Final Report
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Conservation of South Carolina Coastal Plain Reptiles and Amphibians

Submitted By:

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Need and Approach:
South Carolina is home to 143 species of reptiles and amphibians, making it exceptionally diverse for its size. Excluding the sea turtles, 11 reptile and amphibian species are listed as threatened or endangered by the State of South Carolina and 48 are identified in the 2015 revision of the State Wildlife Action Plan. Many of these species are also included as species petitioned for protection under the Endangered Species Act (ESA) or are candidate species. The South Carolina Department of Natural Resources (SCDNR) has made reptile and amphibian conservation a priority and initiated studies on many imperiled taxa (Eastern Diamondback Rattlesnake, Pine Barrens Tree Frog, Flatwoods Salamander, Gopher Frog, and Gopher Tortoise). Though SCDNR has maintained an active herpetological research and survey program, several species are in need of additional survey and monitoring and additional research into their conservation biology. Much of our understanding of species distributions via element occurrences reflects survey effort at specific locations and not equal effort across the range of potential habitat. We conducted 4 individual projects, described below, focused on at-risk species in the Coastal Plain of South Carolina.

Amphibians of isolated wetlands

Objectives: Isolated freshwater wetlands in the coastal plain of South Carolina are home to a unique suite of amphibians that rely on these landscape features for breeding. Several of the most imperiled southeastern amphibians including state and federally listed Frosted Flatwoods Salamander (*Ambystoma cingulatum*), and state endangered Carolina Gopher Frogs (*Lithobates capito*) are only reliably detected during breeding activity at these wetlands through calling or larval surveys. Seasonal variation in rainfall and climatic patterns makes detection difficult and highlights the need for survey efforts across longer time scales to account for years when breeding conditions are not met. Additionally, isolated freshwater wetlands receive no protection through the Clean Water Act or at the state level and are easily lost to development and/or degraded through land conversion, draining, and fire suppression. Historically, most survey and monitoring efforts have focused exclusively on public lands with historic records

(Francis Marion National Forest, Santee Coastal Reserve, Savannah River Site, and Webb Wildlife Center). Over the past 3 years, we have included surveys at several additional public and private lands. In 2014, we detected a calling male gopher frog at Bonneau Ferry, indicating that this species may occur in other undocumented or under-surveyed locations. An unusual rain event in late 2015 prompted a large breeding event for gopher frogs at Francis Marion National Forest (NF), outside of the normal breeding season, highlighting the need to monitor this species at a wider temporal scale. Under a previous State Wildlife Grant (SWG), T-57-R, we deployed automated recorders extensively and developed an acoustic recognizer to help speed data processing and analysis that is an effective tool in identifying chorus activity in large datasets.

We focused surveys for Gopher Frogs, Tiger Salamanders, Flatwoods Salamander, and Dwarf Sirens; all of these are species identified in the 2015 State Wildlife Action Plan (SWAP) Supplemental Volume under the Pond Breeding Amphibians Guild. In this project, we continued to monitor historic ponds and populations, survey public lands for additional breeding ponds, and expand survey efforts to private landowners as permission dictated. We extended survey efforts beyond properties covered in past projects. Information of this sort is imperative for understanding the breeding biology and chronology of these species and will help us to understand their distribution in South Carolina.

Methods: We selected isolated ephemeral wetlands on SCDNR, Federal, and private lands based on appropriate habitat characteristics and historic records for Carolina Gopher Frog (*Lithobates capito*), Frosted Flatwoods Salamander (*Ambystoma cingulatum*), Tiger Salamander (*Ambystoma tigrimum*), and Dwarf Siren (*Pseudobranchus striatus*). Ponds were identified utilizing historic and current aerial imagery, LiDAR, visual surveys of properties, and local knowledge of isolated wetland features. During the 2016-2020 report period, we focused our survey efforts at Francis Marion NF, Donnelly Wildlife Management Area (WMA), Santee Coastal Reserve WMA, Bonneau Ferry WMA, Cathedral Bay Heritage Preserve (HP), Yawkey Wildlife Center, a private plantation in Berkeley County, Poinsett Electronic Combat Range (Shaw Air Force Base), Victoria Bluff HP, Private land owned by the BP Corporation, Tillman Sand Ridge HP, Lynchburg Savannah HP, Longleaf Pine HP, Crackerneck WMA, Palachucola WMA, Webb Wildlife Center, Hamilton Ridge WMA, a private plantation in Cordesville, SC and a private plantation in Allendale County (Figure 1).

We established coverboard transects comprised of 2'x2' plywood sheeting at Donnelly WMA, Santee Coastal Reserve WMA, Bonneau Ferry WMA, Cathedral Bay HP, Yawkey Wildlife Center, and Tillman Sand Ridge HP. We periodically surveyed ponds at all properties using dipnet surveys and aquatic funnel traps baited with glow sticks (Bennett et al. 2011). In 2016-2020, SongMeter SM2+ and SM4 automated recording units were deployed at Bonneau Ferry WMA, Hoover Plantation, Poinsett Electronic Combat Range, a private plantation in Allendale County, and a private plantation in Berkeley county, in addition to monitored ponds at: Webb Wildlife Center, Hamilton Ridge WMA, Palachucola WMA and all historic ponds within the

Heritage database (Savannah River Site, Crackerneck WMA, Francis Marion NF, and Santee Coastal Reserve WMA). We also conducted Gopher Frog egg mass surveys in Francis Marion NF and Santee Coastal Reserve WMA after suspected breeding events. All automated recordings were processed using an acoustic recognizer developed in program RAVEN.

Accomplishments: We detected no Flatwood Salamanders or Dwarf Sirens during all survey activities at any site. We observed Carolina Gopher Frogs (breeding, choruses, and egg masses) at Francis Marion NF and the Savannah River Site each year of the survey (2016, 2017, 2018, 2019, 2020). Tiger Salamanders were detected at the Savannah River Site (David Scott pers. comm.), Crackerneck WMA (2016, 2017), near the Turtle Survival Center in Cross, SC (Cris Hagen, pers. Comm., 2017, 2018) and at a private plantation in Allendale County (2016, 2018-2020; Table 1; Figure 2).

Significant Deviations: None

References:

Bennett, S., J. Waldron, and S. Welch. 2011. Light bait improves capture success of aquatic funnel-trap sampling for larval amphibians. Southeastern Naturalist. 11:49-58

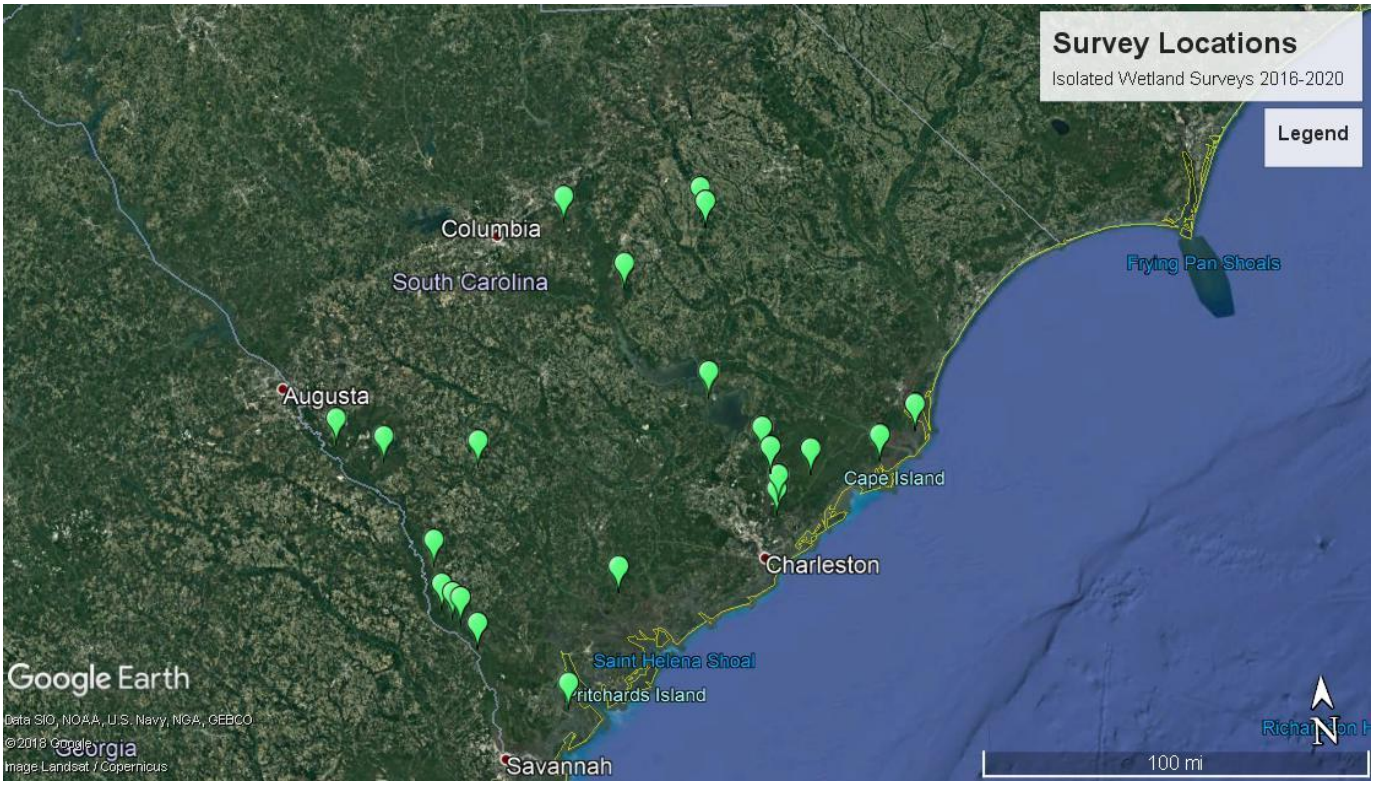

Figure 1. Isolated Wetland Survey Locations for Winter Breeding Amphibians 2016-2020.

Table 1. Observations of Wetland Breeding Amphibians by Year

Species	**2016**	**2017**	**2018**	**2019**	**2020**
Frosted Flatwoods Salamander (*Ambystoma cingulatum*)					
Dwarf Sirens (*Pseudobranchus striatus*)					
Gopher Frog (*Lithobates capito*)	X	X	X	X	X
Tiger Salamander (*Ambystoma tigrinum*)	X	X	X	X	X

Surveys for all species were conducted in all years

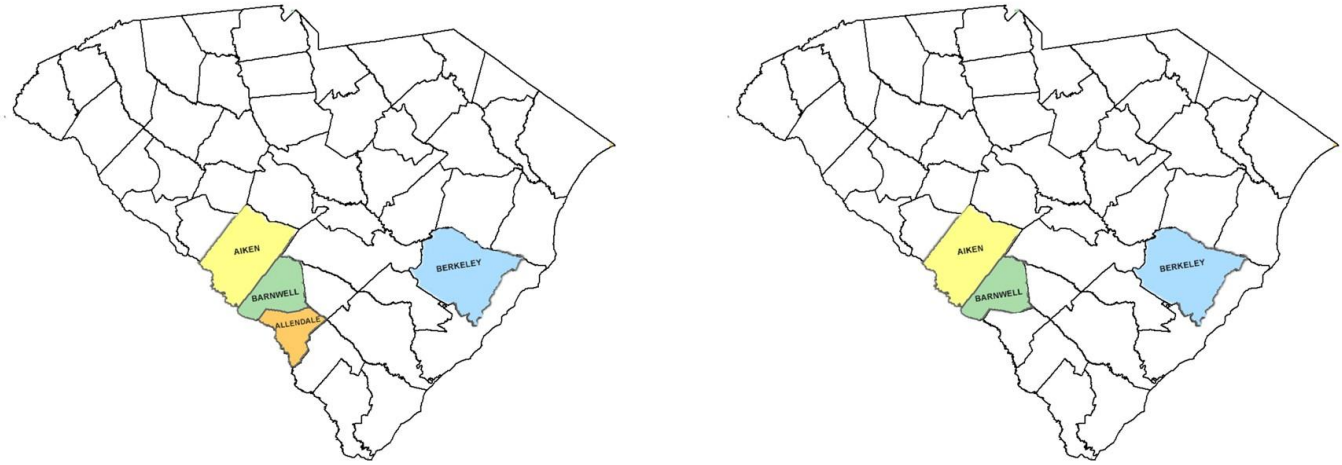

Figure 2. Counties where presence of *Ambystoma tigrinum* (left) and *Lithobates capito* (right) were detected during wetland surveys 2016-2020.

Pine Snake and Southern Hognose Snake Surveys

Objectives: Pine Snakes and Southern Hognose Snakes are included in the Longleaf Pine Reptiles and Amphibian Guild in SCDNR's 2015 SWAP Supplemental Volume. These two species utilize similar habitats and are difficult subjects for survey efforts due to their fossorial habits and low detection probabilities. Southern Hognose Snakes are listed as threatened in South Carolina, and both species have been petitioned for protection under the ESA. Little information on the current distribution and updated presence records at historic localities exists. Most information on these species is restricted to opportunistic observation with little systematic survey performed in the last decade. As the species often co-occur, it is possible to maximize survey effort by including both species in searches. Range maps indicate a vast area of co-occurrence on the coastal plain, but element occurrence (EO) records for each species are limited.

We conducted surveys of public and private lands (as permission dictated) using active searches, artificial cover, driving surveys, and a novel camera trap (Martin et.al. 2017). Sites for survey were selected based on appropriate habitat, and efforts to confirm historic element occurrences were given preference.

Methods: We compiled historic occurrence data from the SCDNR Heritage Database, Carolina Herp Atlas, and Herp Mapper for Pine Snakes (*Pituophis melanoleucus*) and Southern Hognose Snakes (*Heterodon simus*). We identified SCDNR properties, other public properties, and private properties to which we have access that had records for both species. Preference was given initially to properties with records for both species, but without verifications within the last 10 years. Secondary priority was given to properties with records for one of the species, with nearby records for the other. Lastly, we identified properties with suitable habitat for both species (see Figure 1) but lacked confirmation records.

We identified 5 initial properties to target survey efforts and to evaluate survey techniques. Tillman Sand Ridge HP, Aiken Gopher Tortoise HP, the Slater Tract, Carolina Sandhills National Wildlife Refuge (NWR), and Lewis Ocean Bay HP were selected as the primary sites. Secondary sites were selected based on appropriate habitat and to evaluate the techniques described below in areas where records do not exist. Secondary properties included Yawkey Wildlife Center and Hamilton Ridge WMA.

We utilized a variety of techniques—both traditional and new—in order to best detect these species if/when present. We used road survey routes to encounter moving snakes in the general areas of our selected sites, artificial cover, active searching, and AHDRifT camera traps (Martin et. al. 2017). For these sites, we established a minimum of one camera trap, an artificial cover site, conducted active searches when onsite, and conducted road survey routes when conditions were favorable.

We installed a total of 14 camera trap drift fences using the AHDRifT methods (Martin et. al., 2017; Table 1). The fences were constructed with 24" aluminum flashing sunk approximately three inches into a hand-dug trench in the ground (3 inches wide by 3 inches deep). The fences were secured with steel rebar of approximately 36 inches in length, driven into the ground, and attached with wire strung through the flashing and tied onto these rebar posts. Rebar was attached at each end and every 10-15 feet as needed (alternating sides) for structural support. Trenches were filled with removed material back to original ground level. Fences were constructed between 35 feet and 50 feet in length. We modified 5-gallon buckets, per Martin et. al. 2017, and used plexiglass lids covered with shade cloth to cover the trap tops. We used Stealthcam® and Moultrie™ brand cameras set to record 3-shot bursts upon each trigger event with a 10-second delay between each trigger event before firing again. Some cameras (Moultrie™) were modified with duct tape covers on the flash bulbs to eliminate glare and washout in nighttime pictures. Each camera was loaded with 32g ScanDisk® SD cards and downloaded periodically. Photos were examined individually, and all photos where a reptile or amphibian triggered the camera were recorded. All herps were identified to species, unless distinguishing characters were obscured. In those cases, we identified each herp to the most specific taxonomic unit possible. All other observed animals were recorded and identified when possible.

Accomplishments: We installed a total of 14 camera trap fences during the study period (Table 1). The fences at the Slater Tract and Carolina Sandhills NWR were removed in 2018 after consistently identifying Pine Snakes. Additionally, in 2018, we erected new camera trap drift fences at Marsh WMA, Woodbury WMA and a private property located in Aiken County. Due to extremely high water in the Pee Dee River system, resulting from Hurricanes Florence and Michael, the fences at Marsh and Woodbury WMAs were destroyed in the fall of 2018 but rebuilt in the spring of 2019. On Marsh WMA, we constructed an additional two fences in 2018 post-hurricanes. We established road cruising routes to be used on several properties and public roadways in areas of good habitat to encounter migrating reptiles and amphibians.

In total we detected 29 Pine Snakes and 10 Southern Hognose Snakes from 2017-2020. Of the 29 Pine Snakes, 16 were detected with camera traps, 6 during driving surveys, and 7 records that were either detected during other survey work or reported to SCDNR (Table 2). The camera traps in the Francis Marion NF were part of a separate study conducted by the Amphibian and Reptile Conservancy. We detected Southern Hognose Snakes 3 times during this survey. One was found dead on the road during a road cruising survey near the Slater Tract; one was found dead on the road in route to Aiken Gopher Tortoise HP; and one found alive on the road during a road cruising survey in Allendale County, SC. Additionally, we received reports of seven additional Southern Hognose snakes: two at the Slater Tract (K. Bulmann, pers. comm. 2017) and five were detected during road cruising surveys at Francis Marion NF (J. Holmes, pers. comm. 2017(1), 2018(3) and 2019 (1)).

In total, 182,587 individual images were collected and processed from 2017-2020. From those we detected 2,262 reptiles and amphibians consisting of at least 35 species (Table 3). We detected Pine Snakes 16 times at five properties: Aiken Gopher Tortoise HP, Tillman Sand Ridge HP, the Tract, Carolina Sandhills NWR, and the Francis Marion NF during a separate study (Table 2). No Southern Hognose Snakes were detected using the camera traps. Artificial covers were checked 206 times during this project consisting of 1,382 individual flips resulting in 108 individual reptile and amphibian detections of 11 species (Table 4). No Pine Snakes or Southern Hognose Snakes were identified under coverboard arrays including those coverboards that were paired with a camera fence that successfully detected Pine Snakes. 1,087 miles of driving surveys were conducted during this project resulting in the detection of 22 reptile and amphibian species (Table 5). Overall, camera traps have been far more effective in detecting Pine Snakes than any other method and have detected a higher diversity of species than other techniques. Tillman Sand Ridge HP had the highest observed Shannon Diversity of all surveyed sites (Table 6) for camera trap data.

Significant Deviations: None

References:

Martin, S., R. M. Rautsaw, F. Robb, M. R. Bolt, C. L. Parkinson, R. A. Seigel. 2017. Set AHDriFT: applying game Cameras to Drift Fences for Surveying Herpetofauna and Small Mammals. Wildlife Society Bulletin. DOI: 10.1002/wsb.805

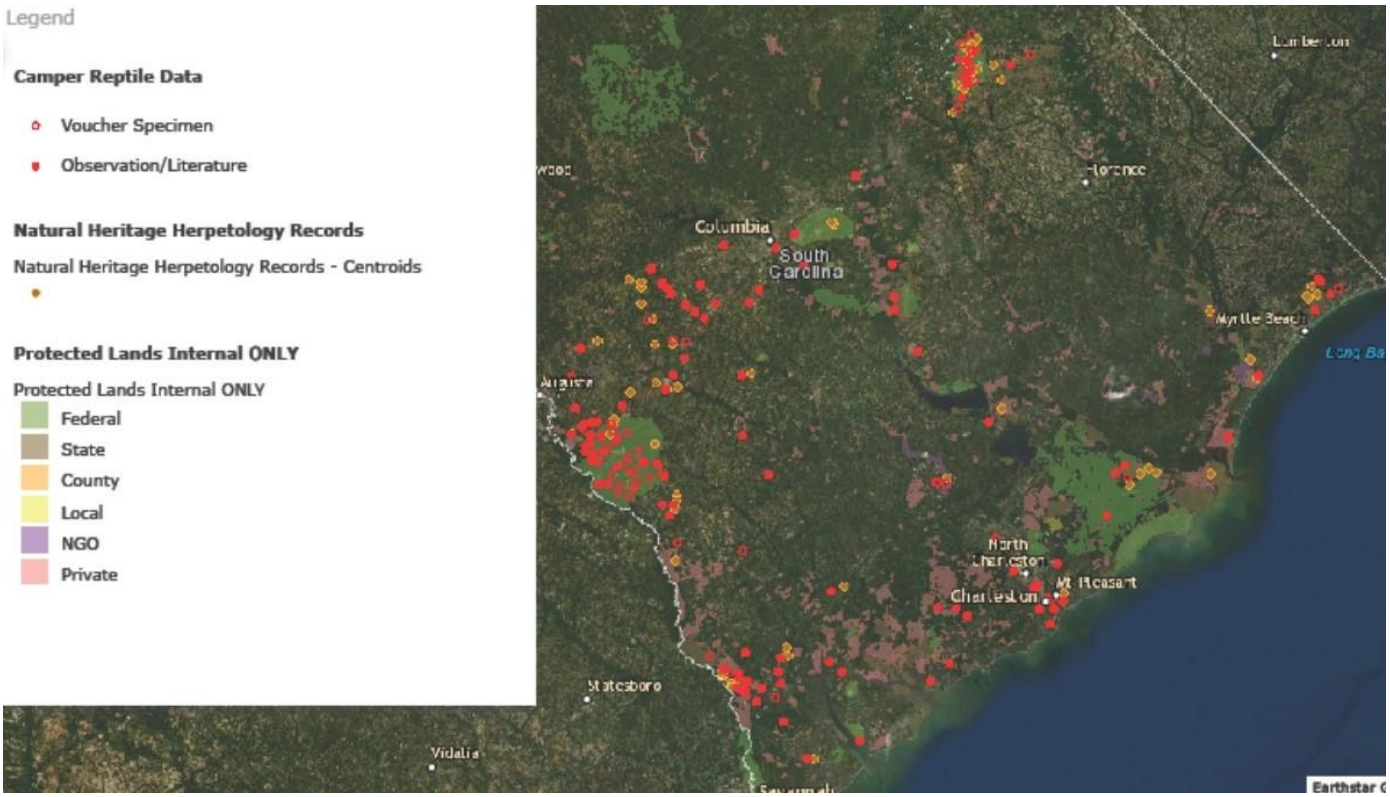

Figure 1: Historic Records and Survey Locations

Table 1. Location of Camera Trap Fences, Time Frame Fences were Surveying, Number of Fences Present and Detection of Target Species.

Property	Year Erected	Year Removed	Number fences	*P. melanoleucus* detected?	*H. simus* detected?
Tillman Sand Ridge HP	2017	N/A	1	Y	N
Aiken Gopher Tortoise HP	2017	N/A	2	Y	N
Slater Tract	2017	2018	1	Y	N
Carolina Sandhills NWR	2017	2018	1	Y	N
Lewis Ocean Bay HP	2017	N/A	2	N	N
Hamilton Ridge WMA	2017	N/A	1	N	N
Yawkey Wildlife Center	2017	2019	1	N	N
Marsh WMA	2018	N/A	3	N	N
Private Property Aiken Co.	2018	N/A	1	N	N
Woodbury WMA	2018	N/A	1	N	N

Table 2. Properties with Pine Snake Detections, Number of Detections and Methods Used.

Property	Camera Fence	Road Surveys	Other
Tillman Sand Ridge HP	3	1	1
Aiken Gopher Tortoise HP	2	1	1
Slater Tract	7		
Carolina Sandhills NWR	3		
Lewis Ocean Bay HP		3	1
Hamilton Ridge WMA			
Yawkey Wildlife Center			
Marsh WMA			
Private Property Aiken Co.			
Woodbury WMA		1	
Francis Marion National Forest	1*		2
Other			2

*The camera traps in the Francis Marion NF were not part of this study.

Table 3. Species Detected in Camera Traps

Species	Scientific Name	Detections
Six-lined Racerunner	*Cnemidophorus sexlineatus*	925
Coachwhip	*Masticophis flagellum*	331
Black Racer	*Coluber constrictor*	310
Southern Toad	*Anaxyrus terrestris*	216
Unidentified Skink Species	*Eumeces sp.*	203
Broadhead Skink	*Eumeces laticeps*	34
Eastern Fence Lizard	*Sceloporus undulatus*	31
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	20
Ground skink	*Scincella lateralis*	17
Pine Snake	*Pituophis melanoleucus*	16
Unidentified Frog Species	*Lithobates sp.*	16
Gopher Tortoise	*Gopherus polyphemus*	14
Unidentified Snake Species		14
Eastern Spadefoot Toad	*Scaphiopus holbrooki*	13
Ratsnake	*Pantherophis obsoleta*	13
Oak Toad	*Anaxyrus quercicus*	12
Copperhead	*Agkistrodon contortrix*	9
Unidentified Glass Lizard Species	*Ophisaurus sp.*	8
Green Anole	*Anolis carolinensis*	9
Southern Leopard Frog	*Lithobates sphenocephalus*	7
Corn Snake	*Pantherophis guttata*	6
Eastern Garter Snake	*Thamnophis sirtalis*	6
Eastern Hognose	*Heterodon platirhinos*	5
Canebrake Rattlesnake	*Crotalus horridus*	4
Banded Watersnake	*Nerodia fasciata*	3
Eastern Box Turtle	*Terrapene carolina*	3
Yellow-bellied Slider	*Trachemys scripta*	3
Bullfrog	*Lithobates catesbeianus*	3
Dwarf Salamander	*Eurycea quadridigitata*	2
Eastern Mud Turtle	*Kinosternon subrubrum*	2
Eastern Ribbon Snake	*Thamnophis sauritis*	2
Eastern Glass Lizard	*Ophisaurus ventralis*	2
American Alligator	*Alligator mississippiensis*	1
Chicken Turtle	*Dierochelys reticularia*	1
Red spotted Newt	*Notophthalmus viridescens*	1

Table 4. Species Identified Under Artificial Cover

Species	Scientific Name	Detections
Skink Species	*Eumeces sp.*	34
Eastern Fence Lizard	*Sceloporus undulatus*	17
Ground Skink	*Scincella lateralis*	14
Coachwhip	*Masticophis flagellum*	14
Black Racer	*Coluber constrictor*	8
Six-lined Racerunner	*Cnemidophorus sexlineatus*	6
Copperhead	*Agkistrodon contortrix*	4
Corn Snake	*Pantherophis gutattus*	4
Dwarf Salamander	*Eurycea quadridigitata*	3
Green Anole	*Anolis carolinensis*	3
Southern Toad	*Bufo terrestris*	1

Table 5. Species Detected During Road Surveys

Species	Scientific Name
Pine Snake	*Pituophis melanoleucus*
Eastern Box Turtle	*Terrapene carolina*
Yellow-bellied Slider	*Trachemys scripta*
Six-lined Racerunner	*Cnemidophorus sexlineatus*
Eastern Hognose	*Heterodon platirhinos*
Black Racer	*Coluber constrictor*
Green Anole	*Anolis carolinensis*
Southern Toad	*Bufo terrestris*
Southern Leopard Frog	*Rana sphenocephala*
Eastern Garter Snake	*Thamnophis sirtalis*
Eastern Glass Lizard	*Ophiosaurus ventralis*
Banded Watersnake	*Nerodia fasciata*
Frog Species	*Rana sp*
Treefrog Species	*Hyla sp.*
Ratsnake	*Pantherophis obsoleta*
Gopher Tortoise	*Gopherus polyphemus*
Coachwhip	*Masticophis flagellum*
Southern Hognose	*Heterodon simus*
Copperhead	*Agkistrodon contortrix*
Rough Green Snake	*Opheodrys aestivus*
Pygmy Rattlesnake	*Sistrurus miliarius*
Canebrake Rattlesnake	*Crotalus horridus*

Table 6. Shannon Diversity and Species by Property for Camera Traps

Property	Tillman	Ham Ridge	Marsh Mule Pen	Marsh	LOB EDGE	Marsh LLP Stand	Woodbury	AGTHP2	Slater	CSNWR	Yawkey	LOB Quail	Whits	AGTHP 1
Shannon Diversity	1.80502128	1.685802093	1.734825879	1.555530456	1.541758824	1.427061043	1.353737048	1.285308858	1.257638954	1.209883134	1.177915012	1.127357789	0.997180402	0.654413684
P. melanoleucus	x							x	x	x				x
B. quercicus	x				x						x			
B. terrestris	x		x	x	x		x	x			x	x		x
H. platirhinos					x			x						x
C. sexlineatus	x	x	x	x	x		x	x	x	x	x	x	x	x
C. constrictor	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P. gutatta	x	x						x						
E. sp	x	x			x	x	x	x			x	x		x
G. carolinensis	x	x	x	x					x					
G. polyphemus	x													x
T. scripta				x										
Lithobates sp.	x										x			x
L. sphenocephala	x			x										
M. flagellum	x	x	x	x	x	x	x	x	x	x		x	x	x
S. undulatus	x									x				x
A. carolinensis			x		x			x			x	x		
S. lateralis	x				x		x	x				x		
N. viridescens		x												
O. ventralis		x												
T. sirtalis		x			x									
T. sauritis											x			
P. obsoleta		x				x	x					x		
S. holbrooki			x	x										
A. mississippiensis											x			
N. fasciata					x									
C. horridus						x			x					
A. contortrix												x		
T. carolina					x									
Unk Frog					x									

Gopher Tortoise: Survivorship and movements of juveniles at Aiken Gopher Tortoise HP

Objectives: The Aiken Gopher Tortoise Heritage Preserve (AGTHP) in SC was established in 1995 to protect Gopher Tortoises at the northern-most extent of their historic range. From an original small population (n=<12), the population has been supplemented through the use of released "waifs"—tortoises from unknown origins, former captives, etc. To date, more than 300 tortoises have been released on site, and of these, approximately 160 were adult tortoises. The property is managed specifically for the purpose of attaining a minimum viable population (MVP) of tortoises of at least 250 adult tortoises (The Gopher Tortoise Council 2013). Evidence of reproduction has been observed (eggs and eggshell fragments) on the site; however, the degree to which these individuals contribute to the population is unknown, and hatchling/juveniles are rarely observed. This is particularly critical given that juveniles are far more prone to hazards and predation than adults. In order to meet the MVP criteria, reproduction and recruitment must occur within a population. At this time, 20 years since the Preserve's inception, we do not know about the survival and movements of juvenile tortoises at this site, and we have not observed the numbers of juvenile age classes expected in a population of this size and observed at other 'natural" populations in well-managed habitat.

We planned to utilize radio telemetry to follow the survivorship and movements of at least 15 tortoises during the project period. We were to collect eggs from nests at AGTHP and hatch them in the laboratory to provide the tortoises needed for this study.

Methods:

Egg Collection- We collected Gopher Tortoise eggs from nests at the AGTHP in the summers of 2015, 2016, 2017 and 2018 to provide juvenile tortoises for this project. We searched for eggs by excavating sand in and around the entrance of each burrow (Smith et al. 2005) and used hand spades to carefully remove the first 10 to 15 cm of soil in layers. Then, using our hands, we continued to remove further soil up to approximately 30 cm in depth. We excavated into the burrow tunnel as far as possible and out into the apron about one meter from the entrance. For the 2015 and 2016 collections, eggs were collected upon discovery and placed in plastic containers filled with sand from the burrow. The 2015 and 2016 eggs were removed immediately and taken to Savannah River Ecology Lab (SREL) for incubation and hatching. In 2017 and 2018, we began nest searching earlier and protected eggs in place to be removed after the majority of incubation had occurred. Nests located in 2017 and 2018, were marked with flagging tape in the nest chamber to help find the eggs upon final excavation. We covered the nest with a layer of sand, and hardware cloth of an appropriate size was placed over each nest and staked into place with landscaping stakes. The remaining soil was replaced covering the nest and all parts of the hardware cloth. Care was taken to maintain the original depth of soil in the nest chamber, and we recreated proper floor depth within the burrow.

Eggs were carefully excavated between July and September of each collection year, before the eggs hatched (Landers et al. 1980), and taken to SREL to complete incubation. Upon removal, the top of each egg was marked with a soft-tipped pencil to ensure each egg stayed upright; eggs are sensitive to rotation and movement (Bustard 1972). Each clutch was maintained in a separate container in the field. At SREL, each clutch was placed in a separate Sterlite® container in a moist perlite mixture (1:1 water: perlite mass ratio) and incubated at 30°C based on previous studies (Burke et al. 1996; Demuth 2001). Eggs were checked daily for hatching, and hatchlings were placed into individual bins to allow them to absorb their yolks.

Rearing and Headstarting- 2015, 2016, 2017 and 2018 tortoises were reared for one year at SREL in small groups. Each group was housed in a large Rubbermaid® Stock Tank with lights for basking and nighttime heat. They were kept on a sand substrate and had a halved corrugated drainage pipe for cover. Tortoises were fed a mix of greens and commercial tortoise diet throughout each week and allowed to eat freely. In 2016, the remaining 2015 cohort that was not released was transferred to Riverbanks Zoo and reared in a similar manner for an additional year. Hatchlings from the 2016 cohort were also reared at Riverbanks zoo for one year prior to release.

Release and Radio Telemetry- In October 2016, we released the first cohort of tortoises to be followed via radio telemetry. We released a group of five 2016 hatchling tortoises and twenty 2015 one-year-old headstarted tortoises. A second cohort of tortoises was released in late September and early October 2017. This group contained fifteen 2017 hatchling tortoises, seven one-year-old headstarted tortoises, and eight two-year-old headstarted tortoises. In late September and early October 2018, we released eleven two-year-old headstarted tortoises from the 2016 cohort that was transferred to Riverbanks Zoo. Additionally, fifteen one-year-old headstarted tortoises were released into pen 12, but only five had transmitters attached and were tracked weekly. In September-October 2019, we released seven two-year old headstarted tortoises as well as 44 one-year-old headstarted tortoises (Table 1). In 2016, each hatchling was randomly paired to a one-year-old headstart and released at an adult burrow. In 2017, each hatchling was randomly paired to either a one-year-old headstarted tortoise or a two-year-old headstarted tortoise and released to an adult burrow. In 2018, each tortoise was released at an adult burrow. In 2019, headstarted tortoises were released at both juvenile starter burrows in pen 13 and at the entrance of adult burrows on the property. Hatchling tortoises were outfitted with Advanced Telemetry Systems R1655 Glue-on transmitters (1.2g) and attached with Permatex® 5-minute epoxy, and the one- and two-year-old headstarted tortoises were outfitted with Advanced Telemetry Systems R1680 Glue-on transmitters (3.6g) attached with JB Weld® WaterWeld Epoxy Putty. Transmitters were attached to the rear of the plastron on the penultimate vertebral scute, and total weights of the transmitter package were kept to less than 10% of the total body weight of the tortoise.

We conducted radio telemetry surveys on annual cohorts of juvenile Gopher Tortoises during 2016-2019. We radio located tortoises twice weekly until hibernation occurred and movements stopped, approximately the first 12 weeks after their release. We made every attempt to visually identify tortoises upon each radio location to accurately record predation events. We attempted to maintain a 1-3 m distance from telemetry-equipped tortoises to minimize disturbance. If a tortoise could not be visually located, we used a small handheld metal detector swept gently above the suspect area to identify the tortoise. We recorded coordinates using a Garmin^{TM} hand-held GPS (model: GPSMap 62s) accurate to within 4 meters. When a tortoise established a new burrow, we labeled the burrow with an individual metal tag to the right of each burrow mouth. We recorded new locations if the tortoise moved more than 4 meters (i.e. the accuracy of our GPS). If a tortoise's transmitter began to fail, we captured the animal if it was on the surface, or when feasible, trapped the individual and then re-attached a new transmitter.
We noted predation events and attempted to determine the cause of death. If we suspected predation, we considered the recovery of any part of the tortoise, the presence of visible wounds and the distance between the recovery location and the previous radio location. We would assume mammal predation if we found tooth marks on the recovered tortoise. Starvation was assumed when the tortoise had no external damage indicative of a predation event.

Data analysis **-** We used known fate models in Program Mark (White and Burnham 1999) to calculate survival throughout this study for hatchling, one year and two year headstarted tortoises released since 2016.

Accomplishments: During the course of this project (2016-2020), we released a total of 132 gopher tortoises, 71 of which were equipped with transmitters and tracked using radio telemetry to document movements and survival. Of the 132 individuals, 20 were hatchlings, 86 were one-year-old headstarts, and 26 were two-year-old headstarts (Table 1). The average mass of radio tracked Gopher Tortoises prior to transmitter attachment was 29.6 g for hatchlings (n=20, min=21.5g, max=33.7g), 136.5 g for one-year-old headstarts (n=30, min=54g, max=364g) and 357.5 g for two-year-old headstarts (n=21, min=150g, max=622g).

We used data collected from 71 juvenile Gopher Tortoises that were monitored over the course of 3.5 years. Our preliminary analyses showed that estimated overall survival was 19% (SE: 0.058, 95% CI: 0.102 to 0.329). Estimated hatchling survival was 9% (SE: 0.081, 95% CI: 0.013 to 0.411), one-year headstart survival was 11% (SE: 0.061, 95% CI: 0.036 to 0.298) and two-year headstart survival was 41% (SE: 0.138, 95% CI: 0.183 to 0.679). Overall, 23 individuals (32%) of all age classes survived the first year after release and of those, seven individuals (10%) survived for two years following release. All seven of these individuals were lost due to transmitter failure and are presumed to be alive. Of these seven individuals, five were two-year-old headstarts and two were one-year-old headstarts. Of all age classes, 30 individuals (42%) were known to have died; the other 41 were right censored and lost due to transmitter failure throughout the study. For hatchlings, only seven (35%) of the 20 hatchlings were observed dead,

five in the same month they were released, while the other 13 were eventually lost to transmitter failure. Two (10%) individuals survived the longest—14 months—prior to their transmitter failing (Table 2). For one-year headstarts, 16 individuals (53%) were observed dead, while the other 14 were eventually lost due to transmitter failure. Two individuals (6.6%) survived the longest—26 months—prior to their transmitter failing (Table 2). For two-year headstarts, seven individuals (33%) were observed dead while the other 14 were eventually lost due to transmitter failure. There were five two-year-old headstarts that survived 30 months before their transmitters finally failed, and unfortunately, we were not able to capture those individuals and replace their transmitters. However, they were last observed alive and in their burrows at the end of March and are presumed alive. Fates of tracked individuals can be seen in Table 2 below.

Significant Deviations: None

References:

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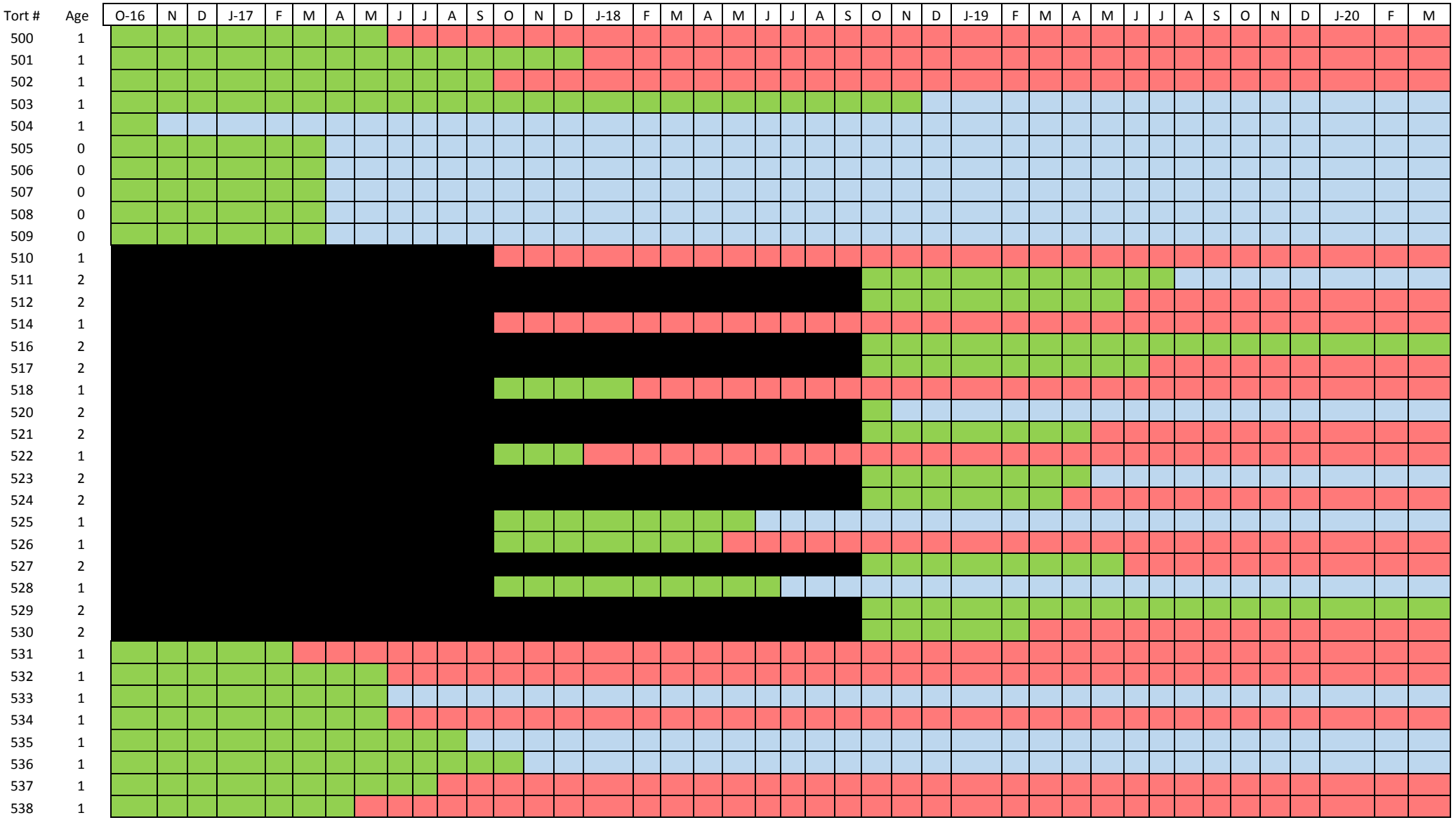
Table 1. Tortoises released at the AGTHP as part of the survivorship study

Year	Hatchlings	Yearlings	Two-year olds	Total
2016-17	5	20		25
2017-18	15	7	8	30
2018-19		59	18	77
Total	20	86	26	132

Table 2. Fates of all gopher tortoises tracked using radio telemetry. Green represents an individual that is actively being tracked, blue represents transmitter or attachment failure and red represents an individual is known to be deceased.



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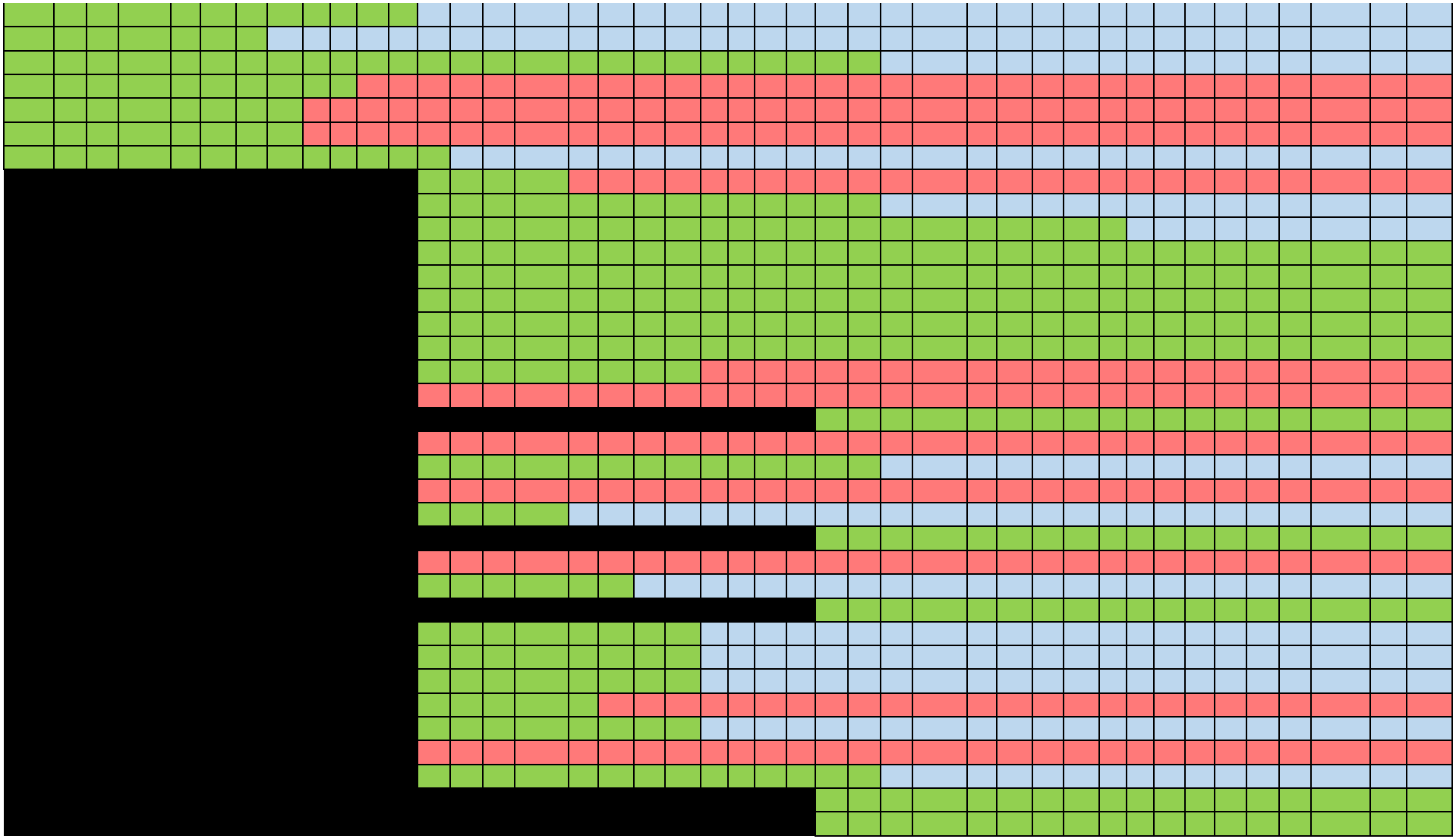
371	2



Reptile and Amphibian Response to Restoration and Land Use History

Objective 1: A subset of isolated freshwater wetlands on each of the three properties were to be selected based on index value and land-use history and sampled utilizing automated recording, dip-netting, and funnel trapping for amphibians. Upland areas associated with these wetlands were to receive coverboards to be sampled for EDBs.

Methods: We sampled herpetofauna at ponds and associated upland habitats (Fig. 1-3) on Hamilton Ridge, Webb, and Palachucola WMAs. We sampled for aquatic amphibians using dip nets and funnel traps baited with glowsticks. We trapped each pond annually (or when ponds held sufficient water for sampling) using 10 funnel traps baited with glowsticks. Ponds were sampled for at least two nights during spring. We used a dip net to sample larval amphibians when we pulled funnel traps from wetlands. Dip net surveys were conducted for 20 person minutes per wetland.

We used digital audio recorders to sample breeding anurans yearly between January and June. In addition to spring/early summer sampling, we used digital audio recorders to sample anurans at historic and potential Carolina Gopher Frog breeding locations in the fall (i.e. September – December) during rain events. We focused our fall gopher frog sampling efforts on four ponds: Peanut Pond, Mike's Gopher Pond, Big Gopher Pond, and Jay's Bay), but we included high integrity ponds on Hamilton Ridge WMA (Gin Jug Pond) and Palachucola WMA (Polk Borrow Pit, Big Parker Bay) in our acoustic recognizer analysis. We used an acoustic recognizer (Table 1) in Raven 1.4 to identify gopher frog calls that were recorded by audio recorders (Wildlife Acoustic Models SM2, SM4). We programmed the recorders to record in five-minute intervals every hour from 17:00 to 9:00 hours daily but restricted our analysis to nocturnal intervals (i.e. 21:00-5:00). We periodically visited sampled locations to download digital data and replace audio recorder batteries. We deployed recorders when ponds held water, i.e., we did not deploy acoustic data loggers in dry ponds, and thus some ponds were not sampled in all years.

We placed coverboard arrays at seven sites/ponds on Hamilton Ridge WMA (Gin Jug Pond, Hate Pond, HR Avenue Pond, Gum Pond, Gregory Arrowhead Pond, Palmetto Pond, and Middle Pond), eight sites/ponds on Palachucola WMA (Big Parker Bay, Jones Pond, Quail Road Pond, Polk Borrow Pit, Campground Pond, Stuckey Pond, and Woman's Dam Pond), and nine ponds on Webb WMA (Pignut Mud Turtle Pond, Jay's Bay, Webb's Last Chance, Wade's Bay, Upper Avenue Pond, Peanut Pond, Back Woods O'Henry Pond, Quail Straddle Pond, and Fig Tree Pond). We deployed 10-20 cover boards in upland terrestrial habitats within 300 m of long-term monitoring ponds. We focused cover board sampling during spring emergence (February – April), ensuring we sampled each site at least three times annually between February and November. We were unable to analyze acoustic data collected during Spring 2020 due to travel restrictions associated with SARS Covid19.

We measured and marked snakes and turtles upon capture. We processed turtles by measuring carapace length (CL), carapace width (CW), and plastron length (PL) using a pair of calipers. We weighed turtles using a digital scale or a Pesola hanging scale. Each turtle was given a unique identification number via notching of marginal scutes.

We recorded snake snout-vent length (SVL), total length (TL), and mass. We determined sex using cloacal probes. We marked all snake species using scale cauterization. Eastern diamondback rattlesnakes and canebrake rattlesnakes were given PIT tags in addition to scale cautery marking.

Accomplishments: We failed to detect Carolina Gopher Frog choruses using any of the methods employed in this study. We detected 25 amphibian species and 21 reptile species (Table 2).

Table 1. Parameters used in the Carolina Gopher Frog (*Rana capito*) recognizer.

Parameter	Value
Minimum Frequency	160 Hz
Maximum Frequency	1100 Hz
Minimum Duration	0.704 s
Maximum Duration	7 s
Minimum Separation	0.08 s
Minimum Occupancy	5.0 %
SNR Threshold	8.0 dB
Block Size	4 s
Hop Size	1 s
Percentile	70 %

Table 2. Species richness estimates, by pond, using acoustic anuran surveys, dipnetting, minnow trapping, and coverboard surveys and long-term monitoring plots, based on data collected and analyzed between 2016 and 2020. Property = W (Webb), HR (Hamilton Ridge), and PWMA (Palachucola).

Property	Pond/Site	Reptiles[1]	Amphibians[2]	Richness
W	Back Woods O'Henry	Ac, Ca, Cc, Ch, Lt, Mf, Pob, Tsi*	Ac/Ag, At, Amo, Amm, Hc, Hch, Hf, Hg, Pb, Pc, Pn, Po, Poc, Rc, Rcl, Rh, Rs, Sh	26
W	Big Gopher Pond		Ame, Amo, Amt, Aq, At, Hch, Hf, Pb, Pc, Pn, Po, Poc, Rs, Rh, Sh	15
W	Fig Tree House	Ac, Ca, Ch, Cc, Lt, Nf, Pob, Pg, Tc, Tsi	Ac/Ag, Amo, Aq, Pc, Pn, Po, Poc, Rs	18
W	Grassy Chicken Bay	Ca*, Cc, Dr*, Ks, Pg, Vs, Lt	Amt, Amo, Amm, Aq, At, Pb, Pc, Pn, Po, Poc, Ac/Ag, Rs, Hc, Hch, Hf, Hg, Hs, Sh	25
W	Jay's Bay	Cc, Ks, Tc, Tsi*	Amm, Pb, Pn, Po, Poc, Ac/Ag, Rs, Hf, Hs, Hg, At	15
W	Peanut Pond	Ac*, Ca*, Ch*, Cc, Hp, Lt, Pg, Pob	Amo, Amm, At, Aq, Pn, Pc, Po, Poc, Hf, Hc, Hch, Rs	20

W	Pignut Mud Turtle	Ac, Cc, Ch, Dp, Hp, Ks, Lt, Pg, Pob, Tsi	Amo, Hc, Hch, Nv, Pb, Pc, Pn, Rs, Sm	19
W	Quail Straddle	Ac, Ca, Cc, Ch, Hp, Lg, Lt, Mf, Pg, Tc	Amo, At, Aq, Pb, Pn, Pc, Rs	17
W	Upper Avenue	Ca, Cc, Ch, Ks, Mf, Lg, Lt, Pob, Tc*	Amm, Amo, Amt, Aq, Pc, Pn, Po, Poc, Rs	18
W	Wade's Bay	Cc	Amm Amo, Amt, Aq, Pc, Pn, Rs	8
W	Webb's Big Pond	Ap, Cc	Ac/Ag, Amo, Amma, At, Aq, Hf, Hc, Hch, Hg, Hs, Pb, Pc, Pn, Po, Poc, Rs, Sh	19
W	Webb's Last Chance	Cc, Hp, Ks, Pg	Amm, Aq, Rs, Pc, Pn	8
W	Mike's Gopher Pond	Ca, Ch, Tsi	Ac/Ag, Amm, Amo, Aq, At, Gc, Hch, Hf, Hg, Pc, Po, Poc, Pn, Rs, Sh	18
HR	Boring Pond		Gc, Hch, Hf, Pb, Pc, Pn, Rs	7
HR	Smith/Dead Turtle	Ks	Pb, Pc	3
HR	Four House		Ac/Ag, Amo, Pc, Pn, Rs	5
HR	Gin Jug	Ca, Cc, Dr*, Pob, Tc	Ac/Ag, Amt, At, Gc, Hc, Hch, Hf, Pb, Pc, Pn, Po, Poc, Rc, Rcl, Rs, Sh	21
HR	Gregory Arrowhead	Am, Cc, Hp, Ks, Mf	Pc, Pn, Rc, Rs	9
HR	Gum	Hp	At, Pb, Pc, Pn, Rs	6
HR	Hate	Cc, Ks, Lg, Pg	Pb, Pc, Pn, Hch, Rs	9
HR	HR Avenue	Ac, Ks, Pob	Ac/Ag, Hch, Pb, Pc, Rs	8
HR	Jordan		Pc, Pn, Rs	3
HR	Middle		Pb, Pc, Pn, Rs	4
HR	Palmetto	Cc, Lg, Ks	Ac/Ag, At, Gc, Hc, Hch, Hf, Pb, Pc, Pn, Poc	13
HR	Timmerman		Pb, Pc, Pn, Hf, Rs	5
PWMA	119/321 Pond	Am	Pc, Pn, Po, Rc, Rs, Hc, Hg	8
PWMA	Big Parker Bay	Ac, Cc, Hp	Ac/Ag, At, Gc, Hf, Pb, Pc, Pn, Po, Poc, Rcl, Rs	14
PWMA	Big Polk Bay	Am	Ac/Ag, Hc, Hch, Pb, Pc, Pn, Rs, Rc, Rcl	10
PWMA	Black Swamp	Ac, Cc, Nf, Oa	Pb, Pc, Pn, Po, Rcl, Rs, Sh	11
PWMA	Campground	Ac, Cc, Ch, Ts	Ac/Ag, Gc, Hc, Hch, Hf, Pb, Pc, Pn, Po, Rc, Rcl, Rs	16
PWMA	Jones	Cc, Ks	Pc, Pn, Po	5

PWMA	Juke Joint	Ac	Amm, Amo, Amt, Aq, Ec, Hf, Hch, Hs, Pb, Pc, Pn, Po, Rs, Sh	15
PWMA	Polk Borrow Pit	Cc, Ch, Pg, Tsi	Ac/Ag, Amo, Amm, At, Ec, Gc, Hc, Hch, Hf, Hg, Hs, Pb, Pc, Pn, Po, Poc, Rc, Rs	12
PWMA	Quail Road	Ac, Cc, Mf	Ac/Ag, At, Gc, Pb, Pc, Pn, Po, Hc, Hch, Hf, Hg, Hs, Rcl, Rs	17
PWMA	Stuckey	Cc, Pob	Ac/Ag, Hch, Hf, Pb, Pc, Pn, Po, Rc, Rcl, Rs, Sh	13
PWMA	Womans Dam	Ac, Cc	Ac/Ag, Amo, Amt, At, Aq, Gc, Hc, Hch, Hf, Pb, Pc, Pn, Po, Rc, Rcl, Rs, Sh	19

[1]Ac = *Agkistrodon contortrix*, Am = *Alligator mississipiensis*; Ap = *Agkistrodon piscivorus*, Ca = *Crotalus adamanteus*, Ch = *Crotalus horridus*, Cc = *Coluber constrictor*, Dp = *Diadophis punctatus*; Dr = *Deirochelys reticularia*, Hp = *Heterodon platirhinos*, Ks = *Kinosternon subrubrum*, Lg = *Lampropeltis getula*, Lt = *Lampropeltis Triangulum*, Mf = *Masticophis flagellum*, Nf = *Nerodia fasciatus*, Pob = *Pantherophis obsoleta*, Pg = *Pantherophis guttata*, Oa = *Opheodrys aestivus*, Tc = *Terrapene carolina*, Ts = *Trachemys scripta*, Tsi = *Thamnophis sirtalis*, Vs = *Virginia striatula*

[2] Ac = *A. crepitans*, Ag = *Acris gryllus*, Ame = *Amphiuma means*, Amo = *Ambystoma opacum*, Amt = *A. talpoideum*, Amm = *A. mabeei*, Amma = *Ambystoma maculatum*, Aq = *Anaxyrus quercicus* At = *Anaxyrus terrestris*, Ec = *Eurycea chamberlaini*, Hc = *Hyla cinerea*, Hch = *Hyla chrysoscelis*, Hf = *Hyla femoralis*, Hg = *Hyla gratiosa*, Hs = *Hyla squirella*, Nv = *Notophthalmus viridescens*, Pb = *Pseudacris brimleyi*, Pc = *Pseudacris crucifer*, Pn = *Pseudacris nigrita*, Po = *P. ornata*, Poc = *Pseudacris ocularis*, Rcl = *Rana clamitans*, *Rh* = *Rana heckscheri*, Rc = *R. catesbeianas*, Rs = *R. sphenocephala*, Sh = *Scaphiopus holbrookii*, Sm = *Stereochilus marginatus*

*Visual Observation

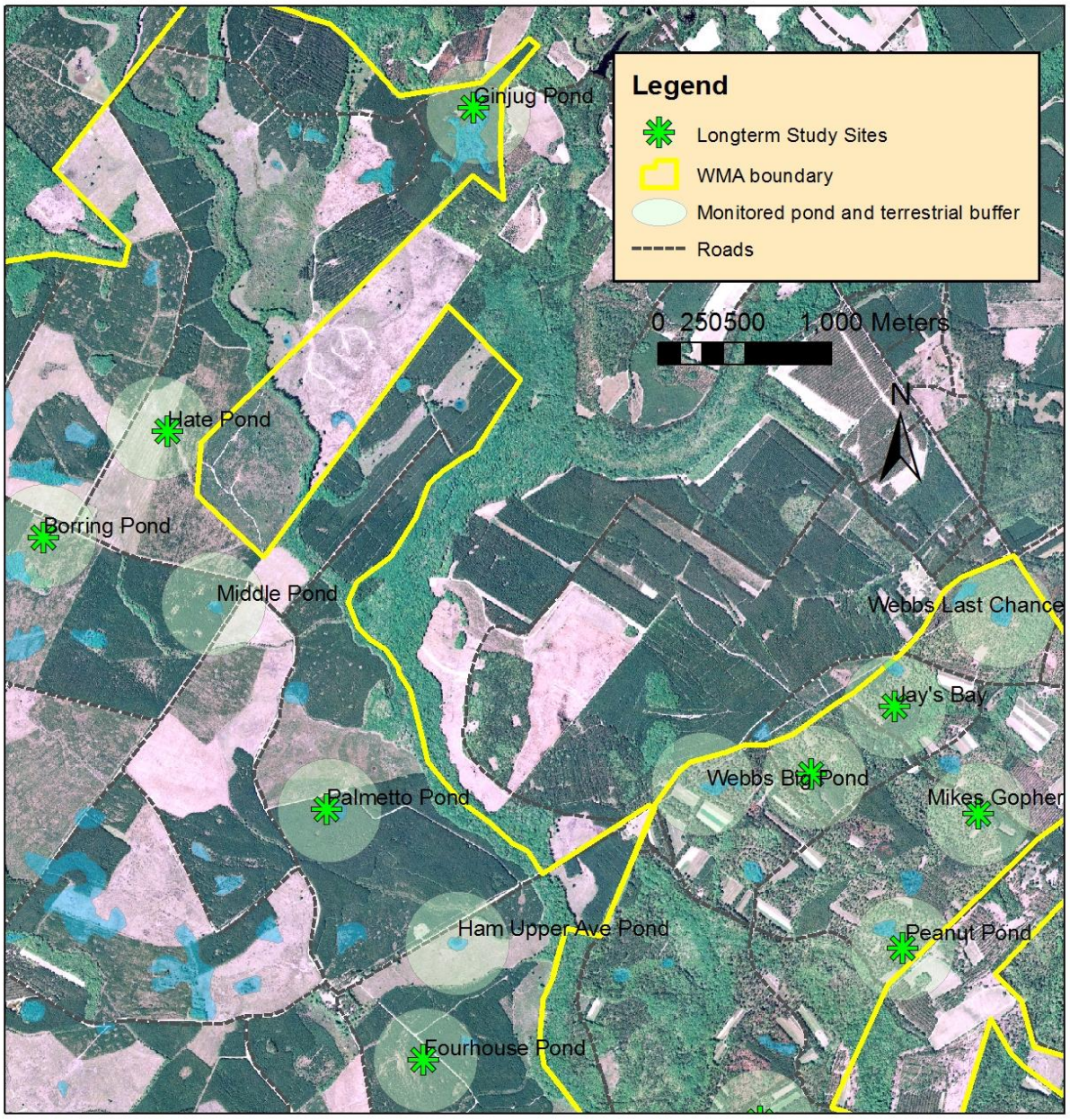


Figure 1. Map depicting long-term sampling locations on Hamilton Ridge WMA and Webb WMA.

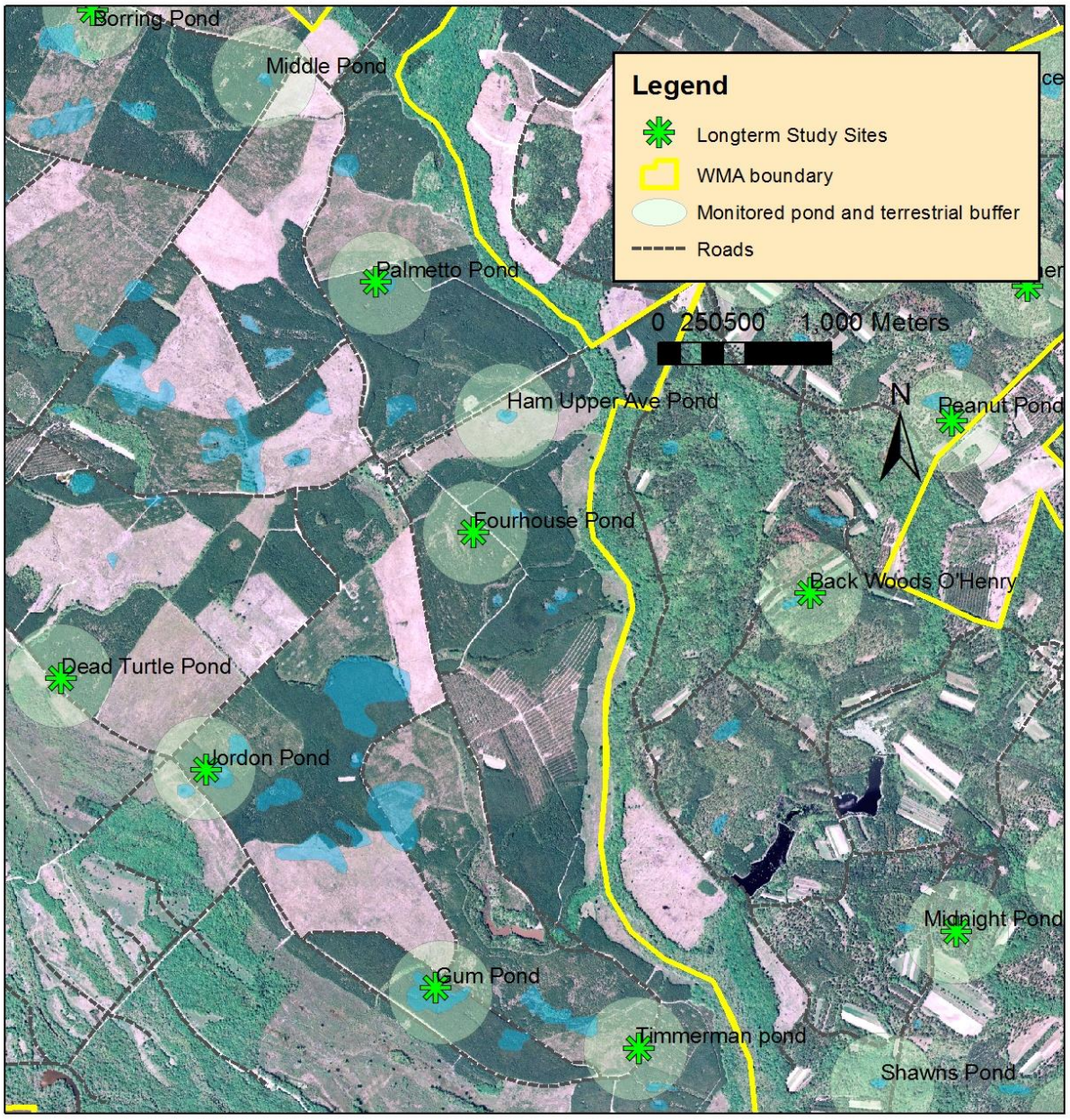


Figure 2. Map depicting long-term sampling locations on Hamilton Ridge WMA and Webb WMA.

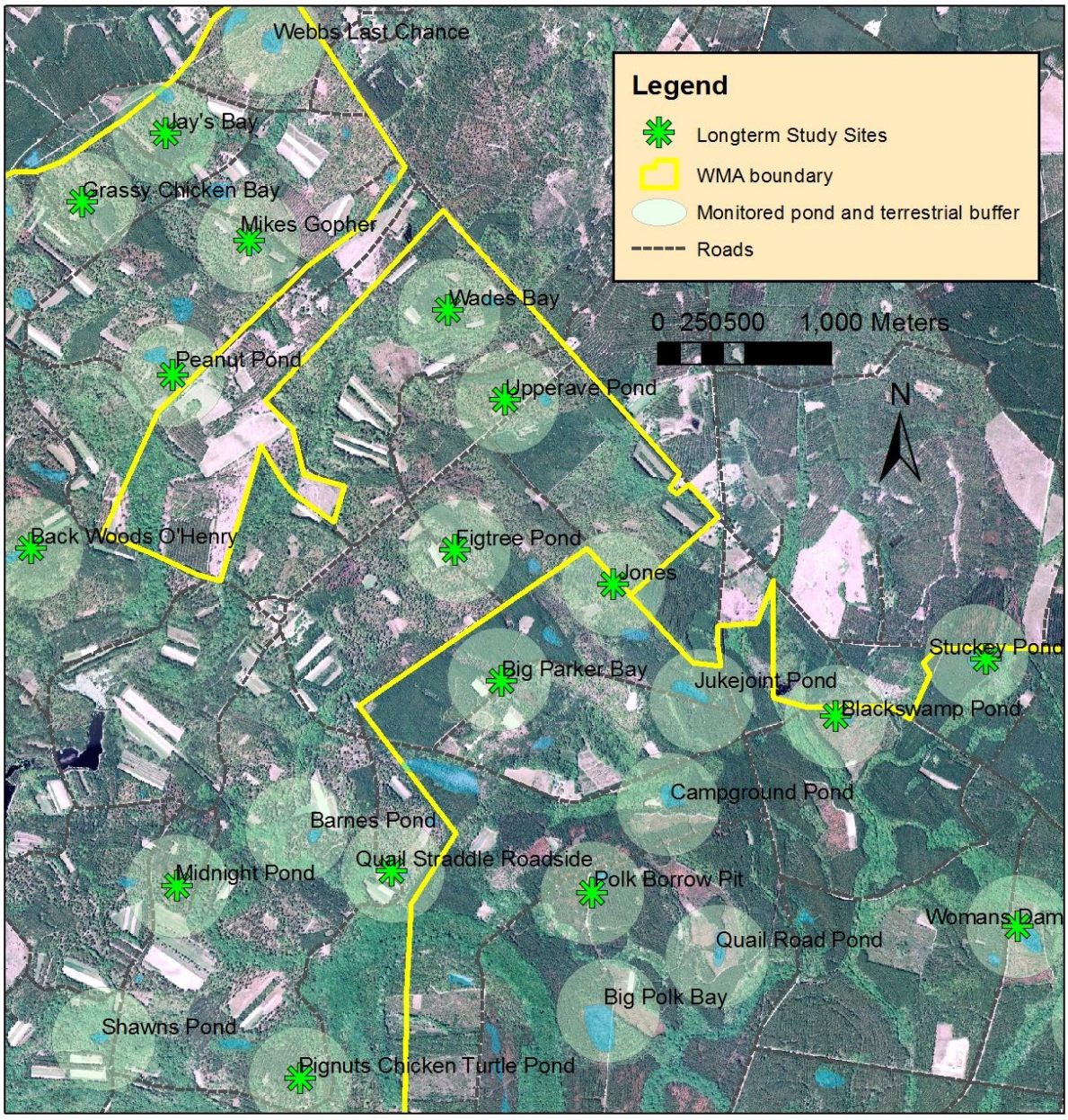


Figure 3. Map depicting long-term sampling locations on Webb WMA and Palachucola WMA.

Objective 2: The mark-recapture protocol for the eastern diamondback was to be expanded to include locations on Hamilton Ridge and Palachucola WMAs. Rattlesnakes were to be field collected, checked for PIT tags, measured, and weighed. The sex of new, unmarked snakes were to be determined. Their overall health would be assessed and they would be marked using a combination of PIT tag and cautery marking on ventral scales. All animals were to be released at their site of capture within 1-2 days of capture.

Methods: We conducted snake surveys (i.e. cover board surveys, road surveys, and visual encounter surveys) between March and November at historical monitoring sites (i.e. those that have been monitored continuously since 2001 and those deployed near ponds (i.e. Job 1) at Webb, Palachucola, and Hamilton Ridge WMAs. Upon capture, we processed snakes (measured, weighed, and marked) before releasing study animals at their capture locations. We marked captured rattlesnakes using scale cauterization and passive integrative transponders (PIT tags). We marked all other snake species using scale cauterization. Captured snakes and turtles were measured and weighed to assess body condition. Table 4 summarizes captures by species (snakes and turtles), including data from 2016-2019.

We did not sample during spring emergence 2020 due to travel restrictions associated with SARS Covid19.

Accomplishments: Annual surveys yielded captures of 19 snake species and five turtle species. Capture-recapture data (Table 4) for five species (eastern diamondback rattlesnake, canebrake rattlesnake, southern racer, southern copperhead, and corn snake) are suitable for demographic analysis. Snake demographics are largely lacking due to low detection probabilities that make demographic analysis difficult. This study demonstrates the necessity of long-term efforts for accumulating survey data for use in population-scale analyses. While outside the scope of the current project, we plan to analyze these data to calculate parameters (e.g. survival probability) that can be used in population viability studies.

Table 4. Snake and turtle species captured during visual, cover board, and road surveys conducted at the Webb Wildlife Center (including Palachucola WMA and Hamilton Ridge WMA). Snakes marked with an asterisk (*) include captures recorded since 1994, as well as EDBs that were translocated to the study area from other properties as part of a concurrent translocation study. All other captures were tallied using captures between 2010 and 2019.

Species	New	Recaptures	Total Captures
Canebrake Rattlesnake*	250	62	312
Eastern Diamondback Rattlesnake*	146	52	198
Southern Copperhead	174	18	192
Eastern Cottonmouth	6	0	6
Corn Snake	143	14	157
Eastern Hognose Snake	38	1	39
Eastern Kingsnake	8	0	8
Garter Snake	28	0	28
Rough Green Snake	6	0	6
Black Racer	382	30	412
Yellow Rat Snake	49	2	51
Rough Earth Snake	10	0	10
Redbellied Snake	2	0	2
Banded Water Snake	1	0	1
Redbelly Water Snake	3	0	3
Scarlet Snake	4	0	4
Scarlet Kingsnake	28	1	29
Southern Ringneck Snake	5	0	5
Eastern Coachwhip	20	1	21
Eastern Box Turtle	31	0	31
Chicken Turtle	2	0	2
Mud Turtle	20	0	20
Snapping Turtle	1	0	1
Spotted Turtle	2	0	2

Significant Deviations: As part of Objective 1, we were unable to analyze amphibian acoustic data collected during Spring 2020 due to travel restrictions associated with SARS Covid19. As part of Objective 2, we did not sample for snakes during spring emergence 2020 for the same reason.

Overall Recommendations for SC-T-F16AF00708: Close the grant.

Expenses: See final 425.