDB) is a declining North American reptile that is considered endangered, threatened, or is listed as a species of special concern in all but one state in which it occurs. Population declines have been attributed to habitat loss (Timmerman and Martin 2003, Martin and Means 2000, Waldron et al. 2008), over-collection (Means 2009), and a lack of public policy regarding the species’ protection (Martin and Means 2000). Habitat loss is likely the main driver of the species’ decline (Martin and Means 2000, Timmerman and Martin 2003), given that EDB distribution is congruent with the historic range of southeastern longleaf pine (*Pinus palustris*) savannas and woodlands. Specifically, EDBs are dependent on pine savanna structure at multiple spatial scales (Waldron et al. 2006, Waldron et al. 2008, Hoss et al. 2010), and thus the imperilment of the longleaf pine ecosystem is considered the main cause of EDB population declines.

An increasing number of EDB population monitoring studies and life history data are making it possible to assess management strategies relative to the EDB and its habitats. Current efforts to review the status of EDBs (spearheaded by the IUCN's Viper Specialist Group, IUCN 2011; see Conservation Action Plan) are underway, and one goal of this assessment is to evaluate management strategies that will benefit EDB conservation. The success of this endeavor depends on the applicability of specific management strategies. Translocation, defined as deliberately moving individuals from one part of their distribution to another location within their distribution where the species either historically or currently occurs (IUCN 1998), is a conservation strategy that needs to be assessed for managing EDB populations. Translocations have been successfully used in other taxa to ease human-wildlife conflicts (Massei et al. 2010), move animals away from sources of imminent danger (e.g., Tuberville et al. 2005), and to re-establish populations (e.g., Moorhouse et al.2009).

Previous efforts to translocate snakes have yielded mixed results, and generally indicate that success is contingent on the spatial ecology of the species. For example, Reinert and Rupert (1999) found that translocated timber rattlesnakes had higher mortality than resident rattlesnakes partly because the translocated snakes moved more erratically and traveled longer distances than resident snakes; consequently, these behaviors placed translocated rattlesnakes at greater risk of mortality. Similarly, Roe et al. (2010) documented abnormal movement behavior and lower survival in translocated snakes relative to resident water snakes, and concluded that prior experience and site familiarity were significant contributors to successful translocations. Therefore, EDB translocation success is likely dependent on the species' spatial awareness, behavioral plasticity, and its ability to habituate to changes at the home range scale.

The EDB is characterized by slow life history traits and a spatial ecology that contributes to the species imperilment (Waldron et al. 2008, Waldron et al. *in review* (Appendix 2-1)). These traits include delayed maturation, greater than 30-yr maximum longevity, high habitat specificity, and low dispersal among larger, older individuals at the landscape-scale. While these traits limit the species' ability to redistribute in respond to rapid and expansive landscape change, they suggest that tradeoffs among delayed maturation / longevity and habitat specificity selected for increased spatial awareness and behavioral plasticity at the home-range scale. These tradeoffs imply that EDB spatial ecology includes a degree of spatial awareness at the home range-scale, and behavioral plasticity that likely allows the species to respond to changes in predator and prey distributions over decades. Combined, these traits suggest that EDBs are unlikely to respond to translocation by dispersing at the landscape scale and thereby leaving a translocation site, but rather we suspected that EDBs were likely to habituate to a new landscape over time (years). Therefore, we hypothesized that, over multiple years, translocated EDBs would 'learn' the translocation site and that their movement behaviors would trend toward pre-translocation behaviors or converge with those of resident snakes.

The objective of this study was to examine the utility of long-distance translocation as a conservation strategy to manage EDB populations. We conducted a translocation study that incorporated multi-year movement data of resident EDBs along with movement data and body conditions of pre- and post-translocated EDBs. We used home range size and the spatial overlap of home ranges among different years for individual EDBs as a measure of inter-annual home range fidelity to examine translocation effects on the spatial ecology of the species. A body condition index was used to monitor the general health of translocated snakes over the course of the study and to examine potential correlations between the home-range size, initial body size, and body condition. This approach allowed us to assess direct and indirect effects associated with translocation while controlling for differences in habitat quality among donor sites and the translocation site.

Methods and Materials

Study areas.—This study was conducted at five locations (Table 1). Four locations were used as donor sites from which EDBs were translocated. The fifth site, James W. Webb Wildlife Center, was used as the recipient site for translocations. Study site descriptions are summarized below.

Cheehaw Combahee Plantation: Consisting of approximately ca. 9,300 hectares of coastal habitats, Cheehaw Combahee Plantation (Cheehaw) is a privately owned and managed quail plantation located in Colleton County, SC. The property is bordered by the Cheehaw and Combahee Rivers, and supports an abundance of upland pine savanna habitats that are dominated by loblolly (*P. taeda*) and longleaf pines. Other dominant habitat types on Cheehaw include maritime forests, remnant rice fields, tidal marsh, and hardwood forests. Wildlife management activities include dormant and growing season fire prescriptions, silviculture, and food plot maintenance.

Nemours Wildlife Plantation: Located in the ACE Basin, the Nemours Foundation (Nemours) is a nonprofit organization that manages ca. 4,000 hectares for wildlife and their habitats. The plantation supports upland pine habitats (including longleaf, loblolly, and slash pines), cypress/tupelo forests, mixed pine-hardwood forests, hardwood forests, and bottomland hardwood forests.

Hoover Plantation: Located in Tillman, SC, Hoover Plantation (Hoover) is privately owned and managed for wildlife, particularly bobwhite quail. The plantation is approximately 930 hectares acres and is dominated by open canopy, longleaf pine savannas. Growing-season fires are the primary management tool for maintaining quail habitat. Hoover Plantation borders the Okeetee Hunt Club (> 20,234 hectares); together, the two properties encompass over 6,070 contiguous hectares in the Tillman area.

James W. Webb Wildlife Management Area: Located in Garnett, SC., the James W. Webb Wildlife Management Area (Webb) is owned and managed by the South Carolina Department of Natural Resources. The WMA is situated along the Savannah River and contains a mosaic of habitats that include longleaf pine flatwoods and savannas, loblolly pine forests, oak-hickory mixed-pine hardwoods, hardwood bottoms, and cypress-tupelo swamp forests associated with the Savannah River floodplain. The 2374-ha property area is managed with growing- and dormant-season prescribed fires, which maintain high vascular plant diversity (Porcher and Rayner 2001) and supports habitats for longleaf-pine endemic wildlife, including colonies of federally endangered red-cockaded woodpeckers. Game management, which includes maintenance of agricultural food plots in upland habitats, focuses on bobwhite quail and white-tailed deer. Webb was chosen as the recipient site because it supported an EDB population that had been extensively studied by the authors since 1997 (e.g., Waldron et al. 2006; Waldron et al. 2008; Waldron et al. *manuscript in review*). We relied on long-term monitoring data from Webb, including radio telemetry data collected between 1997 and 2006 on 21 adult EDBs, for our data analysis. Specifically, a subset of Webb EDBs was radio telemetrically monitored for multiple years, and we used these data to make comparisons to movement patterns exhibited by translocated EDBs (see data analysis).

Table 1. Study areas where eastern diamondback rattlesnakes were monitored for one year prior to translocation to the James W. Webb Wildlife Management Area (Webb). Distance = kilometers between respective study area and Webb.

Property	Location	County	Distance (km)
Hoover Plantation	Tillman, SC	Jasper	20

Cheehaw Combahee Plantation	Wiggins, SC	Colleton	70
Nemours Wildlife Foundation	Yemassee, SC	Beaufort	60
Okeetee Hunt Club	Tillman, SC	Jasper	20
Donnelly Wildlife Management Area	Green Pond, SC	Colleton	75

Study species and monitoring.—The EDB is endemic to the imperiled longleaf pine ecosystem (Means 2006). Dependent on savanna structure at multiple spatial scales, the EDB is considered a remnant of the historical southeastern woodland-savanna landscape (Martin and Means 2000; Waldron et al. 2006; Waldron et al. 2008). It occurs in the southeastern Coastal Plain, ranging from southeastern North Carolina south through Florida, and west through eastern Louisiana (Martin and Means 2000; Timmerman and Martin 2003), and it is listed as a species of conservation concern in Alabama, Mississippi, and South Carolina. North Carolina populations are endangered, and EDBs are critically imperiled in Louisiana. In addition to habitat loss, over-collection, rattlesnake round-ups (Means 2009), indiscriminant killing by humans, and a lack of public policy regarding protection have accelerated the species' decline (Martin and Means 2000).
We conducted visual searches for EDBs and used snake hooks and clear snake tubes to safely constrain individuals while we collected morphological data. We determined sex by counting subcaudal scales and using cloacal probes. We measured snout-vent-length (SVL; cm) and subcutaneously injected a passive integrative transponder (PIT) tag ca. 13 ventral scale rows above (cephalad) the cloaca. After 2005, we used portable cauteries to mark ventral scales according to Winne et al. (2006), in addition to injecting PIT tags.

We used radio telemetry to monitor resident (1997-2006) and translocated EDBs (2006-2009). We implanted radio transmitters (SI-2, 11-13 g, Holohil Systems, Carp. ON) into EDBs using modified surgical procedures outlined by Reinert and Cundall (1982). We monitored individuals for up to three years using a radio receiver (Telonics, TR-2, Mesa, AZ). During the active season (Mar-Nov), we radio located resident EDBs three to five times each week, and located translocated EDBs weekly. During the inactive season (Dec-Feb), we located resident snakes and relocated snakes biweekly. We used a hand-held GPS unit to record snake locations (spatial error < 5 m) while maintaining 2.5-5 m between observer and rattlesnake, allowing visual assessment of snakes. These spatial data were used assess movement behaviors via home-range size and home-range overlap.

Prior to translocation, we monitored EDBs for one year at the study area from which they were captured. After the initial year of radio telemetry monitoring, we re-captured study animals when they emerged from hibernation and transported them to Webb. We monitored translocated snakes for up to four years to assess post translocation movement behaviors. We re-captured telemetered EDBs annually to collect morphological data.

Table 2. Snout-vent length (SVL; cm), total length (TL; cm), mass (g), sex (F = female; M = male), capture year, and capture location (i.e., property) of eastern diamondback rattlesnakes used in radio telemetry study.

Snake ID	Year	Sex	SVL	TL	Mass	Capture Property
CRL	2006	F	127	136	1899	Hoover / Okeetee
STV	2006	M	108	120	1224	Hoover / Okeetee
BDG	2007	F	122	130	1750	Cheehaw Combahee
EDA	2006	F	115	122	1049	Cheehaw Combahee
JAC	2006	M	91	99	737	Cheehaw Combahee
JOD	2006	M	95	104	651	Cheehaw Combahee
SSE	2006	M	126	139	2012	Cheehaw Combahee
LCR	2007	M	131	144	1400	Cheehaw Combahee
HLN	2007	F	123	131	2100	Cheehaw Combahee
PGG	2007	F	130	138	2358	Cheehaw Combahee

EIE	2007	M	126	138	2050	Nemours
ADR	2006	M	137	149	1361	Nemours

Data analysis. We used minimum convex polygons (MCPs; ha) to quantify EDB home ranges and used home-range size and overlap as generalized measures of movement behavior. Home ranges were calculated based on an annual cycle relative to spring egress. We classified home ranges of translocated snakes as, year1 (pre-translocation home range), year2 (first-year post-translocation home range), and year3 (second-year post translocation). We classified resident EDB home ranges as first- and second-year home ranges, which represented two sequential years of data.
We used these groups (i.e., resident and translocated EDBs) to conduct within and among comparisons to elucidate the effects of translocation on movement behavior. To ensure that resident and translocated EDBs exhibited comparable movement behaviors, we used a factorial two way ANOVA to compare year1 home-range size (log-transformed to meet normality assumptions) between resident and translocated EDBs with sex as the interaction term.

We had insufficient sample size to compare home-range size among study sites or to test for an effect of Julian year. We used a repeated measures approach to compare home-range size across years for translocated EDBs. Specifically, we used generalized estimating equations (Liang and Zeger 1986) in PROC GENMOD (SAS Institute Inc. 2008), including year as a categorical predictor variable with three classes (year1, year2, year3). We identified snake as the repeated subject and excluded the intercept term. Additionally, we assessed whether translocated EDBs exhibited evidence that they habituated to their new environment by comparing multi-year post-translocation home ranges and comparing them to resident home ranges based on two consecutive years of telemetry data. We quantified home-range size change (HRchange) to examine whether, 1) post-translocation home ranges (year2 and year3) differed, and 2) whether differences in post-translocation home ranges were similar to those exhibited by resident EDBs. For translocated and resident EDBs, respectively, we calculated HRchange as,
$\text{HRchange}_{\text{trans}} = \text{Home-range size}_{\text{year3}} - \text{Home-range size}_{\text{year2}}$
$\text{HRchange}_{\text{res}} = \text{Home-range size}_{\text{year2}} - \text{Home-range size}_{\text{year1}}$,
with positive and negative values indicating an increase and decrease in home-range size over time, respectively. For the translocation group, we used a paired t-test to compare first- and second-year post-translocation home ranges. We used a pooled t-test to compare HRchange between translocated and resident EDBs.

We calculated home-range overlap to examine patterns of inter-annual home range fidelity. We assumed that home-range overlap served as a coarse measure of site fidelity, and as such could be used to make comparisons between resident and translocated EDBs. We calculated home-range overlap by calculating the percent of each snake's home range that overlapped the prior-year's home range. For translocated EDBs, we calculated home-range overlap between year two and year three, and we calculated home-range overlap between two consecutive years for resident EDBs. We compared home range overlap between resident and translocated EDBs using a pooled t-test.

We used body condition as a general measure of health. To assess the impacts of translocation on EDB body condition, we conducted two analyses. First, we compared the change in body mass, calculated as,

$\mathrm{Mass}_{\mathrm{year2}} - \mathrm{Mass}_{\mathrm{year1}} = \mathrm{MassChange}_{1}$

$\mathrm{Mass}_{\mathrm{year3}} - \mathrm{Mass}_{\mathrm{year2}} = \mathrm{MassChange}_{2}$,

between residents and translocated EDBs. Using a paired approach controlled for the effects of transmitter implantation surgery. Secondly, we calculated body condition using residuals from ordinary least squares regression of body mass on SVL, such that positive values indicated high relative body condition and negative values indicated lower relative body condition (Jakob et al. 1996, Schulte-Hostedde et al. 2001). We used a paired t-test to compare body condition before and after translocation. Lastly, we used correlation analysis to determine if, 1) changes in body condition (positive or negative) were associated with initial SVL or post-translocation home-range size, and 2) post-translocation home-range size was associated with initial SVL.

Results

First year home-range size averaged 102.77 ha (SD = 97.81) across all telemetered EDBs included in our analysis (N = 38). We failed to detect a difference ($F_{3,34} = 0.19$, $P = 0.8199$) in first-year home-range size between Webb EDBs (N = 19) and pre-translocation EDBs, but males (N = 16; untransformed mean = 146.41, SD = 97.66) had larger first-year home ranges than females (N = 22; untransformed mean = 71.02, SD = 86.81; two-way ANOVA, $F = 3.29$, $P = 0.0322$).
Home ranges of translocated snakes varied across years ($\chi^2 = 8.91$, DF = 3, $P = 0.0305$; Figs. 1 and 2; Table 3), but appeared to stabilize during year three. Pre-translocation home ranges (untransformed mean = 115.92 ha, SD = 111.65) were significantly smaller (estimate = -0.3305 ± 0.1079, $\chi^2 = 9.38$, $P = 0.0022$) than the first-year post-translocation home ranges (untransformed mean = 195.48 ha, SD = 118.82), but did not differ (estimate = 0.2010 ± 0.2034, $\chi^2 = 0.98$, $P = 0.3232$) from second-year post-translocation home ranges (untransformed mean = 60.10 ha, SD = 38.45). Second-year post-translocation home ranges were significantly smaller than first-year post-translocation home ranges (estimate = -0.5315 ± 0.1789, $\chi^2 = 8.83$, $P = 0.0030$).

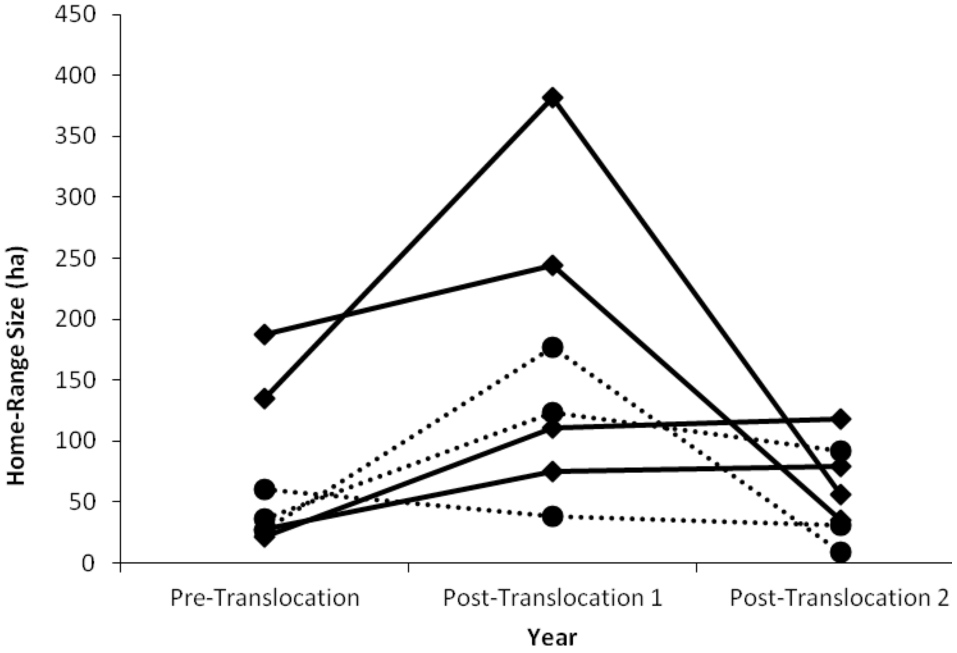

Figure 1. Eastern diamondback rattlesnake home-range size (minimum convex polygon; ha) for seven individuals monitored using radio telemetry, 2006-2009. Each line (females = dashed line; males = solid line) illustrates changes in EDB movement patterns in response to translocation (i.e., snakes were translocated at the beginning of year 2 = Post-Translocation 1). Thus, three home-range sizes are represented: one for pre-translocation

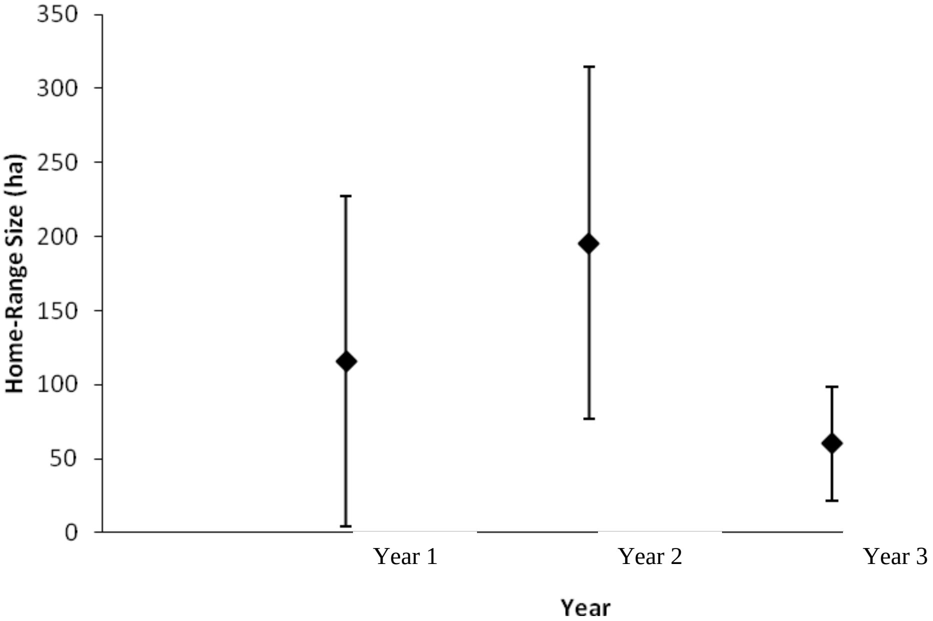

Figure 2. Mean eastern diamondback rattlesnake home-range sizes (minimum convex polygons, ha) with standard deviations. Year 1 = pre-translocation home range; Year 2 = first year post-translocation home range; Year 3 = second year post-translocation home range.

Table 3. Regression coefficients from generalized estimating equations used to examine the effect of year (i.e., pre-translocation, first-year post translocation, and second-year post translocation) on eastern diamondback rattlesnake home-range size.

Effect	Estimate ± SE	χ^2	*P*
Pre-Translocation (year 1)	1.8667 ± 0.1418	173.37	< 0.0001
First-year Post-Translocation (year 2)	2.1973 ± 0.1037	449.27	< 0.0001
Second-year Post-Translocation (year 3)	1.6658 ± 0.1352	151.70	< 0.0001

We failed to detect a difference in HRchange between first- and second-year post-translocation home-range size (paired t-test; N = 7, t = 2.10, *P* = 0.0804). Further, we failed to detect a difference (t = -1.94, *P* = 0.0737) in HRchange between resident (N = 8; mean HRchange= 10.22, SD = 96.03) and translocated snakes (N = 7; mean HRchange= -104.10, SD = 131.20), although our results approached significance.

We failed to detect a difference in inter-annual home-range fidelity among resident and post-translocated snakes, based on home range overlap (t = 1.45, *P* = 0.1694). Inter-annual home-range fidelity averaged 65.12 % (SD = 26.60 %) for the translocation group (N = 7) and 48.36 % (SD = 17.71 %) for the resident group (N = 8).

Neither EDB body mass (t = 1.96, DF = 7, *P* = 0.0907, Fig. 3) nor body condition (t = 0.00, DF = 7, *P* = 1.00; Fig. 4) was affected by translocation body condition. Change in body condition was not correlated with post-translocation home-range size (*r* = -0.02, *P* = 0.9726) or initial SVL (*r* = 0.11, *P* = 0.8156); however, initial SVL was positively correlated with post-translocation home-range size (*r* = 0.80, *P* = 0.0293).

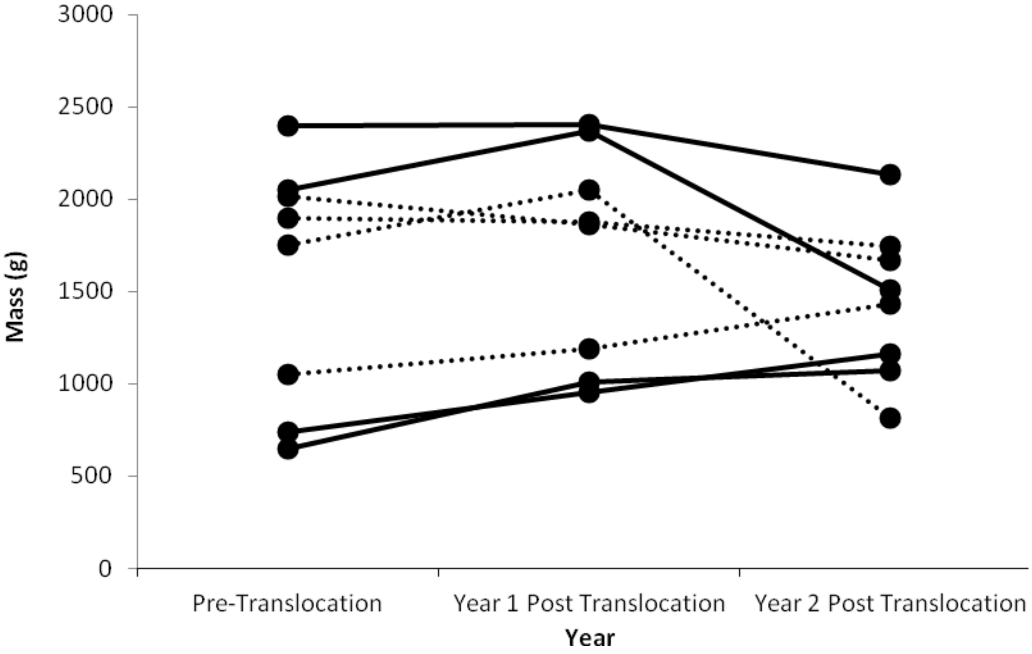

Figure 3. Eastern diamondback rattlesnake body mass (g) by year. Lines (dashed line = female; solid line = male) represent individuals monitored between 2006 and 2009 and illustrate changes in body mass patterns in response to translocation (i.e., snakes were translocated at the beginning of year 2 = Post-Translocation 1).

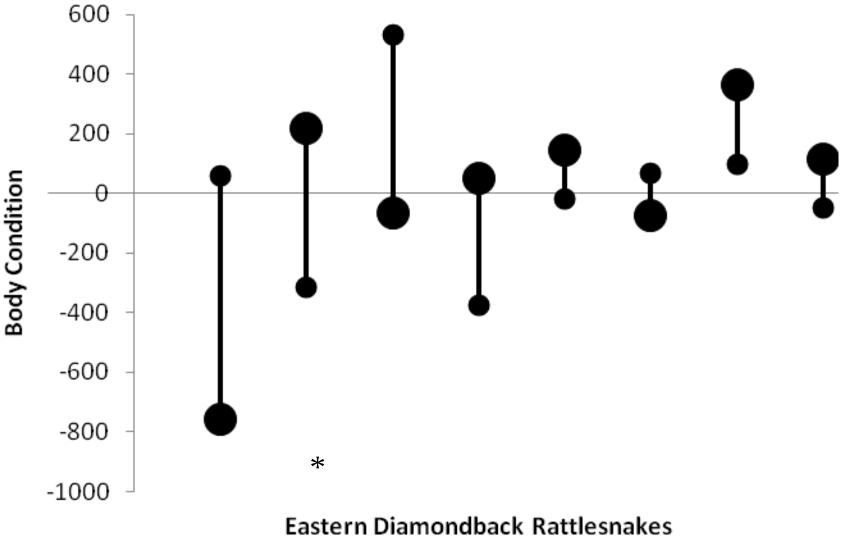

Figure 4 Changes in eastern diamondback rattlesnake body condition (calculated as residuals from sex by mass regression) in response to translocation. Large circles indicate directional change in body condition following translocation. The asterisk represents body condition from a gravid female that gave birth following translocation.

Discussion

The results of this study indicate that EDB translocation has the potential for use as a conservation management strategy. The movement patterns exhibited by translocated EDBs support our hypothesis that tradeoffs among life history traits enhance the success of translocation. Specifically, translocated EDBs did not attempt long-distance dispersal at the landscape scale. Rather, home-range sizes tended to normalize to pre-translocation levels during second-year post-translocation. Therefore, we do not expect abnormal movement behaviors to significantly increase mortality among translocated EDBs, as observed in other snake translocation studies (e.g., Reinert and Rupert 1999).

Despite the apparent success of this study, we caution against casual use of EDB translocations. Because this study used only one recipient site, our results may be partly related to the attributes of the James W. Webb WMA. We believe it is important to ensure that the recipient site is large enough to encompass an initial increase in EDB home-range size, which was exhibited by our translocated study animals. Given the location of our recipient site, our translocated EDBs had little exposure to paved roads and high-speed vehicular traffic. Additionally, we suspect habitat quality likely played a role in response of our translocated snakes and the rate at which their home ranges normalized. Our initial optimism regarding the utility of translocation in EDB management was predicated on the hypothesis that tradeoffs among delayed maturation / longevity and habitat specificity selected for increased spatial awareness and behavioral plasticity at the home-range scale. Under this scenario, it would be expected that high-quality habitat would tend to anchor a translocated snake and stabilize its movement patterns, thereby increasing the success of translocation.

An additional consideration is the reproductive condition of EDBs prior to translocation. Although we were unable to quantify the effects of reproductive condition (e.g., gravid) due to small sample size, it is important to note the reproductive behaviors observed during this study. All but two translocated males exhibited breeding behavior in the second post-translocation year, suggesting that normalized movement behavior was indicative of normal reproductive behavior. We did not detect breeding activity in females in post-translocation years; however, one female that bred prior to translocation gave birth during first-year post-translocation. This female suffered from poor body condition following parturition (Fig. 4), and eventually died in 2010 (carcass was found in road). Thus, we recommend translocating gravid females until more information regarding the effects of reproductive condition on translocation success is available.

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Significant Deviations: None

Objective 3. Conduct research and monitoring at the Webb Wildlife Center (continuation of ongoing monitoring) and at least three other public properties in the S.C. coastal plain that support longleaf pine habitat. Determine the potential distribution of longleaf pine habitat on public properties in the S.C. coastal plain using a qualitative vector GIS model.

Accomplishments:
Beginning in January 2010, we expanded the long-term rattlesnake monitoring study to include all snake species. Surveys included visual searches (including burned and unburned habitats), tin surveys, and road observations. Rattlesnakes were marked using scale cauterization and tagged with passive integrative transponders (PIT tags), but all other species were only marked using scale cauterization. All captured snakes and turtles were measured and weighed to assess body condition. Table 1 summarizes captures by species (for snakes and turtles). All non-venomous snake and turtle captures did not include recaptures.

Our surveys yielded 14 new canebrake rattlesnakes and 2 new eastern diamondback rattlesnakes. To date, we have marked 111 eastern diamondback rattlesnakes and 205 canebrake rattlesnakes. In 2010, we recaptured 7 canebrake rattlesnakes that were originally captured in previous years, and growth data obtained from these individuals were used to update growth models developed in 2009. We

A pair of canebrake rattlesnakes (Crotalus horridus) found under a sheet of tin used as a passive trap for monitoring rattlesnake populations.

currently have 27 growth intervals (i.e., males = 9, females = 18) for canebrake rattlesnakes, which have been incorporated into growth models originally developed in 2009 (see SWG report from 2009). Preliminary results suggest that canebrake rattlesnakes reach maturity between 5 and 6 years. Neither of the eastern diamondback rattlesnakes encountered during 2010 surveys had been previously captured; thus, their respective growth models could not be updated.

We continued to monitor translocated eastern diamondback rattlesnakes via radio telemetry (N = 4). We observed our first mortality of a translocated individual during summer 2010. The individual was found dead on a road, and thus we assume the snake was hit by a vehicle. We conducted a preliminary analysis of the short-term effects of translocation (i.e., comparing pre-relocation home-range size to home range used during the initial year following translocation) on eastern diamondback rattlesnake home-range size (N = 9), based on minimum convex polygons. Our results indicated that post-translocation home-range size (mean = 202.19 ± 113.17) was larger than pre-translocation (mean = 115.89 ± 111.60 ha) home-range size (paired t-test; t = -2.97, df = 8, $p < 0.05$). Despite the increase in home-range size, translocated eastern diamondbacks (N = 10) did not lose significant body mass (paired t-test; t = -1.46, df = 9, $p > 0.05$). We are currently analyzing data collected from year 3 and 4 of the study to assess whether rattlesnake movement patterns continued to change over time following translocation.

Table 1. Snake and turtle species captured during visual, tin, and road surveys conducted in 2010 at the Webb Wildlife Center.

Species	**Total Captures**
Eastern diamondback rattlesnake	2
Timber rattlesnake, a.k.a. canebrake rattlesnake	22
Southern copperhead	27*
Cottonmouth	4
Corn snake	22*
Rat snake	13
Eastern hognose	4
Rough green snake	2
Eastern garter snake	4
Rough earth snake	5
Eastern kingsnake	2
Scarlet kingsnake	9
Black racer	53*
Mud turtle	6
Box turtle	1
Snapping turtle	1
Pine snake	1*

*Numbers include captures from Tillman Sand Ridge Heritage Preserve (TSR). Specifically, captures include 1 pine snake, 7 copperheads, 2 corn snakes, and 1 black racer from TSR.

Comparable surveys were conducted at the Webb Wildlife Center in 2011 and data from these surveys is currently being analyzed and will be presented as an addendum to this report.

Significant Deviations: None

Job3. Timber Rattlesnake

Objective 1. Determine the distribution of the montane phase and the coastal plain phase of this species in the region.

Accomplishments:
The study primarily focused on timber rattlesnake populations in upstate South Carolina. Specifically, our main study location is Table Rock State Park. Of the current 15 snakes captured, 4 of them are classified as the coastal plain phase and exhibit light coloration and the remaining 11 are classified as the montane phase with subsequent dark coloration. Moreover, of the 11 montane phase individuals, 3 of these are further classified as being the yellow coloration of the montane phase. Currently, there appears to be no elevation, habitat, or sex differences in either phase of the upstate timber rattlesnake.

Six new timber rattlesnakes were added to the study in 2008. Two male rattlesnakes were both montane phase animals, one of which was the yellow montane phase. Of the four new female rattlesnakes three were montane phase and one was a coastal (canebrake) phase. To date twenty two timber rattlesnakes have been collected, or observed during this study. Sixteen of these are montane phase animals, four of which are the yellow phase and five animals are coastal phase animals. While the montane phase is the more common phase in this area, it is important to note that the coastal phase does occur at these sites, which are within the upper Piedmont and lower Blue Ridge provinces of South Carolina.

No new rattlesnakes were added to the study in 2009. Overall, the study consisted of monitoring twenty-one timber rattlesnakes. Sixteen of these are montane phase animals, four of which are the yellow phase. There were five animals classified as coastal phase animals. While the montane phase is the more common phase in this area, it is important to note that the coastal phase does occur at these sites, which are within the upper Piedmont and lower Blue Ridge provinces of South Carolina.

In conclusion the timber rattlesnake, in South Carolina, comprises two “forms” previously considered sub-species, the timber “form”, which is restricted to higher elevations in SC, and the canebrake “form” which occurs throughout the state. In neighboring Georgia the timber form is found at elevations above 2460 feet (Jensen et al, the Amphibians and Reptiles of Georgia). In SC the canebrake form can be common to abundant, especially in the Coastal Plain. The timber from is not common in SC and has been considered a “species of concern” historically, and is currently considered a Species in Need of Conservation.

The data from this study suggests that we do need to treat the mountain form as a separate “form” of the timber rattlesnake. A wide range of morphological variation was observed during this study from animals that looked like canebrakes to a few yellow individuals and two black individuals were captured as well. However, that is more of the exception than the norm. Most of timber rattlesnakes (80-90%) were what was termed a “muddy” phase and were a blackish/yellowish/tannish morph. They did not have the nice yellows and blacks of northern timbers and they also were not those clean and crisp grays and pinks you see with canebrakes. They were definitely a mountain form.

Based on basic biology the animals in this study do not seem to differ much from the canebrakes with the individual dens that range in assortment from crevices to stumps to creek beds. However, the investigator believes there is adequate data to support the conclusion that they are separate enough to warrant separation from canebrakes as far as protection. The numbers are not large and they are very difficult to sample. Only 30individaul adult rattlesnakes were captured and marked during this study. This leads the investigator to the conclusion that the population is very small as the same individuals are being seen over and over again with very few new ones found outside of the population we are studying. Case in point, the investigator was able to capture 2 individuals that had transmitter failures a year after they went missing, at the same time finding no new ones in those same areas.

The principle investigator (Jeff Mohr) received his doctorate in August 2010 and a copy of the dissertation was submitted with the FY10 Interim Report.

Significant Deviations. None

Objective 2. Develop a management strategy for the timber rattlesnake on public lands in South Carolina; Determine the population size and demography at selected sites to include: population structure, sex ratios, mortality, reproductive success, survivorship and mortality. Determine home range size, habitat use and seasonal activity patterns for both "forms" of this species, in this region, using radio telemetry.

Accomplishments:

In late September 2006, 3 timber rattlesnakes were captured and implanted with radio transmitters. All three were males and represented 2 phases (2 montane, 1 coastal) of the species. These three snakes were monitored throughout the fall and tracked to their place of hibernation. Two snakes hibernated in areas of rocks and boulders, while the third snake overwintered in the bank of a small creek. Last activity for two of them was at the end of November and the other was on December 18th, 2006. All three began to move again in late March 2007.

Two of the snakes emerged from hibernation fine and are currently being tracked. One snake, however, made one movement in early spring and went under a set of rocks whereupon a few weeks later the radio transmitter was found on the surface. This rattlesnake was not sighted during its single spring movement. The transmitter had bite marks and it and it is presumed the rattlesnake perished underground and a rodent carried the transmitter out. No signs of skin or bone were found. It should be noted, this snake was one that was found trailside in the park and it had been reported that people were throwing rocks/sticks at it. During the surgical implantation of the transmitter, a wound was found on the snake where the ventral meets the side. This wound was debrided of skin and dirt but there could have been internal damage that was not visually detectable. It is thought that this injury combined with a late freeze in April may have contributed to the snake’s death as this snake spent the longest time above ground (last sighting Dec. 18th) before going underground for winter. The time above ground could have been used to gain heat to assist in repairing the injured area. Although it is possible there could have been problems associated with the transmitter surgery, no post-op complications were observed and the other two snakes that were implanted with transmitters during the same time have had no problems.

In the spring/summer/fall of 2007, 12 new snakes were captured and implanted with radio transmitters. There were 5 males and 7 females. Most of the snakes were randomly encountered but a few were found in the course of tracking radio transmittered snakes. Two females were found a month apart being courted by the same radio transmittered male. Moreover, one of these females was found mating with a new male about a month later.

Mating and courting were observed in the fall with all observations (3 courting, 1 mating) taking place in September and October. Three of the females in the study (not ones that were captured courting/mating) gave birth in late August/early September. One litter yielded at least 5 young. The number of young in the other two litters is unknown as only one snake was found from one and no babies were observed at the third probable birthing. However, with the latter, the female lost weight and baby timber rattlesnake shed skins were found at the area she occupied shortly after her observed weight loss.

Movements for males appear greater than females but no statistical analysis has been preformed yet. Both males and females seem to have an affinity for wooded areas and are often found in the vicinity of fallen logs and branches. Two females captured at a rocky outcrop stayed in the vicinity of the outcrop the majority of the time, however, both females were ones that gave birth. Another female (non-pregnant) and two males that were captured in rocky outcrop areas spent very little time in the rocky outcrop area post surgery release. All of the other captured rattlesnakes were found in wooded areas.

Of the 12 new snakes captured in spring/summer/fall of 2007, one death was observed. This mortality was of one of the females that had given birth in late August/early September but the mortality was not due to birthing or a natural predator. Unfortunately, this snake was most likely killed by humans. This female was very site specific and had extreme preference to a rocky outcrop area where there were numerous crevices. The area was off the main trail and most hikers (probably all) would miss this area as they would hike along the trail. However, a group decided to camp illegally (no camping allowed in park along trails) and have a campfire (also unlawful) near this area. Hatchet marks were found on a fallen log less than 10 feet from a crevice this snake was fond of using and less than 2 days after the group had been there, the snake was found dead and missing the head and tail. It is very unlikely a natural predator would eat only the head and tail and leave the rest of the body intact. Instead, it is much more likely that a human used their hatchet to take care of the head and keep the rattle as a souvenir. No other mortalities of radio tagged animals were observed. (Note: Three additional timber rattlesnakes were found dead during part of this study. None of these animals had been marked, and all were human related mortalities. One was found trailside with no head or tail near a popular bathing place in the creek. The other two were road-kills of which one was deliberate according to the park staff member who witnessed the event. The other one was not witnessed and therefore we cannot determine whether it was deliberate or accidental.)

As of December of 2007, there were fourteen timber rattlesnakes implanted with radio transmitters in the field. Of the two snakes captured in 2006 that overwintered again in 2007, one returned to the exact hibernaculum as in 2006, the other was in the same ravine system but approximately 150m downhill from its winter 2006 hibernaculum.

Sometime in the early spring of 2008, three rattlesnakes were killed by unknown predators. It is hypothesized they came out to warm up, yet were too cold to fend off a predator. One of the rattlesnakes predated upon was pregnant and was most likely warming herself for the sake of the embryos/babies. In addition to the three predation events, another snake in the study had a transmitter battery failure sometime in the early spring of 2008 before emergence. An attempt to capture the animal during spring emergence failed due to the fact the animal was never seen emerging from its hibernaculum.

During the spring/summer/fall of 2008, three new snakes were captured in the main corridor of the study. An additional three were captured in campgrounds/public use areas and per park rules, were trans-located to the other side of the park away from facilities and visitors. Both of the corridor captures were found because a female rattlesnake who was part of the study brought us to a male (who we implanted with a transmitter) and he consequently brought us to a female three weeks later (who was also implanted and became part of the study).

No breeding activity was observed in 2008, however, it is believed three females gave birth. One was thought to have given birth in the late spring and the other two were in the fall. In none of the cases were babies observed but the females did appear to have lost weight. The snakes were not weighed due to the erratic change in movements and behavior seen in previous manipulations. In the summer of 2008, one male died of unknown causes and in November, a female was predated upon by a raccoon.

Two instances of prey capture were observed during the 2008 year. One involved a squirrel (*Sciurus carolinensis*) and the other was an eastern chipmunk (*Tamias striatus*). In both instances, the meal left a noticeable lump, days after eating. Lumps were also observed in at least three other instances, one of which was a squirrel sized lump in the same snake that was seen eating the squirrel. Let it be noted the snake that ate the squirrel was also a relocation animal and thusly appears that to have successfully adapted its hunting technique to its new location.

Of the nine living rattlesnakes in the study that overwintered in 2007, six returned to the same hibernaculum, one overwintered 15m from the 2007 hibernaculum, and two have unknown hibernaculum due to the rough terrain and inaccessibility of their overwintering areas. The hibernacula vary from creek banks to rock crevices to old stumps. It is believed that although the timber rattlesnakes appear to have overwinter site fidelity, they do not a have a communal hibernaculum. That being said, however, one of the female relocation rattlesnakes did use the same stump as a male relocation animal from 2007. Although he returned to the same spot in 2008 as he did the winter of 2007, he did so weeks after she found it this winter in 2008. It is unknown whether she used cues given by the male during his 2007 overwintering to establish whether this foreign area was a suitable hibernaculum. Due to her being a relocation animal, no conclusions can be made on communal denning behavior as a natural condition in this area.

In December of 2008 there were thirteen timber rattlesnakes implanted with radio transmitters in the field and as of December 2009, twelve of those remain. One of the original snakes captured in 2006 has returned to the exact hibernaculum in 2006, 2007, 2008, and is currently heading that direction for 2009. Four of the 2007 captures have returned to their exact hibernacula in 2008 and now again in 2009. Two of the 2007 captures have returned to slightly different areas (150m) from their 2007 and

2008 overwintering den sites. The last living timber rattlesnake captured in 2007 does not have a known hibernaculum due to the rough terrain and inaccessibility of the area. The hibernacula vary from creek banks to rock crevices to old stumps. It is believed that although the timber rattlesnakes appear to have overwinter site fidelity, they do not a have a communal hibernaculum. That being said, however, one of the female relocation rattlesnakes did use the same stump as a male relocation animal from 2007. Although he returned to the same spot in 2008 as he did the winter of 2007, he did so weeks after she found it in 2008. It is unknown whether she used cues given by the male during his 2007 overwintering to establish whether this foreign area was a suitable hibernaculum. Due to her being a relocation animal, no conclusions could be made on communal denning behavior as a natural condition in this area. Furthermore, we experienced transmitter failure on this female during overwintering 2008-09 and consequently no more data has been obtained.

During the spring/summer/fall of 2008, no new snakes were captured. However, one snake which experienced a battery failure in April was recaptured in August and implanted with a new transmitter. In fact, this snake was captured because he was found breeding one of our transmittered females (pictured). It should be noted that this pairing was how we originally caught the female in 2007 when the male brought us to her. That 2007 location of breeding was approximately 200m away from the 2009 location. This was the only instance of breeding activity observed in 2009. It is believed two females gave birth in the fall but in neither case were babies observed. The females were robust and sunning often and then suddenly visually lost weight. The snakes were not physically weighed due to the erratic change in movement and behavior seen in previous manipulations. No specific predation events were recorded in 2009 but lumps were noticed several times during the year. No mortalities were observed in the 2009 field season but one animal (relocation female mentioned above) was lost due to transmitter failure. Movements of most of the snakes appeared to mirror similar movements in previous years and analysis will be conducted using ARC GIS in the spring of 2010.

The principle investigator (Jeff Mohr) received his doctorate in August 2010 and a copy of the dissertation was submitted with the FY10 Interim Report.

Significant Deviations: None

Job 4. Seepage Slope Salamanders

Objective 1. Develop a predictive model for coastal plain seepage slope habitat as a means of identifying potential habitat for the southern dusky salamander and Chamberlain's dwarf salamander. Survey potential habitat for presence absence of the target species. Collect specimens of the southern dusky salamander, when present, for genetic analysis to determine if there are "cryptic' species of this complex found in South Carolina.

Accomplishments:

During FY07 we initiated a molecular phylogeny study involving the two focal species of this project the Southern Dusky Salamander (*Desmognathus auriculatus)* and Chamberlain’s Dwarf Salamander (*Eurycea chamberlainii)*. The goal of this study was to resolve the phylogenetic, and eventually the taxonomic status of these two species and their “closest” relatives in South Carolina. We contracted with East Carolina University for assistance with this portion of the project. A secondary goal of this objective was to document the species of Plethodontid salamanders co-occurring with the

In FY09 we expanded the scope of the salamander surveys to include the piedmont of South Carolina. We expanded the scope of the project due to preliminary results of the molecular phylogeny research, which indicates that the focal species, *Desmognathus auriculatus*, does not occur in South Carolina. And, the Fall Line plays no apparent role in the distribution of the Desmognathus "lineages" identified as a result of the study.

Survey Results


Patchnose Salamander (*Urspelerpes brucei*)

One site surveyed during FY09, a SCDNR property located in Oconee County, produced very interesting results. The Patchnose Salamander (*Urspelerpes brucei*) the new genus and species of salamander, described recently from a site in Georgia the site, was discovered at this site. This is the first time this species has been documented outside of Georgia. Additional salamander species documented at this site include the Blackbelly Salamander (*Desmognathus quadramaculatus*), Dwarf Blackbelly Salamander (*Desmognathus folkertsi*), a recently described species, Seal Salamander (*Desmognathus monticola*), Ocoee Salamander (*Desmognathus ocoee*) and the Blueridge Two-lined Salamander (*Eurycea wilderae*).

Beginning in FY07 through FY11 187 sites in South Carolina were surveyed for the presence of Plethodontid salamanders (Figure 4-1), in particular the Southern Dusky Salamander and Chamberlain's Dwarf Salamander. Sites were selected based on several criteria including historic locations for the Southern Dusky Salamander, secondary roads crossing small streams, sites with potential seepage wetlands (seeps, springs etc.) as determined by use of ArcGIS, known or historic sites with springs and properties, both public and private where access was available.
The location of every site is archived in a geo-referenced database, using ArcGIS as a platform. The database contains the dates of survey, all species of amphibian and reptile observed, number of individuals and the major habitat and micro-habitat in which each species was observed.

One or more species of Plethodontid salamander was documented for 136 (73%) of the 187 sites sampled. The most commonly observed species was the Southern Dusky Salamander at 55 sites. The Northern Dusky Salamander (*Desmognathus fuscus*) was observed at 15 sites. However, as will be discussed later in the report, these species were initially distinguished only by their location, and proved to very difficult to distinguish morphologically. Concurrent phylogenetic research indicated that *Desmognathus auriculatus* does not occur in South Carolina. For purposes of the survey effort both species are grouped together. As such Dusky Salamanders currently assigned either to *Desmognathus auriculatus* or *Desmognathus fuscus* were observed at 70 sites in South Carolina, 38% of the sampled sites Figure 4-2).

Pseudotriton ruber

Six additional salamander species in the family Plethodontidae were documented at the sample sites. The Three-lined Salamander (*Eurycea guttolineata*) was observed at 44 sites, 23% of the sites sampled (Figure 4-3). The Southern Two-lined Salamander, (*Eurycea cirrigera*) was observed at 38 sites, 20% of the sites sampled (Figure 4-4) and the Dwarf Salamander, (*Eurycea quadridigitata*) was observed at 39 sites, 20% of the sites sampled (Figur3e 4-5). These two species were observed to co-occur at only three of these sites. The Mud Salamander, (*Pseudotriton montanus*) was observed at 17 sites, 9% of the sites sampled (Figure 4-6), the Red Salamander (*Pseudotriton ruber*) was observed at 16 sites, 8% of the sites sampled (Figure 4-7) and Chamberlain's Dwarf Salamander (*Eurycea chamberlaini*) was observed at 9 sites, 5% of the sites sampled (Figure 4-8), one of which was a historic location. Eight of these sites represent new locales for this species, in effect tripling the known sites for this species in South Carolina.

Chamberlain's Dwarf Salamander, a recently described species and target of this project, proved to be a difficult species to survey. This species was typically associated with seepage wetlands, unlike the Dwarf Salamander which occurs in a number of habitat types including isolated ponds and flood plains. Chamberlain's Dwarf Salamander was most commonly observed with leaf or pine straw litter along the edge of seep streams, or small debris piles in the terrestrial uplands adjacent to seepage wetlands. This species was observed on at least two occasions at depths of 15cm. or greater in stream-side leaf debris. It was seldom encountered under larger woody debris which was a preferred microhabitat of the Dwarf Salamander. Chamberlain's Dwarf Salamander breeds in late fall through early winter and adults were found at two sites, at that time of year, under leaf litter adjacent to seepage wetlands.

Based on the results of our survey efforts the investigators feel that Chamberlain's Dwarf Salamander is under-represented in collections due to low detection probability, not rarity necessarily. We recommend maintaining this species as a conservation priority, and continuing survey efforts and life history research.

Desmognathus Phylogeny

Based on preliminary analysis of the molecular phylogeny data we have identified five separate lineages of dusky salamanders in South Carolina, which would have previously been identified as either southern dusky salamander (*Desmognathus auriculatus*) or northern dusky salamander (*Desmognathus fuscus*). These lineages appear to be distinct from these two known species, neither of which has been documented as occurring in South Carolina as a result of this research.

One of the lineages is a known species, the spotted dusky salamander (*Desmognathus conanti*), which had previously been considered a subspecies of the northern dusky salamander, and only recently documented to occur in South Carolina. Three of the lineages show greatest affinity with *Desmognathus fuscus* and one with *Desmognathus carolinensis*. The preliminary results reported here represent a portion of one of the author's (Dave Beamer) doctoral dissertation. Neither the research nor the dissertation have been finalized, and no results have been published to date, as such these results may change and therefore should not be disseminated until all research is final and published in a peer reviewed journal.

The results of the Desmognathus phylogeny research that are presented in Appendices 4-2 and 4-3.

Seepage Slope Predictive Model

Based on the results of our survey efforts and research the best predictor of seepage wetlands which support Plethodontid salamanders is a combination of topography and aerial photography, which depicts canopy condition. Seepage wetlands are represented on topographic maps as incisions which cut back into bluffs along larger stream valleys (figure 4-9). Seeps which were sampled during the survey portion of this project were selected by comparing topography to canopy condition, as indicated by digital orthophoto quarter quads (DOQQs) using ArcGIS. Figure 4-9 illustrates of seep topography and canopy conditions that would have been accepted and rejected as potential sample sites. In general seepage wetlands where the canopy had been removed, or severely altered did not provide habitat for Plethodontid salamanders. This predictive model, used for this study, was employed manually due to lack of GIS support. However, the site-specific data from this study and protocols for identifying seepage wetlands for sampling could be developed into a GIS based model for predicting the presence and quality of these wetlands in South Carolina.

Salamander Sampling at Coastal Plain Study Seeps

In 2007 two additional seeps were added to the seep project initiated under T-15 (see T-15 Final Report for details). These two additional seep study sites were added to the water quality, hydrology and salamander-monitoring portion of this study during FY 07. Each new site comprises two seeps, bringing the total number of seeps monitored to eight. Water sampling wells and cover-board transects were placed at the new seeps following the same protocols used for the original seeps. One new site is located on Beidler Forest approximately five miles from the original Beidler study site. The other new site is located in Calhoun County, on private property, approximately five miles from the original Calhoun County site.

In 2008 six additional sample sites were added to the study. All of these sites were located in the Upper Coastal Plain (Level IV Ecoregion), with three located in the northern portion of this region and three in the southern region. In 2010 six additional seeps, all located in Calhoun County, were selected for inclusion in this study. All of these seeps are located on one private property and consisted of pairs, two of which are downslope of an ongoing agriculture operation, two are downslope of ongoing silviculture and two are downslope of an old-field that has been allowed to convert to native vegetation. Ten coverboards and five dataloggers that record temperature and light were deployed at each study seep. The objective of this study is to determine if any effects of agriculture or silviculture can be detected in amphibian communities and water chemistry or quality associated with these seeps.

Each sample site contained two seeps, separated by a distance of at least fifty meters, and each seep was equipped with an array of ten coverboards (2’X2’ squares of ¾” untreated plywood). Boards were numbered sequentially from the head of the seep to the tail, and separated by approximately 3-5 meters. Coverboards at all study sites were sampled four times a year, upon establishment, and all amphibians and reptiles observed were identified to species, counted and sorted into three classes, adult, juvenile and larva, based on size and physical characteristics.

All salamander data collected is archived in a database at SCDNR. Analysis of this large dataset is not finished, however several publications representing initial analysis are underway. Appendix 4-1 includes the outline of a publication summarizing salamander data from the original study sites and statistical models of the data examining occupancy and phenology of board use by Plethodontid salamanders.

Salamander data from the six Upper Coastal plain study sites, established in FY08 (Figure 4-12) is summarized in Table 4-1 and Figures 4-13 through 4-18. The range of values for both Simpson's Diversity Index and the Shannon-Weiner Diversity Index suggest that, while there are similarities in the species composition among sites the overall assemblages differ among sites. The investigators are initiating an analysis of this data, comparing it to the physical data collected at each study site to determine what variables are important in shaping the Plethodontid salamander assemblages associated with seepage wetlands.

Significant Deviations: None

Objective 2. Develop a management plan for seepage slope habitats, specific to amphibians, for South Carolina. This is an expansion of an existing pilot project in cooperation with the University of South Carolina and National Audubon Society.

Accomplishments:
During winter 2009 we initiated a phase of this project in which we selected six study sites across the inner Coastal Plain, three each on the east and west sides of the Santee River (Table 1). The basic purpose was to further evaluate habitat variability at the seep-, site-, and large-scales that may influence salamander species occurrence. At each site we selected two headwater seeps and deployed cover boards and continuous light/temperature loggers along the seep run. During seasonal sampling we enumerated salamander species and counts. We also collected point data on temperature, pH, and specific conductance at the groundwater discharge point and at the end of the string of cover boards. The purpose was to detect additional data on potential seasonal or site differentiation and longitudinal gradients.

During summer 2010 we initiated another phase of the project in which we selected a study site in Calhoun County that had several different agricultural land use activities upslope of headwater seepage wetlands. The three uses are row crops, silviculture, and old field succession. At each location we selected two seeps and deployed a sting of cover boards and data loggers and a shallow well at the seep head with continuous data logger. Seasonally we will sample similar to the earlier phase.

This analysis incorporates all the water quality data collected during sampling trips at all sites from summer 2009 through autumn 2011 (Table 2). At each site a water sample was analyzed at the downstream end then the upstream end, ensuring undisturbed analysis at each end. Data were recorded by hand in a field notebook then transferred to an Excel spreadsheet in the lab. Statistical analysis was performed using the SAS package on a PC. SAS procedures used were UNIVARIATE, TTEST, and GLM.

Mean water temperature in the headwater seepage sites ranged from 16.1 – 19.2 ^{o}C (Table y). There was significant seasonality ($p<.01$) although the difference between spring and autumn was not significant. There were several temperature differences among sites when accounting for seasonality.

The Luther Wannamaker site was significantly cooler (p<.01) than every other site except Barnwell State Park. Barnwell State Park was cooler than Bates Mill Creek, Healing Springs, and Poinsett State Park although the relationship was not as strong (p<.05). These sites all occur in the same climate zone, some within several kilometers of each other, suggesting that temperature differences relate to groundwater residence time and perhaps other unmeasured factors.

Mean pH of the water among sites ranged from 4.9 – 7.0 (Table 3). There was no overall seasonality in pH but there were significant differences among sites. Barnwell State Park and Louthers Lake were not different and the Luther Wannamaker site was not difference from Poinsett State Park. All other comparisons had significant differences (p<.01).

Mean specific conductance of the water among sites ranged from 37.8 – 152.7 µS/cm (Table 3). Winter and autumn were weakly different (p=.0271); there were no other seasonal differences. There were many differences among sites when accounting for seasonality. Bates Mill Creek was different (p<.0001) from every site except Healing Springs. Barnwell State Park was different from Bates Mill Creek, Healing Springs, and Louthers Lake (p<.01). Healing Springs was different (p<.0001) from every site except Bates Mill Creek. Lee State Park, the Luther Wannamaker site, and Poinsett State Park were different from Bates Mill Creek and Healing Springs (p<.0001).

Clearly seasonality exists at these sites and is a factor in differences among sites. Whether these differences have ecological significance in terms of salamander habitat requires additional analysis. This is especially the case with specific conductance. Although many differences existed the measured values were all very low so whether salamanders are sensitive to differences in dissolved salts at this scale is an unanswered question from these results.

Another analysis was to assess if there is longitudinal variability in the headwater seep ecosystems. On a site basis all showed upstream/downstream differences on at least one parameter, four had differences for two parameters, and Bates Mill Creek had this differential for all three parameters (Table 4). On a parameter basis, specific conductance had significant upstream/downstream differences at five sites (weak at two), pH at six sites, and temperature at two sites (weak at one) (Table 4). In all cases except temperature at Lee State Park (not significant) and the Luther Wannamaker site (weakly significant) the downstream values were greater than the upstream values, e.g. pH was larger at the tail of the seep than the head.

Analysis of the longitudinal data by seep suggests difference within sites (Table 4). For example, at Bates Mill Creek there is a significant temperature gradient at BC01 but not at BC02; there was a significant specific conductance gradient at BC02 but not at BC01. Similar comparisons exist at other sites. As with the analysis of seasonality, further analysis is needed to determine if these longitudinal differences (or lack of) have ecological significance in terms of habitat suitability for salamanders.

The final data analysis considered whether there are differences among sites located east and north or west and south of the Santee/Congaree/Wateree Rivers. This stratification was based on the differences in stratigraphy between the two general regions. The analysis shows that mean pH in the western part of the study area was significantly less than mean pH in the eastern part (4.96 vs. 5.93; p<.01). Similarly, mean specific conductance in the western part of the study was significantly more than in the eastern part (87.8 vs. 48.5 µS/cm; p<.01). There was not a significant difference in temperature. The temperature result raises the question of whether the east/west stratification has ecological significance. The earlier discussions of temperature showed significant differences among sites and in some cases within sites. This suggests that for temperature at least, local rather than synoptic factors are the drivers. This may also be the case for pH and specific conductance which, if so, suggests these results are artifacts and not meaningful in the context of this study.

Table 1. The study sites and location information. The E/W of Santee R column indicates if the site is east and north (East) or west and south (West) of the Santee River.

Site name	Site mnemonic	County	Watershed	E/W of Santee R	Seeps
Barnwell State Park	BP	Barnwell	Savannah	West	BP01, BP02
Healing Springs	HS	Barnwell	Edisto	West	HS01, HS02
Bates Mill Creek	BC	Calhoun	Congaree	West	BC01, BC02
Poinsett State Park	PP	Sumter	Wateree	East	PP01, PP02
Lee State Park	LP	Lee	Lynches	East	LP01, LP02
Louthers Lake	LL	Darlington	Great Pee Dee	East	LL01, LL02
Luther Wannamaker	LW	Calhoun	Santee	West	LW01-LW06

Table2. Sampling dates included in this analysis.

Date	Season	Notes
15-Apr-09	Spring	
15-Jun-09	Summer	HS01, PP01 no water
1-Sep-09	Autumn	HS01, PP01, LP01, LP02 no water
15-Dec-09	Winter	
15-Apr-10	Spring	HS01 no water
24-Aug-10	Autumn	HS01 no water
6-Oct-11	Autumn	LW site only, LW01, LW02 no water
6-Jan-11	Winter	HS01 no water
25-Jan-11	Winter	LW site only, LW01 no water
6-Apr-11	Spring	LW site only
19-May-11	Summer	HS01, LP01, LP02 no water
2-Aug-11	Summer	LW site only, LW01, LW02 no water
30-Nov-11	Autumn	LW site only, LW01, LW02 no water

Table 3. Mean measured values of the parameters at each site.

Site	Temperature °C	pH	Specific Conductance µS/cm
BC	18.4	7.02	146
BP	17.1	4.95	38
HS	19.2	6.66	153
LL	18.1	4.93	67
LP	18.1	4.53	48
LW	16.1	5.47	52
PP	18.5	5.46	43

Table 4. p-values for the test of difference in downstream vs upstream values. The upper portion is by site, the lower portion is by seep. Yellow cells indicate the downstream value was less than the upstream value. For all other comparisons the relationship was the opposite. nd = insufficient data for this analysis.

Site name	Seep	Temperature °C	pH	Specific Conductance µS/cm
Bates Mill Creek	Both	0.003	<.0001	0.0011
Barnwell State Park	Both	0.929	0.1217	0.0268
Healing Springs	Both	0.164	0.0003	0.0064
Louthers Lake	Both	0.771	0.0014	0.0282
Lee State Park	Both	0.565	0.0006	0.6312
Luther Wannamaker	All	0.043	0.0004	0.3635
Poinsett State Park	Both	0.898	<.0001	0.0156
Bates Mill Creek	BC01	0.0029	0.0077	0.2172
	BC02	0.2283	<.0001	0.0003
Barnwell State Park	BP01	0.5057	0.0705	0.1326
	BP02	0.2837	0.4478	0.1081
Healing Springs	HS01	0.4631	0.0371	0.2954
	HS02	0.2347	0.0003	0.0124
Louthers Lake	LL01	0.9001	0.0001	0.5092
	LL02	0.2061	0.2862	0.0031
Lee State Park	LP01	0.3138	0.0146	0.2615
	LP02	0.8276	0.0371	0.8493
Luther Wannamaker	LW01	0.0434	0.0004	0.3635
	LW02	nd	nd	nd
	LW03	nd	nd	nd
	LW04	0.1766	0.0035	0.6913
	LW05	0.4625	0.6442	0.5637
	LW06	0.3805	0.0001	0.3213
Poinsett State Park	PP01	0.7705	0.0165	0.0137
	PP02	0.3248	<.0001	0.1333

The investigators will continue analysis of this data set with the goal of incorporating the salamander data and determining the relationship between seep characteristics and the salamander assemblage present.

Significant Deviations: None

Job 5. Gopher Frog Conservation and Habitat Management

Objective 1. Determine the current extent and status of the Gopher Frog in South Carolina based on breeding surveys and landscape analysis, and develop management recommendations for this species and its habitat.

Accomplishments:

Gopher Frog research was conducted primarily at two sites, one a known historical site, the Savannah River Site in Barnwell County the other a newly confirmed site, Webb Wildlife Center in Hampton County.


Adult male gopher frog from Webb Wildlife Center

Gopher Frogs were first documented at the Webb Wildlife Center in April of 2009, following a significant rainfall (10”+) event. There were historic records for this species from Hampton County, in the vicinity of the Webb Center, but no exact location was given. In 2009 four male Gopher Frogs were heard calling in a small depressional wetland. Two males were captured and out-fitted with radio transmitters, however they were able to slip out of the radio harnesses within a few days. Subsequent larval surveys were unsuccessful so there’s no evidence that they successfully reproduced that year.

In 2010 we initiated auditory surveys (i.e., time-constrained nocturnal surveys) for anurans with the primary objective of detecting Gopher Frog breeding ponds at three adjoining wildlife management areas, Webb Wildlife Center, Palachucola WMA and Hamilton Ridge WMA, with differing land-use histories. All of these properties are located in Hampton County, S.C. Historical USDA aerial photographs from March 1948 and 3.75 minute Digital Orthophoto Quarter Quads (DOQQs) (SC DNR GIS 2010) were used to identify and select upland isolated wetlands (i.e., amphibian breeding ponds) on the James W. Webb Wildlife Center, Palachucola Wildlife Management Area, and Hamilton Ridge Wildlife Management Area.

Inundations from 1948 winter and spring rainfalls provided high contrast between upland isolated wetlands and surrounding terrestrial habitats and enabled the visual identification of historical amphibian breeding ponds within the study area. A subset of ponds (n=42) were randomly selected and multiple 5 minute frog-call surveys were conducted at each pond (range = 2-8 surveys per pond). We conducted all surveys between February and May 2010.

We detected 18 Anuran species (Table 5-1) during surveys. We detected Gopher Frogs at three ponds at which we heard multiple calls. Further, we heard single calls at two additional ponds, but additional surveys have yet to confirm Gopher Frog breeding activity at these locations.

Isolated ponds at Webb, Hamilton Ridge, Palachucola, Bonneau Ferry and Donnelley WMAs were monitored during the late winter and spring of 2011 but no Gopher Frog breeding activity was documented at any of these sites. Automated recording devices, Song Meter SM2, available from Wildlife Acoustics Inc. were deployed at several of these sites but no calling male Gopher Frogs were recorded. While many of these ponds held water in the early winter a lack of late winter and early spring rains resulted in dry, or nearly dry breeding ponds, and as such a poor to moderate year for amphibian breeding.

Savannah River Site

The Carolina Gopher Frog (*Rana capito*) is a terrestrial ranid frog that inhabits coastal plain forests in a landscape that includes seasonal wetlands, including Carolina bays. The Gopher Frog requires seasonal wetlands for breeding and appears to prefer those with hydroperiods that retain water from the winter months through early summer, and are characteristically open canopy, grass-dominated, and have hard pan bottoms. The Gopher Frog is currently a State Endangered Species in South Carolina and is ranked S1/G3G4 and the species was identified as a priority species for conservation in South Carolina's State Wildlife Plan.

Craig's Pond (large bay in center), Sarracenia Bay (small bay, upper left) Long Bay (linear wetland, lower right) and the terrestrial vicinity

Craigs Pond is a large, ecologically intact Carolina Bay located partially on the U.S. Department of Energy's Savannah River Site and partially on private property belonging to Energy Solutions, Inc. These properties are currently being considered for inclusion in the Craigs Pond State Heritage Preserve and collectively contain at least nine shallow isolated depression wetlands (most of them Carolina bays). Craigs Pond is recognized as one of the finest remaining examples of an herbaceous depression meadow Carolina bay (Harry Shealy, Craigs Pond Advisory Committee, pers. comm.) and is considered to have significant conservation value. The U.S. Forest Service (USFS-SR) conducts prescribed burns on the U.S. Department of Energy's Savannah River Site. Prescribed burning has occurred periodically in both the Craigs Pond wetland and the surrounding uplands. Burning has also occurred on the Energy Solutions portion of the property by a hired consultant forester. More recently, the DOE Set-Aside Committee has become more interested in actively managing Set-Aside Research Areas on the SRS (L. Lee, Set-Aside Coordinator, pers comm). Although originally "set-aside" for research purposes and for use as environmental control sites, it is becoming apparent that many of the plant and animal features that were important considerations when the Set Aside areas were first designated may disappear without management intervention. These factors include landscape scale habitat fragmentation and interruption of natural fire regimes. As such, conservation attention is beginning to focus on the type, quality, condition, and management of the diverse upland habitats that surround Craigs Pond and the other bays.

Gopher Frogs are known to breed in Craigs Pond and a very large breeding chorus was heard at Craigs Pond on 26 February 2008 (K.A. Buhlmann and T. Luhring, pers obs). However, little information exists regarding the habitat, and micro-habitat, use of adult and metamorphic Gopher Frogs in the non-breeding season. Research conducted in North Carolina indicates that adult Gopher Frogs have terrestrial refugia that may be located between 505 m and 3470 m from the breeding wetland (J. Humphries and M. Sisson, SE PARC presentation, pers comm). Terrestrial refugia consist of small mammal burrows and burned out long-leaf pine stump and root holes. Where they occur, burrows of Gopher Frog (*Gopherus polyphemus)* are used.

Prescribed burning of upland pine forests is also suspected to improve terrestrial habitat conditions for Gopher Frogs. However, questions have been raised as to whether the timing of prescribed burning may adversely affect Gopher Frogs, either metamorphosing juveniles if the timing coincides with their emergence (presumably May or June), or the arrival of adults for breeding during the winter. Humphries and Sisson (unpublished data) have also documented mortality of radio-tracked adult Gopher Frogs during winter prescribed burns. During breeding migrations between stump-hole refugia and the wetland, frogs will temporarily reside and shelter in clumps of wiregrass and other bunch grasses where they may be vulnerable to prescribed winter fires. Thus, information is needed about Gopher Frog terrestrial habitat use for the overall management of the Craigs Pond Set-Aside and Natural Area.

Dip-netting and use of Fyke Nets

Our plan was to capture Gopher Frogs in the wetland by dipnet during their February-March breeding season, as we had done in February 2008. However, the appropriate climatic conditions (i.e., warm rains on warm winter nights) did not occur in 2010, and although several trips were made to listen for calling males, only two male Gopher Frogs were briefly heard calling (but not captured) in Craigs Pond on 21 March 2010 during a University of Georgia Herpetology class field trip. Thus, we knew that at least a few Gopher Frogs arrived at Craigs Pond for breeding in winter 2010. SREL purchased radio-transmitters with funding from SCDNR to attach to adult Gopher Frogs post-breeding season in 2010, but we were not able to use them. We erected several fyke nets in Craigs Pond in an effort to intercept adult Gopher Frogs, but were not successful. The nets did capture other amphibians, notably greater sirens (*Siren lacertina*), tiger salamanders (*Ambystoma tigrinum*, larvae), barking treefrogs (*Hyla gratiosa*, adults), southern toads In 2011 Craigs Pond did not fill with water for the February-March breeding season. However, the southern portion of Craigs Pond (located on Energy Solutions property) contains the deepest portion of the bay. In fact, this deep section appears to resemble a bay-within-a-bay and exhibits an extended hydroperiod, often holding water year round. We obtained permission from Energy Solutions (Mr. Jim Lathum) to listen for breeding Gopher Frogs and dipnet this section of the wetland. We found several paedomorphic mole salamanders (*Ambystoma talpoideum*), but did not detect


Carolina Gopher Frog at Craigs Pond during a breeding chorus on 26 February 2008

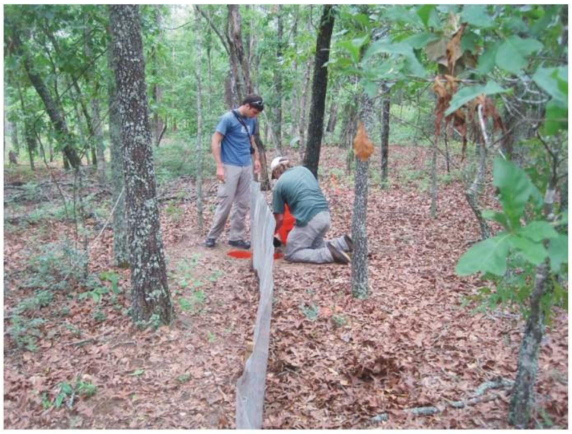

Fyke net set in Craigs Pond March-April 2010


The southern portion of Craigs pond that is semi-permanent 5 January 2011

Drift Fencing

Location of four drift fences erected May 2010 to intercept Gopher Frog metamorphs exiting Craigs pond

We erected four sections of drift fence, one each in four distinctly different terrestrial habitat types in the DOE Set-Aside portion of land surrounding Craigs Pond in April 2010. These habitats included 1) a predominantly scrub oak and pine woodland, 2) a mature loblolly stand, 3) a plantation loblolly stand, and 4) a sparsely forested longleaf pine stand that had been thinned by several hot prescribed burns and is approaching savanna-like conditions. These fences were constructed with aluminum flashing with five-gallon buckets to capture juvenile Gopher Frogs as they exited Craigs Pond during metamorphosis. These drift fences were operated for a short period of time when metamorphosis was occurring (24 May-11 June 2010) and did capture 18 metamorphic Gopher Frogs, indicating that at least some reproduction occurred in 2010, in spite of the very minimal breeding choruses detected and the lack of tadpoles found by dip-netting or use of the fyke net. The majority of Gopher Frog metamorphs found in June 2010 (N=11) were captured in the drift fence indicated as “burned thinned pine fence” in the right side of the Figure above. Four metamorphs were captured in the oak-pine fence, three metamorphs were captured in the young longleaf fence, and none in the plantation pine. A total of 17 amphibian and reptile species were captured in the drift fences during May-June 2010 (Table 5-2).


One of four 50 foot sections of drift fence erected in terrestrial habitat on DOE Set Aside land bordering Craigs Pond

During 2011, the drift fences were operated during February and March with the intent of intercepting migrating adult Gopher Frogs on their way to Craigs Pond from the surrounding uplands. One adult (sex uncertain, 100 mm) Gopher Frog was captured 5 April in a pitfall trap at the 1st drift fence in the oak-pine, NW side of Craigs Pond on its presumed migration towards the bay. It was fitted with a radio and entered the pond (which was dry), and slipped out of its radio 20 days (25 April) later in the center of the pond.

Head-starting 2011

We retained 11 of the 18 metamorphic Gopher Frogs captured in May-June 2010 in Animal Care facilities at the Savannah River Ecology Laboratory. Recently, both state and federal agencies have taken notice of

declines occurring within the gopher/crawfish frog (*Rana capito, R.. sevosa*, and *R.. areolata*) species complex and various reintroduction protocols have been established. We evaluated the use of head starting as a tool for population recovery and conservation of the Carolina Gopher Frog (*Rana capito*).
Our objectives were as follows:

1) Rear field collected metamorphic Gopher Frogs in captivity to larger, sub-adult size, presumably increasing their long-term probability of survival once released back into the wild.
2) Release the head-started sub-adult frogs back into natal wetland boundary and radio-track them in order to increase understanding of dispersal behavior and habitat use.
3) Monitor condition and survival of the released frogs to better understand future practices of Gopher Frog repatriation and reintroduction efforts in restored habitats.

The metamorphic Gopher Frogs (N = 11) were individually housed in 9.5 inch wide x 21.5 inch deep x 3.65 inch tall shoebox style plastic tubs and held in a stacked rack system (Freedom Breeder, Turlock, CA). Tubs were initially maintained with a wet, unbleached paper towel substrate and hiding cover. Frogs were fed twice weekly with captive reared crickets, dusted in a calcium/mineral supplement (small-medium sized).


Newly metamorphosed Carolina Gopher Frogs captured at Craigs pond drift fences in May and June 2010

Six of the metamorphic Gopher Frogs died within two weeks of being placed in captivity. These deaths were believed to be due to bacterial/fungus complications caused by excessive moisture in the rearing enclosures. We then changed the conditions of the plastic tub containers to approximate terrestrial conditions. Specifically, we cleaned and re-filled the tubs with sandy soil and organic leaf litter. We provided a glass bowl with water that the frogs could immerse themselves in, if they desired. Discussions with Mr. Scott Pfaff (Riverbanks Zoo) lead to the realization that housing conditions for these Ranid frogs, unlike other con-specifics, i.e., *Rana spenocephala*), needed to be regarded as *terrestrial* with the ability to access water, rather than *aquatic/moist* with ability to access land.

Following the change of substrate to a dry sand/organics mixture, no further deaths from health complications were observed. The head-started frogs were maintained in captivity for approximately 8 months (July 2010-February 2011).The remaining five frogs were reared from an average metamorphic size of 37 mm SUL, 3.4 g mass to 57.4 mm SUL, 35.4 g mass. The change in size represents a 55.1% increase in SUL, but a surprising 941.2 % increase in mass, indicating that recent metamorphs are thin (skinny) relative to sub-adults. Another way of looking at this data is through body condition which indicates that head-started Gopher Frogs had a body condition index of .092 (initial g / mm) at the time of metamorphosis but an index of .617 (final g / mm) after head-starting in captivity, representing a 6.71-fold increase (initial / final) in body condition index by the time of release.

Head-started Carolina Gopher Frog outfitted with radio transmitter

Radio-tracking of Head-started Frogs

The sub-adult frogs (N = 5) were fitted with 1.3 g BD-2, externally attached, radio transmitters (Holohil Systems, LTD). Radio belts were composed of plastic bead chains and a metal clasp (IndentiSys Inc, Eden Prairie, MN), attached to the transmitter with an epoxy bonding agent (Multi-purpose repair putty, Loctite, Rocky Hill, CT). Frogs were kept in captivity and monitored for a proper belt fit for~36 hours prior to release. Release occurred at the perimeter of their natal wetland (Craigs Pond), one hour after sunset under rainy conditions on 5 March 2011.

Individuals were located daily for the first 10 days following release and every third day thereafter via the homing technique. Individuals were visually inspected once a week, or as close to this as was possible. Tracking was conducted with a hand held, electronic receiver (R-1000, Communications Specialist Inc., Orange, CA) and a “rubber ducky” H-antenna (Telonics, Mesa, AZ). Locations were recorded with a hand held GPS (Garmin, Olathe, KS) and processed in ArcGIS (Esri Products, Redlands, CA).

Figure 10. Radio tagged gopher frogs, as located *in situ*. The top frog is characteristic of individuals prior to their locating a burrow or other type of refugia. In order to confirm condition and health, subterranean frogs were on occasion excavated from burrow systems, such as this one near the wetland boundary. Though burrow entrances were difficult to visually locate within the habitat, frogs that had been located at a burrow demonstrated a strong fidelity to their refugia sights, which included small mammal burrows, root holes and log cover.

Following release, one frog slipped out of its radio attachment and was lost from the study within the first 3 days (light-red dots east side Craigs Pond; Figure 5-1). The remaining frogs (N = 4) demonstrated 100% survival during the first 40 days of tracking, while making movements of up to 150 m from their release locations into the surrounding uplands (Figure 5-1). Frog movements in the wetland were often characterized as short and appeared random in direction. When individuals were found in the more upland type habitats, movements were away from the pond edge and into the surrounding forest. Individuals were located both above and below ground, using vegetative cover as well as small mammal burrows, root holes and cover logs. All animals maintained healthy body conditions throughout the study. One individual (dark-red dots in Sarracenia Bay; Figure 5-1) was consumed by a banded water snake (*Nerodia fasciata*) 45 days after release. The snake was captured by tracking the radio signal and was found under water and vegetation in Sarracenia Bay. Another head-started frog was found dead (skeleton) outside a burned out stump hole (blue-dot; Figure 5-1) after 50 days. On 25 April (50 d after release), the transmitter of a head-started frog (orange-dots; Figure 5-1) was found inside a burned-out stump; the frog was presumed still alive. We note that the orange-dot frog had also visited Sarracennia Bay on 19 April and was observed alive beneath 3 inches of water. On 25 April, a head-started frog (green-dots; Figure 5-1) was found alive in a small burrow at the interface of the Craigs wetland and upland. Thus, after 50 days from release, 2 of the 5 head-starts were known dead (blue-dots-skeleton found; dark red-dots snake eaten), 2 had lost their transmitters (light red-dots and orange-dots), and 1 (greendot) was known alive. The green-dot head-start was still in that burrow on 6 June (91 d after release), but could not be located one week later.

Discussion

Our drift fence sections were successful at intercepting metamorphic Gopher Frogs as they exited Craigs Pond in May-June 2010. We note that the 4t drift fence segment on the northeastern side of Craigs Pond (easternmost side of Craigs Pond) captured the greatest number of Gopher Frogs and was the most frequently burned and had the sparsest canopy cover of any portion of the north-side of the bay. If metamorphic Gopher Frogs exit the wetland and immediately seek long-leaf pine savanna habitat (i.e., Roznik and Johnson 2007), then the area would be attractive and preferable compared to the other terrestrial forested choices.

We also note that only one adult Gopher Frog was captured at the drift fence in 2011, as it presumably migrated towards Craigs Pond. The capture of one individual suggests that at least a few adults may move towards the pond for breeding even if the wetland does not contain standing water. We hope that in future years we may be able to radio-track several adults back to their terrestrial refugia, and then determine when and under what conditions they migrate towards the wetland for breeding. Given that the Southeast U.S. in currently in a severe drought (Figure 13), we are not certain when the opportunity may

Our preliminary head-starting results suggest that rearing young frogs in captivity may be a viable method to increase survivorship of individuals used in reintroduction programs. Future projects should experiment with additional release techniques, such as releasing frogs directly into upland habitats or refugia, and including much more long-term field observations (i.e., one year or more) to determine whether head started frogs return to their release wetlands for breeding. Also, we released head-started frogs in March, which is not a month when metamorphic frogs would normally be entering the terrestrial habitat for the first time. In the future we might conduct releases in the months of May or June when Gopher Frogs typically migrate from wetlands.

Habitat Management for Gopher Frogs on the Savannah River Site may be entering a new phase of opportunity. Recently, the USFS-SR hired a new prescribed fire manager who is particularly interested in long-leaf pine/wiregrass restoration, as well as exploring wetland restoration through use of fire. In autumn 2011, and in collaboration with SREL, another Gopher Frog breeding site, Mona Bay, was burned to reduce sapling pine encroachment and reduce transpiration by trees. Mona Bay is located one thousand meters north of Craigs Pond and given the known distances that Gopher Frogs are known to move terrestrially, is likely part of the same metapopulation of Gopher Frogs as the Craigs Pond population. Concurrently, discussions regarding the need for habitat management actions in DOE Set-Asides (including Craigs Pond, Mona/Woodward Bays complexes, Dry Bay, and Ellenton Bay) have recently begun. In conclusion, we are hopeful that management on the SRS may be entering a new phase where endangered and threatened amphibian and reptile species may be beneficiaries of new initiatives.

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T-26 Final Report

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FIGURES
And
TABLES
(not included in report text)

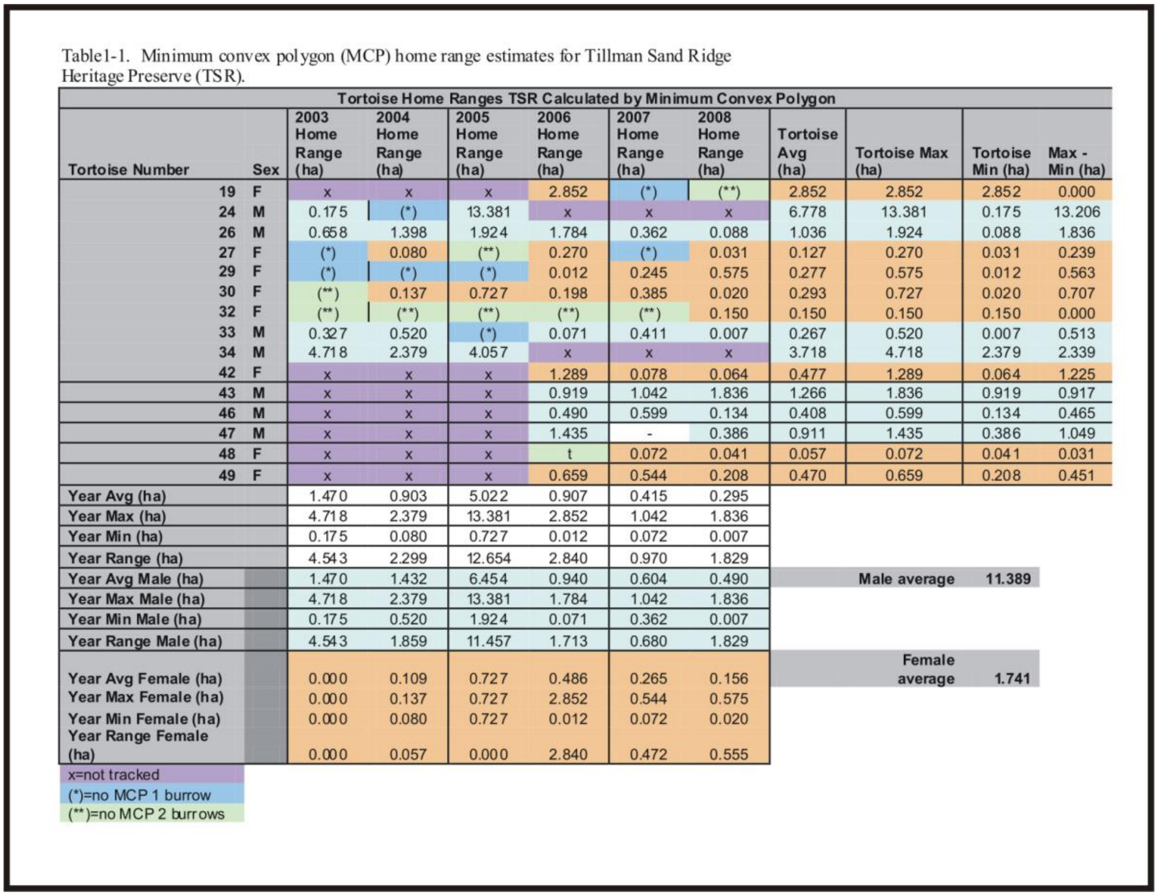


Table1-1. Minimum convex polygon (MCP) home range estimates for Tillman Sand Ridge Heritage Preserve (TSR).

Tortoise Home Ranges TSR Calculated by Minimum Convex Polygon												
Tortoise Number	Sex	2003 Home Range (ha)	2004 Home Range (ha)	2005 Home Range (ha)	2006 Home Range (ha)	2007 Home Range (ha)	2008 Home Range (ha)	Tortoise Avg (ha)	Tortoise Max (ha)	Tortoise Min (ha)	Max - Min (ha)
19	F	x	x	x	2.852	(*)	(**)	2.852	2.852	2.852	0.000
24	M	0.175	(*)	13.381	x	x	x	6.778	13.381	0.175	13.206
26	M	0.658	1.398	1.924	1.784	0.362	0.088	1.036	1.924	0.088	1.836
27	F	(*)	0.080	(**)	0.270	(*)	0.031	0.127	0.270	0.031	0.239
29	F	(*)	(*)	(*)	0.012	0.245	0.575	0.277	0.575	0.012	0.563
30	F	(**)	0.137	0.727	0.198	0.385	0.020	0.293	0.727	0.020	0.707
32	F	(**)	(**)	(**)	(**)	(**)	0.150	0.150	0.150	0.150	0.000
33	M	0.327	0.520	(*)	0.071	0.411	0.007	0.267	0.520	0.007	0.513
34	M	4.718	2.379	4.057	x	x	x	3.718	4.718	2.379	2.339
42	F	x	x	x	1.289	0.078	0.064	0.477	1.289	0.064	1.225
43	M	x	x	x	0.919	1.042	1.836	1.266	1.836	0.919	0.917
46	M	x	x	x	0.490	0.599	0.134	0.408	0.599	0.134	0.465
47	M	x	x	x	1.435	-	0.386	0.911	1.435	0.386	1.049
48	F	x	x	x	t	0.072	0.041	0.057	0.072	0.041	0.031
49	F	x	x	x	0.659	0.544	0.208	0.470	0.659	0.208	0.451
Year Avg (ha)		1.470	0.903	5.022	0.907	0.415	0.295				
Year Max (ha)		4.718	2.379	13.381	2.852	1.042	1.836				
Year Min (ha)		0.175	0.080	0.727	0.012	0.072	0.007				
Year Range (ha)		4.543	2.299	12.654	2.840	0.970	1.829				
Year Avg Male (ha)		1.470	1.432	6.454	0.940	0.604	0.490		Male average	11.389	
Year Max Male (ha)		4.718	2.379	13.381	1.784	1.042	1.836				
Year Min Male (ha)		0.175	0.520	1.924	0.071	0.362	0.007				
Year Range Male (ha)		4.543	1.859	11.457	1.713	0.680	1.829				
Year Avg Female (ha)		0.000	0.109	0.727	0.486	0.265	0.156		Female average	1.741	
Year Max Female (ha)		0.000	0.137	0.727	2.852	0.544	0.575				
Year Min Female (ha)		0.000	0.080	0.727	0.012	0.072	0.020				
Year Range Female (ha)		0.000	0.057	0.000	2.840	0.472	0.555				

x=not tracked

(*)=no MCP 1 burrow

(**)=no MCP 2 burrows

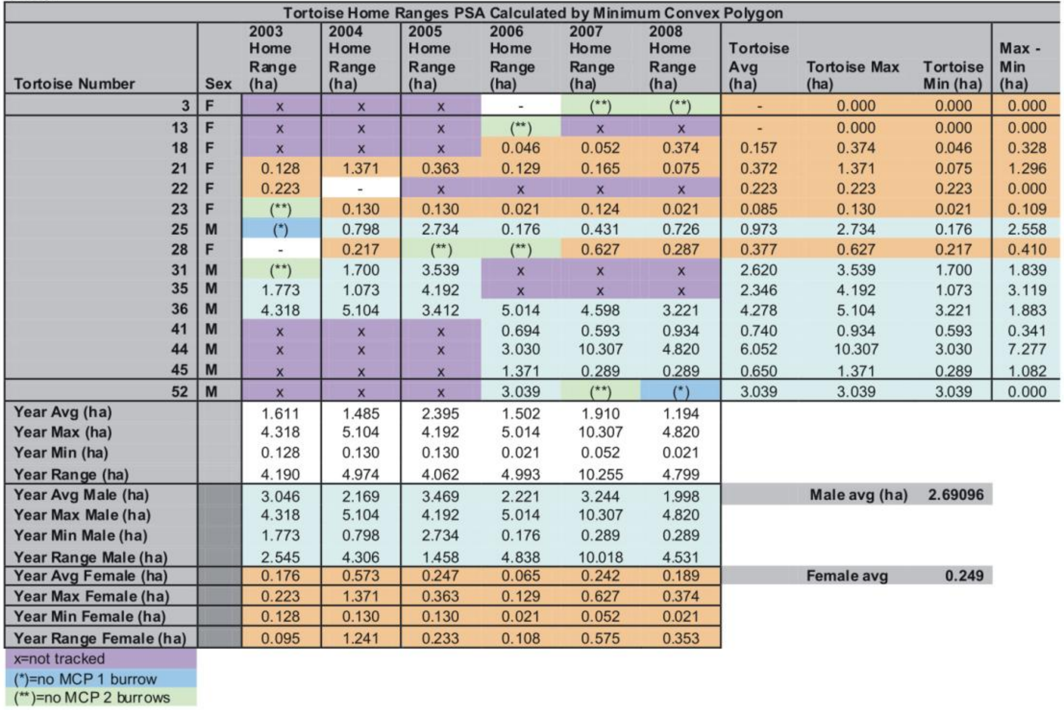


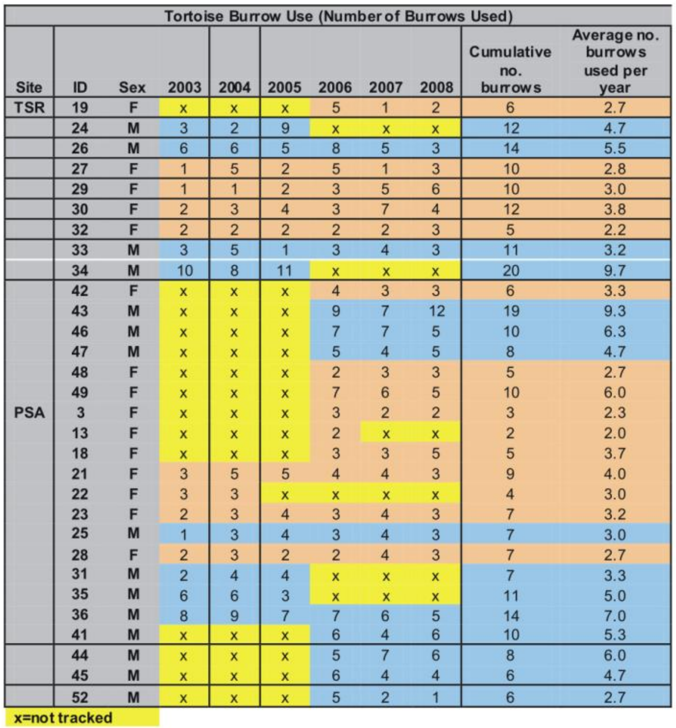
Table 1-2. Minimum convex polygon (MCP) home range estimates for Public Service Authority (PSA).

Tortoise Home Ranges PSA Calculated by Minimum Convex Polygon

Tortoise Number	Sex	2003 Home Range (ha)	2004 Home Range (ha)	2005 Home Range (ha)	2006 Home Range (ha)	2007 Home Range (ha)	2008 Home Range (ha)	Tortoise Avg (ha)	Tortoise Max (ha)	Tortoise Min (ha)	Max - Min (ha)
3	F	x	x	x	-	(**)	(**)	-	0.000	0.000	0.000
13	F	x	x	x	(**)	x	x	-	0.000	0.000	0.000
18	F	x	x	x	0.046	0.052	0.374	0.157	0.374	0.046	0.328
21	F	0.128	1.371	0.363	0.129	0.165	0.075	0.372	1.371	0.075	1.296
22	F	0.223	-	x	x	x	x	0.223	0.223	0.223	0.000
23	F	(**)	0.130	0.130	0.021	0.124	0.021	0.085	0.130	0.021	0.109
25	M	(*)	0.798	2.734	0.176	0.431	0.726	0.973	2.734	0.176	2.558
28	F	-	0.217	(**)	(**)	0.627	0.287	0.377	0.627	0.217	0.410
31	M	(**)	1.700	3.539	x	x	x	2.620	3.539	1.700	1.839
35	M	1.773	1.073	4.192	x	x	x	2.346	4.192	1.073	3.119
36	M	4.318	5.104	3.412	5.014	4.598	3.221	4.278	5.104	3.221	1.883
41	M	x	x	x	0.694	0.593	0.934	0.740	0.934	0.593	0.341
44	M	x	x	x	3.030	10.307	4.820	6.052	10.307	3.030	7.277
45	M	x	x	x	1.371	0.289	0.289	0.650	1.371	0.289	1.082
52	M	x	x	x	3.039	(**)	(*)	3.039	3.039	3.039	0.000
Year Avg (ha)		1.611	1.485	2.395	1.502	1.910	1.194				
Year Max (ha)		4.318	5.104	4.192	5.014	10.307	4.820				
Year Min (ha)		0.128	0.130	0.130	0.021	0.052	0.021				
Year Range (ha)		4.190	4.974	4.062	4.993	10.255	4.799				
Year Avg Male (ha)		3.046	2.169	3.469	2.221	3.244	1.998		**Male avg (ha)**	**2.69096**	
Year Max Male (ha)		4.318	5.104	4.192	5.014	10.307	4.820				
Year Min Male (ha)		1.773	0.798	2.734	0.176	0.289	0.289				
Year Range Male (ha)		2.545	4.306	1.458	4.838	10.018	4.531				
Year Avg Female (ha)		0.176	0.573	0.247	0.065	0.242	0.189		**Female avg**	**0.249**	
Year Max Female (ha)		0.223	1.371	0.363	0.129	0.627	0.374				
Year Min Female (ha)		0.128	0.130	0.130	0.021	0.052	0.021				
Year Range Female (ha)		0.095	1.241	0.233	0.108	0.575	0.353				

x=not tracked
(*)=no MCP 1 burrow
(**)=no MCP 2 burrows

Table 1-3. Burrow use by radio-tracked gopher tortoises at TSR and PSA.



Tortoise Burrow Use (Number of Burrows Used)										
Site	ID	Sex	2003	2004	2005	2006	2007	2008	Cumulative no. burrows	Average no. burrows used per year
TSR	19	F	x	x	x	5	1	2	6	2.7
	24	M	3	2	9	x	x	x	12	4.7
	26	M	6	6	5	8	5	3	14	5.5
	27	F	1	5	2	5	1	3	10	2.8
	29	F	1	1	2	3	5	6	10	3.0
	30	F	2	3	4	3	7	4	12	3.8
	32	F	2	2	2	2	2	3	5	2.2
	33	M	3	5	1	3	4	3	11	3.2
	34	M	10	8	11	x	x	x	20	9.7
PSA	42	F	x	x	x	4	3	3	6	3.3
	43	M	x	x	x	9	7	12	19	9.3
	46	M	x	x	x	7	7	5	10	6.3
	47	M	x	x	x	5	4	5	8	4.7
	48	F	x	x	x	2	3	3	5	2.7
	49	F	x	x	x	7	6	5	10	6.0
	3	F	x	x	x	3	2	2	3	2.3
	13	F	x	x	x	2	x	x	2	2.0
	18	F	x	x	x	3	3	5	5	3.7
	21	F	3	5	5	4	4	3	9	4.0
	22	F	3	3	x	x	x	x	4	3.0
	23	F	2	3	4	3	4	3	7	3.2
	25	M	1	3	4	3	4	3	7	3.0
	28	F	2	3	2	2	4	3	7	2.7
	31	M	2	4	4	x	x	x	7	3.3
	35	M	6	6	3	x	x	x	11	5.0
	36	M	8	9	7	7	6	5	14	7.0
	41	M	x	x	x	6	4	6	10	5.3
	44	M	x	x	x	5	7	6	8	6.0
	45	M	x	x	x	6	4	4	6	4.7
	52	M	x	x	x	5	2	1	6	2.7

x=not tracked

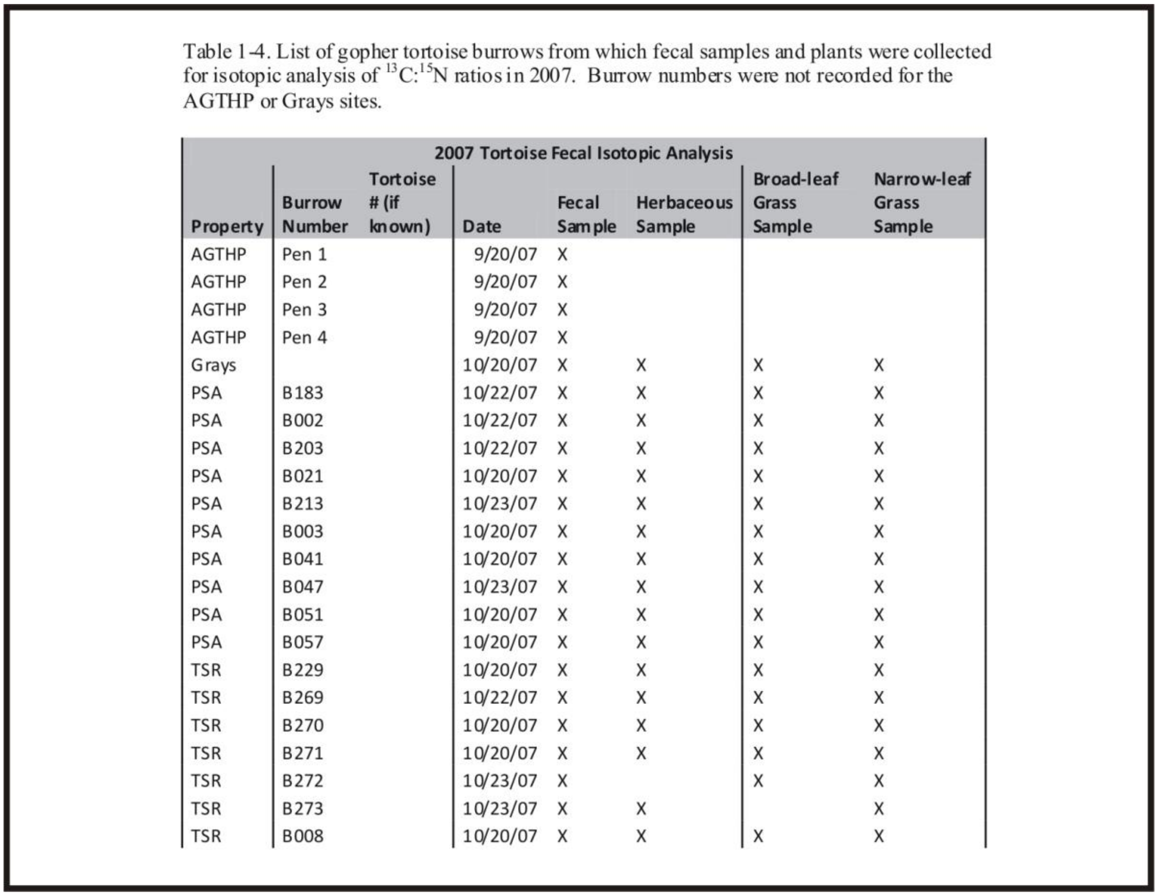


Table 1-4. List of gopher tortoise burrows from which fecal samples and plants were collected for isotopic analysis of ^{13}C:^{15}N ratios in 2007. Burrow numbers were not recorded for the AGTHP or Grays sites.

2007 Tortoise Fecal Isotopic Analysis							
Property	Burrow Number	Tortoise # (if known)	Date	Fecal Sample	Herbaceous Sample	Broad-leaf Grass Sample	Narrow-leaf Grass Sample
AGTHP	Pen 1		9/20/07	X			
AGTHP	Pen 2		9/20/07	X			
AGTHP	Pen 3		9/20/07	X			
AGTHP	Pen 4		9/20/07	X			
Grays			10/20/07	X	X	X	X
PSA	B183		10/22/07	X	X	X	X
PSA	B002		10/22/07	X	X	X	X
PSA	B203		10/22/07	X	X	X	X
PSA	B021		10/20/07	X	X	X	X
PSA	B213		10/23/07	X	X	X	X
PSA	B003		10/20/07	X	X	X	X
PSA	B041		10/20/07	X	X	X	X
PSA	B047		10/23/07	X	X	X	X
PSA	B051		10/20/07	X	X	X	X
PSA	B057		10/20/07	X	X	X	X
TSR	B229		10/20/07	X	X	X	X
TSR	B269		10/22/07	X	X	X	X
TSR	B270		10/20/07	X	X	X	X
TSR	B271		10/20/07	X	X	X	X
TSR	B272		10/23/07	X		X	X
TSR	B273		10/23/07	X	X		X
TSR	B008		10/20/07	X	X	X	X

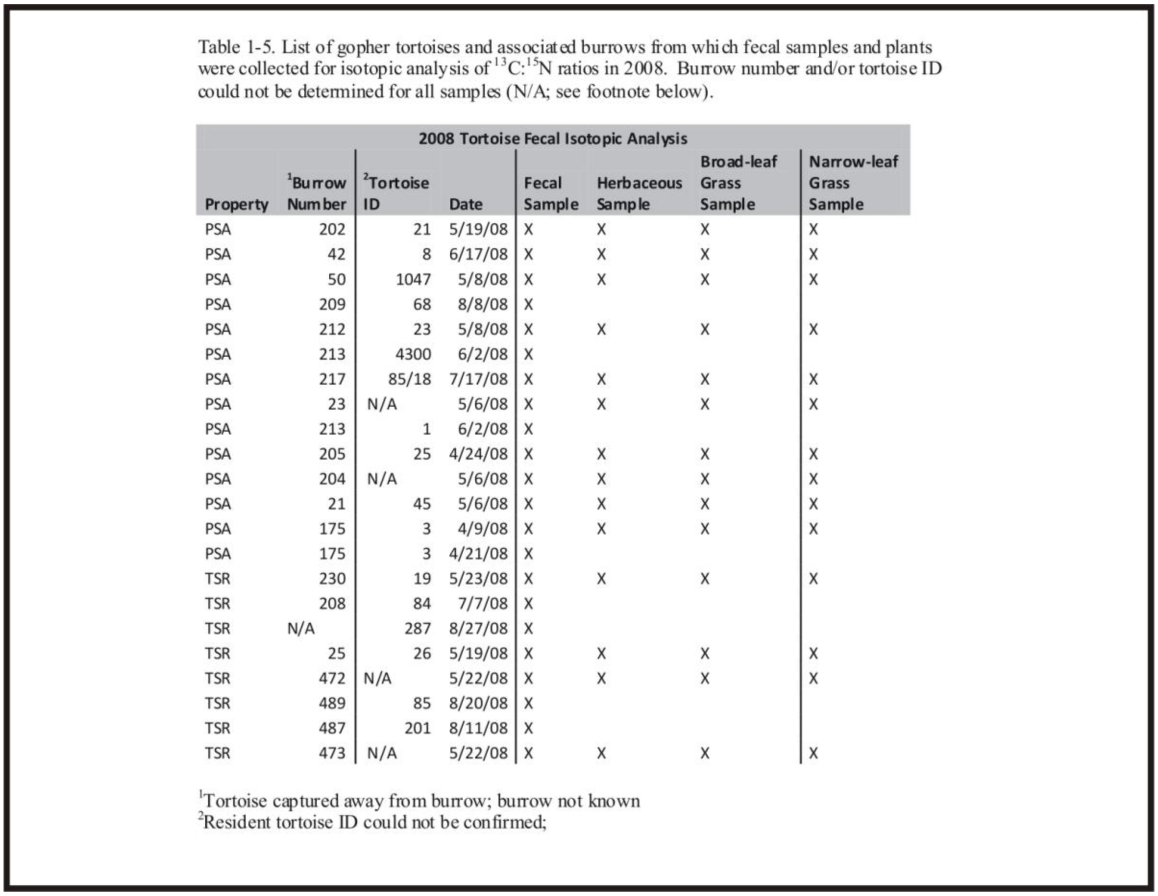


Table 1-5. List of gopher tortoises and associated burrows from which fecal samples and plants were collected for isotopic analysis of ^{13}C:^{15}N ratios in 2008. Burrow number and/or tortoise ID could not be determined for all samples (N/A; see footnote below).

2008 Tortoise Fecal Isotopic Analysis

Property	[1]Burrow Number	[2]Tortoise ID	Date	Fecal Sample	Herbaceous Sample	Broad-leaf Grass Sample	Narrow-leaf Grass Sample
PSA	202	21	5/19/08	X	X	X	X
PSA	42	8	6/17/08	X	X	X	X
PSA	50	1047	5/8/08	X	X	X	X
PSA	209	68	8/8/08	X			
PSA	212	23	5/8/08	X	X	X	X
PSA	213	4300	6/2/08	X			
PSA	217	85/18	7/17/08	X	X	X	X
PSA	23	N/A	5/6/08	X	X	X	X
PSA	213	1	6/2/08	X			
PSA	205	25	4/24/08	X	X	X	X
PSA	204	N/A	5/6/08	X	X	X	X
PSA	21	45	5/6/08	X	X	X	X
PSA	175	3	4/9/08	X	X	X	X
PSA	175	3	4/21/08	X			
TSR	230	19	5/23/08	X	X	X	X
TSR	208	84	7/7/08	X			
TSR	N/A	287	8/27/08	X			
TSR	25	26	5/19/08	X	X	X	X
TSR	472	N/A	5/22/08	X	X	X	X
TSR	489	85	8/20/08	X			
TSR	487	201	8/11/08	X			
TSR	473	N/A	5/22/08	X	X	X	X

[1] Tortoise captured away from burrow; burrow not known

[2] Resident tortoise ID could not be confirmed;

Figure 1-1. Graphical representation of isotopic analysis of 2007 fecal, broadleaf grass (BLG), narrowleaf grass (NLG), and herbaceous plants (H) collected from gopher tortoise populations in South Carolina. Polygons have been drawn around symbols associated with each sample type to aid in visualization of isotopic signature of each group and identify those groups that overlap.

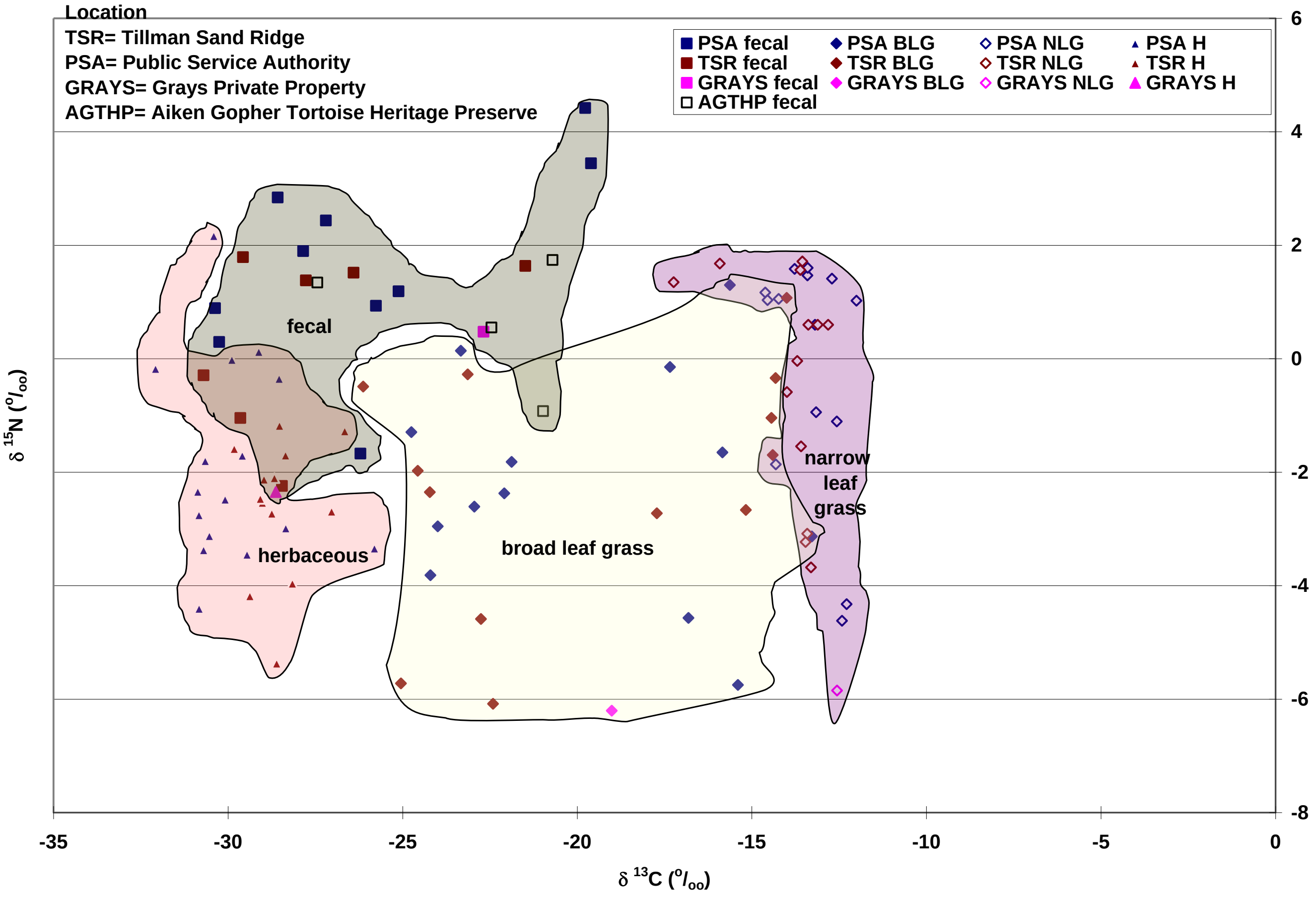


Figure 1-2. Graphical representation of isotopic analysis of 2007 and 2008 fecal, broadleaf grass (BLG), narrowleaf grass (NLG), and herbaceous plants (H) collected from gopher tortoise populations in South Carolina. Polygons depicted in this figure are based on 2007 data only.

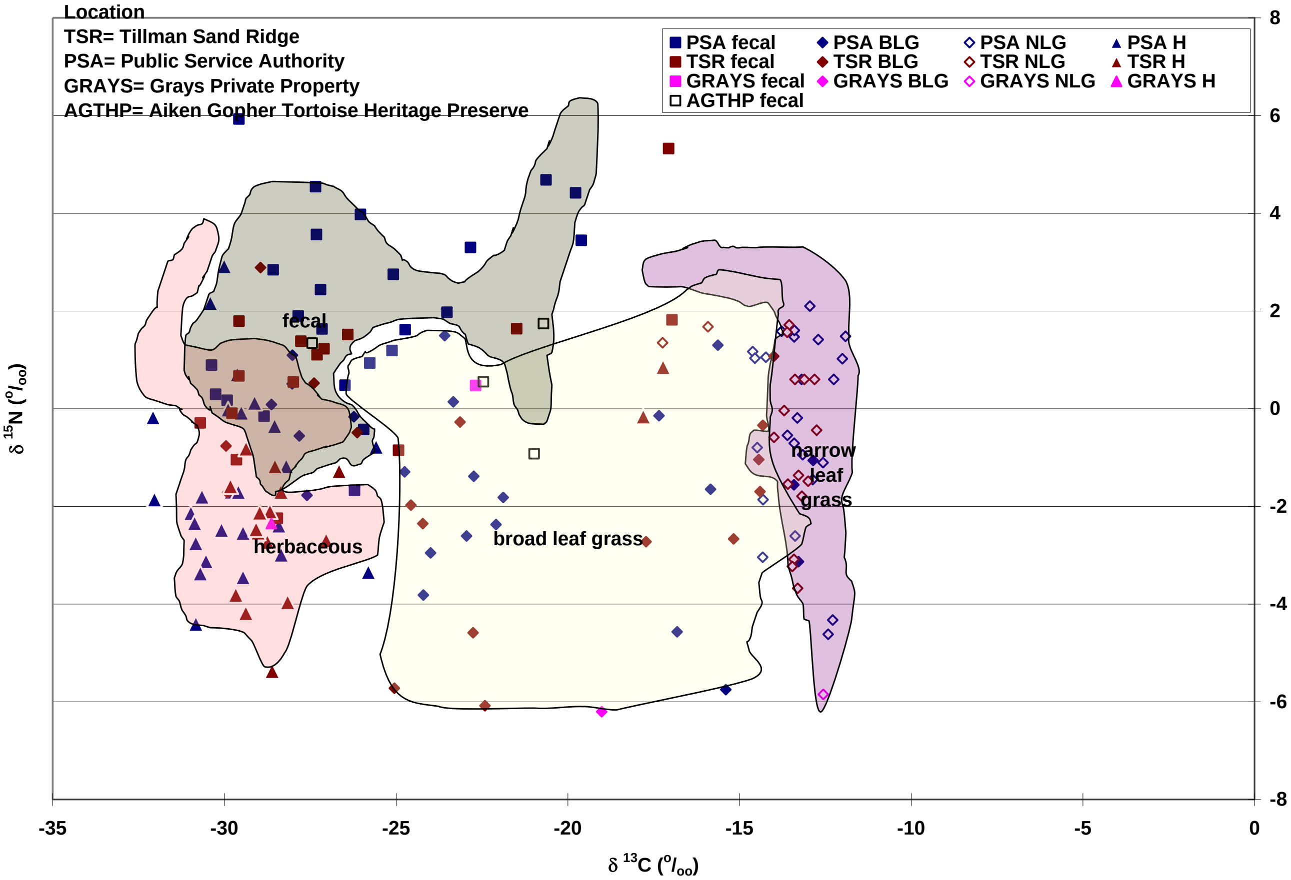
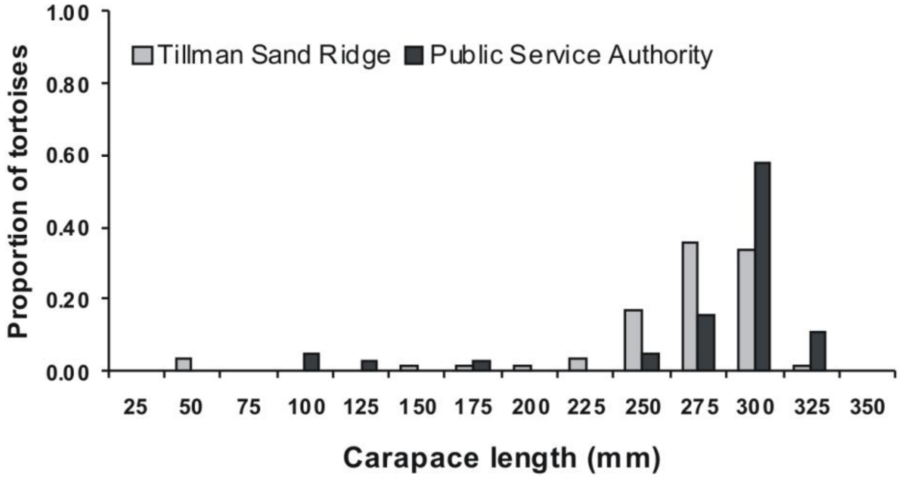


Figure 1-3. Size distribution of gopher tortoises captured at Tillman Sand Ridge (n=59) and Public Service Authority (n=38) during based on first capture. Hatchlings released from protected nests are not included.



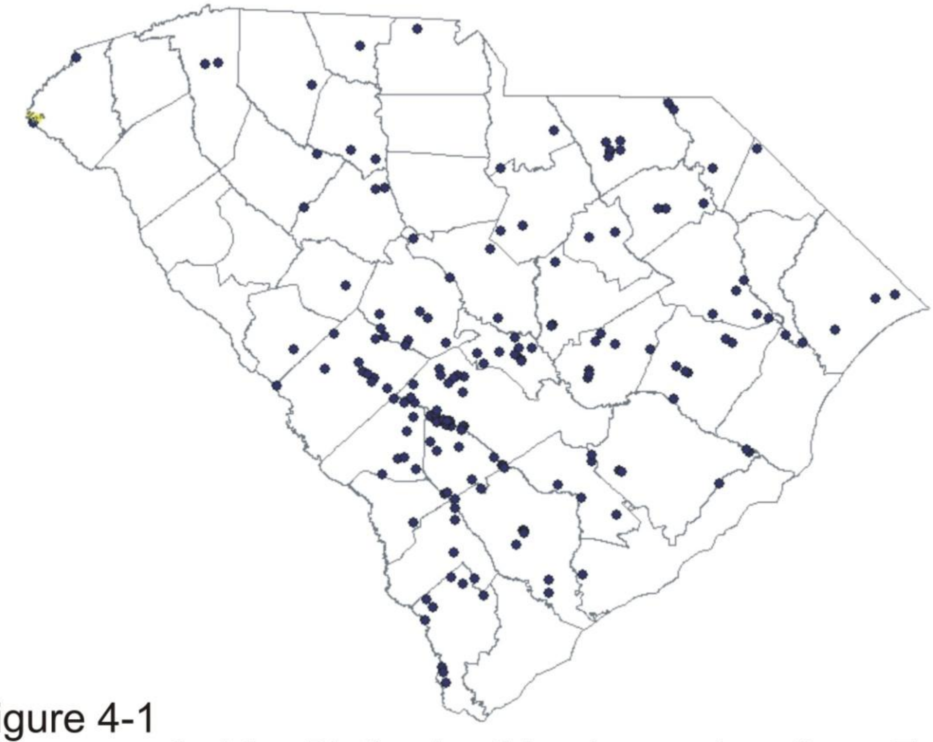


Figure 4-1
Sites sampled for Plethodontid salamanders from Fy07 through Fy11

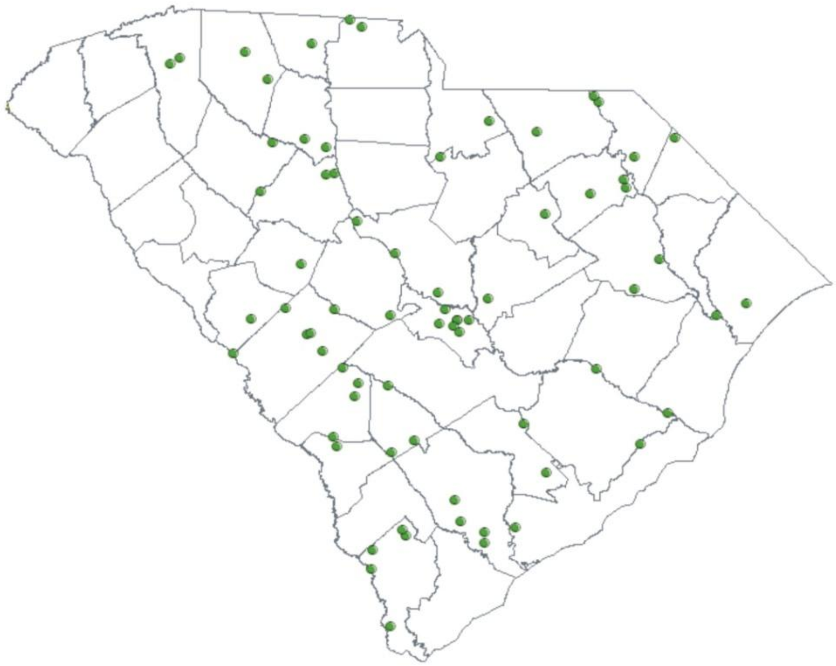


Figure 4-2 Sites where the Dusky Salamanders currently assigned to either *Desmognathus auriculatus* or *D. fuscus* were documented

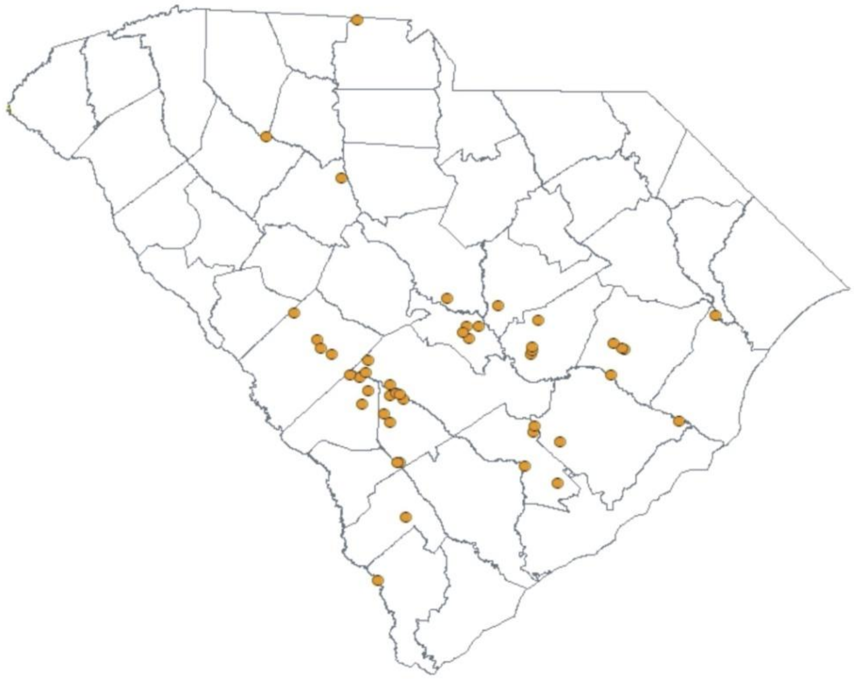


Figure 4-3 Sites where the Three-lined Salamander (*Eurycea guttolineata*) was documented

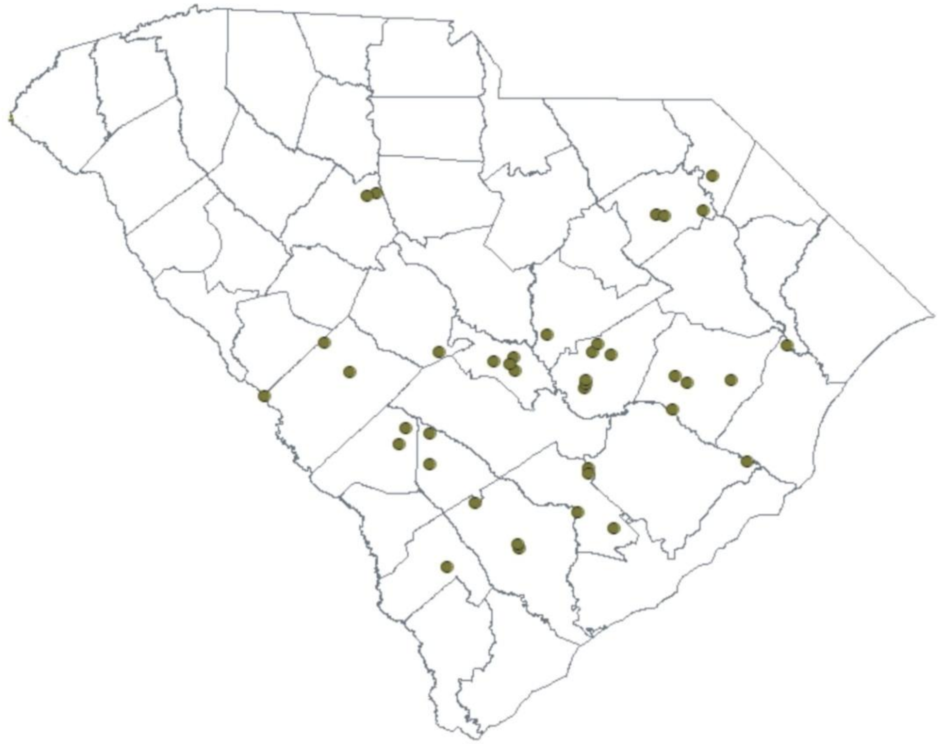


Figure 4-4 Sites where the Southern Two-lined Salamander (*Eurycea cirrigera*) was documented

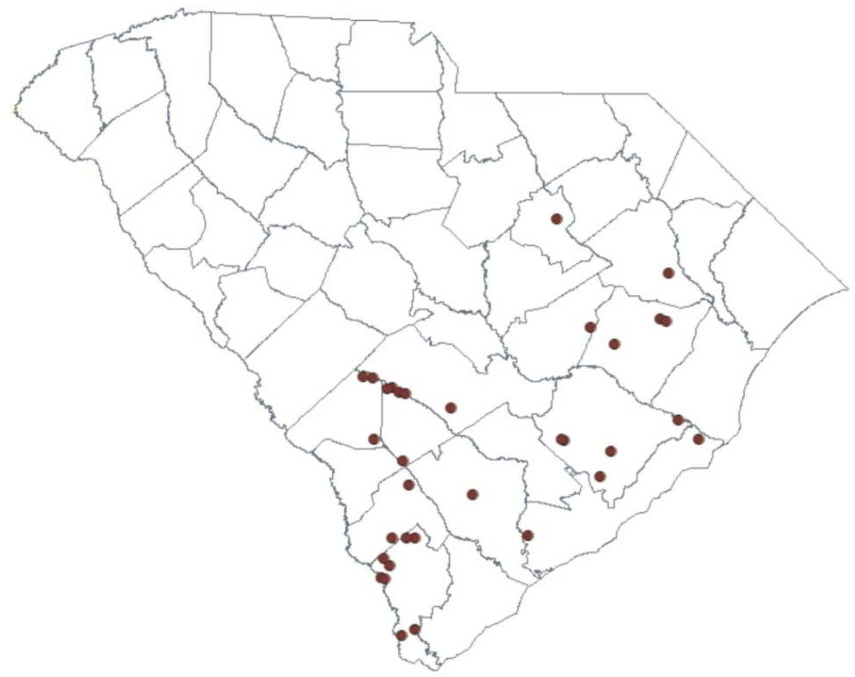


Figure 4-5 Sites where the Dwarf Salamander (*Eurycea quadridigitata*) was documented

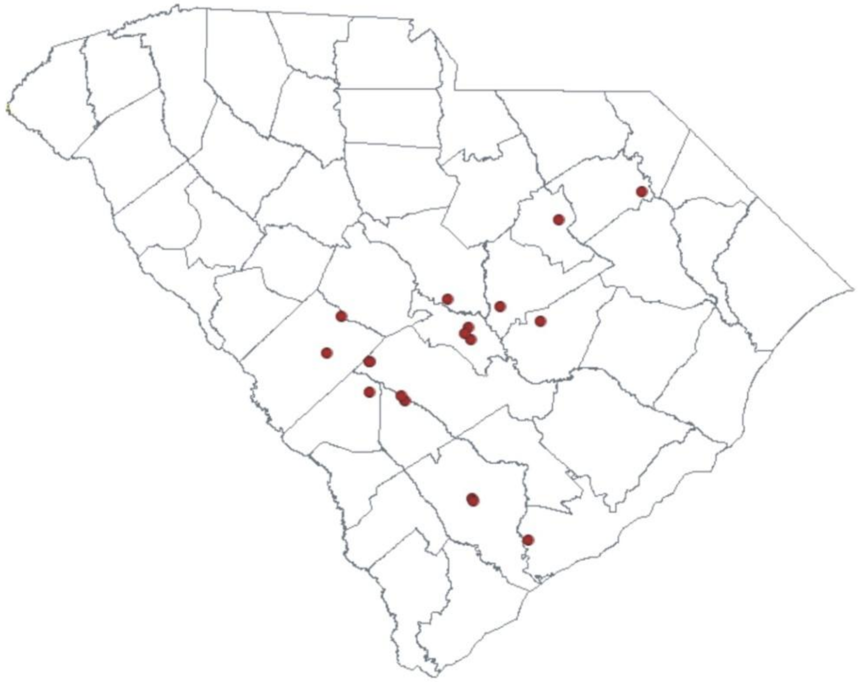


Figure 4-6 Sites where the Mud Salamander (*Pseudotriton montanus*) was documented

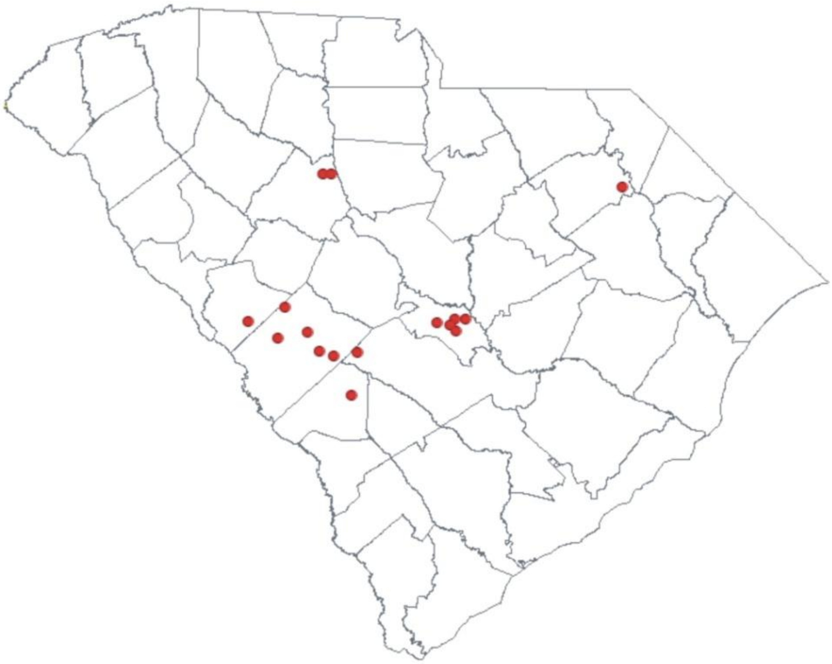


Figure 4-7 Sites where the Red Salamander (*Pseudotriton ruber*) was documented

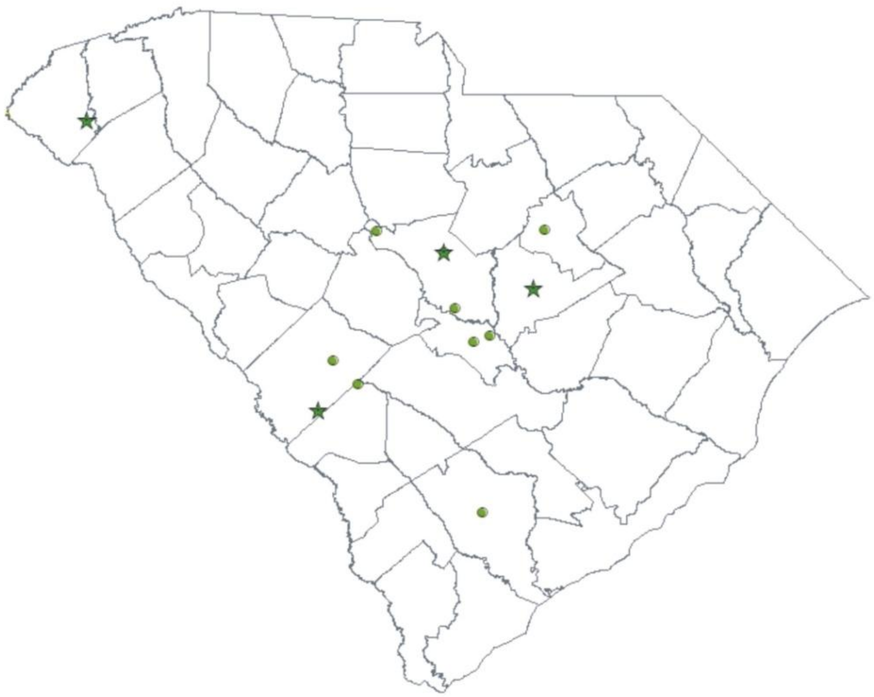


Figure 4-8 Sites where Chamberlain's Dwarf Salamander (*Eurycea chamberlaini*) was documented, stars are historic locations

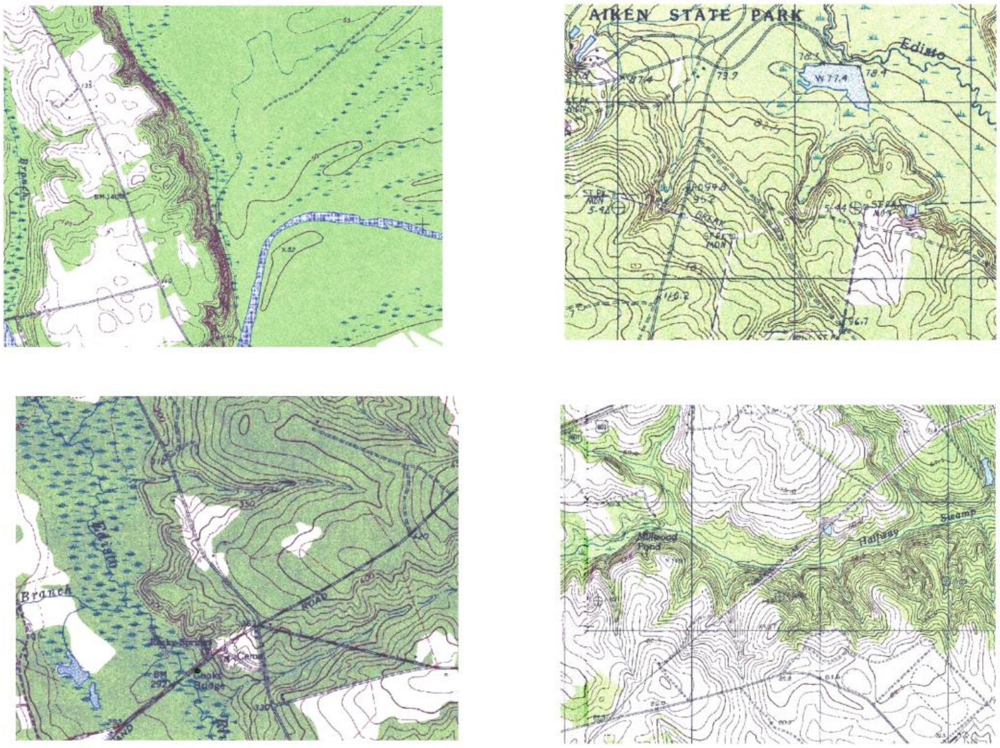
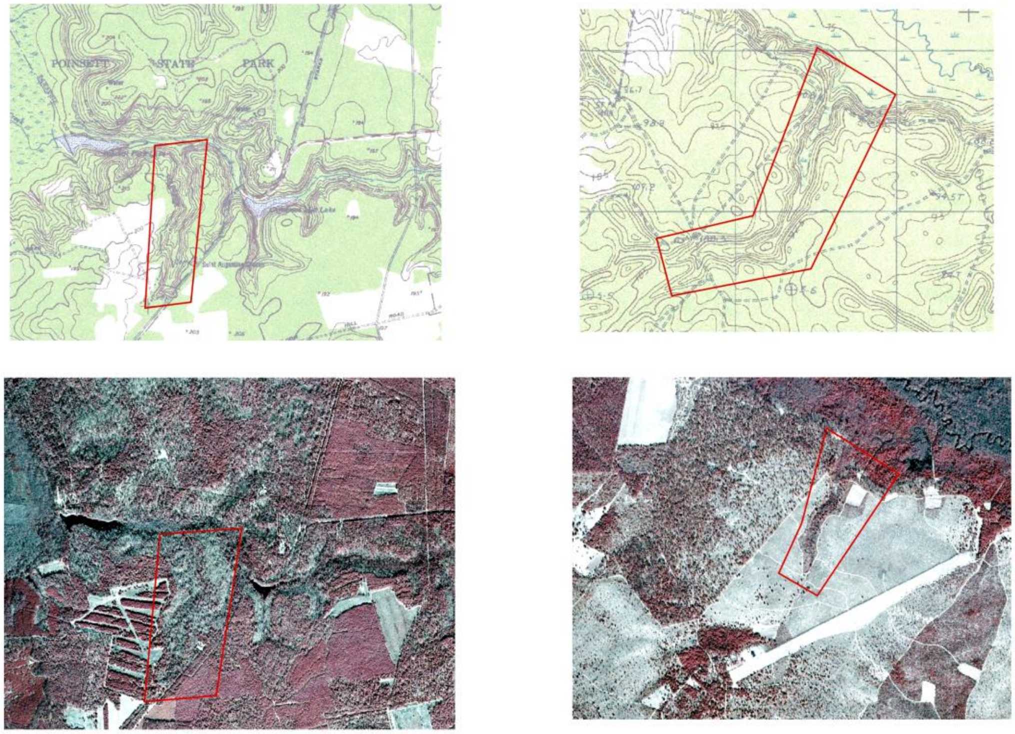


Figure 4-9 Seep topography



Acceptable

Not acceptable

Figure 4-10 Comparison of seep topography and aerial photography

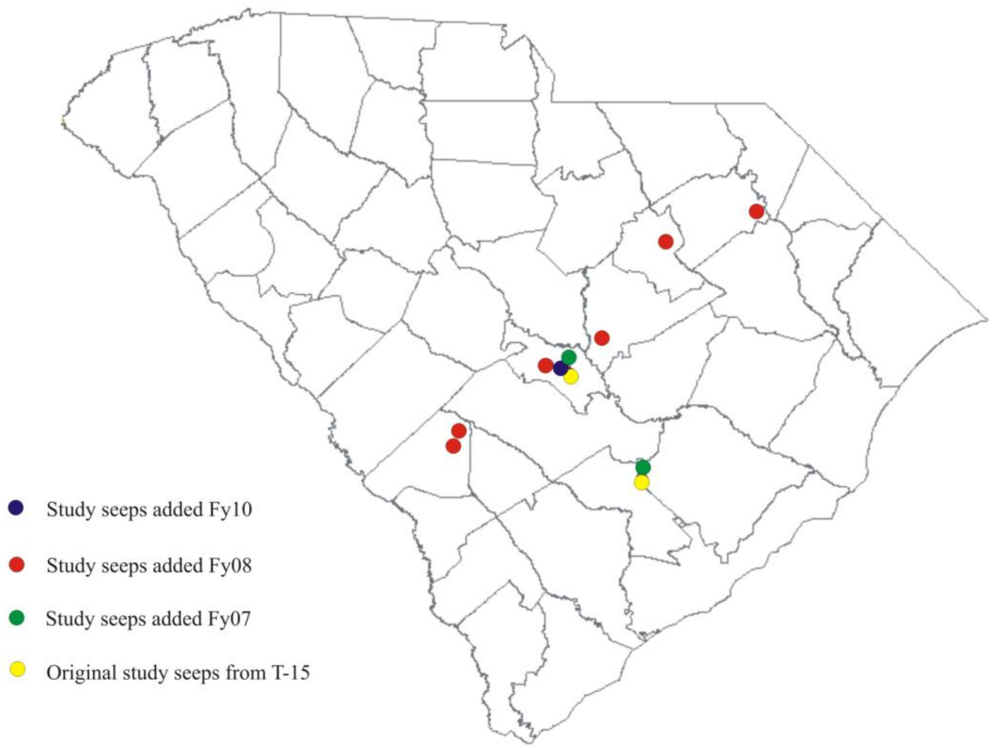


Figure 4-11 Location of all study seeps sampled FY07-FY11

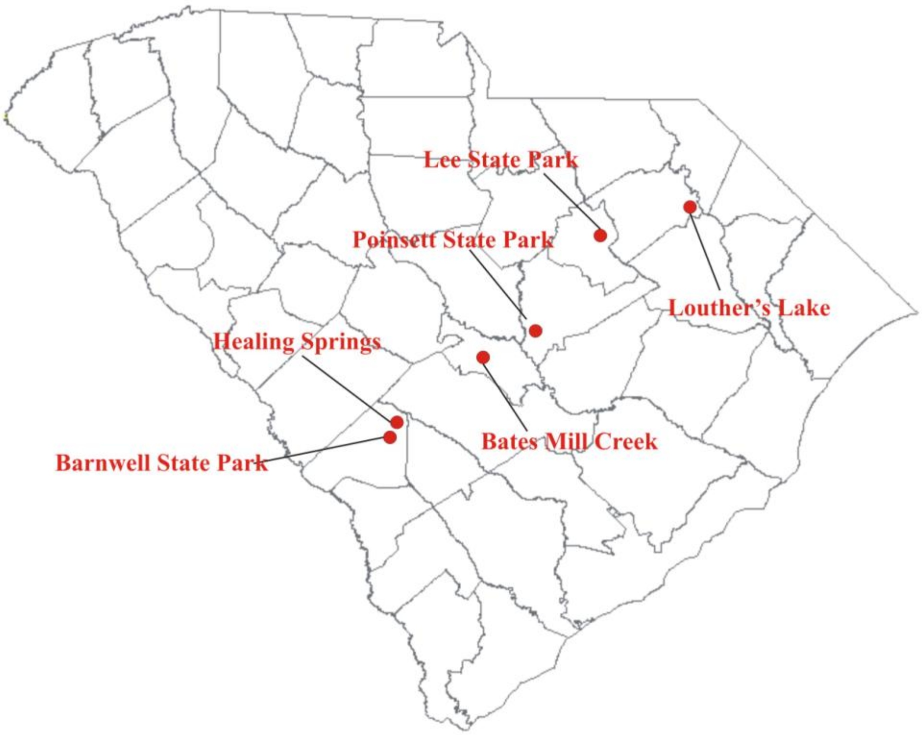
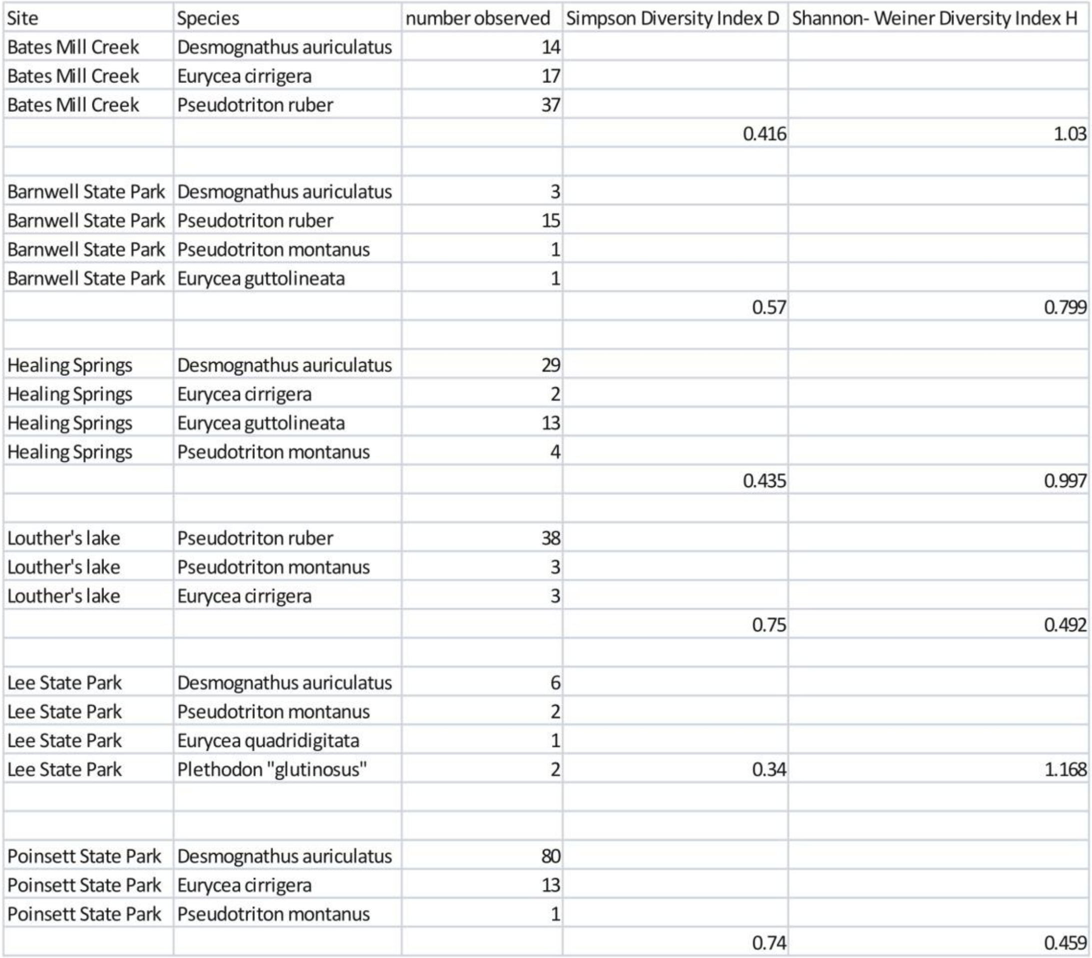


Figure 4-12 Upper Coastal Plain study seeps established in Fy08



Site	Species	number observed	Simpson Diversity Index D	Shannon- Weiner Diversity Index H
Bates Mill Creek	Desmognathus auriculatus	14		
Bates Mill Creek	Eurycea cirrigera	17		
Bates Mill Creek	Pseudotriton ruber	37		
			0.416	1.03
Barnwell State Park	Desmognathus auriculatus	3		
Barnwell State Park	Pseudotriton ruber	15		
Barnwell State Park	Pseudotriton montanus	1		
Barnwell State Park	Eurycea guttolineata	1		
			0.57	0.799
Healing Springs	Desmognathus auriculatus	29		
Healing Springs	Eurycea cirrigera	2		
Healing Springs	Eurycea guttolineata	13		
Healing Springs	Pseudotriton montanus	4		
			0.435	0.997
Louther's lake	Pseudotriton ruber	38		
Louther's lake	Pseudotriton montanus	3		
Louther's lake	Eurycea cirrigera	3		
			0.75	0.492
Lee State Park	Desmognathus auriculatus	6		
Lee State Park	Pseudotriton montanus	2		
Lee State Park	Eurycea quadridigitata	1		
Lee State Park	Plethodon "glutinosus"	2	0.34	1.168
Poinsett State Park	Desmognathus auriculatus	80		
Poinsett State Park	Eurycea cirrigera	13		
Poinsett State Park	Pseudotriton montanus	1		
			0.74	0.459

Table 4-1 Summary of salamander observations at Upper Coastal Plain study sites

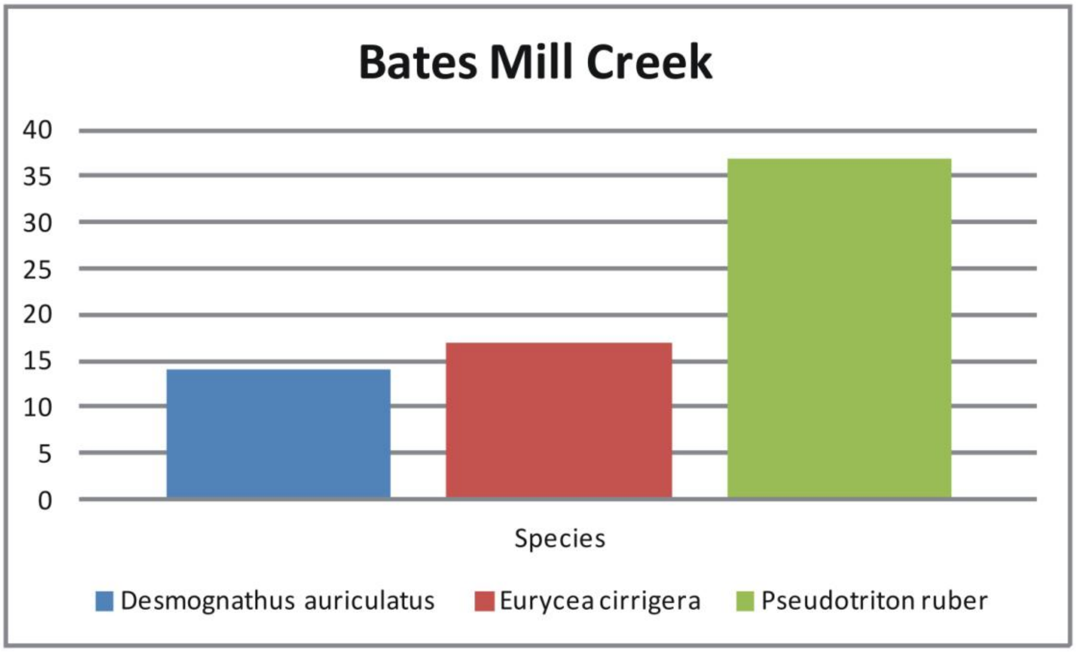


Figure 4- 13 Species counts for Plethodontid salamanders observed under coverboards at both seeps for the Bates Mill Creek study site

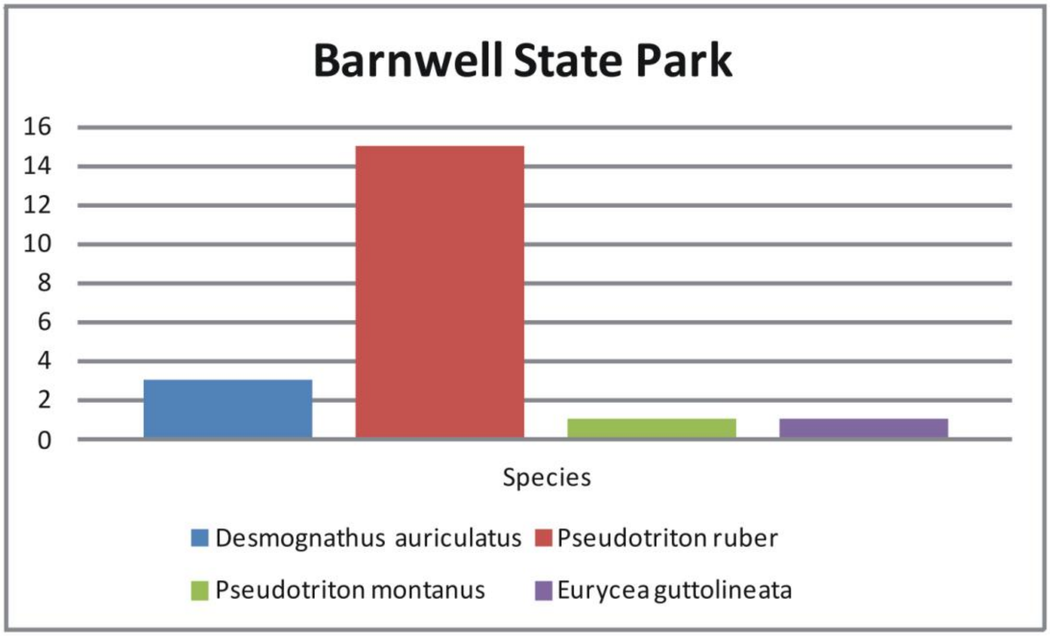


Figure 4- 14 Species counts for Plethodontid salamanders observed under coverboards at both seeps for the Barnwell State Park study site

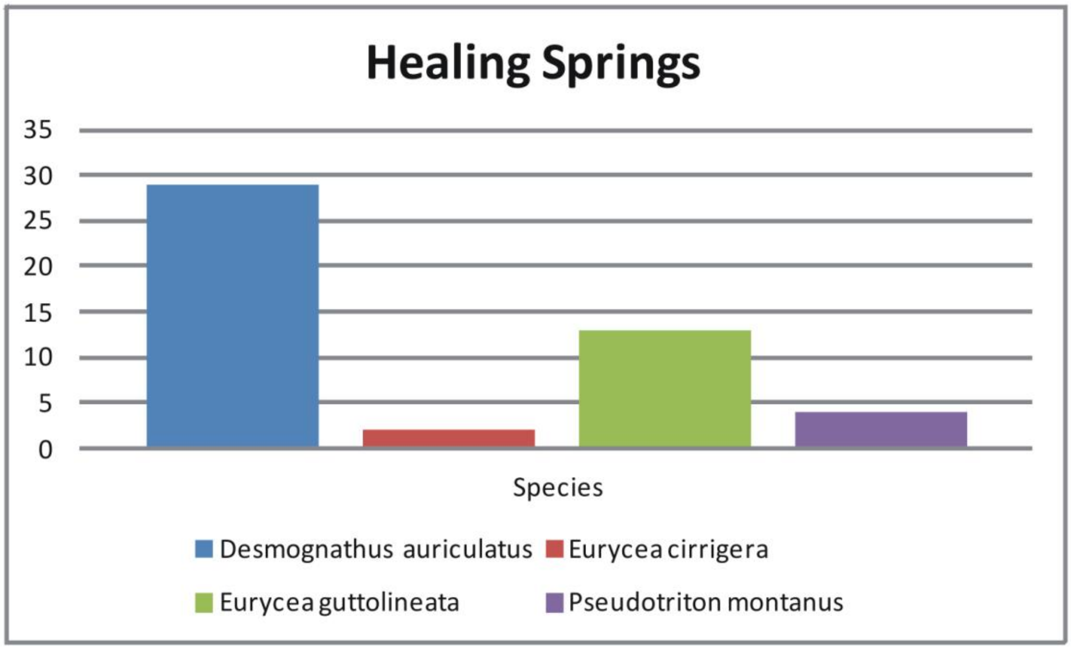


Figure 4- 15 Species counts for Plethodontid salamanders observed under coverboards at both seeps for the Healing Springs study site

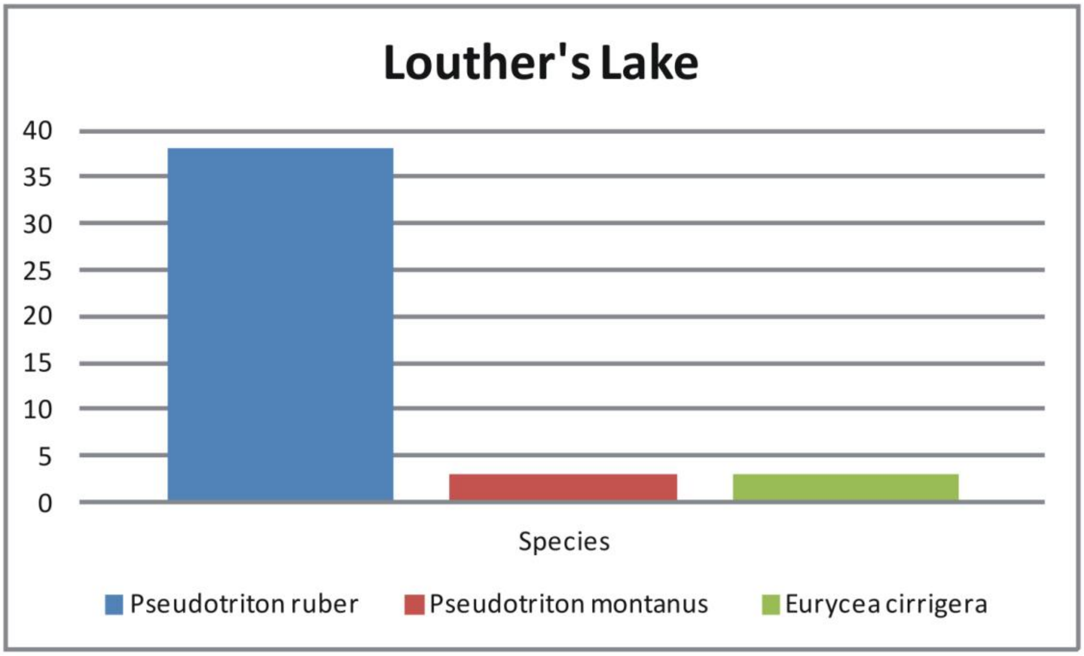


Figure 4- 16 Species counts for Plethodontid salamanders observed under coverboards at both seeps for the Louther's Lake study site

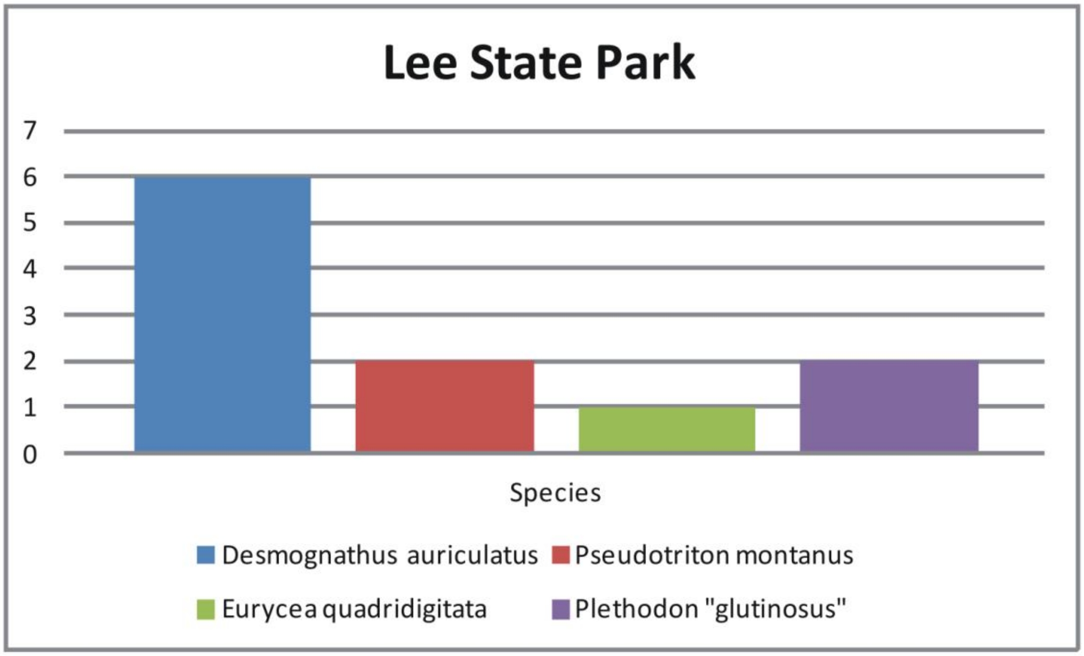


Figure 4- 17 Species counts for Plethodontid salamanders observed under coverboards at both seeps for the Lee State Park study site

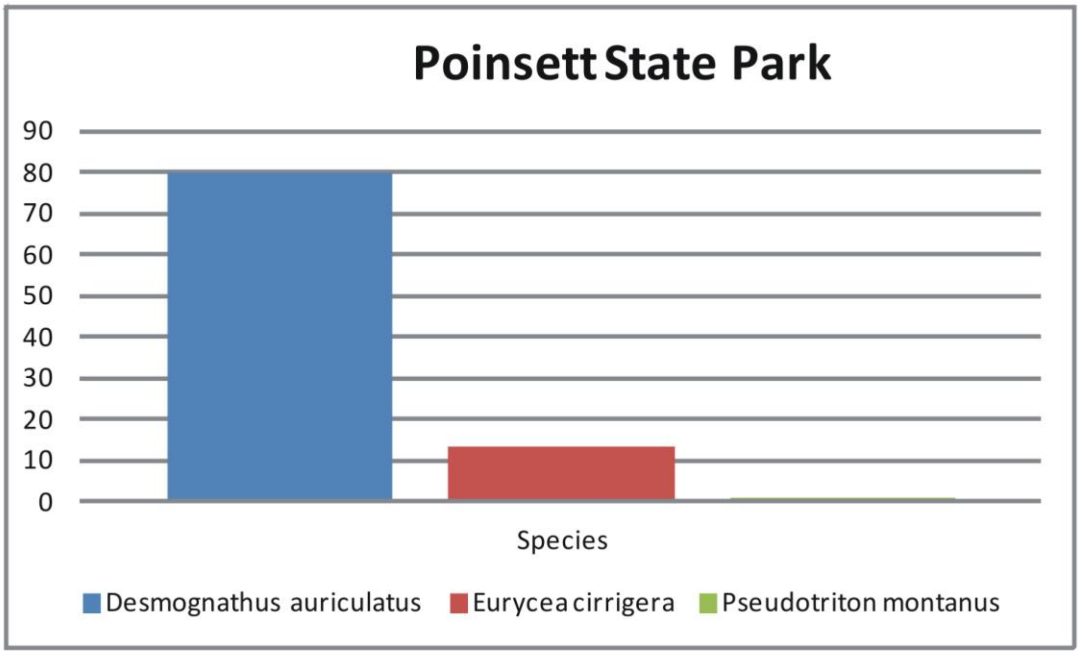
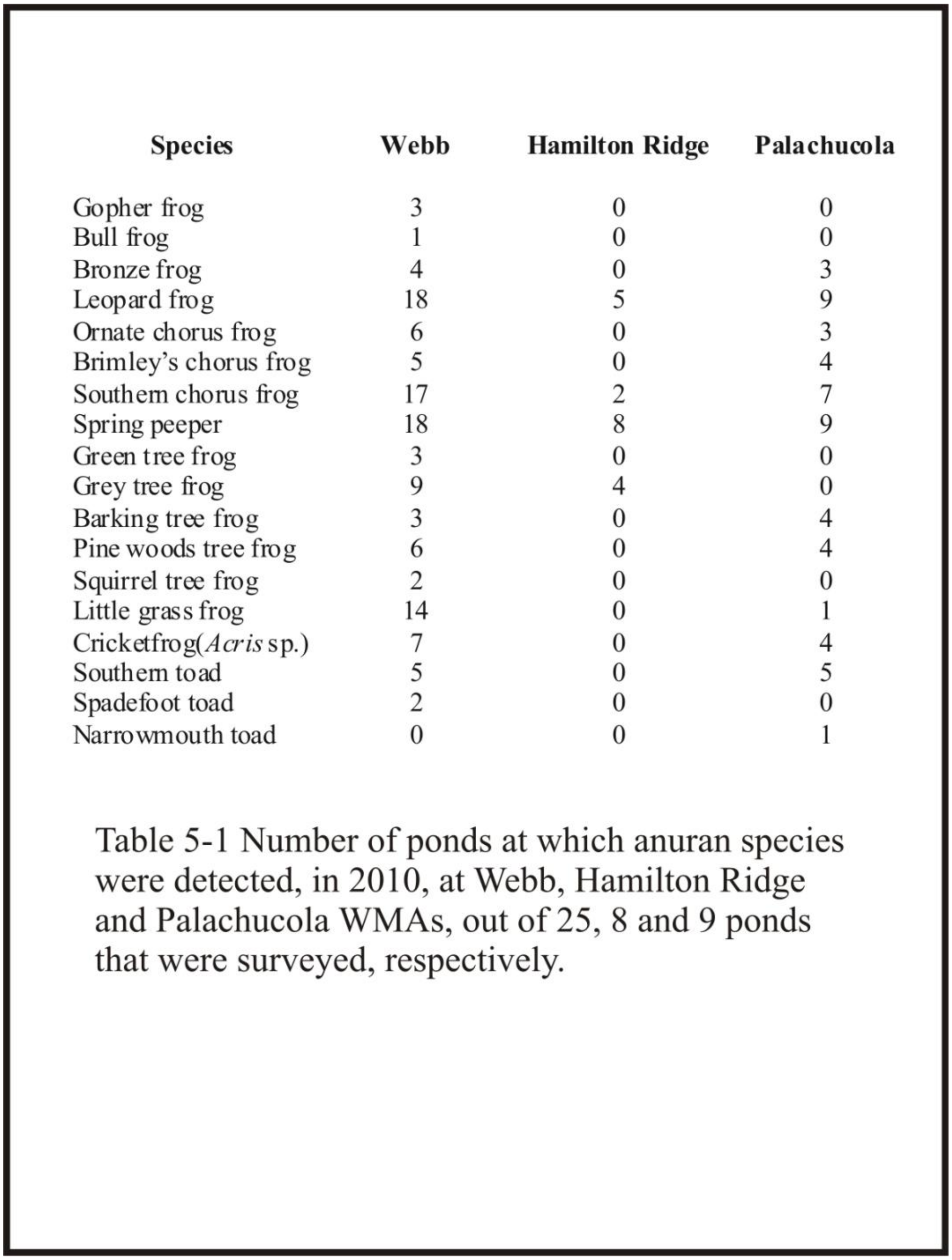
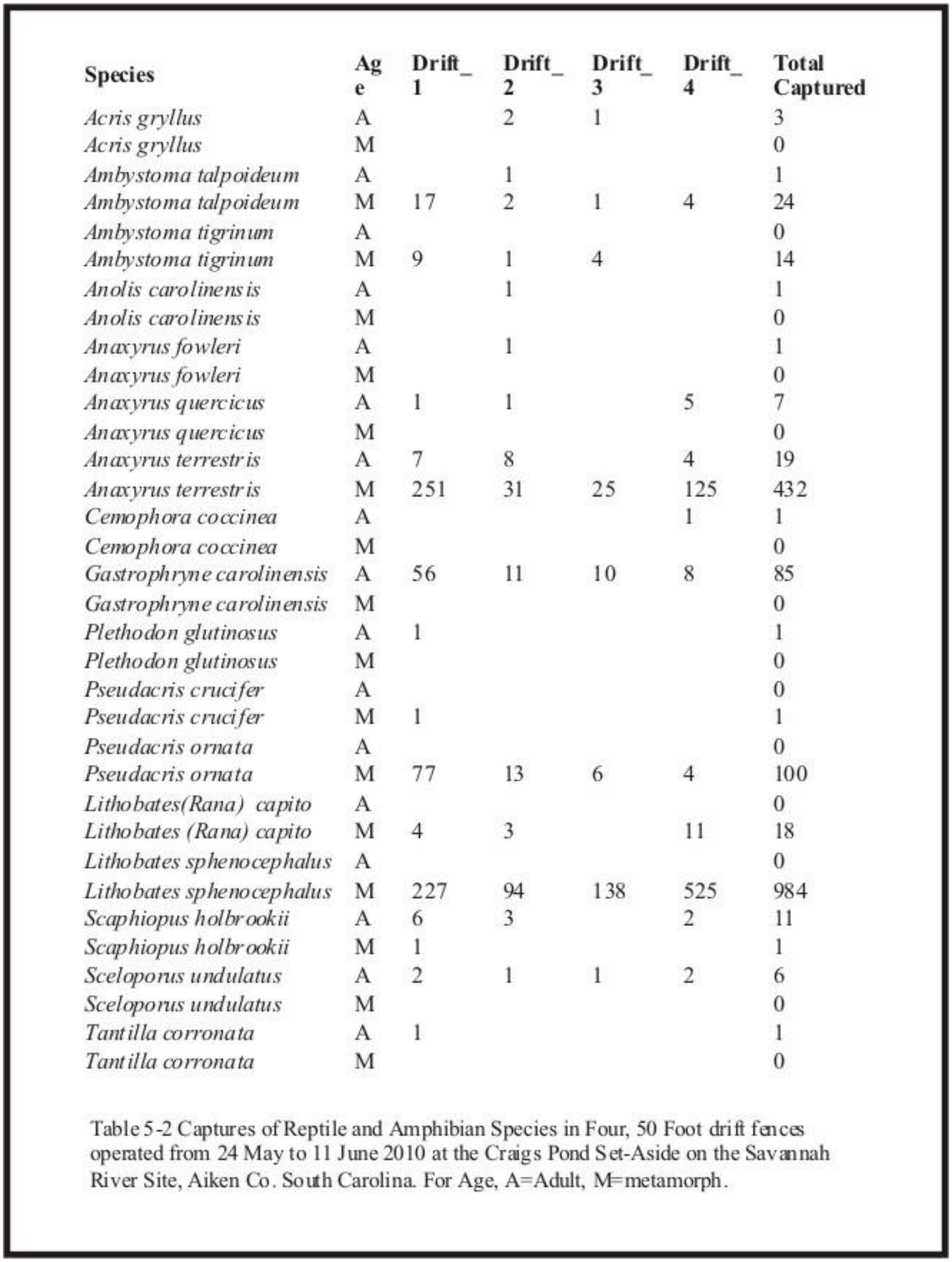


Figure 4- 18 Species counts for Plethodontid salamanders observed under coverboards at both seeps for the Poinsett State Park study site



Species	**Webb**	**Hamilton Ridge**	**Palachucola**
Gopher frog	3	0	0
Bull frog	1	0	0
Bronze frog	4	0	3
Leopard frog	18	5	9
Ornate chorus frog	6	0	3
Brimley's chorus frog	5	0	4
Southern chorus frog	17	2	7
Spring peeper	18	8	9
Green tree frog	3	0	0
Grey tree frog	9	4	0
Barking tree frog	3	0	4
Pine woods tree frog	6	0	4
Squirrel tree frog	2	0	0
Little grass frog	14	0	1
Cricketfrog(*Acris* sp.)	7	0	4
Southern toad	5	0	5
Spadefoot toad	2	0	0
Narrowmouth toad	0	0	1

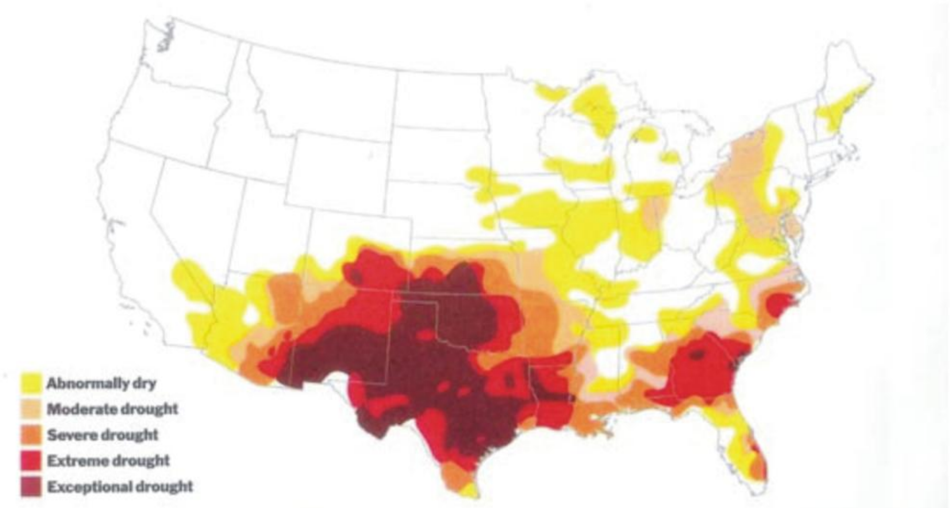
Table 5-1 Number of ponds at which anuran species were detected, in 2010, at Webb, Hamilton Ridge and Palachucola WMAs, out of 25, 8 and 9 ponds that were surveyed, respectively.



Species	Age	Drift_1	Drift_2	Drift_3	Drift_4	Total Captured
Acris gryllus	A		2	1		3
Acris gryllus	M					0
Ambystoma talpoideum	A		1			1
Ambystoma talpoideum	M	17	2	1	4	24
Ambystoma tigrinum	A					0
Ambystoma tigrinum	M	9	1	4		14
Anolis carolinensis	A		1			1
Anolis carolinensis	M					0
Anaxyrus fowleri	A		1			1
Anaxyrus fowleri	M					0
Anaxyrus quercicus	A	1	1		5	7
Anaxyrus quercicus	M					0
Anaxyrus terrestris	A	7	8		4	19
Anaxyrus terrestris	M	251	31	25	125	432
Cemophora coccinea	A				1	1
Cemophora coccinea	M					0
Gastrophryne carolinensis	A	56	11	10	8	85
Gastrophryne carolinensis	M					0
Plethodon glutinosus	A	1				1
Plethodon glutinosus	M					0
Pseudacris crucifer	A					0
Pseudacris crucifer	M	1				1
Pseudacris ornata	A					0
Pseudacris ornata	M	77	13	6	4	100
Lithobates(Rana) capito	A					0
Lithobates (Rana) capito	M	4	3		11	18
Lithobates sphenocephalus	A					0
Lithobates sphenocephalus	M	227	94	138	525	984
Scaphiopus holbrookii	A	6	3		2	11
Scaphiopus holbrookii	M	1				1
Sceloporus undulatus	A	2	1	1	2	6
Sceloporus undulatus	M					0
Tantilla corronata	A	1				1
Tantilla corronata	M					0

Table 5-2 Captures of Reptile and Amphibian Species in Four, 50 Foot drift fences operated from 24 May to 11 June 2010 at the Craigs Pond Set-Aside on the Savannah River Site, Aiken Co. South Carolina. For Age, A=Adult, M=metamorph.

Figure 5-1. Satellite view of Craig's Pond (center) and Sarracenia Pond (left) complex and surround upland. Five head-started gopher frogs were released along the northern parameter of the pond, at the interface of the hydric and upland habitats. We note that the Craigs Pond wetland did not contain standing water in 2011, except in the baywithin-a-bay in the southern portion. The yellow dots represent an adult gopher frog captured in the 1st drift fence (oak-pine fence; Figure 5) on 5 April 2011, entered the pond, but slipped its radio in the middle of the pond 20 days later.



Dry Nation. Nearly 12% of the country is in the most severe state of "exceptional drought"—the biggest area on record

Source: U.S. Drought Monitor, as of Aug. 2

Figure 5-2 Drought severity index for U.S. (From Time Magazine September 2011)

Appendices

Appendix 1-1 PDF, attached as hard copy

Tuberville et al. Translocation as a conservation tool: site fidelity andmovement of re[atriated gopher tortoises (*Gopherus polyphemus*). 2005. Animal Conservation

Appendix 1-2

Nest Guarding in the Gopher Tortoise (*Gopherus polyphemus*)

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Abstract – Nest guarding is rarely observed among reptiles. Specifically, turtles and tortoises are generally perceived as providing no nest protection once the eggs are laid. Here we describe observations of nest guarding by female gopher tortoises (*Gopherus polyphemus*).

Nest guarding among reptiles is considered uncommon (Reynolds et al. 2002). While many crocodilians are known to protect their nests and offspring from potential predators, turtles and tortoises are generally perceived as providing no parental care once the egg laying process is complete. One exception, the Asian brown tortoise (*Manouria emys*), behaves similarly to crocodilians with regard to nest construction and protection, and has been observed guarding its nest from intruders (Eggenschwiler 2003; Bonin et al. 2006). During the nesting season, female *M. emys* will gather nearby debris and vegetation and construct a small mound. Once completed, the female digs a small compartment in the center and deposits her eggs. The female remains near the nest and when a potential threat appears, she will move toward the threat in an attempt to bite and push the intruder away with her carapace (Eggenschwiler 2003; Bonin et al. 2006). Documented observations have shown this behavior to be successful in deterring intruders from investigating the nest more closely, thus protecting the eggs from depredation (Bonin et al. 2006).

Two North American tortoise species, the desert tortoise (*Gopherus agassizii*) and gopher tortoise (*Gopherus polyphemus*), live in underground burrows they excavate. Females of both species are also known to construct their nests in close proximity to their burrows. During burrow excavation, excess sand is shoveled to the surface and deposited outside the burrow entrance, creating a large mound of sand called the apron. As a result of tortoises digging their burrows where the canopy is relatively open, the apron is often used as a basking platform. The apron's deep sandy soils also provide an ideal nesting site. In addition, by nesting at the burrow entrance or apron, females may reduce their own vulnerability to potential predators or extreme heat during nesting because they can rapidly retreat into the shelter of the burrow, if necessary. Additionally, by laying eggs at the entrance of their burrow, tortoises may be providing their nests with some degree of protection, though this has never been confirmed.

The Aiken Gopher Tortoise Heritage Preserve (AGTHP) is a 656 ha preserve managed by the South Carolina Department of Natural Resources (SCDNR) primarily for gopher tortoises and their habitat. The AGTHP is located in Aiken Co., South Carolina, USA and marks the location of the northern-most known native population of gopher tortoises (Clark et al. 2001, SCDNR 2005). As part of an on-going population augmentation project (Buhlmann et al., unpubl. data), native tortoises on the preserve, as well as waif tortoises (i.e., formerly captive or confiscated individuals with no locality data or rehabilitated injured tortoises) from around the southeastern US, have been translocated and penned in 1-ha enclosures for at least one year to increase site fidelity by limiting dispersal after pen removal (Tuberville et al. 2005). One such pen was removed in July 2009 and all tortoises (n=14) were equipped with Holohil (Ontario, Canada) AI-2F transmitters. Following release from their pens, tortoises were radio-tracked weekly, during which time individuals were frequently seen basking on burrow aprons. In every such observation, tortoises retreated into their burrows once observers were close enough to be seen or heard. In August 2010, we searched for nests in aprons of burrows used by females to document natural reproduction in the translocated animals. At one burrow we observed what we consider to be nest guarding behavior by a female gopher tortoise and describe our observations below.

On 25 August 2010, we arrived at a gopher tortoise burrow that had been established almost a year prior by an adult female tortoise (#10). Upon our arrival, this tortoise was not visible at the burrow entrance and was presumed to have retreated further into her burrow. We began our nest searching by digging with hand trowels at the burrow entrance. At the onset of digging, we were unable to see the tortoise within two meters of the burrow entrance. However, almost immediately the female tortoise emerged from the burrow and began hissing and lunging toward the shovel. As we continued digging she moved closer to the shovel, but did not venture further than the burrow entrance, but continued hissing and lunging forward. This behavior of lunging forward and hissing loudly continued for almost 20 minutes while we continued to carefully excavate the apron digging backwards from the burrow entrance. At 0.5 meters from the burrow entrance, sand was removed to reveal a clutch of nine eggs—seven live hatchlings and two unhatched eggs. As the hatchlings and eggs were carefully removed, the resident female tortoise continued to lunge forward toward us hissing loudly. KAB put his hand in front of the tortoise (< 8 cm), in response to which she quickly opened her mouth and made a deliberate lunging attempt to bite his hand (Figure 1). Once the clutch of hatchlings and eggs were successfully removed from the nest, we retreated, and the tortoise continued to loudly hiss from the safety of her burrow. On a return trip the following week to radio-track tortoises, this female was observed basking at the entrance of the same burrow. Upon our arrival, she slid back into her burrow and only retreated further when attempts were made to entice her to the burrow entrance again (e.g. digging at the apron and patting the sand at the entrance).

To our knowledge, this is the first written account describing what we consider to be nest guarding in the gopher tortoise. One of us (TDT) has also observed this behavior on two other occasions in a gopher tortoise population on St. Catherines Island (SCI), Georgia, USA. On 13 September 2006, female #152 was observed 0.3 meters inside the burrow entrance and hissed, advanced toward the burrow entrance, and started bobbing her head once apron excavation was initiated. A clutch of 11 eggs was subsequently discovered in the apron. On 23 June 2007, this same female (#152) was observed inside a different burrow, where a clutch of 12 eggs was detected during apron excavation. The female exited the burrow to investigate as the protective nest cage was installed. Despite the more than 200 burrow aprons collectively searched for nests by the authors, we only observed these behaviors on these three occasions. Tortoise #10 from AGTP is the only gopher tortoise that has ever attempted to bite one of us. In each case, we confirmed that a nest had been deposited in the apron. Further, by comparing the genotypes of the successfully hatched offspring to the female residing in the burrow, we were able to confirm that the hatchlings were in fact the resident female's offspring (SCI - Tuberville et al. *in press*; AGTHP - Tuberville et al., unpublished data). Based on our field observations and the genetic data, we think that in each occasion reported above, the female gopher tortoise was clearly protecting her nest from a perceived predator. We suggest that other researchers who are studying tortoises be observant for other instances of nest guarding.

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Appendix 2-1

Life History Attributes and Eastern Diamondback Rattlesnake Imperilment

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Suggested Running Head: Rattlesnake life history

Key words: *Crotalus adamanteus*, survival, age at maturation, longevity, growth, site fidelity

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We combined mark-recapture and radio telemetry data collected over 17 years from an inland peripheral Eastern Diamondback Rattlesnake (*Crotalus adamanteus*; EDB) population in South Carolina to, 1) examine survival and site fidelity, 2) assess causes of mortality, 3) model growth (asymptotic size and age at maturation), and 4) use derived parameters and mark-recapture intervals to examine potential longevity. We used a combined recapture/recovery model to estimate monthly survival (S) and site fidelity ($F = 1 -$ permanent emigration), which we interpreted as the probability that EDBs did not redistribute at the landscape scale, from encounter histories that were defined by seasonal patterns of rattlesnake behavior (i.e., egress, foraging season, reproductive season, and hibernation). We examined the influence of sex, body size (snout-vent length; SVL), and body condition on model parameters. Survival (98.5 ± 0.9 %) did not vary seasonally and was not influenced by model covariates. Site fidelity was high (96.1 ± 3.2 %) and was positively associated with body size, indicating that large individuals were less likely to redistribute at the landscape scale. Growth models indicated that female asymptotic size was 136.8 cm and that maturity was reached in 7.24 years (95 % CI: 6.65 – 7.85). Maximum recapture intervals averaged 3.11 years (range = 0.05 − 13.38), and longevity likely exceeded 20 years. The results of this study indicate that, in addition to the species' high habitat specificity to the imperiled Longleaf Pine (*Pinus palustris*) ecosystem, EDBs exhibit high survival, slow growth, and limited dispersal ability. Life history traits that include delayed maturation often require high adult survival to maintain viable

populations, and thus management efforts should reduce threats to adult survival. Further, adult EDBs exhibited high spatial fidelity and were unlikely to redistribute at the landscape scale, suggesting that adaptive traits may limit the ability of adult EDBs to redistribute in response to landscape change. This observation, combined with the species' high habitat specificity and the fragmented distribution of remnant pine savannas and woodlands, sheds further light on EDB imperilment.

Populations located along the periphery of a species' distribution (i.e., peripheral populations) are of conservation interest for many reasons. In general, peripheral populations tend to be constrained by genetics (Vucetich and Waite 2003), resources, and conditions (Dussault et al. 2005; Koprowski et al. 2008), which can negatively affect a population through decreased survival (Carrascal and Seoane 2009), reduced recruitment, and low population densities (Lawton 1993; Sagarin and Gaines 2002). Thus, peripheral populations are often more sensitive to ecological perturbations and prone to extirpation (Griffith et al. 1989; Wolf et al. 1996). For imperiled species, the ecological sensitivity of peripheral populations may act to amplify signals of those factors contributing to overall population declines, making them well-suited to examine specific hypotheses concerning the cause of declines. Thus, peripheral populations of imperiled species can serve important conservation roles by providing critical insight into how life history constraints interact with human activities and land use practices to affect population viability (Ferguson et al. 1999).

Within certain constraints, life history traits (e.g., age at maturation, dispersal rates) naturally vary across populations, reflecting environmental variation and local selection that shift the balance of tradeoffs to maximize fitness and contribute to population viability (Stearns 1992). Tradeoffs link competing life history traits to maximize fitness, but can constrain the ability of a population to respond to abrupt changes in selection (Roff 2002). For instance, species with low dispersal abilities are highly susceptible to local extirpation in rapidly deteriorating environments (Parvinen 2004). Life histories (e.g., generation time) can interact with the spatial and temporal dimensions of a disturbance, resulting in a lag time (e.g., the extinction debt) between anthropogenic activities and extirpations (reviewed in Jackson and Sax 2010). These interactions are extremely important for conservation because wildlife biologists can focus efforts on specific demographics to increase population viability. For example, long-lived wildlife species are more likely to suffer population declines that are triggered by a decrease in adult survival, regardless of changes in juvenile survival (Crone 2001; Legendre 2004); in this scenario, biologists can influence population viability by managing/manipulating adult survival (Crone 2001).

Interest in observational studies of wildlife populations has increased, particularly in light of concerns about the potential impacts of climate change and wide-scale habitat loss on wildlife conservation (Dayton et al. 1998), and demographic data derived from single populations are considered critical for making broad-scale comparisons and assessing population constraints (Sagarin et al. 2006). Unfortunately, demographic data are often lacking for wildlife populations, and understudied taxa present difficult conservation challenges. For example, snake population declines (Dodd 1987; Gibbons et al. 2000) are a conservation challenge because snake ecology studies have been rare relative to other taxa (Shine and Bonnet 2009), forcing biologists to rely on incomplete demographic data. Further, long-term population monitoring is often required to gain insight into population status (Shine and Bonnet 2009). These limitations are due, in part, to low detection probabilities that often yield insufficient data for estimating demographic parameters (Dorcas and Willson 2009; Steen 2010) and relatively long life spans. Thus, snake population studies benefit from using multiple sampling and analytic approaches that are relatively robust to low detection probabilities (Dorcas and Willson 2009).

In this study, we combined mark-recapture and radio telemetry data collected over 17 years from an inland peripheral Eastern Diamondback Rattlesnake (*C. adamanteus*; EDB) population to examine demographic parameters. Insight into EDB demography is important in light of documented population declines (e.g., Means 2009), which motivated the IUCN viper specialist group to review the species' status (IUCN 2011) and triggered a petition for protecting EDBs under the Endangered Species Act. Our specific objectives were to, 1) estimate survival rates, 2) examine EDB site fidelity at the landscape scale, measured as the probability of remaining in the study area (1− permanent emigration), 3) use growth models to estimate asymptotic size and age at maturation, and 4) use derived parameters and mark-recapture intervals to examine potential EDB longevity.

MATERIALS AND METHODS

Study area.—This study was conducted in the South Carolina southeastern Coastal Plain on property managed by the South Carolina Department of Natural Resources. The study area is located within ca. 18 km of the western limit of the species' distribution in South Carolina. The 2374-ha property is located along the Savannah River, and contains a mosaic of habitats that include longleaf pine (*P. palustris*) flatwoods and savannas, loblolly pine (*P. taeda*) forests, oak-hickory mixed-pine hardwoods, hardwood bottoms, and cypress-tupelo swamp forests associated with the Savannah River floodplain. The area is managed with growing- and dormant-season prescribed fires, which maintain high vascular plant diversity (Porcher and Rayner 2001) and supports habitats for longleaf-pine endemic wildlife, including colonies of federally endangered red-cockaded woodpeckers (*Picoides borealis*). Game management, which includes maintenance of agricultural food plots in upland habitats, focuses on bobwhite quail (*Colinus virginianus*) and white-tailed deer (*Odocoileus virginianus*).

Study species.—The EDB is endemic to the imperiled Longleaf Pine (*Pinus palustris*) ecosystem (Means 2006). Dependent on savanna structure at multiple spatial scales, the EDB is considered a remnant of the historical southeastern woodland-savanna landscape (Martin and Means 2000; Waldron et al. 2006; Waldron et al. 2008). It occurs in the southeastern Coastal Plain from southeastern North Carolina through eastern Louisiana, including Florida (Martin and Means 2000; Timmerman and Martin 2003), and it is listed as a species of conservation concern in Alabama, Mississippi, and South Carolina. North Carolina populations are endangered, and EDBs are critically imperiled in Louisiana. In addition to habitat loss, over-collection, rattlesnake round-ups (Means 2009), indiscriminant killing by humans, and a lack of public policy regarding protection have accelerated the species' decline (Martin and Means 2000).

Data collection.—We monitored EDBs between 1994 and 2011 using mark recapture surveys and radio telemetry. We sampled the study population using cover board surveys and visual searches for rattlesnakes. Although visual surveys were conducted year-round, we allocated disproportionate sampling effort during spring emergence, which coincided with availability of experienced volunteers. During the active season (Mar-Nov), we conducted periodic visual surveys, but the majority of captures outside of spring emergence were incidentally obtained while conducting radio telemetry surveys.

We captured rattlesnakes using snake hooks and used clear snake tubes to safely constrain individuals while we collected morphological data. We determined sex by counting subcaudal scales and using cloacal probes. We measured snout-vent-length (SVL; cm) and subcutaneously injected a passive integrative transponder (PIT) tag ca. 13 ventral scale rows above (cephalad) the cloaca. After 2005, we used portable cauteries to mark ventral scales according to Winne et al. (2006), in addition to injecting PIT tags.

We implanted 29 adult EDBs (21 females, 8 males) with radio transmitters (SI-2, 11-13 g, Holohil Systems, Carp. ON) between 1997 and 2005 using modified surgical procedures outlined by Reinert and Cundall (1982). We monitored individuals for up to three years using a radio receiver (Telonics, TR-2, Mesa, AZ), and thus some individuals required multiple transmitter implantation and removal surgeries. We located individuals three to five times each week during the active season (Mar-Nov), and biweekly during winter months. Our goal was to visually detect EDBs upon each radio location while maintaining 2.5-5 m between observer and rattlesnake, allowing visual assessment of whether snakes were above or below ground, in ecdysis, in ambush posture, if a bolus was visible, and to observe breeding activity.

Survival.— We analyzed mark-recapture and radio telemetry data using Burnham's combined recapture/recovery model (Burnham 1993) in program MARK (White and Burnham 1999) to estimate survival (S), site fidelity (F = 1 – permanent emigration), capture probability (p), and probability of recovering (r) dead individuals (White and Burnham 1999). We modeled survival using encounter histories that were defined by seasonal patterns of rattlesnake behavior (e.g., Waldron et al. 2006). The first interval represented egress (Mar-Apr), the second represented the foraging season (May-Jul), the third represented the breeding season (Aug-Nov), and the fourth represented overwintering (Dec-Feb). We accounted for differences between time interval length in our analysis, and derived estimates of monthly survival probability. We included all size/age classes in our encounter history file, including neonates (young-of-year). Pregnant EDBs gave birth between Aug and Sep, and thus we coded neonates so that they were unavailable for capture prior to the breeding/birthing season interval (3rd interval). Many EDBs were telemetered for multiple years and others were captured across multiple years. Our encounter history file included multiple entries for these individuals, and thus 'EDB-years' determined sample size.

Estimates of true survival (*S*) require that recovery data are collected from a larger geographic area than the capture area, otherwise datasets that use identical study locations for dead recovery and recaptures can only estimate apparent survival (Francis and Saurola 2002). It was unlawful to kill or harm rattlesnakes at our study area, thus none of our dead recovery data were derived from rattlesnake hunts. Rather, we obtained dead recoveries during rattlesnake surveys by visually detecting dead individuals, and from biologists and land managers that found dead EDBs in neighboring wildlife management areas. Because we collected recovery data from a larger geographic area than we sampled, we assumed that our estimates of *S* represented true survival.

We constructed candidate models that included sex, body size (SVL), and body condition as covariates. Body size and body condition were represented by measurements taken upon first capture within the corresponding year in the encounter history file. We calculated body condition using residuals from ordinary least squares regression of body mass (log transformed) on SVL, such that positive values were indicative of high relative body condition, and negative values indicated lower relative body condition (Jakob et al. 1996; Schulte-Hostedde et al. 2001). For the body size covariate, we normalized SVL using a z-transformation. Ideally, we would have included age classes (i.e., year classes or cohorts) in our analysis to examine potential differences in age-specific survival, but we were unable to reliably age individuals with this level of detail for several reasons, 1) rattlesnakes exhibit indeterminate growth that asymptotes following maturation, which made it impossible to use body size as a direct measure of age, and 2) we were unable to use rattle segments to age individuals due to a high incidence of broken rattles. Further, size-frequency histograms are often unreliable for herpetofauna because variance in body size within a particular age class can be high (Halliday and Verrell 1988). However, we assumed body size captured coarse patterns in snake maturation (e.g., adult and subadult).

Our candidate models included survival as constant ($S(.)$) and as time-dependent ($S(t)$), with time defined by behavioral seasons. We also included body size, body condition, and sex as survival covariates. All survival covariates were included alone and as additive effects in time-dependent models, and we included body size and body condition as interactive effects in time-dependent models. Even though survival likely varied over the course of the study, we had insufficient sample size to test for an effect of year on EDB survival. We hypothesized that EDB survival would be high and exhibit time-dependent variation reflective of seasonal differences in snake behavior (e.g., Bonnet et al. 1999). We suspected that EDB survival would be lowest during egress and ingress (winter), particularly for individuals with low body condition. In other *Crotalus* species, adult survival is often higher than juvenile survival (e.g., Northern Pacific Rattlesnakes, *C. viridis oreganus*, Diller and Wallace 2002; Timber Rattlesnakes, *C. horridus*, Brown 2008); thus, we expected that EDB survival would increase with body size. Further, we expected that differences between male and female reproductive behavior would affect survival. For example, male survival could suffer during the breeding season due to increased movement activity associated with mate searching.

We modeled site fidelity at the landscape scale as a constant ($F(.)$), and with body size and sex as covariates. Given limited insight into rattlesnake site fidelity, we included sex and body size as covariates to assess whether site fidelity was sex- or size-biased, assuming that small snakes, on average, were younger than large snakes. Thus, we used site fidelity to examine the probability of EDB redistribution across the landscape as a function of body size and sex. We fixed $p = 1$ and $r = 1$ for encounter histories corresponding to telemetered EDBs, and right-censored encounter histories for telemetered EDBs when we could not determine their fate. For EDBs that were monitored with mark-recapture surveys, we modeled p and r as constants ($p(.)$, $r(.)$). For individuals that were captured as neonates, we fixed all parameters equal to zero for the first and second intervals, when neonates were not yet available for capture (i.e., neonates were born during interval three).

We assessed goodness-of-fit (GOF) by dividing the observed deviance by the mean deviance derived from 200 bootstrap simulations of a fully time-dependent, interactive model. We assessed the sensitivity of our model ranking to adjustments in $\hat{c}$ by manually increasing $\hat{c}$ to 2.0 by 0.05 increments. We used Akaike's Information Criterion adjusted for small sample size and overdispersion ($QAIC_c$) to compare candidate models and used models with $\Delta QAIC_c \leq 2.00$ for inference (Burnham and Anderson 2002). We adjusted $QAIC_c$ model weights (w) so that weights of supported models summed to 100%. We assessed covariate performance by summing adjusted model weights across supported models that corresponded to each covariate, and we used 90 % confidence intervals of model-specific beta estimates and, where appropriate, model-averaged beta estimates ($\hat{\bar{\beta}}$) to examine covariate effects. We averaged parameter estimates across supported candidate models and present weighted-average parameter estimates (based on $QAIC_c$ weights) with unconditional standard errors, which accounted for model-specific variation as well as variation from model selection uncertainty (Burnham and Anderson 2002).

When possible, we determined cause of death for recovered EDBs. For telemetered EDBs, we used our knowledge of rattlesnake behavior (e.g., time since last meal and reproductive condition) and carcass condition to assess cause of death. Specifically, we distinguished between starvation and predation using our knowledge of snake body condition and feeding success prior to death. For example, we assumed that starvation caused death when snakes in poor body condition failed to acquire a meal, despite numerous feeding attempts (based on our observations of the snake in ambush posture). In these cases, we found whole carcasses that had no external signs of trauma. For snakes with normal body condition, we assumed predation caused death when carcasses had external signs of trauma. For non-telemetered EDBs, we could only determine cause of death from carcass condition and location. We categorized mortality as the result of starvation, predation, vehicular injuries, dormant-season prescribed fire, and intentional killing by humans. We categorized EDB mortalities as occurring during the active (Mar-Oct) or inactive season (Nov-Feb) and tested for equal proportions using a chi-square test.

Growth.—We modeled growth for female EDBs; males were excluded due to insufficient recapture data. We calculated growth rate as the percent change in SVL, divided by the time interval (years) between captures. To examine the assumption that growth rates slowed with increasing size, we assessed the slope of growth rate regressed against initial SVL. With the exception of neonates, all EDBs captured in this study were of unknown age. Thus, we used interval equations of the von Bertalanffy and logistic growth models to estimate asymptotic size because neither required knowledge of age (Fabens 1965; Frazer and Ehrhart 1985; Frazer et al. 1990). Both equations use length at first capture (L_1), length at recapture (L_2), and the time between captures (d) to derive two parameters, 1) a = asymptotic size, and 2) r = the growth coefficient (Fabens 1965, Schoener and Schoener 1978, Frazer and Ehrhart 1985). The von Bertalanffy and logistic growth models use the following two equations, respectively,

$L_2 = a - (a - L_1)e^{-rd}$,

$L_2 = a\,L_1 / [L_1 + (a - L_1)e^{-rd}]$,

where e is the base of the natural logarithm. We used SVL as our length measurement, and only included one growth interval per individual (i.e., each snake represented one degree of freedom). When snakes were captured more than once, we used the growth interval between the first and last capture in our analysis.

We analyzed growth data using SAS software, version 9.2.1. We used nonlinear least squares regression with the Marquardt algorithm (PROC NLIN) to fit the recapture data to the growth models and estimate asymptotic SVL (a) and the characteristic growth parameter (r). We assessed model fit by comparing the residual error mean square (REMS), i.e., the model with the lowest REMS was considered the best fit to the recapture data (Schoener and Schoener, 1978). Because the estimate of a should be slightly larger than the average size of the largest individuals in the population (Frazer et al. 1990), we calculated the average SVL of reproductively mature females in our study population (N = 42) and used SVL measurements taken at first capture of the largest 50% to determine the average size of the largest individuals.

We included estimates of a and r from the best fitting growth model to estimate age at first reproduction (t). We used a modified version of the von Bertalanffy growth interval equation,

$L = a(1 - be^{-rt})$,

which required estimates of a and r, knowledge of hatchling size (h), and knowledge of average adult body size (Frazer and Ehrhart 1985). In this equation, e is the base of the natural logarithm, and we used estimates of a and r derived from the growth model that provided the best fit for our data (see above). We solved for b using average female hatchling size (mean SVL = 40.34 cm, N = 22) from three clutches. Thus, we solved for b using the following equation,

$$b = 1 - \left(\frac{h}{a}\right)$$

$$b = 1 - \left(\frac{40.34}{136.8}\right),$$

and estimated age at maturation by solving for t in the following equation,

$$L = 136.8\,(1 - 0.71e^{-0.20t}).$$

We solved for t at given values of L = L_m, in which we used average adult female SVL in the study population (mean SVL = 125.14 cm, SD = 13.61, Range = 102-163) as the upper limit for L_m, and the smallest recorded SVL of a reproductively mature female (i.e., 102 cm) as the lower limit for L_m.

RESULTS

Out of 144 captures between 1994 and 2011, we marked 115 unique EDBs (48 males and 67 females). The majority of captures resulted from visual searches (N = 64). While tracking telemetered individuals, we captured 23 neonates born to telemetered females, and three adults that were breeding (either courting, paired, or copulating) with telemetered snakes. Incidental road observations were few (N = 8), and cover board surveys were ineffective, yielding only three captures. Maximum recapture intervals for individual rattlesnakes averaged 3.11 years (SD = 3.43, N = 19), ranging from 0.05 to 13.38 years. Recapture intervals for males (mean = 1.44, SD = 0.59, N = 7) and females (mean = 1.54, SD = 1.12, N = 12) did not differ (Pooled T-test; t_{17} = 0.21, P = 0.83).

Survival.—Merged telemetry and mark recapture data yielded 155 EDB-years for survival analysis. Survival data were overdispersed ($\hat{c}$ = 1.43), but model ranking was not sensitive to increased $\hat{c}$ values. Thus, we adjusted $\hat{c}$ to 1.43 and used QAIC_c for model selection. Five out of thirty candidate models received support (Table 1), none of which included seasonally-defined, time-dependent survival (S(t)). Model-averaged monthly survival was 98.5 ± 0.9 %. Our top two supported models contained survival as a function of body size, accounting for 51 % of adjusted model weights (Table 1). Although beta estimates indicated that body size was negatively associated with survival, these results were inconclusive because 90% confidence intervals of beta estimates for S(size) contained zero (top model β = -0.928 ± 0.595; second model β = -0.922 ± 0.588). Two supported models contained constant survival ($\hat{\beta}$ = 3.850 ± 0.363), and accounted for 39 % of model weights (Table 1). The lowest-ranking supported model included survival as a function body condition (Table 1), but the effect of body condition was T1 negligible (β = 0.001 ± 0.001).

Model-averaged site fidelity was 96.1 ± 3.2 %. Three supported models contained constant site fidelity ($\hat{\beta}$ = 4.012 ± 0.492), accounting for 56 % of model weights (Table 1). Two supported models contained site fidelity as a function of body size and accounted for 44 % of model weights (Table1). Site fidelity was positively associated with body size ($\hat{\beta}$ = 1.796 ± 0.646), indicating that larger, older individuals were less likely to disperse from the study area (Fig. 1).

Model-averaged capture probability was low 7.6 ± 3.6 % ($\hat{\beta}$ = -2.541 ± 0.416). Recovery probability ($\hat{\beta}$ = -0.397 ± 1.21) was highly variable, and our model-averaged estimate was 44.5 ± 36 .0 %. We recovered 18 dead EDBs, 13 of which were equipped with radio transmitters at time of death (Table 2). Mortalities were equally distributed F1 across the active and inactive seasons (χ^2 = 0.20, P = 0.65). We determined cause of death for all but five individuals and for two additional individuals that had not been captured previously (Table 2). Death occurred as the result of vehicular traffic (N = 5, including one EDB that was killed by farm equipment), dormant-season prescribed fire (N = 3), starvation (N = 4, including one postpartum female), predation (N = 3, including one postpartum female), and intentional killing by humans (N = 1). On two occasions, we found radio transmitters without carcass remains, and thus we were unable to determine if either snake was predated, or whether they expelled transmitters via incorporation into their alimentary tract, as described by Pearson and Shine (2002). We were unable to determine cause of death for one individual that did not emerge from its overwintering site. We were unable to distinguish between predation and starvation for two individuals that were in poor body condition prior to death. In both cases, our inspection of carcass remains was inconclusive due to our inability to determine whether the snakes were predated or scavenged.

T2

Growth.—Growth rates slowed in older, reproductively mature individuals ($R^2 = 0.88$, $F = 84.20$, $P < 0.001$). Both growth models provided similar estimates of asymptotic size (von Bertalanffy, $a = 136.8 \pm 3.9$ cm; logistic, $a = 136.3 \pm 5.0$), but the von Bertalanffy model provided a smaller estimate of r (von Bertalanffy, $r = 0.16 \pm 0.05$; logistic, $r = 0.20 \pm 0.10$). We used estimates of a and r obtained from the von Bertalanffy model to estimate age at maturation because it provided the best fit to our mark-recapture data, based on REMS (von Bertalanffy = 21.58; logistic = 40.11). The general von Bertalanffy growth model estimated that females reached reproductive maturity at 7.24 ± 0.29 years (95% CI: 6.65-7.85; Fig. 2).

DISCUSSION

Snake vulnerability to natural (e.g., starvation) and anthropogenic (e.g., intentional killing by humans) mortality varies seasonally and with respect to life history strategy (Bonnet et al. 1999). In this study, EDBs exhibited high monthly survival, but contrary to our expectations, survival did not vary seasonally. We suspected that survival would reflect seasonal behavioral patterns that place snakes at greater risk of mortality, e.g., during breeding activity (mate searching, parturition, neonate dispersal prior to overwintering). Our dead recovery data supported these findings, as dead EDBs were found in similar proportions during winter and summer.

F2

Our monthly survival estimate (98.5 %) corresponds to 83.4 % annual survival probability (i.e., annual survival = monthly survival to the twelfth power, S^{12}), which is similar to the estimate given by Parker and Plummer (1987) for late-maturing viperids in their review of snake survival studies. We failed to detect an effect of body condition or sex on EDB survival. We detected a negative relationship between body size and survival, but the effect of body size was inconclusive due to large standard errors associated with beta estimates. However, rattlesnakes typically have lower relative survival during their first year of life (e.g., Diller and Wallace 2002, Martin 2002, Brown 2008), after which survival increases and varies little between immature individuals and adults. For example, Diller and Wallace (2002) detected an increase in western rattlesnake (*C. viridis oreganus*) survival with age class, but survival was similar between adult (95 % CI: 75.4 − 88.6 %) and immature (second year and older; 95 % CI: 65.2-88.3) snakes. It is uncertain whether we would have detected reduced neonate survival had we sufficient sample size to estimate age-specific survival, but our observations support the assumption of high adult survival. We assume that EDBs are similar to other crotalids, and thus it is likely that adult and immature (excluding neonates) EDBs exhibit similar survival probabilities. Further, we recognize that other factors could have influenced EDB survival, including year, environmental conditions, and prey abundance, but the inclusion of these variables was beyond the scope of this study.

Our use of Burnham's model for combined live-dead encounter data allowed us to examine the probability that EDB redistributed at the landscape scale, expressed as the probability of remaining in the study area (i.e., site fidelity; F = 1-permanent emigration). Given limited insight into snake dispersal patterns, in general, our goal was to examine the effects of sex and body size, as a coarse measure of age, on EDB redistributions at the landscape scale. We used this approach to provide evidence of size/age or sex-biased dispersal patterns. Several snake species exhibit sex-biased dispersal, but whether snake dispersal is female or male-biased can vary intraspecifically to reflect resource availability (Lane and Shine 2011). We failed to detect an effect of sex on site fidelity, but our estimates were robust, indicating that EDBs were unlikely to disperse from the study area. Site fidelity was positively associated with body size, suggesting that younger individuals were more likely to disperse from the study area. Juvenile crotalids exhibit variable movement patterns (e.g., Cobb et al. 2005), with neonates using conspecific trailing of adults to locate overwintering locations (Reinert and Zapalorti 1988; Cobb et al. 2005). Fidelity to overwintering sites, birthing areas, and basking areas has been well documented in *Crotalus*. However, these examples are less applicable to EDBs and potentially other crotalids that occur at southern latitudes, where basking site availability is less critical due to warmer environmental conditions, and structures used for overwintering sites and birthing sites (e.g., stumpholes and slashpiles; Means 2005) are more readily available and temporary in nature (e.g., they decay or burn in prescribed fires).

Our growth models indicated that our study population exhibited delayed maturation, providing further evidence that EDBs have a slow life history. We estimated that female EDBs reached maturity in 7.24 years, and the smallest reproductively mature female we observed was 102 cm SVL. Given that our study site was positioned along the inland periphery of the species' range, it is possible that our estimates were high relative to core, coastal, or more southerly populations. Geographic variation in growth, survival, and other demographic parameters is an important theme in studies of life history evolution (Roff 2002), and snakes exhibit some degree of life history variation at multiple scales (e.g., Bronikowski and Arnold 1999; Jenkins et al. 2009; Ashton 2001). Such variation is often explained by thermal reaction norms that influence growth and survival of ectotherms (Angilleta et al. 2004), and rattlesnake populations are more likely to delay maturation in colder environments (e.g., Ashton 2001). However, EDBs inhabit a subtropical climate, and thus the geographic range of EDBs does not include cold climates that necessitate short growing seasons, likely precluding a conditional response that drives delayed maturation. Life history theory predicts that the benefits of delaying maturation must outweigh costs associated with reduced survival in immature individuals (Stearns 1992). Delayed maturation often reflects an increase in fecundity with age, requiring that adults have a high probability of surviving to the next breeding attempt in iteroparous species (Stearns 1992; Roff 2002). This scenario seems applicable to our study population, given that, 1) fecundity increases with SVL in *Crotalus* species (e.g., Davis 2008; Jenkins et al. 2009), 2) our estimates of EDB survival were high, and 3) EDBs are long-lived. Timmerman and Martin (2003) speculated that wild, free-ranging EDB longevity reached 15-20 years. In our study population, maximum longevity appeared to exceed 20 years. The largest recapture interval observed (13.5 yr) was for a female that was initially captured as an adult, placing her at $\geq$ 20 yrs (assuming maturation occurred in 7.2 years).

Life history traits that include delayed maturation often require high adult survival to maintain viable populations (Stearns 1992). Our findings that adult EDBs exhibited higher spatial fidelity and were less likely to redistribute at the landscape scale, relative to younger, immature individuals, suggest that adaptive traits may limit the ability of adult EDBs to redistribute in response to landscape change. In addition to a high degree of habitat specificity (Waldron et al. 2006; Waldron et al. 2008; Hoss et al. 2010) and the fragmented distribution of remnant longleaf pine savannas and woodlands, low adult dispersal at the landscape scale likely places EDB populations at even greater risk of extirpation, further contributing to the species' imperilment.

The results of this study have important conservation implications for EDBs, particularly in light of heightened concern over population declines. Efforts to conserve the EDB are hampered by limited insight into its population ecology, and the results of this study provide initial estimates of demographic parameters that are currently needed to manage EDB populations and prevent further declines. The EDB appears to harbor many life history characteristics make it vulnerable to declines (see Webb et al. 2002), including high habitat specificity (Waldron et al. 2006, Waldron et al. 2008, Hoss et al. 2010), delayed maturation (this study), and long birthing intervals (Timmerman and Martin 2003). More EDB populations must be studied to assess inter-population variation in demography, which will help identify specific drivers of population decline. Further, future research should focus on 1) EDB reproductive ecology, which will identify factors that influence fecundity, and 2) habitat management, particularly in reference to the effects of pine savanna management and restoration on resource availability. This information is essential for assessing EDB population viability, which is necessary to effectively conserve remaining EDB populations.

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Fig. 1. Predicted relationship (solid lines) and 95% confidence intervals (dashed lines) between Eastern Diamondback Rattlesnake body size and site fidelity (i.e., 1 – probability of dispersing from study area), based on estimates from $\{S(\text{size}), p(.), r(.), F(.)\}$.

Fig. 2. Predicted von Bertalanffy growth curve (in snout-vent-length; SVL) for a South Carolina Eastern Diamondback Rattlesnake population. The vertical dotted line corresponds to estimated age at maturation.

Table 1. Supported ($\Delta QAIC_c < 2.00$) Eastern Diamondback Rattlesnake survival models, based on Akaike's Information Criterion adjusted for small sample size and overdispersion ($QAIC_c$). $\Delta QAIC_c$ = the difference between the model with the lowest $QAIC_c$ score and the present model, *w* = adjusted model weights, and K = number of parameters. Parameters were modeled as constant (.), and with the following covariates: body size (size), body condition (bc), and sex.

Model	$QAIC_c$	$\Delta QAIC_c$	*w*	K
S(size), *p*(.), *r*(.), *F*(.)	188.24	0.00	0.26	5
S(size), *p*(.), *r*(.), *F*(size)	188.30	0.06	0.25	6
S(.), *p*(.), *r*(.), *F*(.)	188.70	0.45	0.21	4
S(.), *p*(.), *r*(.), *F*(size)	188.88	0.63	0.19	5
S(bc), *p*(.), *r*(.), *F*(.)	190.21	1.97	0.10	5

Table 2. Description and cause of death for 20 Eastern Diamondback Rattlesnakes, recovered between 1997 and 2011, South Carolina, USA. Class indicates whether individual was an adult (A) or reproductively immature (I). Cause is a categorical representation of cause of death: vehicle = found dead on road (DOR) or killed by agricultural equipment, DSF = dormant-season (winter) prescribed fire, human = intentionally killed by human, starvation, and unknown. Telemetry indicates whether the individual was equipped with a radio transmitter (T) when it was recovered (NT = no transmitter). Season corresponds to egress (Mar-Apr), foraging season (Forage; May-Jul), breeding season (Breed; Aug-Nov), and overwintering (Winter; Dec-Feb).

Sex	Class	Telemetry	Cause	Season	Description
F	A	NT	Vehicle	Breed	DOR
F	A	NT	DSF	Egress	Snake was found coiled, within 10 m of overwintering site.
F	A	T	Unknown	Winter	Found transmitter (no carcass).
F	A	T	DSF	Egress	Found carcass within 10 m of overwintering site.

F	A	T	Starvation	For	Postpartum female. Found emaciated carcass with no external signs of trauma.
F	A	T	DSF	Winter	Found carcass within 10 m of overwintering site.
F	A	T	Unknown	Egress	Found transmitter (no carcass).
	A	T	Unknown	For	Unable to distinguish between starvation, disease, or predation.

F	A	T	Predation	Winter	Postpartum female. Found portions of trachea and fresh blood was visible on ground and on woody vegetation within 0.25 m of ground. Multiple signs of fresh coyote scat around carcass remains.
F	A	T	Human	For	Found carcass in ditch with head injuries that indicated blunt force trauma and rattles were missing (portion of tail below basal rattle was severed)
M	A	NT	Starvation	Egress	Poor body condition. Found carcass with no external signs of trauma. Necropsy did not detect evidence of disease or infection.
M	A	NT	Vehicle	Egress	DOR
M	A	T	Predation	Breed	Snake had normal body condition; found fresh carcass with evidence of predation.
M	A	T	Unknown	Winter	Snake did not emerge from overwintering site.
M	A	T	Unknown	For	Could not distinguish between predation and starvation.
M	A	T	Vehicle	For	Found carcass in habitat; snake was killed by agricultural equipment.
M	A	T	Starvation	For	Poor body condition was coupled with unsuccessful feeding attempts. Found whole carcass with no external signs of trauma.

Table 2, continued.

M*	I	NT	Vehicle	For	DOR
M	I	NT	Predation	Breed	Snake had normal body condition; found fresh carcass with evidence of predation.
U*	I	NT	Vehicle	For	DOR

* First sighting of individual (i.e., was not captured prior to death and thus was not included in survival models)

Appendix 4-1

Draft section of article summarizing salamander data from original study sites

Descriptive ecology of seepage slope wetlands from two sites on the South Carolina Coastal Plain

Introduction

Salamander species of the family Plethodontidae (lungless salamanders) occurring in the South Carolina Coastal Plain have not, until recently, been identified as requiring conservation research. Little attention, in general, has been paid to this family throughout the southeast Coastal Plain (Means 1974), despite the high number of genera present in this region.

Historically, research and conservation efforts for plethodontids have focused primarily on montane species and habitats. One notable exception is Highton's work on molecular phylogeny in the *Plethodon glutinosus* species complex, which included southeast Coastal Plain populations (Highton 1962a,b; Highton et al 1989). This research established the concept that one species, widely accepted as such based on classic taxonomy, can comprise many genetically distinct, yet morphologically indistinct species or, at least, subgroups.

Recently the South Carolina Department of Natural Resources (SCDNR) completed their Comprehensive Wildlife Conservation Strategy (CWCS). This document identifies the species of wildlife and habitats in South Carolina that are in need of conservation. Two species of plethodontid salamanders, associated with seepage slope wetlands in the South Carolina Coastal Plain, have been identified as priorities for conservation action through this planning process.

Chamberlain's dwarf salamander, (*Eurycea chamberlainii*), is a recently described species that was separated from its closest relative based on morphology and genetics (Harrison and Guttman 2003). Little is known about the life history and distribution of this species in South Carolina, but it is believed to be closely associated with seepage slope wetlands.

The southern dusky salamander, (*Desmognathus auriculatus),* is one of three, currently recognized, members of the genus Desmognathus that occur in the southeast Coastal Plain and the only species historically known to occur in South Carolina. The species is largely restricted to the southeast Coastal Plain, occurring in a variety of wetland habitats including the margins of slow moving, or stagnant bodies of water with muck, acidic soils. Specimens have also been found in springs, cypress swamps, sloughs, mud-bottomed pools in floodplains and slowly moving muddy streams (Neil and Rose 1949; Robertson and Tyson 1950; Rossman 1959; Means 1974). With the exception of Means this species has been virtually overlooked by researchers for the past five decades.

The southern dusky salamander was identified as a conservation candidate in the South Carolina CWCS due to concerns about population and taxonomic status throughout its range. The species has either disappeared or drastically declined at historic sites in the southeast (Means and Travis 2006; Graham 2006). No one cause explains these declines or extinctions, but feral pigs and forestry practices may have played a role in the loss of this species from steephead habitats in northwest Florida (Means and Travis 2006).

Beamer and Lamb (2007) have determined recently through phylogenetic analysis that *Desmognathus auriculatus* is not monophyletic. They propose that the species currently known as *Desmognathus auriculatus* comprises four independent lineages of remarkably similar ecomorphs. Based on these authors analysis of samples from South Carolina it is likely that three of these independent lineages occur in South Carolina. In addition they have documented the occurrence of *Desmognathus conanti* in South Carolina. This species has not been previously reported from this state.

The objectives of the herpetofaunal component of the seeps project were to: 1. Document and quantify the amphibians and reptiles associated with two seepage wetlands in the Coastal Plain of South Carolina, with particular attention given to the salamander species identified as conservation candidates. 2. Compare amphibian and reptile assemblages among seeps. 3. Compare amphibian and reptile assemblages living in seep habitat with the assemblages living in terrestrial habitat adjacent to seeps. 3. Begin correlation of amphibian and reptile data with plant distribution and water chemistry/quality data as a means of understanding the ecological relationships within these wetland systems.

3) Methods

d. Amphibian and Reptile Sampling

Characterization of the amphibian and reptile assemblages associated with seeps within the study sites was accomplished through the use of artificial cover objects (ACO), in this case cover boards. The use of artificial cover is relatively new and has not been fully tested (Fellers and Drost 1994). Artificial cover has been used successfully to monitor terrestrial salamanders in the northeast (Monti et al 2000), but has proven less successful for terrestrial salamanders in the southeast (Houze and Chandler 2002). The issues identified by Houze and Chandler as affecting performance of ACO in the southeast included the inability to maintain steady temperature and moisture regime under the objects. The authors chose to test ACO for sampling seep herpetofauna believing these issues would be negated by using ACO directly in a seep.

Cover-board transects were established at each study seep within both sites (Figure 1). Cover-boards consisted of 60.9cm square sheets of 1.9cm thick untreated plywood. Two transects, totaling 20 boards were established at each seep. Ten boards were placed in, or on the edge of each seep, parallel to the flow of the seep beginning at the head of the seep (seep transect). This transect was established to sample amphibians and reptiles inhabiting the seep. An additional 10 boards were placed perpendicular to the seep, at the approximate mid-point of the seep (terrestrial transect). This transect was established to sample terrestrial amphibians and reptiles living adjacent to the seeps. All transect boards were spaced approximately 5 meters apart.

Cover-boards were checked quarterly as follows: 27 July 2005; 3 November 2005; 7 February 2006; 27 April 2006. All amphibians and reptiles observed under a board were identified to species, assigned to a size class (adult, juvenile, larval) based on visual inspection, and totals for each species by board were recorded. Animals were not measured, weighed or removed unless required for identification. Animals were not marked for individual or cohort identification. All data from the cover-board sampling is stored in an MS Access database.

Results; Amphibians and Reptiles

1. Species Occurrence and Abundance

Data presented herein consist of individual observations of animals, not individual animals. Comparisons between sites are based on observations, not on individual animals as would result from a mark-recapture study. Combined observations of 282 amphibians and reptiles were observed at both sites across 9 species (Table 1). The Beidler Forest (BF) seeps produced 232 observations across 6 species and the Wannamaker Nature Preserve (WNP) seeps produced 50 observations across 7 species.

Figures 2 and 3 present the total observations for all species for both sites. The amphibian assemblages at both sites appear comparable, with some small differences.

Two currently recognized salamander species, the three-lined salamander, *Eurycea guttolineata,* and the southern dusky salamander, *Desmognathus auriculatus*, comprise 85% of the total observations at both sites combined (85% at BF and 64% at WNP). The southern two-lined salamander, *Eurycea bislineata cirrigera (Eurycea cirrigera),* comprised 5% of the total observations, and was observed more frequently at WNP (22%) than at BF (2%). The southern red salamander, *Pseudotriton ruber vioscai*, was observed at WNP, which is within the range of the species, but not at BF, which is outside the known range of this species. All of these species are members of the family Plethodontidae and all have an aquatic larval stage.

Two related salamander species, *Plethodon chlorobryonis* (WNP) and *Plethodon variolatus* (BF) were observed at the respective study sites. These two species are members of the slimy salamander complex (formerly all known as *Plethodon glutinosus*). They are morphologically indistinguishable and are likely to share similar life histories and ecological roles. These two species are members of the family Plethodontidae, they both are completely terrestrial, and undergo direct development with no aquatic larval stage. Both of these specie were observed primarily under boards in the terrestrial transects.

Two frog species were observed during the study period, the bronze frog, *Rana clamitans* (BF-8 observations, WNP-3 observations) and the pinewoods treefrog, *Hyla femoralis* (1 observation BF). All individuals of *Rana clamitans* observed were newly metamorphosed. No frog larvae were observed within the seeps during the study period and it is likely that this species bred in deeper ponds in the floodplain, or the adjacent stream and used the seeps as corridors for post-metamorphosis dispersal.

One reptile, the banded watersnake, *Nerodia fasciata,* was observed (WNP) during the study. This is a common species of water snake throughout the SC coastal plain, and would be expected to occur within the floodplain forest adjacent to the seeps.

2. Comparison of observations within study site:

At this time insufficient data exist for statistically meaningful comparisons within or between study sites. All comparisons presented will be based on data summaries.

There are obvious differences between observations made at boards within seep transects compared to boards within the terrestrial transects at both sites. Terrestrial transect observations comprise 13% of the observations at BF and 6% of the observations at WNP. Two species of salamander, *Plethodon chlorobryonis* (WNP) and *Plethodon variolatus* (BF), both of which are terrestrial were most commonly observed under terrestrial transect boards. Distance from seep does not appear to affect the number of observations within the terrestrial transect at BF (insufficient data exists for WNP) (Figure 4).

No strongly apparent difference was detected for observations between seeps within a study site (Figure 5.) Based on this assumption data from both seeps will be combined for further analysis.

Figure 6 indicates that sample date did not obviously affect the number of observations at either site. There may be some differences in size or age class of individuals observed, but there is insufficient data at this time to address this issue.

There was no apparent difference in the number of observations within the seep transects at either site based on location of the cover board within the seep (Figure 7). There is a slight trend for more observations under boards near the head of the seep (boards 1-2) at BF, but this may not be significant.

3. Comparison of study sites.

While insufficient data exist at this time for statistical comparison there are some obvious similarities and obvious differences in the amphibian assemblages observed at both sites. Only one reptile species was observed at one site, and will not be considered in the following comparisons.

The amphibian assemblages found at both sites are markedly similar. There’s a 66% overlap in species composition, when species observed only in seeps are considered. This overlap would not change if the terrestrial species were included, but were considered as ecological correlates. One complicating factor is the taxonomic status of *Desmognathus auriculatus,* which may actually be two morphologically similar, by genetically distinct species at these two sites (Beamer and Lamb 2007). If that is the case these two species, much like the two species of *Plethodon* are most likely ecological correlates.

The most striking difference between the two sites is in the absolute number of observations (Figure 8). The Wannamaker site accounted for only 17% of the total observations during the study period, while the Beidler Forest site accounted for 83% of the total observations.

5) Discussion.

Similarities in the amphibian assemblages might be explained by similarities in the habitat and the habitat requirements of the dominant species. Within the coastal plain, seepage slope wetlands are likely a preferred habitat for plethodontid salamanders. Most Coastal Plain plethodontid salamanders are either semi-aquatic, or have aquatic larval stages requiring them to live in close proximity to wetland habitat (Gordon, R.E. 1953; Conant and Collins 1991; Petranka 1998). Many of these species have affinities with montane plethodontid salamanders, species that are associated with cool, running water, typically found in springs and seeps. It is probable that seepage wetlands in the southeast Coastal Plain are the ecological equivalents of the montane habitats preferred by plethodontid salamanders.

Seepage wetlands provide a stable habitat for salamanders throughout the year, and provide adequate breeding habitat. Therefore the numbers of observations at seeps varied little over the sampling events and within the seeps themselves. The difference in observations between the seep transects and terrestrial transects was obvious. This difference is most likely due to the lack of completely terrestrial salamanders in the southeast Coastal Plain and indicative of the importance of seep habitat to Coastal Plain plethodontid salamanders.

Two possible explanations may account for the obvious difference in the absolute number of animal observations between study sites. The seeps at Beidler Forest may provide more suitable habitat than those at Wannamaker Nature Preserve, and therefore are capable of supporting greater numbers of animals. It is possible that the Beidler seeps have greater prey abundance for salamanders, provide more natural cove and refugia, or contain fewer predators.

An alternative, and more likely, explanation may be that the seeps at Wannamaker provide equally suitable habitat for salamanders, but sampling bias has affected the number of observations at this site. The morphology of the seeps at each site may account for the possible sampling bias. The seeps at Beidler are linear, occur along a slightly steeper gradient, and are more distinct from the surrounding upland. The seeps at Wannamaker are not necessarily linear, they are less “steep” and tend to merge with the surrounding floodplain wetlands. Therefore salamanders at Beidler seeps may tend to concentrate in the seeps, while those at Wannamaker are dispersed over a greater area of suitable habitat, which includes the floodplain habitat.

There is a need to resolve the taxonomic status of *Desmognathus auriculatus.* This "species" may represent multiple species, or lineages, distinguishable only through genetic analysis. The species has been identified as "in need of conservation" in SC, and unresolved taxonomic issues must be addressed to facilitate conservation planning for the species or species complex. The "species" has declined, or has been locally extirpated in recent years (Means and Travis 2006, Graham 2006) yet remains common in our two study sites. These sites are separated by96 kilometers, are located within different watersheds and unlikely to be affected by the same local conditions or perturbations. According to Beamer and Lamb (2007) these two study sites represent two distinct lineages of Desmognathine salamanders and visual inspection by the author indicates that there are morphological differences between the two populations.

Eurycea chamberlainii, a species identified as "in need of conservation" has not been observed at either site during the study period. *Eurycea guttolineata* and *Eurycea bislineata,* two related species have been observed at both sites. One possible explanation for the lack of this species is a misunderstanding of its habitat requirements. The seeps at both study sites are exfiltrating seeps, flowing from fissures and cracks in a subsurface rock (limestone or diagenetic). These types of seeps may not provide suitable habitat for this species.

Eurycea chamberlainii has been documented recently at Congaree National Park (CNP), Richland County, SC (author's note). A single specimen was found under natural cover adjacent to a seepage pool at the base of a bluff at this site. The seepage pools at CNP differ from those at the two study sites. These seepage pools result from upwelling seeps, groundwater that moves, or is forced upward through unconsolidated sediments, not through a subsurface rock. *Desmognathus "auriculatus"* has also been documented from the seepage pools at CNP and this population may be distinct from the populations in the Calhoun county seeps, which are across the Congaree River, 15 Km from CNP, based on initial field observations.

Conclusions:

1. Seepage wetlands in the Coastal Plain provide important habitat for plethodontid salamanders. These systems are relatively stable throughout the year and do not appear to support populations of predatory fish.

2. The assemblage of salamanders at both study sites is remarkably similar and differences in absolute numbers of animals observed may be explained by sampling bias caused by differences in the geomorphology of each site.

3. There is a need to clarify the taxonomic status of the *Desmognathus "auriculatus"* complex in South Carolina and address the conservation implications inherent in such a revision.

4. There is a need to understand and quantify the different types of seepage wetlands found in the South Carolina Coastal Plain, the amphibian species associated with them and develop a conservation strategy for these systems.

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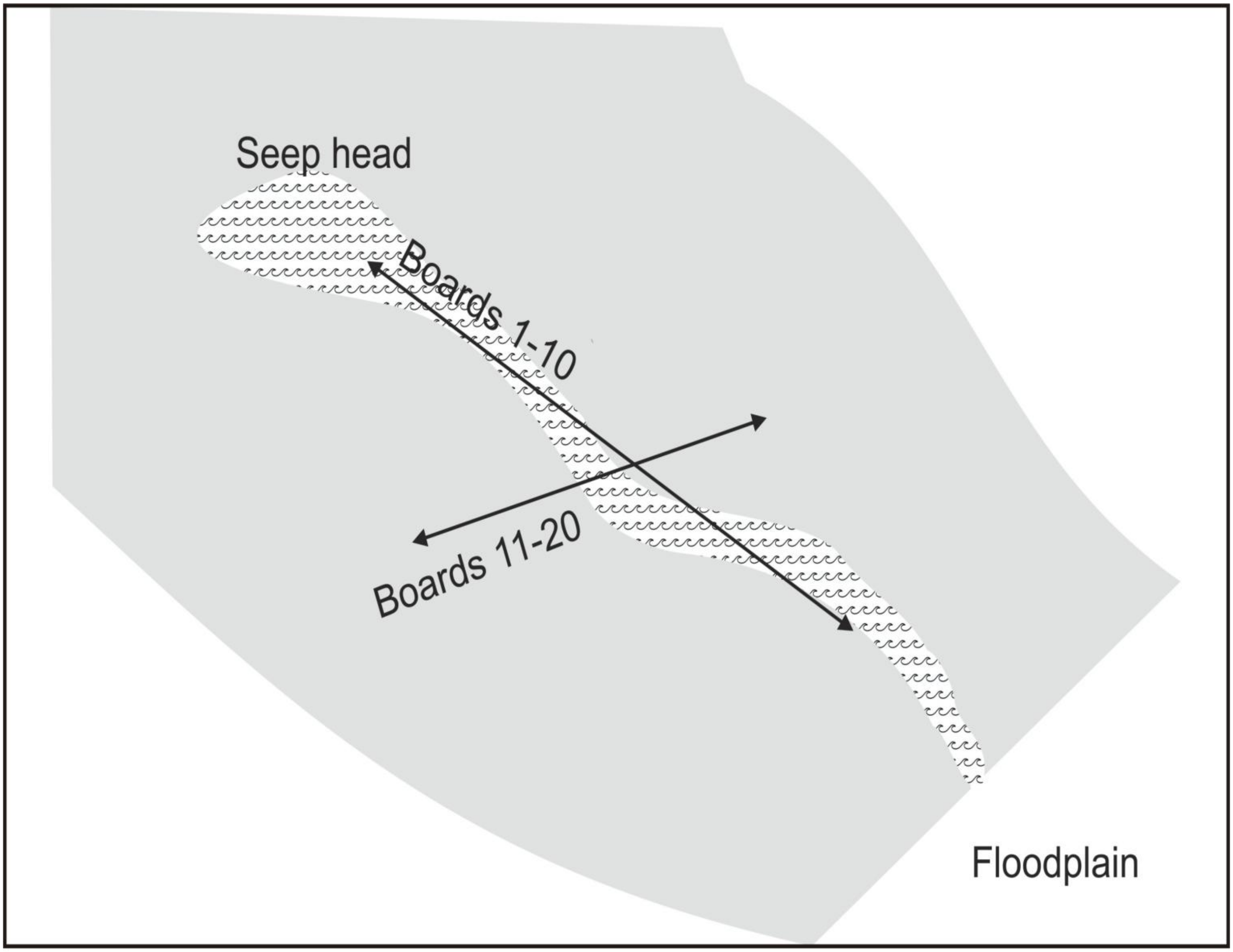
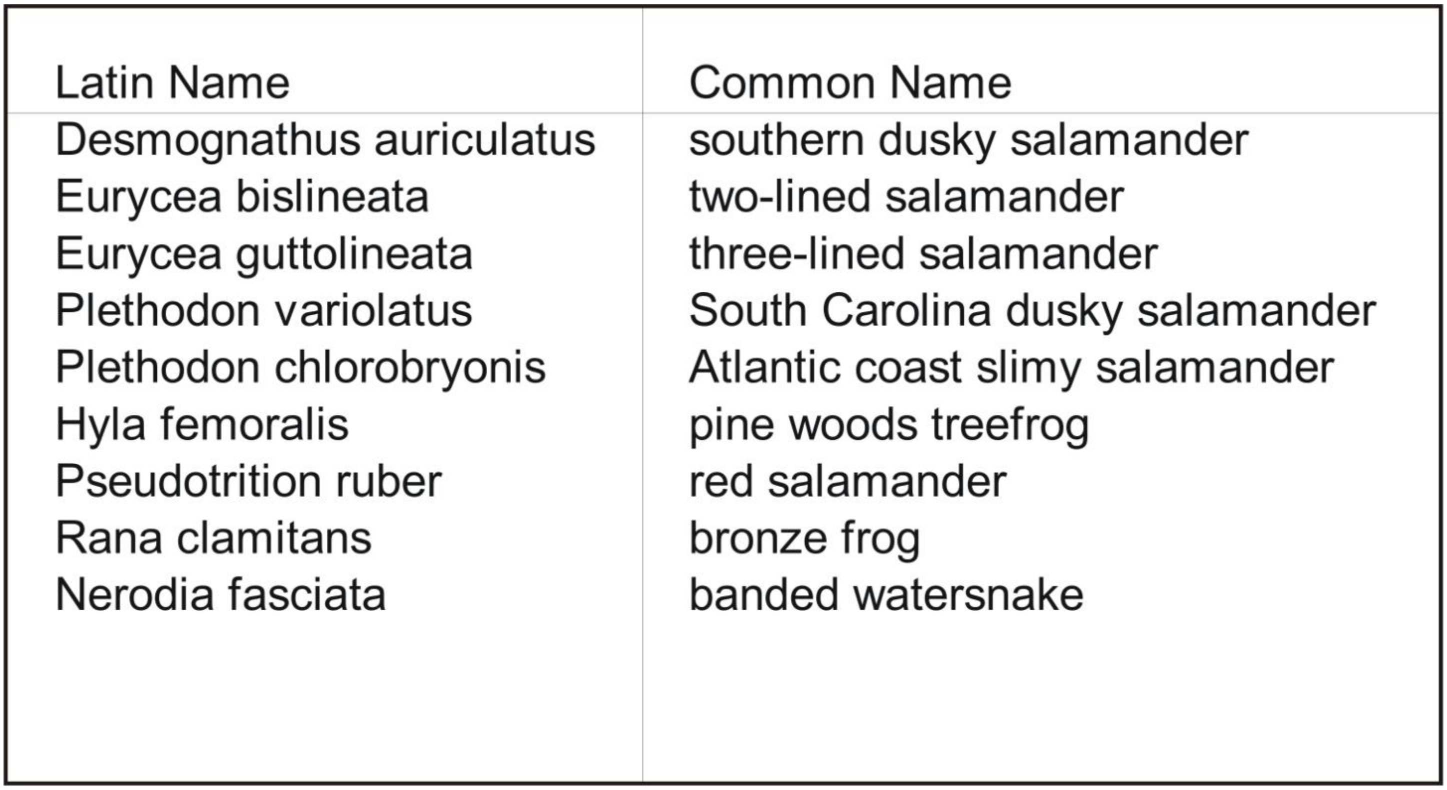


Figure 1. Placement of cover-board transects at seep.



Latin Name	Common Name
Desmognathus auriculatus	southern dusky salamander
Eurycea bislineata	two-lined salamander
Eurycea guttolineata	three-lined salamander
Plethodon variolatus	South Carolina dusky salamander
Plethodon chlorobryonis	Atlantic coast slimy salamander
Hyla femoralis	pine woods treefrog
Pseudotrition ruber	red salamander
Rana clamitans	bronze frog
Nerodia fasciata	banded watersnake

Table 1. Amphibian and reptile species observed at both seep sites during study period.

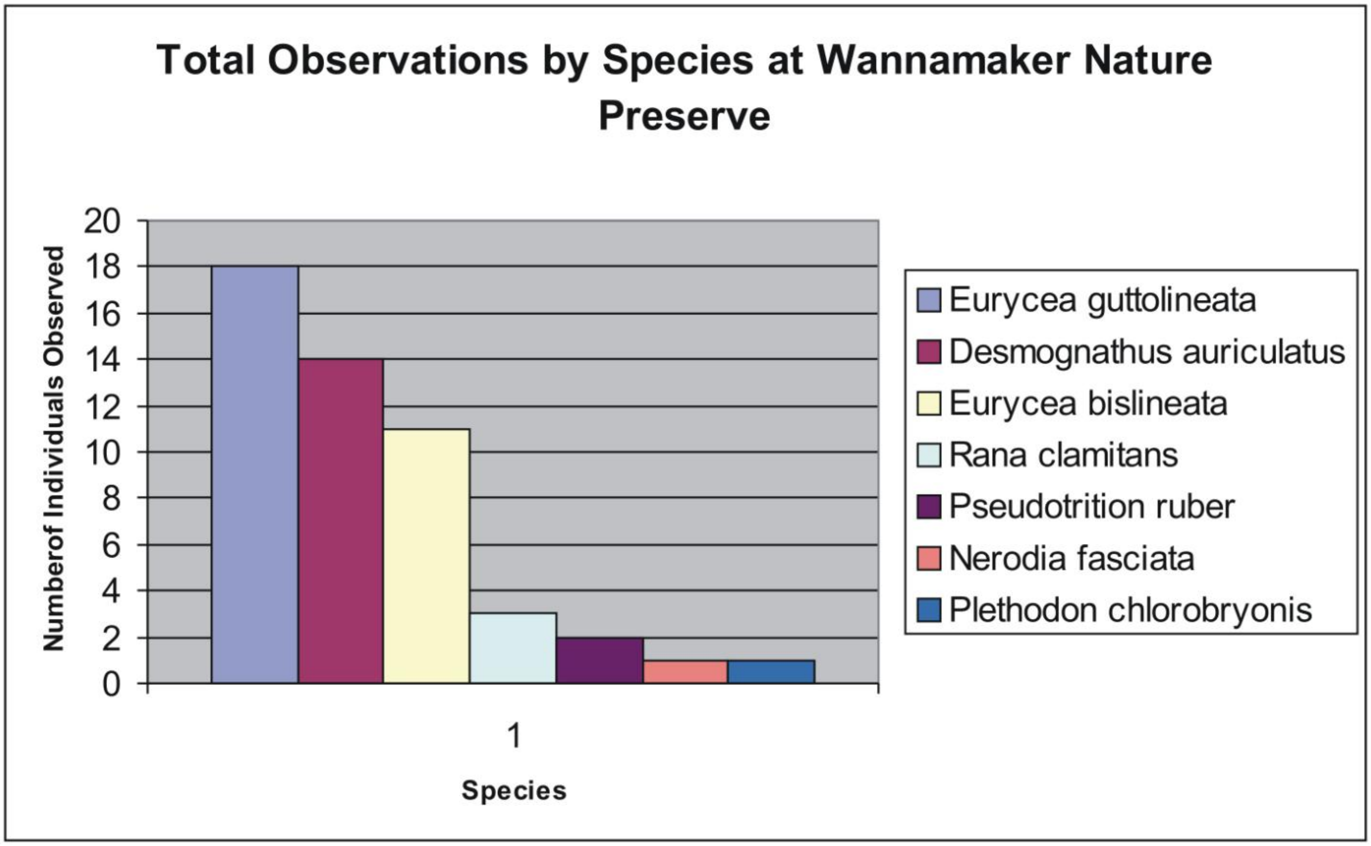


Figure 2. Total observations at WNP

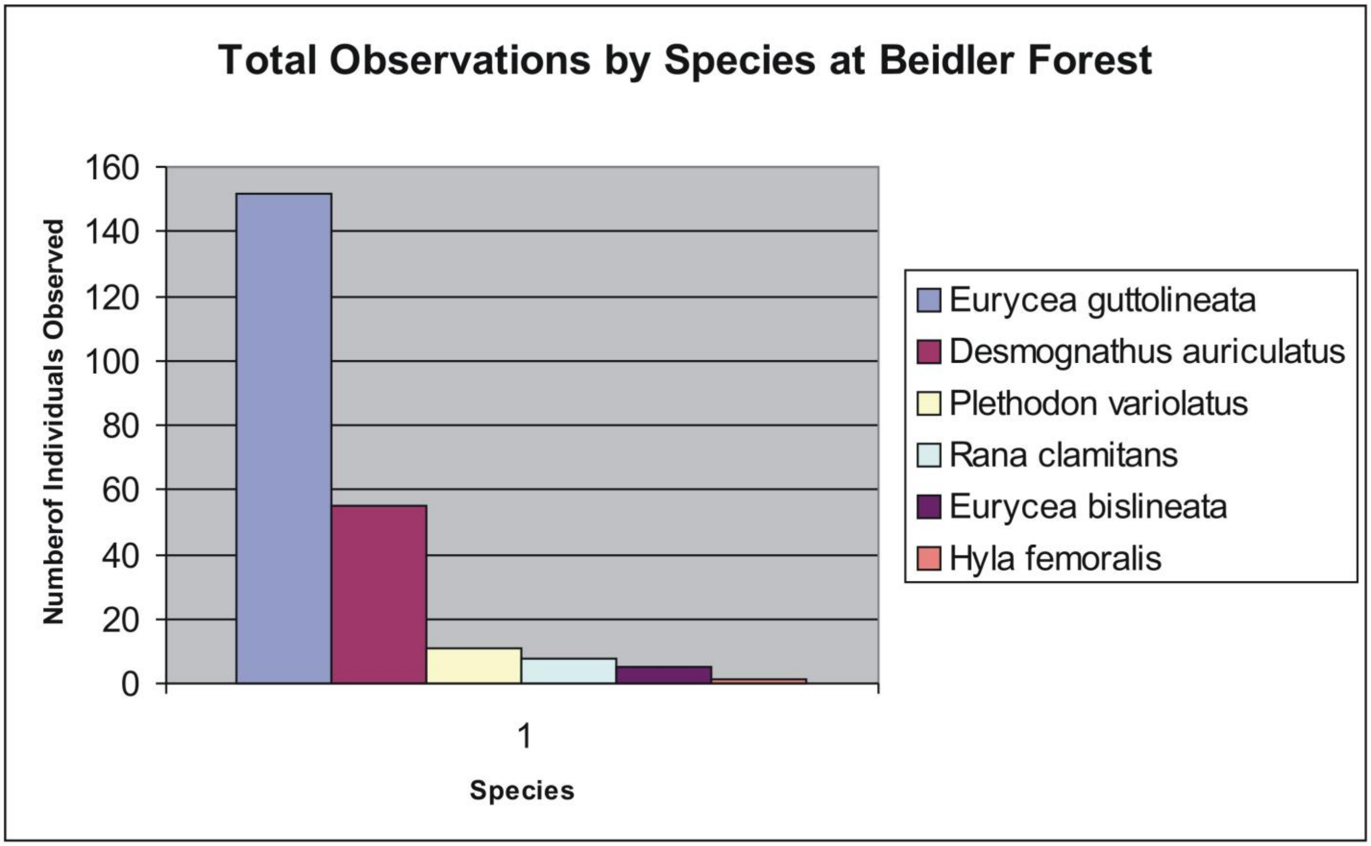


Figure 3. Total observations BF

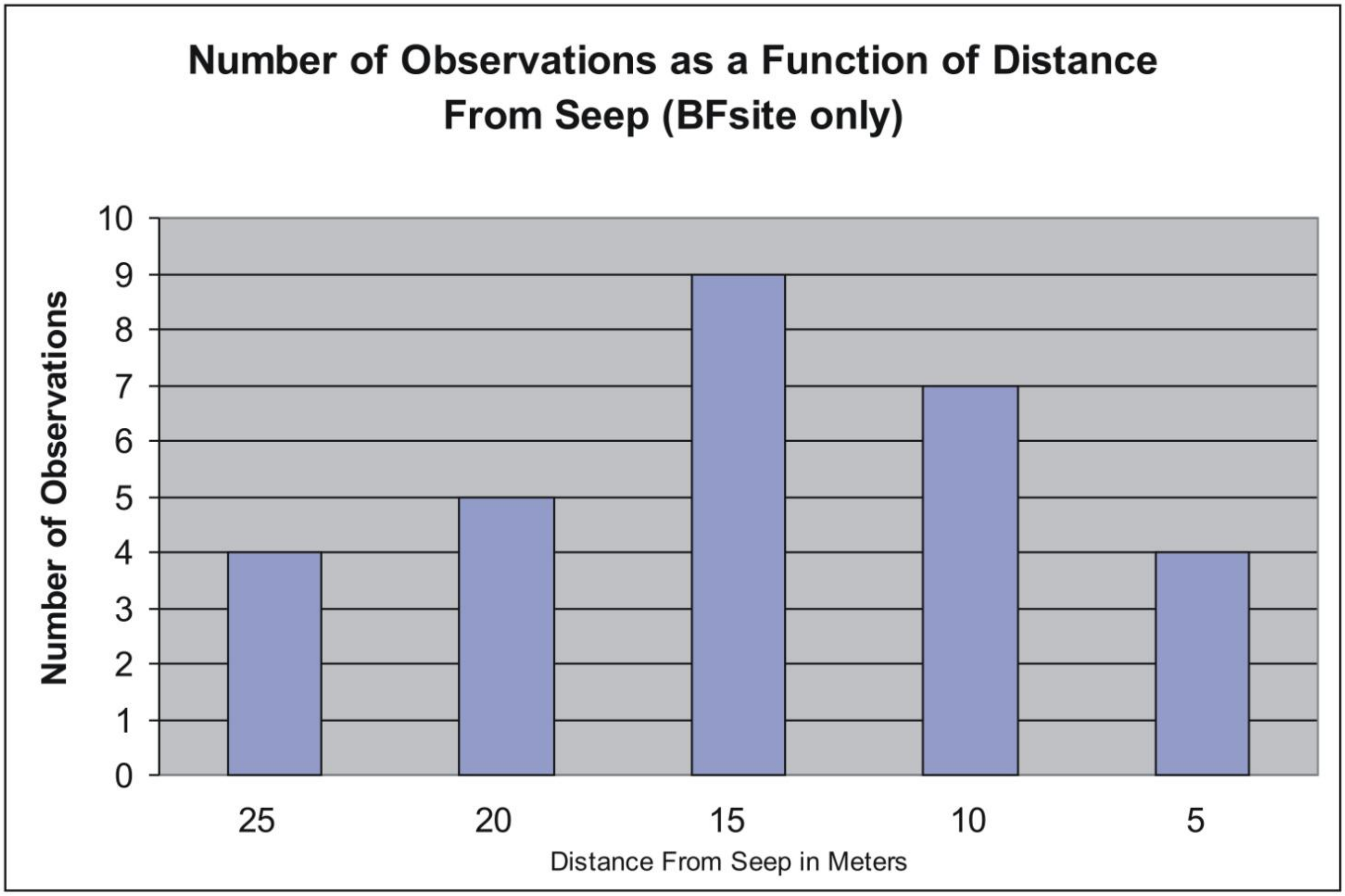


Figure 4. Number of observations at BF by distance from seep

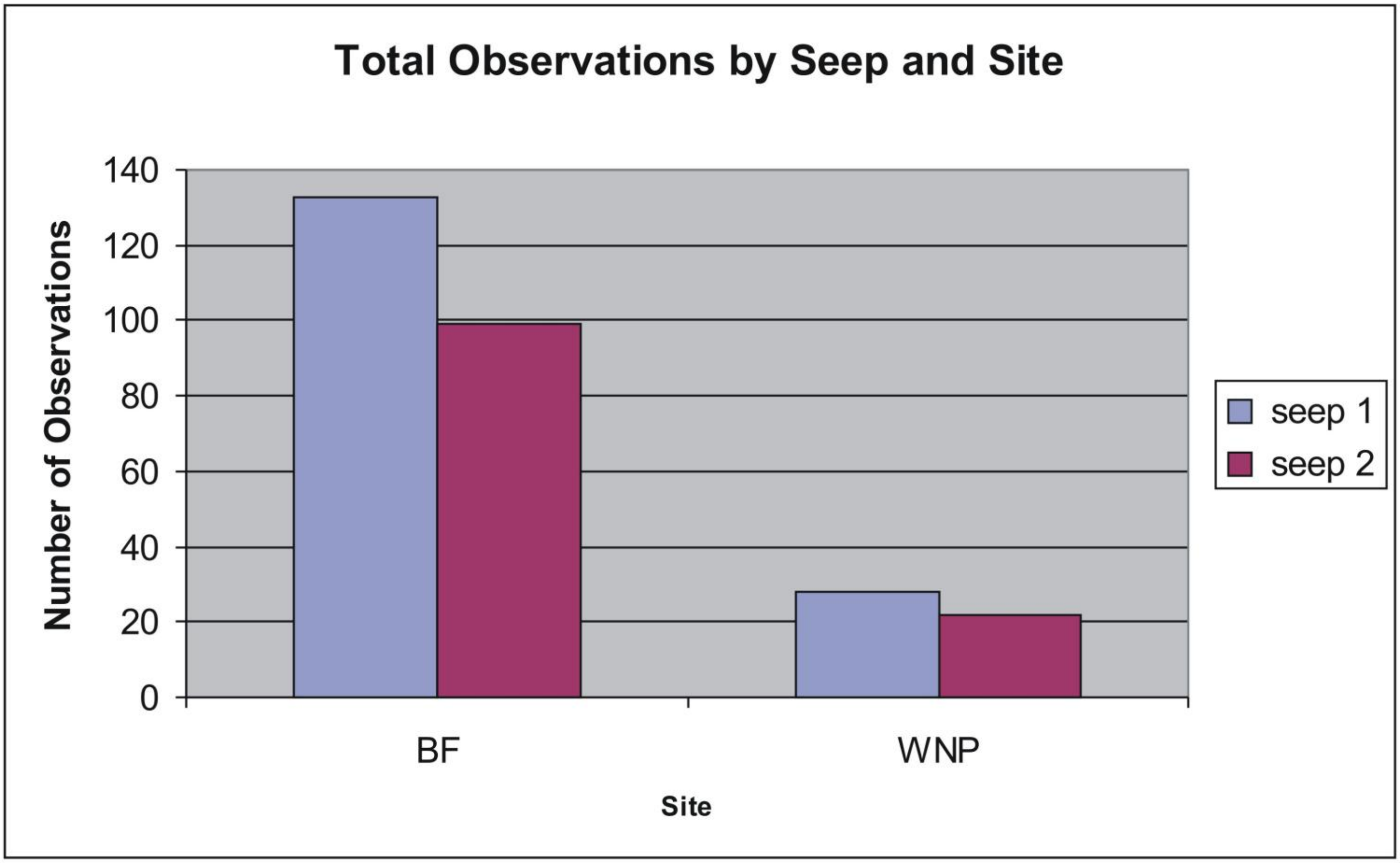


Figure 5. Comparison of observations by seep within site

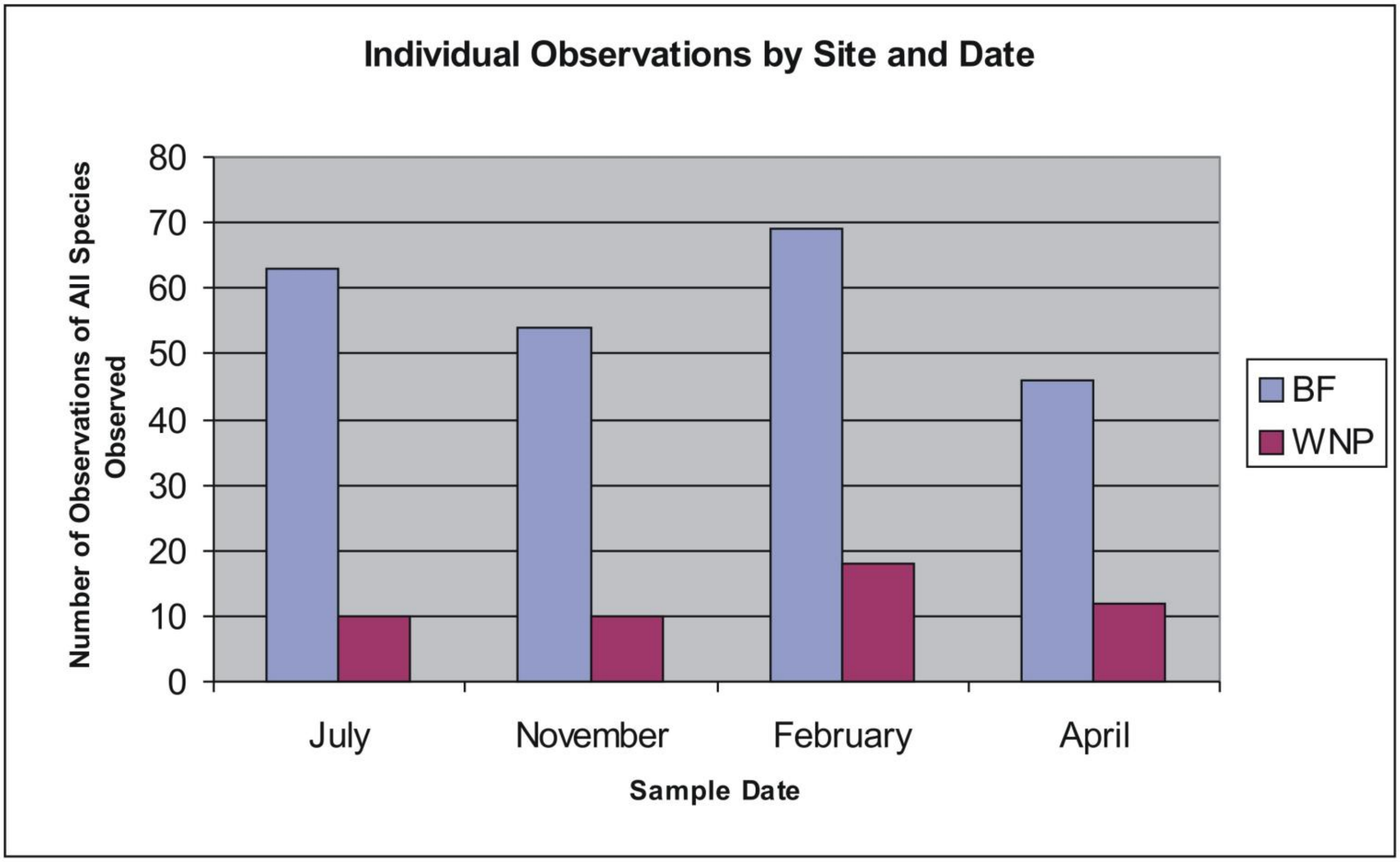


Figure 6. Comparison of observations by date

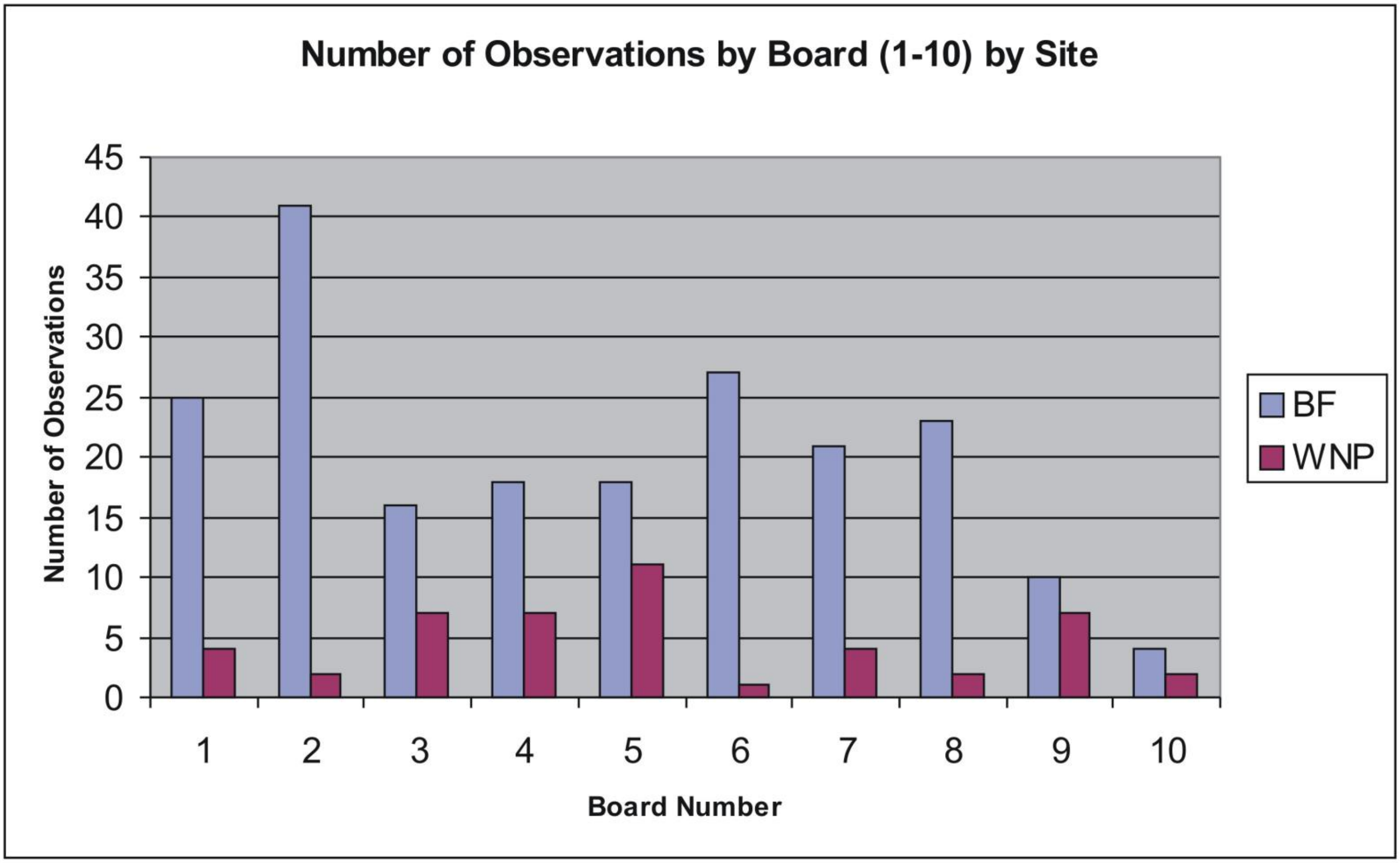


Figure 7. Comparison of observations by seep board

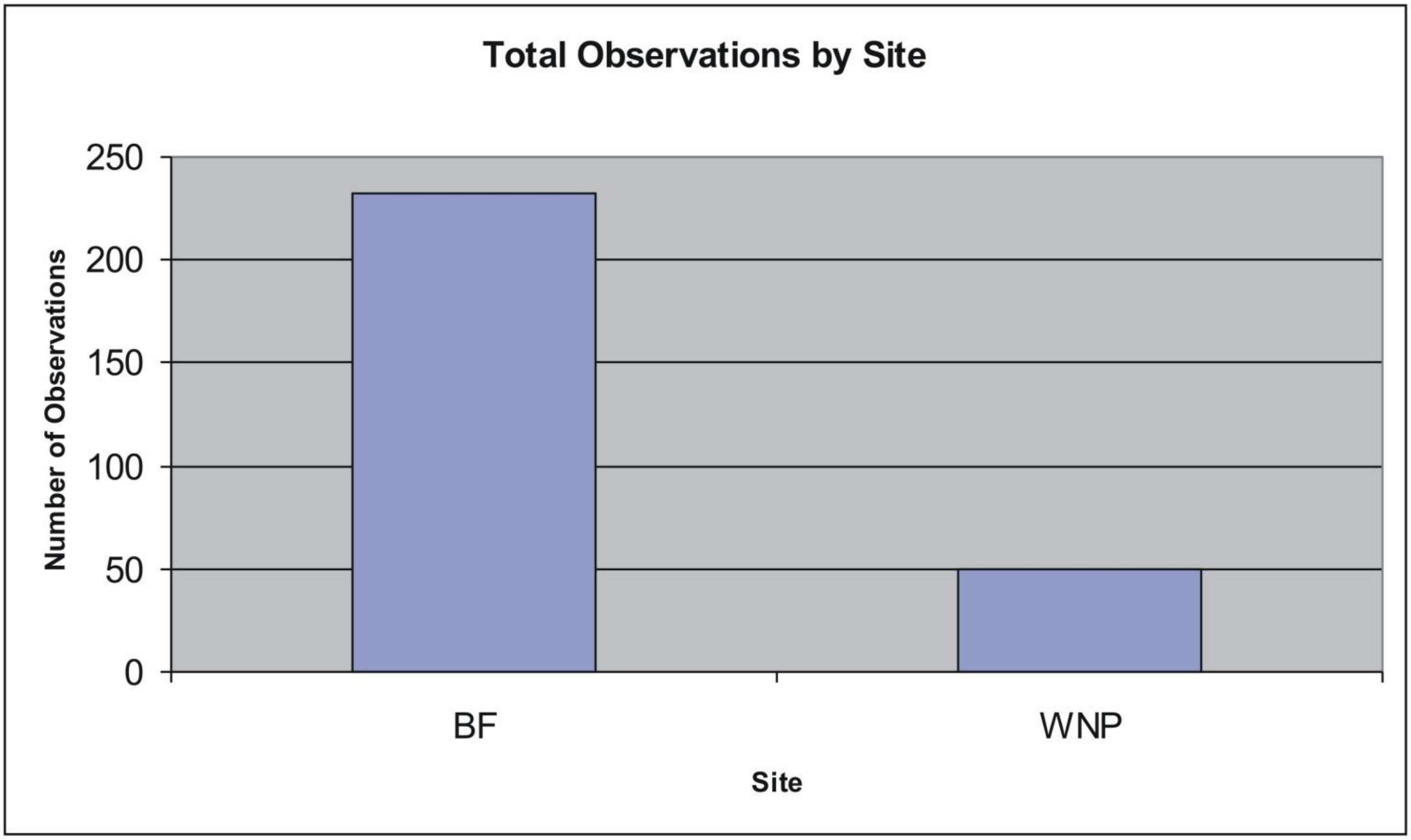


Figure 8. Comparison of total observations by site

```
Matrix 1: rows=3, cols=8
-,a1,a2,a3,a4,a5,a6,a7,
p  1 0 Summer Fall Winter 0 0
lambda  0 1 0 0 0 Distance WNP
======================
Number of parameters          = 7
Number of function calls      = 258
Final function value          = 198.188447
-2log(likelihood)             = 396.376894
AIC                           = 410.376894
Naive occupancy estimate      = 0.400000
```

```
Untransformed (beta) parameters:
Estimated parameter                estimate  std.err
--------------------------          --------  -------
```

beta0 = -1.1340 0.3864 – this means that detection probability intercept had a significantly negative slope (95% confidence intervals = -1.89, -0.37)

beta1 = 0.4075 0.2776 – this means that detection probability was positively associated with summer, but this was not significant (i.e., 95% CI contained zero)

beta2 = -0.7507 0.3177 This means that there was a negative relationship between detection probability and fall (Significant – 95% CI = -1.37, -0.13) – I have to run spring separately, but I suspect there will be a positive relationship with spring)

beta3 = -1.0475 0.4107 This means there was a significant negative relationship between detection probability and winter (95% CI = -1.85, -0.25)

beta4 = 0.2093 0.3244 This means there was a nonsignificant positive slope for abundance – don't worry about this one.

beta5 = -0.6883 0.2319 this means there was a negative relationship between abundance and distance from seep (i.e., salys were more abundant in seep: significant: 95% CI = -1.14, -0.23)

beta6 = -1.0971 0.3082 this means there was a negative relationship between abundance at WNP as compared to FB (i.e., fewer at WNP – Significant: 95% CI: -1.70, -0.49)

```
beta var-cov matrix:
   0.1493  -0.0690  -0.0672  -0.0699  -0.0497   0.0113   0.0115
  -0.0690   0.0771   0.0089   0.0109  -0.0039   0.0157  -0.0320
  -0.0672   0.0089   0.1009   0.0570   0.0544  -0.0015  -0.0015
  -0.0699   0.0109   0.0570   0.1687   0.0542  -0.0018  -0.0018
```

```
  -0.0497   -0.0039    0.0544    0.0542    0.1052    0.0006    0.0007
   0.0113    0.0157   -0.0015   -0.0018    0.0006    0.0538   -0.0009
   0.0115   -0.0320   -0.0015   -0.0018    0.0007   -0.0009    0.0950

==============================================================================
```

Individual Site estimates of c: this means detection probability was the same across sites, which is good

```
      Site        Survey          c     Std.err     95% conf. interval
    1       1    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    2       2    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    3       3    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    4       4    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    5       5    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    6       6    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    7       7    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    8       8    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
    9       9    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   10      10    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   11      11    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   12      12    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   13      13    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   14      14    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   15      15    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   16      16    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   17      17    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   18      18    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   19      19    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   20      20    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   21      21    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   22      22    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   23      23    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   24      24    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   25      25    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   26      26    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   27      27    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   28      28    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   29      29    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   30      30    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   31      31    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   32      32    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   33      33    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   34      34    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   35      35    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   36      36    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
   37      37    1        1-1:  0.1319   0.0390     0.0723 - 0.2284
```

```
   38     38   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   39     39   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   40     40   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   41     41   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   42     42   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   43     43   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   44     44   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   45     45   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   46     46   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   47     47   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   48     48   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   49     49   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   50     50   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   51     51   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   52     52   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   53     53   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   54     54   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   55     55   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   56     56   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   57     57   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   58     58   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   59     59   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   60     60   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   61     61   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   62     62   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   63     63   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   64     64   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   65     65   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   66     66   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   67     67   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   68     68   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   69     69   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   70     70   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   71     71   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   72     72   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   73     73   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   74     74   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   75     75   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   76     76   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   77     77   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   78     78   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   79     79   1       1-1:  0.1319   0.0390     0.0723 - 0.2284
   80     80   1       1-1:  0.1319   0.0390     0.0723 - 0.2284

=================================================================
```

Individual Site estimates of Lambda: These are the abundance estimates with SE and 95% CI for every board!

```
      Site        Survey      Lambda     Std.err     95% conf. interval
    1       1    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    2       2    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    3       3    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    4       4    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    5       5    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    6       6    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    7       7    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    8       8    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
    9       9    1        1-1:  2.5015   0.7219     2.1247 - 6.5861
   10      10    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   11      11    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   12      12    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   13      13    1         1-1:  0.4997   0.2572     0.2973 - 2.2361
   14      14    1         1-1:  0.6906   0.2940     0.4841 - 2.5684
   15      15    1         1-1:  0.9478   0.3314     0.7458 - 2.9369
   16      16    1         1-1:  1.3098   0.3830     1.1077 - 3.4853
   17      17    1         1-1:  1.8101   0.4875     1.5702 - 4.5127
   18      18    1         1-1:  1.8101   0.4875     1.5702 - 4.5127
   19      19    1         1-1:  1.3098   0.3830     1.1077 - 3.4853
   20      20    1         1-1:  0.9478   0.3314     0.7458 - 2.9369
   21      21    1         1-1:  0.6906   0.2940     0.4841 - 2.5684
   22      22    1         1-1:  0.4997   0.2572     0.2973 - 2.2361
   23      23    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   24      24    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   25      25    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   26      26    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   27      27    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   28      28    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   29      29    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   30      30    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   31      31    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   32      32    1         1-1:  2.5015   0.7219     2.1247 - 6.5861
   33      33    1         1-1:  0.4997   0.2572     0.2973 - 2.2361
   34      34    1         1-1:  0.6906   0.2940     0.4841 - 2.5684
   35      35    1         1-1:  0.9478   0.3314     0.7458 - 2.9369
   36      36    1         1-1:  1.3098   0.3830     1.1077 - 3.4853
   37      37    1         1-1:  1.8101   0.4875     1.5702 - 4.5127
   38      38    1         1-1:  1.8101   0.4875     1.5702 - 4.5127
   39      39    1         1-1:  1.3098   0.3830     1.1077 - 3.4853
   40      40    1         1-1:  0.9478   0.3314     0.7458 - 2.9369
   41      41    1         1-1:  0.2305   0.1056     0.1527 - 0.9208
   42      42    1         1-1:  0.1668   0.0903     0.0940 - 0.7839
   43      43    1         1-1:  0.8350   0.2840     0.6657 - 2.5249
```

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    44      44   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    45      45   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    46      46   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    47      47   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    48      48   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    49      49   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    50      50   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    51      51   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    52      52   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    53      53   1      1-1:  0.1668   0.0903     0.0940 - 0.7839
    54      54   1      1-1:  0.2305   0.1056     0.1527 - 0.9208
    55      55   1      1-1:  0.3164   0.1233     0.2349 - 1.0827
    56      56   1      1-1:  0.4372   0.1490     0.3483 - 1.3242
    57      57   1      1-1:  0.6042   0.1949     0.4928 - 1.7447
    58      58   1      1-1:  0.6042   0.1949     0.4928 - 1.7447
    59      59   1      1-1:  0.4372   0.1490     0.3483 - 1.3242
    60      60   1      1-1:  0.3164   0.1233     0.2349 - 1.0827
    61      61   1      1-1:  0.2305   0.1056     0.1527 - 0.9208
    62      62   1      1-1:  0.1668   0.0903     0.0940 - 0.7839
    63      63   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    64      64   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    65      65   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    66      66   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    67      67   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    68      68   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    69      69   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    70      70   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    71      71   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    72      72   1      1-1:  0.8350   0.2840     0.6657 - 2.5249
    73      73   1      1-1:  0.1668   0.0903     0.0940 - 0.7839
    74      74   1      1-1:  0.2305   0.1056     0.1527 - 0.9208
    75      75   1      1-1:  0.3164   0.1233     0.2349 - 1.0827
    76      76   1      1-1:  0.4372   0.1490     0.3483 - 1.3242
    77      77   1      1-1:  0.6042   0.1949     0.4928 - 1.7447
    78      78   1      1-1:  0.6042   0.1949     0.4928 - 1.7447
    79      79   1      1-1:  0.4372   0.1490     0.3483 - 1.3242
    80      80   1      1-1:  0.3164   0.1233     0.2349 - 1.0827
```

Occupancy model analysis for Plethodontid salamanders at two Coastal Plain seepage wetland sites.

1. Aquatic versus terrestrial analysis for the Southern Dusky Salamander (*Desmognathus auriculatus*) (Da) (also covers phenology)

Methods (analysis): We used program Presence to assess the effects of board location (aquatic versus terrestrial), and site (Beidler Forest seep 1 (BF1), Beidler Forest seep 2 (BF2), Wannamaker Nature Preserve seep 1 (WNP1), Wannamaker Nature Preserve seep

2 (WNP2)) on Da occupancy of cover boards. We modeled detection probability as a function of season (phenology). Using data from aquatic and terrestrial cover boards, we used presence absence data (taken upon each visit to the study sites) to compare four candidate models (Table 1). We treated board location as a continuous variable, which we normalized using a z-transformation, that represented distance from water. We did a post hoc comparison (t-test) of cover boards located in aquatic and terrestrial transects using board-specific abundance estimates derived from the top occupancy model.
Results: One candidate model received support (Table 1). We had a 13 % (95 % CI = 7 – 23 %) probability of detecting Da under cover boards. Da abundance did not vary seasonally (β = 0.4075 ± 0.2776; 95% CI = -0.1366 – 0.9515), but decreased as distance from water increased (β = -0.6883 ± 0.2319; 95% CI = -1.1428 – -0.2338; Figure 1). Further, Da were more abundant at WNP than FB (β = -1.0971 ± 0.3082; 95% CI: -1.7012 – -0.0.4930). Da abundance was significantly higher under cover boards located in aquatic transects than in those located in terrestrial transects (Figure 2; DF=72.5; t = 3.01, *P* = 0.0036).

Table 1. Candidate models of Da occupancy.

Model	AIC	ΔAIC	K	Model Likelihood
p(season), λ(distance+site)	410.38	0.00	0.99	1.00
p(season), λ(site)	419.58	9.2	0.01	0.01
p(season), λ(distance)	422.71	12.33	0.00	0.00
p(season), λ(.)	430.21	19.83	0.00	0.00

2. Aquatic versus terrestrial analysis for Southern Two-lined Salamander (*Euryces cirrigera*) (Ec), Three-lined Salamander (*Eurycea guttolineata*) (Eg), and Slimy Salamander (*Plethodon "glutinosus"* complex) (Pg)

We had insufficient data to use program Presence for Ec, Eg, and Pg using methods outlined above (for Da). Thus, we used generalized estimating equations as an extension of negative binomial regression to model relative abundance for Ec, Eg, and Pg as a function of four candidate models (Table 2). In this analysis, treatment was a binary predictor variable that corresponded to captures either the terrestrial or aquatic transect, and site was a binary predictor variable that represented FB or WNP. We used seep as the repeated subject and identified an auto-regressive working correlation structure for each model.

Results:

Ec.—The only model that adequately fit the Ec capture data included site as the only predictor variable of Ec abundance, but regression coefficients (Table 3) indicated that we failed to detect a significant site effect for Ec.

Pg.— The top ranking model included site as the sole predictor of Pg abundance, but we failed to detect a significant effect of site (Table 3). The second-ranking model included site and treatment as predictors, and regression coefficients from this model indicated that Pg abundance was higher in the terrestrial transect (estimate for terrestrial transect = 2.0487; SE = 0.7907; Z = 2.59; *P* = 0.0095).

Eg.—We failed to detect a treatment effect in Eg abundance; however, Eg abundance was greater at FB than at the WNP (Table 3).

Table 2. Ranking of candidate negative binomial regression models for Ec, Eg, and Pg. We used salamander count data as the response and site (FB, WNP) and treatment (Terrestrial, Aquatic) as predictors. NA indicates model lack of fit.

Species	Model	QICu	Model Rank
Ec	Site + Treatment + Site*Treatment	NA	NA
	Treatment + Site	NA	NA
	Treatment	NA	NA
	Site	34.00	1
Eg	Site + Treatment + Site*Treatment	-523.29	4
	Treatment + Site	-569.38	2
	Treatment	-565.83	3
	Site	-580.83	1
Pg	Site + Treatment + Site*Treatment	NA	NA
	Treatment + Site	35.20	2
	Treatment	52.75	3
	Site	33.59	1

Table 3. Output from top-ranking negative binomial regression models for Ec, Eg, and Pg.

Species (Model)	Parameters	Estimate ± SE	Z	*P*
Ec (Site)	Intercept	0.7500 ± 0.5301	1.41	0.1571
	FB	-0.2974 ± 0.7496	-0.40	0.6915
Eg (Site)	Intercept	0.1747 ± 0.3970	0.44	0.6600
	FB	2.1913 ± 0.5112	4.29	< 0.0001
Pg (Site	Intercept	-1.6743 ± 0.7890	-2.12	0.0338
	FB	1.5406 ± 0.9183	1.68	0.0934

3. Phenology for Ec, Eg, and Pg

We illustrated seasonal variation in Ec, Eg, and Pg abundance (Fig. 2) from capture data collected at WNP seeps 1 and 2 and BF seeps 1 and 2. These data will be used in future analyses aimed at modeling occupancy for each species at the landscape scale (using data from multiple study sites and scales).

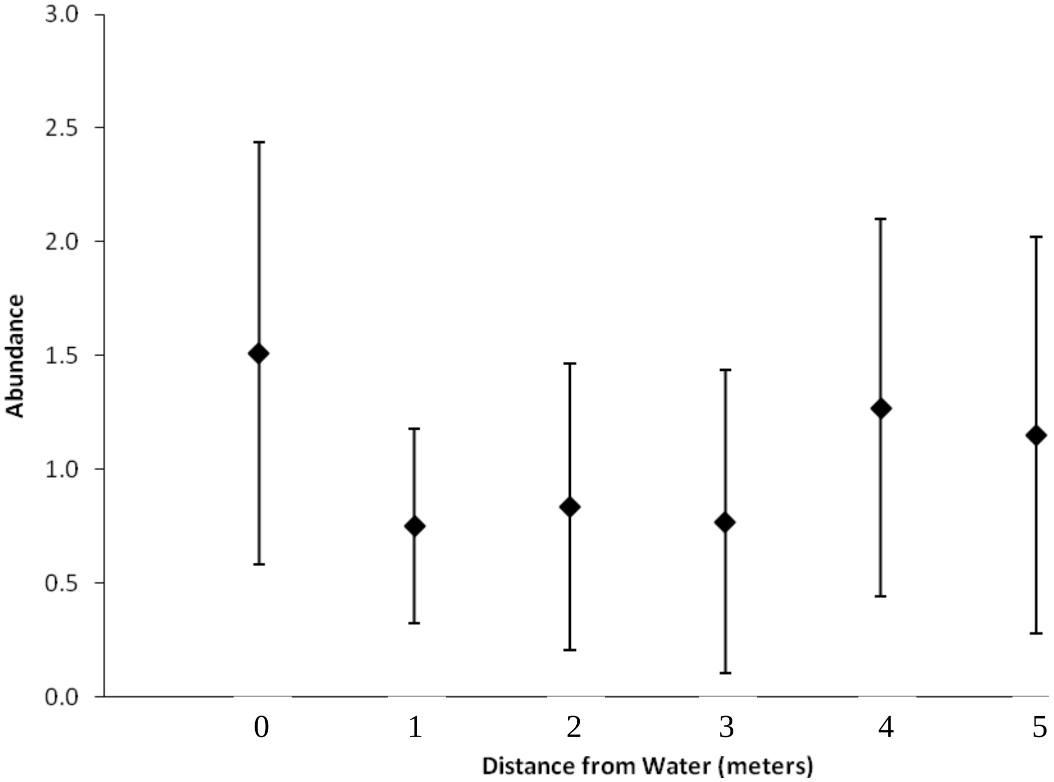


Figure 1. Average abundance and standard deviation of Da under coverboards located 0-5 meters away from center of seep.

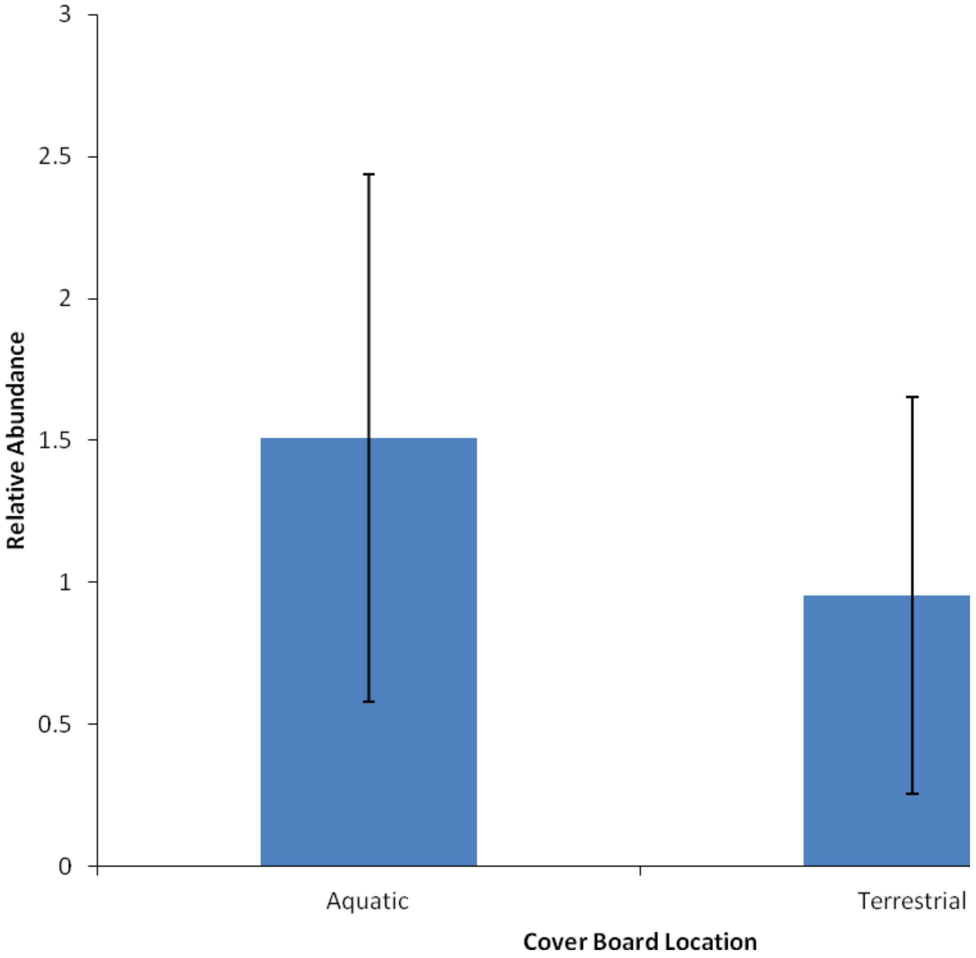


Figure 2. Average Da abundance ± standard deviation under cover boards located in aquatic and terrestrial transects.

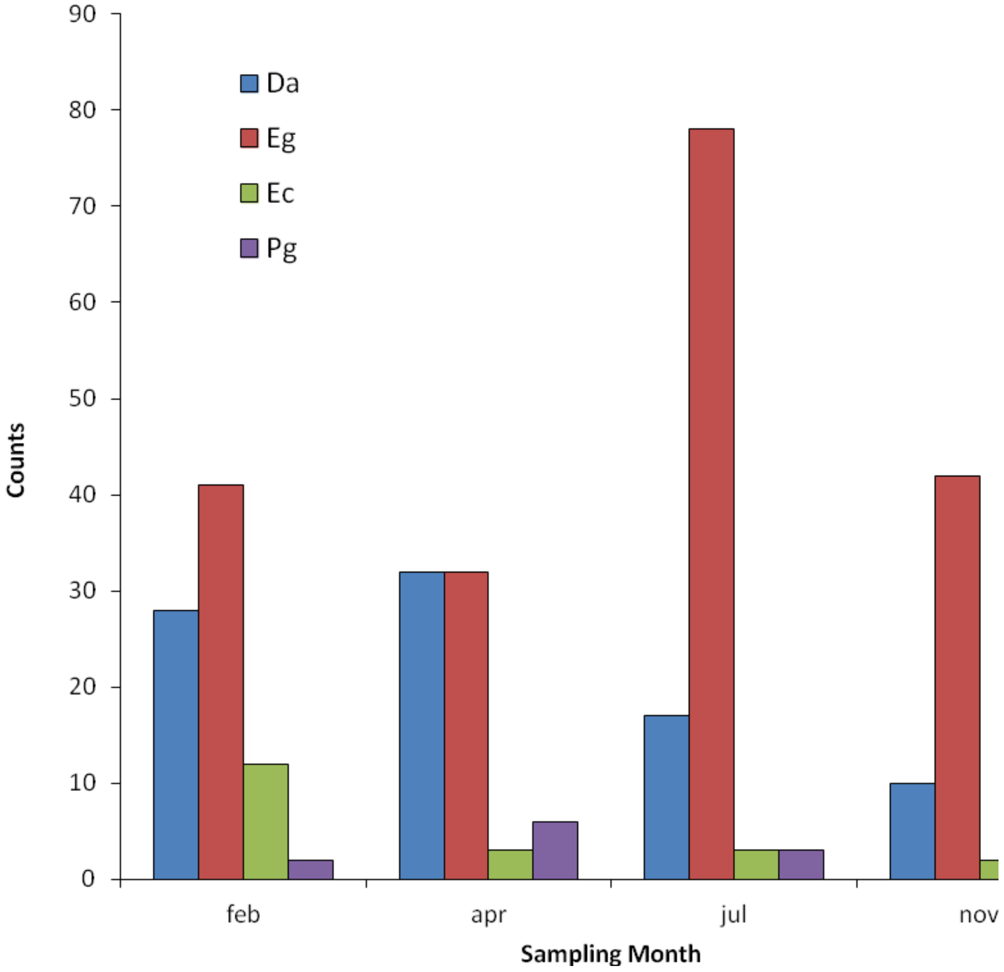


Figure 3. Counts of Da, Eg, Ec, and Pg sampled using cover boards in terrestrial and aquatic transects at FB and WNP.

Appendix 4-2

auriculatus A

This lineage includes specimens collected from near the type locality of *Desmognathus auriculatus*, while Kozak et al. (2005) questioned the validity of *D. auriculatus* Beamer and Lamb (2008) demonstrated that populations from the vicinity of the type locality had mtDNA haplotypes that were very divergent from other coastal plain *Desmognathus* populations. The only other published molecular data that likely pertains to *Desmognathus auriculatus* is for seventeen polymorphic proteins from a population in Liberty County, Florida that also supported species status for *D. auriculatus* (Karlin & Guttman, 1986).

Phylogenetic analysis of this dataset also supports specific status for *D. auriculatus*. In the mtDNA reconstruction the *auriculatus* A clade is sister to a clade of all populations currently recognized as *D. fuscus* and some other populations of D. *auriculatus* (*auriculatus* B & C). A sister relation is recovered between *D. auriculatus* and *D. planiceps* in the three species tree reconstructions, the clade containing *D. auriculatus* and *D. planiceps* is sister to a clade of all populations currently recognized as *D. fuscus* and some other populations of D. *auriculatus* (*auriculatus* B & C).

This lineage appears to have declined since the mid-1970's (Dodd, 1999; Means & Travis, 2003; Graham, 2006) and as a result the range extent of *Desmognathus auriculatus* and the other coastal plain endemic lineages is imprecisely understood. One of the outstanding problems regards those populations that formerly existed west of the Okefenokee Plains. Means (FWS report) states that populations become more lightly pigmented in Alabama and he reports a habitat hiatus across the lower Gulf Coastal Plain between the dark pigmented Florida specimens and the light pigmented Alabama specimens. Means hypothesized that if Escambia Bay or Mobile Bay interrupted gene flow between swamp dwelling *Desmognathus* populations for a sufficiently long time the pale western populations might represent a separate species. While I am lacking samples between the Okefenokee Plains and the western Florida panhandle, a swamp dwelling Coastal Plain population from near Grand Bay, Alabama is not referable to *D. auriculatus* (see *auriculatus* D account).

Means (1975) hypothesized that continuous swampy habitat along the coast might provide ready dispersal opportunities for swamp dwelling *Desmognathus*. Patterns of genetic differentiation in another swamp dwelling coastal plain lineage and to a lesser extent with *D. auriculatus* support this hypothesis (Beamer & Lamb, 2008). While the current configuration of habitat precludes continuous gene flow, Means points out that in the Pleistocene and at other lower seal level stands, swampy habitat may have been more continuous near the two embayments. An alternative hypothesis for the low genetic variability of swamp dwelling Coastal Plain *Desmognathus* is that despite being a moderately old lineage their swampy coastal plain habitats have been repeatedly reconfigured by rising and falling sea levels. As a result modern populations have only recently occupied their present distribution.

Beamer and Lamb (2008) did not publish a revised distribution map of *D. auriculatus* mainly because they only had three populations and they lacked specimens of "*auriculatus*" from west of the Okefenokee Plains. Nonetheless they suggested that this name be restricted to the lineage occupying swamps west of the Ogeechee River to at least the Okefenokee Plains. My samples of this lineage now total four populations, three from Georgia and one from Florida. The specimens were all collected in mucky aquatic microhabitats from the Sea Island Flatwoods and Okefenokee Plains ecoregions in the Suwannee, Altamaha and the Ogeechee (west of the river) drainages. My single additional sample does not extend the known range since it is from a locality that lies between those published in Beamer and Lamb (2008).

Means (1974 & 1975) worked extensively with populations in the Florida panhandle and based on comparison to freshly collected topotypic material from Riceboro, Georgia he referred these Florida populations to *D. auriculatus*. I have examined specimens from the Florida panhandle housed in the Field Museum of Natural History and at the Coastal Plains Institute and concur with Means that these populations are likely referable to *D. auriculatus*. However it is worth pointing out that all of the populations of *auriculatus* A found during this study have been found sympatrically with *Stereochilus*. *Stereochilus* does not occur south or west of the Okefenokee Plains therefore it is possible then that the Florida panhandle populations represent a separate lineage. These Florida panhandle specimens occupy the Gulf Coast Flatwoods in the Aucilla, Ochlockonee, Apalachicola, Econfina, Choctawhatchee and Yellow River drainages.

Rossman (1959) and Means (1974) assigned populations previously recognized as *Desmognathus fuscus carri* to *D. auriculatus*. No specimens from within the range of *D. f. carri* where available for DNA sequencing so the phylogenetic position of these populations is unknown. If these populations are referable to *D. auriculatus* then the range also included the Southwestern Florida Flatwoods, Central Florida Ridges and Uplands, Eastern Florida Flatwoods in the Waccasassa, Tampa Bay and St. Johns River drainages.

Graham (2006) presented a map of the distribution of *Desmognathus auriculatus* in Georgia based on examination of museum specimens and his collections of fresh material. His map depicts populations of *D. auriculatus* from the following level iv ecoregions; Okefenokee Swamp, Bacon Terraces, Vidalia Upland, Tifton Upland, Tallahasee Hills/Valdosta Limesink and adds the Satilla River drainage. He demonstrated that many of the Georgia populations previously called *D. auriculatus* were morphologically and ecologically distinct and should instead be referred to either *D. conanti* or *D. apalachicolae*.

The range of *Desmognathus auriculatus* contacts at least four of the lineages recovered in my phylogenetic analysis. Means (1975) and Means and Karlin (1986) discussed the contact between *D. auriculatus* and *D. apalachicolae* in the Florida panhandle. In this area D. auriculatus is excluded from preferred seepage habitats by the smaller *D. apalachicolae*. *Desmognathus auriculatus* is apparently excluded from all low order streams occupied by *D. apalachicolae*. In adjacent drainages that lack *D. apalachicolae*, *D. auriculatus* is found in low order streams all the way to their heads. In areas where low order streams enter the floodplain of the Apalachicola River, both *D. auriculatus* and *D. apalachicolae* were found together (Means, 1974). There is no evidence of hybridization between *D. auriculatus* and *D. apalachicolae*. *Desmognathus auriculatus* displays a similar microhabitat shift farther west in the Florida panhandle where it contacts the *conanti* B& C lineage (Means, 1975). In the Yellow River drainage *D. auriculatus* is found along the swampy river floodplain and it is also found all the way to ravine heads in small tributary streams that lack the *conanti* B&C

lineage. However in small tributary streams where the *conanti* B&C lineage is present, *D. auriculatus* is apparently excluded.

Farther east in the Florida panhandle the *Desmognathus auriculatus* and the *conanti* B&C lineages are distributed parapatrically in two adjacent and small drainages that enter Choctawhatchee Bay; here *D. auriculatus* is entirely absent from the drainage basin that contains the *conanti* B&C lineage. Means (1975) did not report any sites of direct contact between *D. auriculatus* and the *conanti* B&C lineage but presumably there was contact in the Yellow River drainage where the small tributary streams containing the *conanti* B&C lineage enter the Yellow River floodplain swamp. There is no evidence of hybridization between *D. auriculatus* and the *conanti* B&C lineage.

The range of *Desmognathus auriculatus* and the *conanti* A lineage are adjacent one another where escarpments along the western sides of the Altamaha and Ogeechee Rivers abut the swampy river floodplain. My samples of the *conanti* A lineage from the lower Altamaha drainage near Doctortown, Georgia were collected in seepages along a river bluff while my nearby Altamaha drainage *D. auriculatus* samples were collected from cypress swamp habitat. It is not known if *D. auriculatus* inhabits ravine habitats in this portion of the range and if the distribution patterns between *D. auriculatus* and congeners noted by Means (1975) in the Florida panhandle are paralleled in this area. There is no published data on the contact zone between these two lineages and likewise there is no evidence of hybridization.

The eastern edge of the range extent of *D. auriculatus* is adjacent to the western edge of the range extent of *auriculatus* B. My closest samples of these two lineages lie less than 35 km apart separated by the Ogeechee River. Both populations inhabit similar cypress-gum swamps and there are not any obvious biogeographic barriers. Both lineages are distributed across river drainages and river channels that are larger than the Ogeechee River. Nonetheless the exact same geographic break is also present in co-distributed populations of *Eurycea quadridigitata* although the populations of *E. quadridigitata* are sister clades and *D. auriculatus* and *auriculatus* B are only distantly related. These populations were considered conspecific until Beamer and Lamb (2008) demonstrated their distinctness, as a result nothing is known regarding the interactions of these two lineages where they contact. *Desmognathus auriculatus* might contact *auriculatus* D somewhere in the western panhandle of Florida or in the vicinity of Mobile Bay but nothing is known of their interactions.

As mentioned above populations of *Desmognathus auriculatus* (and other swamp dwelling lineages) have apparently declined. Dodd (1998) published the first account of declines or extirpations of populations of *D. auriculatus*. He reported on greatly reduced populations at Silver Glen Springs (type locality of *D. f. carri*) and the apparent extirpation of populations at the Devil's Millhopper State Geological Area in Alachua County, Florida. The population in Devil's Millhopper was last reported from that site in 1971 and a vertebrate survey of the site in 1976 failed to reveal the presence of this species that had previously been characterized as common.

Graham (2010) reported on apparent declines in *D. auriculatus* populations in Georgia. He was only able to locate populations at two historical sites and one new site (one additional site he reported on is apparently referable to the *conanti* A lineage. Means (1974) had no problem collecting a series of topotypes near the LeConte Woodmanston but the species has not been found there despite several recent attempts. In the past the species was evidently common as several moderate single day collections in the Georgia State Museum collection attest.

Means and Travis (2007) reported on the apparent extirpation of populations of *Desmognathus auriculatus* from ravines on Eglin Airforce Base. They also reported the absence of the species from the Ochlockonee River Floodplain in Leon County, Florida since 1971, from the Telogia Creek floodplain in Liberty County, Florida since 1974 and Deep Springs Canyon, Bay County, Florida since 1976. The species was very common at all three sites prior to those dates. At the time of their publication they were only aware of two extant populations in the Florida panhandle. Means and Travis (2007) also reported a decline in a population from Irwin County, Georgia. During the course of this study many attempts were made to locate this species in suitable habitats in both Florida and Georgia. My searches included many historical localities as well as newly identified sites and I was only able to locate two extant populations. My remaining two samples from Georgia collected by Sean Graham and Dirk Stevenson are the only additional extant populations known from Georgia. I am aware of only seven localities where this species has been collected in the last ten years and I have a reliable report of one other Florida locality.

***fuscus* C**

The populations comprising the *fuscus* C lineage have been considered to represent both *Desmognathus fuscus* and *D. auriculatus*. This lineage corresponds to the C3 clade in Beamer and Lamb (2008). Aside from that publication, this mtDNA lineage has not been sampled in any other molecular phylogenetic studies. There are no available names for *D. fuscus*-like salamanders from the area where these samples were collected.

My Bayesian mtDNA phylogenetic reconstruction recovers a genealogically exclusive clade comprised of fifteen haplotypes representing *fuscus* C from thirteen populations. This clade is sister to another clade containing all other populations of *Desmognathus fuscus* as well as some populations that have been referred to as *Desmognathus auriculatus* (*auriculatus* B & C lineages). The concordance and concatenated species tree reconstructions recover the same topology but the BEST species tree differs slightly. The BEST analysis recovers a sister relation between *fuscus* C and a clade containing *D. auriculatus* (*auriculatus A*) and *D. planiceps*; this clade then forms the sister group of all other populations currently recognized as *D. fuscus* as well as the *auriculatus* B & C lineages.

My collections of the *fuscus* C mtDNA lineage are from portions of the Mid-Atlantic Floodplains and Low Terraces, Atlantic Southern Loam Plains, Kings Mountain, Northern Inner Piedmont, Southern Lower Piedmont ecoregions in the Broad-St. Helena Sound, Edisto, Santee and Pee Dee drainages. All of the samples are from South Carolina aside from a single specimen from Valdese, North Carolina; the samples from Kings Mountain are only 3.2 km south of the North Carolina state line. There are two major clades within *fuscus* C; one occupies the southwestern extent of the distribution and is restricted to the coastal plain and the other clade is found both above and below the fall line. The coastal plain clade occurs in swamps and springs while the second clade has only been collected from springs and stream habitat.

I have not found any *Desmognathus* lineages sympatrically with the *fuscus* C lineage but a population of the *conanti* A lineage is found within 14 km of a *fuscus* C population in Barnwell County, South Carolina. Both of these populations are from small tributaries that enter the Edisto River from the southwest. The population of *conanti* A is from the southern edge of the Sand Hills ecoregion while the *fuscus* C population is from the Atlantic Southern Loam Plains. Farther downstream in the Atlantic Southern Loam Plains on the same side of the Edisto River I have two additional populations of *fuscus* C. It appears that these two species replace one another in this area with *conanti* A being restricted to more headwater portions of the drainage.

The *fuscus* C and *auriculatus* B lineages are apparently parapatrically distributed in the lower coastal plain of South Carolina. In the Broad-St. Helena Sound drainage *fuscus* C occupies the headwaters in the Atlantic Southern Loam Plains ecoregion while *auriculatus* B is found in the Carolina Flatwoods further downstream. A similar pattern is observed in the Edisto, Santee and Pee Dee drainages with *fuscus* C populations occurring inland of *auriculatus* B populations. My single sample from the Ashley drainage, which has its headwaters in the Carolina Flatwoods, is referable to *auriculatus* B.

The *fuscus* C lineage approaches *auriculatus* C near the Pee Dee River in the Atlantic Southern Loam Plains ecoregion. In this area the river channel may act as a barrier to contact between these two lineages as *fuscus* C is known only from localities west of the river. Aside from a single population, *auriculatus* C has only been found east of the Pee Dee; the population from west of the river is 19.7 km from the nearest *fuscus* C population. In South Carolina *fuscus* C also appears to be separated from *fuscus* D by the Pee Dee River channel. Both lineages occur in the Pee Dee drainage portion of the Sand Hills; the closest populations are approximately 36 km from one another.

Where the Congaree and Wateree rivers confluence to form the Santee River, populations of *fuscus* C and members of the *carolinensis*/*fuscus* lineage occur in close proximity to one another. I have five samples from near the river confluence including two populations of *fuscus* C and three of the *carolinensis*/*fuscus* lineage. The closest populations of the two lineages are 5.4 km apart. Closer to the headwaters of the Santee drainage two other populations of *fuscus* C and *carolinensis*/*fuscus* are found within 15 km of one another. Other populations of these two lineages are likely to approach one another closely in the Southern Lower Piedmont of North Carolina and South Carolina. Both lineages have typical *fuscus* morphology and there are not any apparent differences in microhabitats.

One population of *carolinensis*/*fuscus* from the coastal plain of North Carolina was sequenced for three nuclear genes. In each case the phylogeny reconstructed from the nuclear DNA dataset recovers this population in a clade with two samples of *fuscus* C. It seems likely that the *carolinensis*/*fuscus* populations are the result of past hybrid introgression between two lineages.

As mentioned above *fuscus* C is comprised of two geographically partitioned clades. The clade restricted to the coastal plain is common in spring and seepage habitats but is generally much harder to find in swamps. It has been collected in large cypress/gum swamps that are similar to the lentic habitats occupied by *Desmognathus auriculatus*. These habitats are generally harder to search effectively than stream habitats so it is unknown whether population densities in these areas are lower than in lotic habitats. The clade that has only been found in streams and seepages is common at several sites. The population at Kings Mountain is especially robust and greater than ten specimens have been observed in an hours search on more than one occasion.

auriculatus **B**

The *auriculatus* B lineage corresponds to the Ashepoo and Santee populations from the C2 clade in Beamer and Lamb (2008). Aside from that publication, this mtDNA lineage has not been sampled in any other molecular phylogenetic studies. There are no available names for *D. auriculatus*-like salamanders from the area where these samples were collected.

My Bayesian mtDNA phylogenetic reconstruction recovers a genealogically exclusive clade comprised of haplotypes representing fourteen populations from South Carolina and Georgia. This clade is sister to *auriculatus* C and together *auriculatus* B and *auriculatus* C form the sister group to a clade containing the *fuscus* B and *fuscus* D lineages. The concordance and concatenated species tree reconstructions recover the same topology as the mtDNA reconstruction but the BEST species tree differs by recovering a sister relation between *auriculatus* B and the clade containing *fuscus* B and *fuscus* D. A sister relationship was not recovered between *auriculatus* B and *auriculatus* C in a phylogenetic reconstruction based on the RAG-1 gene. While there is not much variation in RAG-1 a similar pattern was found in one of the mtDNA analyses. These results combined with the long branches between *auriculatus* B and *auriculatus* C as well as a pattern of geographic cohesiveness within each of the separate lineages lead to my decision to consider each lineage separately.

My collections of *auriculatus* B are from the Sea Island Flatwoods, Carolina Flatwoods and portions of the Sea Islands/Coastal Marsh ecoregions in the Ogeechee, Savannah, Broad-St. Helena Sound, Edisto, Ashley, Santee and PeeDee drainages. All of my samples are from South Carolina except for one from Georgia. This region has been sampled intensively and the range extent of this lineage is pretty clearly defined. This lineage has only been found in lentic swamp habitats of the lower coastal plain.

A population of the *auriculatus* B lineage is found within 10 km of a *conanti* A lineage population in Jasper County, South Carolina. Both populations are from swampy habitats but the *conanti* A population was collected immediately adjacent to a sand ridge that runs parallel to the Savannah River. The sand ridge rises out of Black Swamp where the *auriculatus* B sample was collected. It is very likely that these populations might come into direct contact with one another and this area would be good site to investigate interactions between these lineages.

The *auriculatus* B lineage and *auriculatus* C lineage appear to be parapatrically distributed. Both lineages occur in the Pee Dee drainage, in general *auriculatus* B appears to occupy swampy habitats south of the Pee Dee river channel while *auriculatus* C occupies similar areas north of the river. As mentioned in the *fuscus* C account, a population with *auriculatus* C haplotypes is found south of the Pee Dee River; this population is about 55 km from the nearest *auriculatus* B population. It is possible that these lineages contact one another somewhere in the intervening area.

The distribution of the *auriculatus* A and *auriculatus* B lineages was already discussed in the *auriculatus* A account. Briefly, the two lineages are distributed parapatrically and are apparently separated by the Ogeechee River. More sampling in the Ogeechee drainage will be required to determine if these lineages come into direct contact with one another.

As discussed in the *fuscus* C account, *auriculatus* B and *fuscus* C are distributed parapatrically in the Pee Dee, Santee, Edisto and Broad-St. Helena drainages. The closest populations of the two lineages are 29 km apart but lie in different drainages; the closest populations in the same drainage are 35 km apart. Most of the range of the *auriculatus* B lineage lies in the Carolina Flatwoods ecoregion; it is not known to occur farther inland in the Atlantic Southern Loam Plains where *fuscus* C occurs. One population of *fuscus* C penetrates into Carolina Flatwoods where the Mid-Atlantic Floodplains and Low Terraces descend into that ecoregion. It is possible that the *fuscus* C and *auriculatus* B lineages might come into close contact in this area.

In order to ascertain the population status of "*Desmognathus auriculatus*" in South Carolina, Steve Bennett and myself revisited most of the historical records for this lineage. We found populations persisted at most localities and that the *auriculatus* B lineage was still present across the known range extent in South Carolina. In addition to recording the presence of this lineage at historical sites we also revealed its presence at a number of newly identified sites. At most sites it was dug out of deep muck and only rarely were specimens found beneath superficial cover. One of the most striking results of our South Carolina survey was that none of the populations of "*Desmognathus auriculatus*" are actually referable to that name (*auriculatus* A). A large number of the historical records for "*Desmognathus auriculatus*" in South Carolina were not from swampy habitats and are referable to the *conanti* A, *fuscus* C and *carolinensis*/*fuscus* lineages.

In Georgia *auriculatus* B is known with certainty only from a site near the Little Ogeechee River. At this site Sean Graham collected a single specimen in 2006, I visited this site with Dirk Stevenson on May 4, 2008 and we were not able to find any specimens despite considerable effort spent raking through muck. This same site yielded nineteen specimens on November 18, 1967 and specimens were found nearby crossing Quacco Road at night on several occasions in 1969, 1970 and 1971. Taken together these records suggest that this lineage may have declined at the extreme southwestern limits of its distribution in Georgia.

auriculatus C

With the exception of the Ashepoo and Santee samples, all of the populations in the C2 clade of Beamer and Lamb (2008) are referable to *auriculatus* C. Based on their geographic provenance the samples from population 23 in Karlin and Guttman (1986) almost certainly belong to this lineage. They found this population to be highly divergent from their samples of *Desmognathus fuscus* and D. *conanti*. As a result they omitted this population from all of their analyses and stated that it would be discussed along with additional populations from the Atlantic Coastal Plain in a separate paper. There are no available names for *D. auriculatus*-like salamanders from this region.

The *auriculatus* C lineage is comprised of sixteen populations that are recovered as a genealogically exclusive group in a Bayesian phylogenetic reconstruction. As previously discussed in the *auriculatus* B account, my mtDNA phylogenetic reconstruction recovers a sister relationship between *auriculatus* B and *auriculatus* C which together form the sister group to a clade containing the *fuscus* B and *fuscus* D lineages. The concordance and concatenated species tree reconstructions recover the same topology as the mtDNA reconstruction while the BEST species tree differs by recovering a sister relation between *auriculatus* C and *fuscus* A.

I have collected *auriculatus* C from the Atlantic Southern Loam Plains, Mid-Atlantic Flatwoods, Carolina Flatwoods and Rolling Coastal Plain ecoregions in the Roanoke, Pamlico, Neuse, White Oak, Cape Fear and Pee Dee drainages. Most of the range of this lineage lies in North Carolina but populations also occur in South Carolina to the Pee Dee River. At one site immediately west of the Pee Dee River, a population with an *auriculatus* C haplotype has been found; another population in a similar situation farther downstream is referable to the *carolinensis*/*fuscus* lineage.

All of the populations that have been sampled from the lower coastal plain of Virginia have mtDNA haplotypes and the pigmentation patterns of the *fuscus* B lineage. There is still a small hiatus between the *fuscus* B populations in Virginia and *auriculatus* C populations in North Carolina and collections from this area are important for clarifying the edge of the range extent of these two lineages.

Three disjunct populations of *auriculatus* C have been collected from a small area in the headwaters of the Pee Dee River from the border between the Southern Crystaline Ridges and Mountains and New River Plateau ecoregions. Two of these populations (their populations 18 and 19) comprise clade C in Tilley et al. (2008). They reported large genetic distances based on their allozyme dataset between their clade C and *Desmognathus fuscus* and *D. planiceps*; in their STRUCTURE analyses 91% of the individuals from population 18 and 19 where placed into the same cluster and most of the time individuals from those populations were essentially the only populations in those clusters. In their maximum-likelihood analysis, Tilley et al. (2008) recovered a sister relationship between clade C (*auriculatus* C) and clade B (*fuscus* B) though this relation had only moderate bootstrap support (64% ML, 88% MP). Their clade C and B cyt-b haplotypes were 7.68% divergent.

Bonett (2002) also sampled one of the Virginia mountain populations; his population 25 is from the same site as population 19 in Tilley et al. (2008). In an allozyme based phylogenetic reconstruction, Bonett's population 25 was nested within his Group C; Group C consists mainly of populations occupying the North Carolina Piedmont that correspond to at least two mtDNA lineages (Tilley et al. 2008).

I sampled two populations of the *auriculatus* C lineage in Virginia, Tilley et al.'s population 18 and another site located a short distance away. My Bayesian reconstruction recovers these two Virginia populations as the sister group to a clade containing all other populations of *auriculatus* C. The Virginia *auriculatus* C sequences are most similar to the nearest populations below the fall line in the Pee Dee drainage of South Carolina. A pattern of headwater populations being more closely related to lowland populations is also found in the *conanti* A lineage, *Desmognathus apalachicolae* and in *Eurycea chamberlaini*.

The existence of a tiny group of populations of *auriculatus* C in the headwaters of the Pee Dee when most populations of the lineage are confined to areas below the fall line begs explanation. One possibility is that the populations where introduced, possibly as released fish bait. However this seems unlikely because populations of coastal plain *auriculatus* C are typically hard to collect and I am not aware of their use as fish bait anywhere in the North Carolina coastal plain.

This lineage is characterized by low genetic diversity over relatively great distances so if the mountain populations were introduced I would expect the haplotypes to be nearly identical to coastal plain populations. Instead the mountain populations occupy a basal position in the clade. *Desmognathus planiceps* occurs just to the north of the Virginia mountain populations of *auriculatus* C and even farther north on the Blue Ridge escarpment is an apparently relict population of *fuscus* D. This portion of the Blue Ridge escarpment may be serving as a haplotype museum, preserving ancestral populations while the surrounding areas have been inhabited by other lineages. Addressing this hypothesis will require more intensive sampling along the Blue Ridge escarpment.

In the lower Coastal Plain the ranges of the *auriculatus* B and *auriculatus* C lineages approach one another near the Pee Dee River in South Carolina. It appears that the river channel might define the limits of each lineage; *auriculatus* B is found southwest of the Pee Dee River and *auriculatus* C is found northeast of the river. These two lineages are very similar to one another in both habitat preference and in morphological characters, intensive surveys of sites near the Pee Dee River will be required to determine whether these two lineages should be considered separate species.

As mentioned in the previous paragraphs the ranges of *auriculatus* C and *fuscus* B approach one another in the Coastal Plain near the North Carolina-Virginia state line. Virginia Coastal Plain populations have been considered to represent both *Desmognathus auriculatus* and *D. fuscus* (Mitchell and Reay, 1999) and although all of my samples are referable to the *fuscus* B lineage it is possible that populations of *auriculatus* C are present in Virginia. It is worth mentioning that my southern most Coastal Plain *Desmognathus* population in Virginia was collected from cypress swamp habitat and where it was collected sympatrically with *Stereochilus*. This is the type of habitat where *auriculatus* C would be expected but this population apparently is *fuscus* B. More collecting in the Albemarle-Chowan drainage will be required to understand the contact zone between these two lineages in this portion of their distribution.

The populations of *auriculatus* C in the mountain headwaters of the Pee Dee River are found in close proximity to populations of *fuscus* B. My closest populations of these two lineages are 27.5 km apart; the closest known population of *fuscus* B is Kozak et al. (2005) population 63 that lies approximately 13 km to the south. Populations of *fuscus* B occupy the two adjacent ecoregions, flanking the mountain populations of *auriculatus* C; thus it appears that the range extent of *auriculatus* C in the Pee Dee River headwaters is very small.

The *auriculatus* C and *fuscus* C lineages approach one another in the Atlantic Southern Loam Plains near the Pee Dee River in South Carolina. In general *auriculatus* C appears to largely restricted to areas east of the Pee Dee River, but one population in South Carolina occurs on the west side of the river. The individuals at this site occupy seepages above the river floodplain swamp; morphologically they appear very different from other populations of *auriculatus* C. In fact they appear identical to populations of *fuscus* C that occur nearby. It seems likely that introgression has occurred between these lineages in this area. The site does contain parapatric populations of *Pseudotriton ruber* and *P. montanus*. The *P. ruber* populations are common in the same seeps that contain *Desmognathus* while *P. montanus* are found in the swampy habitat below. While *P. montanus* is often found together with *auriculatus*, to date no specimens of *Desmognathus* have been found in these swamps. The proximity of these two different habitats suggests the potential for contact between two lineages.

The *auriculatus* C and *fuscus* D lineages are parapatrically distributed; in general *auriculatus* C occurs in lowland habitats while *fuscus* D occurs at more inland localities. All populations of *fuscus* D have been found in streams or spring run habitats; areas where these types of habitat descend into the Coastal Plain allow populations of *auriculatus* C and *fuscus* D to occur in close proximity to one another. For instance in

North Carolina, a population of *auriculatus* C is found within 18 km of a *fuscus* D population. These two populations occur at roughly the same latitude but the *fuscus* D population is in a spring run that cuts through an escarpment above the Cape Fear River while the *auriculatus* C populations is found in cypress swamp habitat in the adjacent Pee Dee drainage. It is likely that these two lineages might directly contact one another either where swampy rivers approach the Fall Line or farther down in the Coastal Plain where small seepage streams descend into swampy habitat.

The closest contact between *auriculatus* C and another lineage in the coastal plain occurs along Otter Creek in Pitt County, North Carolina. In this area *auriculatus* C is fairly widespread in swampy habitats, the *carolinensis*/*fuscus* lineage on the other hand is markedly restricted in distribution. The *carolinensis*/*fuscus* lineage is relatively widespread at localities above the Fall Line but it is known from only a single site in the Coastal Plain of North Carolina (it is also known from the Coastal Plain of South Carolina where it contacts *auriculatus* B). At the confluence of Otter Creek and Kitten Creek is a typical cypress/gum swamp that is inhabited by *auriculatus* C (and other swamp dwelling salamanders like *Amphiuma*). Less than 1 km away a population of the *carolinensis*/*fuscus* lineage occurs in deep ravines formed in sandy soils that bear strong similarities to Florida steepheads. The two lineages are distinct in morphology; *auriculatus* C is much more slender in build and tends to be more darkly pigmented. I have visited both of these sites many times over the past 10 years and I have never found an individual of the *carolinensis*/*fuscus* lineage in the swamps and conversely I have never found an individual of *auriculatus* C in the ravines. These two populations appear to parallel the distributional patterns observed by Means (1975) in the Florida panhandle. Near the headwaters of the Pee Dee River, *auriculatus* C occurs within 44 km of a population of the *carolinensis*/*fuscus* lineage. It is possible these lineages might contact one another near the interface between the Northern Inner Piedmont and the Southern Crystaline Ridges and Mountains in the headwaters of the Pee Dee drainage.

Desmognathus quadramaculatus was found sympatrically (this is just wrong) with individuals of the *auriculatus* C lineage at one of the Virginia mountain sites. In fact an adult *D. quadramaculatus* and a juvenile *auriculatus* C were raked out of the same pile of vegetative debris; an adult *auriculatus* C was found nearby in a mucky area with skunk cabbage. At another site 0.2 km farther up the road *D. monticola* and *auriculatus* C were found together.

Eaton (1953) described populations of *auriculatus* C from the Pitt County area as abundant and widely distributed. He stated that it locally it outnumbered all other salamanders combined. During my surveys of the area I found this lineage to be widespread but only locally common and I certainly did not find it more than all other salamanders combined. This species does not appear to need pristine habitat as I have found it in second growth swamps and at the edges of highly disturbed areas. Nonetheless I did not find it at many sites that appeared to have high quality habitat including several sites were it occurred historically. From speaking with people who collected this lineage before the mid-1970’s, it appears that in specimens of this salamander are seen less frequently than they were in the past. Apparently a similar decline to that in *auriculatus* A has occurred in this lineage; though a much larger number of populations of *auriculatus* C is extant.

Appendix 4-3

ABSTRACT

Previously we identified 50 geographically concordant lineages among 530 sampled populations of *Desmognathus*. We sequenced three nuclear regions (RAG-1, POMC and IEBF-3) for individuals representing 45 of these lineages. We conducted Bayesian phylogenetic analyses of each gene region individually; the resulting topologies were notably incongruent with one another. We used three different methods (total evidence, Bayesian untangling of concordance knots and Bayesian estimation of species trees) in an attempt to resolve the gene tree heterogeneity we observed. The results of these three methods are markedly congruent with one another and we conclude that they represent a reasonable approximation of the true species tree.

INTRODUCTION

Desmognathus large/species rich group evolutionary/taxonomy has been addressed in piecemeal fashion. A few seminal papers Titus & larson derived-basal, Chippindale reversal, Kozak begin to see lineage rich and convergence and homoplasy are more widespread than previously believed. All of these have served to recognize how complex desmognathus is. Demonstrate the need for more comprehensive sampling. In the following paragraphs we discuss the troubled taxonomic history

The plethodontid genus *Desmoganthus* (Gr. desmos ligament & Gr. gnathos jaw = ref. bundle of ligaments holding jaw), commonly known as dusky salamanders, represents a radiation of particular interest; following a major life-history reversal (the re-evolution of an aquatic larval stage from a direct-developing ancestor), they surpassed all other plethodontid genera in adaptive diversity (Chippindale et al 2004). Otherwise morphologically conservative, dusky salamander species demonstrate significant variation in body size (38–210 mm total length), a feature stressed to account for their high ecological diversity (Hairston 1986), which ranges from permanently-aquatic riffle specialists to high elevation, terrestrial forms inhabiting spruce-fir forests. Although *Desmognathus* is indisputably monophyletic (Chippindale et al 2004; Mueller et al 2004), species delineation within the genus has always been challenging and remains controversial.

Twenty nominal species are currently recognized, but recent molecular phylogenetic surveys (Beamer & Lamb 2008; Kozak et al 2005) have identified > 25 additional independent evolutionary lineages, disclosing levels of diversity far greater than previously thought and underscoring the need for a comprehensive taxonomic revision. Many of these additional lineages have been considered to represent widespread species, but most exhibit substantive genetic divergence and occupy topologically disparate positions across our respective phylogenetic trees (Beamer & Lamb 2008; Kozak et al 2005).

These “new” lineages are based solely on mitochondrial gene sequences. While the merits of mtDNA for delineating recent radiations have long been recognized (Avise et al 1987; Moore 1995), recent empirical and theoretical work has identified many instances where gene tree incongruence exists or is expected (Degnan & Rosenberg 2006). In an effort to more rigorously evaluate the potential specific status of these lineages, we sampled many additional populations (Chap 1) and sequenced additional mtDNA as well as three nuclear regions.

It is widely acknowledged that species trees and gene trees may differ for a number of reasons including incomplete lineage sorting, introgression, gene duplication and in the case of organellar regions, differences in male and female-mediated gene flow (Edwards 2009; Jockusch & Wake 2002; Maddison 1997). Recently, several approaches have been proposed for reconciling these differences (Ané et al 2007; Kubatko et al 2009; Liu 2008 ; Oliver 2008). Although they share the common goal of reconciling gene trees with species trees, each differs with respect to methodological approach. As described below in the methods section we utilized three different approaches in our attempts to produce a species tree. Below we will briefly review these methods, and then we will compare and contrast the results produced by each of the methods. Finally we will discuss their ramifications for a species level phylogeny of *Desmognathus*.

METHODS

We used a total evidence approach (Kluge 1989) by concatenating our sequence data for the four different genomic regions (one mitochondrial, three nuclear). Concatenation may reveal a topology congruent with the species tree (i.e., if the signal supporting the species tree is the strongest signal in the data) despite the presence of data conflict, especially if large numbers of loci are included (Rokas et al 2003). On the other hand, it is now well established that significant heterogeneity can exist among gene trees, resulting in conflicting topologies (Edwards 2009). As a result, many genes will likely be required to account for gene tree variation (Liu et al 2008) because concatenation of only a few genes has potential to generate topologies that are highly incongruent with the species tree.

We also used Bayesian estimation of concordance as implemented in the software BUCKy 1.4.0 (Ané et al 2007) to test for concordance among three of our gene trees (mtDNA, RAG-1, and interleukin enhancement binding factor 3). BUCKy uses the trees sampled from the posterior distribution of a Bayesian analysis of individual genes and then builds a primary concordance tree based on the dominant history of sampled individuals. This method also provides a measure (concordance factor) of how much of the genome supports each relationship in the primary concordance tree. Bayesian estimation of concordance is able to accommodate gene tree heterogeneity because it does not assume that genes all have the same topology. Another strength is that no assumption is made regarding the reason for discordance among gene trees.

Finally we used a Bayesian estimation of species trees approach implemented in the software BEST 2.3. BEST accommodates gene tree heterogeneity by estimating individual gene trees and then uses a vector of all of these gene trees to estimate the joint posterior distribution of gene trees and species trees. It estimates a species tree based on the constraint that all divergences of species pairs must occur after the respective gene divergences occur. This method can account for incomplete lineage sorting (deep coalescence) but does not account for the issues of hybrid introgression or gene duplication.

In review the total evidence approach does not account for gene tree heterogeneity and as such it may not accurately recover the species tree when biological processes such as incomplete lineage sorting, hybridization and gene duplication have been common. The Bayesian estimation of concordance approach can accommodate all three of these processes while the Bayesian estimation of species trees approach can accommodate gene tree heterogeneity due to incomplete lineage sorting. These last two methods then explicitly attempt to account for the issue of gene tree heterogeneity.

RESULTS

Individual gene trees

In this section I will first compare the mtDNA tree produced by the analysis of 45 taxa with the topology obtained by the analysis of mtDNA for all 530 taxa. Then I will compare each of the individual gene trees recovered from the analysis of nuclear regions with this 45 taxa mtDNA tree.

mtDNA

The topologies of the individual gene trees vary widely and outwardly appear to exhibit little congruence. The 45 taxa mtDNA gene tree is mostly congruent with the 530 taxa gene mtDNA presented in chapter 1. Differences include the placement of *planiceps* and *auriculatus* as sister to one another in this tree, *santeetlah* being sister to all remaining *conanti* populations and *aeneus* rather than *imitator* being sister to all *Desmognathus* exclusive of *wrighti*. It is also of note that the members of the clade including X6, X8, X7/*fuscus* B, *fuscus* B and *fuscus* A have a different branching order. Most of the clades have strong support but some of the clades have lower values than for the corresponding clade in the 530 taxa analysis.

IEBF-3

The topology recovered for IEBF-3 has many differences from the mtDNA topology and has very low support for most nodes. One of the most striking departures from the mtDNA gene tree is that *aeneus*, *imitator* and *carolinensis* are nested within the clade containing all samples of *quadramaculatus*, *marmoratus* and *folkertsi*. Another notable feature is that the samples of conanti are scattered throughout the tree whereas in the mtDNA tree they are all contained in a single highly supported clade (pp = 1.0). As already mentioned most nodes receive very low support, for example a clade consisting of *orestes*, *ochrophaeus* and lineage X9 is recovered but with a posterior probability of 0.06 and with a different branching order. There are some strongly supported clades that are congruent with equivalent clades in the mtDNA reconstruction; a clade containing some of the populations of *quadramaculatus*, *marmoratus* and *folkertsi* is recovered with very high support (pp = 1.0). Another interesting finding is that a clade containing lineages X6 and X8 has very strong support in this gene tree (pp = 0.99) but was not recovered in the mtDNA (though this clade also has moderate support in the 530 taxa mtDNA gene tree (pp = 0.85)). There are also some strongly supported nodes that do not have a counterpart in the mtDNA phylogeny. For example, there is a strongly supported clade (pp = 1.0) containing *abditus*, *welteri*, *fuscus* B, *fuscus* A, *planiceps*, *ochrophaeus*, *orestes* and X9.

RAG-1

The topology produced by the analysis of RAG-1 is greatly at odds with the mtDNA gene tree. As in the IEBF-3 gene tree, populations of *quadramaculatus*, *marmoratus* and *folkertsi* do not form a genealogically exclusive clade. However, rather than being paraphyletic with respect to three other taxa (carolinensis, aeneus and imitator) as for IEBF-3, they occupy disparate positions in the topology. Samples of *conanti* are again scattered throughout the tree rather than forming a single highly supported clade (pp = 1.0) observed in the mtDNA gene tree. A very striking feature of the RAG-1 tree is that certain taxa (*aeneus*, *quadramaculatus*, *marmoratus* and *folkertsi*) that occupy basal positions in the mtDNA tree are deeply nested within other clades. In general support values are very low. The most obvious exception is a strongly supported clade containing southern populations of *quadramaculatus*, *marmoratus* and *folkertsi* (pp = 1.0) that is also identified in the mtDNA and IEBF-3 trees.

POMC

The POMC gene tree provides little resolution and is very comb-like. The support values in general are exceptionally low, with many posterior probabilities < 0.1. As in the IEBF-3 and RAG-1 trees (and in marked contrast to the mtDNA gene tree), a genealogically exclusive clade comprising all samples of *quadramaculatus*, *marmoratus* and *folkertsi* is not recovered. However, a strongly supported clade consisting of southern populations of *quadramaculatus*, *marmoratus* and *folkertsi* is recovered with relatively high support (pp = 0.98). This clade is the only clade that is well supported in all four gene trees though branching order differs here, and the internal nodes are not well supported. One other moderately supported clade is of note. Lineages X6 and X8 form a clade with moderate support (pp = 0.84), this clade is also recovered in the IEBF-3 and 530 taxa mtDNA gene trees but not in the RAG-1 and 45 taxa mtDNA gene trees.

Species trees

Total evidence

The species tree produced by the total evidence approach is generally well resolved with moderate to high support for most clades. The topology is similar to that of the mtDNA gene tree as would be expected given than roughly half of the total sequence data consists of mtDNA, moreover the mtDNA is more variable than the nuclear regions. One of the major topological differences between the species tree produced by total evidence and the mtDNA gene tree is the nested position of a clade containing *ocoee* A, *ocoee* B and *ocoee* D. This clade is placed sister to most *Desmognathus* lineages in the mtDNA gene tree with very strong support (pp = 1.0). Another difference is the placement of *brimleyorum* as sister to most *Desmognathus* lineages whereas *brimleyorum* is sister only to all populations of *conanti* in the mtDNA gene tree (though this is not strongly supported (pp = 0.40). Lineages X6 and X8 form a strongly supported clade (pp = 1.0), this relationship was recovered in the IEBF-3 and POMC gene trees as well as the 530 taxa mtDNA gene tree but not the 45 taxa mtDNA gene tree. A sister relationship between *auriculatus* and *planiceps* has considerably more support in the concatenated species tree (pp = 0.89) compared with the mtDNA gene tree (pp = 0.43). Most of the remaining topological differences are minor and did not receive strong support in the mtDNA gene tree. Likewise the remaining clade posterior probabilities are similar.

Bayesian estimation of concordance

The species tree estimated via Bayesian concordance analysis is topologically identical to the mtDNA tree with two exceptions. In the species tree, *imitator* is sister to all other lineages whereas *aeneus* is sister to the remaining lineages in the mtDNA tree. The concordance factors, which are estimates of the proportion of the sampled genes for which the clade is true, are generally quite low (~ 33%). Concordance analysis produces a primary concordance topology by proposing clades that are found most commonly in the sampled genes. One third of the dataset is composed of mtDNA and most clades were very weakly supported in the IEBF-3 and RAG-1 trees, yet most clades were strongly supported in the mtDNA gene tree. It is clear then that most of the time the clades are supported primarily from the mtDNA alone. On the other hand there are several clades that are supported by both mitochondrial and nuclear gene data. In fact a few clades are supported by all of the gene samples, including the clade comprising the southern populations of *quadramaculatus*, *marmoratus* and *folkertsi* (noted in the individual gene tree accounts). .

Baum (2007) stressed that concordance factors are not estimates of support analogous to bootstrap percentage or Bayesian posterior probabilities and therefore can not be interpreted as statistical support. He further suggested that any attempt to identify a cutoff value that is "significant" is doomed to failure. Nonetheless, higher concordance factors clearly indicate that the topology is more likely to be the real species topology. Baum (2007) also urges caution against using mitochondrial genes in Bayesian concordance analysis because of the different inheritance pathways between organellar and nuclear genes. The true concordance factor of a clade could be different for nuclear versus cytoplasmic genomes. This is clearly a concern in our analysis and deserves strong consideration. However, he also states that in cases where there has been little hybridization, cytoplasmic gene data would help infer the primary history of the entire genome.

Bayesian estimation of species trees

The species tree produced by our BEST analysis has many similarities with the mtDNA gene tree and the species trees produced by both the total evidence and Bayesian estimation of concordance approaches. The biggest departure is the lack of resolution at the base of the tree. The BEST tree has a three-way polytomy involving a clade consisting of *imitator* and *aeneus*, a second consisting of all populations of *quadramaculatus*, *marmoratus* and *folkertsi* and a third comprising all remaining lineages. In common with the total evidence tree and in contrast to species tree produced by concordance analysis, the nested position of the *ocoee* A, *ocoee* B and *ocoee* D clade in the BEST tree is depicted in the total evidence tree but not the concordance tree. The *ocoee* ABD clade is sister to all *conanti* populations though this relationship is weakly supported (pp = 0.7). Likewise *santeetlah* occupies a position sister to the remaining conanti populations (pp = 0.55), the same topology as recovered in the total evidence species tree. A notable difference between all gene trees and all species trees is a sister relationship between *brimleyorum* and *carolinensis*. The branching order of a strongly supported clade (pp = 1.0) that contains fuscus A, fuscus B, X7/fuscus B, X6 and X8 is different from both of the other species trees though two of the nodes are weakly supported (pp = 0.41 and 0.63).

DISCUSSION

Have we found the *Desmognathus* species tree?

The three methods we used to infer a species tree produced remarkably congruent topologies that are quite similar to the mtDNA gene trees. This is not surprising in the case of the total evidence species tree as mitochondrial gene sequence represents half of the data matrix and generates the strongest signal. It is also not surprising that the primary concordance tree has a topology essentially identical with that of the mtDNA gene tree. In general the concordance factors are low and many times are close to 0.333, a value that represents the proportion of mtDNA in our genome sampling. Moreover, most clades in the mtDNA gene tree have very high support (pp = 1.0), which demonstrates that a concordance factor of 0.333 reflects minimal concordance between the mitochondrial and the remaining nuclear genes.

As Edwards (2009) noted, the topologies generated by concatenation are probably reasonable approximations of reality. However some results call into question the total evidence approach, especially when the group in question exhibits patterns of rapid divergence (Kubatko & Degnan 2007), a situation exemplified by *Desmognathus* as evidenced by the large amounts of divergence following very short branches.

If it is true that gene trees resolved by multilocus concatenation should be congruent with the species tree, except in cases where incomplete lineage sorting, hybridization and gene duplication are common (Edwards 2009) one might conclude that these three issues are not a problem with *Desmognathus*.

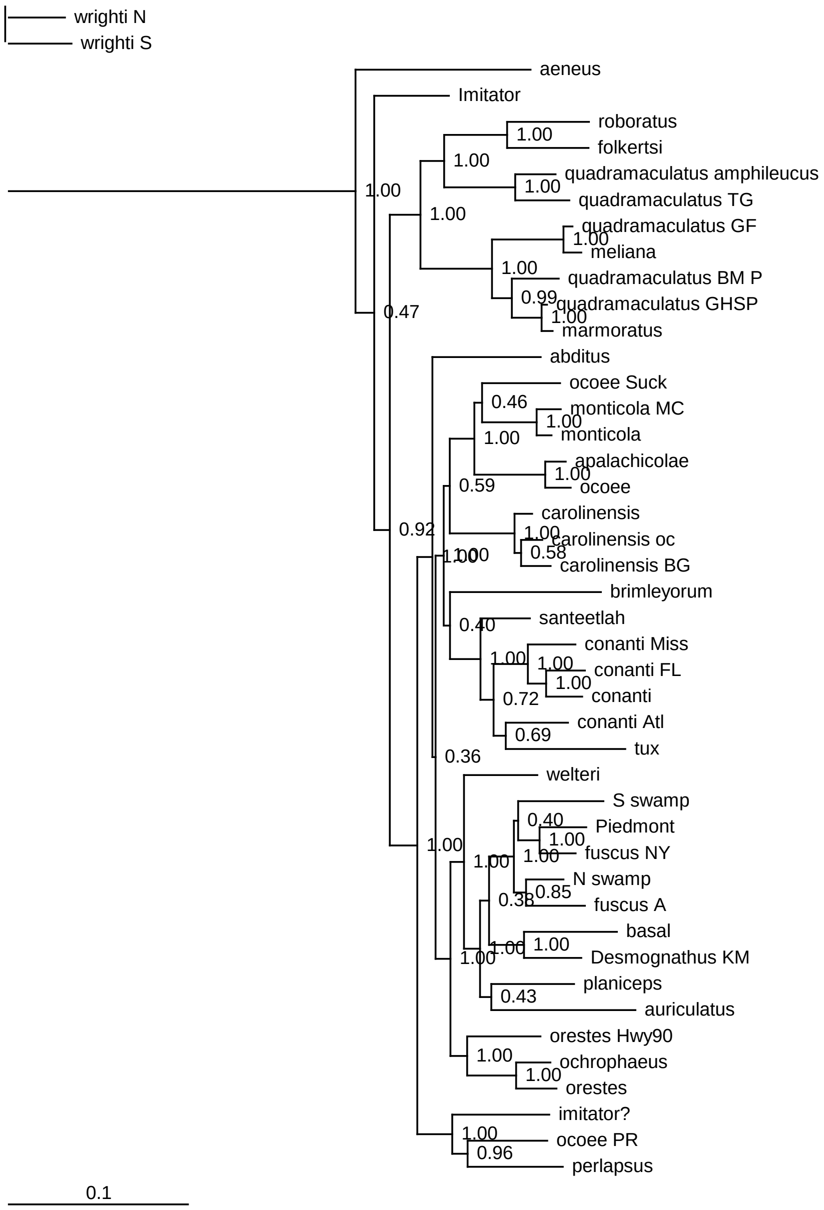
Hybridization and incomplete lineage sorting

There are several reports of hybridization in *Desmognathus* (Bonett 2002; Karlin & Guttman 1986; Tilley & Schwerdtfeger 1981) however in each of the cases it did not appear to be extensive and widespread. We did not detect any instances of hybrids in our mtDNA analysis, that is we did not find discordance between the position of haplotypes in the mtDNA gene tree and our a priori identification based on morphology. It is also worth noting that *Desmognathus* communities are often comprised of many congeneric species (Bruce 1991) which allows for the potential for hybridization over a large proportion of the area inhabited by this genus yet hybridization is either absent or occurs rarely. For these reasons we do not believe hybridization is likely to be a large concern in our attempts to recover a *Desmognathus* species tree

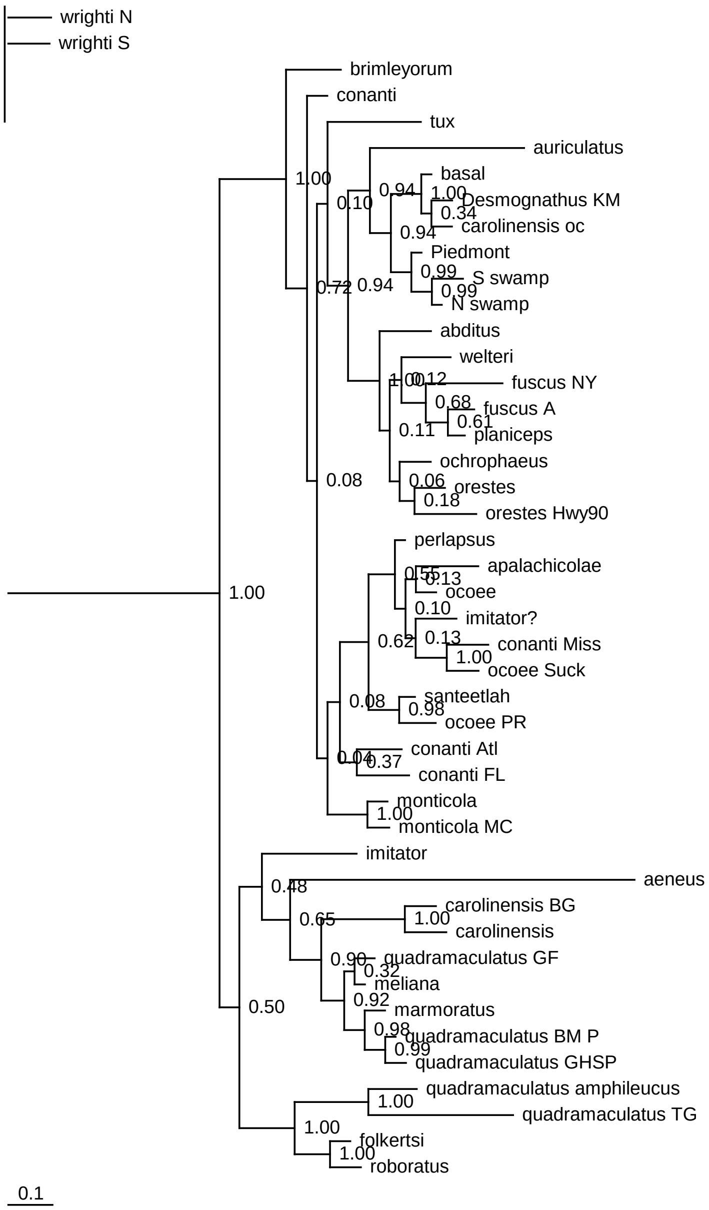
The recent and explosive radiation of *Desmognathus* sets the stage for the conditions in which incomplete lineage sorting would be likely. Incomplete lineage sorting is expected to be most common when branches are short and wide (Maddison 1997). Although little is known about ancestral population sizes in *Desmognathus*, modern populations are generally quite large. Given that large population sizes characterize most species of *Desmognathus*, it seems reasonable to assume that ancestral populations were of comparable size. Additionally most lineages within *Desmognathus* follow a series of very short branches. These two factors are reasons for major concern regarding the confounding effects of incomplete lineage sorting in our species tree.

One striking aspect of our phylogenetic reconstructions is the pronounced pattern of concordance displayed between lineages and geography. It is in strong opposition to the limited geographic concordance predicted for extensive hybridization or incomplete lineage sorting (Wiens & Penkrot 2002).

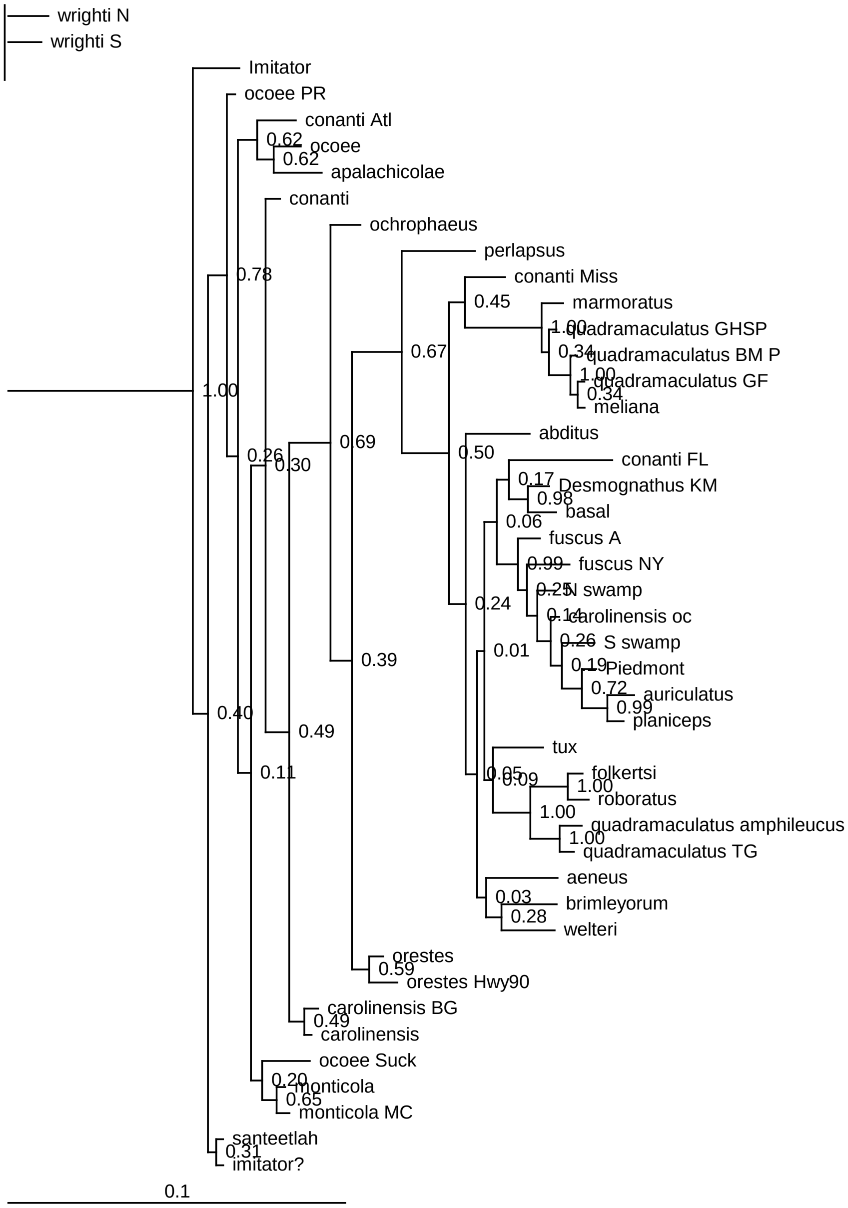
Given pervasive gene tree heterogeneity, it seems likely that many genes will be required for species tree reconstruction to account for gene tree variation (Liu et al 2008). We have sampled only four regions of the genome (three nuclear and one mitochondrial), yet despite significant substitution rates variation and incongruent gene trees, the three methods we used to construct a species tree for *Desmognathus* produced remarkably similar topologies Thus, we conclude that our tree is a reasonable approximation of the true species tree.



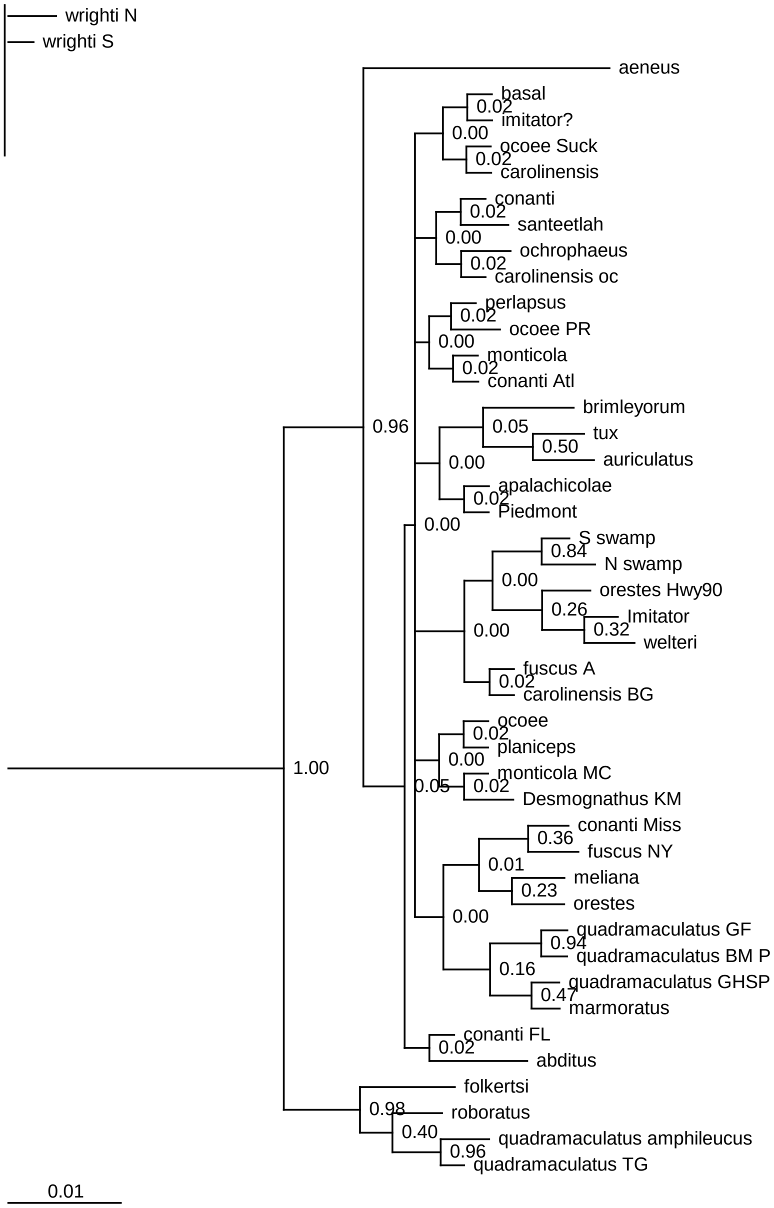
mtDNA gene tree



IEBF-3 gene tree

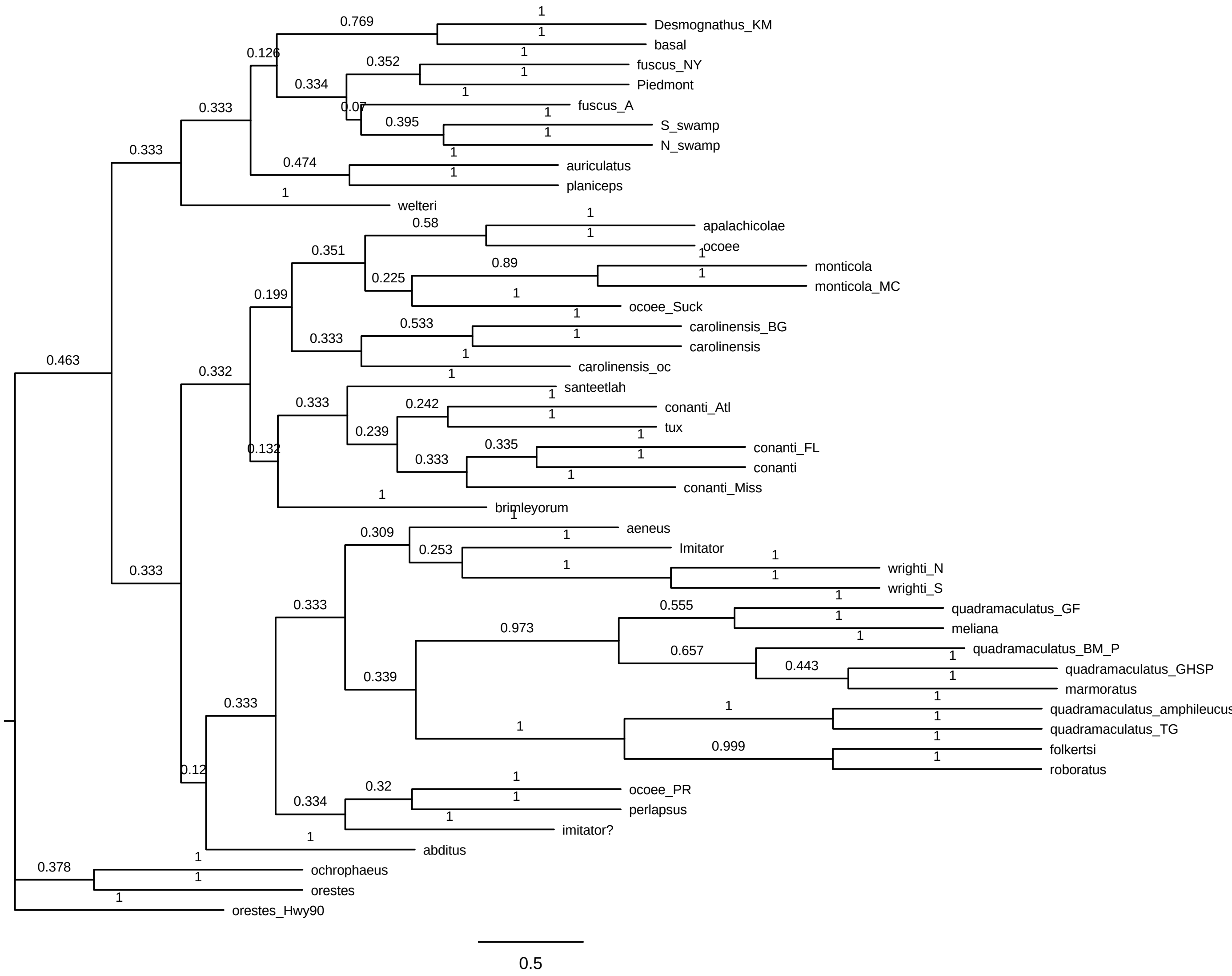


RAG-1 gene tree

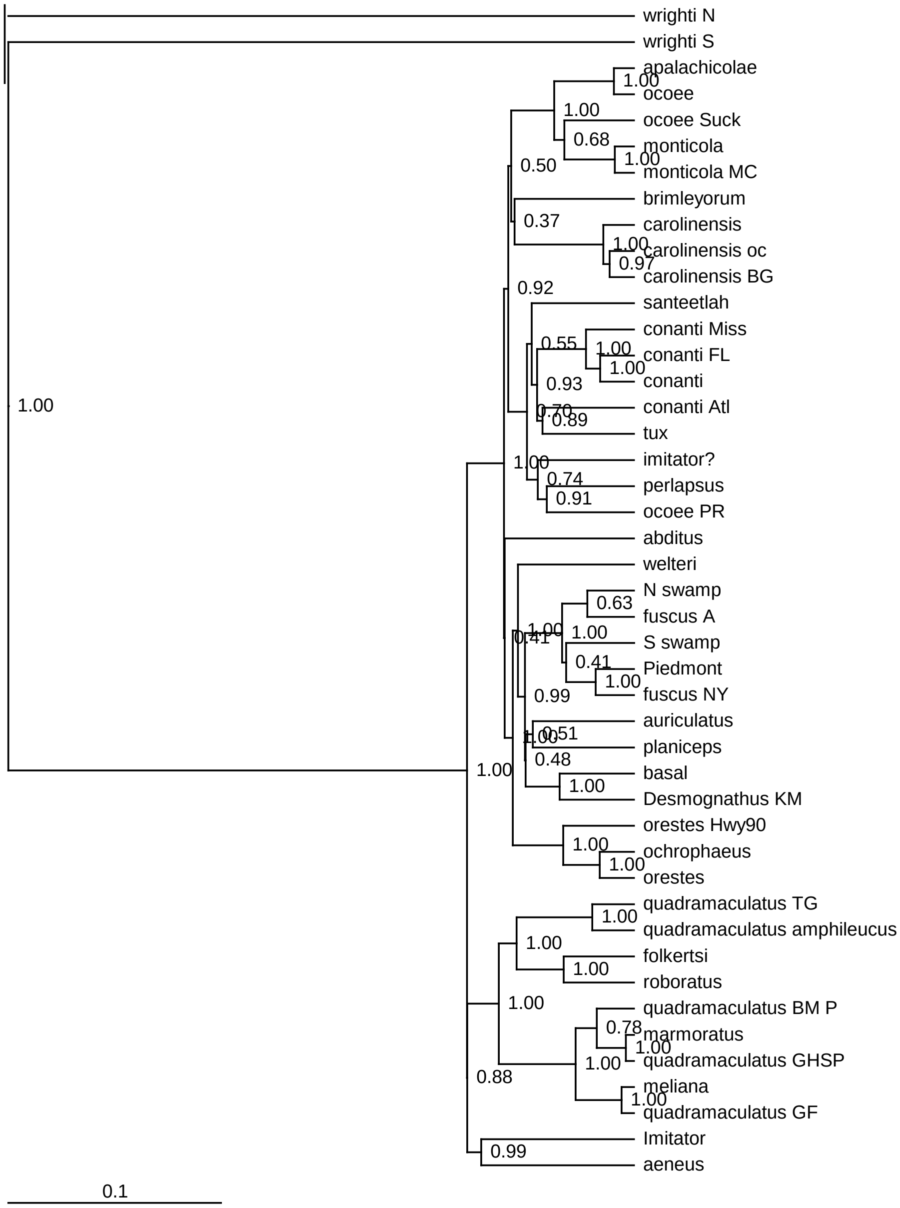


POMC gene tree

Total evidence species tree



Primary Concordance species tree



BEST species tree

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