Survival assessment and movement analysis of gopher tortoises (*Gopherus polyphemus*) in south Georgia, USA

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ABSTRACT

The keystone and threatened status of the gopher tortoise (*Gopherus polyphemus*) makes them a species of interest for wildlife management. Two studies were conducted on gopher tortoises in south Georgia, USA. The first study assessed the survivability of 174 passive integrative transponder (PIT) tagged gopher tortoise hatchlings released in 2008 and 2009 at Reed Bingham State Park (RBSP), Cook County, Georgia. It was hypothesized that 10% (17/174) would be recaptured. From March 2020 to July 2021, release sites Gopher Tortoise Management Area and Pioneer Site were surveyed. Zero (N = 0) of the 174 PIT-tagged hatchling tortoises were recaptured. Suggestions for recapture failure were predation, human presence, detection difficulty, dispersal away from release sites, and Upper Respiratory Tract Disease. The second study analyzed movement activity of an adult male gopher tortoise utilizing Global Positioning System (GPS) technology at Moody Air Force Base, a military installation in Lowndes and Lanier Counties, Georgia. Movement was GPS-tracked from September 2020 to January 2021. GPS data contained location, temperature, and satellite-specific acquisition information. A total of 263 (N = 263) Fixed Locations (FL) were acquired. FL were analyzed for home range, core area, distance >300 m, and nocturnal movement (20:00 – 05:00). Home range increased 10-fold with increasing horizontal dilution of precision (HDOP) (range: 3.55 – 15.86 ha, HDOP ≤ 1.5 – 9.9), core area was 0.13 ha, greatest straight-line distance was 345 m, and 41 nocturnal events occurred. GPS unit troubleshooting and gopher tortoises' fossorial nature attributed to early battery life depletion and FL accuracy and precision analysis. Most FL occurred in the core area; long distance movement outside the core area was exhibited five times, one included nocturnal movement. Suggestions for long distance movement were habitat quality, overwinter burrowing, reproduction, social interaction, and energy expenditure recovery.

Keywords: gopher tortoise, survival, hatchling mortality, movement, GPS technology, HDOP, home range, core area, nocturnal activity, spatial ecology, military

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DEDICATION

This thesis is dedicated in loving memory to Barry C. Weaver.

An opinionated man who told me to finish what I started.

A loving dad who told me not to be so hard on myself.

My original mentor who loved to ask the question:

“May I make a suggestion?”

Here’s to you.

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Chapter I

BACKGROUND

I.I. Species Status

The gopher tortoise is a large, semi-fossorial reptile found in the southeastern United States (USA). The species is a keystone species because of their burrows, which are utilized by over 300 other species often used as shelter with a stable microclimate, feeding, mating, or nesting ground (Catano & Stout, 2015; Jackson & Milstrey, 1989).

Gopher tortoises are federally listed as “Threatened” by the United States Fish and Wildlife Service (USFWS) and afforded federal protection west of the Mobile and Tombigbee Rivers in Alabama, Mississippi, and Louisiana (USFWS, 1987). Their eastern range is across Florida, Georgia, and South Carolina, where they are a “Candidate” species for listing as “Threatened” or “Endangered” (Figure 1). State listing of the tortoise varies by state: Mississippi and South Carolina list them as endangered, while Florida lists them as a species of special concern (Epperson & Heise, 2003, Wilson et al., 1997). On October 12, 2022, a Proposed Rule by the Fish and Wildlife Service determined that listing the gopher tortoise as threatened or endangered across their entire range was not applicable and only the populations in the western range met the threatened or endangered species criteria under the Endangered Species Act (USFWS, 2022a).

The USFWS completed a Species Status Assessment (SSA) Report for the gopher tortoise consisting of a comprehensive review of the tortoise’s biology, ecology, factors influencing their viability, current population and species needs, and future viability (USFWS, 2022b). Based on future projections, the SSA predicted low extinction risk to gopher tortoise populations but acknowledged their adaptive capacity and population distribution likely decreased significantly relative to their historical distribution (USFWS, 2022b). Auffenberg and Franz (1982) assessed the distribution of gopher tortoises’ entire range and estimated 80% of extant gopher tortoise colonies were in south Georgia and north Florida and concluded they were being extirpated at an “alarming rate.” A study by McCoy et al. (2006) compared gopher tortoise populations a decade apart in 10 protected sites in Florida and concluded populations declined in 8 of those sites, suggesting that components related to habitat quality and other causes like Upper Respiratory Tract Disease transmitted by *Mycoplasma agassizii* (Brown et al., 1999) were potential factors for population decline. Infectious (viral, bacterial, mycotic, parasitic) and noninfectious diseases (metabolic bone disease, vitamin A deficiency, hypothyroidism, toxicosis, etc.) were reported as contributors to tortoise mortality (Jacobson, 1994). Other factors that contribute to gopher tortoise decline include high early age mortality rate, low reproductive potential, habitat destruction and degradation, and human activity (*i.e.*, urbanization) (Auffenberg & Franz, 1982; Diemer, 1986; Epperson & Heise, 2003; McCoy et al., 2006; Wilson et al., 1997; USFWS, 2022b).

In addition to high mortality rates in clutches and hatchlings, human activity has had serious impacts on gopher tortoise populations (Auffenberg & Franz, 1982; Wilson et al., 1997). Human actions that influence habitat include urbanization, agricultural clearing, vehicle fatality, fire suppression, mining, and human predation (Auffenberg & Franz, 1982; Bond & Keane, 2017; Czech et al., 2000). Poor habitat management can lead to vegetative overgrowth, influencing sexually mature adults to leave the population for a better area to forage, reducing recruitment into the population (Wilson et al., 1997). Planting of fire intolerant species such as sand pine (*Pinus clausa*) or loblolly pine (*Pinus taeda*) yield dense forests with minimal understory, providing low understory forage for tortoises (Auffenberg & Franz, 1982; Diemer, 1986).

I.II. Habitat and Diet

Gopher tortoises occupy xeric environments with well-drained sandy soils suitable for burrowing (Diemer, 1986). Common habitats used by gopher tortoises are longleaf pine ecosystems (*Pinus palustris*), oak woodlands (*Quercus* spp.), xeric hammocks (evergreen forests on well-drained sandy soils), pine flatwoods, dry prairies, sandhills and ruderal communities (roadsides, grove edges, and clearings) (Auffenberg & Franz, 1982). These habitats provide suitable areas for burrow construction, abundant herbaceous vegetation, and open sunny areas for nesting and thermoregulation (Wilson et al., 1997). Gopher tortoises avoid densely canopied habitats because they reduce sunlight exposure needed for thermoregulation (Wilson et al., 1997). Low sunlight diminishes ground vegetation and can influence the growth, development, and reproduction of the gopher tortoise (Wilson et al., 1997).

Gopher tortoises construct deep burrows, averaging approximately 3 – 6 m in length and 2 m in depth (Smith et al., 2005). Burrows are characterized by the apron, formed from the tortoise digging the burrow and a half–moon shaped entrance, which represents the size and shape of the tortoise shell (Carthy et al., 2005; Guyer & Hermann, 1997). The tortoises spend most of their time in these burrows due to its ability to maintain a stable temperature and humidity year-round, acting as shelter for shade and escape from extreme weather, fire, desiccation, and predators (Diemer, 1986; Douglass & Layne, 1978; Guyer & Hermann, 1997; Smith et al., 2005).

Gopher tortoises are herbivores with a preference for forbs, wiregrass (*Aristidia stricta*), and blue grasses (*Andropogon sp.*), but may also consume charcoal, seeds, and insect parts (Aresco & Guyer, 1999; MacDonald & Mushinsky, 1988). Garner and Landers (1981) discovered that grass-like plants like Asteraceae, Cyperaceae, and Fabaceae were an important part of the tortoises' diet in southwest Georgia. In a sandhill community in west-central Florida, MacDonald and Mushinksy (1988) found the plant taxa Poaceae, Asteraceae, Fabaceae, Pinaceae, and Fagaceae were the main components of a gopher tortoise's diet. Gopher tortoises act as seed dispersal agents for plants such as wiregrass (*A. stricta*), Bahiagrass (*Paspalum notatum*), and slender paspalum (*P. setaceum*) (Carlson et al., 2003).

Chapter II

SURVIVAL ASSESSMENT OF 2008-2009 GOPHER TORTOISE HATCHLINGS AT REED BINGHAM STATE PARK, COOK COUNTY, GEORGIA, USA

II.I. Introduction

The purpose of this study was to assess the survivability of gopher tortoise (*Gopherus polyphemus*) hatchlings more than a decade after release. In 2008 and 2009, Valdosta State University (VSU) researchers released 174 passive integrative transponder (PIT)-tagged gopher tortoise hatchlings at Reed Bingham State Park (RBSP), Cook County, Georgia, USA (Chessler, 2010). The goal was to determine survival success of hatchlings a decade post-release and factors that contribute to their survivability. Mortality is high in gopher tortoise hatchlings (Auffenberg & Franz, 1982; Butler & Sowell, 1996; Epperson & Heisse, 2003; Pike & Seigel, 2006; Quinn et al., 2018) and studying those that survive to subadult age could improve our understanding of juvenile mortality and growth rates. Other tortoises captured in this survey were identified as unique individuals through body size measurements and carapace and plastron physical characteristics to establish the relative abundance of gopher tortoises present at RBSP.

II.I. I. Reproduction and Growth

There are 5 age classes of gopher tortoises identified by Wilson (1991): eggs, hatchlings, juveniles, subadults, and adults (Table 1.). They are a long-lived species with low reproductive potential because they do not reach sexual maturity for close to a decade and maturation varies by state: in Mississippi, 15 – 20 years for both sexes; in Florida, 9 – 18 years for males and 10 – 21 years for females; in southern Alabama, about 20 years for both sexes (Epperson & Heise, 2003).

The gopher tortoise breeding season starts in March and ends in October (Landers et al., 1980). Males will perform head-bobbing behaviors at the entrance of a female-occupied burrow when they are looking for a mate. If the female exits the burrow, the male will mount for copulation. Females lay only one clutch per year; average annual clutch size is 5 – 8 eggs but this varies across the tortoises' range (Epperson & Heise, 2003). Eggs are deposited in open locations where they can receive heat from the sun, and they are buried in the burrow apron (Epperson &

Heise, 2003). Incubation period varies by latitude: 110 days in South Carolina, 102 days in southwest Georgia, and 80 – 90 days in northern Florida (Diemer, 1986).

There is low survivorship for early life stages of the gopher tortoise. There is an estimated 90 – 94% mortality rate for both eggs and hatchlings between the time eggs are laid and the end of the first year of life due to predation, which results in an average of one successful clutch every 9 – 10 years (Alford, 1980). Hatchlings and juveniles have softer shells and bask at the mouth of the burrow during the spring and fall, both of which increase their vulnerability to avian and mammalian predation (Wilson, 1991). Documented predators include small mammals: raccoons (*Procyon lotor*), opossums (*Didelphis virginianus*), nine-banded armadillos (*Dasypus novemcintus*); avian species: red-tailed hawks (*Buteo jamaicensis*), American crows (*Corvus brachyrhynchus*); snakes: cottonmouths (*Agkistrodon piscivorous*), eastern indigo snakes (*Drymarchon corgis*); and insects: red imported fire ants (*Solenopsis invicta*) (Butler & Sowell, 1996). As gopher tortoises age from juvenile to subadult, survival against predators increases as their shells harden and turn brown or tan, and from subadult to adult, the carapace is completely hardened and dark, leading to fewer natural predators (Wilson, 1991).

II.I. II. Marking Techniques

Mark-recapture studies can be used to assess population dynamics, movement, behavior, and density estimates. There are multiple methods used to mark gopher tortoises. Silvy (2012) noted that markings can be natural, invasive, or non-invasive. Natural markings used to identify reptile species are patterns present on the body, invasive markings including chemical injections, passive integrative transponder (PIT) tags, external tags (*i.e.*, metal tags), branding (tortoises/turtles branded on their carapace), and tissue removal (toe clipping and shell notching), and noninvasive markings involving bands, adhesive with numbers, and colored paints/dyes. Cagle (1939) described a system for marking turtles for future identification using a consistent scute marking scheme; the system is used often in mark-recapture studies. Gibbons and Andrews (2004) published a thorough review of PIT tags and their applications in various animal species.

There are limitations to external marking techniques. External tags could be lost, rendered unidentifiable, or introduce uncertainty whether an individual has been previously marked (Gibbons & Andrews, 2004). Notches or scute marking systems are not standard across all North

American genera and could lead to identification misinterpretation, could injure an individual and result in death, and may be limited to age classes where the shell is well ossified (Cagle, 1939; Gibbons & Andrews, 2004). PIT tags are advantageous because they are internal, permanent, function across temperature ranges, and seldom impact an animal's behavior, movement, physiology, and ecology (Gibbons & Andrews, 2004). Gopher tortoises at Reed Bingham State Park and Moody Air Force Base have been PIT-tagged and drill marked on their marginal scutes similar to methods in Cagle (1939).

II.II. Hypothesis

It was hypothesized that 10% (17/174) of the gopher tortoise hatchlings released in 2008–2009 at Reed Bingham State Park (RBSP) would be captured based on the estimated 90–94% mortality rate in previous studies analyzing gopher tortoise hatchling survival (Alford, 1980) and maintenance of suitable habitat for gopher tortoises by RBSP staff. This equated to a 6–10% survivability for hatchlings. Previous studies thoroughly documented high mortality in gopher tortoise hatchlings. Butler and Sowell (1996) captured and released 31 gopher tortoise hatchlings in two cohorts in 1991 and 1992 and by June 1993, all the tortoise hatchlings were reported dead, likely predated by snakes, hawks, raccoons, armadillos, and other mammalian predators. Epperson and Heise (2003) released 48 gopher tortoise hatchlings and tracked them with radio-transmitters, all but one was killed, with the last individual surviving up to 736 days (2.01 years) at the conclusion of the study. Pike and Seigel (2006) released 20 gopher tortoise hatchlings and found that all were predated and none survived past 335 days.

II.III. Materials and Methods

II.III. I. Study Site

The study site was Reed Bingham State Park (RBSP), Cook County, Georgia, USA (Latitude 31.171330°, Longitude 83.540550°) (Figures 2 – 3). The park consists of 653 hectares (ha) of land with 152 ha comprising RBSP Lake. The two specific areas of interest within RBSP were the gopher tortoise hatchling egg collection and release sites (Figure 4): Gopher Tortoise Management Area (GTMA) and Pioneer Site (Pioneer).

GTMA is a 4.20 ha area comprised of sandy soil, almost evenly spaced long leaf pine tree (*Pinus palustris*) canopy, and wiregrass (*Aristida stricta*) understory (Figure 5). Along the north, south, and west edges of GTMA is a maintained, sandy right-of-way (ROW) used by RBSP personnel, while the east side borders County Road 258, a paved road separated by a strip of mowed grass. Along with a wiregrass-dominated understory were shrubs and herbaceous plants such as bluejack oak (*Quercus incana*), dog fennel (*Eupatorium capillifolium*), and prickly pear cacti (*Opuntia humifusa*). Burrows were abundant throughout this area.

Pioneer is a 2.67 ha area similar to GTMA, but with less vegetation, more open space, and presence of hardwood trees (Figure 6). The west end of Pioneer borders County Road 258, a paved road separated by a strip of mowed grass and the north end is adjacent to a cattle pasture. In the east end of Pioneer is dense vegetative brush and forest that transitions into an open area dominated by wiregrass understory. Burrows were distributed throughout the range of Pioneer but visually observed at a lower density than GTMA.

The Web Soil Survey (WSS), produced by the National Cooperative Soil Survey and operated by the United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS), provides soil maps and data to 95% of the nation's counties (Soil Survey Staff, 2020). An NRCS WSS was performed on GTMA and Pioneer to evaluate soil suitability for gopher tortoises. GTMA was comprised of 91.4% Kershaw sand, 0 to 5 percent slopes (soil code: KdB) and 8.6% Osier-Pelham complex, 0 to 2 percent slopes, frequently flooded (soil code: OP) (Figure 7) (Soil Survey Staff, 2020). The WSS assessed the soil for gopher tortoise burrowing suitability for GTMA, and rated Kershaw sand as "Highly suited" for burrowing suitability, while areas with Osier-Pelham complex were rated as "Unsuitable" for burrowing suitability due to poor drainage (Figure 8). Pioneer was made up entirely of Kershaw sand (Figure 9) (Soil Survey Staff, 2020) and gopher tortoise burrowing suitability was rated as "Highly suited" (Figure 10) (Soil Survey Staff, 2020). These WSS for GTMA and Pioneer indicated that these areas were suitable for gopher tortoise burrowing behavior.

RBSP staff performed management practices for gopher tortoises and the surrounding park. They conducted prescribed burns every 1 to 2 years to prevent the habitat from becoming unsuitable for the current tortoise population. Two prescribed burns occurred during the sampling period, one in early 2020 and one in February 2021. Vegetation was occasionally mowed to minimize overgrowth.

II.III. II. Survey Method and Data Collection

From March 2020 to July 2021, a pedestrian line sweep survey (Figure 11) (Stack & Smith, 2003), was performed in Gopher Tortoise Management Area (GTMA) and Pioneer Site (Pioneer). The surveys occurred at a minimum of two days per month with one to four sweeps conducted throughout each survey day. To increase detection of gopher tortoises, noise was kept to a minimum by slow walking and avoiding noisy vegetation (dry leaves or fallen branches). These methods were employed to avoid initiating tortoises' flight into a burrow (Radzio & O'Connor, 2017). The sampling period consisted of consecutive surveys of both GTMA and Pioneer.

Prior to each survey, the date and weather conditions including temperature, wind, humidity, dew point, pressure, UV index, and visibility from weather.com (The Weather Channel) were recorded. The Weather Channel uses data from a weather system known as the IBM POWER9 (supercomputer) to deliver accurate weather forecasts (Sibley, 2019). This data was used to determine time, month, temperature range of captures and weather tortoises were typically captured.

When a gopher tortoise was encountered, the following information was recorded: time of capture, GPS coordinates, PIT-tag number (if applicable), picture of carapace and plastron, straight carapace length (SCL), carapace width, plastron length, plastron width, shell height, weight, and sex (Figures 12-14). SCL and growth ring annuli were used to approximate age (Aresco & Guyer, 1998).

A Google Pixel 3A (2019 smartphone) and the app "GPS Coordinates" by Financept (2020 version) were used to record GPS coordinates and export data. The GPS accuracy of the smartphone had a mean accuracy of 4.9 meters or 16 feet (Van Diggelen & Enge, 2015). These coordinates were used to determine where tortoises were frequently detected. A handheld PIT tag Pocket Reader ™ (scanner) was used to scan tortoises for PIT tags (Figure 15). Calipers were used to take the tortoises' shell measurements, Haglöf Mantax Blue Calipers were used for tortoises that were greater than 150 millimeters (mm) SCL, max caliper measurement length was 762 mm, and Blue-Point® Electronic Digital Calipers were used for tortoises less than or equal to 150 mm SCL, max caliper measurement length were 150 mm. The weight was measured with an EXTECH Instruments weight scale, measurement units in pounds and ounces.

Sex of tortoises was determined through visual examination of plastron concavity (Figure 16), length of gular projection (Figure 17), width opening of rear carapace (Figure 18), and overall body size and SCL (females haver greater SCL than males) (McRae et al., 1981b). Measurement combinations of plastral concavity, anal shields/notch/width, and overall body size were the best method to determine gopher tortoise sex (McRae et al., 1981b). Subjective visual analysis was the primary method used to sex tortoises, but this method can lead to sex misclassification (McRae et al., 1981b), therefore, tortoise sex ratios in this study were considered as visual estimations rather than quantified determinations.

If a PIT tag was not scanned, then body measurements other than SCL, presence or absence of growth ring annuli (Figure 19), and black markings on individual scutes of the plastron (Figure 20) from each gopher tortoise were compared to others to determine if an individual was a unique capture or a recapture from an earlier sampling survey.

II.IV. Results

Over a period of 16 months, there were 71 sampling surveys completed in the sampling period either alone or with field assistants. A minimum of two sample surveys were done every month, except April 2020, when only one survey was completed due to the Coronavirus Disease (COVID-19) pandemic leading to a lockdown with travel restrictions. Thirty-six of the 71 sampling surveys occurred between July and August 2020. There were 8 days where two to four sampling surveys were completed within the day, starting early morning (07:52 hrs) to late evening (18:08 hrs), totaling 22 of the 71 sampling surveys. Three sample surveys were completed in one overnight period, starting on July 21, 2020, at 20:55 hrs and ending on July 22, 2020, at 05:50 hrs. Only one sampling survey per day was completed for the other 46 days.

Zero of 174 PIT-tagged hatchling tortoises (N = 0) released in 2008 and 2009 at Reed Bingham State Park (RBSP) were recaptured. There were 42 unique, non-replicated individuals out of the 95 total gopher tortoises captured, 35 were adults, 1 was a subadult, and 6 were juveniles according to Straight Carapace Length (SCL) classifying their age (Table 1). Nineteen adults were female, 16 adults were male, the sex of the remainder were unable to be determined due to size. The mean adult SCL was 302.38 mm, range: 238.76 – 347.98 mm (males mean SCL 264.92 mm, range: 261.62 – 330.20 mm, and females mean SCL 266.36 mm, range: 238.76 – 347.98); subadult SCL was 182.88 mm; mean juvenile SCL was 70.15 mm, range: 60.91 – 78.33 mm. Seven of the

42 individuals were PIT-tagged in the right anterior ventral leg (Figure 15), but none of their ID's matched the 174 PIT-tagged hatchlings from 2008 and 2009. In addition, six adult tortoises, including two PIT-tagged, had drill holes resembling the marking system from Cagle (1939) (Figure 21).

Seventy-eight captures occurred in Gopher Tortoise Management Area (GTMA), 7 captures were at Pioneer Site (Pioneer), and 10 incidental captures were outside of the study sites (Figure 22). Most captures of unique individuals occurred in GTMA, most were adults, 6 unique captures were at Pioneer, and 7 unique captures were incidental (Figure 23). Unique subadult and juvenile tortoises were captured exclusively in GTMA. The time and weather conditions that captures occurred most were between 12:00 hrs and 17:00 hrs, 26.66 – 31.66°C (80 – 89°F), and typically during sunny weather. Sixty-four of the 95 total tortoise captures occurred between July and August 2020 with 1 to 5 captures per sampling survey. The remaining 31 captures occurred in the months before July 2020 and after August 2020. The number of captures decreased after August 2020 due to availability of field aides, reducing sampling effort; and gopher tortoise response to colder weather, reducing detection.

II.V. Discussion

Multiple studies over the last four decades documented low survivability in early life stages of gopher tortoises and this study's results aligned with other studies that followed up on hatchlings released (Auffenberg & Franz, 1982; Butler & Sowell, 1996; Epperson & Heise, 2003; Pike & Seigel, 2006; Quinn et al., 2018). Chessler's (2010) 2008 – 2009 recapture effort consisted of 83 field trips to the Gopher Tortoise Management Area (GTMA) and Pioneer Site (Pioneer), 4 – 6 times a month, lasting 3 – 4 hours and had varying availability of field assistants and Reed Bingham State Park (RBSP) staff. Only 6% (11/174) of the tortoises were recaptured over an 18-month period (Chessler, 2010). In comparison, this study's survey effort was from 2020 – 2021, consisting of 71 sampling surveys over a 16-month period, a minimum of 2 times per month, lasting 1 – 3 hours per sampling survey with availability of field assistants in summer. This study's recapture effort and availability of field aides may have played a role in zero recaptures.

Multiple mark-recapture studies conducted follow-ups on previously tagged and released gopher tortoises. Diemer (1992a) captured and marked 372 gopher tortoises from 1982 – 1986 at three sites in northern Florida, recapture rates (marked tortoises captured during year/all previously marked tortoises) of marked individuals were 46 – 75% at one site and 30 – 37% at the other site; juveniles had the lowest recapture rate. Tuberville et al. (2014) assessed differences in survival, demography, and growth of three gopher tortoise populations with different management histories in southwest Georgia (two sites) and south-central Alabama (one site), using 5 – 11 years of mark-recapture data, survivorship was found to be high for adult tortoises and lower for immature tortoises with recapture rates ranging from 21 – 97% based on number of traps set and number of days traps were deployed per sampling year and site. Berish and Leone (2014) conducted a follow-up survey in 1995 on one of the sites from Diemer's (1992a) study and recaptured 18 tortoises previously marked with recapture rates of 3% for formerly immature tortoises, 10% for adult females, and 22% for adult males.

RBSP is a near ideal environment for gopher tortoises. Gopher Tortoise Management Area (GTMA) and Pioneer Site (Pioneer) were areas maintained by prescribed fires, mowing, and brush removal. The sites consisted of limited canopy cover for basking behavior and grasses that are preferred by tortoises. The Soil Survey Staff's assessment of the soils in GTMA and Pioneer indicated that the soils were well-drained/porous sandy soil, which are preferred by gopher tortoises to allow them to dig burrows. Although the habitat of the sites was optimal, there were other factors that affected the habitat's suitability and possibly explained the recapture failure of the PIT-tagged tortoises released in 2008 and 2009.

Predated gopher tortoise clutches were evident throughout Gopher Tortoise Management Area (GTMA). Fire ants were observed at burrow entrances and may have decreased hatchling survival (Dziadzio et al., 2016). In addition, if released hatchlings used a conspecific adult burrow, then there was a chance of predation by a commensal predator as observed by Pike (2006). Humans were another possibility for recapture failure. RBSP is a state park, which meant there were people visiting and camping at the park and driving vehicles on County Road 258 bordering the release sites, leading to vehicle mortality. One juvenile tortoise carcass was discovered on County Road 258 with a fractured and almost flattened shell. Besides road fatality (Bond & Keane, 2017), people may have taken or transported tortoises to different areas of the park or unknowingly dropped off tortoises in the nearby lake by mistaking them for a freshwater turtle. Subadult tortoises and their burrows may have also been harder to detect since smaller burrows had a propensity to fill faster than adult burrows due to burrow size (Guyer & Hermann, 1997).

PIT-tagged tortoises may not have stayed within release sites and made residence in other parts of the park as evident from the tortoises captured outside of release sites. This notion is not attributed to population density or habitat quality since gopher tortoises only increase movement distance relative to lower density areas (Guyer & Hermann, 1997) and do not move far from high quality habitat, where understory forage and open canopy are available (McCoy et al., 2013). Pike (2006) tracked gopher tortoise hatchling movement and suggested that hatchlings and juveniles traveled to satisfy energy requirements, exploring an area for resources, minimize genetic mixing, and spread out to avoid predation.

A final suggestion for recapture failure was disease related to Upper Respiratory Tract Disease (URTD). URTD is caused by contagious pathogens such as *Mycoplasma agassizii* and *Mycoplasma testudineum* and implicated in reducing tortoise populations (McGuire et al., 2014). RBSP has tortoises with a high prevalence of *M. agassizii* antibodies and presence of URTD lesions (McGuire et al., 2014).

Seven of the tortoises that were captured had a PIT-tag. These tags did not match any of the ones from Chessler's (2010) study. By the time of this study, the released PIT-tagged tortoises would have been subadult age (5 – 15 years old) and their SCL would have been 120 – 230 mm (Table 1). PIT-tagged tortoises that were captured in this study had a mean SCL of 297.18 mm (males) and 325.12 mm (females). These measurements were outside the subadult SCL range. An expected size of a subadult PIT-tagged tortoise was observed once with SCL of 182.88 mm (Figure 24). RBSP staff tagged some adult tortoises captured incidentally but did not maintain records (Lockhart pers. comm.).

There were 42 unique, non-replicate individuals identified at Reed Bingham State Park from 2021 to 2022 in Gopher Tortoise Management Area, Pioneer Site, and areas that surrounded the sites. For the 35 individuals that were not PIT-tagged, the following characteristics were used to determine if the individual was a unique individual or a prior capture: carapace and plastron natural markings, presence or absence of growth rings, shell measurements, sexual morphologies, and presence or absence of drill holes. Upon every capture, a picture of the tortoise's carapace and plastron were taken. These pictures were used to compare with other captures. Something noted as a distinct feature were black patterns that seemed ingrained into the tortoise's scutes (Figure 20). Not all tortoises had these black marks, but they were present in adults and juveniles. There is a lack of literature on the subject of these black markings in gopher tortoises and it is difficult to determine if they are naturally produced or caused by extraneous factors. These black markings could possibly be from the tortoise's nesting substrate (Dunn, 1982), for thermoregulation selection to either increase or decrease body temperature depending on region (Willemsen & Hailey, 1999), or a result of pheomelanin production from diet, natural and sexual selection, or behavioral traits related to color (Roulin et al., 2013). Further study is required to understand the role of these markings in the gopher tortoise's biology and ecology.

Chapter III

ANALYSIS OF GOPHER TORTOISE ACTIVITY ON MOODY AIR FORCE BASE, LOWNDES COUNTY, GEORGIA, USA

III.I. Introduction

As of 2023, there are only two publications tracking gopher tortoise (*Gopherus polyphemus*) activity with Global Positioning System (GPS) technology (Paden & Andrews, 2020; Stemle et al., 2022), and neither tracked tortoises on a military installation. Military installations are known to carry a high number of threatened and endangered species (Theodorakis et al., 2017), including gopher tortoises in the southeastern United States. Installations in these regions would benefit the most from studying gopher tortoise movement since the species is present throughout the southeast US and have an important role in southeastern US ecosystems as a keystone species. Studying gopher tortoise movement would assist in habitat mitigation planning and protect the integrity of military land's local ecosystems. A common tool used to demarcate tortoise home range is radiotelemetry, but radiotelemetry can have issues with accuracy and precision in its data (Kolodzinski et al., 2010). GPS technology should be considered as a tool to be used to track movement to solve these data issues. To further investigate gopher tortoise activity, GPS technology was field-tested on an adult male gopher tortoise at Moody Air Force Base (MAFB), Lowndes and Lanier Counties, Georgia, USA. The study had two objectives: monitor gopher tortoise movement through GPS technology as a novel approach and assess the practicality of using GPS technology as an alternative monitoring tool for military land and natural resource managers.

III.I. I. Home Range, Movement, and Activity Patterns

Gopher tortoises home range, movement, and activity varies based on age class, individual size, sex, location, season, habitat, population density, burrow density, and social interactions (Diemer, 1992b; Eubanks et al., 2003; McRae et al., 1981a). Regarding annual home range, one study reported greater range in adult males than females at The Jones Center at Ichauway, a private ecological reserve in southwestern Georgia; males home ranged 0.0 – 4.8 ha, females ranged 0.0 – 3.4 ha (Eubanks et al., 2003). In a northern Florida population, adult mean home range was found to be larger than immature individuals (Diemer, 1992b). In general, male gopher tortoises had larger home ranges and traveled longer distances compared to females (Diemer, 1992b; Eubanks et al., 2003).

Gopher tortoises limit time outside burrows in the winter but increase activity as the seasonal temperatures increase (McRae et al., 1981a; Wilson et al., 1997). Based on a North Florida tortoise population, signs of recent activity in burrows increase in April, peak in July, and stay high in October (Diemer, 1992a). Mating activity peaks in the summer months and median distance movement increases as population densities decreases (Eubanks et al., 2003; Guyer et al. 2012; McRae et al., 1981a). Gopher tortoises have a specific home range that increases in size as they age but decreases in size as herbaceous ground cover (*i.e.*, food resources) increases (Diemer, 1992b; McRae et al., 1981a). Tortoises move long or short distances depending on population density, with one adult male gopher tortoise traveling up to 500 m to visit a female as burrows became more isolated (Guyer et al., 2012).

Gopher tortoises are diurnal species, and like most reptiles, are ectothermic, or an organism that relies on its external environment to regulate its body temperature (Douglass & Layne, 1978; Zug et al., 2001). Reptiles are dependent on temperature for their physiological processes (development, growth, reproduction) (Zug et al., 2001). An issue that reptiles encounter daily is a need to focus their thermoregulatory behavior in a specified temperature range to meet physiological function, while at the same time, reducing predation risk (Zug et al., 2001). Nocturnal activity in gopher tortoises has been mentioned and presented in several publications. Douglass and Layne (1978) discussed gopher tortoise nocturnal activity, indicating that historically, the species was once described as a nocturnal animal back in the early 19th century and observations of nocturnal movement have been documented since then, however, their study supported gopher tortoises being diurnal. Pike and Grosse (2006) were the first to document nocturnal activity in hatchling and juvenile gopher tortoises, showing tortoises emerged after a heavy summer rainstorm to drink water collected on the ground. In other species, Nordberg and McKnight (2020) found evidence of nocturnal basking behavior in Krefft's river turtles (*Emydura maquarii krefftii*) in Queensland, Australia; Hendrickson (1958) was one of the first to document hatchling green sea turtles (*Chelonia mydas*) emerging at night as a mechanism of avoiding extreme heat and using the protection of darkness to avoid predators as they approach the sea; Hjort et al. (2021) documented cathemeral activity patterns in mature spotted turtles (*Clemmys* *guttata*) and hypothesized the species exhibited nocturnal movement for mate-seeking and foraging and diurnal activity was reserved for basking.

III.I. II. GPS Technology and Applications in Wildlife Monitoring

The threatened status of gopher tortoises and their ecological role make them a species of interest to wildlife managers and military installations (Theodorakis et al., 2017). A common method for monitoring gopher tortoise movement is radio telemetry (Eubanks et al., 2003; Guyer et al., 2012; McRae et al., 1981a; Rautsaw et al., 2018; Tuberville et al., 2021). Radio telemetry is a remote monitoring technique that gathers location information on an individual or population and uses the data to understand behavior, movement, and demography (Silvy, 2012). The method involves attaching an individual with a radio-tag (transmitter) that emits a very high frequency (VHF), which is detected by an antenna and hand-held receiver (Dahlgren et al., 2018). The most common method for locating VHF radio-tagged individuals is triangulation, an intersection of radio signals that estimated the location of an animal (Silvy, 2012). The disadvantage of using radiotelemetry on gopher tortoises is that it required researchers to be near the tortoise for detection, which could influence the tortoise's movement (Radzio & O'Connor, 2017), thus impacting natural movement data. GPS technology uses satellites to record spatial data, allowing for more natural movement to be recorded.

GPS transmitters have been used recently to monitor gopher tortoise movements (Paden & Andrews, 2020; Stemle et al., 2022). GPS units determine location by calculating the time required for a satellite to transmit a signal to a receiver on earth (Silvy, 2012). There are 24-32 US satellites orbiting the earth, broadcasting their location and time of transmission (Silvy, 2012). A geographic location is determined when signals from 3 satellites overlap, allowing the GPS unit to pinpoint their coordinates on earth (Silvy, 2012). There are three types of errors that can occur with GPS positioning accuracy: errors with signal propagation, positional error from the geometry of satellite constellations known as Dilution of Precision (DOP), and errors from the unit itself (Specht, 2022). DOP is a non-dimensional coefficient that indicates the impact of satellite distribution on the precision of a GPS unit; lower DOP means better precision and accuracy (Specht, 2022). Horizontal Dilution of Precision (HDOP) is a type of DOP that measures position and time on a horizontal plane (Specht, 2022). A low HDOP is generated when widely distributed satellite signals overlap, allowing the GPS unit to receive transmissions from different angles versus closely spaced satellites transmitting a signal from one angle, thus improving precision and accuracy and reducing Location Error (Specht, 2022). Location error is a common error that occurs in GPS technology and radiotelemetry and is defined as a distance between a recorded position and the actual position of the recording unit (Paden & Andrews, 2020; Silvy, 2012). Location error can be quite large in radiotelemetry, averaging 128 m (Gilsdorf et al., 2008), compared to GPS units, where location error can average 10.9 m (Stemle et al., 2022).

III.II. Hypothesis

The first hypothesis was that the GPS-tracked gopher tortoise would exhibit a home range size greater than 1.00 ha based on home range sizes of adult male gopher tortoises (Diemer, 1992b; Eubanks et al., 2003; McRae et al., 1981a). The second hypothesis was that straight-line distance movement would be observed to be greater than 500 m since male gopher tortoises were documented to exhibit long distance movement (Guyer et al., 2012). The third hypothesis was that the gopher tortoise would exhibit nocturnal movement between 20:00 hrs – 05:00 hrs. The hypotheses were based on three premises:

1. Adult gopher tortoises, especially male, have been documented to travel long distances of up to 2.6 km (McRae et al., 1981a) and move 267 m within 24 hours (Diemer, 1992b). The tortoise tracked at Moody Air Force Base (MAFB) was an adult male.
2. Theodorakis et al. (2017) analyzed the effect of military activity and habitat quality on DNA damage and oxidative stress on gopher tortoises. They found lower quality habitat increased stress with increased military activity, while *higher quality* habitats decreased stress with increased military activity. The area of interest in this study was near areas of loud noise: 1,300 m northeast of an active air strip, 35 m west of a designated training area for response to chemical, biological, radiological, and nuclear threats, where explosions were simulated, and two miles east, A-10 Warthog gunfire for military training. The gopher tortoise at MAFB may move to other areas of the base to find better habitat relative to quality and military activity.
3. Pike and Grosse (2006) were the first to document nocturnal activity in hatchling and juvenile gopher tortoises, showing tortoises emerged after a heavy summer rainstorm to drink water collected on the ground.

III.III. Materials and Methods

III.III. I. Study Site

The study site was in Moody Air Force Base (MAFB), Lowndes and Lanier Counties, Georgia, USA, 10 miles northeast of Valdosta (30.969722°N, 83.196583°W) (Figures 25 – 26). The site is a military installation that is approximately 4856 ha with gopher tortoises distributed throughout its range (Figure 27). The area of interest (AOI) was 500 m around the capture and release point of the GPS-tracked tortoise (30.9873530°N, 83.1788890°W) in 23 Civil Engineering Squadron (CES) / Civil Engineering Operation (CEO) vegetation debris drop-off site, north of 23 CES/CEO separated by a fence is the Atkinson's Property (AP) with resident tortoises, east of 23 CES/CEO is an open field used as an area for designated training, southeast of 23 CES/CEO is an open clear-cut area surrounded by forest, south of 23 CES/CEO is Eismann road and MAFB Natural Resources Support Facility, and west of 23 CES/CEO is an additional forested area and an agricultural field (Figure 28). The AOI contained a variety of vegetation but was dominated by loblolly pine (*Pinus taeda*), brambles (*Rubus* spp.), and greenbriers (*Smilax* spp.).

III.III. II. GPS Backpack Installation and Tortoise Release

The equipment used to track the gopher tortoise was a waterproof Q4000ER small GPS collar (Backpack) (Figure 29a) and a long-range Base Station transceiver (Transceiver) to download data (Figure 29b), software called "Collar SW v2.92" (CollarSW) programmed the Backpack, downloaded data from the Transceiver and exported data (Telemetry Solutions, Concord, California). Telemetry Solutions noted that as of May 2018, the Transceiver had an effective download radius of up to 30 km away in ideal conditions (unobstructed, open sky) (Q4000ER, 2019).

The test subject used for this study was an adult male gopher tortoise, named "TnT" (Figure 30). TnT was an adult when he was first captured on July 4, 2001, at Moody Air Force Base (MAFB) and was PIT-tagged. He was last handled in 2003 and has not grown since his last capture. He was estimated to be greater than 35 years old at the time of this study. Body measurements: straight carapace length = 289.56 mm, carapace width = 218.44 mm, plastron length = 304.80 mm, plastron width = 185.42 mm, shell height 134.62 mm, bodyweight = 4.98 kg.

On September 22, 2020, the Backpack was attached to TnT's carapace. The Backpack was placed on the left anterior costal scutes 1 and 2 and the top of left anterior marginal scutes 21 and 22 and set with Gorilla Weld Heavy Duty Mix and Gorilla Epoxy (Gorilla Glue Inc., Cincinnati, Ohio) (Figure 31a). The Backpack's antenna was super glued (The Original Super Glue Corporation) around the carapace from left marginal scutes 23 and 25 and anterior vertebral scute 1 to right costal scute 3 (Figure 31b). Then, in three areas along the carapace, starting with the first 25 mm of wire from the base of the Backpack to the end of the antenna, the following scutes had the wire Gorilla Epoxied and Gorilla Welded: (1) left anterior marginal scutes 23 and 24 and anterior vertebral scute 1, (2) right anterior costal scute 1, and (3) between right anterior costal scutes 2 and 3. After Backpack installation, TnT was released back to the burrow where he was initially captured for this study.

The Backpack was programmed with CollarSW and multiple settings were applied before GPS deployment. There were two modes that could be used, Template and Repeat mode. Template mode acquired locations on a unique schedule, while Repeat mode acquired locations based on an interval between 2 minutes up to 48 hours 49 minutes, frequent intervals affected battery life; Repeat mode was used with a 2-hour interval. GPS Timeout was a function that turned off the GPS after a set amount of time if GPS satellites had not been acquired and if GPS location was not calculated, timeout could affect the GPS success rate; 60 seconds was used for GPS Timeout, and it was recommended for open habitat (Q4000ER, 2019). The Backpack also had a Temperature Sensor that recorded temperature in Celsius every time a GPS location was attempted; the sensor was active during deployment. A pre-test for GPS accuracy (location error) was recommended prior to deployment, but it was not required, therefore, it was not performed. A magnet on the Backpack was the on/off switch, removing the magnet turned on the Backpack. The magnet was removed from the Backpack on September 22, 2020 at 06:19 hrs. Based on the GPS schedule applied to the Backpack, CollarSW projected battery life to be between 6 to 9 months.

To substitute location error determination from Backpack pre-test, literature on Horizontal Dilution of Precision (HDOP) and gopher tortoise studies factoring GPS location error were reviewed to approximate location error. Specht (2022) attempted to establish a numerical relationship between HDOP and GPS position error (Location Error) using 900,000 GPS fixes. The study found that in optimal conditions, where the GPS unit was unobstructed by its surrounding environment, GPS position error values correlated with HDOP values. HDOP 1.0 was maintained with a mean of 8.749 satellites and had a R95 (radius of a circle where 95% of values fall within) accuracy of 2.889 m. Paden and Andrews (2020) found that the mean location error for GPS loggers used on gopher tortoises increased from a mean Location Error of 17.34 m from burrow surface to 69.98 m at 2.0 m burrow depth, and the success rate of GPS fixed locations decreased the deeper a tortoise burrowed. Stemle et al. (2022) reported their GPS tags to have a mean location error of 10.9 m after filtering out fixed locations with elevation error <17.2 m, minimum of five satellites and HDOP values ≤ 2. TnT's total fixed locations had a mean HDOP 3.25 and mean number of satellites fixed (SattFix) of 4.73, but if fixed locations were filtered by HDOP ≤ 1.5, then the mean HDOP was 1.259 and mean SattFix of 6.851. Therefore, when compared to the literature, location error was estimated to be between 2.88 m to 17.34 m depending on the fixed locations' HDOP and SattFix.

III.III. III. GPS Backpack Retrieval, Removal, Data Extraction and Analysis

Around January 2021, the Transceiver stopped collecting data from the Backpack. It was later assumed the battery had been depleted and TnT needed to be recaptured to remove the Backpack and retrieve any remaining data. The last set of fixed locations that were collected from TnT was between September 23 to November 12, 2020. The dataset was mapped on ArcGIS Pro 3.1.2 and TnT's last known fixed location was in a forested area adjacent to the open clear-cut land southeast of 23 CES/CEO, which was surveyed for his presence. TnT was found in a burrow at 30.984611°N, 83.177083°W, recaptured on April 29, 2021 and placed in a holding crate outside of the Moody Air Force Base's Natural Resources Support Facility. The Backpack was removed from TnT's carapace with a DREMEL® 300 Series, Model 300-25 Hobby (Dremel). TnT was released back to the burrow where he was recaptured.

The Backpack was returned to Telemetry Solutions to extract any remaining data. A text file after data extraction was received with raw data containing the following information: date, time, ID (fixed location identification number), time to fix (TTF), latitude and longitude in degrees/minutes/seconds, altitude, maximum satellite strength (Maxsnr), horizontal dilution of precision (HDOP), vertical dilution of precision (VDOP), number of satellites (SattFix), 2D/3D location acquisition, GPS battery voltage (V1), temperature (T1) (Table 2). The data was stored in a World Geodetic System, 1984 (WGS-84) format. The coordinates were converted from DMS to decimal degrees to allow for analysis in ArcGIS Pro version 3.1.2. *Note:* The Backpack was not pre-tested for precision and accuracy prior to Backpack installation, thus, data collected from the Backpack was examined as location estimations.

Data was analyzed for home range greater than 1.00 ha, straight-line and non-straight-line distances greater than 300 m in a 24-hour period, and nocturnal movement based on time between sunrise and sunset (20:00 hrs – 05:00 hrs) with ArcGIS Pro version 3.1.2, geographic coordinate system: World Geodetic System, 1984 (WGS-84).

GPS fixed locations were subsampled by Horizontal Dilution of Precision (HDOP) values less than or equal to 1.5, 2.0, 3.0, 5.0 and by all values (range: 0.9 – 9.9) to approximate data precision. Fixed locations were plotted, and minimum convex polygons (MCP) were created to evaluate home range extent based on HDOP subsamples. The "Minimum Bounding Geometry" tool (toolbox category: "Data Management Tools", toolbox: "Features") was used to create all MCPs; Parameters - Geometry type: "Convex hull", Group Option: "All". Acreage in hectares (ha) of MCPs was calculated by adding a field ("Add Field") in the MCP's attribute table, Parameters - Field Name: "Acres", Data Type: "Double", then "Calculate Geometry" in "Acres" field column; Parameters - Property: "Area (geodesic)", Area Unit: "Hectares".

Kernel density estimation (KDE) was used to evaluate the core area within the home range based on all fixed locations. The "Kernel Density" tool (toolbox category: "Spatial Analyst Tools", toolbox: "Kernel Density") was used to create the KDE plot; Parameters – Population field: "None", Output cell size: automatically generated, Search radius: "8.8", Area units: "Square meters", Output cell values: "Densities", Method: "Geodesic"; Environments – Processing Extent: "Current Display Extent". KDE symbology was reconfigured; Primary symbology: "Stretch", Mask column: toggle "Display background value" with no color, Color scheme: "Spectrum by Wavelength – Full Bright", Stretch type: "Standard Deviation", Number of standard deviations: "2". KDE plot was smoothed by "Raster Layer" tab, Rendering - Resampling Type: "Bilinear".

Straight-line and non-straight-line distances were measured using "Measure" tool in "Map" tab, Mode: "Geodesic", Display unit: "Meters". Radial buffers around fixed locations were created using the "Buffer" tool (toolbox category: "Analysis Tools", toolbox: "Proximity"); Parameters – Distance: Linear Unit "Meters", Method: "Planar", Dissolve Type: "No Dissolve".

III.IV. Results

The Backpack attempted a total of 2298 GPS fixed locations between September 22, 2020 and January 4, 2021; 1206 of the attempts contained the correct date/time/ID/TTF/V1/T1, and 263 (N = 263) of those attempts were complete lines of data that had GPS fixed location data (latitude and longitude coordinates), altitude, Maxsnr, HDOP, VDOP, and SattFix (Table 2). The fixed location success rate of the Backpack was 11% (263/2298). Only the complete lines of data were considered for data analysis. The successful FL data ranged from September 23, 2020 to January 2, 2021.

The amount of fixed locations (FL) collected varied in date, time, and temperature. October and November contained the most FL, N = 131 and N = 94, respectively, while readings were drastically reduced by December (N = 9) and terminated the first week of January 2021 due to battery depletion. In a 24-hour period, most FL (N > 20) occurred between 16:50 hrs and 22:50 hrs, peaking at 18:50 hrs (N = 46), mean 37 FL, and the least amount of FL (N < 20) occurred between 00:50 hrs to 14:50 hrs, peaking at 00:50 hrs (N = 16), mean 14.37 FL. Temperature at FL collection was mean 26.78°C (80.22°F), most FL were in warmer temperature ranges, 21.11 – 26.11°C (70 – 79°F) (N = 123) and 26.66 – 31.66°C (80°F – 89°F) (N = 55). Warmer temperature ranges (temperature >21.11°C (70°F)) were recorded mostly in October and November.

The location data had mean time to fix of 35.05 seconds, mean Horizontal Dilution of Precision (HDOP) 3.25, and mean number of satellites fix number of 4.73. The number of fixed locations and home range size increased proportionately to HDOP (Table 3). Minimum convex polygons (MCP) were created to visualize home range extent and variation in fixed location coordinates for each HDOP value cutoff (Figure 32). MCP home range size of TnT was 3.55 hectares based on HDOP ≤ 1.5, doubling with HDOP ≤ 5.0, and increased fourfold with all fixed locations were considered (Table 3).

A kernel density estimation was generated to visualize the density of fixed locations across the area of interest using all fixed locations. A majority of the fixed locations were concentrated in a forested area with an approximate center at 30.9846611°N, 83.1771028°W (Figure 33) and occurring from October 1 – 21, 2020, to October 21, 2020 and November 7, 2020 – January 2, 2021. This was presumed as his core area, an area within a home range where an animal used the most (Feldhamer at al., 2015), and henceforth referred as "Residence". The Residence was approximately 0.13 ha. There were five periods of time where TnT was not within his Residence and traveled straight-line and non-straight-line distances greater than 300 m. From September 23, 2020, at 18:50 hrs to September 24, 2020, at 20:50 hrs, TnT traveled the first time to his Residence (Figure 34), non-straight-line distance 408 m (mean HDOP 1.8). On September 26, 2020, at 14:50 hrs, he traveled 361 m non-straight-line distance north (mean HDOP 1.78) and crossed Atkinson's Property line, stayed until October 1, 2020, 18:50 hrs, where he traveled 291 m straight-line distance south across Atkinson's Property line and stopped at his Residence at 22:50 hrs (Figure 35). On October 21, 2020, from 18:50 hrs to 20:50 hrs, he traveled 345 m straight-line distance north, crossed Atkinson's Property line, no Fixed Locations occurred between October 28 and November 5, then on November 6, 2020, at 18:50 hrs, TnT traveled 392 m non-straight-line distance south (mean HDOP 1.56), his final time across the Atkinson's Property line and back to his Residence (Figure 36).

The data was filtered for nocturnal activity. Nocturnal activity was defined as movement occurring between 20:00 hrs and 05:00 hrs in a 24-hour period, or time between sunset to sunrise. Any set of fixed locations that occurred within this period were defined as a "Nocturnal Event" (NE). A max of 5 fixed locations were possible within one NE based on the GPS schedule programmed into the Backpack. NE was not filtered by HDOP values. There were 41 NE that occurred between September 23, 2020 and January 1, 2021, totaling 102 fixed locations. The overall average temperature for NE was 26.06°C (78.92°F), the highest temperature was 37.15°C (98.88°F) on October 13, 2020, at 20:50 hrs, and the lowest temperature was 14.07°C (57.34°F) on December 6, 2020, at 22:50 hrs. The mean temperature for NE in September was 29.37°C (84.87°F) (N = 10), October was 27.90°C (82.23°F) (N = 51), November was 23.35°C (74.04°F) (N = 36), December was 18.83°C (65.91°F) (N = 3), and January was 22.45°C (72.41°F) (N = 2).

To gain a broader understanding and to illustrate TnT's movement activity, fixed locations before and after these Nocturnal Events (NE) were included to see if TnT was moving within his Residence, possibly moving between burrows, or traveling beyond his Residence. Minimum convex polygons (MCP) were used to define the area that TnT moved within each NE. Only 24 of the 41 NE could produce an MCP, other NE had less than or equal to 2 fixed locations, failing to meet the MCP minimum requirement of 3 fixed locations to produce an MCP. Most of the NE occurred within TnT's Residence, but there was one NE that occurred beyond his Residence (Figure 37). Nocturnal movement beyond TnT's Residence was on September 26, 2020, at 14:50 hrs, TnT traveled north, and stopped at 22:50 hrs, arriving on Atkinson's Property (Figure 38). The maximum estimated location error of 17.34 m was applied to all fixed locations to illustrate non-overlap between fixed locations.

III.V. Discussion

The objective of this study was to field test GPS technology on an adult gopher tortoise, particularly on a military base, and evaluate the practicality of using this tool to monitor gopher tortoise activity. The Q4000ER small GPS collar used on TnT had a battery life that lasted approximately 3 months after deployment, 3 – 6 months short of the projected battery life. The Backpack had 2 built-in mechanisms that ended the battery's life prematurely. The first mechanism was time to fix (TTF). All fixed locations that did not contain location data (latitude and longitude coordinates) had a max TTF of 60 seconds, while the average TTF for all fixed locations with location data was 35.05 seconds. This meant that every 2 hours, when the Backpack attempted a fixed location, it waited 60 seconds searching for a fixed location before turning off, whereas it turned off at an average of 35.05 seconds once a fixed location with location data was recorded. If TnT was above ground more often, more fixed locations with location data could have been collected, reducing fixed locations with max TTF, and prolonging battery life, but it was most likely TnT's burrowing that led to the high frequency of fixed locations with max TTF. Paden and Andrews (2020) demonstrated that Fixed Success Rate (equivalent to fixed locations) decreased as gopher tortoises moved deeper into their burrow and contributed to battery depletion.

The second mechanism influencing battery life was the Backpack synchronizing its internal clock to satellites. It automatically attempted fixed locations every 20 minutes until fixed locations was acquired, which in turn, allowed its internal clock to sync with data. The Backpack's internal clock was disrupted after December 25, 2020 at 14:49 hrs, then corrected itself on December 28, 22:05 hrs, then final a disruption occurred after January 04, 2021 at 06:05 hrs. The Backpack attempted a GPS position every 20 minutes afterwards until the battery expired. It was unknown what caused the internal clock disruption. These were the only mechanisms recognized causing early battery life termination, but there could have been other unknown malfunctions. Despite the short battery life, a total of 263 fixed locations with location data were collected.

The analysis of the 263 fixed locations revealed movement behavior that exceeded the first hypothesis of home range greater than 1.00 ha, failed to meet the second hypothesis of long distance movement greater than 500 m, and meeting the third hypothesis of observation of nocturnal movement between 20:00 hrs and 05:00 hrs. Most fixed locations occurred between 16:50 hrs and 18:50 hrs, this was expected since gopher tortoises avoided extreme temperatures that occurred at 13:00 hrs – 15:00 hrs (McRae et al., 1981a), but a higher number of fixed locations at 20:50 hrs (N = 37) and 22:50 (N = 24) was an unusual observation because nocturnal movement activity exhibited by gopher tortoises is uncommon. Except for some of the Nocturnal Events (NE) discussed later in this section, most of these NE were possibly short distance movements between burrows or idle presence on burrow openings. TnT exhibited more movement activity in October and November than December, which was expected as frequency of activity has been documented to decrease with cooling temperatures (McRae et al., 1981a).

Burt (1943) described home range as the area an individual uses for its normal activities of food gathering, mating, and caring for young, where occasional explorations outside of this range should not be considered as part of their range. It was hypothesized that TnT's home range would be greater than 1.00 ha. TnT's home range was revealed to be 3.55 ha based on a minimum convex polygon generated by fixed locations with HDOP ≤ 1.5. Multiple studies on gopher tortoises reported adult male home ranges as low as 0.06 ha (McRae et al., 1981a) to as high as 5.3 ha (Smith et al., 1997), while other studies had shown home ranges between 0.22 – 2.90 ha (Diemer, 1992b; McLaughlin, 1990; Wright, 1982).

Variations in home range estimations across gopher tortoise studies could be attributed to tracking equipment, sampling intensity (rate), and home range analysis method. Often occurring in radiotelemetry, low sample sizes (<100 locations) could lead to underestimations in minimum convex polygons (MCP) generated for home ranges (Kolodzinski et al., 2010). Stemle et al. (2022) concluded that GPS technology had a 10-fold increase in data collection compared to radiotelemetry and revealed home ranges 6.6-fold larger than what was previously reported in other studies for immature gopher tortoises. Kolodzinski et al. (2010) asserted that MCP required a large sample size (≥100 locations) to accurately define a home range. In this study, GPS technology recorded 263 fixed locations, a sample size greater than what was required to accurately define a home range according to Kolodzinski et al. (2010), but it was also demonstrated that when data was filtered by HDOP, MCP's generated for home ranges increased four-fold from the lowest HDOP cutoff (Figure 32 and Table 3). This suggests possible home range overestimations if data collected from GPS technology is not filtered for accuracy or precision since greater inaccuracies in GPS position occur at higher HDOP; HDOP values less than one were the best indicator for lower Location Error (Moore et al., 2023; Specht, 2022). In cases of low sample availability and MCP cannot be implemented, kernel density estimations could be used as alternative, where a low sample size (≥15, ≥30, ≥50 locations) could accurately estimate home range size, but could be prone to overestimation at lower sample sizes (Kolodzinski et al., 2010; Seaman et al., 1999).

A kernel density estimation (KDE) of all fixed locations revealed a core area (Residence) in the southeastern portion of the area of interest. A core area is defined as a space utilized the most within a home range containing a nest, sleeping area, water source, or feeding site for an animal (Feldhamer et al., 2015). KDE is a nonparametric method, may be argued a better method for home range analysis since an animal's location data may not fit a set of assumptions in parametric methods (Seaman et al., 1999; Worton, 1989). In home range analysis using KDE, density is the amount of time an animal spends in an area (Seaman & Powell, 1996). Although minimum convex polygons can outline the extent of a home range, it may not be as biologically relevant as KDE, *i.e.*, showing a core area, and revealing areas of use and non-use (Row & Blouin-Demers, 2006).

Minimum convex polygons (MCP) and kernel density estimations (KDE) analyses are consistently used in home range studies across species, but there have been growing concerns with their use over the past two decades. MCP and KDE methodologies vary heavily in their application to movement data (or lack thereof) in herpetofauna studies (making inter-study comparisons ineffective) (Laver & Kelly, 2008). MCP and KDE were also posited as outdated approach to home range analysis (Crane et al., 2021; Row & Blouin-Demers, 2006) with the introduction of new movement analysis models (Brownian Bridge Movement Models, dBBMM) to accommodate newer technology (Silva et al., 2020). Additionally, the novel approach of GPS technology on gopher tortoises in this study was limited in comparison to other studies using similar methods. Therefore, in this study, KDE of TnT's fixed locations were included as an alternative method to data visualization and interpretation of movement behavior compared to MCPs. The search radius of 8.8 m was applied to all FL based on the mean daily movement results of adult male gopher tortoises in Metcalf et al. (2023) (mean 8.8 m), creating a density relative to the overlap of fixed locations, or ratio of points present in a radius (Figure 33).

It was hypothesized that TnT would travel a straight-line distance greater than 500 m from his release location based on the two premises: (1) previous literature of long-distance movement greater than 500 m exhibited by adult male gopher tortoises and (2) seeking different habitat relative to habitat quality and presence of military activity (Figure 28). The first premise was not met, the greatest straight-line distance TnT traveled was approximately 350 m from his release location at 23 CES/CEO (30.9873528°N, 83.1788333°W; HDOP 1.3) to the southern edge of his Residence (30.9845833°N, 83.1770861°W; HDOP 1.4). However, when fixed locations with HDOP > 1.5, non-straight-line distances, and initial starting points unrelated to release location were considered, there were a total of five periods of time TnT traveled greater than 300 m, where one non-straight-line distance was 408 m (Figure 34). Travel distances greater than 500 m in adult male gopher tortoises have been reported in previous studies. Eubanks et al. (2003) documented two migrant gopher tortoises traveling long distances, one moving 346 m between two consecutive locations ending with a straight-line distance of 1.2 km, while the second tortoise traveled 1085 m between two consecutive locations ending with a straight-line distance of 1.5 km. Eubanks et al. (2003) suggested this movement could be related to patterns of reproductive behavior and hormone cycles as established in previous studies (Diemer, 1992b; McRae et al., 1981a; Ott et al., 2000), where males try to maintain contact with females for reproductive purposes. Guyer et al. (2012) made some observations regarding long distance movement: (1) movement increased with decreasing burrow density to maintain contact with neighbors; (2) capacity for strong spatial memory and knowing locations of their distant neighbors, especially females, where one female isolated up to 800 m from their nearest 3 neighbors was observed mounted by a male. These suggestions (Guyer et al., 2012) may be plausible reasons for TnT's long-distance travel behavior.

The first period of long-distance travel was September 23-24, TnT traveled south from his release site to his Residence (Figure 34). Approximately 400 m south of his Residence, a high density of gopher tortoise burrows was documented (Figure 27). TnT may have made this expedition to interact with neighbors, especially females, for breeding purposes, but there was no fixed locations in the high burrow density area. Most of the fixed locations were between October and November, outside of the peak breeding season, which was April to June (McRae et al., 1981a). It is possible that this behavior is related to maintaining contact rather than breeding with females. Another suggestion could be that TnT was moving to a burrow he only occupied in the winter season. McRae et al. (1981a) documented adult tortoises migrating to winter burrows in the autumn, suggesting TnT's Residence was an area only occupied towards the fall and winter months before returning north to his original capture location during the breeding season. GPS monitoring in the months following winter would reveal more insights into TnT's movement behavior.

Another suggestion for TnT's long-distance movement south between September 23-24, 2020, was seeking higher quality of habitat. Theodorakis et al. (2017) measured genotoxicity and oxidative stress (lipid peroxidation, glutathione and glutathione dimer ratios, DNA damage) in gopher tortoises at Camp Shelby, Mississippi, an approximately 52,500 ha military installation. At sample sites where gopher tortoises were captured for the study, the habitat was rated as either high quality or low quality and level of military activity was rated high to no activity. They characterized "high quality" habitat as open canopy, abundant herbaceous understory, and sandy soils for burrowing, while the "low quality" habitat was the opposite: closed canopy, absence of herbaceous understory, and non-sandy soils. "High" military activity were areas with active artillery ranges or demolition areas (those that discarded unused ammunition and explosives), "Low" activity were areas adjacent to "High" activity and had increased noise levels, and "No" activity were restricted access areas that were approximately 12 km away from military activity. Their results showed that tortoises' oxidative stress increased with military activity in low-quality habitats, but also a counterintuitive result showed stress levels decreased in higher quality habitats with increasing military activity (Theodorakis et al., 2017). Theodorakis et al. (2017) suggested that this may be a compensatory response, or adaptation to military activity because needs were met in a preferred habitat where conditions were ideal for survival (*i.e.*, increased resource access).

The immediate surrounding environment of TnT's release site and Residence were compared. Upon reviewing both sites in-person, through photos and historical aerial imagery, it could be suggested that TnT was seeking a "high quality" habitat as defined by Theodorakis et al. (2017). His release site was between the edge of a forest and a debris drop-off site (Figure 28). The forested area had canopy and a dense shrub layer, while the debris site had piles of vegetation, tracks from vehicles and construction equipment, and disconnected patches of dead and growing ground vegetation. Approximately 35 meters east was a designated training area where loud explosions were simulated. On the other hand, TnT's Residence had access to large tracts of connected forestland with herbaceous ground cover, open canopy, less dense shrub layer and was adjacent to an approximate 1.00 ha clear cut land, providing an additional source of forage. The Residence was approximately 230 m south of the designated training area (Figures 32 – 33). An NRCS Web Soil Survey showed that both sites were composed of a majority of Tifton loamy sand, 2 to 5 percent slopes (soil code: TqB) (Soil Survey Staff, 2020) (Figure 39). Tifton loamy sand was classified as a well-drained soil with no frequency of flooding or ponding. Based on the characteristics of both sites and habitat descriptions of Theodorakis et al. (2017), the release site would be a low-quality habitat with low military activity and the Residence would be a high-quality habitat with low military activity.

Gopher tortoises prefer longleaf pine habitats with open canopy, abundant herbaceous ground cover, and well-drained sandy soils (Jones & Dorr, 2004; McCoy et al., 2006). Results suggest that TnT moved south to his Residence in search of higher quality habitat because the release site was poor quality: the dense shrub layer precluded the ability for herbaceous vegetation to grow due to limited sunlight, frequent vehicle traffic dropping off debris coupled with the training area likely responsible for increased ground disturbance and noise levels, and discontinuous forestland from one tract of land to another. These factors may have contributed to burrow abandonment from the initial release site (Jones & Dorr, 2004; Theodorakis at al. 2017). The Residence, on the other hand, had widely dispersed open canopy and less shrub density, leading to more sunlight reaching the understory for herbaceous growth, and increased foraging ability (Jones & Dorr, 2004). Large, continuous tracts of open forestland provide additional habitat availability and foraging opportunity, and in the case of TnT, increased likelihood of social interaction (Guyer et al., 2012) because of the high burrow density south of his Residence. Frequency of vehicle traffic at 23 CES/CEO was unknown and the noise levels from the designated training area were not measured or directly observed.

The other 4 periods of time that TnT exhibited long-distance movement were September 26, October 1, October 21, and November 6, 2020. He either crossed into and stayed or moved out of the Atkinson's Property. Two of these periods, he stayed within Atkinson's Property for a few days to over two weeks. The first stay was from September 26 to October 1 (Figure 35), only 5 fixed locations contained location data between these dates, while the other recorded fixed locations did not have latitude and longitude data. TnT was likely in burrow, preventing fixed locations from recording location data (Paden & Andrews, 2020) or in a location with obstructed view from the sky, preventing fixed locations from gathering data. The next stay was on October 21 to November 6, 2020 (Figure 36), where 16 fixed locations contained location data with up to 3 collected in one day, while the rest of the fixed locations did not have location data, again indicating that TnT was likely in a burrow or obstructed area. No fixed locations with location data were recorded between October 28 and November 6, likely due to previous suggestions of fixed location failure. In the other two periods, October 1 and November 6, TnT leaves Atkinson's Property to go to his Residence, completing his last visits to the Atkinson's Property (Figures 35 – 36).

To understand TnT's motives for crossing into Atkinson's Property, the property line between Moody Air Force Base and Atkinson's Property was investigated on March 21, 2021 and surveyed for gaps along the fence TnT may have used to cross. Then, with permission from Atkinson's Property landowner, the area TnT inhabited on Atkinson's Property was explored. Three gaps along the fence and two gopher tortoises were visually observed, one adult and one subadult gopher tortoise, both in their burrows residing on Atkinson's Property. These tortoises were approximately 100 m from TnT's release site point and appeared adjacent to TnT's fixed locations when he was present on Atkinson's Property (Figure 40). Based on these observations and movement literature (Guyer et al., 2012), the movement periods suggest TnT's fixed locations crossing into Atkinson's Property were possibly related to maintaining contact with other tortoises on Atkinson's Property.

Another suggestion for TnT leaving Atkinson's Property and returning to his Residence in the other two periods was to make up for the energy expenditure of his long-distance movement, accessing more herbaceous vegetation around his Residence versus resources available on Atkinson's Property. Guyer et al. (2012) suggested that male gopher tortoises incurred a movement cost when maintaining social relationships or finding mates in habitats with low burrow density, but it was difficult to quantify the actual energy expired for this travel as there was only one publication that explored the energy expended by gopher tortoises and movement cost was not investigated (Jodice et al., 2006). Vegetation on Atkinson's Property was not assessed, preventing comparison of ground vegetation to Residence, and if vegetation was examined, the energy deficit that needed to be made up for long distance movement by gopher tortoises is unknown. Therefore, it was unclear why TnT returned to his Residence on October 1, but for the November 6 departure, it was possibly related to returning to a preferred overwinter burrow (McRae et al., 1981a).

Evidence of possible nocturnal activity in gopher tortoises was documented in this study based on one movement occurring outside of TnT's Residence between 20:00 hrs and 05:00 hrs. The nocturnal movement was exhibited on September 26, 2020, one fixed location at 20:50 hrs (30.9866778°N, 83.1770306°W; 98.42°F; HDOP 2.4; number of satellites fixed (SattFix) of 4;

Time To Fix (TTF) of 14 seconds) and the other Fixed Location at 22:50 hrs (30.9878861°N, 83.1774111°W; 85.15°F; HDOP 1.6; SattFix of 5; TTF of 43 seconds) (Figure 38). Nocturnal movement was confirmed in this Nocturnal Event based on three reasons: (1) 140 m was the distance between each fixed location and based on HDOP values and its relation to location error provided earlier in the discussion, location error of 140 m is unlikely because HDOP was relatively low in the fixed location, (2) the maximum estimated location error radius of 17.34 m was applied to all fixed locations to illustrate a scenario of all fixed locations exhibiting maximum location error relative to HDOP, but no overlap occurred, indicating there was distance between points rather than concentration of fixed locations in one area like TnT's Residence, (3) temperature associated with each fixed location was in a warmer range that was typical of the range gopher tortoises conducted their daily activities (Douglass & Layne, 1978). When viewing fixed locations prior to the nocturnal event, two fixed locations stand out, one at 14:50 hrs and the other at 18:50 hrs, both fixed locations had HDOP value of 1.0, 7 SattFix, and TTF of 42 and 43 seconds. The distance between these two fixed locations was 123 m. The low HDOP value and high SattFix (> mean 4.73) suggested the locations recorded at both fixed locations were one of the most precise and accurate of fixed locations collected and were on the lower end of the estimated Location Error range (≥ 2.88 m), suggesting that TnT had at least traveled outside of his Residence traveling north.

Nocturnal activity in gopher tortoises has been documented in several studies. Douglass and Layne (1978) discussed gopher tortoise nocturnal activity, indicating that historically, the species was once described as a nocturnal animal back in the early 19th century and observations of nocturnal movement have been documented since then, however, their study also supported gopher tortoise as diurnal species. McRae et al. (1981a) did not observe any crepuscular or nocturnal activity over a 2-year period monitoring gopher tortoise activity patterns and asserted that movement was related to diurnal temperature cycles. Alexy et al. (2003) used infrared trail monitors on active gopher tortoise burrows that recorded 3 nocturnal events (two at 22:00 hrs and one at 23:00 hrs) of tortoises entering or leaving burrows. Pike and Grosse (2006) provided visual documentation of nocturnal activity in gopher tortoises with juveniles drinking water after a rainstorm. In other *Gopherus* species, Douglass and Layne (1978) mentioned observations of nocturnal activity occurring in the desert tortoise (*G. agassiziii*), but not in the Texas tortoise (*G. berlandieri*) or bolson tortoise (*G. flavomarginatus*). More recently, Agha et al. (2015) used motion sensor cameras on active *G. agassizii* burrows and documented 23 occasions of nocturnal activity, but the time frame was not provided. In other turtle species, Hjort et al. (2021) observed nocturnal movement (21:00 hrs – 06:00 hrs) in Blanding's turtles (*Emydoidea blandingii*) and spotted turtles (*Clemmys guttata*) and hypothesized that movements exhibited by males were related to foraging or mate-seeking. This hypothesis may be one reason for TnT's nocturnal movement behavior as it appeared his movement direction was going north to Atkinson's Property, where other gopher tortoises were present. Another suggestion would be avoiding temperature extremes, but the data does not warrant this as a possible explanation as TnT only exhibited long-distance nocturnal movement once.

This study was the first to examine diurnal and nocturnal movement data by a gopher tortoise with GPS technology, revealing more natural movement data. The GPS schedule used in this study monitored a gopher tortoise 24 hours a day at 2-hour intervals for approximately 3 months, while Paden and Andrews (2020) used a 1-hour interval collecting fixed locations between 08:00 hrs – 21:00 hrs for 3.5 – 6 months and Stemle et al. (2022) used a 30-minute interval collecting fixed locations between 09:00 hrs – 13:00 hrs for 40 days. This study only GPS-tracked one adult male gopher tortoise, while Paden and Andrews (2020) tracked 38 adult tortoises of both sexes and Stemle et al. (2022) tracked 6 immature tortoises. Thus, analyzing the movement behavior is limited.

Chapter IV

CONCLUSIONS AND RECOMMENDATIONS

Gopher tortoises are a long-lived species, but they have barriers of entry to long-term survival. The survival assessment at Reed Bingham State Park (RBSP) was one of many studies documenting the low survivability of gopher tortoise hatchlings. RBSP was a relatively ideal environment for hatchlings to survive, however, predators, human presence, and Upper Respiratory Tract Disease may have precluded their ability to reach adulthood. Additionally, it was possible that individuals survived, and recapture failure could have been attributed to their fossorial nature or dispersal to other areas outside of Gopher Tortoise Management Area and Pioneer Site, thus, evading detection. In the future, assessment studies at RBSP should consider exploring other parts of the park for gopher tortoise populations and a long-term monitoring program should be implemented to track population trends over the years.

Military land tends to have a disproportionately high amount of biodiversity. The threatened and keystone species status of gopher tortoises makes it necessary for Moody Air Force Base land and natural resource managers to determine habitat areas to avoid, mitigate, or to translocate gopher tortoises. This would aid in the planning phase of proposed developments on the installation by complying with federal and state conservation laws and preserving the integrity of its local ecosystems. In this study, GPS technology for tracking movement was a novel approach for monitoring gopher tortoise activity. It was advantageous for collecting quantitatively more data, showing movement patterns already established in the current literature, but also demonstrating movement directions through fixed interval location acquisitions and illustrating travel behavior during 24-hour periods, which revealed incidences of movement across property lines and nocturnal movement. GPS technology could be used as an alternative tool by military land managers to decide the extent of a gopher tortoise's home range and core areas as well as using the GPS data to reveal locations of other tortoises that were not previously known or detected in an area. However, more research needs to be done on this technology's practical applications because the benefits of this technology came with the cost of battery life, increased data complexity, and monetary constraint. Future considerations for studies utilizing GPS technology on gopher tortoises include:

1) Prior to deployment, GPS units should be pre-tested for types of data acquired and location error's relationship to other types of data, *i.e.*, Horizontal Dilution of Precision (HDOP).
2) Evaluate GPS unit specifications and features to determine appropriate methodological applications for study objectives.
3) Individuals should be radio-tagged to enable tortoises to be recaptured in the event of GPS battery depletion.
4) GPS data analysis should be handled with care regarding data filtration for accuracy and precision and analytical method chosen.
5) Successive studies focused exclusively on gopher tortoise home range and core area delineation using GPS technology should establish a minimum sample size to avoid under- and overestimations associated with minimum convex polygons and kernel density estimations as well as exploring other, newer movement analytical methods that may accommodate the data gathered from GPS technology.
6) Explore differences in movements by sex, age class, time of year, habitat type, and sampling rate (1-hour interval versus 4-hour intervals).
7) Investigate gopher tortoise movement in areas around Moody Air Force Base where high military activity occurs, *i.e.*, tortoises close to the active air strip and bombing range.

Collection and handling of gopher tortoises was in accordance with Valdosta State University Animal Use Protocol (AUP-00078-2021), Georgia Department of Natural Resources (GDNR) Scientific Collecting Permits 2020 – 2021 & 2021 – 2022, and GDNR State Parks & Historic Sites, Scientific Research and Collection Permit 2021 (Permit #062021) (see Appendix A – E).

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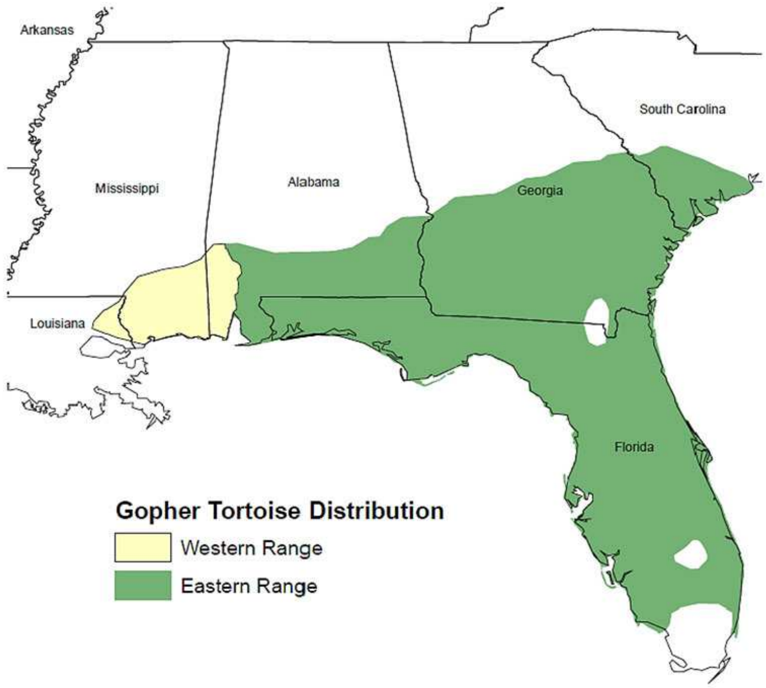

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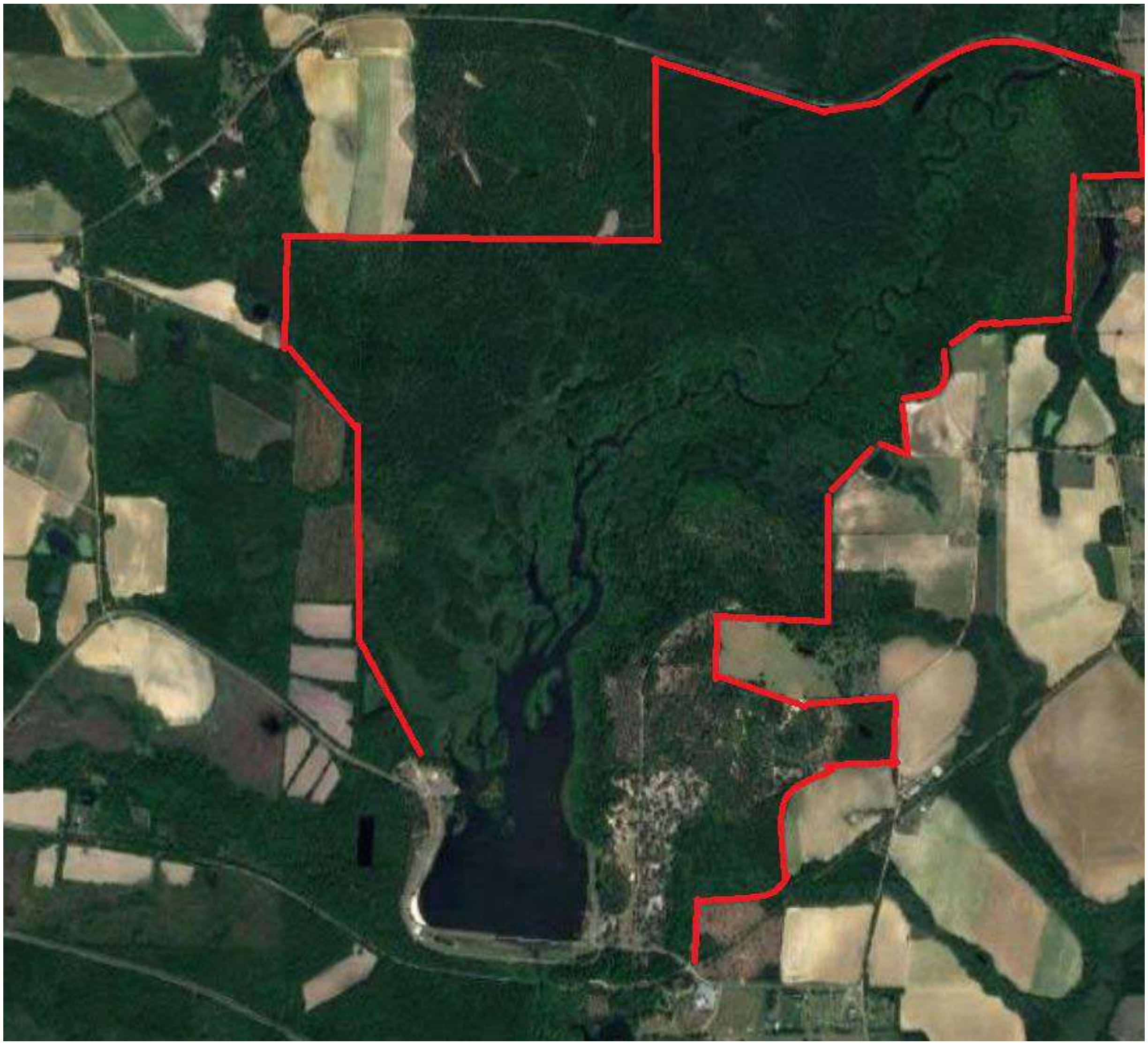

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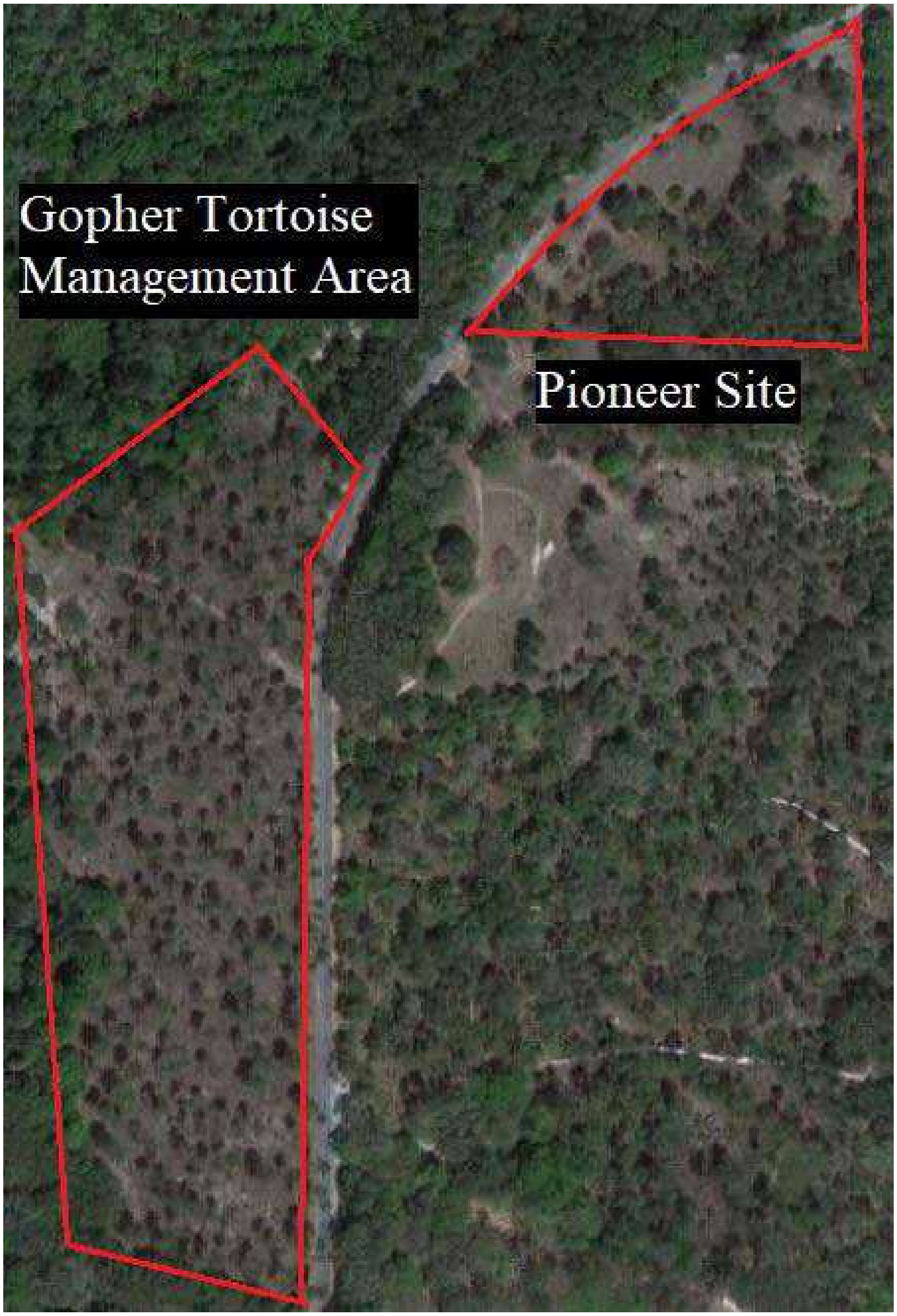

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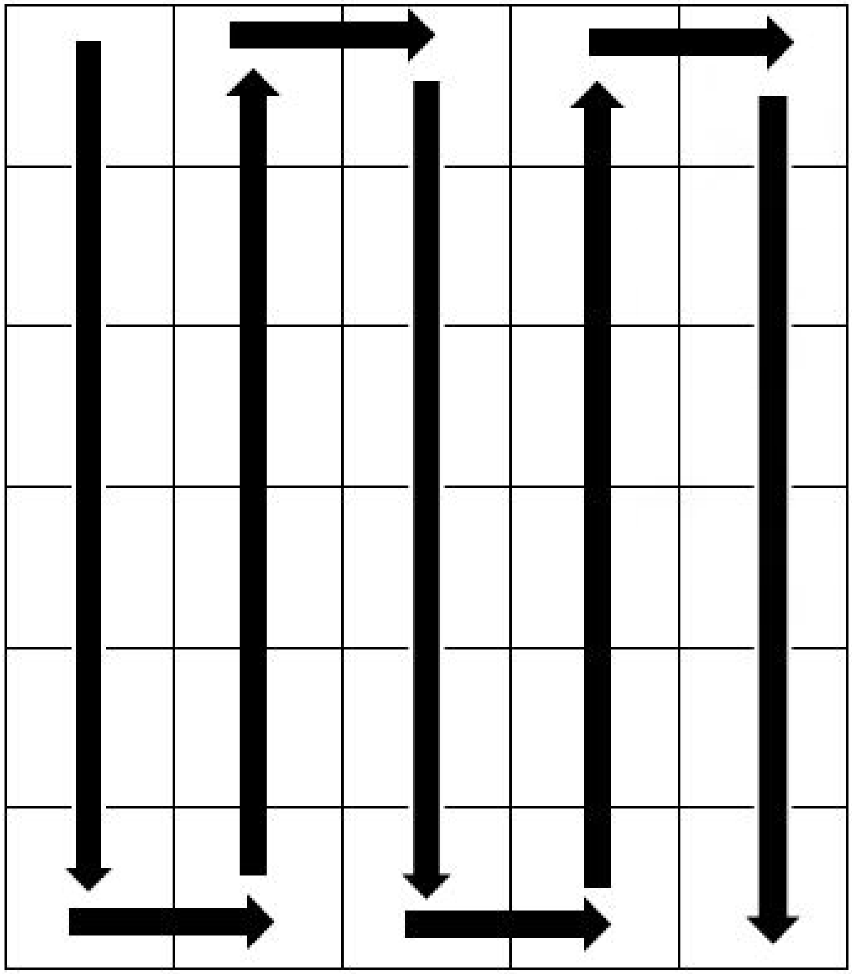

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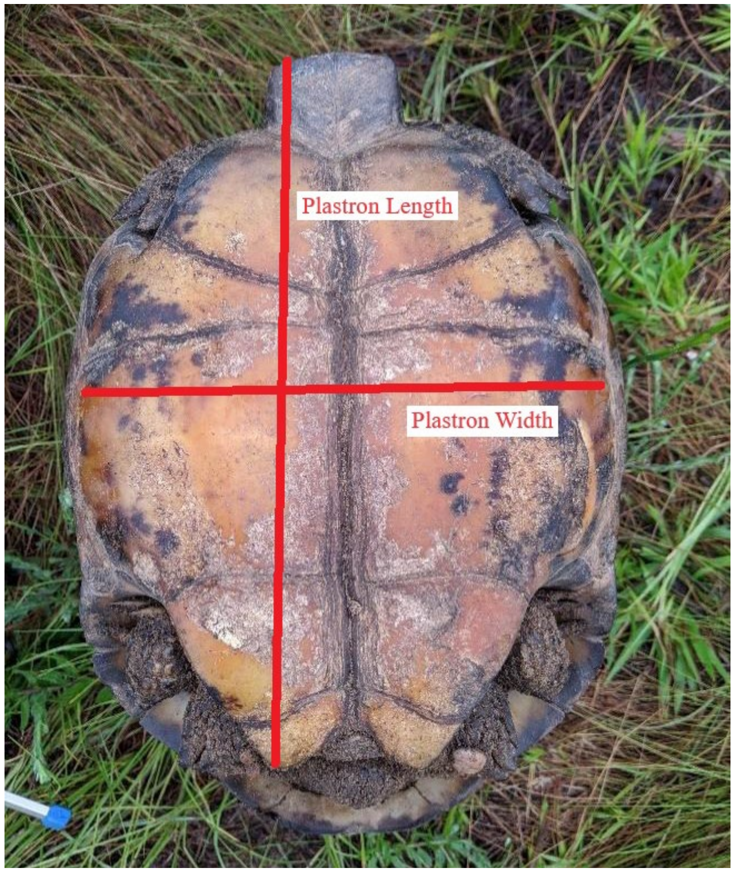
(a) 

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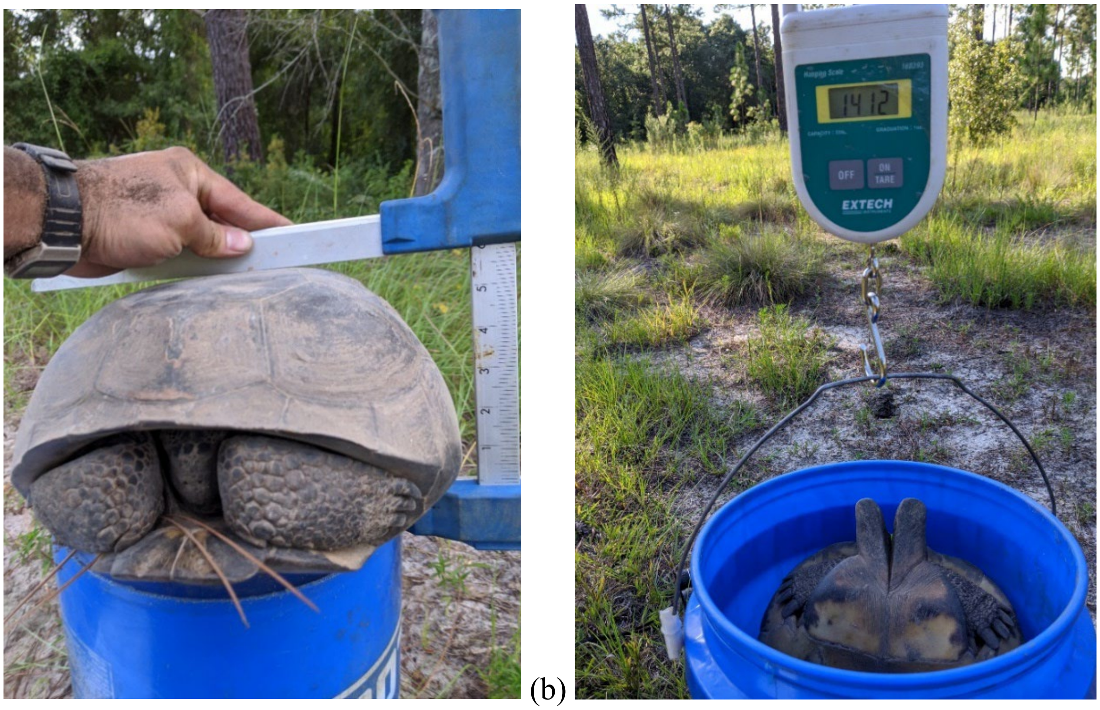

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(a)

(b)

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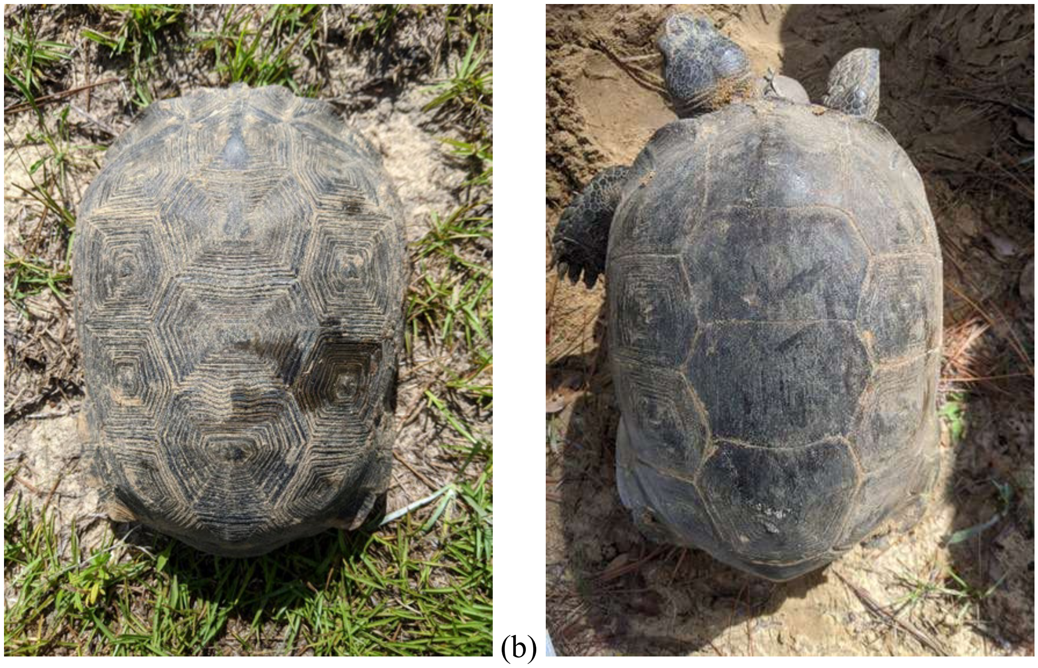

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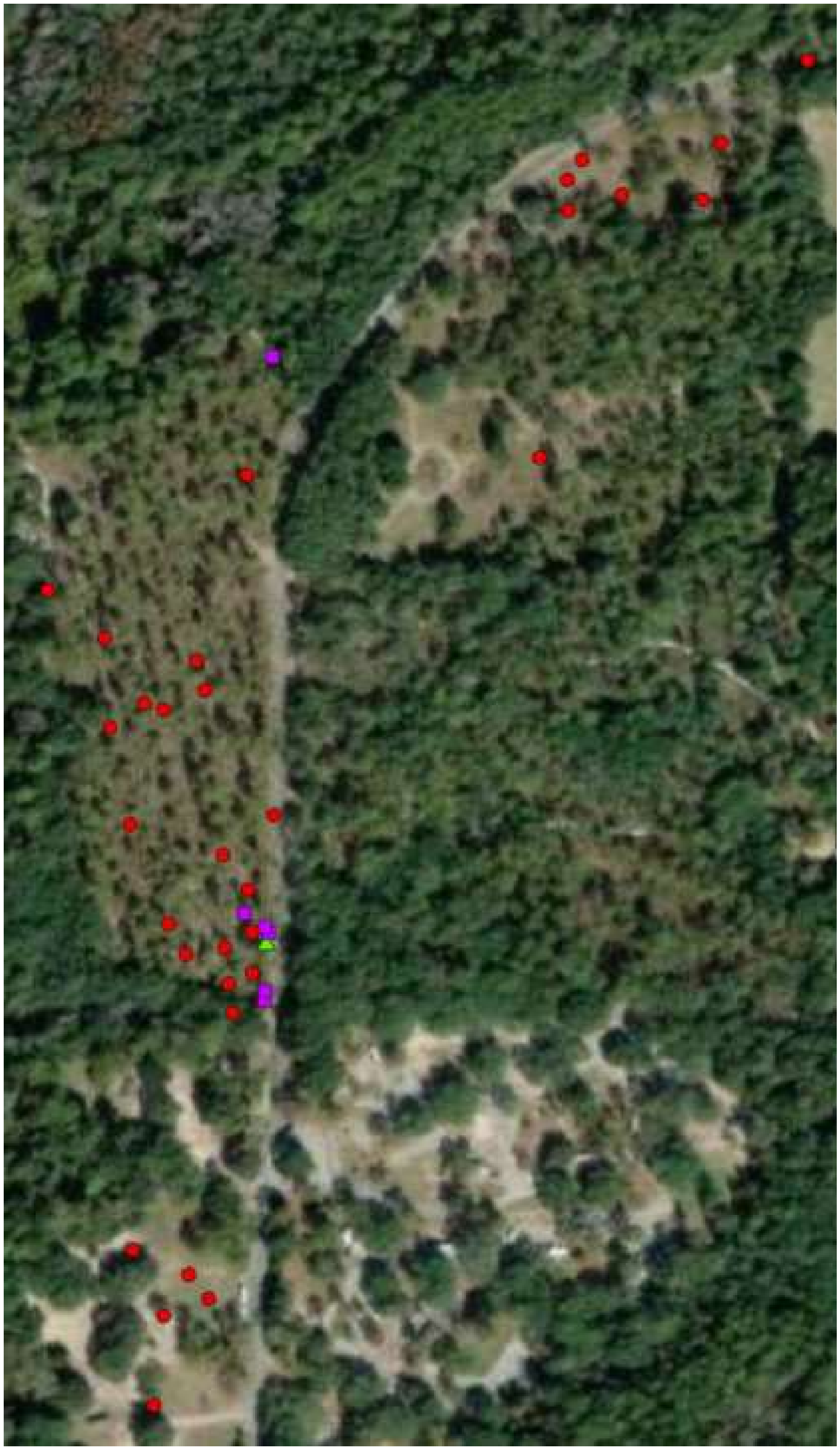

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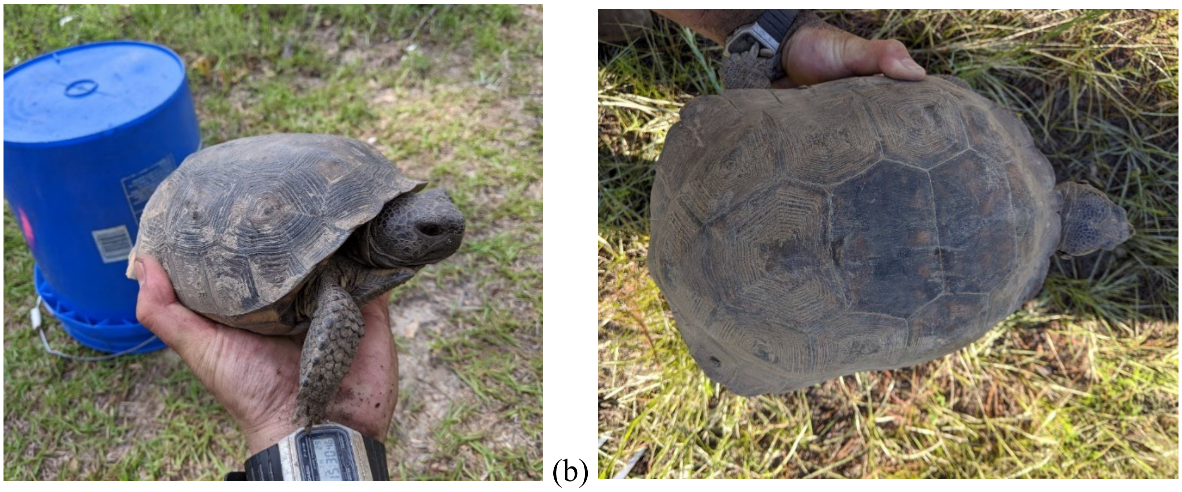
(a)  (b)

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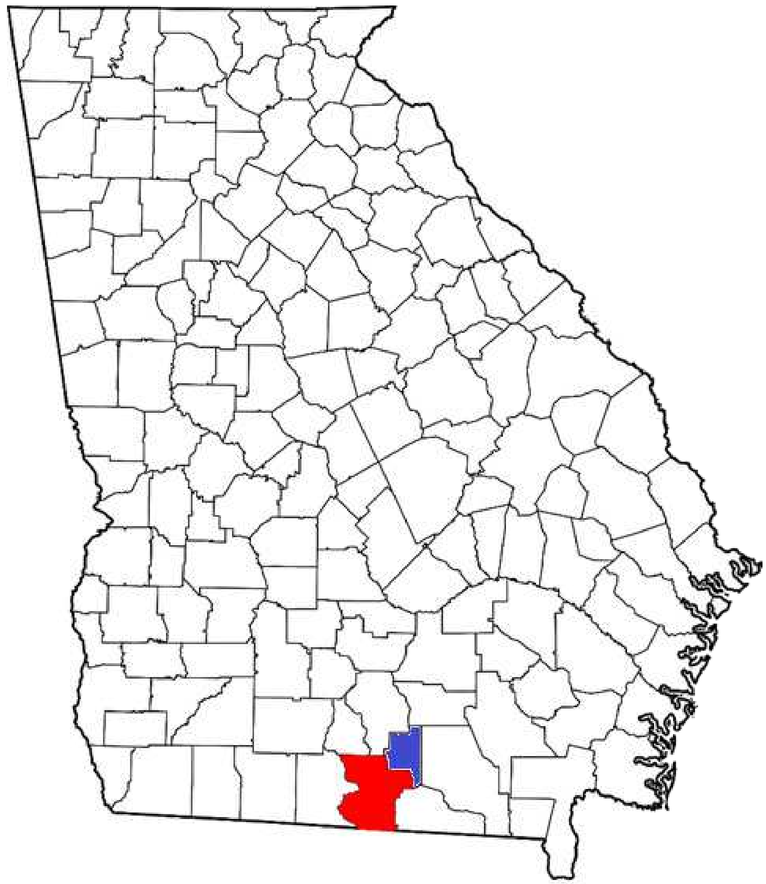

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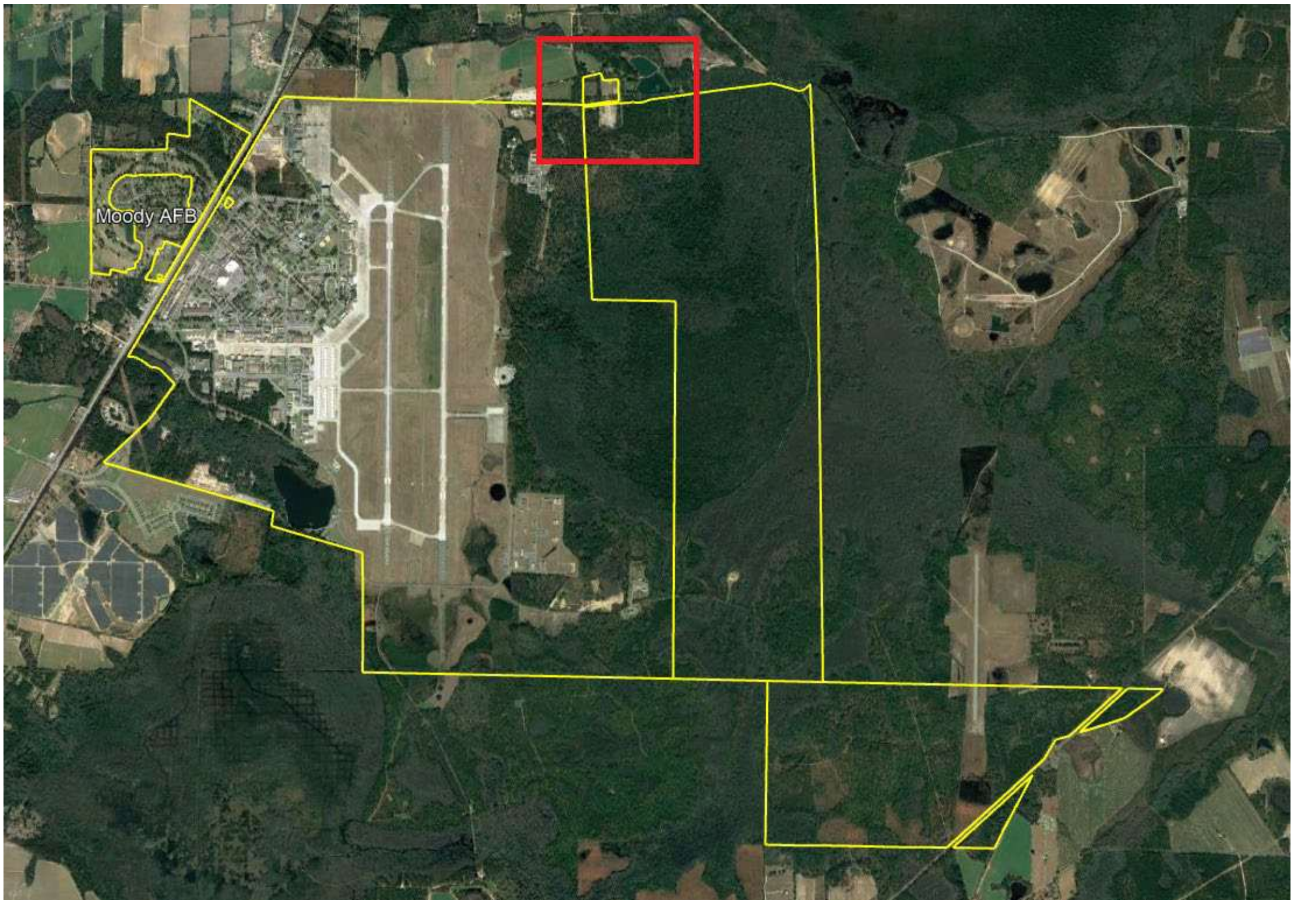

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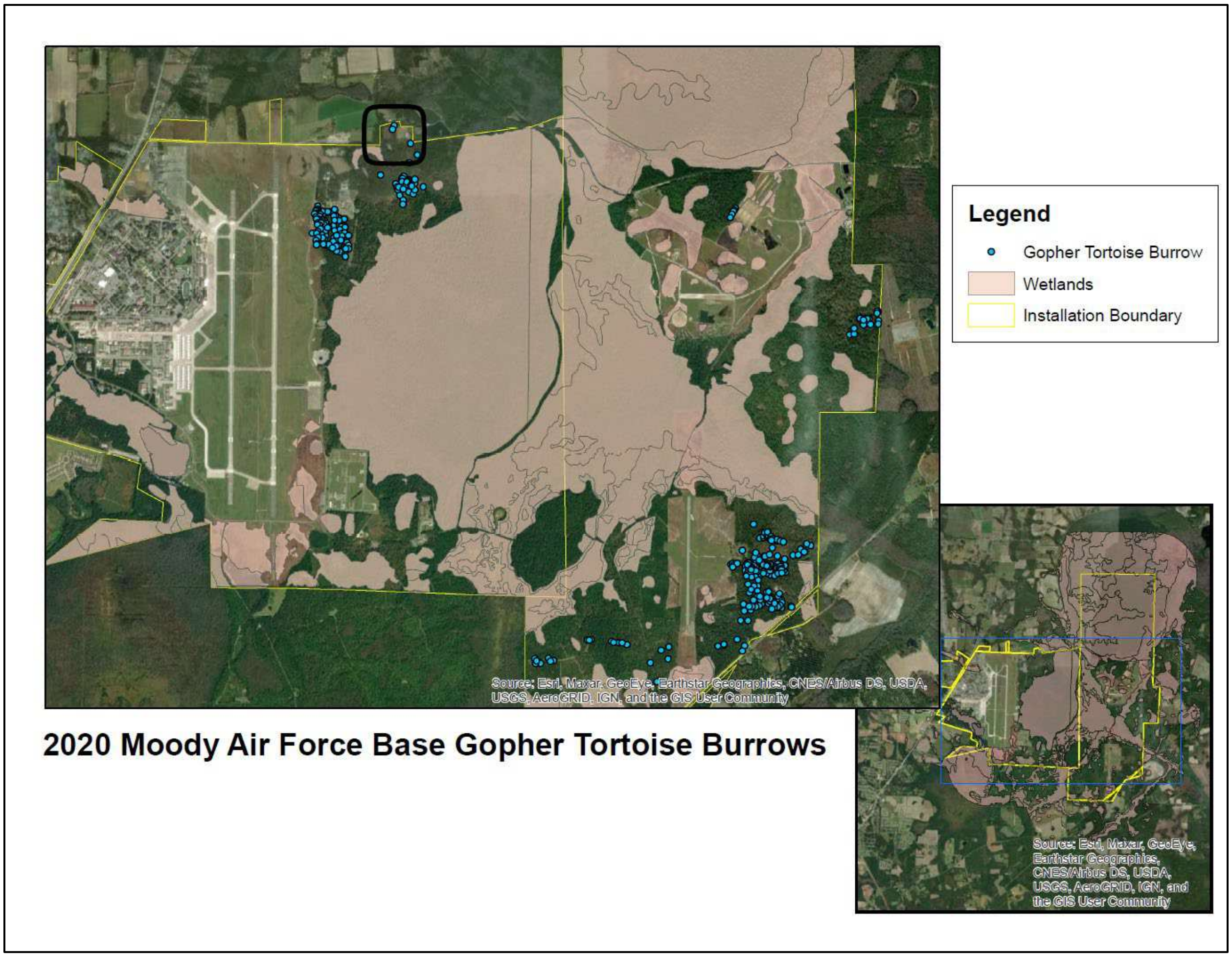

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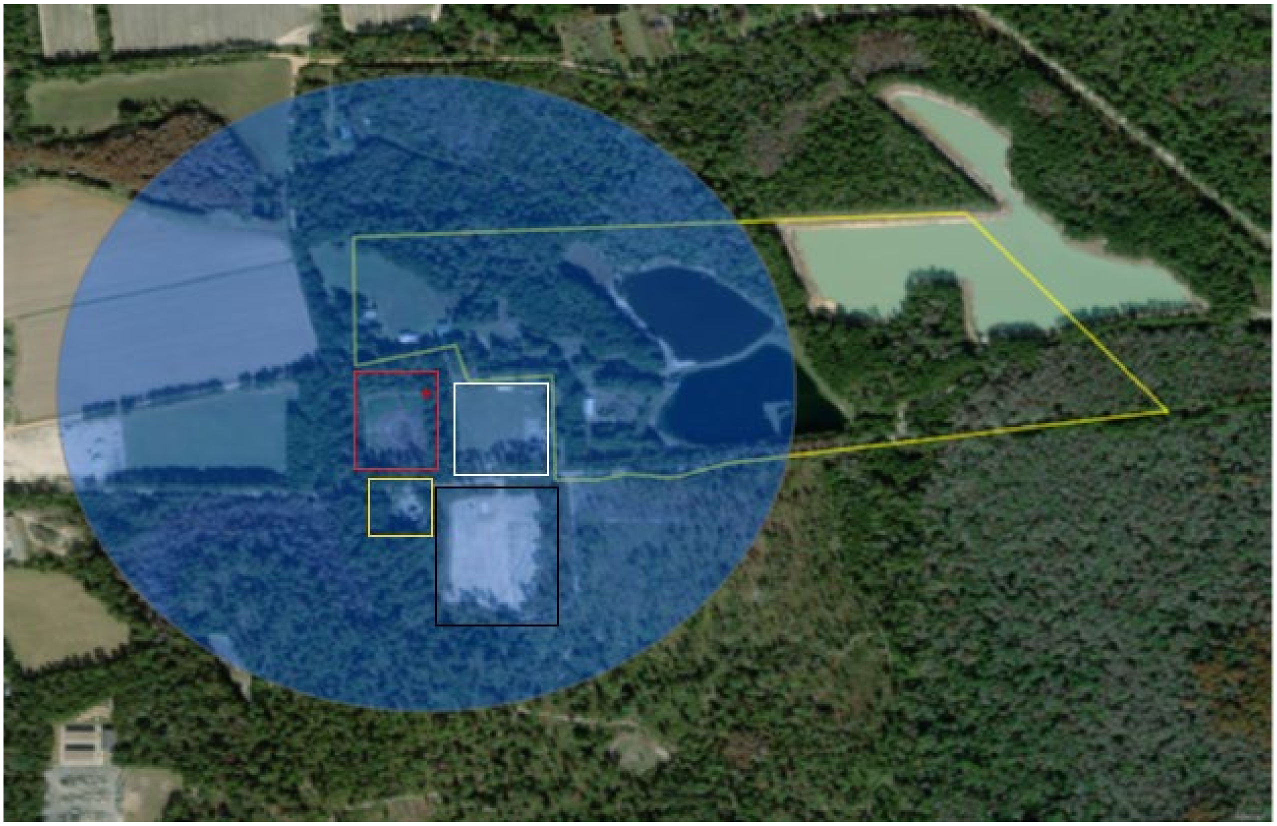

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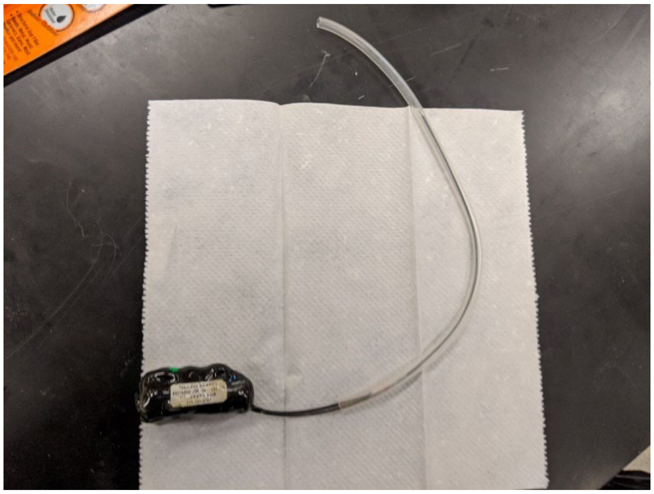
(a)  (b) 

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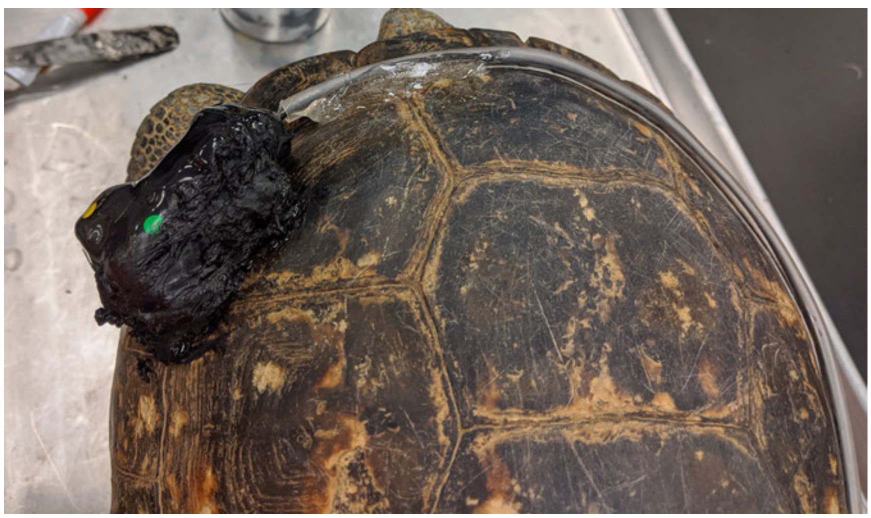
(a) 

(b) 

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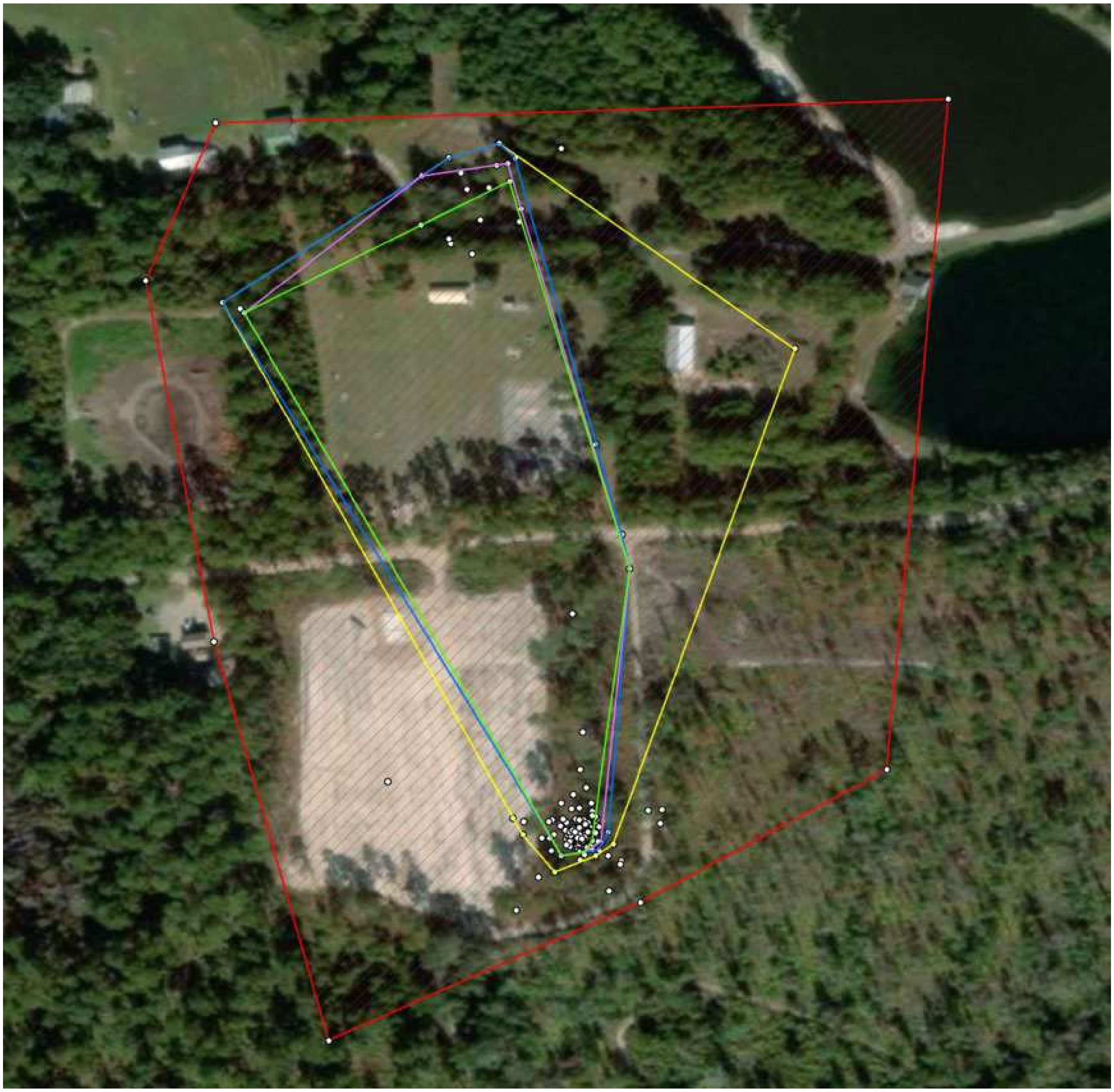

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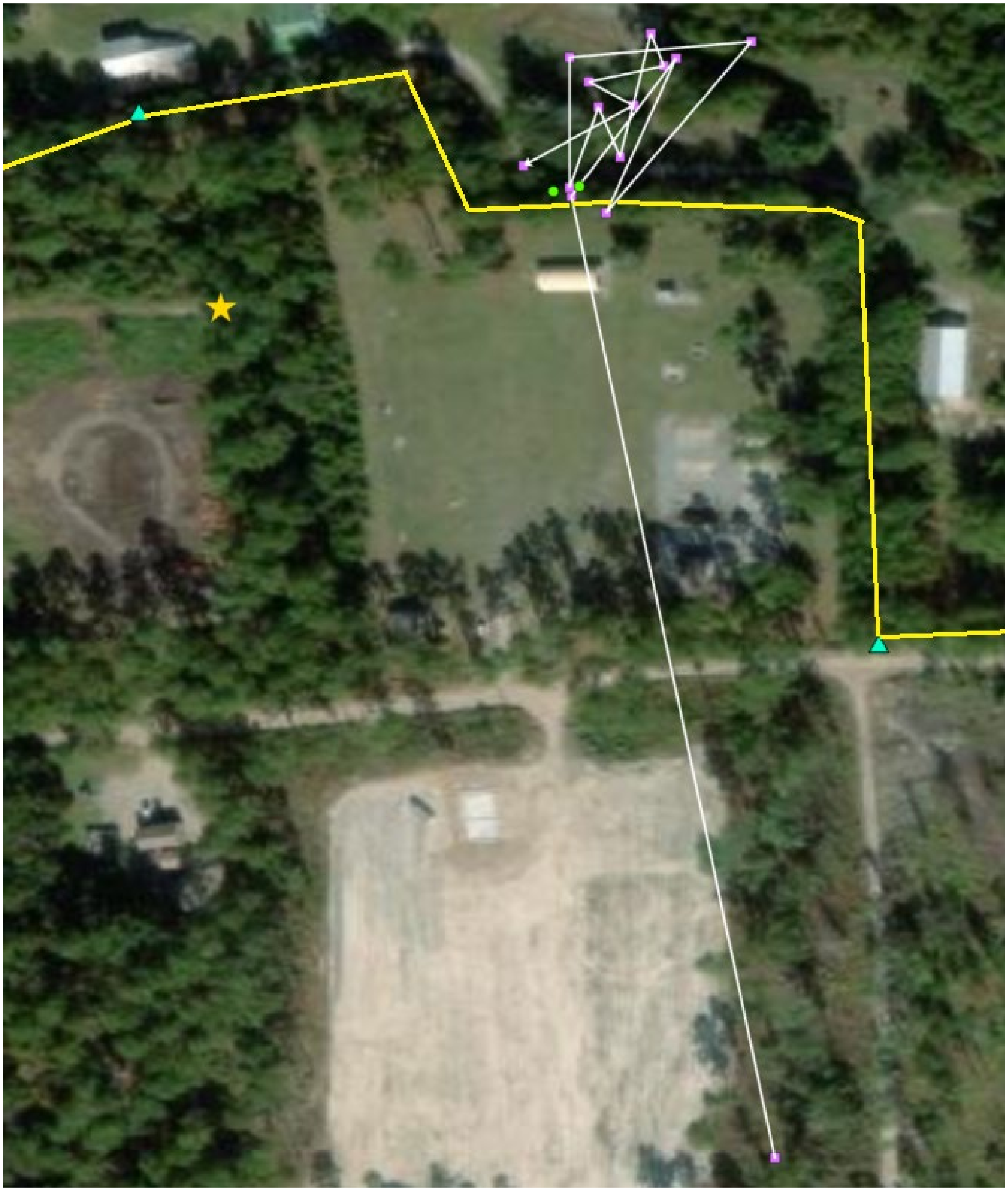

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Table 1: Gopher tortoise age classes (Wilson, 1991).

Age Class	Age (Years)	Approximate Carapace Length (mm)
Eggs	N/A	N/A
Hatchling	≤ 1	≤ 50
Juvenile	1–4	50-120
Subadult	5–15	120–230
Adult	≥ 16	Male: 230–240 Female: 250–265

Table 2: GPS data type definitions (Q4000ER, 2019).

Data Type	Definition
Date	Date that the GPS location was acquired.
Time	Time that the GPS location was acquired. This time stamp reflects the GMT offset that you set.
TTF	Time to fix, the amount of time required to acquire a GPS position plus the GPS additional time.
Altitude	Altitude, please note that this will not be very precise, your maps will have more precise altitude data.
Maxsnr	Maximum satellite signal strength received by the GPS device.
HDOP	Horizontal dilution of precision.
VDOP	Vertical dilution of precision.
Satt	The number of satellites acquired before the GPS module wrote the position to memory. This includes satellites acquired during the GPS Additional Time.
Fix	No means that there was no location acquired. 2D means that it was a two-dimensional location, 3D means that it was a three dimensional location.
V1	GPS battery voltage reading under load at the time the GPS position was recorded.
T1	Temperature at the time the location was recorded. Recorded in Celsius.

Table 3: Horizontal dilution of precision (HDOP) values and number of fixed locations (FL) generated by Q4000ER GPS collar used on GPS-tracked Gopher Tortoise, TnT. Home range values (hectares) calculated based on minimum convex polygons from FL. Moody Air Force Base, Lowndes and Lanier Counties, Georgia, USA.

HDOP Value	Number of FL	Home Range (Hectares)
≤ 1.5	54	3.55
≤ 2.0	101	3.81
≤ 3.0	166	4.12
≤ 5.0	222	6.25
All Values	263	15.86

Appendix A:
Valdosta State University Animal Use Protocol Approval (AUP-00078-2021)

VALDOSTA STATE UNIVERSITY

Institutional Animal Care and Use Committee (IACUC)
Animal Use Protocol Approval

February 3, 2021

Dr. J. Mitchell Lockhart
Department of Biology
Valdosta State University

Dear Dr. Lockhart;

Animal Use Protocol (AUP) *"Gopher Tortoise Survival Survey and Demography at Reed Bingham State Park"* (AUP-00078-2021) has been approved by the Institutional Animal Care and Use Committee (IACUC). This approval is from 01.29.2021 – 01.29.2024. Please note the IACUC Administrator must receive required health screening forms before this research study is permitted to begin.

Each year, an animal report must be submitted to the IACUC to keep your protocol active. You will be contacted by the Office of Sponsored Programs and Research Administration approximately one month before the annual report is due.

Please remember that you must obtain IACUC approval before amending, or altering the scope, or procedures of the protocol. You are also required to report to attending Veterinarian, the IACUC Chair, and the IACUC Administrator any unanticipated problems with the animals that become apparent during the course, or as a result of the research, or teaching activity.

Should you have questions concerning your approved research, please contact Tina Wright, Compliance Officer, phone 229.253.2947, email tmwright@valdosta.edu, or IACUC Alias @ iacuc@valdosta.edu.

Sincerely,
Elizabeth W. Olphie
Elizabeth "Ann" Olphie
IACUC Administrator

Appendix B:
Valdosta State University Animal Use Protocol (AUP-00078-2021)

VALDOSTA STATE UNIVERSITY

ANIMAL USE PROPOSAL
(in accordance with the Valdosta State University Policy on Humane Care and Use of Laboratory Animals)

Final Use Category Assigned (circle): B C D E AUP No. ____________
IACUC Approval Signature: ____________
Date of Approval: ____________
IACUC Reviewer: ____________

This form has been developed to facilitate the review of requests to use animals for specific research, teaching, or biological testing projects. The review process has been designed to communicate methods and materials for using animals through administrative officials and attending veterinarians to the Institutional Animal Care and Use Committee (IACUC). This process will help assure that provisions are made for compliance with the Animal Welfare Act, the *Public Health Service Policy on Humane Care and Use of Laboratory Animals (10/00)*, and the Department of Health and Human Services' 1996 *Guide for the Care and Use of Laboratory Animals*, http://grants.nih.gov.grants/olaw/olaw.htm. This completed form must be approved by the IACUC before the project or course is initiated and before animals can be procured. Approved AUPs and AUP numbers are valid for up to three years subject to approval of modifications and annual reviews.

Use this form only for **NEW** AUPs or for those whose **3-year approval for use has expired.**

For **ANNUAL REVIEWS or MODIFICATIONS** to an approved AUP, use the 1-page "AUP Annual Review or Modification" form. After 3 years a new complete AUP must be submitted and assigned a new AUP number. The number of animals used must be declared annually and their documentation is the responsibility of the Principal Investigator.

MULTIPLE FUNDING AGENCIES: Submit only one AUP for identical procedures, animal species and numbers of animals proposed in grant requests to different funding agencies. Use the same AUP number for each grant application. If funded, submit "AUP Annual Review or Modification" form with changes from original AUP, such as funding agency, procedures, or number of animals. Unless authorized by a Modification, the number of animals used must not exceed the number authorized on the original approved AUP regardless of the sources of funding.

1. Project or Course Title: Gopher Tortoise Survival Survey and Demography at Reed Bingham State Park

2. Name of Principal Investigator: Dr. J. Mitchell Lockhart

E-mail: jmlockha@valdosta.edu

Title of PI: Professor of Biology   Telephone: 229-333-5767

Department: Biology   Bldg./Room: Bailey Sci Room 2060

Please list all persons from your laboratory who will work with animals and the procedures they will perform:

Name: Christopher Le   Procedures: Capture/Handling

Name: To be determined Field Technician   Procedures: Capture/Handling

Name: Procedures:

Name: Procedures:

Name: Procedures:

3. Source of Funding: Proposal Deadline:

Anticipated Starting Date: March 22, 2020 Study Completion Date: March 21, 2021

4. The PI assumes responsibility for compliance with the policies stated above including assuring that the staff are trained and qualified to perform the procedures on animals as indicated in this **Animal Use Proposal** and assures the Committee that this research does not unnecessarily duplicate previous experiments. The PI must hold VSU faculty status. Students, including post-docs, residents and others "in training," and non-VSU employees can only serve as Co-Investigators.

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issues to minimize pain and distress.

Signature of PI: DocuSigned by: Jack Lockhart B918D0B5E6BA45E... Date: 12/9/20

Attending Veterinarian: DocuSigned by: Teresa Doscher 69B339620BD9485... Date: 1/5/2021

Please submit this completed form through the Office of Sponsored Programs and Research Administration: Compliance Coordinator, Suite 3100, Psychology Building, Valdosta State University, Valdosta, GA 31698 Phone: (229) 259-5045 FAX: (229) 245-3853

5. For all proposals submitted, particularly those related to U.S. **PHS or NIH proposals,** please complete this item: The Dean, Department Head, or College Review Committee (Please indicate which by underline) concurs with the scientific relevance of conducting this protocol.

Printed Name: Robert Gannon Signature: DocuSigned by: Robert Gannon 8336203441AE433...

6. Will any aspect of the experimental study (course) or animal husbandry be conducted at another institution? ☐ Yes ☒ No

If Yes, where?

Was the proposal approved by the IACUC of that institution? ☐ Yes ☐ No

7. **Abstract of Proposal**: Describe succinctly, in lay terms, how the animals will be used and what the contribution from using animals will be, or attach your project abstract. (Text box will expand as necessary)

The gopher tortoise (Gopherus polyphemus) is a threatened species in the state of Georgia. Various efforts are aimed at addressing potential weaknesses in the Gopher tortoise life cycle. One significant limitation in tortoise populations is the production and survivability of hatchlings. 174 Passive Integrated Transponder (PIT) tagged hatchling gopher tortoises were released by VSU researchers in 2008 and 2009 at Reed Bingham State Park (RBSP), Cook County, Georgia. Our goal is to determine the survival success and demography of these hatchlings post-release and assess factors that contribute to subadult survivability. Mortality is high in gopher tortoise hatchlings and studying those that survived to subadult age could improve our methods of reducing their mortality. We will be evaluating the viability of PIT tags after a decade, determining if PIT tagging the gopher tortoise hatchlings is a practical tool for monitoring populations, and providing a population estimate to the managers of RBSP at the conclusion of this survey.

8. Does this proposal include the maintenance of a **breeding colony?** ☐ Yes ☒ No

9. Animals to be used in this study per year:

Animal Species (Common name)	Max. No. To be Used Per Year*	Strain	Sex	Facility	Source Location**
Gopher Tortoise	50		Both		Wild Captures

**For breeding colonies, indicate the maximum number to be maintained in the colony at any time.*
Note: Excluding breeding colonies, the number of animals procured and the number of animals used under this AUP must be documented by the investigator each year. By law, this number must not be greater than the maximum approved in this AUP.
***For additional space use bottom of this page or add a page.*

10. a) Explain why each species was selected.

174 PIT-tagged hatchling Gopher Tortoises were released in 2008 and 2009 at Reed Bingham State Park. The goal is to determine how many of those 174 Gopher Tortoises survived and provide a population estimate to the Managers of Reed Bingham State Park.

b) Explain how the numbers needed of each species were determined.

Based on the number of Gopher Tortoise hatchlings released and literature of the low survival rate of hatchlings.

11. Indicate **non-surgical** procedures below and **describe** on a separate sheet.

a) Specimen collection from live animals:

Type and volume of samples: Ticks (Amblyomma tuberculatum) - Amount present

Frequency and duration of collection: Every Gopher Tortoise with ticks present over the duration of the study

Method of collection: Forceps used to collect specimens and placed into a jar of 70% of Rubbing Alcohol.

b) Specimen collection from euthanized animals:

Type: N/A

Source:

c) Induction of neoplasia:

Type: N/A

d) Use of infectious agents *(Request approval from Environmental Health Officer)*

Name of agent: N/A

Amount of dose: Route of administration:

Biosafety level:

e) Use of radioisotopes *(Request approval from Environmental Health Officer):*

Isotope: N/A

Amount/Dose:

Route:

f) Use of carcinogens *(Request approval from Environmental Health Office):*

Carcinogen:

N/A

Amount/Dose:

g) Administration of test substances not listed above:
Amount/Dose:
N/A
Route:

h) Tranquilizers, anesthetics, analgesics or antagonists used in any of the above procedures (specify below):

Species	Agent/Conc.	Dose (mg/kg)	Route
N/A			

NOTE: The Institution does not condone any project involving pain without anesthetic/analgesia.

12. Indicate **Surgical** Procedures:

a) **Type of surgical procedure**. A complete description should include who will administer and what the pre- and post-operative care will consist of, special post-surgical needs of the animal, the length of time the animal will be kept alive following surgery, and any anticipated complications.

Terminal, i.e. animals will be euthanized under anesthesia when surgery is completed. ☐ Yes ☐ No

NOTE: ***Describe fully any euthanasia method to be used and the reasons for the selection (list by name of drug, dosage and route of administration).*** **See question 13.**

Survival, i.e. animals will recover from anesthesia following surgery. ☐ Yes ☐ No

b) Anesthetic and analgesic agents to be used in surgical procedures:

Species	Agent/Conc.	Dose (mg/kg)	Route
N/A			

c) Indicate monitoring and life support systems to be utilized.

☐ Respiratory rate
☐ Mucous membrane color
☐ EKG
☐ Body Temperature
☐ Other:
☐ Heart rate
☐ Reflexes
☐ Blood gases
☐ Blood Pressure

13. Euthanasia procedures:

a) Technique — Species

☐ CO_2 inhalation
☐ Barbiturate overdose
☐ Cervical dislocation
☐ Other Methods (Specify)

b) Name and title of person performing euthanasia:

14. If the answer to any of the following questions is **Yes**, please provide an explanation.

a) Will any technique be performed which would result in prolonged loss of sensation or paralysis?
☐ Yes ☒ No

b) Will any technique be performed which will involve prolonged physical restraint other than routine caging and handling?
☐ Yes ☒ No

c) Will any technique be performed which will involve any non-standard husbandry techniques (feedings, watering, housing, environment)?
☐ Yes ☒ No

d) Will any substance such as **Complete Freund's Adjuvant** be injected which could cause chronic inflammation and/or pain?
☐ Yes ☒ No If yes, describe what will be used and the schedule:

e) Will animals be subjected to potentially painful procedures for identification, i.e. toe clipping, branding, etc?
☐ Yes ☒ No

f) Will it be necessary for live animals to be removed from the immediate care facility?
☐ Yes ☒ No

g) Will this experiment involve the study of stress, pain, or abnormal behavior in live animals which cannot be alleviated with drugs because their use would interfere with the research goal?
☐ Yes ☒ No

h) Will any adverse effects or overt signs of illness be expected?
☐ Yes ☒ No

Please provide explanations related to question 14. a-h).

15. Place proposed animal research/laboratory use into categories according to potential pain and distress. The Animal Welfare Act requires an annual report to the U.S.D.A. For each species of animal involved please read the attached Animal Use Category definitions and indicate which category; B, C, D, or E applies to this project.

Species **Use** **Category**

C	Holding, weighing, measuring

16. What techniques will be used to minimize potential pain and/or distress (e.g. frequency of observations or euthanasia of sick or moribund animals)?

a) The animals will be observed times a day or every hours. Any sick or moribund animals will be treated or euthanized.

b) Other (describe): NA

17. If your study was categorized above as D or E, are there alternatives to the painful procedure?
☐ Yes ☐ No

a) If "Yes" list the alternatives:

b) If alternatives are available but will not be used, justify the procedures used in this proposal.

c) If "No", describe the methods and source you used to determine there are no alternatives to the procedures in this proposal. For example; Biological Abstracts, Index Medicus, AGRICOLA, Animal Welfare Information Center.

18. If applying for a grant, does the information on this form agree with the animal use section of the grant application? ☐ Yes ☐ No

19. If this study involves collections or use of animals requiring governmental permits, please provide the name and number of the permit: See attached State Collecting Permit

USDA Pain Levels:

USDA Category B	USDA Category C	USDA Category D	USDA Category E
Breeding or Holding Colony Protocols	No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions; positive reward projects; routine procedures; injections; and blood sampling.	Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.	Pain or distress or potential pain or distress that is **not** relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.
	Examples	**Examples**	**Examples**
	1. Holding or weighing animals in teaching or research activities. 2. Injections, blood collection or catheter implantation via superficial vessels. 3. Tattooing animals. 4. Ear punching of rodents. 5. Routine physical examinations. 6. Observation of animal behavior. 7. Feeding studies, which do not result in clinical health problems. 8. AVMA approved humane euthanasia procedures. 9. Routine agricultural husbandry procedures. 10. Live trapping. 11. Positive reward projects.	1. Diagnostic procedures such as laparoscopy or needle biopsies. 2. Non-survival surgical procedures. 3. Survival surgical procedures. 4. Post operative pain or distress. 5. Ocular blood collection in mice. 6. Terminal cardiac blood collection. 7. Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with decreased appetite/ activity level, adverse reactions, to touch, open skin lesions, abscesses, lameness, conjunctivitis, corneal edema and photophobia. 8. Exposure of blood vessels for catheter implantation. 9. Exsanguination under anesthesia. 10. Induced infections or antibody production with appropriate anesthesia and post-op/post-procedure analgesia when necessary.	1. Toxicological or micro-biological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs. 2. Ocular or skin irritancy testing. 3. Food or water deprivation beyond that necessary for ordinary pre-surgical preparation. 4. Application of noxious stimuli such as electrical shock if the animal cannot avoid/escape the stimuli and/or it is severe enough to cause injury or more than momentary pain or distress. 5. Infliction of burns or trauma. 6. Prolonged restraint. 7. Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes. 8. Use of paralyzing or immobilizing drugs for restraint. 9. Exposure to abnormal or extreme environmental conditions. 10. Psychotic-like behavior suggesting a painful or distressful status. 11. Euthanasia by procedures not approved by the AVMA.

Guidelines for determining USDA classification in protocols involving tissue collection before/after euthanasia and/or animal perfusion:

If an animal will be euthanatized by an approved physical or chemical method of euthanasia solely for the collection of tissues (after the animal's death), the procedure should be classified as USDA C.

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy), and the animal will then be allowed to recover, the procedure should be classified as USDA D (survival surgery).

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy, etc.); and the animal will then be euthanatized, the procedure should be classified as USDA D (non-survival surgery). In this scenario, it is necessary to justify why the animal couldn't be euthanatized (USDA category C) rather than anesthetized.

If an animal will be anesthetized so that vital tissues can be collected (heart, both kidneys or lungs, whole liver, etc.), the animal will obviously succumb to the procedure. To determine whether this will be euthanasia or non-survival surgery, we must consider the definition of euthanasia. A critical component of this definition is "rapid unconsciousness followed by loss of cardiac, respiratory and brain function". Based on this definition, procedures which require tissue manipulation or other prolonged techniques prior to the animals death (more than a few minutes) should be classified as non-survival surgery (USDA D). Similarly, if an animal will be anesthetized so that the tissue can be collected in the "freshest" possible state (i.e. heart) and the tissues will be rapidly excised, the procedure should be classified as euthanasia (USDA C). (Note: In this scenario, it is difficult to justify why the animal couldn't be euthanatized rather than anesthetized.)

If an animal will be anesthetized so that it can be chemically perfused, the same "test of time" applies (i.e.: long, technical manipulations should be classified as USDA D; while rapid intravascular injection of the perfusate without other manipulations should be classified as USDA C).

NOTE: Because the USDA classification system is based on the "potential for pain, distress or discomfort," the anesthetic/euthanasia drug dose becomes a critical concern. For example, if a known "euthanasia dose" of pentobarbital will be administered, drug irreversibility is assumed. Thus, once the animal is confirmed to be in an anesthetic plane (toe pinch response, etc.), tissues can be collected/ procedures can be performed without the concern about what the animal will be perceiving. This procedure would then be classified as USDA C. The Committee recommends using a euthanizing dose whenever possible. Other methods may be appropriate with proper scientific justification.

Survival and Demography Survey of 2008 and 2009 Released Gopher Tortoise Hatchlings in Reed Bingham State Park, Adel, GA

Abstract

The gopher tortoise (*Gopherus polyphemus*) is a threatened species in the state of Georgia. Various efforts are aimed at addressing potential weaknesses in the Gopher tortoise life cycle. One significant limitation in tortoise populations is the production and survivability of hatchlings. 174 Passive Integrated Transponder (PIT) tagged hatchling gopher tortoises were released by VSU researchers in 2008 and 2009 at Reed Bingham State Park (RBSP), Cook County, Georgia. Our goal is to determine the survival success and demography of these hatchlings post-release and assess factors that contribute to subadult survivability. Mortality is high in gopher tortoise hatchlings and studying those that survived to subadult age could improve our methods of reducing their mortality. We will be evaluating the viability of PIT tags after a decade, determining if PIT tagging the gopher tortoise hatchlings is a practical tool for monitoring populations, and providing a population estimate to the managers of RBSP at the conclusion of this survey.

Appendix C:
Georgia Department of Natural Resources Scientific Collecting Permit 2020-2021

ORDER #: 119101726

GEORGIA
DEPARTMENT OF NATURAL RESOURCES

Scientific Collecting Permit

Valdosta State University, Dept of Biology

Order Date: **4/8/2020** Licensee Customer #: **1001056699** License Name: **ADMA SAFER**

Effective Date: **4/1/2020** Expiration Date: **3/31/2021**

Special Information: **Species Name: NONLISTED SPECIES OF VASCULAR PLANTS, BIRDS, MAMMALS, FISH AND INVERTEBRATES; GOPHER TORTOISE; CRAYFISH; COTTONMOUTH; REPTILES; AMPHIBIANS**

Responsible Person Name: Adam Safer

Above named is hereby permitted, in accordance with O.C.GA 27-2-12 and the regulations of the Georgia Department of Natural Resources subject to the terms, exceptions, and restrictions expressed on the attached ""General Conditions"" and subject to any other applicable state or federal regulations, to take for scientific and educational purposes only in the state of Georgia, wildlife which is listed above.

This permit is conditional and confers NO privileges whatsoever to take, possess, exchange, or transport migratory birds or their parts, nests, or eggs unless the permittee has in his or her possession, while exercising the privilege granted herein a valid subsisting permit to take Migratory Birds and their parts, nests, or eggs for scientific purposes in the state of Georgia issued to him by the U.S. Fish and Wildlife Service, and unless or until that condition is fulfilled, the taking of Migratory Birds, their parts, nests, or eggs is a violation of the regulations as set forth by the State.

Unless otherwise specified, permittee must submit a complete report of all specimens collected under the authority of this permit upon expiration date of permit. This permit (copy and letter of authorization for sub-permittees) must be in possession while collecting.

Signature of Licensee ______________________

Condition: Location: Chattahoochee, Saint Marys, Flint, Satilla and Suwannee Basins
**Subpermittees: Corey Anderson; Brad Bergstrom; Mark Blackmore; Cristina Calestani; Eric Chambers; John Elder; Timothy Fort; Theresa Grove; J.Mitchell Lockhart; Jim Loughry; Colleen McDonough; Ted Uyeno; James Nienow; and Emily Rose

***This permit subject to Conditions numbered 1-12, General Permit Conditions for State Protected Fishes Nongame Conservation Section-Updated May 2017 and Provisions numbered 1-15 as attached. All pages of Conditions and Provisions are part of this license and must be attached hereto (4 pps. including permit)

1) Permittee is authorized to mark, release, recapture freshwater turtles, and temporarily retain non-listed freshwater turtles for eventual release at capture site.
2) Permittee is not authorized for mollusk collecting

Valdosta State University, Dept of Biology
C/O Adam Safer
N PATTERSON ST
DEPT OF BIOLOGY
VALDOSTA, GA 31698

Conditions for Scientific Collection Permit

Valdosta State University
Adam Safer
Department of Biology
Valdosta GA 31698-0015

CN: 1001056699

April 8, 2020

LOCATION: Statewide; Moody Air Force Base and Lake Louise Field Station only for gopher tortoise

1. Authorized to collect the above named species for teaching purposes. Authorized to collect gopher tortoise for disease and population studies.
2. Capture methods may include Sherman live traps, snap traps, trip traps, Ekman dredge, Dendy plate samplers, drift and plankton tow nets, seines, road kills, and cannon nets.
3. All live specimens not needed for teaching purposes are to be released unharmed at capture site.
4. All specimens collected must remain in the Valdosta State College collection and cannot be exchanged with any other institution or synoptic collection.
5. Permittee is authorized to collect no more than 30 gopher tortoise for upper respiratory tract disease data collection:

(a) Collection will occur May through August.
(b) Tortoise will be captured using standard tortoise trapping techniques and hand collection.
(c) Captured specimens shall be marked by notching or drilling holes in rear scutes in accordance with standard tortoise marking techniques. (d) Captured tortoise will be examined for clinical signs of respiratory infection (Mycoplasma sp.) and nasal swabs or nasal lavage (nasal wash) will be collected to culture Mycoplasma sp.
(e) Permittee may use video survelliance to determine if burrows are active to assist in trapping and collection activities.
(f) Tortoise found dead may be necropsied to determine infection with Mycoplasma sp.

6. Permittee is authorized to collect gopher tortoise at the Lake Louise Field Station, Valdosta State University, for populations dynamics studies. Upon collection, data shall be collected and specimens shall be released unharmed at site of capture.
7. Permittee is authorized to collect no more than twenty (20) box turtles from state and county highways in Lowndes County for breeding and educational purposes. Turtles shall be held at 2841 Bud McKey Circle, Valdosta, Georgia (Lowndes County). Data collected from this study shall be used to develop publications for educational science journals. Copies of journals shall be sent to John Jensen, DNR Nongame Program, 116 Rum Creek Drive, Forsyth, Georgia 31029.
8. Permittee is authorized to collect no more than three (3) per species of black and turkey vultures, from the Grand Bay WMA for telemetry studies. Collection shall be by carrion bait and cannon net. Upon collection, specimens shall be marked with feather dye or bleach and fitted with a backpack/harness radio transmitter.
9. Permittee is reminded of the importance of item 2 and all applicable conditions on reverse of permit.
10. WRD has determined, pursuant to O.C.G.A. 27-2-12, that this permit will provide valuable information regarding the species and population proposed for study. WRD has determined that this project is of sound design, does not duplicate previous research, and will not be detrimental to the species or populations proposed for study. O.C.G.A. 27-2-12(e) requires that the permittee submit to DNR, reports detailing the information or data obtained from such collections and in carrying out this permittee is therefore acting as an agent of DNR in furthering the conservation and protection of this state's natural resources.

END OF CONDITIONS

GEORGIA
DEPARTMENT OF NATURAL RESOURCES

WILDLIFE RESOURCES DIVISION

MARK WILLIAMS
COMMISSIONER

RUSTY GARRISON
DIRECTOR

General Collection Permit Conditions for State Protected Fishes
Updated August 2018

During sampling events, the permittee will take appropriate steps to minimize the take of state listed fishes (e.g, use of aerators, limiting the number of fish held in buckets, frequent water changes, careful adjustment of electrofishing settings, etc.). Prior to sampling, permittee should review existing distribution data to identify species that may be encountered (http://georgiabiodiversity.org/) and then review diagnostic characters for each species in their corresponding species profile (see "Profile" link associated with each species) and regional taxonomic guides for fishes. With the exception of the species listed below, **up to 5 voucher specimens of state protected, non-federally listed* fishes** may be retained from each collection site. All collected individuals of the following state protected fishes must be released alive or can be vouchered from each collection site by the number of specimens listed in parentheses. We encourage you to document at least one specimen from each site by photographing them before release.

*Etheostoma brevirostrum (*Holiday Darter, up to 2 voucher specimens)
*Enneacanthus chaetodon (*Blackbanded Sunfish, up to 1 voucher specimen)
Etheostoma vulneratum (Wounded Darter, up to 2 voucher specimens)
Macrhybopsis etnieri (Coosa Chub, up to 2 voucher specimens)
Moxostoma robustum (Robust Redhorse, no voucher specimens from known populations)
Moxostoma sp. ("Sicklefin Redhorse", no voucher specimens)**
Noturus eleutherus (Mountain Madtom, up to 1 voucher specimen)
Noturus munitus (Frecklebelly Madtom, up to 1 voucher specimen)
Percina squamata (Olive Darter, up to 1 voucher specimen)
Percina kusha (Bridled Darter, up to 2 voucher specimens)
Typhlichthys subterraneus (Southern Cavefish, up to 1 voucher specimen)

Electronic submission of data is required. Please include this data with your scientific collecting permit report and email it to zachariah.abouhamdan@dnr.ga.gov. Instructions and a data template are available at http://georgiawildlife.com/conservation/species-of-concern#providing.

*Unless otherwise authorized by the Special Permit Unit of GADNR, sampling in waters with federally listed species is prohibited without a permit from the U.S. Fish and Wildlife Service. This requirement applies to all sampling methods except for visual sampling (i.e., snorkeling, SCUBA diving). Further, harassment of federally protected species during visual surveys is prohibited. This requirement covers the following areas: the mainstem Etowah River upstream of Lake Allatoona, all Etowah River tributary streams that enter the Etowah River within or upstream of Lake Allatoona, Raccoon Creek and its tributary streams in Paulding and Bartow counties, the entire mainstem Conasauga River and all Conasauga River tributary streams entering the Conasauga mainstem from the east, the Coosawattee River and all tributary streams upstream from Carter's Lake Dam, the Coosawattee River between Carters Lake and its confluence with the Conasauga River, the mainstem of Talking Rock Creek, and the mainstem of South Chickamauga Creek downstream from Swanson Mill dam. Outside of these areas, sampling that specifically targets federally listed sturgeon species is also prohibited without a federal permit.

**In order to protect this species during the spawning season, electrofishing may not be carried out in the mainstem of Brasstown Creek between March 1 and June 30 unless this activity has been specifically authorized by the Special Permit Unit of GADNR.

Scientific Collecting Permit General Provisions

1. A Federal Permit is also required for collection of migratory birds or their parts, nests, or eggs.
2. Region or District Law Enforcement office, along with Game Management, Fisheries Management, or the U.S. Forest Service, i f applicable, in each area of collection must be notified at least three (3) days in advance of the date and place of specimens are to be collected, the species to be collected, methods of collection, and permit number.
3. Collectors should be as judicious and humane as possible in collecting and capture.
4. The Department of Natural Resources reserves the right to limit the kind and numbers of specimens collected in order to ensure the conservation of the natural resources of this State. The taking of wildlife at any time by means other than those conditions set forth in this permit is prohibited.
5. This permit does not authorize the taking of any listed (endangered, threatened, rare, or unusual) species unless specifically provided.
6. This permit does not authorize collections on State or Federal Wildlife Refuges, tidal wetlands and beaches, management areas, other sanctuaries, or parks unless specifically provided. If authorized, specific written permission must be obtained from the park manager, area manager, or supervisor prior to any collection activities.
7. Permits are not transferable. However, assistants, employees, or field workers may assist with collections. Designated representatives of the master permittee must possess a copy of the permit and a letter of authorization from the master permittee.
8. Permittees must submit an annual report of specimens collected upon expiration date of said permit.
***** ALL ANNUAL REPORTS SHALL BE SUBMITTED ELECTRONICALLY TO zachariah.abouhamdan@dnr.ga.gov WITH NONGAME, NO PERMITS WILL BE RENEWED UNTIL SUCH REPORT IS RECEIVED. (NOTE: DO NOT SEND YOUR APPLICATIONS OR REQUIRED DOCUMENTS ELECTRONICALLY, PLEASE USMAIL THEM TO THE SPECIAL PERMIT UNIT)**
9. Collections shall be available for inspection at any and all times to duly authorized inspectors or agents of the Department of Natural Resources.
10. Any permit may be revoked at any time for violation of the terms, exceptions, restrictions, or conditions of said permit.
11. The Department may request, when practical, duplicates of any collections taken under authority of this permit for future educational purposes.
12. All permits expire March 31.
13. This permit does not authorize the exchange of fish and wildlife, their parts, nests, or eggs, with persons who do not hold an appropriate permit.
14. Permit (copy and letter of authorization for sub-permittees) must be in possession while collecting.
15. **Encounters with animals listed on the Georgia DNR Nongame Conservation Section's Tracking List of Special Concern Animals must be reported within one (1) year to: GA DNR Nongame Conservation Section, (706) 557-3213 or Fax 706-557-3580 Electronic submission forms may be found at: http://.georgiawildlife.com/conservation/species-of-concern**

Georgia Department of Natural Resources
Law Enforcement Division
Special Permit Unit
2070 U.S. Highway 278, S.E.
Social Circle, GA 30025-4743

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Appendix D:
Georgia Department of Natural Resources Scientific Collecting Permit 2021-2022

ORDER #: 120391704

GEORGIA
DEPARTMENT OF NATURAL RESOURCES

Scientific Collecting Permit

Valdosta State University, Dept of Biology

Order Date:	**4/5/2021**	Licensee Customer #:	**1001056699**	License Name: **ADAM SAFER**
Effective Date:	**4/5/2021**	Expiration Date:	**3/31/2022**	

Special Information: **Species Name: NONLISTED SPECIES OF VASCULAR PLANTS, BIRDS, MAMMALS, FISH AND INVERTEBRATES; GOPHER TORTOISE; CRAYFISH; COTTONMOUTH; REPTILES; AMPHIBIANS**

Responsible Person Name: Adam Safer

Above named is hereby permitted, in accordance with O.C.GA 27-2-12 and the regulations of the Georgia Department of Natural Resources subject to the terms, exceptions, and restrictions expressed on the attached ""General Conditions"" and subject to any other applicable state or federal regulations, to take for scientific and educational purposes only in the state of Georgia, wildlife which is listed above.

This permit is conditional and confers NO privileges whatsoever to take, possess, exchange, or transport migratory birds or their parts, nests, or eggs unless the permittee has in his or her possession, while exercising the privilege granted herein a valid subsisting permit to take Migratory Birds and their parts, nests, or eggs for scientific purposes in the state of Georgia issued to him by the U.S. Fish and Wildlife Service, and unless or until that condition is fulfilled, the taking of Migratory Birds, their parts, nests, or eggs is a violation of the regulations as set forth by the State.

Unless otherwise specified, permittee must submit a complete report of all specimens collected under the authority of this permit upon expiration date of permit. This permit (copy and letter of authorization for sub-permittees) must be in possession while collecting.

Signature of Licensee ____________

Condition: Location: Chattahoochee, Saint Marys, Flint, Satilla and Suwannee Basins
**Subpermittees: Corey Anderson; Brad Bergstrom; Mark Blackmore; Cristina Calestani; Eric Chambers; John Elder; Timothy Fort; Theresa Grove; J.Mitchell Lockhart; Jim Loughry; Colleen McDonough; James Nienow; Emily Rose; and Ted Uyeno

***This permit subject to Conditions numbered 1-12, General Permit Conditions for State Protected Fishes Nongame Conservation Section-Updated March 2021 and Provisions numbered 1-15 as attached. All pages of Conditions and Provisions are part of this license and must be attached hereto (6 pps. including permit)

1) Permittee is authorized to mark, release, recapture freshwater turtles, and temporarily retain non-listed freshwater turtles for eventual release at capture site.
2) Permittee is not authorized for mollusk collecting.

Valdosta State University, Dept of Biology
C/O Adam Safer
N PATTERSON ST
DEPT OF BIOLOGY
VALDOSTA, GA 31698

Conditions for Scientific Collection Permit
Valdosta State University
Adam Safer
Department of Biology
Valdosta GA 31698-0015
CN: 1001056699
April 5, 2021

LOCATION: Statewide; Moody Air Force Base and Lake Louise Field Station only for gopher tortoise

1. Authorized to collect the above named species for teaching purposes. Authorized to collect gopher tortoise for disease and population studies.
2. Capture methods may include Sherman live traps, snap traps, trip traps, Ekman dredge, Dendy plate samplers, drift and plankton tow nets, seines, road kills, and cannon nets.
3. All live specimens not needed for teaching purposes are to be released unharmed at capture site.
4. All specimens collected must remain in the Valdosta State College collection and cannot be exchanged with any other institution or synoptic collection.
5. Permittee is authorized to collect no more than 30 gopher tortoise for upper respiratory tract disease data collection:
(a) Collection will occur May through August.
(b) Tortoise will be captured using standard tortoise trapping techniques and hand collection.
(c) Captured specimens shall be marked by notching or drilling holes in rear scutes in accordance with standard tortoise marking techniques. (d) Captured tortoise will be examined for clinical signs of respiratory infection (Mycoplasma sp.) and nasal swabs or nasal lavage (nasal wash) will be collected to culture Mycoplasma sp.
(e) Permittee may use video survelliance to determine if burrows are active to assist in trapping and collection activities.
(f) Tortoise found dead may be necropsied to determine infection with Mycoplasma sp.
6. Permittee is authorized to collect gopher tortoise at the Lake Louise Field Station, Valdosta State University, for populations dynamics studies. Upon collection, data shall be collected and specimens shall be released unharmed at site of capture.
7. Permittee is authorized to collect no more than twenty (20) box turtles from state and county highways in Lowndes County for breeding and educational purposes. Turtles shall be held at 2841 Bud McKey Circle, Valdosta, Georgia (Lowndes County). Data collected from this study shall be used to develop publications for educational science journals. Copies of journals shall be sent to John Jensen, DNR Nongame Program, 116 Rum Creek Drive, Forsyth, Georgia 31029.
8. Permittee is authorized to collect no more than three (3) per species of black and turkey vultures, from the Grand Bay WMA for telemetry studies. Collection shall be by carrion bait and cannon net. Upon collection, specimens shall be marked with feather dye or bleach and fitted with a backpack/harness radio transmitter.
9. Permittee is reminded of the importance of item 2 and all applicable conditions on reverse of permit.
10. WRD has determined, pursuant to O.C.G.A. 27-2-12, that this permit will provide valuable information regarding the species and population proposed for study. WRD has determined that this project is of sound design, does not duplicate previous research, and will not be detrimental to the species or populations proposed for study. O.C.G.A. 27-2-12(e) requires that the permittee submit to DNR, reports detailing the information or data obtained from such collections and in carrying out this permittee is therefore acting as an agent of DNR in furthering the conservation and protection of this state's natural resources.

END OF CONDITIONS

GEORGIA
DEPARTMENT OF NATURAL RESOURCES
WILDLIFE RESOURCES DIVISION

MARK WILLIAMS
COMMISSIONER

TED WILL
DIRECTOR

General Collection Permit Conditions* for State Protected Freshwater Fishes
Updated March 2021

Survey methods may include snorkeling, scuba, backpack and boat electrofishing, dip netting, seining, trapping, or other alternate survey methods. Methods must be authorized on the permittee's individual permit. During sampling events, the permittee will take appropriate steps to minimize overall mortality and the "take" of state listed fishes (e.g., use of aerators, limiting the number of fish held in buckets, frequent water changes, careful adjustment of electrofishing settings). Prior to sampling, permittee should review existing distribution data to identify species that may be encountered and then review diagnostic characteristics for each species in their corresponding species profile (http://georgiabiodiversity.org/; see "Range Map" and "Profile" links associated with each species) and regional taxonomic guides for fishes.

Photo vouchers should be taken of every state listed species collected at each site. Photo vouchers are preferred over sacrificing individuals. However, except for the species listed below, **up to 5 voucher specimens of state protected, non-federally listed fishes** may be retained from each site. All collected individuals of the following state protected fishes must be released alive or can be vouchered from each site up to the number of specimens listed in parentheses. GADNR may authorize the collection of nonlethal DNA or tissue samples or the retention/vouchering of additional specimens through individual permits.

Macrhybopsis etnieri, Coosa Chub (2)
Moxostoma robustum, Robust Redhorse (0) **
Moxostoma sp., "Sicklefin Redhorse" (0) ***
Noturus eleutherus, Mountain Madtom (1)
Noturus munitus, Frecklebelly Madtom (0)
Enneacanthus chaetodon, Blackbanded Sunfish (1)
Etheostoma brevirostrum, Holiday Darter (2)
Etheostoma vulneratum, Wounded Darter (2)
Percina kusha, Bridled Darter (2)
Percina squamata, Olive Darter (1)
Typhlichthys subterraneus, Southern Cavefish (1)

Unless specifically authorized by the Special Permit Unit of GADNR, sampling in waters with species protected under the U.S. Endangered Species Act is prohibited without a permit from the U.S. Fish and Wildlife Service. This requirement applies to all sampling methods except for visual sampling (i.e., snorkeling, SCUBA diving). Further, harassment of federally protected species during visual surveys is prohibited. In addition to the state permit, a federal permit is needed to collect freshwater fishes in the following areas (Figure 1):

- Etowah River mainstem and its tributary streams upstream of and flowing into Lake Allatoona
- Raccoon Creek and its tributary streams in Paulding and Bartow counties
- Conasauga River and its tributary streams
- Coosawattee River and its tributary streams upstream of Carter's Lake Dam
- Coosawattee River between Carters Lake and its confluence with the Conasauga River
- Talking Rock Creek mainstem
- South Chickamauga Creek mainstem downstream from Swanson Mill dam

WILDLIFE CONSERVATION SECTION
2067 U.S. HIGHWAY 278 S.E. | SOCIAL CIRCLE, GEORGIA 30025
770.557.3333 | FAX 706.557.3030 | WWW.GEORGIAWILDLIFE.COM

Additionally, sampling that specifically targets federally listed sturgeon species requires a separate permit from the National Oceanic and Atmospheric Administration.

All species collection data, including common species, should be submitted to GADNR in the permittee's Scientific Collection Permit Report, emailed to zachariah.abouhamdan@dnr.ga.gov. Electronic submission of data is required. Instructions and a data template are available at http://georgiawildlife.com/conservation/species-of-concern#providing.

*Please see your individual permit for specific conditions that may apply in addition to these general conditions.

**Any Robust Redhorse or Sturgeon encountered shall be documented with GPS location. Collected individuals should be measured, weighed, and returned to point of capture. Mortalities should be put on ice immediately and frozen as soon as possible. Encounters and corresponding data should be reported within one week to paula.marcinek@dnr.ga.gov."

***Electrofishing in the mainstem of Brasstown Creek in Towns County must be specifically authorized in the permittee's individual permit and may only be carried out between July 1st - October 31st.

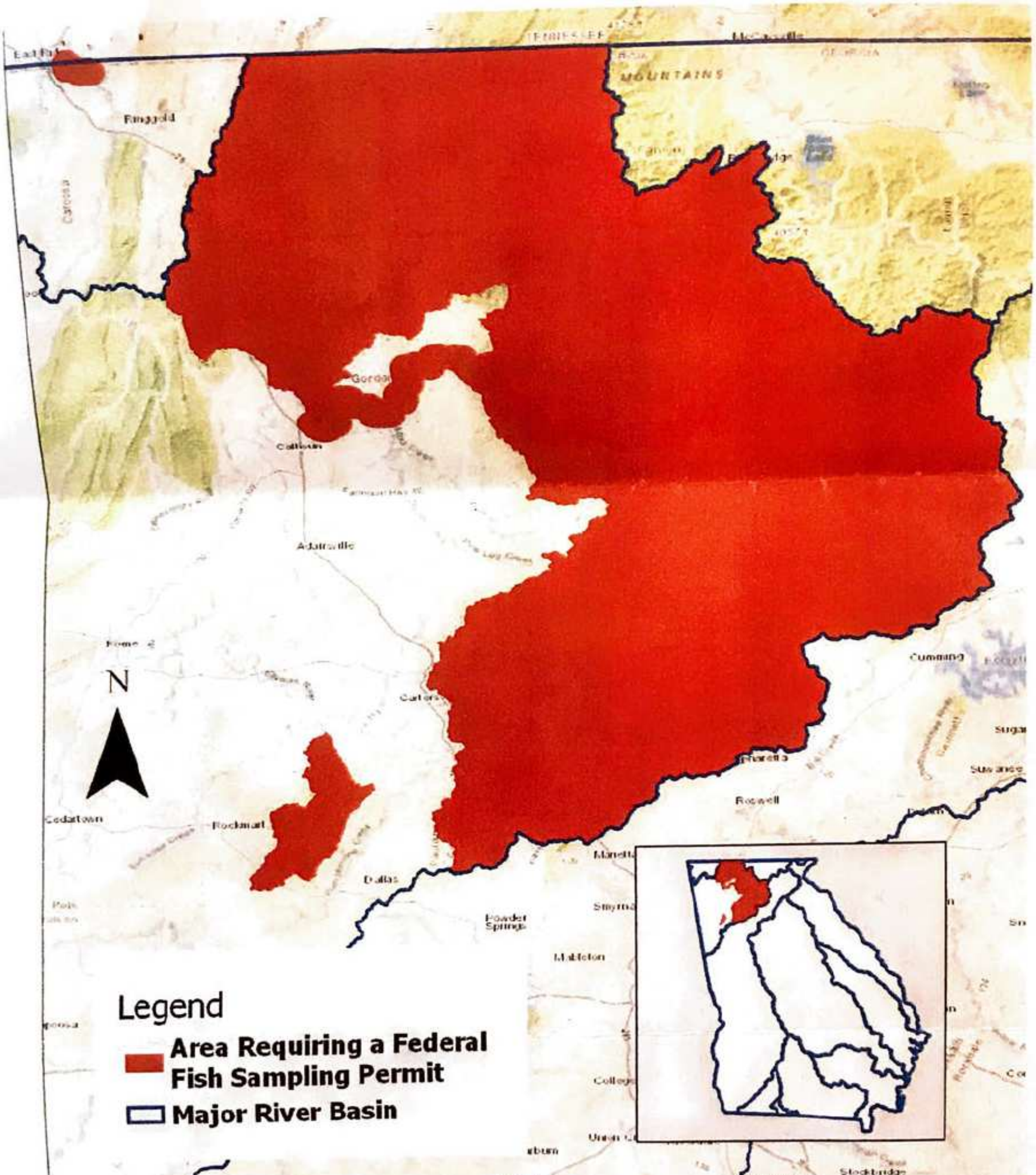

Figure 1: Areas in Georgia requiring a U.S. Fish and Wildlife Service permit in addition to a state permit for the collection of freshwater fishes. A separate permit is needed from the National Oceanic and Atmospheric Administration to target sturgeon (area not delineated on this figure).

Scientific Collecting Permit General Provisions

1. A Federal Permit is also required for collection of migratory birds or their parts, nests, or eggs.
2. Region or District Law Enforcement office, along with Game Management, Fisheries Management, or the U.S. Forest Service, i f applicable, in each area of collection must be notified at least three (3) days in advance of the date and place of specimens are to be collected, the species to be collected, methods of collection, and permit number.
3. Collectors should be as judicious and humane as possible in collecting and capture.
4. The Department of Natural Resources reserves the right to limit the kind and numbers of specimens collected in order to ensure the conservation of the natural resources of this State. The taking of wildlife at any time by means other than those conditions set forth in this permit is prohibited.
5. This permit does not authorize the taking of any listed (endangered, threatened, rare, or unusual) species unless specifically provided.
6. This permit does not authorize collections on State or Federal Wildlife Refuges, tidal wetlands and beaches, management areas, other sanctuaries, or parks unless specifically provided. If authorized, specific written permission must be obtained from the park manager, area manager, or supervisor prior to any collection activities.
7. Permits are not transferable. However, assistants, employees, or field workers may assist with collections. Designated representatives of the master permittee must possess a copy of the permit and a letter of authorization from the master permittee.
8. Permittees must submit an annual report of specimens collected upon expiration date of said permit.

***** ALL <u>ANNUAL REPORTS</u> SHALL BE SUBMITTED ELECTRONICALLY TO <u>zachariah.abouhamdan@dnr.ga.gov</u> WITH NONGAME, NO PERMITS WILL BE RENEWED UNTIL SUCH REPORT IS RECEIVED. (NOTE: DO NOT SEND YOUR APPLICATIONS OR REQUIRED DOCUMENTS ELECTRONICALLY, PLEASE USMAIL THEM TO THE SPECIAL PERMIT UNIT)**

9. Collections shall be available for inspection at any and all times to duly authorized inspectors or agents of the Department of Natural Resources.
10. Any permit may be revoked at any time for violation of the terms, exceptions, restrictions, or conditions of said permit.
11. The Department may request, when practical, duplicates of any collections taken under authority of this permit for future educational purposes.
12. All permits expire March 31.
13. This permit does not authorize the exchange of fish and wildlife, their parts, nests, or eggs, with persons who do not hold an appropriate permit.
14. Permit (copy and letter of authorization for sub-permittees) must be in possession while collecting.
15. **Encounters with animals listed on the Georgia DNR Nongame Conservation Section's Tracking List of Special Concern Animals must be reported within one (1) year to: GA DNR Nongame Conservation Section, (706) 557-3213 or Fax 706-557-3580 Electronic submission forms may be found at: <u>http://.georgiawildlife.com/conservation/species-of-concern</u>**

Georgia Department of Natural Resources
Law Enforcement Division
Special Permit Unit
2070 U.S. Highway 278, S.E.
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Appendix E:
Georgia Department of Natural Resources, State Parks & Historic Sites, Scientific Research and Collection Permit 2021 (Permit #062021)

GEORGIA
DEPARTMENT OF NATURAL RESOURCES
STATE PARKS & HISTORIC SITES

PERMIT # 062021

DATE ISSUED: February 10, 2021
PERMIT EXPIRES: December 31, 2021

SCIENTIFIC RESEARCH AND COLLECTION PERMIT

Permit holders: Christopher Le

The above named is hereby permitted to conduct research and collect at:
Reed Bingham State Park

Species to be collected and method(s):
While performing line sweep surveys, collect and record Gopher tortoise (*Gopherus polyphemus*) data, such as find location, anatomical measurements, PIT tag presence, Gopher Tortoise ticks (*Amblyomma tuberculatum*) - if found, etc. No traps will be used, only hand capture.

Please note, approval for this permit is contingent upon the possession of a current, valid Scientific Collection permit from the GA Department of Natural Resources – Special Permitting Unit, for Gopher tortoise research.

CONDITIONS:

1. PERMIT HOLDER IS REQUIRED TO CONTACT EACH COLLECTION SITE IN ADVANCE.
Contact the site manager at least three days prior to arriving at the park. Please inform the site manager that you hold an active collection permit and explain your needs for collection and/or research. When you arrive at the park, notify the site staff that you are on the property.

2. THIS PERMIT MUST BE IN POSSESSION WHILE CONDUCTING RESEARCH AND/OR COLLECTING.

3. PERMIT HOLDERS ARE REQUIRED TO SUBMIT A WRITTEN REPORT OF THEIR RESEARCH AND/OR COLLECTION ACTIVITY BY **JANUARY 31, 2022.** The written report should include the list of species collected. If you are conducting research, a description of the nature of your research, results and conclusions is required. Publications documenting your research may be submitted in lieu of a report. Renewal permits will be denied until a current report is on file. Reports should be sent to: **brian.nichols@dnr.ga.gov**

Approved: *[signature]*
Permit Coordinator

Scientific Research & Collection Permit Application

Name and Job Title of person requesting a permit (list all if multiple researchers involved):

Christopher Le – Graduate Assistant

Institution(s):_________Valdosta State University______________________

Mailing Address: ____[redacted]_______________

Telephone: ([redacted]___ **E-mail:** ____[redacted]____________

Please note:

1. **Please allow up to two weeks to process your application.**
2. **Permits are valid until December 31 of the current calendar year.**
3. **You may submit a research proposal if available and answer "see proposal" where appropriate on the application form.**

List the species you wish to collect, quantities, and methods of capture to be used (e.g., live traps, nets, etc.)

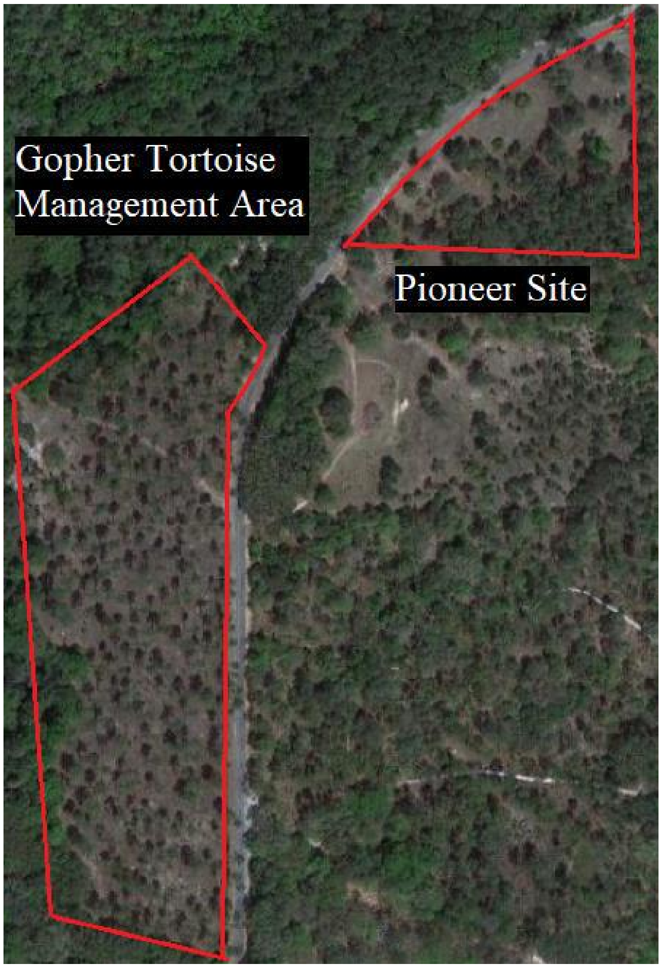
Reference the "2020 Gopher Tortoise RBSP AUP – Signed" pdf file.

50 Gopher tortoises (*Gopherus polyphemus*) max will be used per year. They will be hand captures without the use of traps.

List area (s) and park (s) in which you wish to collect.

Refer to the Google Maps Satellite Picture attached to this section. This is an aerial view of Reed Bingham State Park (RBSP), Cook County, GA.

1. Gopher Tortoise Management Area (GTMA), also known as the Longleaf Pine Restoration Project (located at RBSP)
2. Pioneer Site (located at RBSP)
3. Areas nearby the sites (located at RBSP)



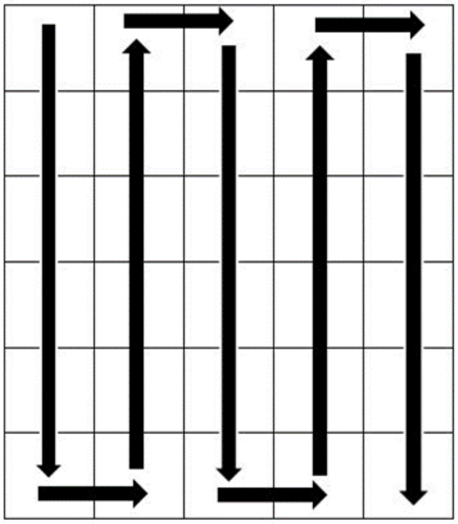
Describe briefly what you plan to do, including problem and methods.

I will focus on the two main release areas: Gopher Tortoise Management Area and the Pioneer Site. I will perform a line sweep survey (also known as a boustrophedon path – Diagram attached below) in each release area at a minimum of two times per month in the morning, afternoon, and/or evening.

Prior to every survey performed, the date and weather conditions including temperature, wind, humidity, dew point, pressure, UV index, and visibility from weather.com will be recorded.

If any gopher tortoise is encountered, then the following information will be recorded: time of capture, GPS coordinates, scan for PIT tag ID number, picture of their carapace and plastron for later Identification, straight carapace length, carapace width, plastron length, plastron width, shell height, weight, and sex. If Gopher Tortoise ticks (*Amblyomma tuberculatum*) are present on the tortoise, then they will be removed with forceps and placed into a jar of 70% alcohol and stored for future study. If a gopher tortoise does not have a PIT tag present, then information recorded from each gopher tortoise will be compared to others to determine if they are a unique individual.

Gopher tortoises are held at site of capture for less than 10 minutes and released after necessary information is recorded.



Boustrophedon pathway

What will be the disposition of your specimens? (All specimens must remain part of the public domain and thus be housed in a museum, college, university, school, park or other such institution.)

Gopher tortoises are released back to their area of capture after necessary information is recorded.

Justification: Describe briefly the reason for your research or collection.

Reference the last page of the “2020 Gopher Tortoise RBSP AUP – Signed” pdf file attached.

Note: If additional space is needed, please insert additional sheets.

I certify that the above information is correct and true to the best of my knowledge.

2/4/2021	
(Date)	**(Researcher's Signature)**

Submit completed permits to:

Sean Crooks
Georgia State Parks and Historic Sites
162 Park Lane
Reidsville, GA 30453
Office: 470-893-7732
Sean.crooks@dnr.ga.gov

To be completed by Parks & Historic Site Division

I recommend that this application be:

Approved ☑ **Not Approved** ☐

02-10-2021	
(Date)	**(Permit Coordinator)**

Date of Issue: 02-10-2021
Date of Expiration: 12-31-2021

Comments or Restrictions:

Please note, approval for this permit is contingent upon the possession of a current, valid Scientific Collection permit from the GA Department of Natural Resources – Special Permitting Unit, for Gopher tortoise research.