ABSTRACT

HUTCHENS, STAN JONATHAN. Inventory and Assessment of the Reptile and Amphibian Community of Bull Neck Swamp, Washington County, North Carolina. (Under the direction of Christopher Shannon DePerno.)

Recent declines in reptile and amphibian populations across the globe have encouraged an increased desire to discover, document, and monitor these taxa. Arguably, the greatest cause is land-use change. Management interests for Bull Neck Swamp (BNS) encouraged research to inventory the reptile and amphibian community and to document possible impacts of land-use practices, such as silviculture and site preparation. Four habitat preserves were delineated based on plant community, leaving 1, 554ha (3, 841ac) available for management. Comparisons between habitat assemblages were used to determine if preserves were occupied by more vulnerable species and land-use effects on these species. However, variations in behavioral or environmental variables, and detection probabilities between capture techniques could provide misleading data for assemblage comparisons of community parameters. Therefore, 11 different capture techniques were employed to obtain better samples of habitat assemblages. To determine the accuracy of sampling techniques at inventorying species, techniques were categorized into primary (i.e., drift fence arrays with pitfall and funnel traps, visual encounter surveys, and coverboard arrays), secondary (i.e., road searches, polyvinyl chloride (PVC) piping grids, auditory surveys, and line transects), and tertiary (opportunistic encounters, aquatic funnel traps, crayfish traps, and basking traps) methodologies. All techniques had variable distributions and were evenly represented in all five areas when possible. All captured individuals were marked; snakes were double-marked with visible implant fluorescent elastomer to augment a concomitant laboratory experiment. Initial capture data were used to derive estimates of species richness (S) and modified Chao -

Jaccard similarity indices (JSI). During May to August, 2005 and 2006, 1, 581 total captures represented 33 species, giving an estimated species richness of 34. Primary techniques sampled an estimated species richness of 14 and two unique species, species detected by only one sampling technique. Estimated species richness for secondary and tertiary techniques was 29 and 25, with three and seven unique species, respectively. If primary techniques alone were used, 59% of the reptile and amphibian community, including 10 unique species, would have been missed. Observed and estimated species richness for habitats ranged from 7 to 32 and 13 to 44, respectively. Chao – Jaccard similarity indices ranged from 0.59 to 1.0, with nine comparisons over 0.75, which indicated high similarity between habitat assemblages. These results suggested that land-use practices should be carefully planned and implemented to reduce effects to the reptile and amphibian community of BNS. Empirical results supported the use of elastomers for snakes. It is recommended that future inventory studies for all taxa employ as many capture techniques as logistically and spatially possible to derive accurate species richness. Also, assemblage comparisons should rely on species composition when determining conservation plans.

Inventory and Assessment of the Reptile and Amphibian Community of Bull Neck Swamp, Washington County, North Carolina

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Fisheries and Wildlife Sciences

Raleigh, North Carolina

2008

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DEDICATION

To Mom and Dad. Here's to the next one.

BIOGRAPHY

Stan Jonathan Hutchens was born in Greensboro, North Carolina on 28 May 1981 to Larry and Carol Hutchens. He grew up in the country, on 40 acres of forested land near Asheboro, North Carolina, approximately 11 miles from the North Carolina Zoo. Through proximity to the zoo, the forest in his backyard, and teachings from his outdoorsman father, Stan began to appreciate and love wildlife and the outdoors. To pursue these interests, he attended North Carolina State University where he graduated with Bachelor's degree in Wildlife Biology (May 2003). During his undergraduate years he nurtured and developed his love for reptiles and amphibians, especially snakes, which was conceived in his adolescence. After nearly 2 years as a research assistant, Stan returned to NC State in 2005 and received his Master's degree, again in Wildlife Biology (May 2008), studying the reptile and amphibian community at Bull Neck Swamp. He intends to pursue his Ph. D. and a career answering questions and creating awareness for the conservation and ecology of his favorite, legless life forms.

ACKNOWLEDGMENTS

First, I would like to thank my chair, Dr. Chris DePerno, for giving me this opportunity, for providing the funds, for answering my stupid questions, and for reading and providing insights on all of my weak drafts. Also, I thank the other members of my committee. Dr. Richard Lancia provided guidance throughout my undergraduate years as my advisor and obliged me with continued advice as a committee member. Dr. Ken Pollock was an excellent advisor for all my statistical questions and tribulations. I shall appreciate my working with these great thinkers, and advocates of wildlife and conservation throughout my life.

Second, I want to thank my friends. My best friend, Adrian Carper, for out-fishing me each time we went, but it kept me going outside to enjoy and foster my wildlife interests. In a long list of acknowledgments for providing entertainment, exercise, and scientific conversation whenever I needed it, I thank the whole Turner House crew: Chris Ayers, Neil Chartier, Colter Chitwood, Kate Golden, Gabe Karns, Tim Langer, Charlotte Matthews, Dr. Nils Peterson, Aimee Rockhill, Liz Rutledge, Mark Sandfoss, Amelia Savage, and James Tomberlin. Special recognition goes to Cindy Burke our administrative assistant, without whom I don't think the Program would accomplish anything and has played foster mother to us all.

Third, I thank Alvin Braswell and Dave "Woody" Woodward for the technical advice and assistance they provided. Also, additional acknowledgments to all who assisted me with chapter revisions during the publication process are provided at each chapter's conclusion.

Finally, I would like to thank my parents for their continued support throughout my education.

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EFFICACY OF SAMPLING TECHNIQUES FOR DETERMINING SPECIES RICHNESS ESTIMATES OF REPTILES AND AMPHIBIANS

Abstract

The ability to detect reptiles and amphibians is influenced by environmental and behavioral variables. Studies to determine species richness of herpetofauna often employ only a small number of sampling techniques, primarily drift fence arrays, visual encounter surveys, and coverboards (i.e., primary techniques). However, using only two or three sampling techniques can underestimate species richness. To evaluate the efficacy of sampling methodologies for determining richness estimates of herpetofauna in a pocosin wetland, I employed 11 different sampling techniques. I hypothesized that adding standardized road searches, polyvinyl chloride (PVC) piping grids, line transects, auditory surveys (i.e., secondary techniques), opportunistic encounters, aquatic funnel traps, crayfish traps, and basking traps (i.e., tertiary techniques) would increase species richness estimates. Species captured (S_{obs}), Chao2 estimates of species richness (S), unique species captured, species-captured success, cost, and cost-per-species-captured for individual techniques and categories (i.e., primary, secondary, and tertiary) were used to determine efficacy. Primary capture methodologies captured 13 species, with a Chao2 estimate of 14. Secondary and tertiary sampling techniques separately captured 18 and 24 species, with Chao2 estimates of 29 and 25, respectively. All sampling methodologies combined captured 33 species with a Chao2 estimate of 34. More unique species (i.e., species detected by only one capture technique) were captured by tertiary techniques than primary or secondary methodologies. Cost and cost-per-species-captured for primary techniques were higher than secondary and tertiary methodologies. To better determine observed and estimated species richness, future

research should incorporate multiple sampling methodologies in addition to more common techniques.

Reptiles and amphibians can be difficult to inventory due to environmental and behavioral variables and differing capture probabilities between sampling techniques (Vogt and Hine, 1982; McKenzie et al., 2002; Williams and Berkson, 2004). Weather variables such as temperature, humidity, wind, and season can influence activity and detectability (Vogt and Hine, 1982; Williams and Berkson, 2004). Similarly, sedentary and fossorial behaviors, and cryptic capabilities can limit detectability of certain species (Fitch, 1992; Flint and Harris, 2005). Sampling techniques can affect the probability of detecting certain species by biasing for or against size (e.g., common snapping turtles would not likely be detected by funnel traps), behavior, or taxon (Gibbons and Semlitsch, 1981; Enge, 1997). However, most studies that inventory species richness of reptiles and amphibians use only two or three methodologies, which limit the reliability of estimates due to low, or zero, detection probabilities for certain species with some techniques (MacKenzie et al., 2002; Bailey et al., 2004).

Methodologies most commonly employed include drift fence arrays (with pitfall and/or funnel traps), visual encounter surveys (VES), and coverboards. I designated these techniques as “primary” due to their prevalence in reptile and amphibian research (Bury and Corn, 1988; Corn and Bury, 1990; Mitchell et al., 1993; Fair and Henke, 1997; Kjoss and Litvaitis, 2001). “Secondary” techniques (i.e., standardized road searches, polyvinyl chloride [PVC] piping grids, line transects, and auditory surveys) are generally used in conjunction with primary capture techniques (Jones, 1988; Lacki et al., 1994; Moulton et al., 1996;

Sullivan, 2000; Turner et al., 2003). Interestingly, "tertiary" techniques (i.e., opportunistic encounters, and aquatic funnel, crayfish, and basking traps) are infrequently reported in studies to determine species richness (Fair and Henke, 1997; Metts et al., 2001; Johnson and Barichivich, 2004). For instance, opportunistic encounters are a versatile capture method but are scarcely reported in sampling studies (Hanlin et al., 2000). Further classification of tertiary techniques was based on the non-standardized and spatially unlimited species abundance distribution sampling design, rather than the quadrat designs of primary and secondary capture techniques (Williams et al., 2002).

Implementing primary capture techniques requires high costs for materials, labor, and time. For example, depending on materials used in trap and array design (e.g., aluminum flashing or silt fencing), drift fence arrays can be expensive to construct, maintain, and operate (Gibbons and Semlitsch, 1981; Bury and Corn, 1987). In contrast, secondary and tertiary techniques are less expensive and require few materials and less labor for maintenance and operation.

I evaluated number of species captured (S_{obs}), species richness estimates (S), unique species captured, total cost, and cost-per-species-captured for primary, secondary, and tertiary techniques in a pocosin wetland. Unique species were defined as those species captured or observed by only one sampling methodology. The objectives of the study were to determine: (1) if primary techniques alone were effective at obtaining an accurate species richness estimate, (2) whether secondary and tertiary techniques increased the species richness estimate enough to justify their time and cost, and (3) the tradeoff of cost versus success between techniques for use in both short- and long-term studies.

Materials and Methods

Study area. – I conducted this study at Bull Neck Swamp (BNS) in Washington County, North Carolina (35.96667° N, 076.41667° W; Figure 1.1). The property is a 2,428ha pocosin wetland owned by North Carolina State University's Department of Forestry and Environmental Resources and managed by the Fisheries and Wildlife Sciences Program. The property created a peninsula in the Roanoke River delta and Albemarle Sound. Bottomland forest and hardwood swamps with patchy cultivated areas comprised the southern border of the property.

Trapping techniques. – During two field seasons (May to August, 2005 and 2006), 11 trapping methodologies were employed to increase the robustness of sampling. I categorized techniques based on their prevalence in published research. Primary capture techniques consisted of drift fence arrays with pitfall and funnel traps, visual encounter surveys (VES), and coverboard arrays. Standardized road searches, PVC piping grids, line transects, and auditory surveys were designated as secondary methodologies. Tertiary techniques consisted of opportunistic road cruises, aquatic funnel traps, crayfish traps, and basking traps. Further distinction of tertiary techniques was made based on their non-standardized nature and disparate sampling design (Williams et al., 2002).

Primary capture techniques. - Drift fences with pitfall traps and/or funnel traps have been widely employed in reptile and amphibian research in several designs (Gibbons and Semlitsch, 1981; Mitchell et al., 1993; Hanlin et al., 2000; Metts et al., 2001; Enge, 2001). In all cases, the objective of fencing was to act as a barricade to movement, encouraging species to travel in the direction of a trap. Ten drift fence arrays (N = 10) were distributed in a systematically random design, and distanced at least 30 m from other capture techniques.

Drift fences were arranged in 'Y'-formations with two funnel traps on each wing and a pitfall trap in the center where possible (e.g., pitfall traps could not be placed in areas inundated with water). Arrays were checked every morning for two 3-week periods during May to August, 2005 and 2006.

Visual encounter surveys were an active capture technique and standardized plots or quadrats were thoroughly searched (Jung et al., 2000; Flint and Harris, 2005). Twenty-five 10 m X 10 m VES plots were established in a systematically random distribution. All natural cover and vegetation was searched for 30 minutes by two observers on perpendicular paths. Captured amphibians were placed in individual plastic bags with substrate and water, and captured reptiles were placed in individual cotton bags until the search time was completed. Surveys were conducted in the morning, between 09:00 and 11:00, and all plots were visited twice during June, 2005 and July, 2006.

Coverboards, or artificial refugia, are passive sampling techniques that use several materials (e.g., plywood sheets, tin, etc…; Mitchell et al., 1993; Reading, 1997) and different designs (Fellers and Drost, 1994), to simulate natural cover, enticing reptiles and amphibians to seek refuge. During this study, coverboard arrays (N = 5) consisted of nine 120 X 120 cm plywood sheets placed flat on the ground and arranged in a "double-diamond" formation. Arrays were established in dry areas during mid-May, 2006 and checked once a week from early-June to mid-August, 2006.

Secondary capture techniques. - Standardized road searches were performed on the four main roads at BNS (i.e., Deep Creek Rd., Old North Bridge Rd., Hufton Rd., and Turtle Point Rd.) selected by the clearest ground visibility (Figure 1.1). Each route was ~2 km long and was surveyed using an all-terrain vehicle (ATV) traveling at 17 to 24 kph. Six searches were conducted per route: three 1-hour before and three 1-hour after sunset during May to June, 2005 and June to August, 2006.

Polyvinyl chloride (PVC) piping grids were established (N = 6) to sample treefrogs. Grids were randomly distributed at least 30 m from other capture techniques. Grids were composed of 12, 3.75 cm PVC pipes that were 1 m in length, and set vertically in the ground 4-pipes wide and 3-pipes deep, spaced 2 m apart. Piping grids were checked weekly from June to August, 2006.

Line transects of 0.8 km in length were established on four roads (Deer Ln., East Juniper Ln., Cypress Ln., and Deep Creek Rd.), or sections of roads not surveyed by standardized road searches. Transects were walked by two observers with each checking opposite sides of the road. Individuals that could be identified to species were counted and their distance from the center of the road was determined. Two searches (N = 8), one in the morning and afternoon, were conducted randomly on each transect from July to August, 2006.

Auditory survey sites (N = 5) were randomly distributed on roads (Hufton Rd., Turtle Point Ln., and Deep Creek Rd.) without regard for distance from other techniques. From June to August, 2006, two surveys were conducted for 20 minutes at each site with number of individuals, species, and distance recorded.

Tertiary capture techniques. - Six aquatic funnel traps constructed of aluminum window screening or hardware cloth were distributed haphazardly in canals and ditches throughout BNS. Traps were checked each morning from June to August, 2005 and May to August, 2006. Two pyramid crayfish traps (Lee Fisher International, Inc., Tampa Bay, FL) were placed in canals and ditches around BNS, much like aquatic funnel traps. Each trap was checked daily from May to August, 2006. Aquatic funnel and pyramid crayfish traps were haphazardly redistributed to new sampling locations to capture new species.

One basking trap (Memphis Net and Twine, Memphis, TN) was deployed at several sites to target turtles. The basking trap could only be placed in wide canals with easy access to banks. The trap was checked daily from May to August, 2006. Opportunistic encounters consisted of species captured at any time, while walking or driving through the study area.

Captured individuals from all techniques were identified to species, measured, weighed, and marked. Passive integrated transponder (PIT) tags were used for snakes (≥300 mm snout-vent length [SVL]), turtles (≥120 mm carapace length), lizards (≥150 mm SVL) and large amphibians (i.e., Two-toed Amphiumas [*Amphiuma means*] and American Bullfrogs [*Rana catesbeiana*]). Visible implant fluorescent elastomer (VIE) was employed to mark all other amphibians and double-mark snakes (S. J. Hutchens, C. S. DePerno, C. E. Matthews, K. H. Pollock, and D. K. Woodward, in review).

Statistical analyses. – I compared species captured (S_{obs}) and species richness estimates (S) for data collected May to August, 2005 and 2006 to evaluate capture efficacy among techniques and categories (i.e., primary, secondary, and tertiary). Also, to evaluate efficacy, I incorporated unique species captured, cost, and cost-per-species-captured. Richness estimates for primary and secondary techniques were obtained from X-matrices of abundance data using the classic Chao2 formula in EstimateS 8.0 (Colwell, 2005). Estimates for the tertiary techniques and total richness were obtained using X-matrices of incidence data (Colwell et al., 2004). Sample-based rarefaction curves of computed species observations (i.e., the Mao Tau) were employed to determine efficacy by comparing asymptotic richness across categories. The Mao Tau, an expected number of species from the pooled samples, is based on collected data for species observed and is used as a representation of accumulated species (Colwell et al., 2004; Colwell, 2005). Sampling units for rarefaction curves were defined as the individual sampling sites for each capture technique. Individual-based curves were employed whenever rescaling of sample-based curves was required (Gotelli and Colwell, 2001)

I evaluated species capture success (i.e., species captured-per-unit-effort) among individual capture techniques. Capture success was calculated by dividing the total number of species captured for a technique by that technique's duration or effort. Also, unique species (i.e., species captured by only one technique) were compared among categories. Set-up and labor costs for all techniques were compared to determine cost-per-species-captured. Set-up costs included all materials; whereas fuel costs were included in both set-up and labor costs. Costs for fuel consumption were calculated based on an estimated 40 mpg for an all-terrain vehicle on dirt roads at $2.80/gallon. Additionally, labor costs were derived as the cost of paying $8.00 an hour for one field technician.

Results

After 151 days encompassing 2 field seasons, 1, 581 individuals were captured, representing 33 species (Table 1.1). Primary techniques sampled 13 species (S_{obs}) with an estimated species richness (S) of 14. In contrast, secondary techniques sampled 18 species with a species richness estimate of 29 and tertiary techniques sampled 24 species for an estimated richness of 25. Numbers of individuals captured with secondary and tertiary techniques were lower, while numbers of species captured were higher as compared to primary methodologies (Table 1.2). Observed species and richness estimates varied between categories. For all 11 techniques, 33 total species were detected for an estimated species richness of 34 (Table 1.2).

Sample-based rarefaction curves of the computed number of species observed (i.e., the Mao Tau) illustrate the efficacy for all categories (Figure 1.2). Primary techniques captured several individuals (N = 1, 068) of only a few species (N = 13), requiring 38 of 45 (84%) sampling units to reach an asymptote. Conversely, secondary and tertiary methodologies captured few individuals (N = 260 and 253, respectively) of many species (N = 18 and 24, respectively) without reaching a clear asymptote (Figure 1.2). To allow easier comparison, these two categories were rescaled using a computed number of individuals captured (Figure 1.3). Together, the Mao Tau for all techniques reached an asymptote in 36 of 76 (47%) sampling units (Figure 1.2). Augmenting primary capture techniques with secondary, and then secondary and tertiary methodologies resulted in large differences in Mao Tau (Figure 1.4) rarefaction curves and, therefore, species richness.

Secondary and tertiary methodologies captured three and seven unique species, whereas primary techniques captured only two unique species (Tables 1.2, 1.3). Interestingly, seven of the 10 unique species captured by secondary and tertiary techniques were detected within seven sampling occasions (Table 1.3). Moreover, secondary and tertiary capture techniques efficiently sampled many of the same species captured by primary techniques (Table 1.4). Species capture success was similar between techniques (Table 1.5). Only line transects (1.25 species/km) and auditory surveys (1.05 species/hour) differed greatly from other techniques. This was likely due to higher species captures with fewer replications for these two techniques.

Materials, set-up, and labor costs were high for the study (Table 1.6). Primary methodologies had the highest costs followed by tertiary and secondary techniques. Interestingly, primary capture techniques accounted for 67% of total costs. Set-up costs and labor for operation drove costs up for PVC piping grids and road searches. Costs for tertiary techniques were primarily from fuel consumption. However, the capture success of some secondary and tertiary techniques lowered costs-per-species-captured (Table 1.6).

Discussion

The evasive nature of reptiles and amphibians (Williams and Berkson, 2004; Flint and Harris, 2005), makes the taxa difficult to detect and requires using several capture techniques to sample all species present in a community. Moreover, capture techniques vary in success of species detection (Bailey et al., 2004). In this study, if only primary capture techniques (i.e., drift fence arrays with pitfall and funnel traps, VES, and coverboard arrays) were used, species richness would have been underestimated by 59% (Table 1.2). The addition of secondary capture techniques more than doubled estimated species richness from 14 to 29. Incorporating tertiary capture techniques increased species richness to 34, adding seven unique species captured by no other methodology (Table 1.2). Thus, I recommend using as many capture techniques as can be afforded by project budgets, personnel, and space to increase the accuracy of species richness estimates.

Similar to other studies, primary techniques captured some, but not all species present (Gibbons and Semlitsch, 1981; Bury and Corn, 1987; Bury and Corn, 1988; Mitchell et al., 1993; Kjoss and Litvaitis, 2001). Unfortunately, most species richness studies rely only on primary methodologies (Gibbons and Semlitsch, 1981; Vogt and Hine, 1982; Mitchell et al., 1993; Flint and Harris, 2005). Primary capture techniques had low initial capture success, added only a few species in several sampling units, and required 33 sampling units to reach asymptotic richness (Figure 1.2). Similarly, the Chao2 estimate of species richness was low, with an estimate of 14 (Table 1.2). Low species captures indicated that primary capture techniques do not provide accurate richness estimates. However, primary methodologies successfully captured the most common species (Table 1.4), implying their usefulness in deriving detection probabilities with mark-recapture or removal designs (Pollock et al., 2002). Two unique species were captured with primary techniques, indicating their value to species richness studies. However, the extra time and costs required to sample these species implies that the effectiveness of primary methodologies would be limited if sampling periods were short or budgets were constrained.

Secondary techniques demonstrated low initial captures for observed species (Figure 1.2), but surpassed primary techniques after 10 of 19 (53%) sampling units. Although the richness curve did not reach a clear asymptote, 18 species were accumulated in less than half the sampling units compared to primary techniques, justifying their incorporation in species richness and monitoring studies. Gotelli and Colwell (2001) determined that curves having not yet reached an asymptote (e.g., curves for secondary and tertiary categories) could be compared after appropriate scaling to individual-based curves (Figure 1.3). The resulting individual-based curves were similar to sample-based curves, with Tertiary techniques accumulating more species faster than Secondary methodologies. High accumulation could be attributed to the success of these techniques capturing more species represented by fewer numbers of individuals (Table 1.2; Figure 1.2). Secondary methodologies augmented species accumulation to 16 when combined with primary capture techniques (Figure 1.4). Moreover, secondary techniques were remarkably versatile, capturing three unique species and securing all but one of the most common species more efficiently than primary techniques (Tables 1.3, 1.4). Greater success was likely due to the active nature of secondary capture techniques, compared to the predominantly passive primary techniques.

Tertiary techniques sampled more species than other techniques and surpassed the success of secondary methodologies in 5 of 12 (42%) sampling units (Figure 1.2). The ability to detect several species with so few individuals resulted in an increased mean Chao2 estimate (Table 1.2). However, after reaching an asymptote the species richness for tertiary methodologies (S = 25) was similar to secondary techniques (S = 29; Figure 1.3). Adding tertiary capture techniques to primary and secondary rarefaction curves demonstrated the importance of employing multiple methodologies (Figure 1.4). It is likely that active trapping was important for the effectiveness of opportunistic encounters. Fair and Henke (1997) determined that opportunistic encounters provided more captures-per-unit-effort than standardized methodologies. In this study, opportunistic encounters captured more species than primary techniques. The importance of tertiary methodologies was emphasized by the detection of 7 unique species. For instance, 32 different Common Kingsnakes (*Lampropeltis getula*) were captured only by opportunistic encounters (Table 1.3).

Opportunistic encounters and aquatic funnel traps captured many of the same species (though both captured high numbers of species with several unique to each technique), resulting in lower diversity (Purvis and Hector, 2000) and a lower Chao2 estimate of species richness when compared to secondary methodologies (Table 1.2). Further, lowered diversity could be attributed to the failure of the basking trap. Despite no success with the basking trap, species captured by tertiary methodologies did not differ from other categories and a low cost-per-species-captured was maintained.

Unique species were detected by some sampling methodologies from each category with varying success (Table 1.3). Unique species were those species detected by only one capture technique and are important in accurately indicating "presence" when deriving species richness estimates (McKenzie et al., 2002; Colwell, 2005). Primary capture techniques sampled fewer unique species than secondary and tertiary methodologies, reiterating the aptitude of primary techniques at capturing the most common species present in an area. Secondary and tertiary capture techniques detected the most unique species while reliably catching common species, supporting the implementation of multiple sampling methodologies in species richness studies and their evaluation for determining efficacy. Also, it should be noted that all unique species captured by secondary and tertiary capture techniques, exclusive of hylid frogs, were capable of being detected by primary methodologies. Detection probabilities for some species can be low to zero (McKenzie et al., 2002), which reinforces the need for several sampling techniques.

When totaled, cost-per-species-captured was high for primary techniques (Table 1.6), resulting in $994 USD for species captured by each technique. For funnel traps (six per array), a considerable portion of the total cost arose from labor/manufacturing costs and establishing arrays, while fuel consumption contributed to high costs for VES, and materials for high costs of coverboard arrays. Conversely, secondary and tertiary capture techniques had low costs-per-species-captured, $452 and $592 USD total, respectively (Table 1.6). However, high capture success made secondary and tertiary methodologies more cost-effective than primary capture techniques. The low cost of both secondary and tertiary techniques suggests their successful application for short- and long-term studies.

When combined, employing 11 capture techniques provided a comprehensive estimate of species richness (S = 34). I acknowledge that these sampling techniques did not detect all species and that non-detection does not discount a species' presence (MacKenzie et al., 2002; Pollock et al., 2002). For example, between trapping sessions and after the conclusion of the study, three new species (Brown Watersnake [*Nerodia taxispilota*], Eastern Mudsnake [*Farancia abacura*], and Worm Snake [*Carphophis amoenus*]) were recorded that had not been sampled by any capture technique during the 2-year study. However, incorporating 11 capture techniques estimated at least 34 species with the three undetected species above included in the upper limit of the estimate's analytical standard deviation (S = 34 + 5). This indicated the estimated species richness closely resembled the community of reptiles and amphibians expected at BNS.

After evaluating species captured, estimated species richness, species capture success, cost, and cost-per-species-captured data for primary (i.e., drift fence arrays with pitfall and funnel traps, VES, and coverboard arrays), secondary (i.e., road searches, PVC piping grids, line transects, and auditory surveys), and tertiary (i.e., opportunistic encounters, aquatic funnel traps, crayfish traps, and basking traps) techniques, I recommend the use of as many techniques as possible to obtain a better measure of species richness. The results determined that (1) primary capture techniques do not capture enough species for an accurate estimate of species richness, (2) secondary and tertiary techniques add enough species to justify their time and cost, and (3) secondary and tertiary techniques are useful for both short- and long-term studies. I recommend arranging capture techniques in a hierarchical design to minimize undetectable species. I believe that the success with multiple technique sampling can be inferred to all habitat types and taxa. Future research should focus on implementing several capture techniques for a variety of habitat types and taxa to determine efficacy, thus enhancing our understanding of species richness and diversity.

Acknowledgments

Procedures for research regarding capture and handling of reptiles and amphibians followed the guidelines for the Institutional Animal Care and Use Committee at North Carolina State University (Approval Number 05-036-0). I acknowledge the North Carolina State University Department of Forestry and Environmental Resources and the North Carolina State University Fisheries and Wildlife Sciences Program for support and funding. Also, I would like to thank K. Golden, C. Matthews, A. Braswell, and K. Pollock for their comments on earlier drafts of the manuscript.

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Table 1.1. Species detected by all capture techniques at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

Common Name	Scientific Name
Yellow-bellied slider	*Trachemys scripta*
Green Frog	*Rana clamitans*
Southern Leopard Frog	*Rana sphenocephala*
Southern Toad	*Bufo terrestris*
Southern Cricket Frog	*Acris gryllus*
Snapping Turtle	*Chelydra serpentina*
Common Kingsnake	*Lampropeltis getula*
Eastern Ratsnake	*Elaphe obsoleta*
Southern Watersnake	*Nerodia fasciata*
Plain-bellied Watersnake	*Nerodia erythrogaster*
Rough Greensnake	*Opheodrys aestivus*
Coastal Plain Cooter	*Pseudemys concinna floridana*
Two-toed Amphiuma	*Amphiuma means*
Eastern Racer	*Coluber constrictor*
Eastern Ribbonsnake	*Thamnophis sauritus*
Cottonmouth	*Agkistrodon piscivorus*
Little Brown Skink	*Scincella lateralis*
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*
American Bullfrog	*Rana catesbeiana*
Striped Mud Turtle	*Kinosternon baurii*
Eastern Mud Trutle	*Kinosternon subrubum*
Eastern Box Turtle	*Terrapene carolina*
Painted Turtle	*Chrysemys picta*
Stinkpot Turtle	*Sternotherus odoratus*
DeKay’s Brownsnake	*Storeria dekayi*
Green Treefrog	*Hyla cinerea*
Gray Treefrog	*Hyla versicolor*
Pine Woods Treefrog	*Hyla femoralis*
Rainbow Snake	*Farancia erytrogramma*
River Cooter	*Pseudemys concinna*
Spotted Turtle	*Clemmys guttata*
Southeastern Five-lined Skink	*Eumeces inexpectatus*
Green Anole	*Anolis carolinensis*

Table 1.2. Total number of individuals and unique species captured, and observed and estimated species richness for all techniques at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

Category	Capture Technique	Total Individuals Captured	S_{obs}[a]	S[b]	Unique Species
Primary	Pitfall traps	489	5		0
	Funnel traps	462	9		1
	Visual encounter surveys	96	7	7	1
	Coverboard arrays	21	4	4	0
	Category totals	1068		14	2
Secondary	Road searches	31	10	22	0
	PVC piping grids	5	1	1	0
	Line transects	164	10	10	1
	Auditory surveys	60	7	7	2
	Category totals	260		29	3
Tertiary	Opportunistic encounters	190	16	17	4
	Aquatic funnel traps	32	17	26	3
	Crayfish traps	31	7	7	0
	Basking trap	0	0	0	0
	Category totals	253		25	7
Totals		1581	33	34	12

[a]All species observed or trapped by a particular technique.

[b]Mean Chao2 estimate of species richness for each technique.

Table 1.3. The duration until capture for unique species by category and technique at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

Common Name	Scientific Name	Category	Capture Technique	Sampling sessions until capture[a]
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	Primary	Visual encounter survey	15
DeKay's Brownsnake	*Storeria dekayi*	Primary	Drift fence array – funnel trap	42
Grey Treefrog	*Hyla versicolor*	Secondary	Auditory survey	1
Pine Woods Treefrog	*Hyla femoralis*	Secondary	Auditory survey	1
Green Anole	*Anolis carolinensis*	Secondary	Line transect	6
Eastern Musk Turtle	*Stenothorus odoratus*	Tertiary	Aquatic funnel trap	3
Eastern Kingsnake	*Lampropeltis getula*	Tertiary	Opportunistic encounter	4
Rat Snake	*Elaphe obsoleta*	Tertiary	Opportunistic encounter	5
Rough Greensnake	*Opheodrys aestivus*	Tertiary	Opportunistic encounter	7
Rainbow Snake	*Farancia erytrogramma*	Tertiary	Aquatic funnel trap	24
River Cooter	*Pseudemys concinna*	Tertiary	Aquatic funnel trap	24

Table 1.3 (continued).

Common Name	Scientific Name	Category	Capture Technique	Sampling sessions until capture[a]
Eastern Mud Turtle	*Kinosternon subrubrum*	Tertiary	Opportunistic encounter	50

[a]Number of sampling sessions for specific techniques until species capture.

Table 1.4. The duration until capture of species detected by more than one capture technique at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

		Days until capture		
Common Name	Scientific Name	Primary	Secondary	Tertiary
Green Frog	*Rana clamitans*	1	1	6
Southern Leopard Frog	*Rana sphenocephala*	1	1	41
Southern Cricket Frog	*Acris gryllus*	1	1	44
Southern Toad	*Bufo terrestris*	1	1	N/A*
Ground Skink	*Scincella lateralis*	6	2	N/A
Southeastern Five-lined Skink	*Eumeces inexpectatus*	6	7	N/A
American Bullfrog	*Rana catesbaeiana*	15	11	136
Banded Watersnake	*Nerodia fasciata*	35	13	7
Red-bellied Watersnake	*Nerodia erythrogastor*	39	15	7
Black Racer	*Coluber constrictor*	42	2	27
Spotted Turtle	*Clemmys guttata*	74	N/A	98
Common Snapping Turtle	*Chelydra serpentine*	N/A	3	1
Cottonmouth	*Agkistrodon piscivorous*	N/A	7	28
Eastern Ribbonsnake	*Thamnophis sauritus*	N/A	11	28
Eastern Box Turtle	*Terrapene Carolina*	N/A	11	52

* N/A = not captured by a technique in that category.

Table 1.5. Capture success and species observed (S_{obs}) for all categories and techniques at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

Category	Capture Technique	S_{obs}	Capture Success[a]	Units
Primary				
	Drift fence arrays	10	0.017	per array night
	Pitfall traps	5	0.015	per trap night
	Funnel traps	9	0.015	per trap night
	Visual encounter surveys	7	0.12	per person-hour
	Coverboard arrays	4	0.013	per array night
Secondary				
	Road searches	10	0.10	per kilometer
	PVC piping grids	1	0.00024	per grid night
	Line transects	10	1.25	per kilometer
	Auditory surveys	7	1.05	per person-hour
Tertiary				
	Opportunistic encounters	16	0.0087	per kilometer
	Aquatic funnel traps	17	0.044	per trap night
	Crayfish traps	7	0.054	per trap night
	Basking trap	0	0.00	per trap night

[a]Total capture success (species-captured-per-unit-effort) is the quotient of species captured by technique duration.

Table 1.6. Materials and labor costs for set-up and operation for all capture techniques at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

Category	Capture Technique	Cost (set-up)	Labor-hours (set-up)	Cost (oper.)	Labor-hours (oper.)	Monthly Costs/Labor	Total Cost	S_{obs}[a]	Unique Species[b]	Total Cost/S_{obs}
Primary	Pitfall traps	$226	$304	$33	$1,137	$671	$1,700	5	0	$340
	Funnel traps	149	1,872	21	711	594	2,752	9	1	306
	Visual encounter survey	0	240	42	480	141	762	7	1	108
	Coverboard arrays	298	80	7	400	501	785	4	0	196
	Category total	672	2,496	103	2,728	1,906	5,999		2	
Secondary	Road searches	0	0	85	176	195	262	10	0	26
	PVC piping grids	111	48	7	240	330	406	1	0	406
	Line transects	0	0	2	80	164	82	10	1	8
	Auditory surveys	0	0	2	53	31	55	7	2	8
	Category total	111	48	97	549	720	806		3	
Tertiary	Opportunistic encounters	0	0	317	201	63	518	16	4	32

Table 1.6 (continued).

Category	Capture Technique	Cost (set-up)	Labor-hours (set-up)	Cost (oper.)	Labor-hours (oper.)	Monthly Costs/Labor	Total Cost	S_{obs}[a]	Unique Species[b]	Total Cost/S_{obs}
Tertiary	Aquatic funnel traps	23	288	159	201	230	670	17	3	39
	Crayfish traps	90	0	159	201	153	449	7	0	64
	Basking trap	100	0	159	201	163	459	0	0	N/A
	Category total	212	288	793	803	608	2097		7	
Totals						$3,235	$8,901		12	

[a]All species observed by a particular technique.

[b]Species captured by only one sampling technique across categories.

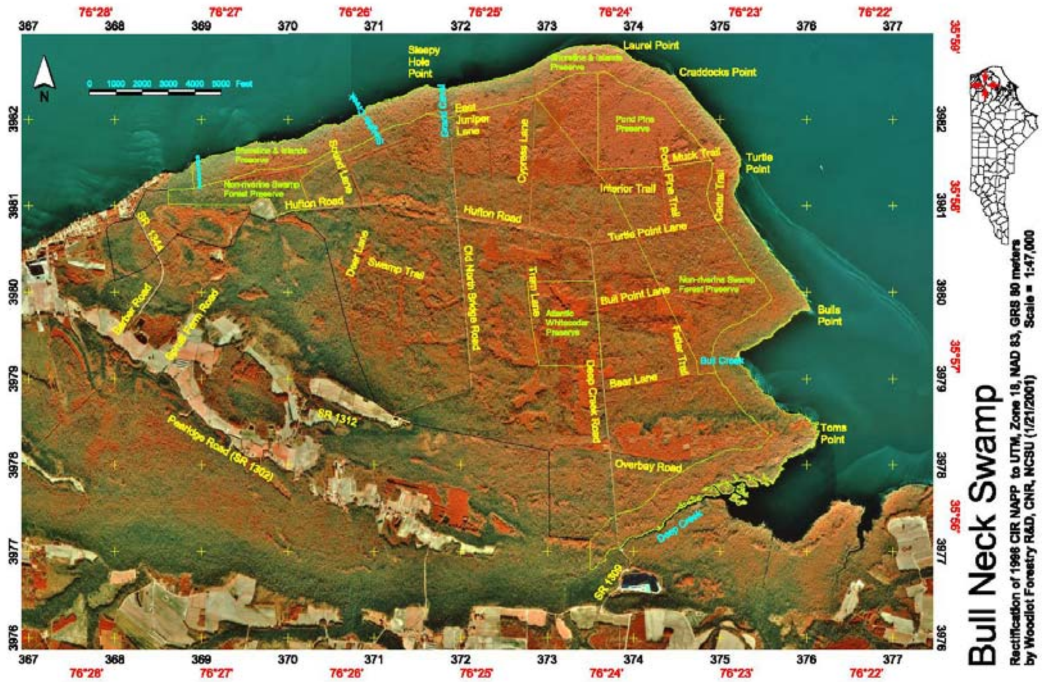


Figure 1.1. Color infrared photography (CIR) of Bull Neck Swamp, Washington County, North Carolina.

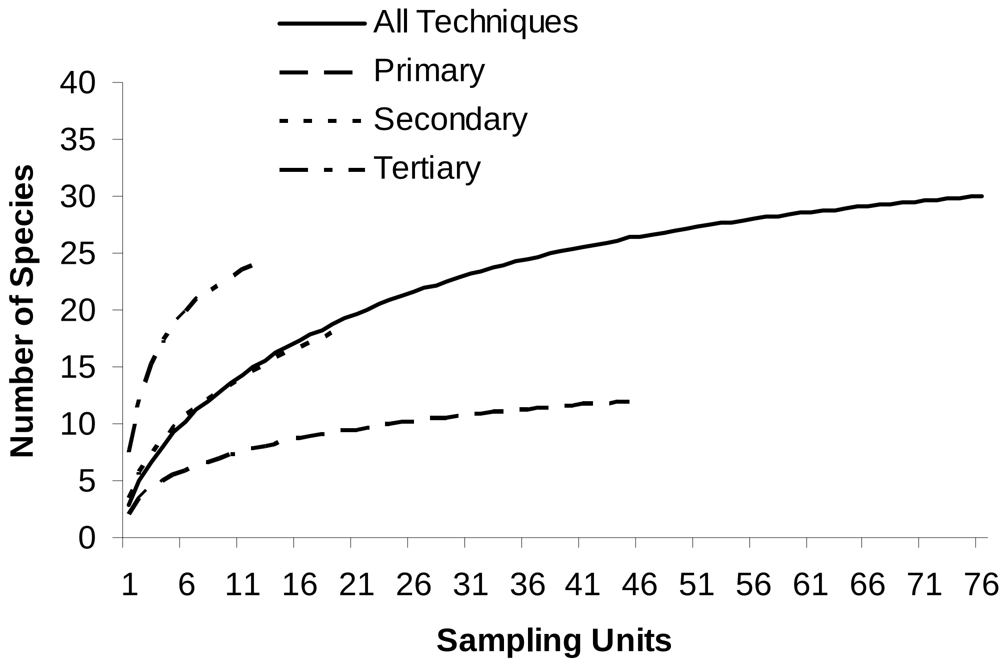
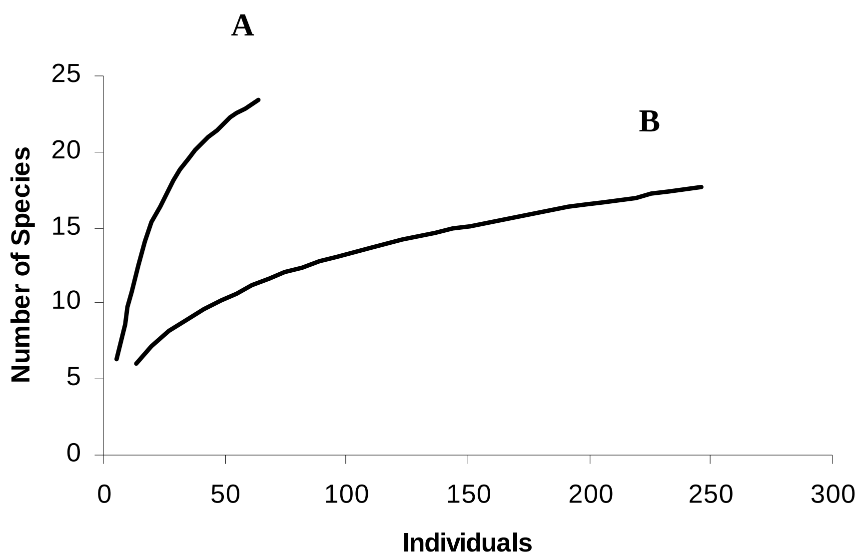


Figure 1.2. Sample-based rarefaction curve of computed species observations, or Mao Tau, for each category and all capture methodologies for data collected May to August, 2005 and 2006. Sampling units were defined as individual sampling sites for all capture techniques.



Figures 1.3. A) Individual-based rarefaction curve of species richness for Secondary techniques. B) Individual-based rarefaction curve of species richness for Tertiary techniques. Because the curves for Secondary and Tertiary capture methodologies did not reach clear asymptotes, curves were rescaled to the computed number of individuals captured for comparison (Gotelli and Colwell, 2001).

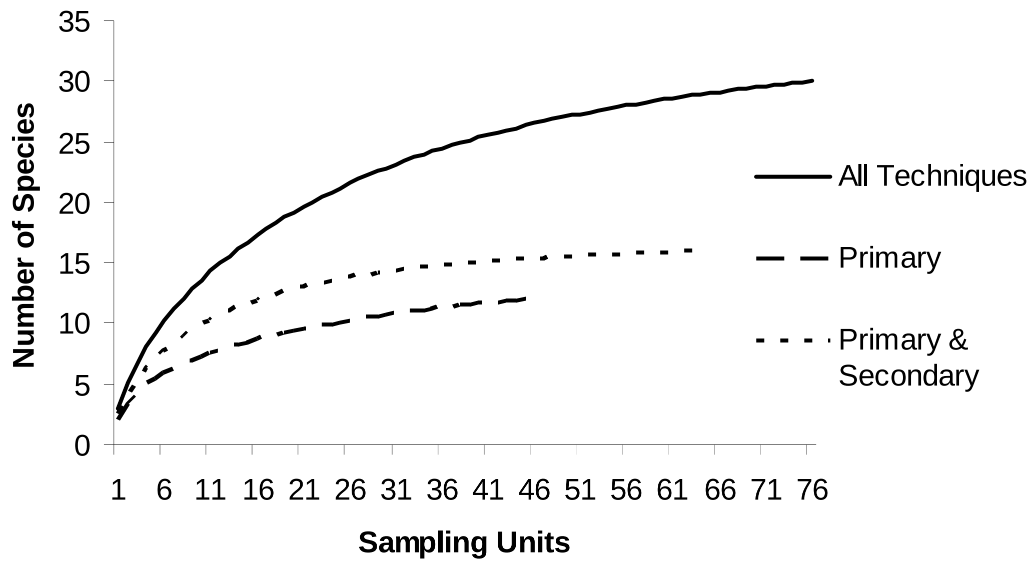


Figure 1.4. Additive sample-based rarefaction curves of Mao Tau estimates. The addition of Secondary and Tertiary categories to Primary methodologies added more accumulated species. The addition of Secondary and Tertiary categories to the estimated richness of Primary methodologies added 44% and 15% more species to the total richness estimate of reptile and amphibian species.

USING COMMUNITY PARAMETERS TO DETERMINE LAND-USE EFFECTS ON REPTILE AND AMPHIBIAN ASSEMBLAGES IN A POCOSIN WETLAND

Abstract

Populations of reptiles and amphibians are declining world wide. There is strong evidence that the primary cause of declines is land-use change (e.g., silviculture or conversion to agriculture). I employed 11 sampling techniques to determine the species richness of the herpetofaunal community at Bull Neck Swamp, a pocosin wetland. Data were used to compare observed (S(obs)) and estimated species richness (S), relative abundance, and species composition among four habitat preserves and a "manageable" area within the wetland. My objectives were to: (1) derive community parameters for each habitat, (2) determine which preserves had species more vulnerable to the effects of land-use practices, and (3) provide recommendations for monitoring and management regimes in the future. Species richness estimates and similarity indices were derived using EstimateS 8.0. A post hoc species distribution for the entire wetland was derived by the Nestedness Temperature Calculator Program. After two field seasons, 1, 581 total captures were recorded for 33 observed species (S = 34). Observed richness ranged from 7 to 32 species across habitats (S = 13 to 44) and abundances ranged from 99 to 873 individuals. Similarity indices were comparable between all habitats, with 90% of comparisons over 0.75 in similarity and nestedness temperature calculation resulted in an even distribution (T = 12.6 ° C). The Manageable area had the highest values of observed and estimated species richness and relative abundance, including 13 species captured only in that area. However, comparable similarity indices between habitats, an even species distribution, and habitat continuity suggested land-use practices would have little impact on the herpetofaunal community.

Future management practices should be carefully considered and planned to mitigate effects to potentially vulnerable species detected within the Manageable area. We recommend other studies employ observed and estimated species richness, relative abundances, and species composition when comparing assemblages.

Reptiles and amphibians populations are declining worldwide (Wake, 1991; Pechman et al., 1991; Heyer et al., 1994; Gibbons et al., 2000). More species of reptile and amphibian are at risk than either birds or mammals, and they have the highest threat status of all terrestrial vertebrates (IUCN, 2006; Gardner et al., 2007a). Reptiles and amphibians hold vital positions in forest and aquatic food webs, are important for nutrient cycling (Pais et al., 1988; Hanlin et al., 2000; Burton and Likens, 1975), are indicators of ecosystem health (Bury and Corn, 1988; Gibbons et al., 2000; Wake, 1991; Dunson et al, 1992; Hanlin et al., 2000), and compose a tremendous portion of the vertebrate biomass (e.g., over 18,000 individuals/ha of terrestrial salamanders in the southern Appalachians; Petranka and Murray, 2001; Pais et al., 1988; Burton and Likens, 1975).

Declines have been attributed to normal population fluctuations (Wake, 1991) and indirect factors (known as Class II hypotheses; Gardner et al., 2007a), such as climate change, pollution, disease, and acidification (Gardner et al., 2007a). However, habitat loss due to land-use change is perhaps the greatest antagonist and is largely accepted as the primary cause of biodiversity loss at such a large scale (Gardner et al., 2007a). Conversion to agriculture, logging, mining, and urbanization are major contributors to land-use change (Pechman et al., 1991; Wake, 1991; Gardner et al., 2007a; Gardner et al., 2007b).

Inventory data from 5 designated habitat types (i.e., four preserves and a "manageable" area) were collected. Observed and estimated species richness, relative abundance, and species composition among habitat types were compared to: (1) derive community parameters for each habitat, (2) determine whether habitat preserves were providing refuge to vulnerable species and the impacts of timber harvest or other land-use practices on species assemblages in the preserves, and (3) provide recommendations for monitoring and management regimes for Bull Neck Swamp in the future.

Materials and Methods

Study area. – I conducted this study at Bull Neck Swamp (BNS; 35° 58' N, 76° 25' W; Figure 2.1), a 2, 491 ha (6, 158 ac) pocosin wetland located in Washington County, North Carolina. Bull Neck Swamp, owned by several timber companies in the past, was purchased by North Carolina State University in 1996 and is managed by the Fisheries and Wildlife Sciences Program. The Natural Heritage Trust Fund established four habitat preserves prior to the acquisition of BNS by the University. Preserved areas totaled 937 ha (2, 315 ac) of habitat, safe from future land management schemes (Table 2.1). The four designated preserves included Atlantic white-cedar (*Chamaecyparis thyoides*), Pond pine (*Pinus serotina*), Non-riverine swamp, and Shoreline/Islands. Although designations were made based on plant community, all of these habitats were contiguous and fairly homogenous sharing many of the same species, such as red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), red bay (*Persea borbonia*), sweet bay (*Magnolia virginiana*), and wax-myrtle (*Myrica cerifera*). Three dry ridges run west to east through the wetland allowing oaks (*Quercus* spp.) and American beech (*Fagus grandifolia*) to grow. These ridges occur only in the "manageable" area.

These remaining "manageable" areas, hereafter the Manageable area, (1, 554 ha; 3, 841 ac) were available for land-use practices, including timber harvest and use as a demonstration forest for wetlands forestry and site preparation applications. The wetland was a small peninsula created by the Roanoke River delta and Albemarle Sound. Bottomland forests and hardwood swamps with patchy cultivated areas comprised the southern border of the property.

Sampling techniques. – Eleven sampling techniques were deployed throughout BNS (Hutchens and DePerno, in review). Techniques employed were drift fence arrays with pitfall and funnel traps, visual encounter surveys, coverboard arrays, standardized road searches, polyvinyl chloride (PVC) piping grids, line transects, auditory surveys, opportunistic encounters, and aquatic funnel, crayfish, and basking traps. All sampling techniques were evenly distributed between habitats when possible (Hutchens and DePerno, in review). All captured individuals were marked.

Analyses. – EstimateS 8.0 (Colwell, 2005) was used to derive Chao2 estimates of species richness for individual habitats and the study area as a whole. Similarly, EstimateS 8.0 was used to compare species composition among the four preserves and Manageable area by deriving Chao - Jaccard Similarity Indices (JSI), employed for their correction for unseen shared species, those species that were likely present in both assemblages but undetected during sampling (Chao et al., 2005; Colwell 2005; Chao et al., 2006). All calculations in EstimateS 8.0 were based on X-matrices of collection data, with species occupying the first column and sampling unit or habitat type occupying the first row. Comparisons of species richness were made with observed species (S(obs)), estimated species richness (S), and proportional species richness (PSR). Proportional species richness was calculated using the formula

$$PSR = S(obs)_H / S(obs)_T$$

where the proportional species richness is equal to the species richness for a particular habitat ($S(obs)_H$) divided by the total species richness ($S(obs)_T$; Cao et al., 2002). This calculation represents the proportion of observed species as compared to the entire species pool. Comparisons of relative abundance were made using raw abundance data and proportional relative abundance (PRA), which follows from the formula above. Comparisons of JSI were made using raw values. Nestedness temperature, a ratio of distribution order where higher temperatures represent greater disorder, was calculated using the Nestedness Temperature Calculator Program (Atmar and Patterson, 1995). Only initial captures (N = 1, 496) were used for calculations.

Results

During May to August, 2005 and 2006, 1, 581 total captures were recorded for 33 species of reptile and amphibian at Bull Neck Swamp (Table 2.2), giving an estimated species richness of 34. Observed species richness values in the different habitats ranged from 7 to 32 species and estimated richness ranged from 13 to 44 species, with the Manageable habitat obtaining the highest values for both (Table 2.3a). Values of observed and estimated species richness increased as habitat area increased except for the Shoreline/Island habitat (Figure 2.2a). Proportional species richness ranged from 21.2% to 96.9% of the total species pool. Relative abundances for all habitats ranged between 99 and 873 individuals (PRA = 6.6% - 58.4%; Table 2.3b). Similarly, relative abundance increased with habitat area with the exception of the Shoreline/Islands habitat (Figure 2.2b).

Most species (N = 19) were detected in at least two habitats, with four species (Green Frog [*Rana clamitans*], Southern Cricket Frog [*Acris gryllus*], Plain-bellied Watersnake [*Nerodia erythrogaster*], and Green Treefrog [*Hyla cinerea*]) detected from all five habitats. Of the 14 remaining species, all occurred only in the Manageable habitat with the exception of DeKay's Brownsnake (*Storeria dekayi*; Table 2.2). Further summaries of species captured within the 5 habitats are provided in Tables 4 to 8.

Chao - Jaccard Similarity Indices were similar between all habitats and most habitats had similarity indices greater than 0.75 (Table 2.9; Figure 2.3). However, the comparison between Atlantic white-cedar and Shoreline/Islands habitats yielded a much lower index (JSI = 0.59; Table 2.9) and could likely be due to the alternating abundances of species common between the two habitats (Chao et al., 2005). Comparison between the Manageable and Non-riverine Swamp habitats produced perfect similarity (JSI = 1.0). Five comparisons had similarity indices greater than or equal to 0.90, with 3 of those possessing an index of 0.97 (i.e., Shoreline/Islands – Non-riverine, Shoreline/Islands – Pond pine, and Non-riverine – Pond pine; Table 2.9). Nestedness temperature calculation yielded a matrix temperature of 12.6° C (Figure 2.4a).

Discussion

Comparisons of observed and estimated species richness, relative abundances, and species composition revealed the five designated habitats of BNS were similar in their reptile and amphibian assemblages. Observed and estimated values of species richness revealed the Manageable habitat to be the most species rich (Table 2.4a). Higher values for these parameters could be expected from the Manageable area due to its larger area when the species-area curve concept is considered (Figure 2.2a; Dunn and Loehle, 1988; Atmar and

Patterson, 1993) or to the diversity of habitats within that area. However, species richness alone could be misleading and could be a poor indicator for conservation value by masking species responses to disturbance (Gardner et al., 2007a).

Similarly, values of relative abundance were dominated by the Manageable area (N = 873), which comprised 58.4% of initial captures (Tables 2.3a, 2.3b; Figure 2.2b). However, care must be taken when using abundance to compare assemblages (van Horne, 1983; Purvis and Hector, 2000; Gardner et al., 2007a). For instance, if the number of individuals detected per species were considered, the probability of randomly selecting two different species would be greater in the Atlantic white-cedar habitat than the Manageable area (Table 2.3a), which confounds this measure of diversity (Purvis and Hector, 2000). Therefore, relative abundances did not provide a basis for quantifying or comparing the similarity of an assemblage to the entire community of reptiles and amphibians at BNS (Cao et al., 2002). Employing similarity indices, such as the Chao - Jaccard Similarity Index, could solve this dilemma.

Similarity indices assess composition between two assemblages based on three variables: the number of shared species between assemblages and the numbers unique to each (Chao et al., 2005; Chao et al., 2006). Abundance-based comparisons among the five assemblages in this study demonstrated high similarity (all JSI > 0.5). Classic, incidence-based estimators are biased against assemblages with several rare species, such as the Manageable habitat (Chao et al., 2006). Therefore, it would be impossible to correct for this bias without employing abundance data. Abundance-based estimators are resistant to undersampling, due to the continued presence of abundant species, and are less likely to be dominated by a particular species (Chao et al., 2005; Chao et al., 2006). This is an important characteristic, given the inability of most studies to sample equally, and the effects on similarity and representations of relative abundance and species richness that undersampling can have (Cao et al., 2002). Additionally, employing an abundance-based similarity index accounted for unseen shared species (Chao et al., 2005; Colwell, 2005; Chao et al., 2006).

Few studies of reptile and amphibian communities can afford or have the space available for large-scale research at the sub-regional level (> 20 km^2; Gardner et al., 2007a). However, access to 4 preserved habitats (> 900 ha) to act as a control of community parameters is important in addressing biodiversity responses to land-use change (Gardner et al., 2007a). Moreover, using 11 sampling techniques provided a better representation of reptile and amphibian assemblages, which will allow for better conclusions regarding the effects of land-use change (Gardner et al., 2007a; Hutchens and DePerno, in review).

In conclusion, the results demonstrated that all designated habitats of BNS were similar assemblages of the reptile and amphibian community. Observed and estimated values of species richness and relative abundance were similar between all habitats except the Manageable area, which had the greatest values (S(obs) = 32 and N = 873, respectively) and 13 species unique to that area. This could be due to the larger size of the Manageable area (Figure 2.2a and b; Dunn and Loehle, 1988; Atmar and Patterson, 1993), or to the range of microhabitats within that area. For instance, the presence of two clear-cuts could increase the number of disturbance-resistant species and dry ridges offer permanently dry substrate for fossorial species, whereas preserves are predominantly inundated. However, similarity of species composition was high among all five assemblages. Nestedness temperature calculation (T = 12.6° C; Figure 2.4a) illustrated that all assemblages were subsets of one another (Atmar and Patterson, 1993), while some species in the Manageable area could be more vulnerable to extirpation, such as by land-use change. This outcome is unlikely for most species given the habitats are contiguous. I recommend that monitoring studies for BNS continue to employ a large diversity of sampling techniques for deriving inventory statistics. Also, silvicultural regimes, although allowable, should be mitigated to prevent loss of biodiversity due to the vulnerability of species. Further, I recommend the use of several sampling techniques and analyses using observed and estimated species richness, relative abundance, and species composition for all studies comparing species assemblages.

Acknowledgments

Procedures for research regarding capture and handling of reptiles and amphibians followed the guidelines for the Institutional Animal Care and Use Committee at North Carolina State University (Approval Number 05-036-0). I acknowledge the North Carolina State University Department of Forestry and Environmental Resources and the North Carolina State University Fisheries and Wildlife Sciences Program for support and funding.

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Table 2.1. Areas of the five designated habitat types of Bull Neck Swamp, Washington County, North Carolina (Figure 2.1).

Habitat Type	Hectares	Acres
Atlantic white-cedar	75	185
Pond Pine	96	237
Non-riverine Swamp	314	777
Shoreline/Islands	452	1,118
Manageable	1,554	3,841
Totals	2,491	6,158

Table 2.2. Habitat occurrences for species detected at Bull Neck Swamp, Washington County, North Carolina from May to August, 2005 and 2006.

Common Name	Scientific Name	# Habitats
Green Frog	*Rana clamitans*	5
Southern Cricket Frog	*Acris gryllus*	5
Plain-bellied Watersnake	*Nerodia erythrogaster*	5
Green Treefrog	*Hyla cinerea*	5
Southern Leopard Frog	*Rana sphenocephala*	4
Yellow-bellied slider	*Trachemys scripta*	3
Southern Toad	*Bufo terrestris*	3
Common Kingsnake	*Lampropeltis getula*	3
Eastern Racer	*Coluber constrictor*	3
American Bullfrog	*Rana catesbeiana*	3
Spotted Turtle	*Clemmys guttata*	3
Snapping Turtle	*Chelydra serpentine*	2
Eastern Ratsnake	*Elaphe obsolete*	2
Southern Watersnake	*Nerodia fasciata*	2
Eastern Ribbonsnake	*Thamnophis sauritus*	2
Cottonmouth	*Agkistrodon piscivorus*	2
Striped Mud Turtle	*Kinosternon baurii*	2
Eastern Box Turtle	*Terrapene carolina*	2
Pine Woods Treefrog	*Hyla femoralis*	2
Rough Greensnake	*Opheodrys aestivus*	1
Coastal Plain Cooter	*Pseudemys concinna floridana*	1
Two-toed Amphiuma	*Amphiuma means*	1
Ground Skink	*Scincella lateralis*	1
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	1
Eastern Mud Trutle	*Kinosternon subrubum*	1
Painted Turtle	*Chrysemys picta*	1
Stinkpot Turtle	*Sternotherus odoratus*	1
Dekay's Brownsnake	*Storeria dekayi*	1
Gray Treefrog	*Hyla versicolor*	1
Rainbow Snake	*Farancia erytrogramma*	1
River Cooter	*Pseudemys concinna*	1
Southeastern Five-lined Skink	*Eumeces inexpectatus*	1
Green Anole	*Anolis carolinensis*	1

Table 2.3a. Community parameters for the assemblages of reptiles and amphibians in the five habitats of Bull Neck Swamp, Washington County, North Carolina.

Habitat	S(obs)	S	# Individuals	# Individuals/S(obs)
Atlantic white cedar	11	13	99	9
Shoreline/Islands	9	17	131	15
Manageable	32	44	873	27
Non-riverine	14	22	275	20
Pond pine	7	13	118	17
All habitats	33	34	1496	45

Table 2.3b. Proportional species richness (PSR) and proportional relative abundances (PRA) for each habitat.

Habitat	S(obs)	PSR (%)	# Individuals	PRA (%)
Atlantic white cedar	11	33	99	6.6
Shoreline/Islands	9	27.3	131	8.7
Manageable	32	96.9	873	58.4
Non-riverine	14	42.4	275	18.4
Pond pine	7	21.2	118	7.8
Totals			1496	100

Table 2.4. Initial captures for species in the Atlantic white-cedar habitat.

Common Name	Scientific Name	# Captured
Green Frog	*Rana clamitans*	55
Southern Cricket Frog	*Acris gryllus*	16
Southern Watersnake	*Nerodia fasciata*	11
Snapping Turtle	*Chelydra serpentine*	4
American Bullfrog	*Rana catesbaeiana*	3
Common Kingsnake	*Lampropeltis getula*	3
Southern Toad	*Bufo terrestris*	2
Green Treefrog	*Hyla cinerea*	2
Yellow-bellied Slider	*Trachemys scripta*	1
Cottonmouth	*Agkistrodon piscivorus*	1
Plain-bellied Watersnake	*Nerodia erythrogaster*	1
Total		99

Table 2.5. Initial captures for species in the Shoreline/Islands habitat.

Common Name	Scientific Name	# Captured
Green Frog	*Rana clamitans*	74
Southern Leopard Frog	*Rana sphenocephala*	32
Southern Cricket Frog	*Acris gryllus*	10
Southern Toad	*Bufo terrestris*	5
Plain-bellied Watersnake	*Nerodia erythrogaster*	3
Eastern Racer	*Coluber constrictor*	3
Green Treefrog	*Hyla cinerea*	2
Dekay's Brownsnake	*Storeria dekayi*	1
Spotted Turtle	*Clemmys guttata*	1
Total		131

Table 2.6. Initial captures for species in the Manageable habitat.

Common Name	Scientific Name	# Captured
Green Frog	*Rana clamitans*	521
Southern Toad	*Bufo terrestris*	96
Southern Leopard Frog	*Rana sphenocephala*	46
Southern Cricket Frog	*Acris gryllus*	38
Yellow-bellied Slider	*Trachemys scripta*	20
Cottonmouth	*Agkistrodon piscivorus*	19
Common Kingsnake	*Lampropeltis getula*	17
Southern Watersnake	*Nerodia fasciata*	14
American Bullfrog	*Rana catesbeiana*	9
Snapping Turtle	*Chelydra serpentine*	9
Little Brown Skink	*Scincella lateralis*	7
Painted Turtle	*Chrysemys picta*	7
Plain-bellied Watersnake	*Nerodia erythrogaster*	6
Eastern Racer	*Coluber constrictor*	6
Eastern Ratsnake	*Elaphe obsolete*	6
Stinkpot Turtle	*Sternotherus odoratus*	6
Two-toed Amphiuma	*Amphiuma means*	6
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	5
Southeastern Five-lined Skink	*Eumeces inexpectatus*	5
Gray Treefrog	*Hyla versicolor*	4
Pine Woods Treefrog	*Hyla femoralis*	4
Eastern Box Turtle	*Terrapene Carolina*	3
Green Treefrog	*Hyla cinerea*	3
Coastal Plain Cooter	*Pseudemys floridana*	3
Striped Mud Turtle	*Kinosternon baurii*	3
Spotted Turtle	*Clemmys guttata*	2
Eastern Ribbon Snake	*Thamnophis sauritus*	2
Rough Greensnake	*Opheodrys aestivus*	2
Green Anole	*Anolis carolinensis*	1
Eastern Mud Turtle	*Kinosternon subrubrum*	1
Rainbow Snake	*Farancia erytrogramma*	1
River Cooter	*Pseudemys concinna*	1
Total		873

Table 2.7. Initial captures for species in the Non-rivierine Swamp habitat.

Common Name	Scientific Name	# Captured
Green Frog	*Rana clamitans*	227
Southern Cricket Frog	*Acris gryllus*	26
Green Treefrog	*Hyla cinerea*	5
Southern Leopard Frog	*Rana sphenocephala*	3
Spotted Turtle	*Clemmys guttata*	3
Eastern Ratsnake	*Elaphe obsolete*	3
Southern Watersnake	*Nerodia fasciata*	1
Eastern Box Turtle	*Terrapene Carolina*	1
Eastern Ribbon Snake	*Thamnophis sauritus*	1
Striped Mud Turtle	*Kinosternon baurii*	1
Common Kingsnake	*Lampropeltis getula*	1
Eastern Racer	*Coluber constrictor*	1
Yellow-bellied Slider	*Trachemys scripta*	1
Plain-bellied Watersnake	*Nerodia erythrogaster*	1
Total		275

Table 2.8. Initial captures for species in the Pond pine habitat.

Common Name	Scientific Name	# Captured
Green Frog	*Rana clamitans*	105
Southern Cricket Frog	*Acris gryllus*	6
Pine Woods Treefrog	*Hyla femoralis*	2
American Bullfrog	*Rana catesbeiana*	2
Plain-bellied Watersnake	*Nerodia erythrogaster*	1
Southern Leopard Frog	*Rana sphenocephala*	1
Green Treefrog	*Hyla cinerea*	1
Total		118

Table 2.9. Numbers of shared species and Chao - Jaccard similarity indices for comparisons of the species assemblages across the five habitats of Bull Neck Swamp, Washington County, North Carolina.

Habitat Comparison	S(obs) First	S(obs) Second	Shared Species	JSI Value
AWC – S/I	11	9	5	0.59
AWC – Man.	11	32	11	0.9
AWC – NR	11	14	7	0.96
AWC – Pond	11	7	5	0.79
S/I – Man.	9	32	8	0.82
S/I – NR	9	14	7	0.97
S/I – Pond	9	7	5	0.97
Man. – NR	32	14	14	1.0
Man. – Pond	32	7	7	0.77
NR – Pond	14	7	5	0.97

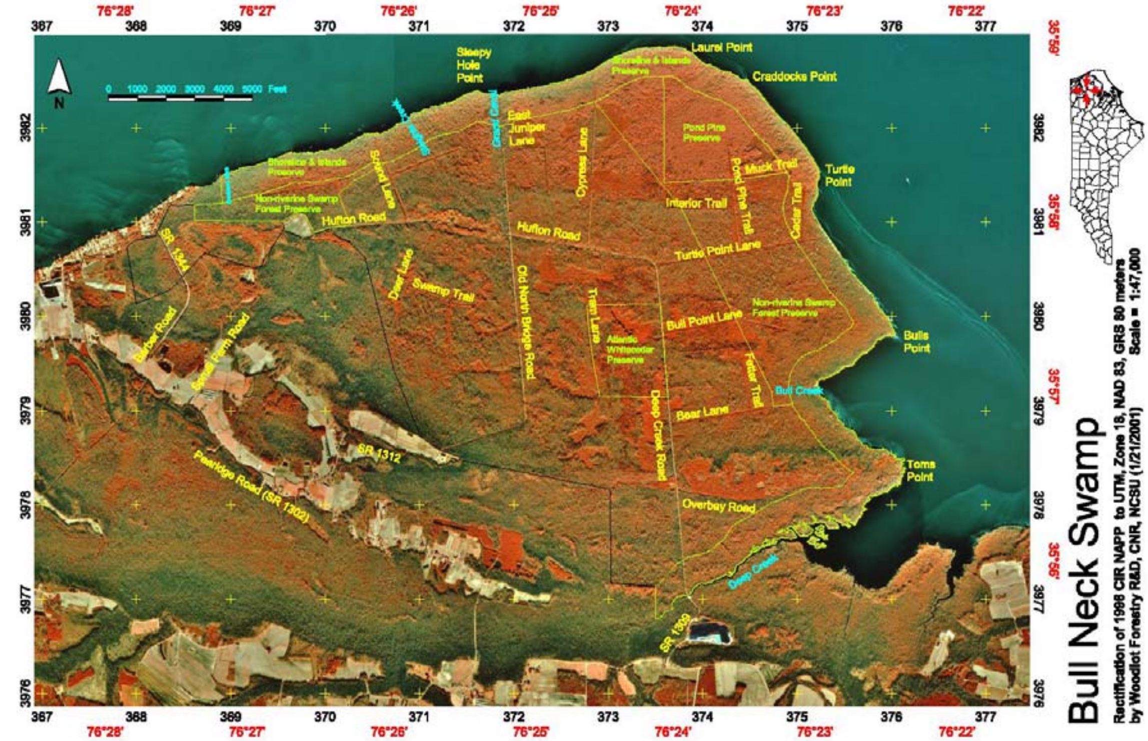
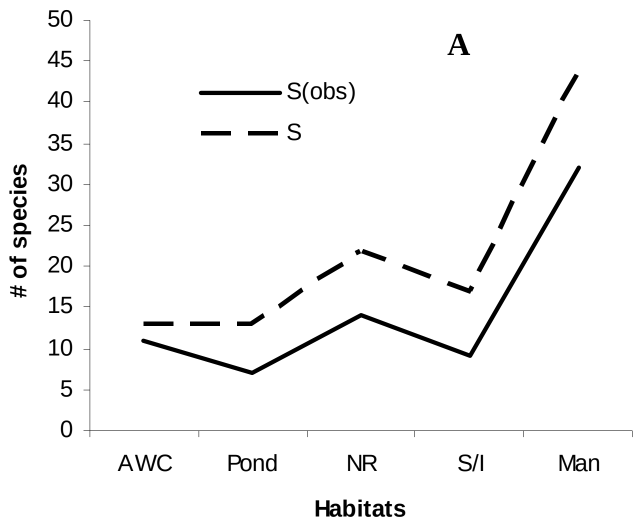


Figure 2.1. Color infrared photography (CIR) of Bull Neck Swamp, Washington County, North Carolina.



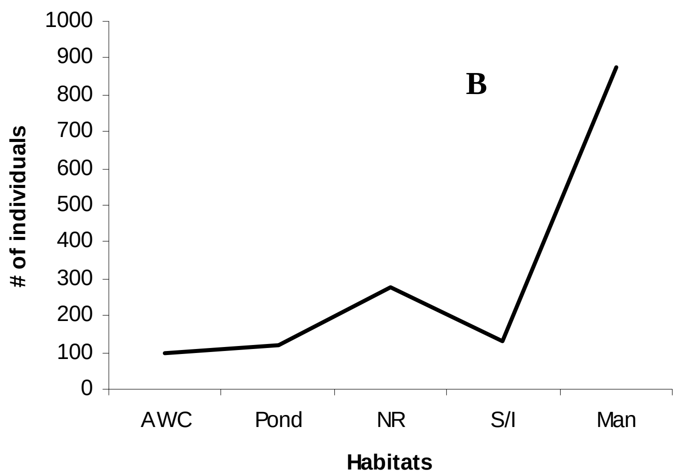


Figure 2.2. A) Observed (S(obs)) and estimated (S) species richness for each habitat of Bull Neck Swamp. B) Number of individuals captured by habitat. Habitats were arranged by increasing area.

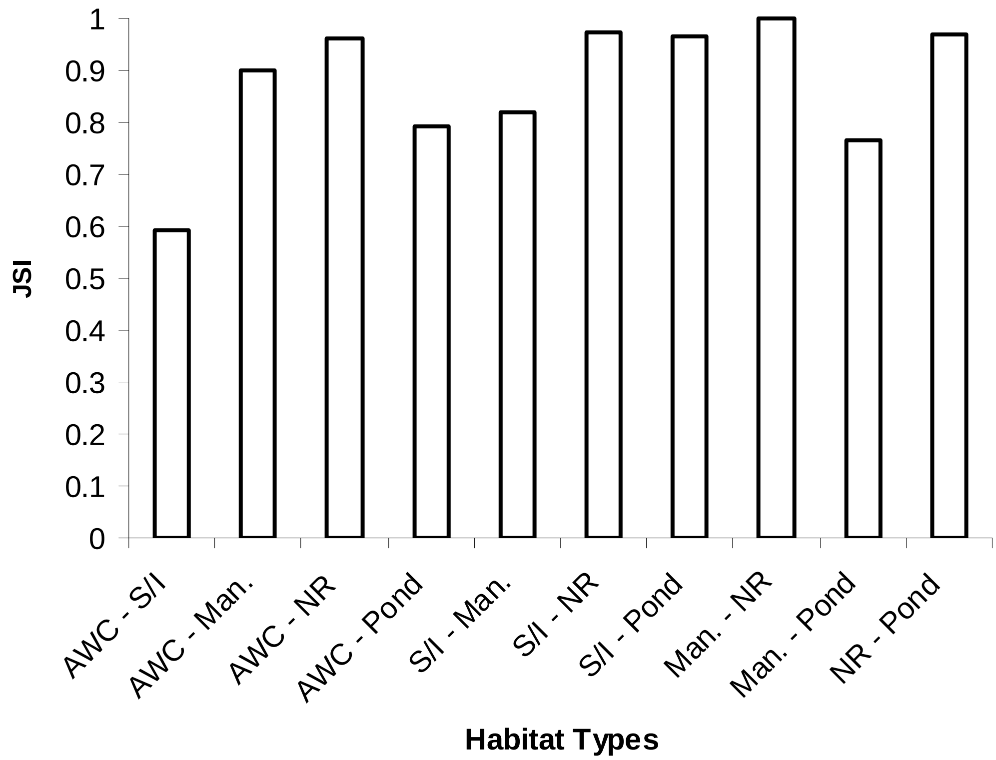
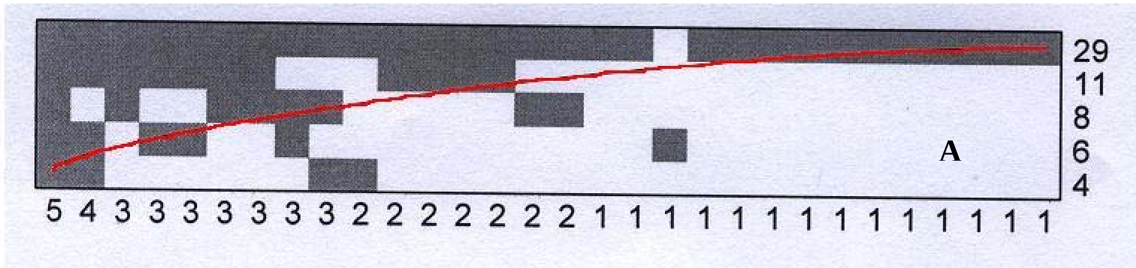


Figure 2.3. Chao - Jaccard similarity indices for comparisons of species assemblages across the five habitats of Bull Neck Swamp, Washington County, North Carolina. Habitat acronyms are as follows: Atlantic white-cedar (AWC), Shoreline/Islands (S/I), Manageable (Man), Non-riverine Swamp (NR), and Pond pine (Pond).



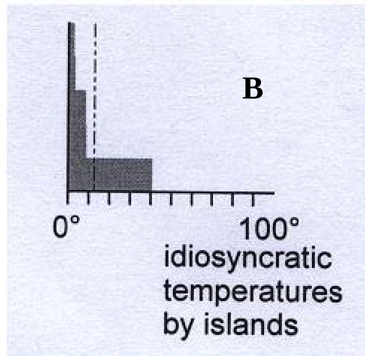


Figure 2.4. A) Nestedness distribution of the species assemblages of each habitat at BNS. Species are plotted along the X axis, with "most stable" species on the left to "most tenuous" on the right. Habitats are plotted along the Y axis, with "most hospitable" at the top and "least hospitable" at the bottom. The "extinction threshold" is represented by the red, curved line. B) Three idiosyncratic species were dropped due to their "warming" effect on matrix temperature (demarcated by the dotted line), which would create more disorder and push more species to the extinction threshold. All three species were sampled in all habitats (see Atmar and Patterson, 1993 and 1995).

VISIBLE IMPLANT FLUORESCENT ELASTOMER:
A RELIABLE MARKING ALTERNATIVE FOR SNAKES

Abstract

I hypothesized that visible implant fluorescent elastomer (VIE) would be a reliable marking alternative to scale clipping and branding. I injected 18 corn snakes (*Elaphe guttata*) with three mark volumes (i.e., 1, 2, and 3 µl) of yellow VIE. After 370 days, 94, 83, and 100% (i.e., 1, 2, and 3 µl, respectively) of marks were retained with no significant differences in retention time between volumes or individuals. Results supported the use of VIE as a reliable marking technique. I recommend future research focus on mark retention and detectability of VIE in the field.

Studies in population ecology require use of reliable marking techniques to estimate various parameters (e.g., population size, density, demographics, movement, or behavior; Penney et al. 2001, Perret and Joly 2002, Walsh and Winkelman 2004, Woods and Martin-Smith 2004). However, it is imperative that marking techniques meet standard assumptions: 1. Marks must remain visible for the duration of the experiment 2. Marks are correctly recorded 3. Marks do not affect the survival of the animal, and 4. Marks do not affect the recapture probability of animals (Otis et al. 1978, Goldsmith et al. 2003). Passive integrated transponder (PIT) tags, scale clipping, and branding are commonly used marking techniques for snake research.

Passive integrated transponder (PIT) tags were a technical advancement in marking snakes (Camper and Dixon 1988, Jemison et al. 1995, Gibbons and Andrews 2004). However, the cost of PIT tags prevents their use in low-budget studies and the injecting needle can cause substantial injury for small-bodied snakes (Keck 1994), necessitating the use of scale clipping and branding. Scale clipping involves removing partial or whole ventral or subcaudal scales to the musculature to produce scarring for identification (Blanchard and Finster 1933, Brown and Parker 1976), potentially exposing individuals to infection (Spellerberg 1977). Similarly, branding marks the ventral and/or dorsolateral scales using either freezing or heat applications; the latter exposes the skin of marked individuals to temperatures between 704° and 1,204° C (Winne et al. 2006).

Scale clipping and branding are considered invasive marking techniques that are not completely reliable (Pough 1970, Spellerberg 1977). For instance, partially removing scales may leave no scar and scars can be overgrown by adjacent scales, limiting future identification (Brown and Parker 1976). Moreover, clipping and branding can lead to recording errors due to miscounting scales or to difficulty in distinguishing marks from natural wear or injuries (Blanchard and Finster 1933, Pough 1970, Brown and Parker 1976).

Visible implant fluorescent elastomer (VIE; Northwest Marine Technology, Inc. [NMT], Shaw Is., Washington, USA) was initially developed for batch marking migratory fish, but has recently been used to mark amphibians and lizards (Nisikawa and Service 1988, Nauwelaerts et al. 2000, Penney et al. 2001, Bailey 2004, Losos et al. 2004). Visible implant fluorescent elastomer consists of a liquid polymer added to a curing agent to create a flexible plastic mark. Color kits are available, capable of marking 15,000 individuals depending on the number of colors used and marking design (NMT). The objective of this study was to determine if VIE was an appropriate marking technique for snake research based on the marking assumptions of Otis et al. (1978) and Goldsmith et al. (2003). I hypothesized that VIE would be a reliable marking alternative to scale clipping and branding techniques.

Materials and Methods

This empirical study was conducted in a laboratory setting at North Carolina State University, Raleigh, North Carolina, USA. I marked corn snakes (*Elaphe guttata*; *N* = 18) between 19 and 29 April 2006. Each snake received three doses (1, 2, and 3 µl) of yellow VIE randomized to the general area of three locations (neck, midbody, and pre-caudal). I injected marks subcutaneously and dorsolaterally on left sides using a graduated 1 cc Luer-lok syringe with a 25 gauge needle (Becton-Dickinson, Franklin Lakes, New Jersey, USA). I used 1 cc syringes to better approximate volumes, which required the 25 gauge needle for a secure fit. I injected additional corn snakes (*N* = 4) and common kingsnakes (*Lampropeltis getula*; *N* = 6) with blue and red to examine VIE color, ground color, and species effects. However, due to small sample sizes, results will not be reported for these additional snakes, but observations will be discussed.

All snakes were captive-raised and housed individually at a constant 26.6°C with food, water, and substrate provided regularly. Facilities and procedures for research regarding captive snakes followed the guidelines for the Institutional Animal Care and Use Committee at North Carolina State University (Approval Number 05-036-0). Snakes were checked for marks every two weeks using a UV-B light (NMT). I collected, dated, and examined sheds to record shedding frequency and expulsion of marks. The study concluded on 4 May 2007 after 370 days.

Retention time was calculated as the median date between when marks were last detected and the next examination. The percentage of marks retained was calculated to demonstrate mark performance by volume. In a 2-way analysis of variance (ANOVA), mark volumes and individuals were tested to determine effects on mean retention time. Similarly, the effects of shedding frequency (i.e., number of sheds/individual) and mark location were tested against mean retention time for all mark volumes with 1-way ANOVA. Analyses were performed using PROC GLM; post hoc analyses were conducted with Fisher's least significant difference (SAS 9.1; SAS Institute, Cary, North Carolina).

Results

All 18 corn snakes used in the experiment were of similar length ($\bar{x}$ = 990.39 ± 79.41mm; snout-vent length) and weight ($\bar{x}$ = 370.33 ± 81.93 g). After 370 days, most marks of all volumes were retained (Table 3.1). No differences in retention time were detected between mark volumes ($F_{2, 34}$ = 1.27, P = 0.2940) or individuals ($F_{17, 34}$ = 0.88, P = 0.6045). Shedding frequency ($\bar{x}$ = 5.05 ± 1.21 sheds/snake) did not have a significant effect on mark retention time at low ($F_{1, 16}$ = 0.79, P = 0.3860), medium ($F_{1, 16}$ = 0.00, P = 0.9501), or high mark volumes (100% retention). Analysis of mark location did not reveal a significant difference in mean retention time ($F_{2, 51}$ = 3.00, P = 0.0588).

Discussion

These data indicated that VIE was a reliable marking technique for snakes, with 83 – 100% retention after 370 days (Table 3.1) and no observed effect on snake survival. The fluorescent colors made elastomer marks easy to identify and record. No significant difference in retention time was observed among mark volumes, individuals, or mark location. However, 3 of the 4 marks lost (1-low and 2-medium volume) were located in the pre-caudal region and were likely lost through expulsion within the first few examinations. If these early expulsions were removed from analyses, retention times after 370 days would be 100%, 94.4%, and 100% for low, medium, and high volumes, respectively.

The results indicated that VIE marks last at least 370 days and satisfy the marking assumptions proposed by Otis et al. (1978) and Goldsmith et al. (2003). Branding and scale clipping have been reported to last ≥3 years (Brown and Parker 1976, Winne et al. 2006) and elastomer marks have been reported lasting well over a year in amphibians (Davis and Ovaska 2001) and are capable of permanence (Kinkead et al. 2006). I acknowledge the short study duration (370 days), but believe VIE satisfies assumptions for correct recording, and survival and recapture effects (Davis and Ovaska 2001, Kinkead et al. 2006).

Scale clipping and branding are invasive procedures that remove or burn ventral or subcaudal scales to produce scarring (Blanchard and Finster 1933, Brown and Parker 1976, Spellerberg 1977, Winne et al. 2006). However, removing small portions of scales, adjacent scale growth, and natural injuries can obscure clipped scars and brands (Blanchard and Finster 1933, Pough 1970, Brown and Parker 1976). In contrast, the fluorescence of VIE helps reduce the obscuring effects of natural injuries and scars. Scale clipping and branding are performed in a numerical sequence (Blanchard and Finster 1933, Brown and Parker 1976, Winne et al. 2006) that can lead to recording errors, precludes using dual numbers (e.g., 44, 77, etc…) and adjacent numbers (e.g., 190s, 1,900s; Brown and Parker 1976), and may be impossible to determine if scars are lost without employing a second marking technique (Winne et al. 2006). However, during this research there were no problems observing marks or recording data.

Stresses incurred by scale clipping and branding could cause infection or mortality resulting in lower recapture probabilities and subsequent biases in population parameter estimates due to increased mortality (Spellerberg 1977, Winne et al. 2006). Parker (1974) reported winter weight loss and mortality increased in newly scale clipped striped whipsnakes (*Masticophis teaniatus*). Further, Davis and Ovaska (2001) noted that toe-clipped salamanders gained less weight and significantly fewer were recaptured than fluorescent marked salamanders. Interestingly, Kinkead et al. (2006) determined no difference in stress levels between clipping and fluorescent marking techniques in salamanders suggesting VIE may have similar effects on survival or recapture rates compared to clipping or branding.

In conclusion, VIE is a reliable alternative to traditional techniques for marking snakes (i.e., scale clipping and branding) due to its retention time and less invasive application. Further, the retention of all mark volumes suggested the usefulness of VIE in snakes of any size. However, problems were encountered with the technique. Pre-caudal marks did not have a significantly lower retention time, but accounted for 75% of marks lost. All mark loss occurred from 23 days to 310 days, and could be attributed to expulsion from the site of injection. Similarly, fragmentation of marks into several pieces could cause detection problems. The application of a liquid bandage product would likely deter expulsion, but further research is necessary.

I recommend future studies evaluate the use and efficacy of VIE in snakes, both in lab and field settings. Future studies should evaluate using VIE in different species of snakes with various ground colors and at various mark locations. Anecdotally, I can report that yellow, blue, and red VIE colors were detectable in corn snakes at all volumes, but blue VIE was difficult to detect in common kingsnakes due to the dark contrast. Mark volume should be studied in the field to better understand mark retention under natural conditions and field and laboratory research should focus on survival and recapture rates for VIE over longer periods. Also, future research should evaluate the effects of growth on mark detectability and compare stress levels incurred by traditional and VIE marking techniques.

Management Implications

Equipment costs for VIE kits ($465 USD) were higher than equipment costs for either scale clipping or branding. Clipping requires only scissors and branding requires cautery units that can be purchased for $20 - $25. However, VIE marking costs per snake were small (i.e., ~$0.10 - $0.29 for marks of 1 – 3 μl). The ability to apply low volume (i.e., ≤1μl) VIE marks is an important element in snake research. Reliable marks can be made *in situ* with the small syringe and needle provided by NMT, precluding the purchase of specialized syringes and needles, as used in this study. Further, small marks can be applied to small-bodied species and individuals (≤26 cm), which may be too small for PIT tags or scale clipping (Spellerberg 1977).

Acknowledgments

I acknowledge my sources of support and funding: the North Carolina State University Department of Forestry and Environmental Resources and the North Carolina State University Fisheries and Wildlife Sciences Program. Also, I would like to thank J. W. Tomberlin, T. J. Langer, R. A. Lancia, and C. Greenberg for comments on earlier drafts of the manuscript.

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Table 3.1. Retention times of visible implant fluorescent elastomer (VIE) mark volumes.

Volume	% Retained	Mean ± SD
Low	94.4	354 ± 66
Medium	83.3	333 ± 98
High	100	370

MANAGEMENT IMPLICATIONS

The similarity of species compositions and even species distributions among the five designated habitats at Bull Neck Swamp indicated that silvicultural regimes, demonstration practices, and site preparation techniques should not adversely affect the reptile and amphibian community. However, because more species were detected in the Manageable area land-use practices could have an effect on species assemblages. Therefore, management decisions require careful consideration before implementation. Further, nestedness calculation determined some species within the Manageable area were more tenuous and vulnerable to land-use practices.

I recommend land-use planning at Bull Neck Swamp consider small-scale practices where regimes have already been implemented. Clearcuts on Hufton and Overbay roads should provide sufficient area to conduct experiments for site preparations or plantings of loblolly pine (*Pinus taeda*) or Atlantic white-cedar (*Chamaecyparis thyoides*). Small-sized, circular-area practices could reduce major land-use effects and patchiness while encouraging habitat heterogeneity. Moreover, land-use practices confined to these areas would protect more vulnerable and sedentary species detected in similar microhabitats of the Manageable area, such as Atlantic Coast Slimy Salamanders (*Plethodon chlorobryonis*). For instance, *P. chlorobryonis* is not a disturbance-resistant species and requires dry, forested areas with sufficient leaf litter or coarse woody debris for cover and reproduction. Therefore, remaining areas of dry forest should be maintained to provide habitat for the species, reducing the danger of extirpation.