# Final Report

**SC-E-F17AP00226**
**Section 6 Reverted Funds – Section 6 Program**
**US Fish and Wildlife Service – South Carolina Field Office**
May 1, 2017 – February 15, 2020

***NOTE: This grant was amended on 12/14/18 to provide a no-cost, 12-month extension.***

**Project Title:** Can waif gopher tortoises be used to restore viability of gopher tortoise populations?

**Species:** Gopher Tortoise (*Gopherus polyphemus*), Federal Status – Warranted but Precluded, SC State Status – Endangered

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**Project Approach and Need**:

Gopher tortoises (*Gopherus polyphemus*) are declining throughout their range and for many populations, habitat management alone is unlikely to ensure persistence. At the same time, species biologists have determined that the minimum viable population size (≥ 250 adults) is larger than previously appreciated. Population augmentations are increasingly being used as recovery tools. However, in some states, particularly South Carolina (SC), there are no suitable donor populations and most other states are reluctant to translocate wild tortoises outside their own state. Thus, the primary source of potential animals for bolstering SC populations is through relocation of waif tortoises (origin unknown, formerly captive or rehabilitated tortoises). With any translocation, there are concerns about the site fidelity, post-release survivorship of released animals. When new animals are used to augment already occupied sites, there are additional concerns of genetic mixing, social integration, and disease transmission among animals from different sources. These concerns are particularly true for waifs, which tend to come from multiple sources whose history is often unknown. Indeed, a population built almost entirely from waifs represents the most extreme scenario and one under which these risks are most likely to be expressed. However, if these risks could be properly managed, waif animals could be used to augment populations without the need to remove individuals from wild donor populations. In addition, the ability to use waifs would address the issue of the increasing number of non-purposed, but ecologically valuable, gopher tortoises in captivity.

The Aiken Gopher Tortoise Heritage Preserve (AGTHP) is a 1,622-acre property in SC where the longleaf pine/wiregrass ecosystem has been restored and managed to promote habitat for gopher tortoises. AGTHP supported a remnant population (N ≤ 10 tortoises) when initially acquired in the early 1990s and was isolated (>80 km) from other native populations, thus precluding natural recolonization, but eliminating risks of waifs contacting other native populations. Since 2006, we (SCDNR and SREL in partnership) have released >280 gopher tortoises (primarily waifs). This project is the first to attempt to establish a viable population almost entirely from waif tortoises, a concept that has emerged as a priority issue by the Gopher Tortoise Council.

We received funding from SCDNR (via a Section 6 grant from USFWS) to evaluate, over a 2 yr period, survivorship and site fidelity (via mark-recapture; TASK 1), disease risks (via pathogen screening; TASK 2), and social integration (via nest searching and parentage analysis; TASK 3), in this population 10 years-post project initiation. AGTHP provides a unique setting to evaluate concerns related to augmentation and translocation of tortoises by providing the extreme case (waif tortoises from multiple sources, differing histories, at the northern extreme of the range) for these concerns to manifest. These data will help determine the effectiveness of using waif tortoises to re-establish a viable population, assist in the recovery of this species in SC, and provide guidance for use of this technique elsewhere in the species' range. The results of each task are summarized below.

**TASK 1 – Site fidelity and survivorship**

All tortoises released to date (2006-2019) on AGTHP were individually marked, measured, and sexed at time of release. In addition, their source population (if known) was noted (Figures 1.1, 1.2; Table 1.1). To encourage site fidelity, tortoises were placed into temporary 1-ha circular enclosures for at least 12 months, after which time enclosures were removed. However, no subsequent systematic monitoring of animals occurred beyond radio-tracking the tortoises from the first release pen. During 2017-2018, we conducted a complete survey of all potential habitat on the preserve via closely spaced transects. We recorded the locations of all encountered burrows using GPS; we marked, assigned a unique ID, and measured the width and height of entrances of all intact burrows. Previously marked burrows were assigned a unique ID regardless of condition; however, collapsed burrows that had not been previously marked were not assigned an ID during the 2017-2018 surveys. We mapped a total of 487 burrows across the Preserve.

We attempted to capture tortoises occupying all intact burrows. Prior to setting traps, we scoped burrows with a burrow camera to verify occupancy. We set live wire traps at occupied burrows, shaded them with burlap and/or live vegetation, and checked them at least twice daily. We transported all captured tortoises to SREL for processing, which included morphometric measurements, palpating females to determine gravidity, visual health assessment, took oral and cloacal swabs for pathogen screening, and conducted blood draw for blood smears (to assess parasites and Heterophil/Lymphocyte ratios), future genotyping, and archiving of plasma. In addition to trying to trap the entire preserve once, we also trapped Unit 16 in both years to be able to estimate detection probability and more accurately model survivorship. Sampling effort increased during 2018 due to both increasing the number of traps and extending the trapping season.

During the 2017-2018 field seasons, we documented a total of 137 individuals, of which 128 were live captures and 9 were shells (Figure 1.3; Table 1.2). Some tortoises were captured in both years, either due to movement of tortoises between units between years or to being captured in Unit 16 in both years.

As expected, annual apparent survival was lowest in juveniles (0.28), with subadult, adult male and adult female annual apparent survival ranging from 0.90-0.96 and not differing significantly from each other (Figures 1.4, 1.5; Table 1.3). Our study provides one of the few estimates for juvenile survival available for any population of gopher tortoises, and survival of adults is similar to those reported for both wild *in situ* populations and for wild-to-wild translocated populations (Tuberville et al. 2008, 2014; Wright 2016). We did not detect a difference in survival due to location from which we obtained waifs (South Carolina, Georgia, Florida, or outside the species' range).

It is difficult to know the fate of the tortoises that were not detected during the 2017-2018 trapping sessions and whether undetected tortoises died between release and re-trapping, dispersed from the Preserve, or resided on the Preserve but were not trapped. However, of those animals we recaptured during 2017-2018, over 75% were recaptured within 400m of their original release location, suggesting strong fidelity to the translocation site (Figures 1.6, 1.7).

Table 1.1. Criteria based on midline carapace length (MCL) used to assign life stage of gopher tortoises released and/or captured at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC during 2006-2018. Secondary sexual characteristics were used to determine sex of mature animals. Individuals smaller than 230 mm were considered to be adult females if they were gravid.

| **Stage** | **Midline Carapace Length (MCL)** | **Additional Characteristics** |
|---|---|---|
| **Hatchling** | < 68 mm | |
| **Juvenile** | ≥ 68 mm, < 130 mm | |
| **Subadult** | ≥130 mm, < 230 mm | Flat plastron |
| **Adult Male** | ≥180 mm | Concave plastron, gular protrusion |
| **Adult Female** | ≥230 mm | Flat plastron |

Table 1.2. Summary of number of individual gopher tortoises captured during the 2017-2018 field seasons at Aiken Gopher Tortoise Heritage Preserve, SC.

| | **Alive** | **Dead** | **Total** |
|---|---|---|---|
| Indiv. captured 2017 only | 12 | 7 | 19 |
| Indiv. captured 2018 only | 70 | 2 | 72 |
| Indiv. captured both years | 46 | -- | 46 |
| **Total individuals captured** | **128** | **9** | **137** |

Table 1.3. Model estimates of mean gopher tortoise apparent survival and transition probabilities to stage class with corresponding SD and 95% Credible Intervals. Model included stage class as a fixed effect and pen as a random effect for survival probabilities. Transition probabilities were estimated for juveniles maturing to subadults (Subadult), and subadults maturing to either adult male (Adult Male) or adult female (Adult Female).

| **Parameter** | **Mean** | **SD** | **2.50%** | **97.50%** |
|---|---|---|---|---|
| *Stage Survival* | | | | |
| Juvenile | 0.25 | 0.17 | 0.03 | 0.67 |
| Subadult | 0.96 | 0.05 | 0.84 | 1.00 |
| Adult Male | 0.91 | 0.07 | 0.70 | 0.99 |
| Adult Female | 0.95 | 0.04 | 0.83 | 0.99 |
| *Stage Transition* | | | | |
| Subadult | 0.35 | 0.16 | 0.09 | 0.69 |
| Adult Male | 0.04 | 0.02 | 0.01 | 0.09 |
| Adult Female | 0.12 | 0.04 | 0.06 | 0.2 |

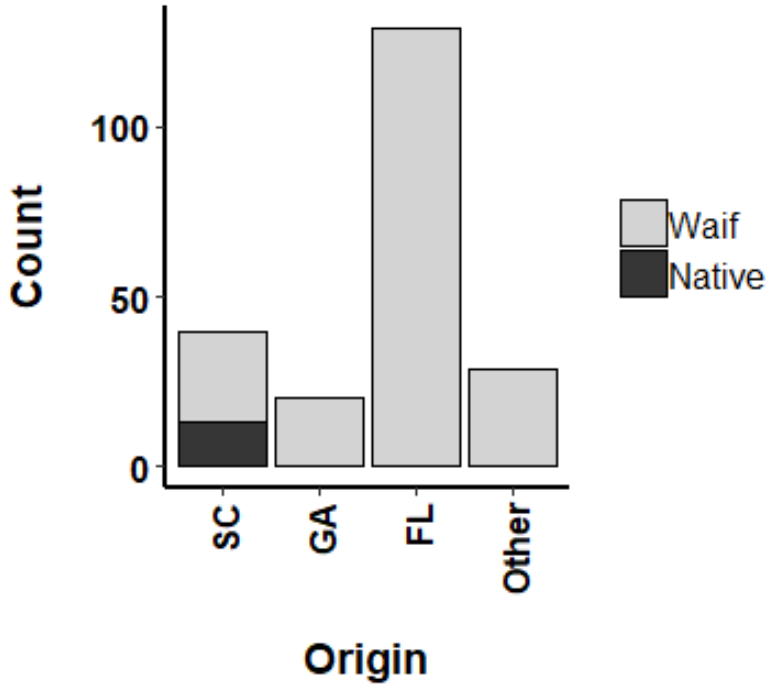



Figure 1.1. Number of gopher tortoises released at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC by tortoise origin. Due to the small number of native animals, native tortoises and tortoises from elsewhere in the state were not differentiated in analysis. The "other" category includes tortoises from states other than SC, GA, and FL; those held outside their native range (usually as illegal pets); and those of unknown origin.

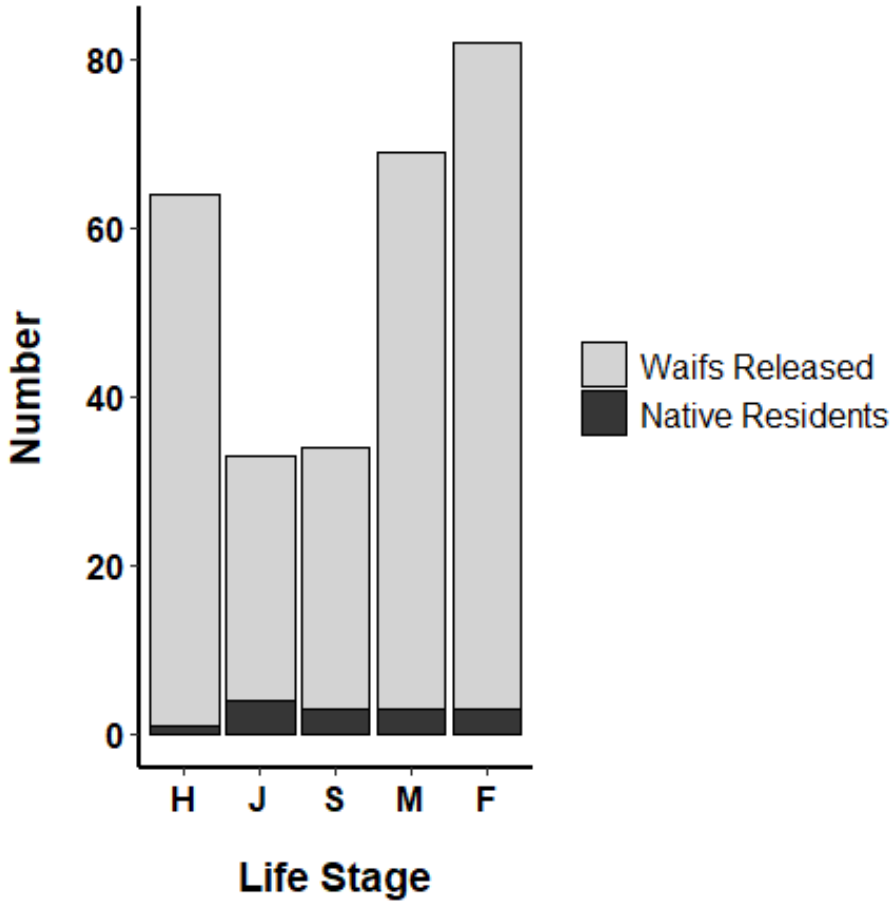



Figure 1.2. Number of waif gopher tortoises and native resident gopher tortoises marked and released at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC between 2006 and 2017. Analyses included juvenile (J), subadult (S), adult male (M), and adult female (F) stage classes, but not hatchlings (H).

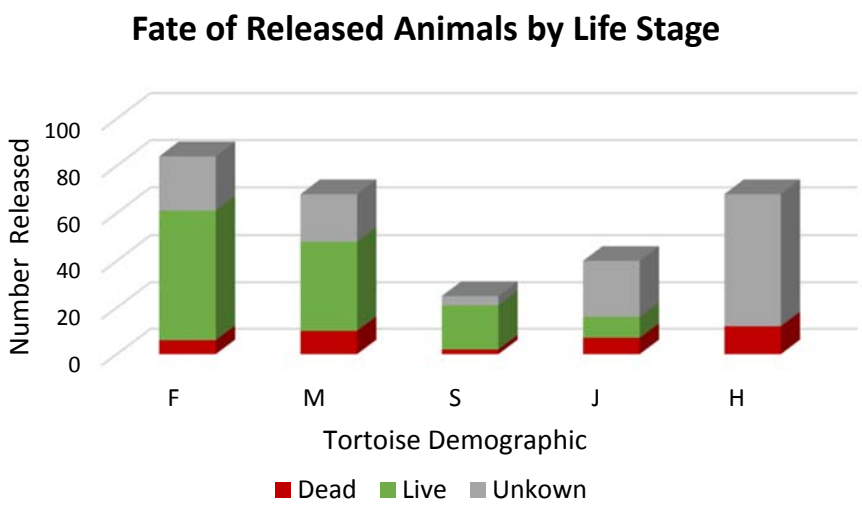



Figure 1.3. Fate of waifs released at Aiken Gopher Tortoise Heritage Preserve based on 2017-2018 live trapping and shells collected throughout the project by stage class at time of release: adult females (F), adult males (M), subadult (S), juvenile (J), and hatchling (H).

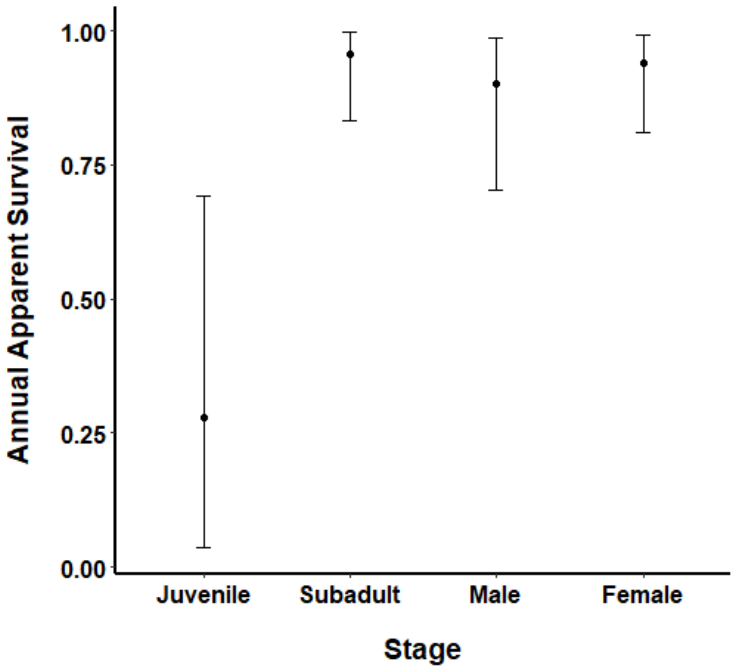



Figure 1.4. Mean annual apparent survival probabilities and 95% Bayesian credible intervals for juvenile (excluding hatchlings), subadult, adult male, and adult female stage classes of gopher tortoises, based on estimates from a joint live-dead multistate Cormack-Jolly-Seber model. Tortoises were marked between 2006-2017 and recaptured 2017-2018 at the Aiken Gopher Tortoise Heritage Preserve, Aiken County, South Carolina.

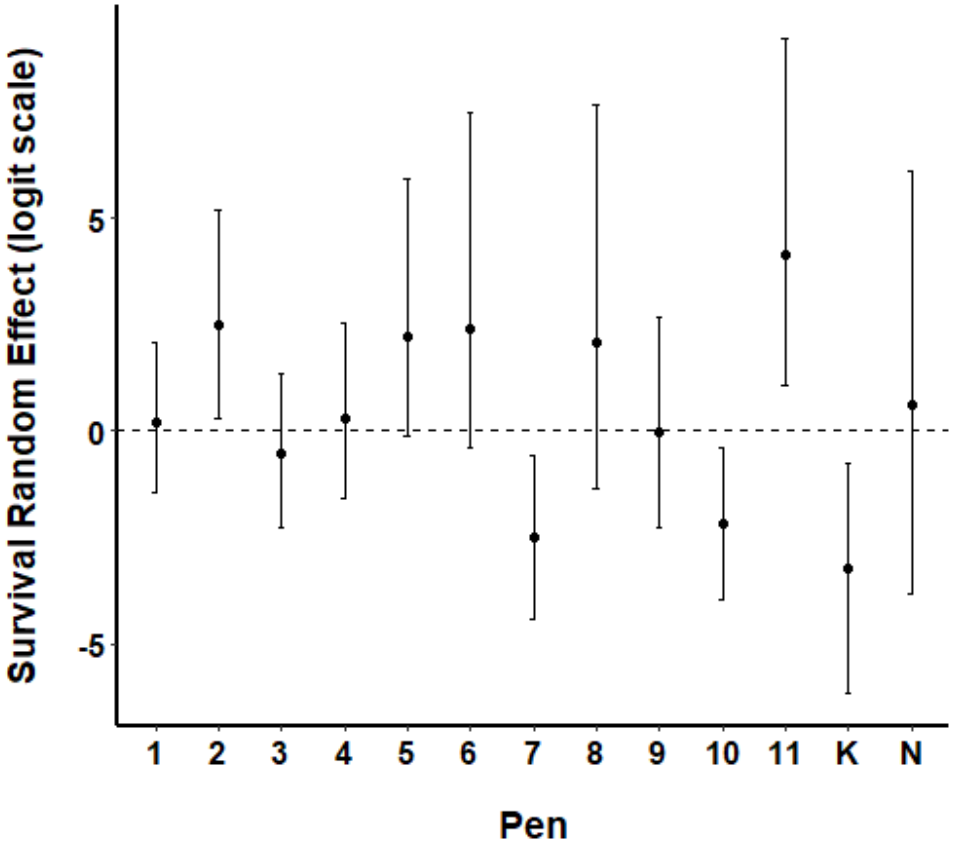



Figure 1.5. Estimated individual pen effects (and 95% Bayesian credible intervals) on annual apparent survival (logit scale) of gopher tortoises marked between 2006-2017 and recaptured 2017-2018 on the Aiken Gopher Tortoise Heritage Preserve, Aiken County, South Carolina. Tortoises were penned for ≥1 year prior to release. Mark-recapture data were analyzed in joint live-dead multistate Cormack-Jolly-Seber models, and pen was included as a random effect in all candidate models. Unmarked tortoises found on site during the 2017-2018 surveys were classified as no pen (N), while all other numbers (1-11) and letters (K) refer to physical pens.

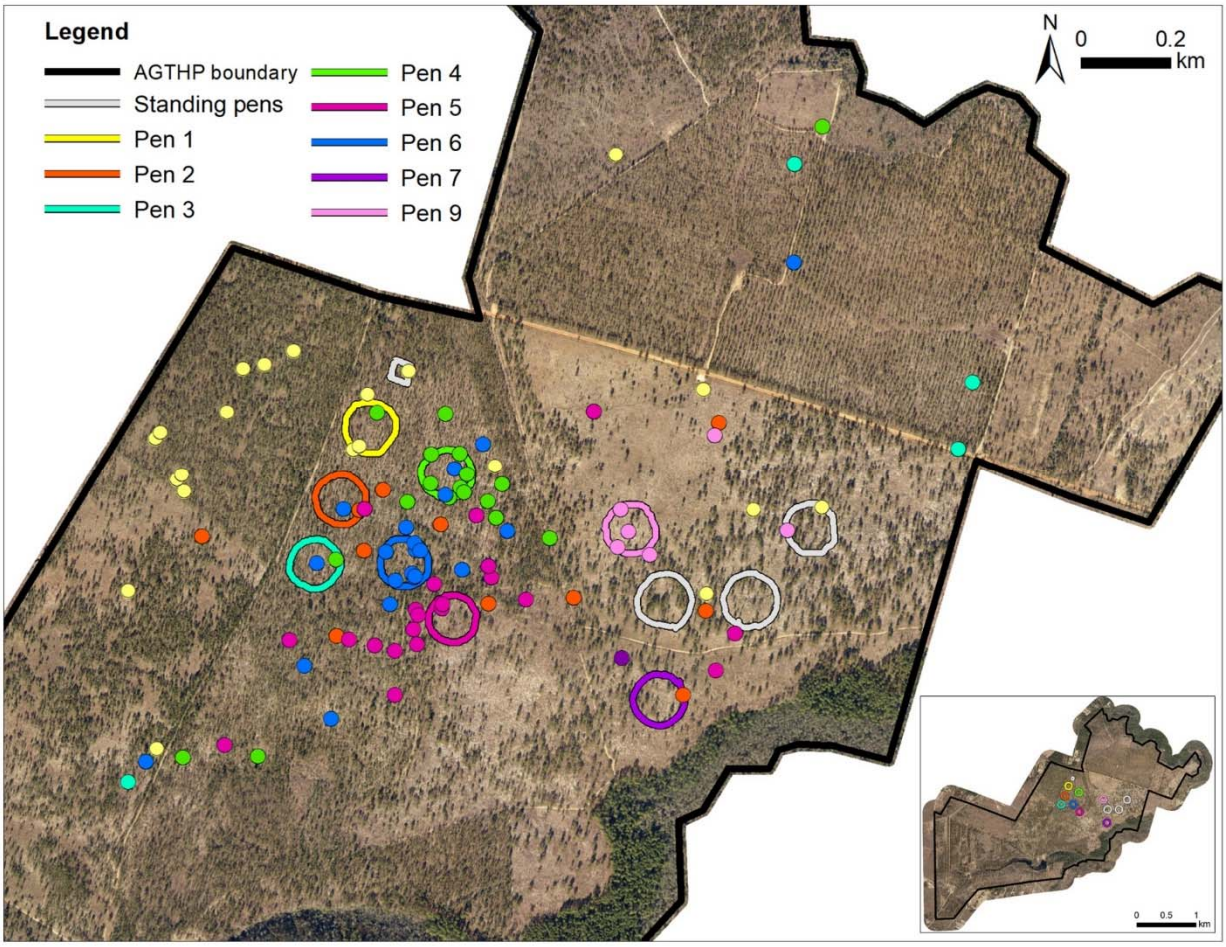



Figure 1.6. Map of gopher tortoise capture locations in 2017-2018 following their release at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC between 2006-2016. All tortoises were penned in 1ha pens for ≥ 1 yr. Capture location of tortoises are color coded with their respective release pen. Gray-outlined pens (representing Pens 8, 10, 11, and K) were still standing at the time of the survey and tortoise capture locations associated with these pens were not included in the site fidelity analysis.

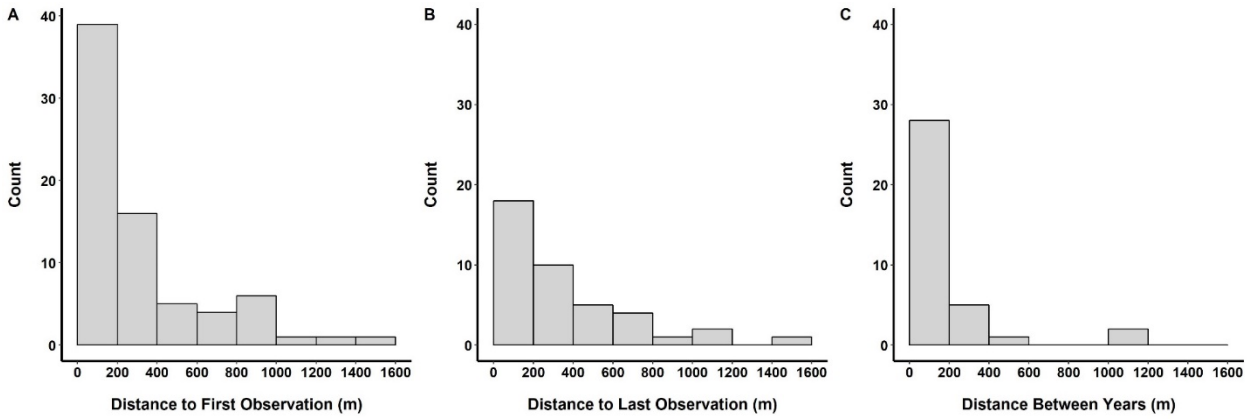



Figure 1.7. Histogram of gopher tortoise dispersal distance following release at the Aiken Gopher Tortoise Heritage Preserve (Aiken County, SC). Distance to First Observation is defined as the Euclidean distance from the center point of the tortoise's release pen to the location of its first observation during 2017-2018. The Distance to the Last Observation is defined as the Euclidean distance between the center point of the tortoise's release pen and its last recapture location. Distance between years refers to the Euclidean distance between a tortoise's first observation in 2017 and first observation in 2018 if it was observed both years.

**TASK 2 – Disease testing**

Due to funding and logistical constraints prior to this grant, disease screening prior to release was precluded. Thus, this grant is the first opportunity to screen for pathogens, the results of which should reveal the current disease and/or infection status within the population.

At the end of each season we submitted oral and cloacal swabs to Dr. Matthew Allender at the University of Illinois Wildlife Epidemiology Laboratory for screening for 13 pathogens (Table 2.1). Some pathogens were specific to wild *Gopherus*, but we also screened for a broader array of herpetofaunal pathogens as these formerly captive waifs could have come into contact with other herp species, depending on their history. During 2017, the only pathogen detected was *Mycoplasma agassizii* and it was only detected in oral swabs (all cloacal swabs were negative; Table 2.2). Six of the 60 individuals tested positive for a prevalence of 10%, which is comparable to wild gopher tortoise populations tested to date. In 2018, we submitted 122 oral swabs and 82 cloacal swabs. The oral swabs represented 78 individuals not tested in 2017 and 44 individuals that were tested in both years, allowing us to evaluate change in infection status between years. In 2018, *M. agassizii* was prevalent in 13.9% of individuals and *M. testudineum* was prevalent in 0.8% of sampled individuals. Of the 42 individuals tested in both 2017 and 2018, six individuals (14.3%) changed infection status between years (Table 2.3).

Table 2.1. Pathogens tested for in gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC based on oral and cloacal swabs submitted to the Wildlife Epidemiology Lab at the University of Illinois. Swabs were tested for a total of 14 pathogens, with 11 pathogens tested in both 2017 and 2018. For those pathogens which had been previously documented in gopher tortoises in other populations, the supporting citation is provided.

| **Pathogen** | **Detection Method** | **Documented in *G. polyphemus*** | **Literature Documenting Gopher Tortoise Infections** |
|---|---|---|---|
| *Mycoplasma agassizii* | qPCR | Yes | Brown et al. 1999 |
| *Mycoplasma testudineum* | qPCR | Yes | Brown et al. 2004 |
| Emydid *Mycoplasma* sp.* | qPCR | No | |
| Frog Virus 3- *Ranavirus* | qPCR | Yes | Johnson et al. 2006, Cozad 2018 |
| Ambystoma tigrinum virus – *Ranavirus* | qPCR | No | |
| Bohle iridovirus— *Ranavirus* | qPCR | No | |
| Epizootic hemorrhagic necrosis virus | qPCR | No | |
| *Salmonella tymphimurium* | qPCR | Yes*** | Lockart et al. 2007; Charles-Smith et al. 2009 |
| *Salmonella enteritidis* | qPCR | Yes*** | Lockart et al. 2007; Charles-Smith et al. 2009 |
| Testudinid herpesvirus 2 | qPCR | No | |
| Tortoise intranuclear coccidia (TINC) | qPCR | No | |
| *Borrelia burdorferi*** | qPCR | No | |
| *Anaplasma phagocytophilum*** | qPCR | Yes | Wellehen et al. 2017, Cozad 2018 |
| Adenovirus** | PCR | No | |

*Tested in 2018, but not in 2017. ** Tested in 2017, but not 2018. ***(*Salmonella* serotype not distinguished (Lockart et al. 2007; Charles-Smith et al. 2009).

Table 2.2. Individual gopher tortoises from the Aiken Gopher Tortoises Heritage Preserve in Aiken County, SC, for which pathogens were detected in oral, cloacal, or naral swabs were submitted to the Wildlife Epidemiology Laboratory at the University of Illinois. Using qPCR, pathogen load was quantified for individuals positive for *Mycoplasma* sp. infections in 2017 and 2018. Two individuals (421 and 436) had relatively low pathogen copy numbers in 2017 (9.09 and 1.36 copies/ng DNA) before testing negative in 2018. *denotes individuals with more than one sample.

| **ID** | **Swab Type** | **Year** | **DNA** (ng/ul) | **Pathogen** | **Pathogen Copy Numbers** | |
|---|---|---|---|---|---|---|
| | | | | | (copies/ uL rxn) | (copies/ng DNA) |
| 19 | Oral | 2017 | 2.25 | *M. agassizii* | 8,967.12 | 1,594.16 |
| 416 | Oral | 2017 | 3.26 | *M. agassizii* | 25,507.59 | 3,129.77 |
| 421 | Oral | 2017 | 5.25 | *M. agassizii* | 119.25 | 9.09 |
| 436* | Naral | 2017 | 2.62 | *M. agassizii* | 8.88 | 1.36 |
| 436* | Oral | 2017 | 4.73 | *M. agassizii* | 178.47 | 15.09 |
| 542 | Oral | 2017 | 5.37 | *M. agassizii* | 88.68 | 6.61 |
| 672 | Oral | 2017 | 1.65 | *M. agassizii* | 14,128.87 | 3,425.18 |
| 16 | Oral | 2018 | 5.92 | *M. testudineum* | 70.57 | 4.77 |
| 19 | Oral | 2018 | 4.45 | *M. agassizii* | 31,262.45 | 2,810.11 |
| 416 | Oral | 2018 | 36.05 | *M. agassizii* | 602,801.5 | 6,688.5 |
| 469 | Oral | 2018 | 11.94 | *M. agassizii* | 23.59 | 0.79 |
| 546* | Oral | 2018 | 38.58 | *M. agassizii* | 14.88 | 0.15 |
| 546* | Naral | 2018 | 5.23 | *M. agassizii* | 75.06 | 5.74 |
| 591 | Oral | 2018 | 5.26 | *M. agassizii* | 10.64 | 0.81 |
| 595 | Oral | 2018 | 10.53 | Emydid *M.* sp. | 11.73 | 0.45 |
| 603 | Oral | 2018 | 29.68 | *M. agassizii* | 33,878.50 | 456.58 |
| 626 | Oral | 2018 | 3.32 | *M. agassizii* | 39.14 | 4.72 |
| 639 | Oral | 2018 | 4.17 | *M. agassizii* | 4,017.15 | 385.34 |
| 645 | Oral | 2018 | 10.37 | *M. agassizii* | 545.37 | 21.04 |
| 646 | Oral | 2018 | 5.43 | *M. agassizii* | 34.33 | 2.53 |
| 666* | Naral | 2018 | 3.97 | *M. agassizii* | 518.24 | 52.22 |
| 666* | Oral | 2018 | 17.89 | *M. agassizii* | 52,879.92 | 1,182.33 |
| 667* | Cloacal | 2018 | 3.67 | *M. agassizii* | 2,541.16 | 276.97 |
| 667* | Naral | 2018 | 3.67 | *M. agassizii* | 40,436.56 | 4,407.25 |
| 667* | Oral | 2018 | 7.31 | *M. agassizii* | 63,708.83 | 3,486.12 |
| 672 | Oral | 2018 | 21.04 | *M. agassizii* | 101,294.6 | 1,925.75 |
| 678 | Oral | 2018 | 11.49 | *M. agassizii* | 2,262.58 | 78.77 |
| 689 | Oral | 2018 | 10.35 | *M. agassizii* | 9.06 | 0.35 |
| 691* | Cloacal | 2018 | 5.1 | *M. agassizii* | 70.95 | 5.56 |
| 691* | Oral | 2018 | 2.1 | *M. agassizii* | 3,370.64 | 642.03 |
| 694* | Cloacal | 2018 | 10.73 | *M. agassizii* | 103.75 | 3.87 |
| 694* | Naral | 2018 | 5.71 | *M. agassizii* | 321.03 | 22.49 |
| 694* | Oral | 2018 | 6.92 | *M. agassizii* | 2,347.44 | 135.69 |

Table 2.3. *Mycoplasma* results for individual gopher tortoises from Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC sampled in both 2017 and 2018, based on oral swabs tested using qPCR. Of the 42 waif gopher tortoises screened for *Mycoplasma* in both years, 6 changed infection status between years for either *Mycoplasma agassizii or Mycoplasma testudineum*. Of the 4 individuals that converted from negative in 2017 to positive to 2018, 3 were positive for *Mycoplasma agassizii* and 1 was positive for *Mycoplasma testudineum.*

| **Resampled animals** | Positive 2018 | Negative 2018 |
|---|---|---|
| Positive 2017 | 3 | 2 |
| Negative 2017 | 4 | 33 |

**TASK 3 – Social integration and parentage analysis**

During the 2017-2018 reproductive seasons, we actively searched for nests at active burrows. We protected nests in the field until shortly before hatching, after which we transferred them to the lab at SREL until hatching. As part of the social integration portion of this grant, we collected shell notches or blood samples from 2017 and 2018 recruits. We also had previously archived samples from several clutches from 2010, 2015, and 2016. From all recruits, we extracted DNA from all blood samples of adults and recruits. We contracted with University of Arizona Core Genetics Laboratory to extract DNA from shell notches of hatchlings and run fragment analysis on all DNA samples (including those we extracted) at 18 loci (Table 3.1). These loci had been previously developed for *Gopherus* or other tortoise species and tested on a subset of 24 gopher tortoise samples from AGTHP to ensure that they would be informative. Genotyping was performed by Dr. Taylor Edwards (University of Arizona) and initial parentage analysis by Dr. Kristina Ramstad (University of South Carolina Aiken). More detailed parentage analysis and interpretation will follow.

After excluding the samples that failed to amplify, we obtained partial or complete genotypes from 201 founders and 116 recruits. We used founder genotypes to characterize the genetic diversity of the population and quantify the power of loci to detect parentage patterns at AGTHP (Table 3.1) using GenAlEx software version 6.5 (Peakall and Smouse 2006, 2012). We created separate lists for recruits, candidate mothers, and candidate fathers. Candidate mothers included all tortoises released at the preserve as an adult female, tortoises released as immatures but known to later mature as an adult female, and all animals released as subadults that could have matured over the course of study but for which sex was unknown. A list of candidate fathers was similarly created.

We used CERVUS (version 3.0; Kalinowski et al. 2007) to assign candidate mothers and fathers to individual recruits. CERVUS assigns the most likely mother to a recruit, the most likely father, and the most likely trio (of recruit, mother, father) while also allowing for genotyping error. However, CERVUS does not consider known sibship or half sibship (i.e. whether or not recruits are clutchmates). Thus, we compared putative assignments across known clutchmates to identify the mother that was consistently assigned as a putative mother and assigned her as the known mother. Based on the identified mother, we then assigned the most likely father to individual recruits within a clutch based on CERVUS output and the minimum number of fathers needed to explain the genotypes of recruits within the clutch.

Our initial parentage analysis included only those individuals that had ≥16 of 18 loci successfully amplify. After excluding eggs that did not hatch, samples that did not amplify, and individuals with less complete genotypes, there were 19 clutches for which candidate parents could be assigned to at least one recruit. Of those, 17 clutches had sufficient number of loci genotyped for at least three recruits included in parentage analysis —the minimum required to detect multiple paternity within a clutch.

We assigned parentage to a total of 88 recruits from 19 clutches: 1 clutch from 2010, 4 from 2015, 4 from 2016, and 10 from 2017. Due to poor amplification of samples, no 2018 clutches were able to be included in the analysis. Of the 17 clutches with at least three recruits, only one clutch required a second male to explain recruit genotypes (i.e. was multiple-sired); the remaining 16 clutches (94%) were sired by a single male. Collectively, at least 15 males contributed to the clutches that were included in parentage analysis.

A total of 17 females were represented by the 19 clutches, including Female #2000 and an unsampled female. The unsampled female was either: a) released by a member of the public without knowledge of

SCDNR/SREL; b) a native AGTHP animal never captured during mark-recapture efforts; or c) one of the few founder females for which a blood sample could not be located or the sample did not successfully amplify. The unsampled female's clutch was sired by male HIMPQ – a native AGTHP animal that was documented on the Preserve prior to the release of any waif gopher tortoises. Male HIMPQ was not captured during the 2017-2018 recapture effort; he was last observed in 2010. Thus, either he was still residing on the Preserve but was undetected during trapping efforts, or he had died and left the Preserve within the last few years but had previously mated with the unsampled female, who stored his sperm. Female #2000 was an unmarked female captured in 2017 and was assigned to a clutch produced in 2017. Her recruits were sired by Male #4, one of the first waifs released at AGTHP (Pen 1).

Two females had clutches represented in more than one year. Female 140 (a native of AGTHP) was represented among the clutches we sampled in 2016 and 2017; for both clutches, Male 4 (a waif from south Georgia) sired all of her offspring. Female 415 was represented in both 2015 and 2016; Male 431 sired all of her offspring from the two clutches. Both Female 415 and Male 431 were waifs from south Florida. As has been previously documented in other studies (Tuberville et al 2011, White et al. 2018), the number of recruits assigned to individual males varies among males. Of those males assigned to recruits, the number of recruits assigned ranged from 1 – 16 (Figure 3.1). Overall, only a small percentage of known adult males (15 of 79; 19.0%) were assigned to any recruit. However, the number of clutches sampled was very low each year and clutches were only sampled in a few years; thus the sampled recruits undoubtedly are representing only a small proportion of recruits produced. Also, additional offspring may be able to be included in parentage analysis if we are able to relax the number of genotyped loci required. Currently, however, our parentage analysis is most meaningful in terms of revealing the social interactions of those males and females represented in our sample rather than for revealing the proportion of founders contributing to the next generation.

By far the most successful male was Male #4 (Pen 1), siring 16 of the 88 recruits (18.2%) included in the parentage analysis (Figure 3.1). Collectively, three males from Pen 1 (#4, 5, 118; all from south Georgia but from at least two different sites) sired 25 (28.4%) recruits (Table 3.2). Of note, two of the native AGTHP males (HIMPQ, 144) sired at least 11 additional recruits (12.5%). Both Pen 1 and native AGTHP males are among the first males to establish residency at the Preserve and, combined, sired 30.7% of recruits included in parentage analysis. This pattern has been previously noted in a translocated population at St. Catherine's Island, GA (Tuberville et al. 2011), where males with longer residency times exhibited higher reproductive success than males more recently released. Although residency time may factor into male reproductive success, we did document successful reproduction from males and females from both different release pens and also different known sources (Table 3.2). Of note, Male 645 was obtained from Rhode Island and was presumably a long-term captive and was documented to have successfully sired offspring. In addition, even animals (both male and female) translocated from the farthest southern extent of their range successfully produced offspring at AGTHP at the northern extent of the species' range. The extent to which residency time, waif source, location within the population, male size, and other factors may influence reproductive success and social interactions will require more detailed analysis.

Table 3.1 Loci characteristics based on analysis of founder representing candidate parents. The following is listed for each microsatellite marker: number of alleles among founders, observed (Hobs) and expected heterozygosity (Hexp), probability of identity, probability of excluding parent with other parent unknown (E-1P) or when known (E-2P), and probability of excluding a parent pair. The source in which each microsatellite was originally published is provided as footnote.

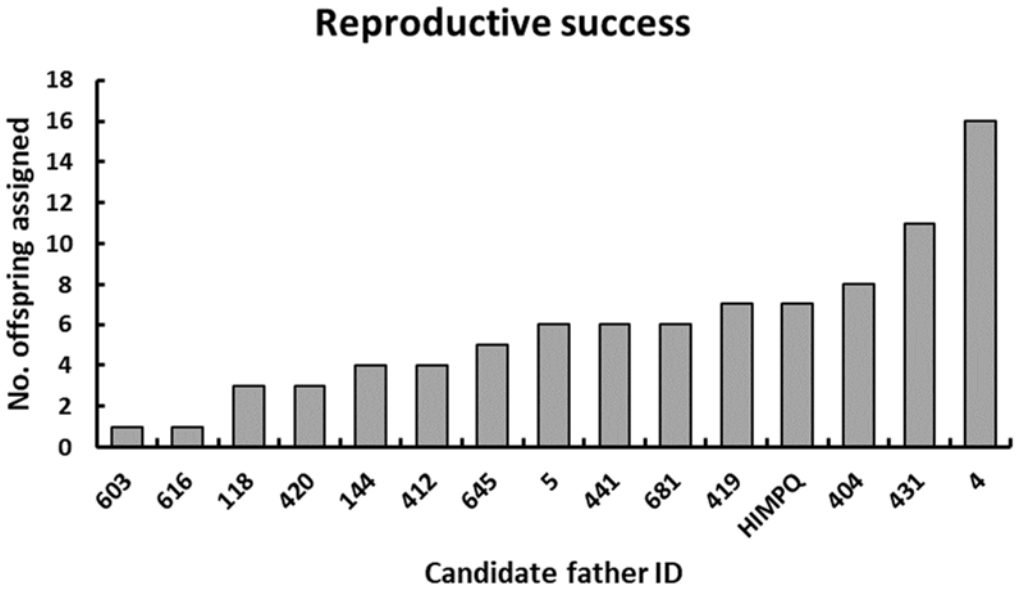
| Locus | k | H(obs) | H(exp) | Prob identity | E-1P | E-2P | Parent Pair |
|---|---|---|---|---|---|---|---|
| [1]GP96 | 7 | 0.418 | 0.471 | 0.31728 | 0.11749 | 0.26813 | 0.43269 |
| [1]GP61 | 7 | 0.478 | 0.581 | 0.24076 | 0.18157 | 0.32363 | 0.48556 |
| [1]GP19 | 3 | 0.431 | 0.533 | 0.30502 | 0.14102 | 0.25376 | 0.38435 |
| [1]GP102 | 15 | 0.333 | 0.816 | 0.05997 | 0.46081 | 0.63484 | 0.81704 |
| [1]GP30 | 11 | 0.440 | 0.598 | 0.23549 | 0.19565 | 0.32940 | 0.48847 |
| [1]GP26 | 6 | 0.390 | 0.487 | 0.28863 | 0.13202 | 0.30026 | 0.48795 |
| [1]GP15 | 20 | 0.720 | 0.829 | 0.05156 | 0.49026 | 0.66090 | 0.83870 |
| [1]GP81 | 8 | 0.577 | 0.729 | 0.12022 | 0.31593 | 0.49000 | 0.67286 |
| [2]Goag6 | 21 | 0.691 | 0.808 | 0.04946 | 0.49069 | 0.66416 | 0.85972 |
| [2]Goag32 | 3 | 0.510 | 0.551 | 0.29587 | 0.15380 | 0.25907 | 0.38761 |
| [3]ROM01 | 10 | 0.637 | 0.765 | 0.07950 | 0.39737 | 0.57975 | 0.77689 |
| [3]ROM07 | 9 | 0.662 | 0.746 | 0.10869 | 0.34160 | 0.51776 | 0.70844 |
| [3]ROM04 | 6 | 0.427 | 0.547 | 0.30511 | 0.14455 | 0.25630 | 0.38972 |
| [4]Test56 | 35 | 0.821 | 0.941 | 0.00690 | 0.77970 | 0.87496 | 0.97556 |
| [3]ROM03 | 3 | 0.132 | 0.15 | 0.71571 | 0.01292 | 0.07576 | 0.13612 |
| [3]ROM02 | 6 | 0.502 | 0.566 | 0.24751 | 0.17580 | 0.31557 | 0.47442 |
| [3]ROM05 | 5 | 0.492 | 0.612 | 0.20429 | 0.20523 | 0.35937 | 0.52521 |
| [5]ROM09 | 32 | 0.815 | 0.931 | 0.00967 | 0.74667 | 0.85418 | 0.96551 |
| | | | | | | | |
| **Multi locus (18)** | | | | **5.5 x 10^-17** | **0.99957** | **1.00000** | **1.00000** |

[1]Schwartz et al. (2003); [2]Edwards et al. (2003); [3]Edwards et al. 2011; [4]Forlani et al. (2005); [5]Davy et al. (2011)

Table 3.2. Known mating pairs of gopher tortoises based on 88 recruits from 19 clutches collected from Aiken Gopher Tortoise Preserve, SC in 2010, 2015-2017 and genotyped at ≥16 loci. Confirmed mothers are listed across the top, along with their corresponding release pen number (above) and population source (below in parentheses). Confirmed fathers are listed in first column. Note: recruits included in analysis represent only a small proportion of recruits produced during four years of the 10+ year project.

| | | PEN 2 | | | PEN 3 | PEN 4 | PEN 5 | PEN 6 | | | | | | PEN 9 | PEN 10 | PEN 11 | NA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 140 (AGTP) | 143 (AGTP) | 148 (AGTP) | 320 (SC) | 406 (FL) | 426 (FL) | 415 (FL) | 418 (FL) | 429 (FL) | 437 (FL) | 463 (FL) | 612 (UNKN) | 630 (FL) | 668 (FL) | 678 (FL) | 2000 (UNKN) | Unsampled (UNKN) |
| PEN 1 | 4 (GA) | X | X | | | | | | | | | | | | | | X | |
| | 5 (GA) | | | | | X | | | | | | | | | | | | |
| | 118 (GA) | | | | X | | | | | | | | | | | | | |
| PEN 2 | 144 (AGTP) | | | X | | | | | | | | | | | | | | |
| | HIMPQ (AGTP) | | | | | | | | | | | | | | | | | X |
| PEN 4 | 412 (FL) | | | | | | | | | | X | | | | | | | |
| | 441 (FL) | | | | | | | | | X | | | | | | | | |
| | 603 (FL) | | | | | | | | | | | | | X | | | | |
| PEN 6 | 404 (FL) | | | | | | | | | | | | X | | | | | |
| | 419 (FL) | | | | | | | | X | | | | | | | | | |
| | 420 (FL) | | | | | | | | | | | X | | | | | | |
| | 431 (FL) | | | | | | X | X | | | | | | | | | | |
| 7 | 616 (UNKN) | | X | | | | | | | | | | | | | | | |
| 10 | 645 (UNKN) | | | | | | | | | | | | | | X | | | |
| 11 | 681 (FL) | | | | | | | | | | | | | | | X | | |

Figure 3.1. Frequency histogram showing reproductive success (i.e. number of offspring assigned) of male gopher tortoises known to have sired offspring based on 88 recruits from 19 clutches collected during 2010 and 2015-2017. Candidate males to which no recruits were assigned are not shown.



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**Dissemination of Results**

Oral Presentations

- "An island of misfit tortoises: assessing the survival and health of translocated waif gopher tortoises." Turtle Survival Alliance, Tucson, AZ. August 2019.
- "An island of misfit tortoises: evaluating the use of waif animals to recover populations from the brink" (exit seminar). UGA's Savannah River Ecology Laboratory, Aiken, SC. May 2019.
- "An island of misfit tortoises: evaluating the use of waif animals to recover populations from the brink" (thesis defense). UGA's Warnell School of Forestry and Natural Resources, Athens, GA. April 2019.
- "Staying alive: waif tortoise survival and population dynamics following translocation" (speed talk). Southeastern Partners in Amphibian and Reptile Conservation, Black Mountain, NC. February 2019.
- "An island of misfit tortoises: estimating survival of translocated waif tortoises." Gopher Tortoise Council, Archbold Biological Station, FL. October 2018.
- "Aiken Gopher Tortoise Heritage Preserve: A project history and overview of success to date." South Carolina Heritage Trust Program Board, Columbia, SC. October 2018.
- "An island of misfit tortoises: health and survival of waif gopher tortoises following translocation." Integrative Conservation Conference, Athens, GA. September 2018.
- "Ecology and conservation of gopher tortoises." Birds and Butterflies Nature Store, Aiken, SC. March 2018.
- "Ecology of gopher tortoises and tools used to recover their populations." Aiken-Augusta Audubon Society. March 2018.
- "Conservation lessons from 20+ years with turtles" (keynote address). Southeastern Partners in Amphibian and Reptile Conservation Annual Meeting. Helen, GA. Feb 2018.
- "Health and stress of waif gopher tortoises following translocation." UGA Student Chapter of Wildlife Disease Association. Athens, GA. April 2018.
- "Waif tortoise health following translocation." Southeastern Partners for Amphibian and Reptile Conservation Meeting. Helen, GA. Feb 2018.
- "Waif tortoise health following translocation." Warnell Graduate Student Symposium. Athens, GA. Jan 2018.
- "An island of misfit tortoises: Using waif animals to recover populations on the brink." Gopher Tortoise Council Meeting. Aiken, SC. Oct 2017.

Poster Presentations

- "An island of misfit tortoises: health and survival of waif gopher tortoises following translocation." The Wildlife Society Meeting, Cleveland, OH. October 2018.
- "An Island of Misfit Tortoises: Using Waif Animals to Recover Populations on the Brink." Turtle Survival Alliance Meeting. Charleston, SC. Aug 2017.

Theses and Publications

McKee, R.K. 2019. An island of misfit tortoises: evaluating the use of waif animals to recover populations from the brink. M.S. Thesis, University of Georgia. 123 pp.

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**Recommendation:** Close the grant.

# Appendix A:
## Master's Thesis

AN ISLAND OF MISFIT TORTOISES: EVALUATING THE USE OF WAIF ANIMALS TO RECOVER POPULATIONS ON THE BRINK

REBECCA K. MCKEE

(Under the Direction of Tracey D. Tuberville and Clinton T. Moore)

ABSTRACT

The gopher tortoise (*Gopherus polyphemus*) is declining throughout its range and is among the most commonly translocated reptile species. While some risk is inherent with any translocation, waif tortoises—animals that were collected illegally or have unknown origins—are generally excluded from translocations due to concerns associated with the health and post-release survival of these individuals. However, waif tortoises could provide the needed numbers to stabilize populations. In 1993, a small isolated population of gopher tortoises ($n \leq 10$) was discovered near Aiken, South Carolina. Since 2006, over 260 waifs were released at the preserve to augment the population. I assessed the annual apparent survival of the waif tortoises and conducted health screenings. The estimated annual apparent survival of tortoises was high (>0.91) for adult and subadult tortoises. We detected two common tortoise pathogens, but overall, the individuals appeared healthy. Survival rates and health profiles were comparable to wild gopher tortoise populations.

INDEX WORDS: Translocation; Reintroduction; Reptile Conservation; Gopher Tortoise; *Gopherus polyphemus*; Apparent Survival; Wildlife Disease; Upper Respiratory Tract Disease

# AN ISLAND OF MISFIT TORTOISES: EVALUATING THE USE OF WAIF ANIMALS TO RECOVER POPULATIONS ON THE BRINK

by

REBECCA K. MCKEE

B.S., Davidson College, 2014

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2019

# AN ISLAND OF MISFIT TORTOISES: EVALUATING THE USE OF WAIF ANIMALS TO RECOVER POPULATIONS ON THE BRINK

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

Habitat loss and fragmentation threaten global biodiversity (Prugh et al. 2008) and contribute to the decline of reptiles (Gibbons et al. 2000). Preserving and restoring habitat is critical to reversing this trend but may not be sufficient to recover populations that have already experienced severe declines. In such cases, translocation— the intentional movement of animals from one location to another—could be a useful way to augment declining populations or reestablish an extirpated population (Griffith et al. 1989). Objectives of translocation efforts vary, but there are many common challenges that can compromise the success of a project, including the introduction of pathogens (Deem et al. 2001; Kock et al. 2010), dispersal of released animals from the intended habitat (Armstrong 1995; Hinderle et al. 2014), and high mortality of translocated animals (Adams 2005; Linnell et al. 1997). Although pathogen introduction to an animal community caused by translocation has always been a risk, emergent diseases linked to the declines of many taxa (Stuart et a. 2004; Daszak et al. 2000) have heightened these concerns (Walker et al. 2008). Because of these risks, it is important to evaluate the outcomes of translocation projects, especially for rare and threatened species. Here I review translocation and disease in the context of the gopher tortoise (*Gopherus polyphemus*), a species that is declining throughout its range (Smith et al. 2006) and is frequently subjected to both conservation- and mitigation-driven translocation (Germano et al. 2015; Tuberville et al. 2008).

## Gopher Tortoise Ecology

The gopher tortoise is a fossorial reptile endemic to the southeastern United States and is among the most commonly translocated reptile species in North America (Tuberville et al. 2008). Because their burrows provide important habitat (Lips 1991; Dziadzio and Smith 2016) and thermal refuge (Pike and Mitchell 2013) for other vertebrates and many invertebrates, the gopher tortoise is considered a keystone species and an ecosystem engineer (Catano and Stout 2015; Kinlaw and Grasmueck 2012). Tortoises also play an important role in shaping plant communities, as their burrow mounds increase seedling recruitment and support high levels of diversity relative to the surrounding habitat (Kaczor and Hartnett 1990).

Like other turtle species, the combination of delayed maturation (Iverson 1980) and low recruitment success (Epperson and Heise 2003; Pike and Seigel 2006; Smith et al. 2013) makes tortoises vulnerable to any threat that increases adult mortality (Heppell 1998). Habitat loss is considered the greatest threat to the species (Smith et al. 2006). Although habitat characteristics vary across the range, tortoises prefer habitats with low canopy cover, sandy soils, and the wiregrass (*Aristida beyrichiana*) understory characteristic of longleaf pine (*Pinus palustris)* savannas (Diemer 1986). Logging and other anthropogenic activities have caused sharp declines in longleaf pine savannas across the southeastern Unites States with less than 3% of the historic range remaining (Outcalt and Sheffield 1996). Additionally, fire suppression can reduce forage, degrade habitat, (Diemer 1986; Yager et al. 2007) and cause declines even on land that is otherwise protected (McCoy et al. 2006).

Collectively, these threats have caused range-wide population declines for the gopher tortoise (Smith et al. 2006). The species has been federally listed as threatened in the western portion of its range since 1987 (US Fish and Wildlife Service 1987). In the eastern portion of its

range, the gopher tortoise is a candidate species for federal listing (US Fish and Wildlife Service 2011).

## Translocation for Non-game and Imperiled Species

Historically translocations were a tool for game management (Hughes and Lee 2015; Martin et al. 2017), but the practice is increasingly used to manage non-game species as well (Seddon et al. 2014). Examples of high-profile translocation efforts in the United States include translocation to increase the genetic diversity of Florida panthers (*Puma concolor coryi*, Johnson et al. 2010), reintroduce river otters (*Lutra canadensis*) to areas of previous extirpation in the Midwest (Raesly 2001), and assist with the repatriation of black-footed ferrets (*Mustela nigripes*) (Biggins et al. 2010).

Although translocation can be a valuable tool for wildlife conservation, there are many challenges to using translocation for conservation efforts, including the high dispersal (Armstrong 1995) and mortality (Adams et al. 2004) of translocated individuals. Therefore, translocating wildlife requires careful preparation and planning (International Union for Conservation of Nature 2013). Clearly defining objectives prior to implementing management actions and monitoring outcomes is essential to the success of translocation projects (Ewen et al. 2014; Gregory et al. 2012, Seddon 1999). For many imperiled wildlife species, common objectives include the augmentation of declining populations (Graf et al. 2006), establishment of new populations to decrease the overall risk of extinction for a species (Priddel et al. 2006), and the reintroduction of a species to an area of previous extirpation (Armstrong and Seddon 2008; Nelson et al. 2002). For all 3 of these objectives, the ultimate goal is establishing a viable population. Because population viability is often difficult to measure in the initial years following translocations, the site fidelity, survival, and reproductive success of translocated

animals can provide a more immediate assessment of the success of a project (McGowan et al. 2014; Tuberville 2008).

The increasing use of translocation for conservation has motivated efforts to measure the overarching success of translocation as a tool for various taxonomic groups (Fischer and Lindenmayer 2000; Germano and Bishop 2009; Miller et al. 1999; Wolf 1998). In the case of reptiles and amphibians, efforts to measure the effectiveness of translocations have been further complicated by the fact that herpetofauna are underrepresented in published translocation studies and in many meta-analyses of translocation literature (Seddon et al. 2005; Germano and Bishop 2009). Literature that has explicitly reviewed amphibian and reptile translocations indicates mixed success (Germano and Bishop 2009; Dodd and Seigel 1991, Sullivan et al. 2015). However, more recently the technique has been successfully applied to imperiled reptile and amphibian species including St. Croix ground lizards (*Ameiva polops,* Fitzgerald et al. 2015) and hellbenders (*Cryptobranchus alleganiensis alleganiensis,* Kraus et al. 2017).

## Translocation of Gopher Tortoises

Because of the pervasiveness of mitigation translocations and the potential role conservation translocations could play in tortoise conservation, numerous studies have attempted to measure the outcomes of gopher tortoise translocation projects (Bauder et al. 2014; Heise and Epperson 2005; Riedl et al. 2008; Tuberville et al. 2008) and to evaluate strategies to improve the success of future projects (Tuberville et al. 2005). One of the most immediate considerations for assessing translocations is the movement patterns of the released animals (Tuberville 2008). Because dispersal of translocated tortoises can potentially reduce the number of released individuals remaining in the recipient population and can also cause stress to the individual, home-range size and site fidelity of translocated populations are life history aspects that are

important to establishment success. Although some studies have found that home range sizes of translocated tortoise are not significantly larger than resident wild tortoises (Riedl et al. 2008), most studies have found translocated tortoises to move more frequently and over larger areas, at least during the first year following translocation (Heise and Epperson 2005; Bauder et al. 2014; Tuberville et al. 2005). However, home range sizes tend to decrease in subsequent years (Tuberville et al. 2005). The increased home range size observed during the first-year post-release does not always result in dispersal from the site, as tortoises can display patterns of increased movement without decreasing overall site fidelity (Bauder et al. 2014). However, the increased dispersal in the first years following translocation can result in fewer than half of the translocated population remaining on site two years following the translocation (Burke et al. 1989; Heise and Epperson 2005). Because high levels of site fidelity are critical to the success of translocation projects, refined release techniques have improved site fidelity of newly translocated tortoises. Long-term penning (≥1 yr) at the release site has been shown to significantly reduce dispersal behavior in gopher tortoises, thereby improving translocation success (Tuberville et al. 2005).

Survival rates of translocated animals are also an important indication of project success and the future viability of the translocated population (Tuberville 2008). Many studies are limited in evaluation to the first few years following release, but long-term studies can best address the question of how translocation affects survival and retention rates over time. These studies are particularly important because apparent survival rates (true survival rate among animals not permanently emigrating off-site) in the first years following release have raised questions about the long-term viability of translocated tortoise populations (Dodd and Seigel 1991; Seigel and Dodd 2000). Despite reduced apparent survival observed in the initial two

years following translocations, the two long-term studies of translocated tortoise populations demonstrate that they maintain very high long-term adult annual survivorship (over 98%; Ashton and Burke 2007; Tuberville et al. 2008).

To achieve population viability following translocation, reproduction and recruitment is essential (Tuberville 2008). Successful reproduction has been documented in newly translocated populations (Riedl et al. 2008). However, parentage analyses of offspring have indicated a differential siring success amongst males, as newly translocated males sired a lower proportion of the offspring than more established males in both gopher tortoises (Tuberville et al. 2011) and desert tortoises (*Gopherus agassizii*, Mulder et al. 2017). This reproductive skew raises important management considerations about effects on social dynamics and genetic diversity following translocation.

Additionally, because gopher tortoises are social animals that live in colonies (McRae et. al. 1981), translocation has the potential to disrupt normal social interactions or established social relationships (i.e., "networks"). Translocation-caused changes in social networks could influence other factors such as disease transmission rates. Although this has yet to be documented for gopher tortoises, there is evidence that translocation creates increased connectivity in the social networks of desert tortoises, potentially facilitating the spread of pathogens (Aiello et al. 2014).

## Disease as a Threat to Conservation

Emergent disease has become a growing concern globally for wildlife (Kilpatrick et al. 2010; Pedersen et al. 2007). Habitat reduction (Holmes 1996), encroachment by domestic animals (Roelke-Parker et al. 1996), climate change (Daszak et al. 2001), and the global wildlife trade (Karesh et al. 2005) have introduced novel pathogens and altered the dynamics of endemic

pathogens. Even if disease is not the principal threat to a species, it can compound other threats and exacerbate declines (Smith et al. 2018). Disease outbreaks can compromise conservation efforts even for populations on protected lands (Bengis et al. 1996; Villafuerte 1994). Consequently, biologists and other parties responsible for wildlife management are increasingly tasked with managing disease in imperiled species, including in the context of reintroduction efforts (Walker et al. 2008). For reptiles and amphibians, recent evidence of declines related to emergent diseases has alarmed scientists (Daszak et al. 2001; Gibbons et al. 2000; Schumacher 2006).

## Pathogens of the Gopher Tortoise and Closely-Related Chelonians

### *Methods of pathogen detection*

Although habitat loss is the primary driver of declines for gopher tortoises (Smith et al. 2006), disease is also considered a threat to the species (Jacobson 1994; McLaughlin 1997; Gates et al. 2002). Numerous techniques have been used to monitor pathogen prevalence in tortoise populations, including enzyme-linked immunosorbent assay (ELISA) to detect antibodies (Johnson et al. 2006; McGuire et al. 2014a; Wendland et al. 2007), traditional polymerase chain reaction (PCR) to detect pathogen DNA (Brown et al. 1995; Wendland et al. 2007), and quantitative PCR (qPCR) to quantify the number of copies of pathogen DNA present (duPré et al. 2011).

All three methods described above have been used to report population prevalence and determine an individual's infection status, but each method provides different information. ELISA tests are indicative of previous exposure to pathogens, but they do not always indicate current disease status (Braun et al. 2014). For chronic diseases, the interpretation of seropositive tortoises is complex, as asymptomatic tortoises can later develop or redevelop clinical signs of

disease (Jacobson et al. 2014). The seroconversion of infected tortoises is often delayed, and an individual can take months to develop antibodies, even if it is displaying clinical signs (Aiello et al. 2016; duPré et al. 2011). In addition, some clinical signs are common to multiple pathogens, further complicating diagnosis. PCR and qPCR techniques detect pathogen DNA that the tortoise host is actively shedding (Braun et al. 2014; duPré et al. 2018). As such, both traditional PCR and qPCR methods can more quickly determine a newly infected tortoise to be positive than the ELISA testing (Aiello et al. 2016; duPré et al. 2011). Although ELISA tests have been used to detect tortoise exposure to *Mycoplasma* spp. and herpesviruses in the *Herpesviridae* family (Wendland et al 2007; Johnson et al. 2006), ELISA tests for many other tortoise pathogens are not readily available. Therefore, the methodological selection for surveillance efforts is often restricted by both the cost of testing and the availability of tests for specific pathogens.

*Pathogens of Concern*

Several pathogens closely associated with chelonians target the respiratory system (Origgi and Jacobson et al. 2000). Herpesviruses have been documented in many turtle species (Greenblatt et al. 2004; Martel et al. 2009), including gopher tortoises (Saldanha 2018) and desert tortoises (Jacobson et al. 2012; Johnson et al. 2006; Origgi and Jacobson et al. 2000). Additional emergent disease concerns, including intranuclear coccidiosis (family *Eimeriidae*) in testudines (TINC) and adenovirus infections (family *Adenoviridae)*, have been identified as potentially serious concerns for chelonians (Gibbons and Steffes 2013). Viruses belonging to the *Ranavirus* genus (family *Iridoviridae*) family are a growing concern for North American herpetofauna including turtles and tortoises (Brenes et al. 2014). *Ranavirus* spp. infections can cause clinical signs such as palpebral swelling and oculonaral discharge often associated with other tortoise pathogens (Johnson et al. 2008). Although there has been only limited documentation of the virus in gopher

tortoises (Cozad 2018, Johnson et al. 2008; Westhouse et al. 1996), *Ranavirus* spp. has been documented in wild box turtle (*Terrapene carolina*) populations (Currylow et al. 2014). In box turtles, infection frequently results in mortality (De Voe et al. 2004). Even with supportive care early in the onset of an infection, 42% of turtles infected with *Ranavirus spp.* died (Sim et al. 2016). Additionally, strains of *Ranavirus spp.* can persist for extended periods in both substrate and water (Nazir 2012). Because the virus is transmissible across taxonomic groups of ectotherms and because the tortoise shares its burrow with many susceptible commensal species like gopher frogs (*Rana palustris*; Hoverman et al. 2011; Brenes et al. 2014), *Ranavirus* spp. infections in tortoises could present a threat to other species as well.

**Upper Respiratory Tract Disease: A Case Study of Disease Investigation and Management**

Although ongoing vigilance for emergent pathogens is important, upper respiratory tract disease (URTD) was the first emergent disease associated with tortoise population declines and is the most characterized chelonian disease (Jacobson et al. 2014; Jacobson1994; McLaughlin 1997; Gates et al. 2002). The desire to reduce the spread of URTD has influenced tortoise management policies over time (Jacobson et al. 2014) and therefore provides an important disease investigation and management case study worthy of review.

Common clinical signs of URTD are similar across turtle species and include oculonaral discharge, periocular swelling, wheezing, and labored breathing (Schumacher et al. 1997; Brown et al. 1999). The first documented observation of URTD in tortoises occurred in a desert tortoise confiscated in California and by the late 1980s, the disease had been observed in wild populations of desert tortoises in California and Nevada (Jacobson et al. 1991). Bacteria belonging to the *Mycoplasma* genus was suggested as the most likely causal agent (Jacobson et al. 1991) and further experiments fulfilling Koch's Postulates confirmed a novel species,

*Mycoplasma agassizii*, as the etiology (Brown et al. 1994). Subsequently, an examination of 24 gopher tortoises from various populations in Florida collected from 1993-1995 documented 10 tortoises with clinical signs of URTD (McLaughlin et al. 2000), and additional studies in the 1990s confirmed seropositive tortoise populations in other states (Smith et al. 1998). *Mycoplasma agassizii* was confirmed as the etiologic agent of URTD of gopher tortoises in experimental trials (Brown et al. 1999). An additional pathogen, *Mycoplasma testudinium*, that causes URTD in both *Gopherus* species was later identified (Brown et al. 2004).

Frequent reports of symptomatic desert tortoises and suspected mass die offs (627 carcasses located in study areas) in the 1980s alarmed biologists and led to further investigative work in both gopher and desert tortoises (Jacobson et al. 1991). The first documentation of URTD in a wild gopher tortoise population occurred on Sanibel Island, Florida in 1989, and further hematological testing confirmed that 80% of the population was seropositive for *Mycoplasma agassizii* (Beyer 1993). Significant gopher tortoise mortality events implicating URTD were also documented in 1998 when 104 individual shells were recovered from a 28-ha section of a 150-ha park in west-central Florida (Gates et al. 2002). In the same year, a marked uptick in tortoise mortality at the Kennedy Space Center was attributed to URTD (Seigel et al. 2003).

Despite the gravity of these findings, the potential threat URTD poses for tortoises is debated. Many argue that the disease can rarely cause dramatic mortality. The force of infections (FOI) appears highly dependent on seroprevalence in the population (Ozgul et al. 2009). Moreover, transmission generally requires prolonged contact with an infected host or shorter contact with a host displaying severe clinical signs (Aiello et al. 2016). Additionally, some data suggest that URTD is a disease of "high morbidity but low mortality" even in populations with

relatively high seroprevalence levels (Diemer Berish 2010). In some populations, seropositive tortoises had higher four-year apparent survival rates than seronegative tortoises, possibly because the antibodies generated during previous exposures to *Mycoplasma* spp. provided some protection against future exposure (Ozgul et al. 2009). Long-term studies of many gopher tortoise populations in Florida found little evidence that URTD was driving declines in populations; many sites with high rates of apparent survival also had high seroprevalence while sites with substantial decline had low seroprevalence (McCoy et al. 2007). Although the higher levels of survival suggest that there may be some long-term immunity due to antibodies, manipulative experiments with seropositive and seronegative tortoises did not reveal any indication of immunological memory generated from previous pathogen exposure (Sandmeier et al. 2017).

To explain the wide variation in population exposure outcomes, some have suggested that URTD is a "context-dependent disease," causing severe adverse impacts only when tortoise populations are coping with infection simultaneously with many other stressors (Sandmeier et al. 2009). Drought (Lederle et al. 1997), cold temperatures (Sandmeier et al. 2013), and heavy-metal exposure (Jacobson et al. 1991) have all been proposed as potential factors that have increased adverse impacts of URTD on tortoise populations.

Incongruities in sampling effort and methods for evaluating prevalence have further complicated the process of understanding the relationship between disease prevalence and its impact on tortoise populations. Detection of pathogens is likely, in part, a reflection of sampling effort (McCoy et al. 2007). Moreover, visual health assessments alone may fail to completely capture the infection status of a population due to subclinical infections (McLaughlin et al.

2000). Even co-infection with both *Mycoplasma* species is not always predictive of clinical disease (Weitzman et al. 2017).

When tortoises are confirmed as positive for infection, it is unclear how to appropriately manage their infection. Medical intervention for wild tortoise populations has not been implemented, partially due to the tremendous logistical challenges associated with administering treatments. Treatment of URTD with antibiotics fails to fully treat the infection but does reduce the clinical signs of symptomatic tortoises (Jacobson et al. 1999). Given that tortoises remain infected, treatment with antibiotics would reduce mortalities associated with outbreaks but is likely ineffective at reducing the persistence of the pathogen in the population. However, additional research on antibiotics that require only a single injection (Kinney et al. 2014) could have applications in the context of tortoise translocation efforts.

Because treatment of the disease in wild populations is not presently considered a viable strategy, most efforts have focused on mitigating the spread of the pathogen to naïve populations. The mortality events of the late 1980s and early 1990s prompted aggressive approaches to preventing the spread of URTD. Because desert and gopher tortoises are commonly translocated to mitigate direct mortality from construction efforts, there were concerns that translocation efforts could inadvertently spread URTD. Historically, translocation efforts for many other species have been compromised due to disease (Kock et al. 2010). Previous research has indicated that tortoises with URTD have stronger dispersal tendencies than healthy tortoises, which could further facilitate transmission of pathogens between populations (McGuire et al. 2014b).

Due to these concerns, scientists working closely with the disease suggested euthanasia for desert tortoises displaying clinical signs of disease if they were previously held as pets or

were wild tortoises slotted for translocation efforts (Jacobson et al. 1995). The state of Nevada enacted the most extreme policy that required tortoises seropositive for *Mycoplasma* spp. to be euthanized, regardless of clinical signs (Rostal et al. 2014). Enacted in 1996, this policy resulted in the loss of many clinically healthy tortoises until the policy was ultimately revised in 2009 following expert recommendations (Jacobson et al. 2014). Policies of only translocating seronegative desert tortoises continued in Nevada and California until 2011, when translocating clinically healthy tortoises regardless of status became more acceptable (Rideout et al. 2011). Because the seropositive status also indicates that a tortoise survived past exposure, seropositive tortoises may be highly valuable from an evolutionary and genetic perspective.

Although no states adopted a policy quite as extreme for gopher tortoises, Florida did initially require disease testing of tortoises prior to translocation (Riedl et al. 2008). Historically, developers were given two options for sites with resident tortoise populations: 1) purchase incidental take permits to entomb tortoises and protect habitat elsewhere; or 2) capture resident tortoises, test them for *Mycoplasma* spp., and translocate them. If a tortoise tested seropositive for *Mycoplasma* spp. exposure, it had to either be left at the development site, quarantined at a licensed rehabilitation facility, donated to a disease research program, or euthanized (Florida Fish and Wildlife Conservation Commission (FFWCC) 2006). As a result of objections from the general public against the large number of tortoises impacted by legal entombment, incidental take permits were discontinued, and the disease testing requirement was dropped for tortoises directly translocated between wild populations (FFWCC 2006).

Although this policy change extends to most translocation activities, gopher tortoises that have been held in captivity or are of unknown origin are considered “waif tortoises” and are generally excluded from traditional translocation projects (FFWCC 2012). The question of what

to do with waifs remains a subject of debate. Many of the concerns with waif tortoises originate from their risk of infection while in captivity. Captive populations of tortoises, particularly when exposed to non-native chelonian species, can have high risk of disease (Johnson et al. 2006) and are sometimes not genetically representative of wild tortoise populations (Edwards and Berry 2013). Because captive desert tortoises in the 1990s displayed similar clinical signs to symptoms that later appeared in wild populations, the unauthorized release of captive tortoises is thought to have played an important role in the increase of URTD in wild populations (Brown et al. 1995). Additionally, some hypothesize that the release of captive pets might partially explain the increased pathogen presence observed in tortoise populations near human-populated areas (Berry et al. 2015). Thus, disease risks are important considerations for any reintroduction effort, but particularly in situations that involve the release of formerly captive animals or other animals of unknown origin.

## Objectives and Outlines of Thesis Research

The objective of my thesis was to assess the survival and health of a gopher tortoise population located in Aiken County, SC, that has been augmented with over 260 waif tortoises over the last decade. As the first effort to recover a relict tortoise population with waif tortoises, the outcomes of this project will provide information important to the future management of waif tortoises and their potential role in the recovery of wild populations. Each chapter addresses the two main concerns about the suitability of waif tortoises for translocation—apparent survival and health. Chapter 2 uses releases and capture records to estimate the long-term annual apparent survival rates for waif gopher tortoises by stage class and origin. Chapter 3 summarizes a comprehensive effort to evaluate the health of the surviving individuals through visual health assessments, pathogen screening, chronic stress evaluations, and quantification of blood parasites. Chapter 4

provides a synthesis of the results of the project to date and makes recommendations for the future management of waif tortoises.

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CHAPTER 2

SURVIVAL OF WAIF TORTOISES FOLLOWING TRANSLOCATION

## Introduction

Translocation, the intentional movement of animals from one location to another, is a common wildlife management technique (Fischer and Lindenmayer 2000; Germano and Bishop 2009; Seddon et al. 2005). With >700 translocation projects carried out annually, the practice has been applied to species across multiple taxonomic groups (Griffith et al. 1989). Historically, game management motivated most translocation efforts (Hughes and Lee 2015; Synder et al. 1999), but there is a growing interest in applying this technique as a conservation measure for imperiled non-game species (Bouzat et al. 2009; Schwartz and Martin 2013; Seddon et al. 2014).

The gopher tortoise is a fossorial reptile endemic to the southeastern United States and is among the most commonly translocated reptiles (Tuberville et al. 2008). Because its burrows provide refuge for diverse taxa, the gopher tortoise is considered a keystone species and an ecosystem engineer (Catano and Stout 2015; Lips 1991; Pike and Mitchell 2013). However, the species is declining throughout its range, predominantly due to habitat degradation or permanent conversion (Smith et al. 2006). The gopher tortoise is federally listed as threatened under the Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana, and is a candidate species for federal listing in the remainder of its range (United States Fish and Wildlife Service (USFWS) 1987, 2011). Although protecting and managing existing habitat is critical to the species' conservation (USFWS 2011), there is a growing interest in whether displaced

tortoises can be used to bolster depleted populations that do not currently meet established criteria for minimum viable populations (MVP; Gopher Tortoise Council 2014).

Because of the widespread use of translocation as a conservation tool for gopher tortoises, numerous studies have attempted to measure the outcomes of wild-to-wild translocation projects (Ashton and Burke 2007; Bauder et al. 2014; Heise and Epperson 2005; Riedl et al. 2008; Tuberville et al. 2008) and to evaluate strategies to improve the success of future projects (Tuberville et al. 2005). Many studies are limited to short-term evaluations of success, but long-term studies are particularly valuable for understanding how translocation affects survival rates of long-lived species (Dodd and Seigel 1991, Germano and Bishop 2009, Sutherland et al. 2010), including gopher tortoises, which can likely live 60 years (Landers 1980). Apparent survival rates (true survival rate among animals not permanently emigrating off-site) recorded in the first years following release are frequently used to assess project outcomes (Burke 1991, Riedl et al. 2008). However, short-term survival metrics may not be indicative of the long-term viability of translocated populations (Ashton and Burke 2007). For example, two long-term studies demonstrated that apparent survival has been shown to be reduced in the initial 1-2 years following translocation, but populations maintain a high level of adult annual apparent survivorship (≥98%) in subsequent years (Ashton and Burke 2007; Tuberville et al. 2008). Reduced rates of apparent survival in the initial 1-2 years has predominantly been attributed to dispersal rather than direct mortality (Tuberville et al. 2005; Heise and Epperson 2005), and this behavior is reduced as tortoises acclimate to their novel environment and social structure.

Despite the potential utility of translocation for managing gopher tortoises and other imperiled species, translocation is not without risk of unintended consequences. Although there are risks associated with any translocation, waif tortoises—any tortoise removed from its natal

site and held in captivity for an extended period or whose collection origin is unknown—pose a unique quandary for their use in translocation. Because of uncertainties about their origin or conditions in captivity, waif tortoises are generally excluded from translocation efforts due to the possible risk of pathogen introduction into the recipient population. Moreover, because captivity can alter behavior, physiology, and nutritional status (Mason 2010, McDougall et al. 2006), formerly captive tortoises may be unable to survive when returned to the wild. However, if these risks could be managed, waif tortoises could provide the needed numbers to stabilize populations that have experienced severe declines.

Studies on the closely related desert tortoise (*Gopherus agassizii)* have questioned the appropriateness of releasing formerly captive tortoises back to wild populations due to concerns of mixing genetically distinct populations (Edwards and Berry 2013), which can in turn, lead to a reduction in the locally adaptive alleles (Edmands 2007). Moreover, released captive desert tortoises exhibited highly variable rates of apparent survival (0% -100%) following release (Field et al. 2007). Similarly, it is unclear if released waif gopher tortoises would exhibit post-release survival as high as documented in wild-to-wild translocated tortoises.

In 1993, a small isolated population of gopher tortoises (n<10) was discovered in Aiken County, South Carolina (Clark et al. 2001) on private property that was later purchased by South Carolina Department of Natural Resources (SCDNR) and designated as the Aiken Gopher Tortoise Heritage Preserve (AGTHP). To achieve a population size goal of 250 adults (Gopher Tortoise Council 2014), the AGTHP population required augmentation. South Carolina is on the periphery of the species' range (Figure 2.1) with few stable populations (Auffenberg and Franz 1982; Tuberville and Dorcas 2001). Acquiring wild tortoises from other states was also not feasible, because many populations within these states were in decline (Ennen et al. 2010;

Hermann et al. 2002; McCoy et al. 2006) and displaced tortoises were needed to augment populations on protected lands within their own state. Given the AGTHP's isolation (Figure 2.2) and urgent need for augmentation, SCDNR, in consultation with University of Georgia's Savannah River Ecology Laboratory (SREL), decided to introduce waif tortoises to recover the resident population, starting in 2006. As the first translocation for the species using primarily waif tortoises, this effort also provided the opportunity to better understand the potential benefits and limitations of using waif tortoises to bolster populations at risk of extirpation. During 2017-2018, after the release of 268 waif gopher tortoises, we conducted mark-recapture to evaluate apparent survival of released waif gopher tortoises a decade after the initial recovery efforts began.

## Study Area

In 1995, SCDNR purchased 148 ha to create the AGTHP. Located 30 km east of Aiken, South Carolina, the AGTHP protects the northern-most extant population of gopher tortoises and is separated from the nearest known gopher tortoise population by >50 km (Figure 2.2; Clark et al. 2001). The site is characterized by xeric soils (Lakeland, Troup, and Fuquay; USDA 1985), an herbaceous understory dominated by wiregrass (*Aristida beyrichiana*) and bluestem (*Andropogon* spp.), midstory dominated by turkey oak (*Quercus laevis*), and a sparse canopy of longleaf pine (*Pinus palustris*). In order to promote herbaceous growth and provide adequate forage for tortoises, SCDNR manages the site with prescribed fire, manual thinning, and periodic applications of broadleaf herbicide (Moule 2013). Since the preserve's establishment, SCDNR purchased additional surrounding properties to enlarge the preserve to its current 656-ha.

Burrow surveys and mark recapture studies were conducted in 1995 and 2001, indicating AGTHP supported <10 native adult tortoises (K.A. Buhlmann, Savannah River Ecology Lab,

unpublished data); these native tortoises were weighed, measured and permanently marked. Introductions of waif tortoises started in 2006 as a collaborative effort between SCDNR and SREL. Waif tortoises were obtained from wildlife rehabilitation facilities, state wildlife agencies, zoos, and other partners throughout the eastern United States, including states outside the species' native range. Prior to release, waif tortoises were also weighed, measured, and permanently and individually marked by scute notching (modified from Cagle 1939). Tortoises were visually inspected and no tortoises displaying clinical signs of upper respiratory tract disease such as ocular or naral discharge (Brown et al. 1999) were released. Any tortoise ticks observed on waifs were also removed prior to release. Waif tortoises and the 14 previously captured native tortoises were provided starter burrows and penned on site (1-ha circular pens) at the AGTHP for ≥1 yr (Figure 2.3; Tuberville et al. 2005) to promote site fidelity. On average, pens were stocked with 13 adult tortoises (range 3-24) and 26 total tortoises including immature classes (range 8-50). Following penning (12-39 months), pen walls were removed to allow tortoises to move beyond the pen footprint. During 2006-2017, 268 waifs were released at the AGTHP. In total, 282 tortoises (14 native, 268 waif) were released into pens, including 64 released as hatchlings, 33 released as juveniles, 34 released as subadults, and 151 released as adults (Figure 2.4).

## Methods

*Sampling Effort*

To locate tortoise burrows, we walked parallel transects spaced 15 m apart in all suitable habitat throughout the preserve during May-June 2017 and Feb-May 2018. We considered flooded habitat and habitat with dense riparian vegetation unsuitable for tortoises (roughly 18% of the site) and therefore excluded it from surveys. We recorded the location of all observed burrows

using Global Positioning System technology (± 5m) and classified them as them active, inactive, or collapsed based on criteria described in Cox et al. 1987. We measured all intact (active and inactive) burrows by recording burrow height and width (cm) at a depth of 0.5 m inside the mouth of the burrow. We marked intact burrows with small stakes and uniquely numbered aluminum tags.

We conducted mark-recapture of tortoises during 22 May – 19 July 2017 and 8 May – 17 July 2018. This effort represented the first systematic attempt to capture all surviving gopher tortoises still residing on AGTHP. We used a burrow camera to determine occupancy of each active burrow (Smith et al. 2005). If we observed a tortoise in the burrow, we immediately placed a live-wire trap covered in shade cloth at the mouth of the burrow (Aresco and Guyer 1999). We checked traps multiple times daily to prevent tortoises from overheating. We also opportunistically captured any tortoise encountered outside of a burrow. We recorded the point of capture for all live tortoises and for tortoise remains (i.e., shells). Due to a concurrent radio-telemetry project designed to assess movement and survival of hatchlings and head-started yearlings, we did not attempt to trap tortoises from these size classes.

Because we needed to collect and immediately freeze blood samples for a related project on tortoise health (see Chapter 3), we brought all captured tortoises to SREL, located approximately 38 km from the AGTHP. We measured, photographed, and identified tortoises by their notched codes. All identifications were referenced against the database and photos of released animals. Tortoises were assigned a stage class at each capture based on their midline carapace length (the distance between the nuchal scute and supercaudal scute) and the presence of secondary sex characteristics (Table 2.1).

Unmarked individuals (possibly native animals undetected in earlier surveys, waifs that were released without authorization by the public, or offspring recruited on-site) were handled similarly but also permanently marked with a unique combination of notches in the marginal scutes (modified from Cagle 1939). Tortoises were returned to their point of capture ≤24 hr. In addition to formal surveys in 2017 and 2018, incidental observations of tortoises and recovery of shells from dead individuals were recorded from 2006-2016. All work was conducted in accordance with appropriate permits (SCDNR Scientific Collection Permit Number #SC-04-2017, #SC-06-2018) and approved University of Georgia IACUC procedures and protocols (AUP# A2017 05-022-Y1-A0).

*Survival Analysis*

We used the information collected at first handling (release for waifs, initial capture for native tortoises and unmarked individuals), subsequent captures, and dead recoveries to construct a capture history for each tortoise. A capture history was an array of 13 digits with each digit representing an individual's capture status for each year *t* of the study (2006-2018). For live captures or release of a tortoise, the corresponding year's digit was assigned a value of 1-4 based on its stage class, *c*, as follows: 1 = juvenile, 2 = subadult, 3 = adult male, and 4 = adult female. We assigned a value of 5 if the tortoise was recovered dead, or 0 if the tortoise was not observed (neither captured alive nor recovered dead). Because we intentionally did not trap hatchling tortoises, our analyses excluded the hatchling stage class. We used a multistate Cormack-Jolly-Seber model (Brownie et al. 1993; Schwarz et al. 1993) for joint live-capture and dead-recovery data (Barker et al. 2005; Burnham 1993) to estimate stage class specific probabilities of: apparent survival ($\varphi_c$), transition to the next stage class given survival ($\Psi_c$, for $c$ = 1 or 2 only), capture of live animals ($p_{c,t}$), and recovery of dead animals ($r_c$). We also estimated 1 parameter

not specific to stage class: probability of transitioning into the adult stage class as male rather than female ($\gamma$; Table 2.2).

Due to sparseness of data, we did not consider temporal variation in any parameter except capture probability (Table 2.3). We assumed the recovery and capture rates varied by size of tortoise, but not by sex of the adults. Therefore, we estimated a common recovery rate of dead adult tortoises ($r_A = r_3 = r_4$) and capture probability for live adult tortoises ($p_{A,t} = p_{3,t} = p_{4,t}$). We modeled annual capture probability as a fixed effect of annual search effort. We defined search effort as whether focused searches for tortoises were conducted in a given year (via burrow surveys or trapping efforts) or not (i.e., tortoises were only discovered incidentally to other field activities). Because tortoises were penned in groups for >1 yr prior to their release, we considered pen number to be a random effect for survival probability within each model. In many cases, tortoises were placed in pens with tortoises with similar captive histories, captive sources, or origins (see details regarding origin below). However, because waifs were often obtained individually or in small groups over many years, penning by history or origin was not always feasible. To separate potential effects of penning group with other effects in subsequent models, we expressed survival probability as a linear-logit function of stage class and the random pen effect. Unmarked tortoises found on site during the 2017/2018 surveys were listed as having no pen (N). We henceforth refer to this as our base model.

Given that our study site is at the northern extent of the species' range, tortoise survival could be influenced by the geographic origin of the tortoise. Therefore, we created a second multistate model that incorporated tortoise origin as an additive (to stage class) categorical effect for survival. Origin was categorized as South Carolina, Georgia, Florida, or Other (Figure 2.5) based on where the tortoise was held while in captivity prior to being transferred to SCDNR.

Because we only observed 13 native tortoises (6 adults, 3 subadults, and 4 juveniles), the South Carolina group included native tortoises and individuals from elsewhere in the state. The Other category described individuals with origins that were completely unknown, were from other states in the range, or were housed in regions that fall outside of the gopher tortoise range; often these were individuals living in captivity for extended periods as pets. Parameter definitions and model structure for recovery, capture, and transition probabilities were implemented as described for the base model.

To allow for the possibility that differences in survival due to origin may differ by stage class, we created a third model that included an interaction term between stage class and origin in the linear-logit model for survival. Again, we maintained the same parameterization for the recovery, capture, and transition probabilities as in the base model. We henceforth refer to this model as our interaction model.

Due to sparsity of the data and desire to incorporate random pen effects, we chose to analyze models in a Bayesian framework following the approach of Kéry and Schaub (2012). The approach uses a state-space representation in which transitions among states through the annual processes of survival, stage class transition, and recovery are modeled as latent mechanisms (Table 2.2) that are probabilistically observable (Table 2.3). Other than our use of the logit scale to model survival as a function of covariates (i.e., stage class, origin, pen random effects), we modeled all parameters directly as probabilities. We converted all predictions of survival from the logit to the probability scale in order to report annual apparent survival probabilities. We used Markov chain Monte Carlo (MCMC) sampling in JAGS (Plummer 2003) via R (version 3.5.1; R Core Team 2018) using package R2JAGS (Su and Yajima 2012) to approximate the posterior distribution of all model parameters. We used non-informative priors,

and we provided random initial values to each of 3 MCMC chains. We performed 10,000 simulations, discarding the first 2,000 as burn-in and retaining every 6th sample, yielding 1,333 simulated values to construct posterior distributions. We checked for chain convergence using the Brooks-Gelman-Rubin statistic (Brooks and Gelman 1998) cutoff of Rhat <1.1 and by visually inspecting the trace plots for evidence of mixing. From the posterior distributions, we reported means ± 1 SD and 95% Bayesian credible intervals (Bayesian analog to the 95% confidence interval).

For each of the survival effects of interest – age, origin, age × origin interaction – we computed sample means and variance-covariance matrices from the MCMC chains for the corresponding model. We used Wald tests (Wald 1943) to sequentially select among the nested models. In each comparison between a model and a more complex alternative, we rejected the simpler model in favor of the more complex model if Wald $Q \geq \chi_k{}^2$ ($\alpha$ = 0.05), the critical value of the chi-square distribution with cumulative probability 1-$\alpha$ and degrees of freedom *k* equal to the difference in number of parameters between models (Table 2.4).

## Results

After excluding hatchlings, there were 13 native and 205 waif tortoises marked and released prior to 2017. During 2017, we captured 58 live tortoises and recovered seven shells. During 2018, we captured 114 live tortoises and recovered two shells. In total, we captured 126 unique, live juvenile, subadult, and adult tortoises with 46 tortoises captured in both 2017 and 2018. All recovered shells were marked tortoises and could be identified as specific individuals. Of the 126 live individuals observed, 119 were previously marked (6 as juveniles, 22 as subadults, and 91 as adults). Of the 119 previously marked tortoises, 10 were native tortoises and 109 were waifs. The remaining 7 animals were not marked prior to 2017 and likely were either undetected in the

initial population survey, naturally recruited, or released at the AGTHP by members of the public.

The interaction between origin and stage did not affect tortoise apparent survival ($Q$ = 8.38, $P$ =0.50), thus we failed to reject the simpler model of additive origin and stage effects (Table 2.4). The origin model estimated the overall apparent survival rate for tortoises to be 0.93 ± 0.09 for South Carolina, 0.82 ± 0.14 for Georgia, 0.80 ± 0.15 for Florida, and 0.87 ± 0.12 for Outside Range. However, variation by origin was not significant ($Q$ = 3.21, $P$ = 0.36), and we found no basis to reject the simpler model of stage class effect only (Table 2.4). However, annual apparent survival probabilities were dependent on stage class ($Q$ = 499.95 $P$ < 0.001). Therefore, we reported parameter estimates from our base model (Table 2.5).

Our base model estimated annual apparent survival probability ($\Phi_c$) to be 0.96±0.04 for females, 0.92±0.07 for males, 0.96±0.05 for subadults, and 0.25±0.18 for juveniles. The 95% credible intervals indicated no significant difference among the annual apparent survival rates for the male, female, and subadult stages (Figure 2.6). However, annual apparent survival for juvenile tortoises was significantly lower than the 3 other stages (Figure 2.7). Among pens, tortoises in pens 2 and 11 exhibited higher average rates of annual apparent survival, while tortoises in pen 7, 10, and K exhibited lower rates of survival (Figure 2.4).

Estimated annual conditional transition probabilities ($\Psi_1$) for juveniles were 0.35±0.17. For subadults the estimated annual probability of transitioning (given survival) to an adult male ($\Psi_2 \cdot \gamma$) was 0.04 ±0.02 and the annual probability of transitioning (given survival) to an adult female ($\Psi_2[1-\gamma]$) was 0.12 ±0.04. Collectively, the estimated conditional transition probability from subadult to an adult regardless of sex ($\Psi_2$) was 0.16±0.04.

In years with focused search effort, our base model determined capture probability ($p_c$) to be 0.57±0.04 for adults, 0.40±0.09 for subadults, and 0.40± 0.18 for juveniles. The 95% credible intervals for these estimates overlapped for all stages. In years without a focused search effort, estimated capture probability was 0.16±0.19 for juveniles, 0.06±0.03 for subadults, and 0.02±0.01 for adults. The recovery rates of dead tortoises ($r_c$) were 0.40±0.09 for adults, 0.40±0.23 for subadults, and 0.22±0.08 for juveniles.

## Discussion

Our study is the first to estimate the survival of waif gopher tortoises following release into the wild. Translocated adult waif gopher tortoises exhibited apparent survival rates similar to those reported for wild *in situ* populations (Ozgul et al. 2009, Tuberville et al. 2014). Our base model estimated annual apparent survival to be 0.95 for females and 0.91 for males. Based on a mark-recapture study conducted on 2 tortoise populations in Georgia and Alabama, estimated adult annual survival rates ranged from 0.87 to 0.98 (Tuberville et al. 2014). A mark-recapture study on 10 tortoise populations in central Florida estimated annual apparent survival probability to be 0.95 ±0.04 for females and 0.89 ± 0.04 for males (Ozgul et al. 2009). The observed difference between male and female survival was not significant in either study (Ozgul et al. 2009, Tuberville et al. 2014), however apparent survival was estimated to be 9% lower for males at the Georgia site (Tuberville et al. 2014). Similarly, although our adult male survival estimate was slightly lower than our adult female survival estimate, the overlap in the 95% credible intervals suggest that this difference is also not statistically different (Figure 2.6).

Previous studies on translocated wild gopher tortoises have observed temporary reductions in survival in the first 2 years after release, followed by high long-term survival rates thereafter (Ashton and Burke 2007, Tuberville et al. 2008). Because the AGTHP population was

not consistently sampled in the years immediately following release, we are unable to determine if released waif tortoises also exhibited a temporary reduction in survival. However, our estimated annual survival rates across all years suggest that released waif tortoises can exhibit high rates of long-term survival. Although many waif tortoises have been held in captivity for extended periods, adult waif tortoises exhibited survival rates comparable to those documented in translocated and *in situ* wild tortoises.

Previous studies on *in situ* tortoise populations have focused on estimating survival of either hatchling (Epperson and Heise 2003, Perez-Heydrich et al. 2012, Pike and Seigel 2006; Smith et al. 2013) or adult stage classes (Ozgul et al. 2009). As a result, very few studies have reported apparent survival rates for the intermediate juvenile and subadult stage classes. A radio-telemetry study on juvenile tortoises used a model (Heisey and Fuller 1985) following the methods of Trent and Rongstad (1974) to estimate survival (Wilson 1991). Bimonthly survival ranged from 0.69-1.0, however, because the data violated the assumption of constant survival across time period of estimation, annual survival rates could not be calculated (Wilson 1991).

Our estimates of annual apparent survival for immature waif tortoises (0.96±0.05 for subadults, 0.25±0.18 for non-hatchling juveniles) are difficult to compare to previous studies because those other studies report only a single survival estimate for all immature classes (i.e., hatchling, juvenile, subadult). In two *in situ* populations in Alabama and Georgia, annual apparent survival for all immature classes was estimated to be between 0.70 and 0.82 (Tuberville et al. 2014). In a wild-to-wild translocated population, annual apparent survival for all immature classes was estimated to be 0.45 ± 0.26 in the first year following release and 0.84 ± 0.05 thereafter (Tuberville et al. 2008).

We obtained waif tortoises from a variety of states to augment the AGTHP population. Because of the northern location of the site, we hypothesized that tortoises from more southern latitudes might survive at lower rates than tortoises originating from locations closer to the release site. However, we did not observe a difference in the apparent survival of tortoises based on tortoise origin. Although our apparent survival estimates were more precise for the older stage classes, the sparseness of the dataset could have prevented us from observing finer patterns in the data, such as slight differences in survival between tortoises of different origins. Additional mark-recapture effort at AGTHP in future years could further illuminate any potential effect of origin on apparent survival.

Due to limited mark-recapture effort prior to the 2017-2018 field seasons, we were unable to estimate temporal factors influencing survival, such as time since release or annual variation in survival. However, pen-level effects partially capture some of the temporal variability. Additionally, the pen random effect helped to account for non-independence of fates of tortoises held in the same pen. Because tortoises were housed in pens for ≥1 year before the pen walls were removed, it is possible that the mortality of 1 individual could affect the survival rates of other tortoises in the pen, particularly if the cause of mortality was an infectious pathogen.

Pens also prevented tortoises from dispersing for their first year on site at AGTHP. Penning has been shown to increase apparent survival of wild-to-wild translocated tortoises (Tuberville et al. 2005), and the use of pens at AGTHP likely contributed substantially to the high rates of apparent survival we observed. Because we penned all tortoises, it remains unclear if released waif tortoises without this intervention would exhibit similar dispersal patterns to

those described for wild tortoises translocated without penning (Heise and Epperson 2005; Tuberville et al. 2005).

Collectively, our study provides insight into an emerging management issue—whether waif gopher tortoises are suitable candidates for release into the wild. As the first study to evaluate waif gopher tortoise translocation, it also provides important considerations for future gopher tortoise management. Due to the sensitivity of population viability to adult mortality in many turtle species (Seigel and Dodd 2000), adult survival following release has historically been an important parameter for determining the success or failure of translocation projects (Ashton and Burke 2007, Dodd and Seigel 1991, Tuberville et al. 2008). Because waif tortoises are often housed in captivity for extended periods, it was uncertain if they would exhibit high levels of survival following release. However, the high rates of apparent survival observed in this study were comparable to *in situ* (Ozgul et al. 2009) and to wild-to-wild translocated tortoise populations (Ashton and Burke 2007; Tuberville et al. 2008). The high survival rates we observed in our study suggest that waif tortoises can be used to augment or re-establish tortoise populations in circumstances where the risk to an existing or neighboring population is low and no other recovery option is possible.

## Management Implications

As gopher tortoises continue to decline, waif tortoises could play an important role in the conservation of isolated populations facing extirpation. However, because waif tortoises have the potential to introduce novel pathogens, extreme caution is still warranted. The use of waif animals to augment populations is likely appropriate only for situations where the recipient site is: 1) not home to a viable population (or a population that could foreseeably become viable with lower risk management interventions such as habitat improvement or nest protection. 2)

geographically isolated from the nearest population by either a distance that greatly exceeds the capacity of a dispersing tortoise and/or is surrounded by features impermeable to a dispersing tortoise (e.g., large bodies of water). Additionally, health assessments prior to release (and when possible, pathogen screening) can minimize the risk of disease introduction. Waif tortoises with clinical signs of infection or permanent injuries that could prevent their survival in the wild are likely unsuitable for release.

Although our results are encouraging, public education efforts are important for reducing the creation of new waif individuals. Currently, the number of waif tortoises is substantial enough to warrant a dedicated management program in the state of Florida (Florida Fish and Wildlife Conservation Commission 2012). Finally, it is important to note that the AGTHP was regularly burned, thinned, and managed for tortoises (Moule et al. 2013). SCDNR efforts to expand and manage the AGTHP likely contributed to the high rates of apparent survival we observed in this study. Because habitat loss and degradation are the main threats to this species (Smith et al. 2006), translocation and population augmentation efforts are likely only effective tools when implemented in conjunction with habitat protection and management. Because this is the first effort to quantify the survival of waif tortoises following translocation, additional monitoring of outcomes for other populations augmented with waifs is important to assessing the broader application of our results.

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## Tables

Table 2.1. Criteria for gopher tortoise stage class assignment. Prior to release at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC, gopher tortoises were measured and assigned to a stage class based on their midline carapace lengths (MCL). Sex of adults was determined by the presence of secondary sex characteristics. Females were considered mature at a smaller MCL if they were gravid. Tortoises were measured and classified at each capture occasion.

| **Stage** | **MCL Length** | **Additional Characteristics** |
|---|---|---|
| Hatchling | < 68 mm | |
| Juvenile | ≥ 68 mm, < 130 mm | |
| Subadult | ≥130 mm, < 230 mm | Flat plastron |
| Adult Male | ≥180 mm | Concave plastron, gular protrusion |
| Adult Female | ≥230 mm | Flat plastron |

Table 2.2. Live-dead multistate Cormack-Jolly-Seber model matrix used to calculate annual apparent survival estimates ($\varphi$) for gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, South Carolina. The matrix of probabilities also was used to model transitions from stage class (juvenile, subadult, adult male, adult female; $c$ = 1-4 respectively) or dead status ($c$ = 5 recently died and discovered; $c$ = 6 other death circumstance) at time $t$ (rows) to corresponding states at time $t$+1 (columns). Probabilities were functions of stage-class-dependent annual survival probability $\varphi_c$, annual probability of transition between size stages given survival $\psi_c$ ($c$ = 1 or 2 only), annual probability of graduating into the adult stage class as male rather than female $\gamma$, and stage-class-dependent annual probability of recovery of the tortoise given its death $r_c$. Recovery probability was constrained $r_3 = r_4$ to permit recovery probability of a dead animal to vary by size stage but not by adult sex. Parameter $\varphi_c$ was further indexed by geographic origin of the individual (South Carolina, Georgia, Florida, and Other) in a model allowing survival to vary by origin. Implausible transitions were fixed to 0.

| | **Juvenile** | **Subadult** | **Adult Male** | **Adult Female** | **Recently Dead** | **Dead** |
|---|---|---|---|---|---|---|
| **Juvenile** | $\varphi_1 * (1-\psi_1)$ | $\varphi_1 * \psi_1$ | 0 | 0 | $r_1*(1-\varphi_1)$ | $(1-r_1)*(1-\varphi_1)$ |
| **Subadult** | 0 | $\varphi_2*(1-\psi_2)$ | $\varphi_2*(1-\psi_2)*\gamma$ | $\varphi_2*\psi_2*(1-\gamma)$ | $r_2*(1-\varphi_2)$ | $(1-r_2)*(1-\varphi_2)$ |
| **Adult Male** | 0 | 0 | $\varphi_3$ | 0 | $r_3*(1-\varphi_3)$ | $(1-r_3)*(1-\varphi_3)$ |
| **Adult Female** | 0 | 0 | 0 | $\varphi_4$ | $r_4*(1-\varphi_4)$ | $(1-r_4)*(1-\varphi_4)$ |
| **Recently Dead** | 0 | 0 | 0 | 0 | 0 | 1 |
| **Dead** | 0 | 0 | 0 | 0 | 0 | 1 |

Table 2.3. Observation model portion of the live-dead multistate Cormack-Jolly-Seber model fit to capture data of gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC. Tortoises were released at the site between 2006 and 2017. Focused search efforts were conducted in 2008, 2017, and 2018. Additionally, intermittent incidental observations were recorded in years without focused search effort. The capture probability (p) for juvenile, subadult, adult male, and adult female stage classes was estimated for years with a focused search effort and for years without a focused effort. We constrained p3,t = p4,t to permit capture probability to vary by tortoise size but not by adult sex.

| | **Juvenile** | **Subadult** | **Adult Male** | **Adult Female** | **Recently Dead** | **Not Seen** |
|---|---|---|---|---|---|---|
| **Juvenile** | $p_{1,t}$ | *0* | 0 | 0 | 0 | $(1- p_{1,t})$ |
| **Subadult** | 0 | $p_{2,t}$ | 0 | 0 | 0 | $(1- p_{2,t})$ |
| **Adult Male** | 0 | 0 | $p_{3,t}$ | 0 | 0 | $(1- p_{3,t})$ |
| **Adult Female** | 0 | 0 | 0 | $p_{4,t}$ | 0 | $(1- p_{4,t})$ |
| **Recently Dead** | 0 | 0 | 0 | 0 | 1 | 0 |
| **Not seen** | 0 | 0 | 0 | 0 | 0 | 1 |

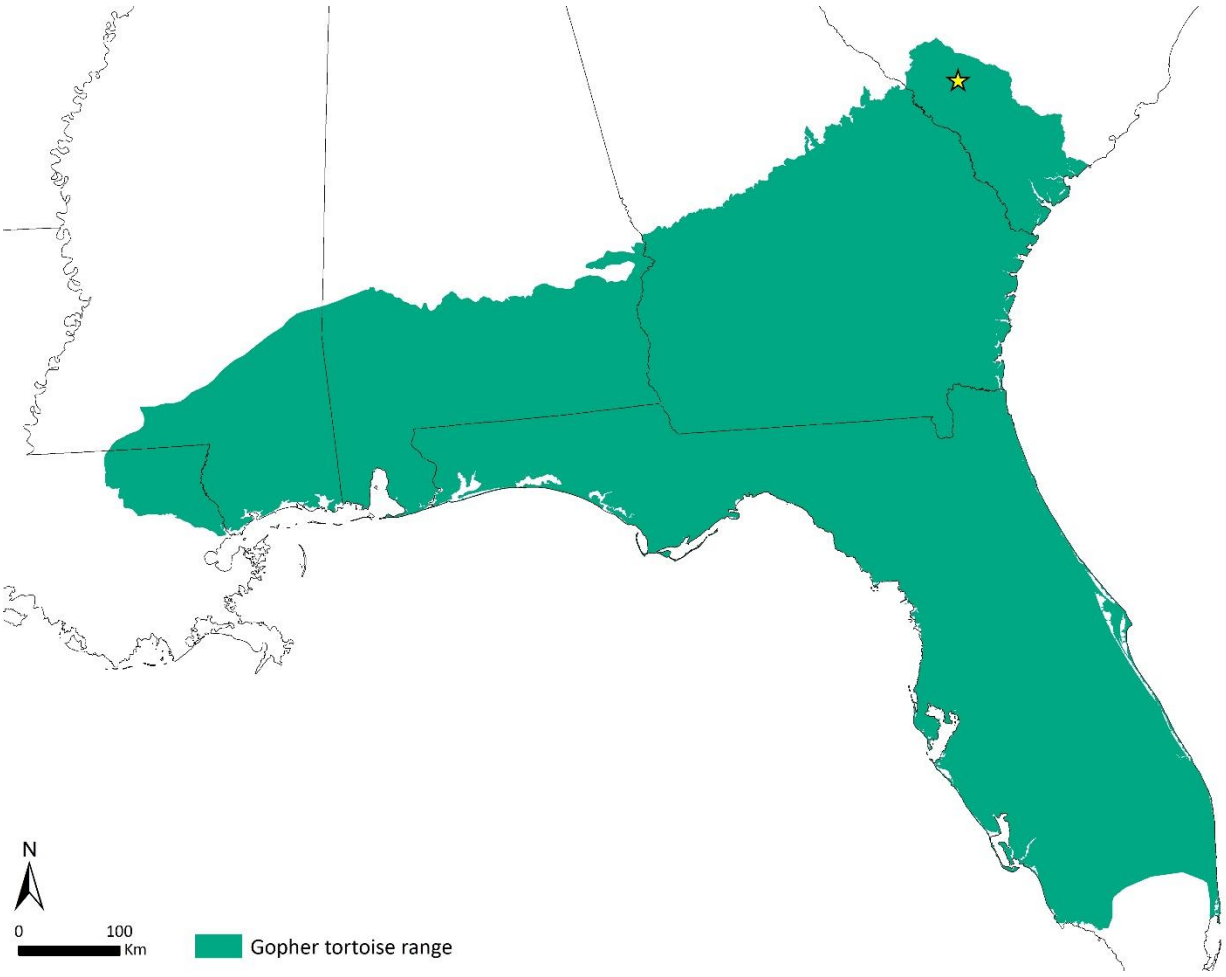
Table 2.4. Candidate models used to estimate annual apparent survival of gopher tortoises released at the Aiken Gopher Tortoise Heritage Preserve. Wald tests were performed to sequentially select among nested models. The simpler model was rejected in favor of the more complex model if Wald $Q \geq \chi_k^2$ ($\alpha$ = 0.05), the critical value of the chi-square distribution with cumulative probability 1-$\alpha$ and degrees of freedom *k* equal to the difference in number of parameters between models.

| **Model Terms** | **Wald *Q*** | **Df** | ***P*** |
|---|---|---|---|
| Stage + Origin + Stage*Origin | 8.38 | 9 | 0.50 |
| Stage + Origin | 3.21 | 3 | 0.36 |
| Origin | 499.95 | 3 | <0.001 |

Table 2.5. Model estimates of mean gopher tortoise apparent survival and transition probabilities to stage class with corresponding SD and 95% Credible Intervals. Model included stage class as a fixed effect and pen as a random effect for survival probabilities. Transition probabilities were estimated for juveniles maturing to subadults (Subadult), and subadults maturing to either adult male (Adult Male) or adult female (Adult Female).

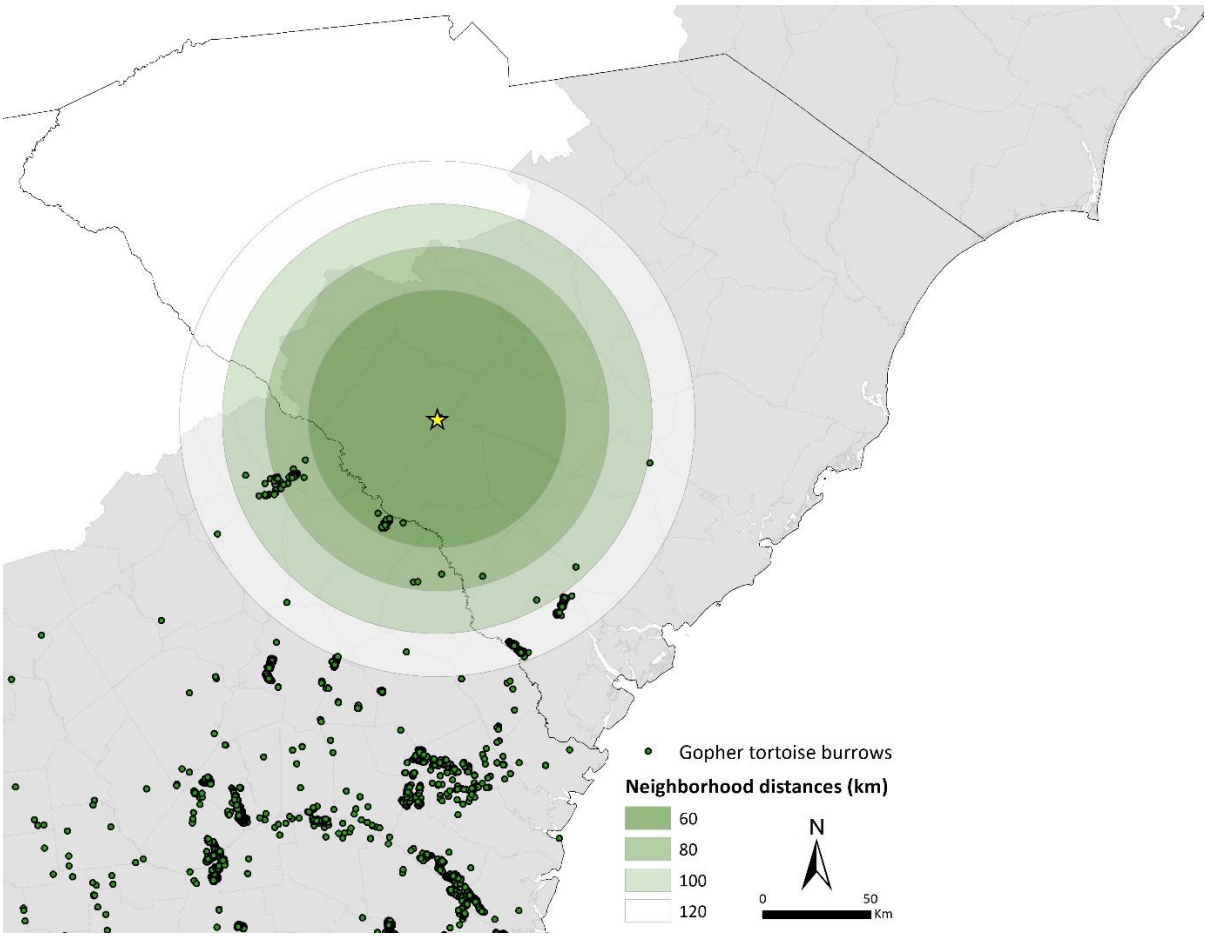
| **Parameter** | **Mean** | **SD** | **2.50%** | **97.50%** |
|---|---|---|---|---|
| *Stage Survival* | | | | |
| Juvenile | 0.25 | 0.17 | 0.03 | 0.67 |
| Subadult | 0.96 | 0.05 | 0.84 | 1.00 |
| Adult Male | 0.91 | 0.07 | 0.70 | 0.99 |
| Adult Female | 0.95 | 0.04 | 0.83 | 0.99 |
| *Stage Transition* | | | | |
| Subadult | 0.35 | 0.16 | 0.09 | 0.69 |
| Adult Male | 0.04 | 0.02 | 0.01 | 0.09 |
| Adult Female | 0.12 | 0.04 | 0.06 | 0.2 |

## Figures




Map credit: Brian Crawford

Figure 2.1. Map displaying the location of the Aiken Gopher Tortoise Heritage Preserve (AGTHP) in the context of the gopher tortoise range. AGTHP is in the northernmost known gopher tortoise population.




Map credit: Brian Crawford

Figure 2.2. Map showing Aiken Gopher Tortoise Heritage Preserve (AGTHP) in context of neighboring gopher tortoise populations. No populations fell within 50km of known tortoise populations. In addition, the Savannah River flows between South Carolina and Georgia, creating an additional barrier.

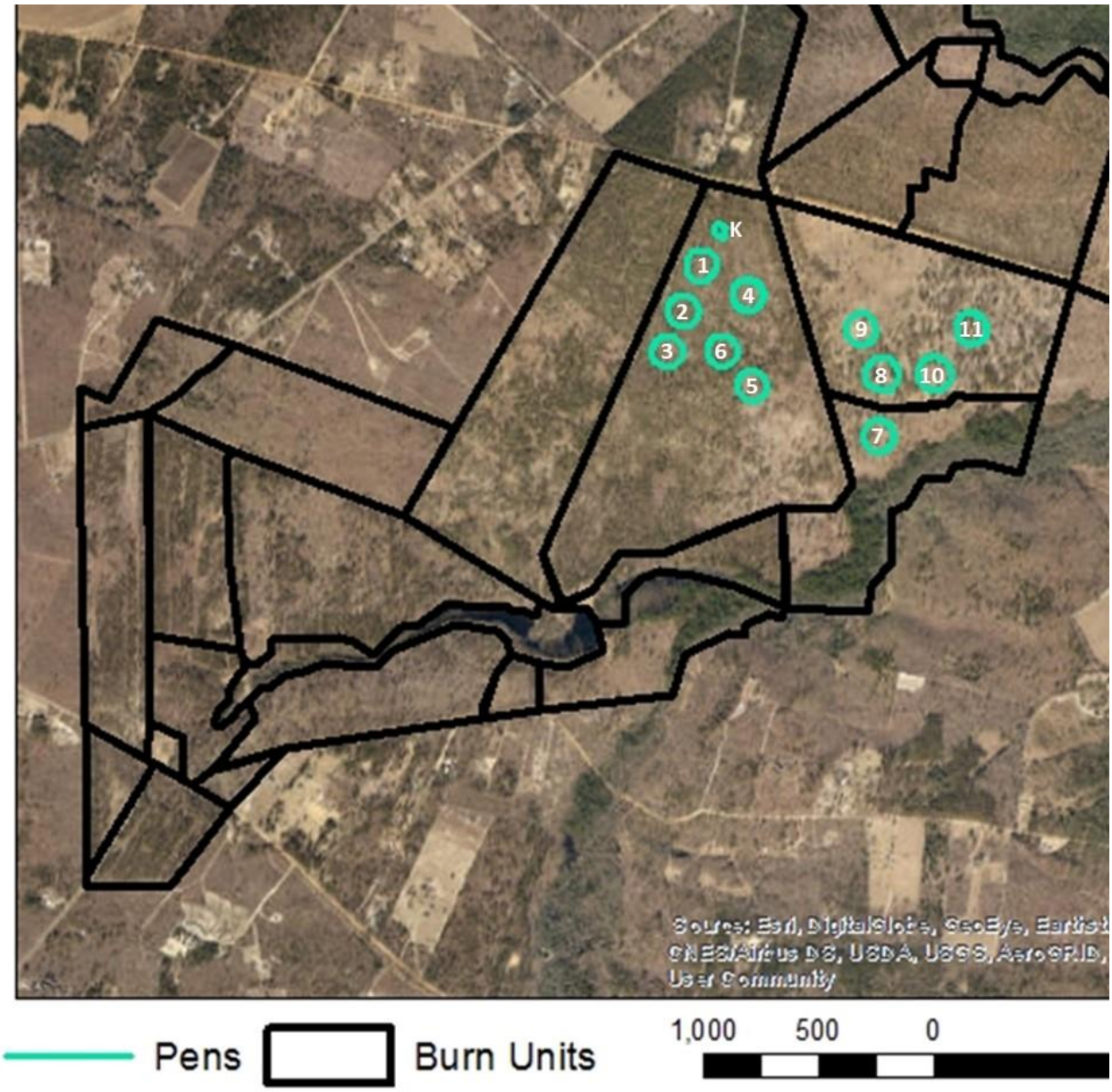



Figure 2.3. Map of burn units and tortoise release pens at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC. Waif tortoises and captured residents were released into pens between 2006-2018. Tortoises were allowed to acclimate >1yr before pen wall were lowed.

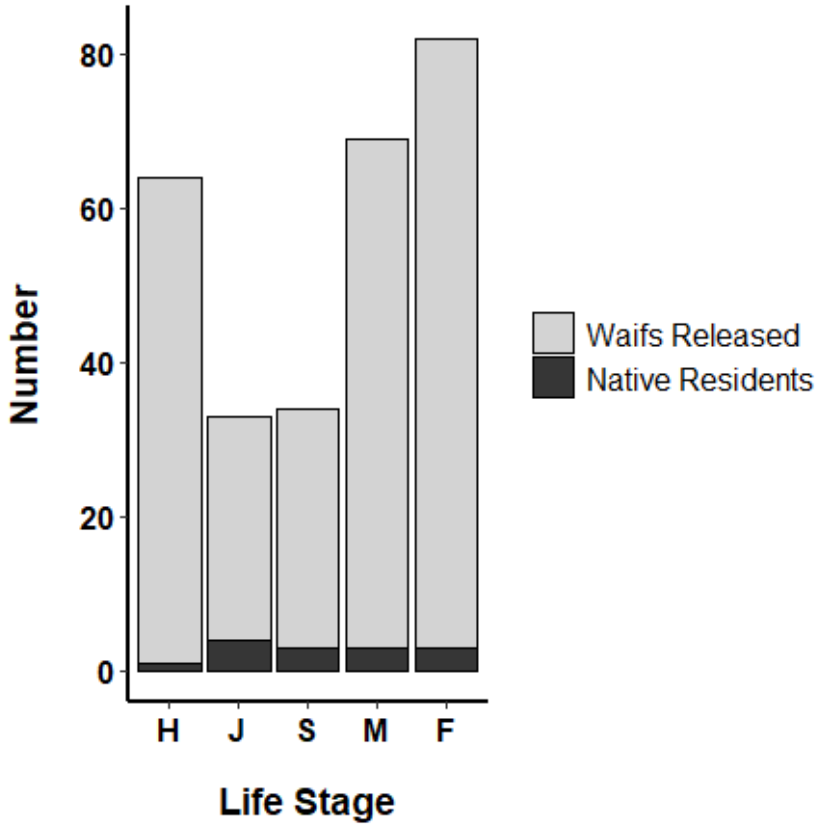



Figure 2.4. Number of waif gopher tortoises and native resident gopher tortoises marked and released at the Aiken Gopher Tortoises in Aiken County, SC between 2006 and 2017. Analyses included juvenile (J), subadult (S), adult male (M), and adult female (F) stage classes, but not hatchlings (H).

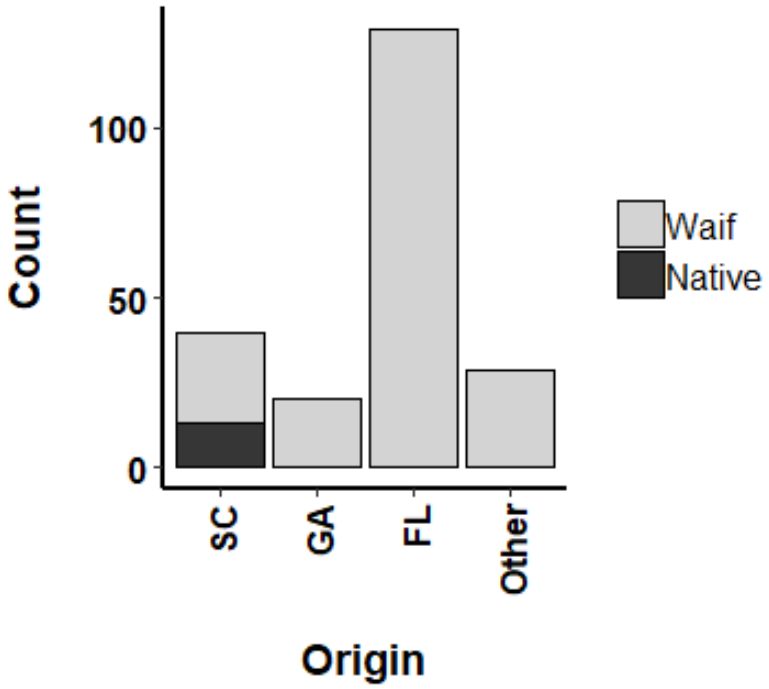



Figure 2.5. Number of juvenile, subadult, adult male and adult female gopher tortoises released at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC by tortoise origin. Due to the small number of native animals, native tortoises and tortoises from elsewhere in the state were not differentiated. The "other" category includes tortoises from states other than SC, GA, and FL and those held outside their native range (usually as illegal pets).

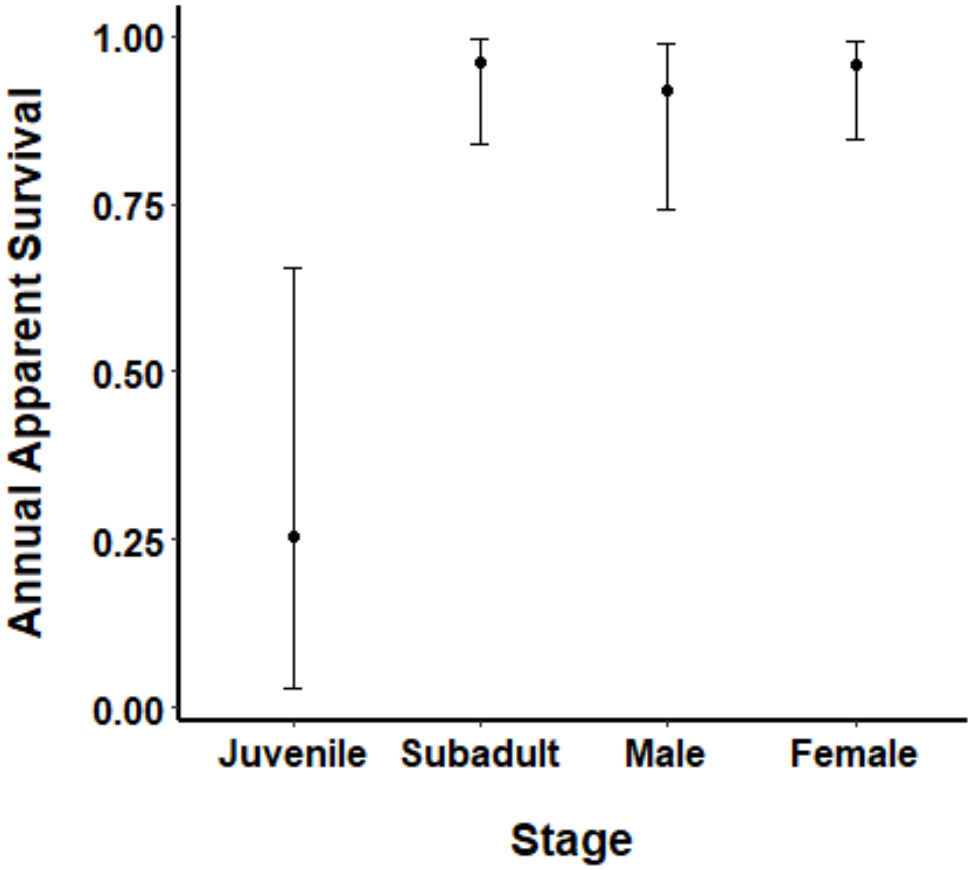



Figure 2.6. Mean annual apparent survival probabilities and 95% Bayesian credible intervals for juvenile, subadult, adult male, and adult female stage classes of gopher tortoises, based on estimates from a joint live-dead multistate Cormack-Jolly-Seber model. Tortoises were marked between 2006-2017 and recaptured 2017-2018 at the Aiken Gopher Tortoise Heritage Preserve, Aiken County, South Carolina.

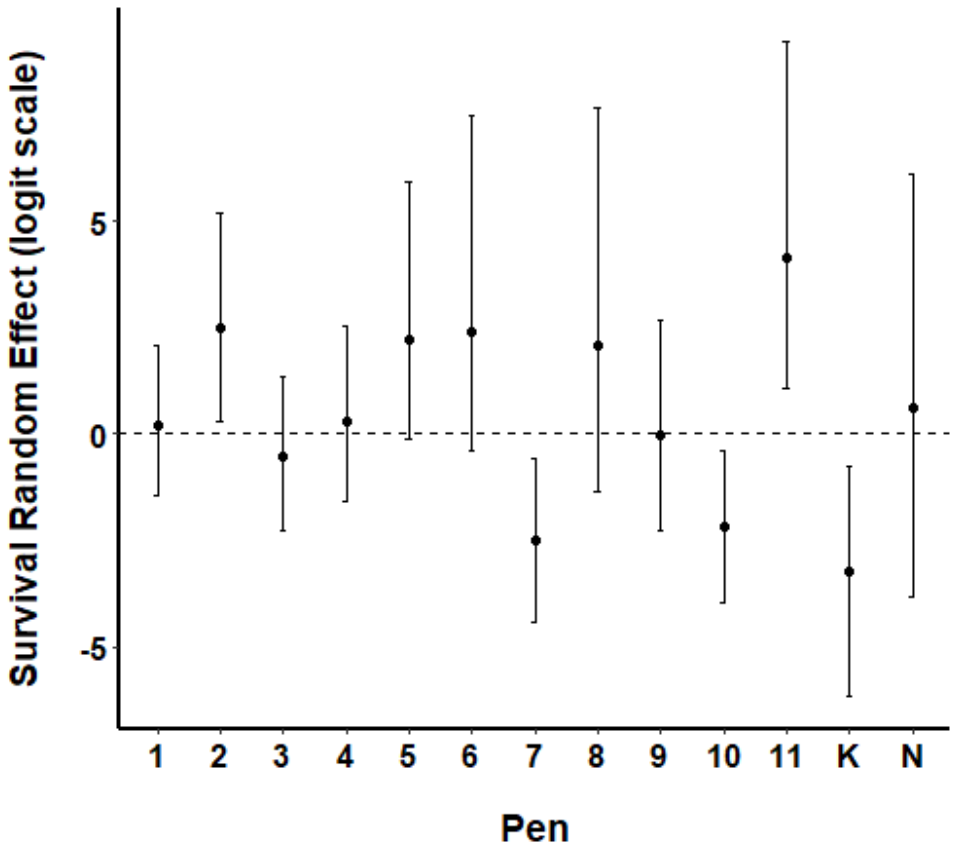



Figure 2.7. Estimated individual pen effects (and 95% Bayesian credible intervals) on annual apparent survival (logit scale) of gopher tortoises marked between 2006-2017 and recaptured 2017-2018 on the Aiken Gopher Tortoise Heritage Preserve, Aiken County, South Carolina. Tortoises were penned for ≥1 year prior to release. Mark-recapture data were analyzed in joint live-dead multistate Cormack-Jolly-Seber models, and pen was included as a random effect in all candidate models. Unmarked tortoises found on site during the 2017-2018 surveys were classified as no pen (N), while all other numbers and letters listed refer to physical pens.

CHAPTER 3

# ASSESSING WAIF GOPHER TORTOISE HEALTH AND STRESS FOLLOWING TRANSLOCATION

## Introduction

Translocation, the intentional movement of animals from one location to another, has become an increasingly important tool for managing imperiled wildlife species (Biggins et al. 2011; Johnson et al. 2010; Kraus et al. 2016). Despite the potential for success and its widespread use (Fischer and Lindenmayer 2000; Germano and Bishop 2009), translocation also carries many risks, including those associated with wildlife health (Deem et al. 2001; Kock et al. 2010). Translocated animals can suffer high mortality rates, especially when naïve individuals are introduced to locations with endemic pathogens (Hurtado et al. 2015; Popp et al. 2014). Translocation efforts can also inadvertently introduce pathogens to naïve resident populations (Deem et al. 2001, Griffith et al. 1993; Kock et al. 2010). In the United States, pathogens and parasites have been spread through the translocation of turkeys (*Meleagris gallopavo*; Castle and Christensen 1990), raccoons (*Procyon lotor*; Schaffer et al. 1981), and whitetail deer (*Odocoileus virginianus*; Cohen et al. 2018; Davidson et al. 1996). Additionally, translocation can induce physiological stress (Dickens et al. 2010, Teixeira et al. 2007), which can in turn can cause mortality directly (Hartup et al. 1999) or can indirectly affect health through immune system suppression (Teixeira et al. 2007).

Because herpetofauna are experiencing dramatic declines (Gibbons et al. 2000; Stuart et al. 2004), conservationists are increasingly tasked with balancing the potential benefits of

management interventions, such as translocation, with potential adverse effects, including the increased risk of pathogen transmission (Aiello et al. 2014; Jacobson 1993; Walker et al. 2008). Numerous translocation efforts have been successful for reptile and amphibian species (Fitzgerald et al. 2015; Jarvie et al. 2016; Kraus et al. 2017). However, emerging infectious diseases present a challenge for both the conservation of *in situ* herpetofauna (Daszak et al. 1999; Tompkins et al. 2015) and translocation programs (Pessier 2008; Walker et al. 2008).

The gopher tortoise (*Gopherus polyphemus*) is a long-lived species endemic to the southeastern United States and is among the most commonly translocated reptile species (Tuberville et al. 2008). Federally listed as threatened in the western portion of its range since 1987 (United States Fish and Wildlife Service (USFWS) 1987), the species has recently also become a candidate for federal listing throughout the remainder of its range (USFWS 2011). Historically, gopher tortoises have been translocated as a mitigation strategy for construction activities, where tortoises might incur direct mortality via crushing from heavy equipment or from entombment—the collapse of tortoise burrows, resulting in the entrapment and suffocation of tortoises (Florida Fish and Wildlife Conservation Commission (FFWCC) 2006; FFWCC 2012; Sullivan et al. 2015). Prior to 2006, incidental take permits allowed developers to pay into a mitigation fund that protected tortoise habitat elsewhere in exchange for not having to relocate tortoises residing on the site slated for development, but discontinuation of that policy in 2006 greatly increased the number of gopher tortoises needing translocation (Florida Fish and Wildlife Conservation Commission 2006; Florida Fish and Wildlife Conservation Commission 2012). Additionally, as development continues to fragment tortoise habitat and populations, there is growing interest in using translocation as a tool to manage for population viability (Gopher Tortoise Council 2014).

Although habitat loss is the primary driver of declines for gopher tortoises (Smith et al. 2006), disease is also considered a risk to the species (Jacobson 1994; McLaughlin 1997; Gates et al. 2002). Upper respiratory tract disease (URTD) has historically been the focal disease of management concern, and as such, it is the best characterized chelonian disease (Jacobson et al. 2014; Jacobson 1994; Gates et al. 2002). URTD outbreaks have been linked to mortality events in both desert tortoises (*Gopherus agassizii*, Jacobson et al. 1991) and gopher tortoises (Beyer 1993, Gates et al. 2002, Seigel et al. 2003). More recently, *Ranavirus* sp. has been documented in gopher tortoises (Cozad 2018; Johnson et al. 2008; Westhouse et al. 1996), and although its effects on tortoise health are poorly understood, infections are often lethal in other chelonians (De Voe et al. 2004; Sim et al. 2016).

There are risks associated with any translocation, however, waif tortoises— any tortoise removed from its natal site and held in captivity for an extended period or whose collection origin is unknown (FFWCC 2012)—are generally excluded from translocation efforts due to heightened concerns of introducing pathogens. In the case of the closely-related desert tortoise, released captive tortoises have been implicated in spreading pathogens, including the causative agents for URTD (Jacobson 1993). Moreover, even short-term captivity can be stressful to wildlife (Gregory et al. 1996), and prolonged captivity can alter behavior, physiology, or nutritional status (Mason 2010, McDougall et al. 2006), potentially making the transition back to the wild even more stressful. However, as the number of waif animals in captivity has increased, finding permanent housing for individuals is often challenging and has strained local and state resources (FFWCC 2012). Moreover, as the species continues to decline throughout its range (Smith et al. 2006), these waif animals could provide the needed numbers to prevent extirpations in isolated wild populations.

In 1993, the discovery of a small gopher tortoise population near Aiken, South Carolina expanded the documented range for the species and inspired the creation of the Aiken Gopher Tortoise Heritage Preserve (AGTHP; Clark et al. 2003). Surveys conducted in 1999 and 2001 determined that only a small relict population of tortoises (n<10) remained on the preserve (K. Buhlmann, unpublished data), and it was concluded that the population was too small to sustain itself long-term without intervention. Due to the site's geographic isolation and lack of suitable donor animals from wild displaced tortoise populations, it was proposed to introduce waif tortoises in an effort to recover the relict population. Since 2006, over 280 waif tortoises, from a variety of origins, have been released in the preserve. No tortoises with clinical signs of infection were released, however, pathogen screening prior to release was not possible due to lack of funding. Because waif tortoises present an ongoing management challenge and their potential contribution to population recovery is unknown, it is important to evaluate the health profile of this uniquely established population. Our primary objective was to assess the health of the translocated waif population by conducting comprehensive health evaluations, including physical exams, hematology, parasite quantification, and pathogen screening, of the surviving individuals after more than a decade of releases.

## Methods

We captured gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve during the summers of 2017 and 2018 (see Chapter 2 for description of survey and capture methods). Once captured, each tortoise was transported to the Savannah River Ecology Lab where we measured and assessed its health and physiological condition. During 2017 and 2018, other wildlife agencies provided additional waif tortoises for release at AGTHP. Prior to their release, we processed and assessed these individuals similarly to tortoises captured in the field.

We recorded the mass of tortoises to the nearest 2 g. We measured the midline carapace length (MCL) from the nuchal scute to the supracaudal scute, the total length from the notch of the gular scute to the supracaudal scute, the width at the widest point of the carapace, and the shell height at the highest point of the shell. We recorded all carapace measurements to the nearest 1 mm. We calculated tortoise body condition by dividing the mass (g) by the body shell volume (Loehr et al. 2004; Daly et al. 2018). To calculate shell volume, we used the formula for the half ellipsoid: shell volume ($cm^3$) = ($\pi \times$ MCL $\times$ width $\times$ height) / 6000.

We classified tortoises as hatchlings, juveniles, subadults, or mature animals by their MCL (Table 3.1). We determined sex for mature animals based on a combination of MCL and secondary sex characteristics (Table 3.1). We considered female tortoises <230mm to be mature adults if they were determined as gravid via palpation, thereby confirming their reproductive maturity.

*Visual Health Assessments*

We assessed each tortoise for obvious signs of injury and trauma. During examination, we also checked for ectoparasites, including the gopher tortoise tick (*Amblyomma tuberculatum)* associated with the transmission of hemogregarines (i.e., blood parasites; Sonderman 2014). We considered the carapace and plastron to be abnormal if they exhibited evidence of previous or recent trauma (e.g., cracks, punctures), irregular "pyramid" growth patterns characteristic of unbalanced diets or inadequate conditions in captivity (Jackson et al. 1976), or flakiness and discoloration characteristic of cutaneous dyskeratosis (Jacobson 1994, Berry and Christopher 2001). We considered skin to be abnormal if we documented lacerations, lesions, discolored scales, pronounced inflammation or other deviations. If the cloaca had signs of inflammation, prolapsed organs, or infection, we considered it to be abnormal. We classified eyes as abnormal

if the eyes were sunken (indicative of dehydration) or if we observed palpebral or periocular swelling, ocular discharge, or ocular discoloration. We defined normal nares as circular and symmetrical in shape, free of current discharge or obstruction, and lacking evidence of previous discharge (bleaching, scale loss, or erosion). We considered respiration to be abnormal if we heard wheezing, whistling, or other sounds during the examination. We characterized the oral cavity as normal if it was pale pink to pink in color, lacked deformities, and was free of crusts or ulcers. We assessed the tympanum for evidence of trauma, abscesses, or aural swelling.

Because many chelonian pathogens target the respiratory tract (Origgi and Jacobson 2000), we carefully scanned each tortoise for evidence of infection. Although we documented any observed abnormality as previously described, we flagged all individuals recorded with naral discharge, eroded nares, ocular discharge, ocular swelling or conjunctivitis as having clinical signs of infection for known gopher tortoise pathogens, such as *Mycoplasma agassizii* sp. or *Ranavirus* sp. (Brown et al. 1999, Brown et al. 2004, Johnson et al. 2008, McLaughlin et al. 2000). If an individual displayed at least one naral clinical sign, we categorized it as naral clinical signs present. If we observed at least one ocular clinical sign, we categorized it as ocular clinical signs present. However, because different pathogens can present with similar clinical signs, additional diagnostic testing is required to confirm infection status and differentiate between pathogens (Cozad 2018).

*Pathogen Screening*

URTD has historically been the primary focus of disease investigations in gopher tortoises (Jacobson et al. 2014; Jacobson 1994; Gates et al. 2002). Experimental trials confirmed *Mycoplasma agassizii* as the etiologic agent of URTD for gopher tortoises in experimental trials (Brown et al. 1999), and a second etiologic agent, *Mycoplasma testudineum*, was later identified

(Brown et al. 2004). More recently, pathogens in the genus *Ranavirus* have emerged as a concern for North American chelonians (De Voe et al. 2004), including gopher tortoises (Johnson et al. 2008, Westhouse et al. 1996). Due to possibility that waif gopher tortoises had been exposed to other testudinid species while in captivity, the panel of pathogens we screened for included some that have not specifically been documented in gopher tortoises but have been known to affect other species (Table 3.2).

We used Costan Diagnostic Flocked Swabs ® to collect a separate oral swab and a cloacal swab from each tortoise. If we observed a tortoise with naral discharge during the visual assessment, we also collected a naral swab. Unlike previous research which used antibody tests to determine exposure (McLaughlin et al. 2000, McGuire et al. 2014), swabs determine if an individual is actively shedding a pathogen. This screening method may also result in an individual's status changing over sampling events. As such, if an individual captured in 2017 was recaptured in 2018, we collected and analyzed oral swabs from both years and calculated prevalence annually. Because we failed to detect pathogens in cloacal swabs in the first sampling period, we did not analyze cloacal swabs for recaptured tortoises in 2018. We stored swabs at -80 °C until analysis. The University of Illinois Wildlife Epidemiology Lab analyzed all collected samples by testing swabs for a panel of pathogens using either quantitative (qPCR) or traditional PCR methods (Table 3.2).

*Hematology and Hemoparasite Quantification*

We collected 0.5-1.5 mL of blood from subadults and adults via the brachial vein using a 25-gauge heparinized needle. If the tortoise was classified as a juvenile, we used a 29.5-gauge needle to collect 0.3-0.6 ml of blood from the subcarapacial vein. We did not collect blood from

hatchling tortoises. To minimize handling time for tortoises, we only collected blood from tortoises during their first capture, resulting in one blood sample per tortoise.

For each tortoise, we filled a micro hematocrit tube with whole blood before centrifuging it to determine packed cell volume (PCV), allowing us to quantify red blood cell percentage. We also used a refractometer to determine the total solids (TS) of the blood sample. Following blood collection, we also made three to five blood smears that were then fixed with methanol. We placed 0.1-0.3 mL whole blood in lysis buffer that we archived for future genetic analysis. Any remaining blood was centrifuged to separate blood cells from plasma and subsequently banked and stored at -80 °C.

A veterinary pathologist at the University of Florida College of Veterinary Medicine stained blood smears using Wright-Giemsa standards. She screened blood smears for hemogregarines, blood-born parasites transmitted by ticks (Cook et al. 2009) that have been previously reported in gopher tortoises (Cooney et al. 2019, Hernandez et al. 2010). Although any ticks observed on waif tortoises were removed prior to their initial release at AGTHP, we assessed current parasitemia because: 1) some ticks may have gone undetected at initial handling; 2) waifs were not screened for hemogregarines at time of initial releases (which occurred during 2006 – 2018); and 3) a previous study of hemogregarine parasitemia in a translocated gopher tortoise population documented change in infection status over time in some released tortoises (Sonderman 2014). We quantified hemoparasite infections by counting the number of red blood cells infected with hemogregarines per 100 red blood cells.

Blood smears were also used to assess chronic stress by determining leukocyte cell profiles – an approach that has been used for a number of wildlife species (Davis et al. 2008), including gopher tortoises (Goessling et al. 2017). In reptiles and birds, stress increases the

relative number of heterophil cells and suppresses the creation of lymphocyte cells, resulting in elevated heterophil:lymphocyte ratios (H:L ratios; Davis et al. 2008, Martinez-Silvestre 2014). The same veterinary pathologist blindly evaluated smears for white blood cell estimates, white blood cell differentials (200 cells), and blood cell morphology. We calculated H:L ratios by dividing the total number of heterophil cells by the total number of lymphocyte cells.

*Modeling Predictors of Infection with Mycoplasma*

We used logistic regression models to identify individual attributes of tortoises useful in predicting infection with *Mycoplasma*. We focused on *Mycoplasma* because: 1) it has historically been considered the primary disease of management concern; 2) its prevalence has been previously reported for *in situ* populations elsewhere in the species' range; and 3) it was the only pathogen we detected with enough frequency to allow statistical analysis. We considered individuals as positive for either *M. agassizii* or *M. testudineum* as infected. Previous research has documented a relationship between infection status and sex/life stage (Wendlend et al. 2010), body condition (Cozad 2018), packed cell volume (Cozad 2018), and stress (McCoy et al. 2005). Therefore, we included stage class (at time of recapture in 2017-2018), body condition, PCV, stress (H:L ratio), naral clinical signs (present/absent), and ocular clinical signs (present/absent). Because an individual's response to infection may depend on its stage class, we also considered interactions between stage class and PCV, body condition, and stress. Additionally, we hypothesized that the relationship between infection status and PCV, body condition, and stress could depend on whether an individual displayed clinical signs of infection, and thus included candidate models with interactions between naral clinical signs and these factors. We did not consider an interaction term for ocular clinical signs because fewer tortoises exhibited these signs (n=7) relative to naral signs (n=17).

Because tortoises were penned on site in groups prior to release to promote site fidelity (Tuberville et al. 2005), we originally included release pen as a random effect in all candidate models. However, because the estimated variance parameter for pen was essentially zero (indicating no detectable difference between pen group), we subsequently removed release pen from all models.

In order to compare candidate models (Table 3.6), we only fit models to data that contained a complete set of individual attributes for each tortoise. Due to a small number of complete records for juveniles (n=2) and subadults (n=15), we combined these stages into a single group classified as "immature" in all candidate models. To more easily make comparisons across models with different continuous variables, we centered and scaled continuous variables (PCV, body condition, H:L ratios) by subtracting the mean and dividing by the standard deviation of each variable. We used corrected Akaike's Information Criteria (AICc) to compare candidate models (Akaike 1974, Hurvich and Tsai 1989). We reported the results from all candidate models with a delta AIC <2. For all averaged observations and estimates, we reported the means ± SD. We conducted all analyses in R (R Core Team, version 3.5.1) and used package Multimodel Inference ('MuMIn') version 1.42.1 for model selection (Bartoń 2018).

## Results

*Visual Health Assessment*

During 2017-2018, we captured a total of 143 unique individual tortoises, comprising 68 females, 50 males, 17 subadults, 5 juveniles, and 3 hatchlings (Table 3.3). We visually assessed 63 tortoises in 2017. Of these, 7 (10.9%) presented with at least one clinical sign. In 2018, we assessed 126 individuals, of which 22 (17.5%) had at least one clinical sign. We did not observe any ectoparasites on tortoises in either year.

*Pathogen Screening*

In total, we screened 142 individuals (139 individuals total with oral swabs). Although we detected *Mycoplasma* spp. in both years, we did not detect any other pathogens (including 3 species of Ranavirus) in our screenings (Table 3.2).

In 2017, we tested a total of 63 individuals using oral (n=59), cloacal (n=63), and/or naral swabs (n=2) for 13 pathogens (Table 3.2). In 2017, *M. agassizii* was the only pathogen detected, with a prevalence of 10.2% in the oral swabs (n=6). We also detected *M. agassizii* in a single naral swab. This individual's oral swab was also positive, with the amount of DNA detected from the oral swab (15.1 copies/ul) over 10 times greater than the amount detected from the naral swab (1.4 copies/ul; Table 3.4).

In 2018, we tested a total of 124 individuals using oral (n=122), cloacal (n=60), and/or naral swabs (n=4) for 11 pathogens (Table 3.2). We detected *M. agassizii* with a prevalence of 13.9% (n=17) and *M. testudineum* with a prevalence of 0.8% (n=1) on oral swabs. All 4 of the naral swabs tested positive for *M. agassizii* and were from individuals whose oral swabs also tested positive for the pathogen. We detected *M. agassizii* in oral swabs from 17 individuals, but the pathogen was only detected in cloacal swabs from 3 of these same individuals (Table 3.4). In 2018, we also detected Emydid *Mycoplasma* sp. in a single individual, a naturally recruited hatchling tortoise (i.e. not a released animal) that showed no clinical signs of infection. Swabs collected from 2017 were not screened for this pathogen.

We collected and analyzed oral swabs from 42 tortoises in both 2017 and 2018. Of these 42 resampled animals, 6 individuals changed infection status between years (Table 3.5). Two individuals converted from positive to negative, and both had low copy numbers of pathogen

DNA detected in 2017 (Table 3.4). Four individuals were negative for all pathogens in 2017 and tested positive for *Mycoplasma* in 2018 – three for *M. agassizii* and one for *M. testudineum.*

*Hematology and Hemoparasite Quantification*

We collected blood from 62 tortoises in 2017 and 81 tortoises in 2018. The average PCV was 27.9±6.2 but ranged from 12-44 among individuals (Table 3.3). The average TS was 3.7±0.8 but ranged from 2.0-5.7 (Table 3.3).

We submitted blood smears for 60 individuals in 2017. All were analyzed for blood parasites and leukocyte cell differentials. No hemoparasites were detected in the blood smears (prevalence = 0%). The average H:L ratio was 1.38± 0.96 but ranged from 0.23-4.63 among individuals. In 2018, we submitted blood smears for 77 individuals. All slides were analyzed for blood parasites, and 75 of the 77 slides were also suitable for leukocyte cell analysis. We detected mild hemoparasite infections (<1 infected cell/100 red blood cells) in two individuals (prevalence = 2.6%). The average H:L ratio was 1.32± 0.69 but ranged from 0.32-4.09 among individuals.

*Modeling Predictors of Infection with Mycoplasma*

We fit data from 124 tortoises (108 negative, 16 positive for either *Mycoplasma agassizii* or *Mycoplasma testudineum*) to 22 candidate models (Table 3.6). We found the presence of naral clinical signs to be an important predictor of infection, with naral clinical signs appearing in all 5 top models (Table 3.6). The presence of naral clinical signs was also the only factor identified as a significant predictor of *Mycoplasma* infection ($P < 0.01$). We observed naral clinical signs in 35.3% of positive animals and 10.2% of negative animals.

The second most supported model included an interaction between naral clinical signs and stress as measured by H:L ratios. This interaction term was not significant ($P = 0.08$), but the

relative weight of this model suggests that probability of infection is dependent on both the stress level of the tortoise and whether it is currently displaying clinical signs. Tortoises with clinical signs and elevated H:L ratios had high probability of being infected. However, tortoises without clinical signs had a low probability of infection, regardless of their H:L ratios, and there even appeared to be a slight negative relationship between elevated stress levels and infection probability.

Body condition appeared in the third model with naral clinical signs, suggesting that there is a benefit to also considering body condition as a predictor of infection (Table 3.6). However, body condition alone is not a reliable predictor and differences in body condition between groups were slight. On average, infected individuals had a body condition of 1.02 ± 0.9, while healthy individuals had an average body condition of 1.07± 0.15.

Tortoise stage class was included in our 4$^{th}$ best model (Table 3.6). Interestingly, this model estimated immature animals to have a slightly higher probability of infection (0.28) relative to adult females (0.06) and to adult males (0.10).

Ocular clinical signs appeared to be less important predictors than naral clinical signs. The presence of naral clinical signs appeared in all five of the top models, while ocular signs appeared only in the fifth model (Table 3.6). Of the 16 animals positive for either pathogen, only 2 exhibited ocular clinical signs and these 2 tortoises also exhibited naral clinical signs.

## Discussion

Our study is the first to provide a comprehensive analysis of individual health of gopher tortoises in a population established almost entirely through the release of waif animals. Overall, captured individuals appeared healthy. Despite the varied, often unknown, histories of individual animals, we detected few pathogens after more than a decade of releases. We documented the presence of

only two known tortoise pathogens, *Mycoplasma agassizii* (with 10.2, 13.9% prevalence) and *Mycoplasma testudineum* (with of 0.0, 0.8% prevalence) in 2017 and 2018, respectively. These pathogens are causative agents of URTD and are believed to be common in wild gopher tortoise populations (McCoy et al. 2007, Diemer Berish et al. 2010). *Mycoplasma* spp. exposure has been documented throughout much of the tortoise range, including Florida (Ozgul et al. 2009; Karlin et al 2010), Georgia (McGuire et al. 2014), Louisiana (Diaz-Figuerosa 2005), and Mississippi (Smith et al. 1998). Surveys across 53 sites in Florida determined 30% of tortoises to be seropositive for *Mycoplasma agassizii*. Exposure varies substantially between populations, however, with some populations having either very low seroprevalence (0-3%) or very high seroprevalence (96%-100%; McGuire et al. 2014). Although pathogen screening is the only way to assess an individual's current infection status, we found that naral clinical signs were an important predictor of infection with *Mycoplasma*.

Despite the attention URTD has received in scientific literature, the disease's overall effect on gopher tortoises is still debated (Deimer Berish et al 2010; McCoy et al. 2007; Seigel et al. 2003). In many cases, URTD appears to have only limited effect on the survival of a population (Karlin et al. 2008; McCoy et al. 2007; Ozgul et al. 2009). Rarely, however, URTD has been implicated in mortality events for some populations (Beyer 1993, Gates et al. 2002, Seigel et al. 2003). In our study, 62.5% of infected individuals had subclinical infections (infected but showing no clinical signs of disease) and were apparently healthy. However, we noticed a high rate of infected tortoises in a single pen (pen 10), with 62.5% of sampled individuals testing positive in at least one sampling period. In addition to the 5 live individuals that tested positive for *Mycoplasma agassizii*, we also recovered 8 shells of dead tortoises from this pen, into which 21 animals had been released during 2016-2017, indicating at least 38.0%.

mortality in this pen. Although we cannot confirm disease as the cause of death, we did not see evidence of predation or obvious signs of other causes of death. We captured seemingly healthy positive animals from other pens, and it is unclear why the morality rate is so high for this pen relative to the high rates of survival estimated for the population as a whole (Chapter 2.) However, a similar variability in host response has been noted in desert tortoises experimentally infected with the pathogen (Aiello et al. 2019) and prior research suggests that high morbidity is related to high prevalence, suggesting an effect of pathogen load in populations (Sandmeirer et al. 2017).

Fortunately, Pen 10 walls are still standing with the animals residing in the pen effectively confined from the rest of the population. Although increasing site fidelity is the primary objective of penning tortoises, penning can also provide an opportunity to monitor translocated individuals for signs of disease before they are integrated into the broader population. Because of the elevated mortality rates and severity of clinical symptoms observed in pen 10, SCDNR decided against lowering the walls for this pen and is currently evaluating management options for these animals.

The presence of naral clinical signs was the only significant predictor of infection, but we did observe some evidence for an interaction between naral clinical signs and stress, as measured by H:L ratios. Previous work has suggested that *Mycoplasma* spp. infections are correlated with elevated H:L ratios (McCoy et al. 2005). We also observed an association between elevated H:L ratios and increased infection probability, but this relationship also depended on whether the tortoise had naral clinical signs (Figure 3.8). Alone, H:L ratios were a poor predictor of *Mycoplasma* spp. infection (Table 3.6). It has been previously suggested that URTD is a context-dependent disease (Sandmeirer et al. 2013), with stressors hypothesized to play a role in its

severity (Sandmeirer et al. 2017). Although individuals with clinical signs and elevated H:L ratios had a higher probability of infection, we cannot determine if individuals were stressed prior to infection (and therefore more susceptible) or if the elevated stress levels occurred in response to infection.

Despite the attention URTD has received in the literature, little is known about *Mycoplasma* spp. infections in immature tortoises. Given the prior finding that immature gopher tortoises are less likely to be infected than adults (Wendlend et al. 2010), it is somewhat surprising that our model estimated infection probability to be higher for younger animals. However, this deviation could be explained by a couple of factors. Because this population is comprised of waif animals that were previously housed in captive facilities, normal infection patterns could have been altered by the increased contact and shared housing and provisioning for individuals across stage classes while in captivity. Additionally, prior studies have used the presence of antibodies to these pathogens to determine infection status of individuals, when antibodies actually detect previous exposure to the pathogen but not necessarily active infection (McCoy et al. 2007, McGuire et al. 2014, Wendlend et al. 2010). Testing for the presence of antibodies in our study population may have revealed more individuals testing "positive" than those we detected through qPCR techniques to be actively shedding pathogens. As pathogen screening using qPCR analysis of swabs becomes more common, we will be able to better compare our results to other gopher tortoise populations.

In addition to the two known tortoise pathogens, we also documented the presence of Emydid *Mycoplasma* sp. in a single individual (an unmarked, naturally recruited hatchling in 2018). This pathogen has been documented in many North American chelonians and usually does not cause disease (Ossiboff et al. 2015). The pathogen copy numbers in this individual were

very low (0.45 copies/ng DNA) and the tortoise showed no signs of clinical disease. Given the low copy numbers, it is possible the individual was not actually truly infected by the pathogen and we detected it due to contamination of the sample or non-infected passage of a *Mycoplasma* from a shared environment with an Emydid host. However, no other known tortoise pathogens, including *Ranavirus* spp, were detected.

Because stress has often been cited as a concern with translocation efforts (Dickens et al. 2010, Teixeira et al. 2007), H:L ratios are important to consider regardless of their correlation with infection status. The H:L ratios documented in AGTHP animals were within the ranges of those reported for wild gopher tortoises throughout their range (Table 3.7). Although there are few values for comparison, particularly from wild, *in situ* populations, comparison with reported values suggests that on average, translocated waif tortoises do not exhibit higher H:L ratios than their wild counterparts. Prior research has indicated that translocation does not induce physiological stress (as measured by plasma corticosterone levels) for the closely related, congeneric desert tortoise (Drake et al. 2012). Additionally, it has been found that gopher tortoises are resistant to the short-term stress associated with capture and handling (Kahn et al. 2007). However, because reptile hematology is often difficult to interpret (Rosenberg et al. 2018) and is affected by factors such as season and temperature (Goessling et al. 2017), additional study regarding the factors influencing variation in H:L ratios is warranted.

Our site had a low hemogregarine prevalence (0% in 2017 and 2.6% in 2018). Both infected individuals (n=2) had the lowest possible categorization of infection (<1 infected cell/100 red blood cells). This low prevalence is likely linked to no observations of the gopher tortoise tick, a vector for hemogregarines (Sonderman 2014). The lack of vectors can most likely be attributed to the manual removal of ticks prior to release (K. Buhlmann, personal

communication). However, previous translocation projects that also removed ticks prior to release continued to observe high parasite prevalence (over 71.4% of individuals infected) in the years following release (Hernandez et al. 2010). Parasite prevalence varies substantially among tortoise populations, with some sites lacking evidence of infection entirely (Cooney et al. 2019). The impact of these parasite infections on tortoise health likely depends on the intensity of the infection and other environmental factors, such as habitat quality (Hernandez et al. 2010).

Overall, waif tortoises exhibited similar health profiles to their wild counterparts (as reported in the literature). As the species continues to decline, waif tortoises may be important for augmentation efforts of isolated populations. Although the population appears healthy, it is important to consider the interim between release and health assessment for many individuals. Due to this delay, infected animals could have died prior to assessment. We cannot determine causes of mortality for these individuals, however, long-term *survival* estimates from this population are comparable to wild *in situ* populations (Chapter 2). Proper risk management is important when considering releasing waif tortoises into the wild and we recommend waif tortoise reintroduction be considered only if populations are 1) far from the threshold of minimum population viability, and 2) completely isolated from other viable populations of tortoises.

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## Tables

Table 3.1. Criteria based on midline carapace length (MCL) used to determine life stage of gopher tortoises captured at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC during 2017-2018. Secondary sexual characteristics were used to determine sex of mature animals. Individuals smaller than 230 mm were considered to be adult females if they were gravid.

| Stage | Midline Carapace Length (MCL) | Additional Characteristics |
|---|---|---|
| **Hatchling** | < 68 mm | |
| **Juvenile** | ≥ 68 mm, < 130 mm | |
| **Subadult** | ≥130 mm, < 230 mm | Flat plastron |
| **Adult Male** | ≥180 mm | Concave plastron, gular protrusion |
| **Adult Female** | ≥230 mm | Flat plastron |

Table 3.2. Pathogens tested for in gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC based on oral and cloacal swabs submitted to the Wildlife Epidemiology Lab at the University of Illinois. Swabs were tested for a total of 14 pathogens, with 11 pathogens tested in both 2017 and 2018. For those pathogens which had been previously documented in gopher tortoises in other populations, the supporting citation is provided.

| **Pathogen** | **Detection Method** | **Documented in *G. polyphemus*** | **Literature Documenting Gopher Tortoise Infections** |
|---|---|---|---|
| *Mycoplasma agassizii* | qPCR | Yes | Brown et al. 1999 |
| *Mycoplasma testudineum* | qPCR | Yes | Brown et al. 2004 |
| Emydid *Mycoplasma* sp.* | qPCR | No | |
| Frog Virus 3- *Ranavirus* | qPCR | Yes | Johnson et al. 2006, Cozad 2018 |
| Ambystoma tigrinum virus – *Ranavirus* | qPCR | No | |
| Bohle iridovirus— *Ranavirus* | qPCR | No | |
| Epizootic hemorrhagic necrosis virus | qPCR | No | |
| *Salmonella tymphimurium* | qPCR | Yes*** | Lockart et al. 2007; Charles-Smith et al. 2009 |
| *Salmonella enteritidis* | qPCR | Yes*** | Lockart et al. 2007; Charles-Smith et al. 2009 |
| Testudinid herpesvirus 2 | qPCR | No | |
| Tortoise intranuclear coccidia (TINC) | qPCR | No | |
| *Borrelia burdorferi*** | qPCR | No | |
| *Anaplasma phagocytophilum*** | qPCR | Yes | Wellehen et al. 2017, Cozad 2018 |
| Adenovirus** | PCR | No | |

*Tested in 2018, but not in 2017. ** Tested in 2017, but not 2018. ***(*Salmonella* serotype not distinguished (Lockart et al. 2007; Charles-Smith et al. 2009).

Table 3.3. Mean gopher tortoise morphometric and hematological measurements by demographic stage class during the 2017-2018 sampling period at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC. If multiple records of the same individual occurred during the sampling period, they were averaged prior to their incorporation into the stage class summary statistics.

| | **Tortoise Stage Class** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Hatchling** | | **Juvenile** | | **Subadult** | | **Adult Male** | | **Adult Female** | |
| **Parameter** | *N* | *Mean±1 SD (range)* | *N* | *Mean±1 SD (range)* | *N* | *Mean±1 SD (range)* | *N* | *Mean±1 SD (range)* | *N* | *Mean±1 SD (range)* |
| ***Morphometrics*** | | | | | | | | | | |
| Midline Carapace Length (mm) | 3 | 51.3±8.0 (43-59) | 5 | 115.0±15.2 (92-128) | 17 | 184.0±26.4 (145-227) | 50 | 279.0±25.1 (225-340) | 68 | 286.0±28.1 (221-355) |
| Mass (g) | 3 | 34±13.7 (22-49) | 5 | 281±105 (135-383) | 17 | 1152±482 (566-1960) | 50 | 4112±1079 (1972-7237) | 68 | 4240±1232 (1910-7428) |
| Body Condition ($g/mm^3$) | 3 | 1.08±0.02 (1.07-1.09) | 5 | 1.04±0.07 (0.95-1.12) | 17 | 1.08±0.08 (0.92-1.21) | 50 | 1.05±0.10 (0.62-1.22) | 68 | 1.05±0.12 (0.63-1.55) |
| ***Hematology*** | | | | | | | | | | |
| Packed Cell Volume (PCV) | | ---- | 4 | 20.2±5.4 (16-28) | 15 | 25.7±7.2 (12-34) | 47 | 29.2±6.2 (14-38) | 66 | 27.8±5.6 (12-44) |
| Total Solids (TS) | | ---- | 2 | 2.2±0.2 (2.0-2.3) | 13 | 3.1±0.8 (2.0-4.3) | 47 | 3.8±0.7 (2.0-5.7) | 64 | 3.8±0.7 (2.0-5.5) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| White Blood Cell Estimate (K/µl) | ---- | 3 | 9.6±2.0 (7.3-11.1) | 15 | 10.6±4.0 (5.6-21.1) | 48 | 9.4±3.9 (3.49-19.8) | 66 | 9.6±3.3 (4.6-21.2) |
| Total Heterophil (K/µl) | ---- | 3 | 4.0±0.1 (3.9-4) | 15 | 4.1±2.2 (1.4-8.3) | 48 | 3.9±1.9 (0.71-8.9) | 66 | 4.1±1.9 (1.4-11.2) |
| Total Lymphocyte (K/µl) | ---- | 3 | 3..8±0.8 (2.9-4.3) | 15 | 3.7±1.2 (2.0-7.0) | 48 | 3.4±1.2 (0.9-5.7) | 66 | 2.3±1.1 (1.5-6.0) |
| H:L Ratio | ---- | 3 | 1.08±0.3 (0.93-1.38) | 15 | 1.24±0.7 (0.23-3.07) | 48 | 1.33±0.9 (0.23-4.63) | 66 | 1.37±0.8 (0.38-4.1) |

Table 3.4. Individual gopher tortoises from the Aiken Gopher Tortoises Heritage Preserve in Aiken County, SC, for which pathogens were detected in oral, cloacal, or naral swabs submitted to the Wildlife Epidemiology Laboratory at the University of Illinois. Using qPCR, pathogen load was quantified for individuals positive for *Mycoplasma* sp. infections in 2017 and 2018. Two individuals (421 and 436) had relatively low pathogen copy numbers in 2017 (9.09 and 1.36 copies/ng DNA) before testing negative in 2018. *denotes individuals with more than one sample.

| **ID** | **Swab Type** | **Year** | **DNA** (ng/ul) | **Pathogen** | **Pathogen Copy Numbers** | |
|---|---|---|---|---|---|---|
| | | | | | (copies/ uL rxn) | (copies/ng DNA) |
| 19 | Oral | 2017 | 2.25 | *M. agassizii* | 8,967.12 | 1,594.16 |
| 416 | Oral | 2017 | 3.26 | *M. agassizii* | 25,507.59 | 3,129.77 |
| 421 | Oral | 2017 | 5.25 | *M. agassizii* | 119.25 | 9.09 |
| 436* | Naral | 2017 | 2.62 | *M. agassizii* | 8.88 | 1.36 |
| 436* | Oral | 2017 | 4.73 | *M. agassizii* | 178.47 | 15.09 |
| 542 | Oral | 2017 | 5.37 | *M. agassizii* | 88.68 | 6.61 |
| 672 | Oral | 2017 | 1.65 | *M. agassizii* | 14,128.87 | 3,425.18 |
| 16 | Oral | 2018 | 5.92 | *M. testudineum* | 70.57 | 4.77 |
| 19 | Oral | 2018 | 4.45 | *M. agassizii* | 31,262.45 | 2,810.11 |
| 416 | Oral | 2018 | 36.05 | *M. agassizii* | 602,801.5 | 6,688.5 |
| 469 | Oral | 2018 | 11.94 | *M. agassizii* | 23.59 | 0.79 |
| 546* | Oral | 2018 | 38.58 | *M. agassizii* | 14.88 | 0.15 |
| 546* | Naral | 2018 | 5.23 | *M. agassizii* | 75.06 | 5.74 |
| 591 | Oral | 2018 | 5.26 | *M. agassizii* | 10.64 | 0.81 |
| 595 | Oral | 2018 | 10.53 | Emydid *M.* sp. | 11.73 | 0.45 |
| 603 | Oral | 2018 | 29.68 | *M. agassizii* | 33,878.50 | 456.58 |
| 626 | Oral | 2018 | 3.32 | *M. agassizii* | 39.14 | 4.72 |
| 639 | Oral | 2018 | 4.17 | *M. agassizii* | 4,017.15 | 385.34 |
| 645 | Oral | 2018 | 10.37 | *M. agassizii* | 545.37 | 21.04 |
| 646 | Oral | 2018 | 5.43 | *M. agassizii* | 34.33 | 2.53 |
| 666* | Naral | 2018 | 3.97 | *M. agassizii* | 518.24 | 52.22 |
| 666* | Oral | 2018 | 17.89 | *M. agassizii* | 52,879.92 | 1,182.33 |
| 667* | Cloacal | 2018 | 3.67 | *M. agassizii* | 2,541.16 | 276.97 |
| 667* | Naral | 2018 | 3.67 | *M. agassizii* | 40,436.56 | 4,407.25 |
| 667* | Oral | 2018 | 7.31 | *M. agassizii* | 63,708.83 | 3,486.12 |
| 672 | Oral | 2018 | 21.04 | *M. agassizii* | 101,294.6 | 1,925.75 |
| 678 | Oral | 2018 | 11.49 | *M. agassizii* | 2,262.58 | 78.77 |
| 689 | Oral | 2018 | 10.35 | *M. agassizii* | 9.06 | 0.35 |
| 691* | Cloacal | 2018 | 5.1 | *M. agassizii* | 70.95 | 5.56 |
| 691* | Oral | 2018 | 2.1 | *M. agassizii* | 3,370.64 | 642.03 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 694* | Cloacal | 2018 | 10.73 | *M. agassizii* | 103.75 | 3.87 |
| 694* | Naral | 2018 | 5.71 | *M. agassizii* | 321.03 | 22.49 |
| 694* | Oral | 2018 | 6.92 | *M. agassizii* | 2,347.44 | 135.69 |

Table 3.5. *Mycoplasma* results for individual gopher tortoises from Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC sampled in both 2017 and 2018, based on oral swabs tested using qPCR. Of the 42 waif gopher tortoises screened for *Mycoplasma* in both years, 6 changed infection status between years for either *Mycoplasma agassizii or Mycoplasma testudineum*. Of the 4 individuals that converted from negative in 2017 to positive to 2018, 3 were positive for *Mycoplasma agassizii* and 1 was positive for *Mycoplasma testudineum.*

| **Resampled animals** | Positive 2018 | Negative 2018 |
|---|---|---|
| Positive 2017 | 3 | 2 |
| Negative 2017 | 4 | 33 |

Table 3.6. Candidate models used to identify predictors of *Mycoplasma* infection in gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve in Aiken County, SC. Categorical variables included naral clinical signs (N), ocular clinical signs (O), and sex and/or stage of the tortoise (Sex). Continuous variables included body condition (BC), packed cell volume (PCV), and H:L ratios (HL). Categorical variables included in each model are denoted by a "●". Continuous variables included in models are represented by their estimated slope on the logit scale. Models also considered several interaction terms, denoted by "*" in the variable name.

| Model | Intercept | Df | AICc | delta | weight | N | O | Sex | BC | PCV | HL | Sex * PCV | Sex * BC | Sex * HL | N * HL | N* PCV | N* BC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | -2.27 | 2 | 92.61 | 0.00 | 0.21 | ● | | | | | | | | | | | |
| N*HL | -2.27 | 4 | 93.19 | 0.58 | 0.15 | ● | | | | | -0.10 | | | | ● | | |
| N+BC | -2.29 | 3 | 93.92 | 1.31 | 0.11 | ● | | | -0.30 | | | | | | | | |
| N+Sex | -2.71 | 4 | 94.13 | 1.52 | 0.10 | ● | | ● | | | | | | | | | |
| N + O | -2.30 | 3 | 94.38 | 1.76 | 0.09 | ● | ● | | | | | | | | | | |
| N+O+Sex | -2.75 | 5 | 95.37 | 2.76 | 0.05 | ● | | ● | -0.34 | | | | | | | | |
| N+O+BC | -2.32 | 4 | 95.59 | 2.98 | 0.05 | ● | ● | | -0.33 | | | | | | | | |
| N*PCV | -2.30 | 4 | 95.71 | 3.10 | 0.04 | ● | | | | -0.24 | | | | | | ● | |
| N+O+BC | -2.72 | 5 | 96.02 | 3.40 | 0.04 | ● | ● | ● | | | | | | | | | |
| N+BC+HL | -2.29 | 4 | 96.04 | 3.43 | 0.04 | ● | | | -0.30 | | 0.04 | | | | | | |
| N*BC | -2.29 | 4 | 96.06 | 3.44 | 0.04 | ● | | | -0.30 | | | | | | | | ● |
| N+O+Sex+BC | -2.77 | 6 | 97.12 | 4.51 | 0.02 | ● | ● | ● | -0.38 | | | | | | | | |
| N+BC+HL+Sex | -2.77 | 6 | 97.46 | 4.85 | 0.02 | ● | | ● | -0.33 | | 0.11 | | | | | | |
| BC | -1.96 | 2 | 97.72 | 5.11 | 0.02 | | | | -0.42 | | | | | | | | |
| O | -2.00 | 2 | 98.18 | 5.56 | 0.01 | | ● | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | -2.29 | 3 | 99.22 | 6.61 | 0.01 | ● | | | | | | |
| PCV | -1.91 | 2 | 99.31 | 6.69 | 0.01 | | | -0.11 | | | | |
| HL | -1.91 | 2 | 99.46 | 6.84 | 0.01 | | | | -0.03 | | | |
| PCV+HL | -1.91 | 3 | 101.41 | 8.79 | 0.00 | | | -0.11 | 0.00 | | | |
| Sex*HL | -2.28 | 6 | 103.37 | 10.75 | 0.00 | ● | | | -0.18 | | | ● |
| Sex*BC | -2.34 | 6 | 103.62 | 11.00 | 0.00 | ● | -0.29 | | | | ● | |
| Sex*PCV | -2.30 | 6 | 105.40 | 12.79 | 0.00 | ● | | -0.23 | | ● | | |

Table 3.7. Average H:L ratios for waif gopher tortoises at Aiken Gopher Tortoise Heritage Preserve (AGTHP), SC compared to values reported for gopher tortoises from previous studies.

| **Population** | **Ratio** (Mean±SD) | **Population Characteristics** | **Citation** |
|---|---|---|---|
| AGTHP (SC) | 1.34±0.81 | Translocated waifs | This study |
| Covington (AL) | 1.83 (winter)<br>0.34 (summer) | Wild tortoises in experimental temperature trials[1] | Goessling et al. 2017a[2] |
| Covington (AL) | 2.1 (control)<br>8.2 (exposed) | Wild tortoises in experimental thermoregulation and immunity trials | Goessling et al. 2017b[2] |
| Hillsdale (MS) | 1.15±0.87 | *In situ* wild | Holbrook 2015 |
| Nokuse (FL) | 1.98±0.96 | Translocated wild | Cozad, unpublished data |
| T44 (MS) | 2.08±1.31 | *In situ* wild | Holbrook 2015 |

[1] The reported values were collected prior to temperature manipulations.
[2] Wild tortoises were captured and housed in captive enclosures for both studies. SD was not reported.

**Figures**

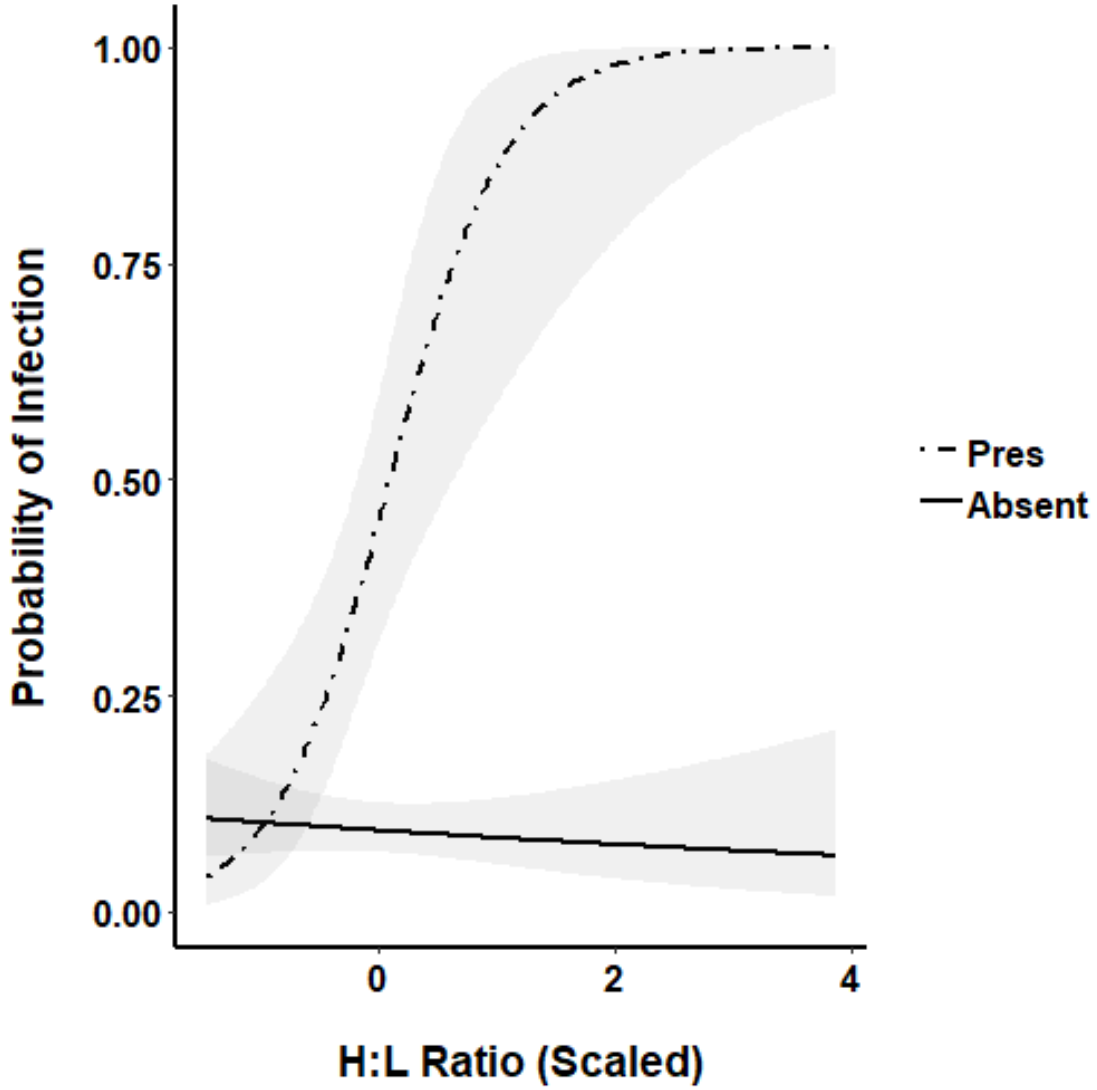


Figure 3.1. Predicted relationship between scaled heterophil:leukocyte (H:L ratios) and probability of infection with *Mycoplasma* in waif gopher tortoises from the Aiken Gopher Tortoise Heritage Preserve in Aiken, SC. Among 22 candidate generalized linear models, the second best model included an interaction between the presence of naral clinical signs and stress levels of tortoises as measured by H:L ratios, which were scaled by subtracting the mean and dividing by the standard deviation. Tortoises with naral clinical signs (Pres) and elevated H:L ratios had a high probability of infection. Grey bars indicate SE for the model predictions.

# CHAPTER 4

# CONCLUSION

Increasingly, wildlife face a number of threats, including habitat loss, population fragmentation, illegal collection, and disease introduction (Liu et al. 2013; Gibbons et al. 2001; Smith et al. 2009). Individually, but especially collectively, these threats pose tremendous challenges to the management and conservation of imperiled species. Many management actions designed to alleviate one threat, can inadvertently increase the risk associated with another. Translocation—the intentional movement of individuals from one location to another—provides a prime example of a management action that has the potential to serve as a tool in wildlife conservation (Griffith et al. 1989; Seddon et al. 2014), but also has the potential to exacerbate other threats, such as those associated with emergent disease (Deem et al. 2001; Kock et al. 2010).

As a commonly translocated species (Tuberville et al. 2008) and a species in decline (Smith et al. 2006), the gopher tortoise (*Gopherus polyphemus*) serves as an important case study in modern wildlife management. The gopher tortoise is federally listed as threatened under the Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana, and is a candidate species for federal listing in the remainder of its range (USFWS 1987, 2011). Habitat degradation, conversion, and fragmentation are the species' primary threats (Smith et al. 2006); however, disease (Jacobson 1994; Seigel et al. 2003) and illegal collection as pets (Auffenberg and Franz 1982) also pose challenges for the conservation of the species. Because of their docile nature, many tortoises worldwide frequently are kept as pets (Edwards and Berry 2013). In the case of the gopher tortoise, once an animal has been held in captivity, it is designated a waif

tortoise and, in many cases, it can no longer be released back into the wild (Florida Fish and Wildlife Conservation Commission 2012). The hesitancy to release waif tortoises is partially motivated by a concern that these individuals could pose a higher disease risk, as a number of pathogens have been documented in captive reptile collections (Johnson et al. 2006; Sim et al. 2016).

As such, waif gopher tortoises pose a unique and ongoing management dilemma. The population recovery efforts at the Aiken Gopher Tortoise Heritage Preserve (AGTHP) in Aiken County, South Carolina, represent the first large-scale effort to translocate waif tortoises as a means to augment a relict wild tortoise population facing extirpation. Over 260 waif tortoises from a variety of origins were released at AGTHP between 2006-2017. This thesis assessed the outcomes of the AGTHP recovery efforts and used the program as a case study to evaluate two of the main concerns associated with releasing waif tortoises—waif tortoise survival and health following release back into the wild.

In Chapter 2, I assessed the long-term apparent survival of translocated waif tortoises. I surveyed the AGTHP for burrows and trapped in 2017 and 2018. I combined release records, intermittent incidental capture records, and data from the 2 years of formal capture efforts to generate capture histories for each tortoise. With these histories, I used a multistate, live-dead mark-recapture model (Barker et al. 2005; Brownie et al. 1993; Burnham 1993; Schwarz et al. 1993) to estimate annual apparent survival probabilities for juvenile, subadult, adult male, and adult female tortoises. I considered models that incorporated an additive effect of tortoise origin and an interactive effect between origin-stage on annual apparent survival probability. There was not support for the inclusion of the effect of origin or the origin-stage interaction. However, apparent annual survival rates varied by tortoise stage class ($P<0.001$). I

estimated the annual apparent survival rates to be 0.96±0.04 for females, 0.92±0.07 for males, 0.96±0.05 for subadults, and 0.25±0.18 for juveniles.

Translocated adult waif gopher tortoises at AGTHP exhibited annual apparent survival rates similar to those reported for wild *in situ* populations (Ozgul et al. 2009, Tuberville et al. 2014) and for wild translocated populations (Ashton and Burke 2007; Tuberville et al. 2008). This finding suggests that waif tortoises have the potential to survive at rates similar to their wild counterparts. Additionally, the waif tortoises released at AGTHP were from a variety of origins. Because AGTHP is the northern-most population, I hypothesized that waif tortoises from the southern portions of the range might survive at lower rates. However, I did not observe significant differences between tortoise annual apparent survival rates based on origin.

As expected, tortoise apparent annual survival rates varied between juveniles and older classes. Although there have been studies to assess the survival of hatchling (Epperson and Heise 2003, Perez-Heydrich et al. 2012, Pike and Seigel 2006; Smith et al. 2013) and adult tortoises (Ozgul et al. 2009; Tuberville et al. 2014), few studies have reported survival rates for intermediate stage classes. Because estimates of all stages are required for accurate modeling of population dynamics and trajectories (Tuberville et al. 2009; Smith et al. 2006), the rates reported in this study can provide needed information on the life histories of these intermediate stage classes.

In Chapter 3, I evaluated the health of the surviving individuals through visual health assessments, pathogen screening, chronic stress evaluations, and quantification of blood parasites. Overall, the individuals we assessed appeared healthy. I did not detect many of the potential pathogens of concern such as *Ranavirus* spp., Testudinid herpesvirus 2, or tortoise intranuclear coccidia (TINC). I did document the presence of two known tortoise pathogens,

*Mycoplasma agassizii* and *Mycoplasma testudineum*. I documented *M. agassizii* in both years with a prevalence of 10.2% in 2017 and a prevalence of 13.9% in 2018. I documented *M. testudineum* only in 2018, with a prevalence of 0.8%. Surveys across 53 sites in Florida determined 30% of tortoises to be seropositive for *Mycoplasma agassizii* (Berish et al. 2000), although exposure varies substantially between populations with some populations having either very low seroprevalence (0-3%) or very high seroprevalence (96%-100%; McGuire et al. 2014). Using logistic regression, I assessed potential visual, physiological and hematological predictors of *Mycoplasma* infection. Although there was substantial model uncertainty, I found the presence of naral clinical signs to be an important predictor of positive infection status. Naral clinical signs was not a perfect predictor of infection, however, and only 35% of positive animals displayed naral clinical signs. AGTHP animals had an average heterophil:lymphocyte (H:L) ratio of 1.34±0.81, which was in the range reported in the literature for gopher tortoises. By this measure, it does not appear translocated waif gopher tortoises are more chronically stressed than their wild counterparts (Holbrook 2015; Goessling et al. 2017).

Overall, my findings provide important information to agencies charged with managing waif tortoises. The survival and health exhibited by the AGTHP population suggest that waif tortoises can be successfully used to augment or reestablish gopher tortoise populations. However, caution is still warranted. Disease screening and visual health assessments can reduce the risk associated with the release of waif tortoises. Additionally, augmentation with waif tortoises is most appropriate for populations that are 1) geographically isolated from neighboring populations 2) are unlikely to achieve viability through lower risk alternatives—e.g. habitat management or nest protection.

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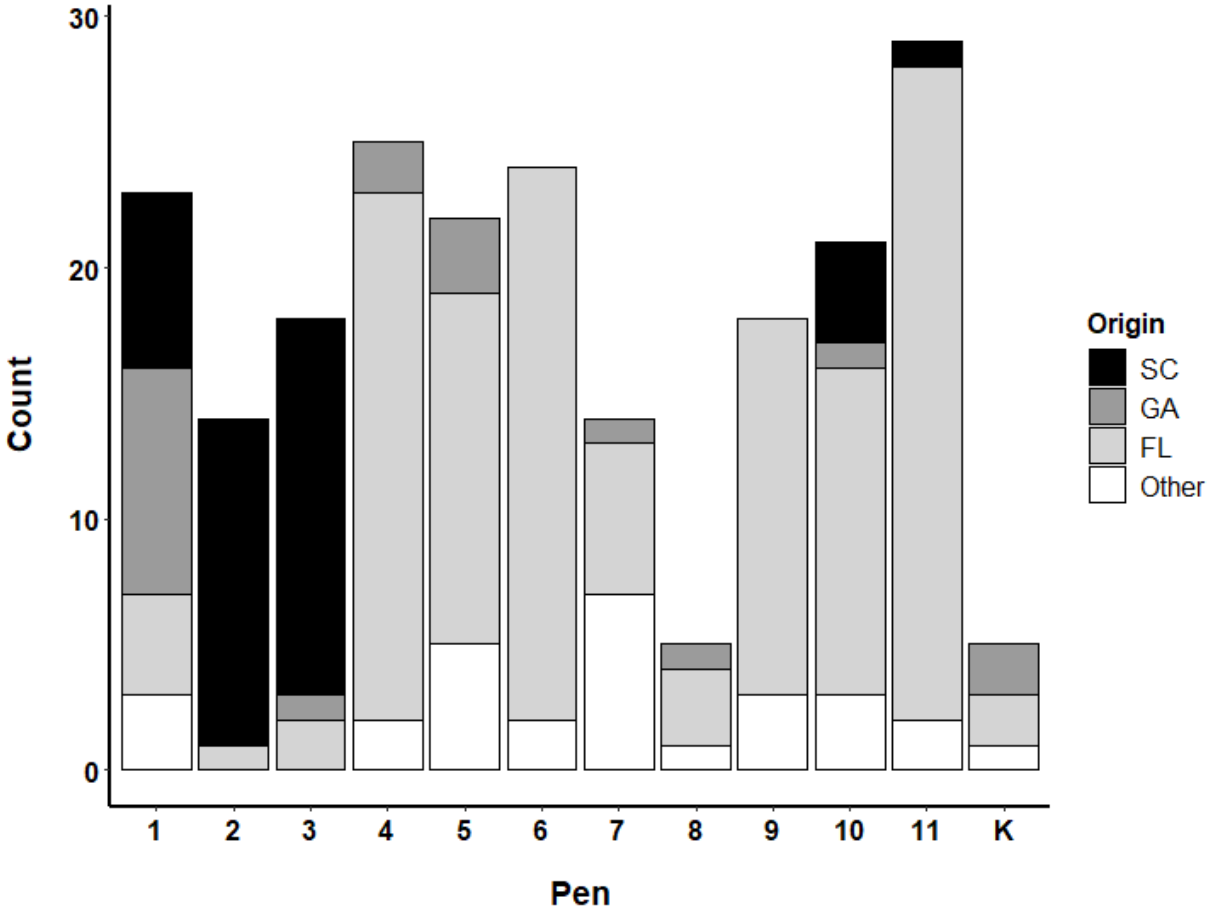
APPENDICES

**A. Breakdown of waif gopher tortoises released into pens at the Aiken Gopher Tortoise Heritage Preserve (Aiken County, SC) by tortoise origin.**



**B. Number of gopher tortoises released at the Aiken Gopher Tortoises Heritage Preserve (Aiken County, SC) by pen and stage class. Tortoises were released into 1-ha circular pens to acclimate for >1yr prior to removal of pen walls. Because tortoises were received intermittently, the number of tortoises placed in each pen varied between pens. Pen K was the first pen constructed at AGTHP. Unlike other pens, "Pen K" was rectangular and 40m x 40m. Normally, it was used as a temporary holding facility while other pens were constructed, however, occasionally these individuals could not be recaptured or they died prior to assignment in a larger pen.**

| Pen | Hatchlings (N) | Juveniles (N) | Subadults (N) | Males (N) | Females (N) | Total | Date first animal released | Date last animal released | Pen walls removed |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 7 | 3 | 1 | 9 | 10 | **30** | Oct-2006 | Jul-2008 | Jul-2009 |
| **2** | 10 | 5 | 3 | 3 | 3 | **24** | Jun-2007 | Oct-2010 | May-2013 |
| **3** | 32 | 11 | 1 | 4 | 2 | **50** | Jul-2007 | Nov-2008 | May-2013 |
| **4** | 11 | 2 | 2 | 8 | 13 | **36** | Aug-2010 | Sep-2012 | Aug-2013 |
| **5** | 0 | 1 | 3 | 7 | 11 | **22** | Oct-2008 | Aug-2012 | Aug-2013 |
| **6** | 0 | 0 | 4 | 11 | 9 | **24** | Aug-2012 | Oct-2012 | Aug-2013 |
| **7** | 1 | 2 | 2 | 4 | 6 | **15** | Aug-2013 | Oct-2013 | Apr-2016 |
| **8** | 0 | 0 | 2 | 0 | 3 | **5** | Apr-2015 | Apr-2015 | Jul-2018 |
| **9** | 3 | 4 | 4 | 5 | 5 | **21** | Oct-2013 | Aug-2015 | Aug-2016 |
| **10** | 0 | 2 | 7 | 6 | 6 | **21** | Aug-2016 | Oct-2016 | |
| **11** | 0 | 3 | 5 | 9 | 12 | **29** | Aug-2016 | Oct-2016 | Jul-2018 |
| **K*** | 0 | 0 | 0 | 3 | 2 | **5** | | | |
| **Total** | **64** | **33** | **34** | **69** | **82** | **282** | | | |

## C. Health Assessment Datasheet used to Evaluate Health of tortoises at the Aiken Gopher Tortoise Heritage Preserve

### Aiken Gopher Tortoise Preserve Datasheet

Tortoise ID:_____ Date _____ Time:_____ ☐New ☐Recap. ☐Dead

Observer(s):_____ Burrow #_____ ☐New Tag

GPS E: _____/N:_____ (if not in burrow)

Capture Method: ☐ Hand ☐ Bucket Trap ☐ Wire Trap ☐Other: _____

Behavior (hand captured):☐ Resting ☐Forage ☐Move ☐Mate ☐Combat ☐Unk. W/ Tort:_____

Notes:

**Tortoise Demographic & Morphometric Data** **NA** ☐

Processor: _____ Life stage: ☐ Adult F. ☐ Adult M. ☐Subadult ☐ Juv. ☐Hatch.

Mass (G):_____Photos: ☐Carapace ☐ Plastron ☐Face ☐Code ☐ Other: _____

MCL(MM): _____ Width(MM): _____ Height(MM): _____ TL(MM)_____

Annuli _____ Age: _____ ☐Estimated ☐Calculated ☐ Not Determined

**Health Assessment** **NA** ☐

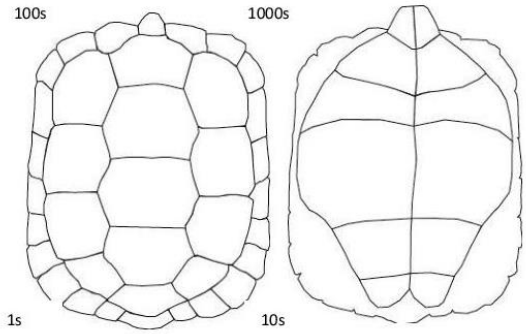
| | Norm. | Abnorm. | Not Assessed | | Norm. | Abnorm. | Not Assessed |
|---|---|---|---|---|---|---|---|
| Eyes | ☐ | ☐ | ☐ | Carapace | ☐ | ☐ | ☐ |
| Nares | ☐ | ☐ | ☐ | Skin | ☐ | ☐ | ☐ |
| Tympanum | ☐ | ☐ | ☐ | Plastron | ☐ | ☐ | ☐ |
| Respiration | ☐ | ☐ | ☐ | Cloaca | ☐ | ☐ | ☐ |
| Musculature | ☐ | ☐ | ☐ | Oral Cavity | ☐ | ☐ | ☐ |

Gravid:☐Yes ☐ No Method:☐ Palp ☐Ultra ☐X-ray

Ectoparasites: ☐Yes ☐No ☐Collected

Describe Abnormalities:

Draw Notches/Injuries




**Tissue Collection and Processing (Date _____)** ☐ **NA**

**Swabs:** ☐Oral ☐Cloacal ☐ Combined **Blood:** ☐ Yes ☐ No ☐Attempt Only **Heparin:**☐Yes ☐No

| **Time** | **Sample Site** | **Vol.** (ML) | **Notes** |
|---|---|---|---|
| | ☐ Brach. ☐Jug ☐Subcarap. ☐ Other | | |
| | ☐ Brach. ☐Jug ☐Subcarap. ☐Other | | |
| | ☐ Brach. ☐Jug ☐Subcarap. ☐Other | | |

**Total Blood:** _____ML **Lysis:** ☐Yes ☐No Aliquots: _____ **Num of Smears**: _____

**PCV:**_____% **TS**_____ **Plasma:** _____ µL **RBC Retained:**☐ Yes ☐ No

Notes:

**D. Detailed pathogen screening methods provided by the University of Illinois Wildlife Epidemiology Laboratory.**

WILDLIFE
EPIDEMIOLOGY LABORATORY
vetmed.illinois.edu/wel

# PATHOGEN DETECTION IN GOPHER TORTOISES IN SOUTH CAROLINA

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## MATERIALS AND METHODS

**Quantitative PCR.**– The DNA in oral and cloacal swab samples was extracted with a QIamp Blood mini Kit (QIAGEN Inc., Redwood City, CA 94063 USA), following the manufacturer protocol. Quantity (ng/µl) and quality (A260:A280 ratio) of DNA was evaluated using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, 02451 USA). Quantitative PCR was performed in a multiplex format to evaluate twelve pathogens simultaneously using published or in house primer-probe assays (Table 1). Initially, Specific Target Amplification was performed on each sample with pooled pathogen Taqman assays and preamp mastermix (Thermo-Fisher, Waltham, MA 02454 USA). Each reaction was performed under the following cycling program on an MJ Tetrad thermocycler: 95°C (10 min), 14 cycles of 95°C (15 sec) and 60°C (4 min). The qPCR assay was then performed in triplicate using 2.25 µl of amplified DNA from the first reaction on a Fluidigm 96.96 Gene Expression IFC and amplified on the Fluidigm Biomark HD Real Time PCR thermacycler (Fluidigm, South San Francisco, CA 94080 USA) using the following cycling protocol: 70°C (30 min), 25°C (10 min), 95°C (1 min), followed by 35 cycles at 96°C (5 sec) and 60°C (20

sec). Serial dilutions of positive controls for FV3-like ranavirus, *Mycoplasma agassizii*, and *Mycoplasma testudineum* were prepared from $10^7$ to $10^1$ copies per reaction. A non-template control was included on each plate. All reactions were then analyzed using Fluidigm Real Time PCR analysis software (Fluidigm, South San Francisco, CA 94080 USA). Following Fluidigm analysis, all positive samples were verified in a simplex reaction. Briefly, qPCR was performed in triplicate on a QuantStudio3 real time thermal cycler. Samples were considered positive if all three replicates had a lower cycle threshold ($C_t$) value than the lowest detected standard dilution.

**Conventional PCR—** with a previously-characterized two-step consensus assay was utilized for adenovirus detection (Wellehan et al. 2004). Products were electrophoresed on a 1% agarose gel and compared to positive and negative controls and a 100bp DNA ladder. PCR products producing appropriately-sized bands (approximately 320 base pairs) were cleaned (Exo-sap it), sequenced in both directions (W.M. Keck Center for Comparative and Functional Genomics, University of Illinois at Urbana-Champaign, Urbana, IL), and compared to known sequences in GenBank using BLASTN to confirm accurate detection