A MULTIFACETED APPROACH TO EVALUATING GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*) POPULATION HEALTH AT SELECTED SITES IN GEORGIA

by

JESSICA LYNN GONYNOR

(Under the Direction of Michael J. Yabsley)

ABSTRACT

Gopher tortoise (*Gopherus polyphemus*) populations are declining across the southeastern United States with an estimated decrease of >80% over the past 100 years. Declines have been attributed to habitat loss, overharvest, and disease. There is debate about the impact of the disease in free-ranging gopher tortoise populations. Cycles of convalescence and recrudescence of upper respiratory tract disease (URTD) have been confirmed in captive gopher tortoises, but to our knowledge, this has not been evaluated in free ranging populations despite URTD related die-offs in Florida. To date, despite extensive surveillance in tortoise populations for *Mycoplasma*, the causative agent of URTD, few evaluations of the survival of clinically ill tortoises have been conducted. Likewise, very little is known about parasites in Georgia tortoise populations. Through surveillance efforts of gopher tortoise populations we found that the prevalence of *Mycoplasma agassizii* and *M. testudineum* varied spatially, as did the diversity of endoparasites. We saw an all or none trend with exposure to *M. agassizii*. *M. testudineum* seroprevalence varied greatly among populations, ranging from 38% to 61%, with only one negative site. Clinical signs consistent with URTD were seen at sites seropositive for both pathogens. One site was selected to evaluate long term (15 year) impacts of URTD, including the

recrudescence of clinical disease and its effects on tortoise behavior. Despite long-term exposure to the pathogen, tortoises in this population demonstrated site fidelity, stable home range size, and tortoise densities that increased through time. However we observed mortality and tortoises with severe clinical signs used significantly larger home ranges than asymptomatic tortoises within this population. These tortoises also made extremely long distance movements. Therefore, we feel that to examine the health of free ranging gopher tortoise populations, it is crucial to monitor the behavior of individuals with and without the disease, and consider other pathogens and parasites. We detected at least eight species of intestinal parasites in Georgia populations of gopher tortoises, including *Cryptosporidium*, a possible pathogen of tortoises. The primary goal of this dissertation was to assess gopher tortoise population health and behavior to provide information to aid in management of the species.

INDEX WORDS: *Mycoplasma agassizii*, *Mycoplasma testudineum*, *Gopherus polyphemus*, gopher tortoise, home range, surveillance, thermoregulation, upper respiratory tract disease

# A MULTIFACETED APPROACH TO EVALUATING GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*) POPULATION HEALTH AT SELECTED SITES IN GEORGIA

by

JESSICA LYNN GONYNOR

BS, Northeastern University, 2001

MS, Northeastern University, 2006

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

DOCTOR OF PHILOSPHY

ATHENS, GEORGIA

2013

A MULTIFACETED APPROACH TO EVALUATING GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*) POPULATION HEALTH AT SELECTED SITES IN GEORGIA

by

JESSICA LYNN GONYNOR

| | |
|---|---|
| Major Professor: | Michael J. Yabsley |
| Committee: | Lora L. Smith<br>Jeff Hepinstall- Cymerman<br>Sonia M. Hernandez<br>John C. Maerz<br>Tracey D. Tuberville |

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

ACKNOWLEDGEMENTS

I have been fortunate to pursue my dreams and have an army to thank for it! I would like to thank my parents, Gary and Linda. My husband Will, who sacrificed to help me get through this and has helped immensely in the field and lab. Thanks to Bryce for the entertainment on class field trips and help in the field. My advisors Michael Yabsley and Lora Smith: You both took a chance and gave me an amazing opportunity.

I would like to thank my committee members for their guidance and collaboration throughout my time at UGA: John Maerz, Sonia Hernandez, Tracey Tuberville, and Jeff Hepinstall-Cymerman. Outside of UGA, Terry Norton at the Georgia Sea Turtle Center, John Jensen at Georgia Department of Natural Resources, Suzanne Passmore at Reed Bingham State Park, Joe Butler, Derry Cannon, Joan Berish at Florida Fish and Wildlife Conservation Commission, Craig Guyer and David Steen at Auburn University, and Mitch Lockhart at Valdosta State University.

I will be forever grateful for the generosity, kindness, and support extended to my family by the Jones Ecological Research Center. I have been fortunate to have been a graduate student at such a wonderful place. I could not have completed this dissertation without the invaluable people there (Lindsay Boring, Jen Howze, Gail Morris, Mike Conner, Jean Brock, Micheal Simmons, Liz Cox, Jessica Hall, Stephanie Allums, Emily Blizzard (at SCWDS too), and my fellow graduate students at JERC).

The Southeastern Cooperative Wildlife Disease Study (SCWDS) has been an amazing resource and a home base. SCWDS kept the project rolling year to year. The support and

camaraderie provided great motivation (John Fischer, Mark Ruder, Justin Brown, Brandon Munk, Page Lutrell, and fellow graduate students). The Warnell School of Forestry and Natural Resources is truly a family that as a group nurtures and invests in graduate research (Steven Castleberry, Bob Warren, Rosemary Wood, Sarah Covert, Maerz Lab, Warnell Graduate Student Association, UGA Herp Society). In addition to support mention previously, work was supported by grants from the Morris Animal Foundation, Gopher Tortoise Council's J.Larry Landers Research Award, Sigma Xi, the Roger Williams Park Zoo's Sophie Danforth Conservation Fund, Orianne Society, Southeastern Cooperative Wildlife Disease Study, and The Wildlife Society: Georgia Chapter.

Finally, I would like to thank my friends, mentors, and graduate students from Northeastern University and Boston University. You have supported me throughout this process and you gave me the confidence to launch into this endeavor: Elizabeth Godrick, Tom Kunz, Missy McElligott, Marianne Moore, Lauren Tereshko, Jeramy Webb, Gail Patt, Miranda Greenhalgh, Emily Freeman, Sherri Goulart, and Ivy Carnabucci. **Boston Strong.**

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

# CHAPTER 1

# INTRODUCTION

"The role of disease in wildlife conservation has probably been radically underestimated."

Aldo Leopold 1933

Disease is not a widespread consideration in wildlife population studies even though it is a crucial aspect of survival and fitness of individuals. It is imperative that baseline information, such as population demographics, is known about the populations before the impact of disease can be measured (Wobeser, 2007). Often, disease in a population is not studied until it is apparent that a population is at risk of extirpation from disease. However, knowledge of disease in a population can help with management decisions involving issues such as harvest rates, and relocation efforts.

Whether or not a disease will impact a population depends on a number of factors, many of which are usually not well understood. It is generally not possible to monitor entire populations of free ranging wildlife over long periods of time. Disease is a normal part of the structure of populations and needs to be considered in population monitoring efforts. Additionally, diseases have been implicated in the extinction or extirpation of populations (Tompkins and Wilson, 1998).

Gopher tortoise (*Gopherus polyphemus*) populations are declining across the southeastern United States and it has been estimated some populations have decreased >80%

over the last 100 years (Auffenberg and Franz 1982). Throughout their range, the primary threats to gopher tortoises are habitat loss, fragmentation, disease, and poor land management (e.g., lack of fire). Considerable research has been conducted on the ecology of gopher tortoises in Florida where upper respiratory tract disease (URTD) is a health concern for the species (Diemer Berish et al., 2000; Wendland, 2007; Berish et al., 2010). In contrast, little is known about the health status of Georgia populations and distribution of URTD despite the fact that the gopher tortoise is state-listed as threatened. Knowledge of disease threats to tortoise populations throughout their range is critical given that the gopher tortoise (including those in Georgia) was recently classified as a candidate for federal listing as a threatened species (USFWS, 2011). This dissertation research was conducted to provide data on the health status of gopher tortoise populations in Georgia to be used in future management and conservation decisions for this species.

*Mycoplasma* related URTD in gopher tortoises, caused by *M. agassizii* or *M. testudineum*, is highly contagious and has been implicated in the reduction of populations in Florida (Brown et al., 1994; McLaughlin, 1997; Brown et al., 1999; Diemer-Berish et al., 2000; Berish et al., 2010). In addition, large scale mortality events, greater than 100 individuals, have been observed in *Mycoplasma* seropositive populations in Florida (Rabatsky and Blihovde, 2002; Gates et al., 2002). However, there is considerable debate about the ecology and impact of the disease in free-ranging tortoise populations (Seigel et al., 2003; McCoy et al., 2007; Sandmeier, 2009). Both *M. agassizii* and *M. testudineum* have been isolated from clinically ill gopher tortoises and have been confirmed experimentally to cause URTD (Brown et al., 1994; Brown et al., 1999). Currently, little is known about the prevalence of *M. testudineum* in free-ranging tortoise populations (Wendland, 2007; duPre' et al., 2011; Jacobson and Berry, 2012) however,

Wendland (2007) found that prevalence was restricted to gopher tortoises in northeast Florida. Clinical signs associated URTD in gopher tortoises include nasal discharge, ocular discharge, nasal cavity obstruction, lethargy, emaciation, periocular and palpebral edema, and conjunctivitis (Jacobson et al., 1995; Shumacher et al., 1993; Shumacher et al., 1997; Seigel et al., 2003; Wendland, 2007). Cycles of convalescence and recrudescence (Brown et al., 1999; Feldman, 2006; Jacobson and Berry, 2012) of URTD have been confirmed in captive gopher tortoises and box turtles, but to our knowledge, this has not been evaluated in a free-ranging gopher tortoise population. It is possible that gopher tortoises are only affected by URTD for a short period of time, after which they exhibit no clinical signs, but due to stress or other factors, can recrudesce at a later point and develop signs and shed bacteria (McLaughlin, 1997). In addition to gopher tortoises, URTD has had a detrimental impact on populations of desert tortoises (*G. agassizii* and *G. morafkai*; Jacobson et al., 1991; Brown et al., 1994; Jacobson et al., 1995; Berry, 1997; Lederle et al., 1997; Brown et al., 1999; Dickinson et al., 2005), close relatives of the gopher tortoise which are found in the western U. S. (Dickinson et al. 2005).

Two common methods to confirm the presence of *Mycoplasma* related URTD in tortoise populations are serologic testing for antibodies indicating exposure and polymerase chain reaction (PCR) testing to detect active shedding of the bacteria (Jacobson et al., 1995). Although *M. agassizii* and *M. testudineum* have been shown to cause clinical disease in gopher and desert tortoises (Brown et al., 1999), however, clinical signs are not always observed in naturally-infected tortoises; therefore it is possible that some as yet unknown factors predispose a tortoise to the development of clinical disease following infection with *Mycoplasma*.

In Florida, tortoises positive for antibodies to *M. agassizii* have been documented throughout the state with a wide range in seroprevalence rates, even between populations in

neighboring counties (Beyer, 1993; Epperson, 1997; Smith et al., 1998; Diemer- Berish et al., 2000; Wendland, 2007; McCoy et al., 2007; Berish et al., 2010). In Georgia, only a limited amount of surveillance for *Mycoplasma* has been done, but previous unpublished work conducted at the J.W. Jones Ecological Research Center at Ichauway in Baker County, Georgia (JERC) indicated that this population had a high prevalence of *Mycoplasma* exposure (J. McGuire, unpublished data), but few signs of clinical disease were reported. Similar results (high seroprevalence, rare clinical disease) were reported from a gopher tortoise population on St. Catherines Island, Liberty County, Georgia (Tuberville et al., 2008). Collectively these data raise questions as to whether tortoises in these populations were infected with a less virulent strain of *M. agassizii* than Florida populations, or a novel and less pathogenic strain of *Mycoplasma*, or that clinical disease is rare because these tortoises inhabit high quality habitats and are minimally stressed (Wendland et al., 2010). If the latter scenario is correct, the likelihood of infected tortoises developing clinical disease may increase as they are exposed to stressors such as habitat change/degradation, drought, or co-infection with other pathogens (Wendland, 2007; Wendland et al., 2009). Interestingly, *Mycoplasma* exposure was rare at Moody Air Force Base in Lowndes County, Georgia where only 1 of 100 tortoises was seropositive (M. Lockhart, Valdosta State University, unpublished data); thus significant spatial variation in *Mycoplasma* prevalence may occur in Georgia.

We hypothesize that gopher tortoise populations with high seroprevalence of *Mycoplasma* (documented over a long period of time) will exhibit low prevalence of clinical disease as long as no environmental changes have occurred. In Georgia, the seroprevalence of *Mycoplasma* in tortoises that were introduced to St. Catherines Island in 1994 was 80% and it is currently 100% and importantly, prevalence of clinical disease has remained low (Tuberville et

al., 2008; T. Norton, Georgia Sea Turtle Center, unpublished data). In contrast, a population in Florida sampled in 1996 had low antibody prevalence but high prevalence of tortoises with clinical signs of disease and high mortality (Berish et al., 2012). In 1996, only *M. testudineum* was documented at the site; however, *M. agassizii* was also detected during a follow-up study (Berish et al., 2010).

To fully understand the effects of URTD on free ranging gopher tortoise populations, it is crucial to monitor the behavior of individuals with the disease (Ozgul and Perez-Heydrich, 2009; Berish et al., 2010). To date, despite extensive surveillance of Florida tortoise populations for *Mycoplasma*, few evaluations of the survival of clinically ill tortoises have been conducted (Berish et al., 2010). Additionally, URTD may not result in acute mortality, but rather could alter movements, home range, foraging, basking, and hibernation that could result in decreased fitness or long-term fecundity. For example, it has been suggested that desert tortoises with URTD bask during unusual times of the day, remain above ground for abnormally long periods of time, and either fail to emerge or emerge late from hibernation (Berry and Christopher, 2001). Thus, it is important to collect data on possible behavioral changes that occur during clinical episodes of URTD.

Although there are currently no known pathogenic parasites of gopher tortoises, few studies have characterized the endo- and ecto-parasite fauna. Limited work has been done to investigate endoparasites of gopher tortoises. In Louisiana, oxyurids (pinworms), *Strongyloides* larvae, and *Strongyle* larvae were reported in tortoises (Diaz-Figueroa, 2005), with no reported consequence. In Georgia, a survey of gastrointestinal parasites from eight tortoises sampled in 1969 and 1970 revealed a high prevalence rate (100%) with strongyloids (*Chapiniella chitwoodae* and *C. gallatii*) and oxyuroids (pinworms) and a low prevalence (25%) of

trichostrongyloids (Lichtenfels and Stewart 1981). At least four pinworm species have been described from gopher tortoises including *Alaeuris gopher macrolabiata. A.* (=*Thelastomoides*) *previcolis, A. longicolils, and A. paramazzottii.* One species of coccidian, *Eimeria paynei,* has been reported from a single tortoise from Tift County, Georgia (Ernst et al. 1971). Endoparasites can be a threat to health in reptiles and burden can fluctuate due to time of year, nutrients, age and stress (Diaz- Figueroa, 2005).

The primary goal of this dissertation was to assess gopher tortoise population health as it relates to URTD (Chapters 3 and 4). As a first step, I evaluated the effectiveness of an anesthesia protocol for gopher tortoises to allow the safe collection of biological samples (Chapter 6). Despite the lack of knowledge regarding the effects of long term exposure in a population to URTD (*Mycoplasma*), I hypothesized that *Mycoplasma* would be distributed throughout tortoise populations across the state, as described in Florida, and that tortoises would survive recrudescence of clinical disease, regardless of overall population rates of exposure. I further hypothesized that tortoises with clinical disease would behave differently from tortoises without clinical signs. Secondarily, I present information on ecto-and endo (intestinal)-parasites of tortoises as part of my comprehensive health assessment of tortoise populations (Chapter 5). Specifically, I addressed four objectives: 1) Conduct surveillance for upper respiratory tract disease and *Mycoplasma* at 10 sites in Georgia, 2)Determine if tortoises in a population with long-term exposure to *Mycoplasma* or those with clinical signs of URTD have altered local and regional movement patterns or behavior, 3) Characterize the intestinal endoparasitic fauna of gopher tortoises in Georgia, and 4) Evaluate the safety and utility of an anesthesia protocol for the collection of biological samples from gopher tortoises.

**Literature Cited**

Auffenberg, W., and R. Franz. 1982. The status and distribution of the Gopher Tortoise (*Gopherus polyphemus*). Pp. 95–126 *In* North American Tortoises: Conservation and Ecology. Bury, R.B. (Ed.). Wildlife Research Report 12. U.S. Fish and Wildlife Service, Washington, D.C., USA.

Berry KH. 1997. Demographic consequences of disease in two desert tortoise populations in California, USA. In *Proceedings of conservation, restoration, and management of tortoises and turtles- an international conference,* Wildlife Conservation Society Turtle Recovery Program and the New York Turtle and Tortoise Society, July 1993, pp. 91-97.

Berry, K.H., and M. M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Diseases 37: 427–450.

Beyer, S. M. 1993. Habitat relations of juvenile gopher tortoises and a preliminary report of upper respiratory tract disease (URTD) in gopher tortoises. M.S. Thesis, Iowa State University, Ames, Iowa, 95 pp.

Brown, M. B., G. S. McLauglin, P. A. Klein, B. C. Crenshaw, I. M. Schumacher, D. R. Brown, and E. R. Jacobson, 1999. Upper Respiratory Tract Disease in the gopher tortoise Is caused by *Mycoplasma agassizii*. J. Clin. Micro. p. 2262–2269.

Brown, M. B., I. M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994. Mycoplasma agassizii Causes Upper Respiratory Tract Disease in the Desert Tortoise. Infection and Immunity 62: 4580-4586.

Diaz-Figueroa, O. 2005. Characterizing the Health Status of the Louisiana Gopher Tortoise (*Gopherus polyphemus*). Thesis, Louisiana State University, Baton Rouge, USA. 119 pp

Dickinson, V.M., I. M. Schumacher, J.L. Jarchow, T. Duck, and C.R. Schwalbe. 2005. *Mycoplasmosis* in free- ranging desert tortoises in Utah and Arizona. J. Wildlife Diseases 41: 839-842.

Diemer Berish, J.E., L. D. Wendland, C. A. Gates. 2000. Distribution and prevalence of upper respiratory tract disease in gopher tortoises in Florida. J. Herpetology 34:1

Diemer-Berish, JE, L.D. Wendland, R.A. Kiltie, E.P. Garrison, and C. A. Gates. 2010. Effects of mycoplasmal upper respiratory disease on morbidity and mortality of gopher tortoises in northern and central Florida. Journal of Wildlife Diseases 46: 695–705.

duPré, S.A., C.R. Tracy, and K.W. Hunter. 2011. Quantitative PCR method for detection of *Mycoplasma* spp. DNA in nasal lavage samples from the desert tortoise (*Gopherus agassizii*). J. Microbiological Methods 86: 160-165.

Epperson, D. M. 1997. Gopher tortoise (*Gopherus polyphemus*) populations: Activity patterns, upper respiratory tract disease, and management on a military installation in northeast Florida. M.S. Thesis, University of Florida, Gainesville, Florida, 61 pp.

Ernst J.V., G.T. Fincher, and T.B. Stewart. 1971. *Eimeria paynei* sp. n. (Protozoa: Eimeriidae) from the Gopher tortoise (*Gopherus polyphemus*). Proceedings of the Helminthological Society of Washington. 38: 223-224.

Feldman, S.H., J. Wimsatt, R.E. Marchang, A.J. Johnson, W. Brown, J.C. Mitchell, and J.M. Sleeman. 2006. A novel *Mycoplasma* detected in association with upper respiratory disease syndrome in free- ranging Eastern Box turtles (*Terrapene carolina carolina*) in Virginia. J. of Wildlife Diseases 42: 279-289.

Gates, C. A., M. J. Allen, J. E. D. Berish, D. M. Stillwaugh, AND S. R. Shattler. 2002. Characterization of a gopher tortoise mortality event in west-central Florida. Florida Scientist 65: 185–197.

Jacobson, ET and Berry KH. 2012. *Mycoplasma testudineum* in Free-ranging desert tortoises,

Jacobson ER, Gaskin JM, Brown MB, Harris RK, Gardiner CH, LaPointe JL, Adams HP, and Reggiardo C. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). J Wildl Dis 27: 296-316.

Jacobson, E.R., M. B. Brown, I. M. Schumacher, B. R.Collins, R. K. Harris, and P. A. Klein. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. Chelonian Conservation and Biology 1: 279–284.

Lederle, P E, K. R. Rautenstrauch, D. L. Rakestraw, K. K. Zander, and J. L. Boone. 1997. Upper respiratory tract disease and mycoplasmosis in desert tortoises from Nevada. J Wildl Dis 33:759-765.

Lichtenfels, J.R. and T.B. Stewart. 1981. Three New Species of *Chapiniella* Yamaguti, 1961 (Nematoda: Strongyloidea) from Tortoises. Proceedings of the Helminthological Society of Washington. 48(2): 137-147.

McCoy, E. D., H.R. Mushinsky, and J. Lindzey. 2007. Conservation strategies and emergent diseases: The case of upper respiratory tract disease in the gopher tortoise. Chelonian Conservation and Biology 6:170-176.

McLaughlin, G. S. 1997. Upper respiratory tract disease in gopher tortoises, *Gopherus polyphemus*: Pathology, immune responses, transmission, and implications for conservation and management. Ph.D. Dissertation, University of Florida, Gainesville, Florida, 110 pp.

Ozgul, A., M.D, B.B, and C. Perez-Heydrich. 2009. Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises. Ecological Applications, 19:786-798.

Rabatsky A., and B. Blihovde. 2002. Gopher tortoise die-off at Rock Springs Run State Reserve, Lake County, Florida. Turtle and Tortoise Newsletter 6:27–28.

Sandmeier F.C., C.R. Tracy CR, S. duPre, K. Hunter. 2009. Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: A reevaluation. Biological Conservation 142: 1255-1268

Seigel, R. A., R.B. Smith, and N. A. Seigel. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. Journal of Herpetology 37:137-144.

Smith, R.B., R.A. Seigel, K.R. Smith. 1998. Occurrence of upper respiratory tract disease in gopher tortoise populations in Florida and Mississippi. J. of Herpetology 32: 426-430.

Tuberville, T.D., T. M. Norton, B.D. Todd, and J. S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: a comparison of newly released and previously established animals. Biological Conservation 141:2690-2697.

U.S. Fish and Wildlife Service (USFWS). 2011. Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To List the Gopher Tortoise as Threatened in the Eastern Portion of Its Range; Final Rule. Accessed online 5 September 2012 http://www.fws.gov/northflorida/GopherTortoise/12-month_Finding/20110726_frn_Gopher-Tortoise_12month_finding.htm.

Wendland, L. D. (2007). Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. PhD Dissertation in *Infectious Diseases and Pathology*. Gainesville, FL,University of Florida: 178 pages.

Wendland,L., H. Balbach, M. Brown, J. Diemer Berish, R. Littell, and M. Clark, 2009. Handbook on gopher tortoise (*Gopherus polyphemus*) health evaluation procedures for use by land managers and researchers. ERDC/CERL TR-09-1. U.S. Army Corps of Engineers. Washington, D.C., 82pp.

Wendland, L, Klein PA, Jacobson ER, and Brown MB. 2010. Strain variation in *Mycoplasma agassizii* and distinct host antibody responses explain differences between ELISA and Western blot assays. Clin. Vaccine Immunol. 17:1739-1745.

# CHAPTER 2

# LITERATURE REVIEW

It is imperative that assessments of wildlife population health include an integrative approach. Factors other than absence or presence of disease, such as habitat quality, need to be investigated (reviewed in Hanisch et al., 2012). Long-term stability of a population requires evidence of recruitment, in addition to the presence of a range of age structures. In concert with the ability to define a healthy population, is the ability to recognize disease (Decker et al., 2006). To understand the impacts of a disease on a population, long term studies need to be initiated (Wobeser, 2006).

The presence of chronic and recurring disease in a population, particularly in species of conservation concern, cannot be overlooked (Perez- Heydrich et al., 2012). Infectious diseases, such as mycoplasmal upper respiratory tract disease in gopher tortoises need to be taken seriously, as the pathogen can potentially impact other the species of concern as well as sympatric related species. Transmission of disease is also impacted by numerous factors related to resource connectivity, social interaction, density, stress, and immunity which are also integral parts of a complete population health assessment (Cowled et al., 2012).

## Gopher Tortoise Natural History

The gopher tortoise (*Gopherus polyphemus*) is one of five extant tortoises in North America and the only native tortoise in the southeast U. S. (Auffenberg and Franz, 1982) with populations in southern South Carolina, Georgia, Florida, southern Alabama, Mississsippi, and southeastern Louisiana. As threatened in the western portion of its range (western Alabama,

Mississippi, and Louisiana) and the eastern population is a candidate for federal listing as threatened (USFWS, 2011). The impetus for the federal listing of the eastern populations of

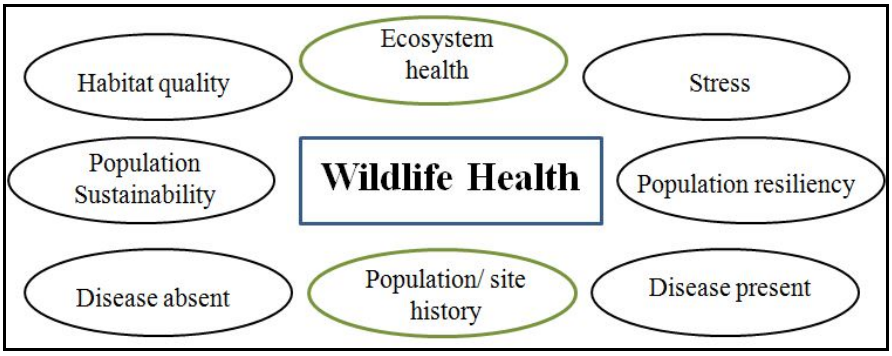


**Figure 2.1. Concepts to consider when describing and investigating the ecological drivers of wildlife health (adapted from Hanisch et al 2012).**

gopher tortoise was the alarming rate of habitat loss and subsequent population declines (Auffenberg and Franz, 1982; Diemer, 1986; Burke, 1989; Burke, 1991; Dodd and Seigel, 1991; McCoy and Mushinsky, 1992; McCoy etal., 2006), and concern regarding disease (Beyer, 1993; Diemer- Berish et al., 2000; Brown, 2002; Wendland, 2007;Diemer- Berish, 2010). Nearly 80% of the remaining populations of gopher tortoises are in Georgia and Florida (Auffenberg and Franz, 1982).

The gopher tortoise is strongly associated with deep, sandy, well-drained soils, historically associated with the longleaf pine (*Pinus palustris*) ecosystem (Auffenberg and Franz, 1982). Gopher tortoises dig long, deep burrows, up to 4.5 m in length. Tortoises are primarily found in dry upland habitats, including longleaf pine and the sandhills or scrub oak woodlands. Longleaf pine once dominated the upland habitats and is estimated to have declined by 98% (Noss et al., 1995). Many other endangered or threatened species, such as the Florida gopher frog (*Rana capito*), eastern indigo snake (*Drymarchon couperi*) and the Florida mouse (*Podomys*

*floridanus*), rely on gopher tortoise burrows, thus, the gopher tortoise is considered a keystone species of the long-leaf pine ecosystem (Eisenberg, 1983; Jackson and Milstrey, 1989).

Gopher tortoises have a reproductive lifespan of approximately 40 years and may produce 6 eggs or more per year (Iverson, 1991; Landers, 1982; Smith et al., 2012). Body size (e.g., carapace length; CL) is correlated with age in tortoises (Auffenberg and Iverson, 1979; Landers et al., 1982). Gopher tortoises are long-lived species and can live more than 60 years and reach sexual maturity between 10 to 20 years of age (> 180 mm CL for males and > 220 mm CL for females) (Landers etal., 1982; Smith 1995). Tortoises lay eggs in May and June, across most of their range. Eggs incubate for approximately 90- 110 days (Landers et al., 1982). Hatchlings have many predators, including mammals and fire ants (Landers et al., 1980; Smith, 1995; Smith et al., 2012).

It has been hypothesized that the minimum viable population size for the gopher tortoise is 50 individual adults (Cox et al., 1987; Eubanks et al., 2002). According to Eubanks et al. (2002), population of 50 adult tortoises requires contiguous habitat of 25- 81ha. Strysky et al. (2010) suggests that a viable tortoise population requires much larger area than that reported by Cox et al. (1987). This discrepancy may be related to the quality of habitat and geographic location of the reserve. Disagreement over population and habitat assessments of this species confounds studies that attempt to elucidate the effect of disease on a population (Eubanks et al., 2002; Styrksy et al., 2010). Until recently, population estimates were unavailable throughout much of the range (Smith et al., 2006). As a result, historic population data is not available for disease investigations.

There has been considerable work on home range size and movement patters of gopher tortoises. In a year-long study in southwest Georgia, female gopher tortoises had a mean home

range of 0.40 ha ± 0.08 (n=53) ha and used a mean of 5.20 ±0.32 burrows (Eubanks et al., 2003). Males had a mean home range of 1.1 ha ± 0.13 (n=70) and used a mean of 10.00 ±0.53 burrows (Eubanks et al., 2003). A shorter study in southwest Georgia documented smaller mean home ranges in males (0.10-1.40, n=8) and females (0.04-0.14, n=5) than the Eubanks et al. (2003) study (McRae et al., 1981). The differences in tortoise movements between the two studies may be due to the differences in land use, as one was an industrial forest and the other was an ecological researve (McRae et al., 1981; Eubanks et al., 2003), or to differences in the length of the two studies. Smith (1995) documented home ranges between <0.10 and 1.44 ha (n=14) during an evaluation of home range size of female tortoises in north central Florida. Diemer-Berish (1992), also in Florida, measured home ranges of 0.88ha (0.20-2.90, n=6) for males, 0.31 ha (0.00-1.20, n=5) for females.

Tortoises exhibit burrow fidelity and, as a result, long distance movements are expected to be rare, and home- ranges small (McRae 1981; Epperson et al., 1997). However, home range size and movements may expand depending on habitat availability, resources, and density (Eubanks et al., 2002; Smith, 1995). The longest short-term movement of a gopher tortoise was 0.74 km by a “dispersing sub-adult” over four days (Eubanks et al., 2003). Eight days after the tortoise was released, the signal was lost. In the same study, the longest known movement in 24h period was 0.27 km by an adult male (Eubanks et al., 2003). Long distance movement in males could be associated with mate seeking (Gibbons, 1986). Maximum movements between recaptures and or tracking events of males in a Florida study were 474 m and 186 m for females (Diemer- Berish 1992). These long-distance movements have serious implications for disease transmission as a result of increased potential contact with other gopher tortoises, in addition to

other chelonians (e.g., Eastern box turtles (*Terrapene carolina*) that may be susceptible to pathogens such as ranavirus (Johnson et al., 2008) and *Mycoplasma agassizii* (Wendland, 2007).

Long distance movements of animals associated with clinical disease can increase the potential for naïve animals to be exposed (Real et al., 2005). Limited work has been done to target movement of tortoises with clinical disease; however, mortality of six radio-instrumented tortoises was documented and 10 marked gopher tortoises were found dead in one study (Diemer- Berish, 2010). It has been hypothesized that chronically infected tortoises are less likely to emigrate (Ozgul et al, 2009); however, no data are available to validate this claim. Examples of long distance movements due to disease or parasites are available in other systems. It is thought that the territorial behavior of the badger (*Meles meles*) limited the spread of tuberculosis (Delahay et al., 2000). However, due to occasional emigration by animals for natural reasons, such as reproduction or foraging, new cases of disease were detected. Movements of badgers between social groups enhanced the risk of disease spread (Roper et al., 2003).

Upper Respiratory Tract Disease (URTD) of Chelonians

*Mycoplasma* related URTD has been implicated in the decline of the desert tortoise (Brown et al., 1994; USFWS, 1994; Brown et al., 1999). *M. agassizii* was identified as the causative agent, but other pathogens such as ranavirus (Johnson et al., 2008) and herpesvirus (Jacobson et al., 1991; Johnson et al., 2005) can cause similar clinical signs as *Mycoplasma* related URTD. Cases of herpesvirus are generally accompanied with caseous plaques and lesions in the oral cavity (Johnson et al., 2005). Additionally, herpesvirus is considered by some to be an emerging pathogen in desert tortoises (Jacobson and Berry, 2012) and should be considered as a potential pathogen in gopher tortoises as well. Recent evidence suggests that many desert

tortoise populations have been exposed to herpesvirus and infections are subclinical or disease is mild (Johnson et al., 2005; Jacobson and Berry, 2012). A parallel hypothesis exists for ranavirus in the gopher tortoise. Box turtles have experienced mass mortality related to ranavirus in the eastern U.S. (Allender et al., 2011). Additionally, *M. agassizii* has been isolated from clinically ill box turtle with URTD (Feldman et al., 2006); box turtles were also reported to be seropositive for *M. agassizii* as well (Wendland, 2007).

Mycoplasmal upper respiratory tract disease was first identified during the early 1990's in desert tortoise populations (Jacobson et al., 1991). It was first identified in gopher tortoises on Sanibel Island, Florida, in1989 (McLaughlin, 1990). URTD in gopher tortoises is highly contagious and has been implicated in the reduction of populations in Florida (Brown et al., 1994; Brown et al., 1999; Diemer-Berish et al., 2000), although there is considerable debate about the ecology and impact of the disease in free-ranging gopher tortoise populations (Seigel et al., 2003; McCoy et al., 2007; Sandmeier, 2009). The bacterium *M. agassizii* has been isolated from clinically ill gopher tortoises and has been confirmed, through transmission studies to be a causative agent of URTD (Brown et al., 1999, Brown et a.l, 1994). Another bacteria associated with URTD is *M. testudinium*, for which an ELISA has been developed, but not validated (Brown et al., 2004). *Mycoplasma* is a genus of bacteria in the class *Mollicutes*, that adheres to the ciliated epithelium of the upper respiratory tract of the tortoise (McLaughlin et al., 2000; Brown et al., 1994; Jacobson et al., 1991). URTD is characterized by mild to severe nasal and ocular discharge, conjunctivitis, and swelling of the eyes and nares (Figure 2).

Experimentally, *Mycoplasma* is transmitted horizontally by respiratory exudates (Brown et al., 1994). There is evidence of the presence of maternal antibodies in hatchlings, antibodies may not persist for more than a year (Schumacher et al., 1999). In adults, clinical signs may

**Figure 2.2. Tortoises with severe clinical signs of upper respiratory tract disease. Both tortoises have cloudy nasal exudate and swollen eyelids.**

appear a couple of weeks after exposure, but it is estimated that it takes more than one month for a tortoise to develop an immune response (Diemer- Berish et al., 2000).

Due to limited reports of mortality events in gopher tortoises, and a number of seropositive populations that are apparently healthy, disease is often not considered a serious threat to gopher tortoise populations (Seigel et al., 2003; Sandmeier et al., 2009). However, several published accounts of mortality events in gopher tortoise populations exist. At a mitigation park in Florida, in the late 1990's, 87 tortoises were found dead and surviving tortoises were seropositive for *M. agassizii*.

(Gates et al., 2002). Although *M. agassizii* has been shown to cause clinical disease in gopher and desert tortoises (Brown et al 1999), clinical signs are not always observed in naturally-infected tortoises. Currently it is unknown what factors predispose a tortoise to the development of clinical disease following infection with *M. agassizii*. Some populations in Florida have a high incidence of clinical URTD while other populations have a high prevalence of antibodies suggesting that exposure has occurred, yet clinical disease and related mortality are thought to be rare. This dichotomy has led researchers to speculate that clinical illness is

triggered by stressors such as habitat change, drought or co-infection with other pathogens (Wendland, 1997; Diemer- Berish et al., 2000; Sandmeier et al., 2013). Another hypothesis is that there are multiple strains of *M. agassizii*, some that cause clinical disease and others that may not (Brown et al. 1999; Wendland et al., 2010). More transmission studies with isolates collected throughout the range of the gopher tortoise are necessary. Loss of individuals to disease can have significant long term impacts on gopher tortoise populations (Perez- Heydrich et al., 2012; Heppell, 1998).

Basking Behavior

Winter thermoregulatory behavior in gopher tortoises can vary greatly based on geography (Carr, 1952). In the northern part of the range, DeGregorio et al., (2012) found that tortoises were rarely active in the winter and seemed to exhibit synchronous basking behavior through the seasons. They did note that young tortoises seemed to bask more often during the winter than adults, which could be attributed to their small body size and higher rate of heat loss. In south Florida, tortoises are active year round, but winter activity is restricted to the warmest days (Douglass and Layne, 1978).

Regulation of temperature is an essential part of a reptile's physiology (summarized in Dorcas et al. 2004). It has been shown in other ectotherms, including chelonians, that sick animals may spend more time basking at warmer temperatures to harness the energy necessary to fight off pathogens (Ouendraogo et al., 2004). It is believed that elevated body temperature, may increase immune response (Swimmer, 2006), thus increased basking (behavioral fever) may be an attempt to overcome infection (Monagas and Gatten, 1983). For example, fish and lizards challenged with *Aeromonas* developed a fever resulting in a 92% survival rate. When a behavioral fever was prohibited, the result was 100% mortality (Kluger, 1978).

Atypical behavior, such as wandering outside of burrows in cold weather or abnormal basking, has been noted in both desert and gopher tortoises with clinical signs of URTD (Berry and Christopher, 2001; Diemer-Berish et al., 2000; McLaughlin, 2000; Brown et al., 1994; Berish et al., 2010). Cold weather basking by an adult female gopher tortoise has been observed in Florida (Diemer, 1992). Tortoises have also been documented to remain in the burrows for extended periods of time (5 months), during what should be an active time (Diemer, 1992). In tortoises experimentally infected with *Mycoplasma*, tortoises have been noted to show reduced foraging and decreased appetite (Brown et al., 1994; McLaughlin, 2000), which can ultimately lead to death.

Parasites

Another important, but often overlooked aspect of wildlife health is the presence and role of parasites. By exploiting resources from their hosts, parasites can reduce host fitness and survival (Heylen and Matthysen, 2008). Tortoises are one of the most commonly relocated animals in the United States (Tuberville et al., 2008). Relocation of animals with parasites can put naïve animals at the recipient site at risk (Sainsbury and Vaughan- Higgins, 2011). However, some studies suggest that parasites might play an important role in density regulations of populations, and not moving associated parasites might have implications as well (Torchin et al., 2003). Additionally, parasites might thrive due to host stress response or new parasites might be introduced to new species in the new area (Anderson and May, 1986). For example, in birds, coccidia infection when introduced can negatively impact young animals and animals under stress (Sainsbury and Vaughan- Higgins, 2011). This potential could be amplified given the number of other species that also use tortoise burrows (Jackson and Milstrey, 1989). There have been events where tortoises have been treated with acaricides prior to relocation (T. Norton,

Georgia Sea Turtle Center, personal communication). However, this is not common practice during most tortoise relocations (personal observation).

Limited work has been done to investigate parasites of gopher tortoises. During studies conducted in Louisiana (one site) and Georgia (two sites), only 7-8 species of parasites in 3-4 taxonomic groups were reported (Walton, 1927; Ernst *et al.* 1971; Petter and Douglass 1976; Lichtenfels and Stewart 1981; Diaz-Figueroa 2005). Collectively, five studies conducted on approximately 42 tortoises have reported 10 species of parasites including two species of Strongylidae (*Chapiniella chitwoodae* and *C. gallatii)* from two sites in Georgia and a single site in Louisiana, a Strongyloididae (*Strongyloides* spp. larvae) from Louisiana, an unidentified Trichostrongylidae in two sites in Georgia, four species of Oxyuridae (*Alaeuris gopher macrolabiata. A.* (*=Thelastomoides*) *previcolis, A. longicolils, and A. paramazzottii)* from Georgia and Louisiana, an acanthocephalan (*Neoechinorhynchus pseudemydis*) (USPNC 82347) from Louisiana, and a coccidian (*E. paynei*) from a single tortoise in Georgia (Walton 1927, Ernst *et al.* 1971; Petter and Douglass 1976; Lichtenfels and Stewart 1981; Diaz-Figueroa 2005).

**Literature Cited**

Allender, M.C., M. Abd-Eldaim, J. Schumacher,D. McRuer, L. S. Christian, and M. Kennedy. 2011. PCR Prevalence of Ranavirus in Free-Ranging Eastern Box Turtles (*Terrapene carolina carolina*) at Rehabilitation Centers in Three Southeastern US States. J. Wild. Dis. 47: 759-764.

Anderson, R.M., and R.M. May. 1986. The invasion, persistence and spread of infectious diseases within animal and plant communities. Philosophical Transactions of the Royal Society London B, Biological Sciences 314:533-570.

Auffenberg, W. and J.B. Iverson. 1979. Demography of terrestrial turtles. Pp 541-569 *In* Turtles: perspectives and research. Harless, M. and H. Morlock (Ed.). Wiley Interscience, New York, New York.

Auffenberg, W., and R. Franz. 1982. The status and distribution of the Gopher Tortoise (*Gopherus polyphemus*). Pp. 95–126 *In* North American Tortoises: Conservation and Ecology. Bury, R.B. (Ed.). Wildlife Research Report 12. U.S. Fish and Wildlife Service, Washington, D.C., USA.

Berry, K.H., AND M. M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. J. Wild. Dis. 37: 427–450.

Beyer, S. M. 1993. Habitat relations of juvenile gopher tortoises and a preliminary report of upper respiratory tract disease (URTD) in gopher tortoises. M.S. Thesis, Iowa State University, Ames, Iowa, 95 pp.

Brown, D.R. 2002. Mycoplasmosis and immunity of fish and reptiles. Frontier in Bioscience 7: 1338-1346.

Brown, D. R., J. L. Merritt, E. R. Jacobson, P. A. Klein, J. G. Tully and M. B. Brown. 2004. *Mycoplasma testudineum* sp. nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. Int J Syst Evol Microbio 54: 1527–1529.

Brown, M. B., I. M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62: 4580-4586.

Brown, M. B., G. S. McLauglin, P. A. Klein, B. C. Crenshaw, I. M. Schumacher, D. R. Brown, and E. R. Jacobson, 1999. Upper respiratory tract disease in the gopher tortoise is caused by *Mycoplasma agassizii*. J Clin Micro 37:2262–2269.

Burke, R.L. 1989. Florida gopher tortoise relocation: overview and case study. Biological Conservation 48: 295-309.

Burke, R.L. 1991. Relocations, repatriations, and translocation of amphibians and reptiles: taking a broader view. Herpetologica 47: 350-357.

Carr, A. 1952. Handbook of turtles: the turtles of the United States, Canada, and Baja California. Cornell University Press, New York. 542 pp.

Cowled, B.D., M.P. Ward, S.W. Laffan,F. Galea, M. G. Garner, A. J. MacDonald, I. Marsh, P. Muellner, K. Negus, S. Quasim, A. P. Woolnough, S. D. Sarre. 2012. Integrating Survey and Molecular Approaches to Better Understand Wildlife Disease Ecology. PLoS ONE 7(10): e46310. doi:10.1371/journal.pone.0046310

Cox, J., D. Inkley, and R. Kautz. 1987. Ecology and habitat protection needs of gopher tortoise (*Gopherus polyphemus*) populations found on lands slated for large-scale development in Florida. Nongame Wildlife Technical Report No. 4. 75pp.

Decker DJ, Wild MA, Riley SJ, Siemer WF, Miller MM, Leong KM, Powers JG, and Rhyan JC. 2006. Wildlife disease management: a manager's model. Hum Dimens Wildl 11:151-158.

DeGregorio, B.A., K. A. Buhlmann, and T. D. Tuberville. 2012. Overwintering of gopher tortoises (*Gopherus polyphemus*) translocated to the northern limit of their geographic range: temperatures, timing, and survival. Chelonian Conservation and Biology 11: 84-90.

Delahay RJ, S.Langton, G.C. Smith, R.S. Clifton-Hadley and C.I. Cheeseman. 2000. The spatio-temporal distribution of Mycobacterium bovis (bovine tuberculosis) infection in a high-density badger population. Journal of Animal Ecology 69:428-441.

Diaz-Figueroa, O. (2005). Characterizing the health status of the Louisiana gopher tortoise (*Gopherus polyphemus*). Thesis, Louisiana State University, Baton Rouge, USA. 109 pp.

Dickinson, V. M., I. M. Schumacher, J. L. Jarchow, T. Duck, AND C. R. Schwabe. 2005. Mycoplasmosis in free-ranging desert tortoises in Utah and Arizona. Journal of Wildlife Diseases 41: 839–842.

Diemer, J.E. 1986. The ecology and management of the gopher tortoise in the southeastern United States. Hepetologica 42: 125-133.

Diemer, J.E. 1992. Demography of the tortoise *Gopherus polyphemus* in northern Florida. Journal of Herpetology 26: 281-289.

Diemer- Berish, J.E., L. D. Wendland, C. A. Gates. 2000. Distribution and prevalence of upper respiratory tract disease in gopher tortoises in Florida. J. Herpetology, Vol. 34:5-12.

Diemer-Berish, J.E., L.D. Wendland, R.A. Kiltie, E.P. Garrison, and C. A. Gates. 2010. Effects of mycoplasmal upper respiratory disease on morbidity and mortality of gopher tortoises in northern and central Florida. J. Wild. Dis. 46: 695–705.

Diemer-Berish, J.E., R.A. Kiltie, and T.M. Thomas. 2012. Long-term population dynamics of gopher tortoises (*Gopherus polyphemus*) in a pine plantation in northern Florida. Chelonian Conservation and Biology 11:50-58.

Dodd Jr., C.K., and R.A. Seigel. 1991. Relocation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica 47: 336-350.

Douglass, J.F., and J.N. Layne. 1978. Activity and thermoregulation of the gopher tortoise (*Gopherus polyphemus*) in southern Florida. Herpetologica 34: 359-374.

Dorcas, M. E., Hopkins, W. A., and J. H. Roe. 2004. Effects of body mass and temperature on standard metabolic rate in the eastern diamondback rattlesnake (Crotalus adamanteus). Copeia 2004:145-151.

Eisenberg, 1983. The common tortoise as a keystone species. Florida State Museum, Gainesville, FL. 4pp.

Ernst J.V., Fincher, G.T. and Stewart, T.B. (1971). *Eimeria paynei* sp. n. (Protozoa: Eimeriidae) from the gopher tortoise (*Gopherus polyphemus*). Proc Helminthol Soc Wash 38, 223-224.

Eubanks, J. O., J. W. Hollister**,** C. Guyer, and W. K. Michener**.** 2002. Reserve area requirements for gopher tortoises (*Gopherus polyphemus*). Chelenion Conservation and Biology 4:464-471.

Eubanks, J. O., W. K. Michener, and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). Herpetologica 59:311-321.

Feldman, S.H., J. Wimsatt, R. E. Marchang, A. J. Johnson, W. Brown, J.C. Mitchell, and J.M. Sleeman. 2006. A novel *Mycoplasma* detected in association with upper respiratory disease syndrome in free-ranging eastern box turtles (*Terrapene carolina carolina*) in Virginia. Journal of Wildlife Diseases 42: 279-289.

Gates, C. A., M. J. Allen, J. E. D. Berish, D. M. Stillwaugh, AND S. R. Shattler. 2002. Characterization of a gopher tortoise mortality event in west-central Florida. Florida Scientist 65: 185–197.

Gibbons, J.W. 1986. Movement patterns among turtle populations: applicability to management of the desert tortoise. Herpetologica 42: 104-113.

Hanisch SL, Riley SJ, and Nelson MP. 2012. Promoting wildlife health or fighting wildlife disease: insights from history, philosophy, and science. Wildlife Soc B 36:477- 482.

Heppell, S. S. 1998. Application of life-history theory and population model analysis to turtle conservation. Copeia 1998(2): 367–75.

Heylen, D.J.A., and E. Matthysen. 2008. Effect of tick parasitism on the health status of passerine bird. Functional Ecology 22: 1099-1107.

Iverson, J.B. 1991. Patterns of survivorship in turtles. Canadian Journal of Zoology 69: 385-391.

Jackson, D.R. and E.G. Milstrey. 1989. The fauna of gopher tortoise burrows. In *Proceedings of the Gopher Tortoise Relocation Symposium*, ed. J.E. Diemer, D.R. Jackson, J.L. Landers, J.N. Layne & D.A. Wood. *Florida Game and Fresh Water Commission, Nongame Wildlife Program Technical Report*. Tallahassee, FL.

Jacobson, E.R. and K.H. Berry. 2012. *Mycoplasma testudineum* in free-ranging desert tortoises, *Gopherus agassizii*. Journal of Wildlife Diseases 48: 1063- 1068.

Jacobson, E.R., Gaskin, J.M., Brown, M.B., Harris, R.K., Gardiner, C.H., LaPointe, J.L., Adams, H.P., Reggiardo, C., 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). J. Wildl. Dis. 27, 296–316.

Johnson, A.J., A. P. Pessier, J. F.X. Wellehan, R. Brown, and E. R. Jacobson. 2005. Identification of a novel Herpesvirus from a California desert tortoise (*Gopherus agassizii*). Veterinary Microbiology 111: 107-116.

Johnson, A.J., A.P. Pessier, J.F.X. Wellehan, A. Childress, T. M. Norton, N.L. Stedman, D.C. Bloom, W. Belzer, V. R. Titus, R. Wagner, J.W. Brooks, J. Spratt, and E.R. Jacobson. 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. Journal of Wildlife Diseases 44: 851-863.

Kluger, M. J. 1986. Is fever beneficial? The Yale Journal of Biology and Medicine. 59: 89-95.

Landers, J.L., J.A. Garner, and W.A. McRae. 1980. Reproduction of gopher tortoises (*Gopherus polyphemus*) in southwestern Georgia. Herpetologica 36:353-361.

Landers, J.L., W.A. McRae, and J.A. Garner. 1982. Growth and maturity of the gopher tortoise in southwestern Georgia. Bulletin of the Florida State Museum 27: 81-110.

Lichtenfels, J.R. and Stewart, T.B. (1981). Three new species of *Chapiniella* Yamaguti, 1961 (Nematoda: Strongyloidea) from tortoises. Proceedings of the Helminthological Society Washington 48: 137-147.

McCoy, E. D., and H.R. Mushinsky. 1992. Studying a species in decline: gopher tortoises and the dilemma of “correction factors”. Herpetologica 48: 402-407.

McCoy, E. D., H.R. Mushinsky, and J. Lindzey. 2006. Declines of the gopher tortoise on protected lands. Biological Conservation 128: 120-127.

McCoy, E. D., H.R. Mushinsky, and J. Lindzey. 2007. Conservation strategies and emergent diseases: The case of upper respiratory tract disease in the gopher tortoise. Chelonian Conservation and Biology 6:170-176.

McLaughlin, G.S. 1990. Ecology of gopher tortoises (*Gopherus polyphemus*) on Sanibel Island, Florida. MS Thesis. Iowa State University, Ames, Iowa. 114pp.

McLaughlin, G. S., E.R. Jacobson, D.R. Brown, C.E. McKenna, I.M. Schumacher, H.P. Adams, M.B. Brown and P.A. Klein. 2000. Pathology of upper respiratory tract disease of gopher tortoises in Florida. 36: 272-283.

McRae, W.A., J.L. Landers, and J.A. Garner. 18981. Movement patterns and home range of the gopher tortoise. American Midland Naturalist 106: 165-179.

Miller, P.S. 2001. Preliminary population viability assessment for the gopher tortoise (*Gopherus polyphemus*) in Florida. PVA workshop report summary. Tallahassee, Florida.

Monagas, W.R. and R.E. Gatten. 1983. Behavioural fever in the turtles *Terrapene carolina* and *Chrysemys picta*. Journal of Thermal Biology 8:285-288.

Noss, R.F., E.T. LaRoe, and J.M. Scott. 1995. Endangered Ecosystems of the United States: a preliminary assessment f loss and degradation. USDI National Biological Service, Biological Report 28.

Ouendraogo, R.M., M. S. Goettel, and J. Brodeur. 2004. Behavioral thermoregulation in the migratory locust: a therapy to overcome fungal infection. Behavioural Ecology 138:312-319.

Ozgul, A., M.D, B.B, and C. Perez-Heydrich. 2009. Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises. Ecological Applications, 19:786-798.

Perez-Heydrich C., M.K. Oli, and M. Brown. 2012. Population- level impacts of a recurring disease on a long-lived wildlife host. Oikos 121:377-388.

Petter, A.J. and Douglass, J.F. (1976). Etude des populations d'Oxyures du colon des *Gopherus* (Testudinidae). Bull Mus Natl Hist Natur, strie 3, no. 389, Zoologie 271, 731-768.

Real, L.A., C. Russell, L.Waller, D. Smith, and J. Childs. 2005. Spatial dynamics and molecular ecology of North American rabies. Journal of Heredity 96:253-260.

Roper, T.J., J.R. Ostler and L. Conradt . 2003. The process of dispersal in badgers *Meles meles*. Mammal Review 33:314-318.

Sainsbury, A.W., and R.J. Vaughan-Higgins. 2011. Analyzing disease risks associated with translocations. Conservation Biology 26:442-452.

Sandmeier F.C., C.R. Tracy CR, S. duPre, K. Hunter. 2009. Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: A reevaluation. Biological Conservation 142: 1255-1268.

Sandmeier, F.C., C. R. Tracy, B.E. Hagerty, S. DuPré, H. Mohammadpour, and L. Hunter. 2013. Mycoplasmal upper respiratory tract disease across the range of the threatened Mojave desert tortoise: association with thermal regime and natural antibodies. EcoHealth. Epub ahead of print 12 April 2013.

Schumacher IM, Brown BR, Jacobson ER, Collins BR, and Klein PA. 1993. Detection of antibodies to a pathogenic mycoplasma in desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. J Clin Microbiol 31: 1454- 1460.

Schumacher IM, Bradford Hardenbrook D, Brown MB, Jacobson ER, and Klein PA. 1997. Relationship between clinical signs and upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Nevada. J Wildl Dis 33: 261-266.

Seigel, R. A., R.B. Smith, and N. A. Seigel. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. Journal of Herpetology 37:137-144.

Smith, L.L. 1995. Nesting ecology, female home range and activity, and population sixe-class structureof the gopher tortoise, *Gopherus polyphemus*, on the Katharine Ordway Preserve, Putnam County, Florida. Bulletin of the Florida Museum of Natural History 73: 97-126.

Smith, L. L., T. D. Tuberville, and R. A. Seigel. 2006. Workshop on the ecology, status, and management of the gopher tortoise (*Gopherus polyphemus*), Joseph W. Jones Ecological Research Center, 16-17 January 2003: final results and recommendations. Chelonian Conservation and Biology 5:326-330.

Smith, L.L., DA. Steen, L.M. Conner, and J.S. Rutledge. 2012. Effects of predator exclusion on nest and hatchling survival in the gopher tortoise. The Journal of Wildlife Management 77: 352-358.

Styrsky, J.N., C. Guyer, H. Balbach and A. Turkmen. 2010. The relationship between burrow abundance and area as a predictor of gopher tortoise population size. Herpetologica 66: 403-410.

Swimmer, J.Y. 2006. Relationship between basking and fibropapillomatosis in captive green turtles (*Chelonia mydas*). Chelonian Conservation and Biology 5: 305-309.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., and Kuris, A.M. 2003. Introduced species and their missing parasites. Nature 421: 628-630

Tuberville, T.D., T.M. Norton, B.J. Waffa, C. hagen, and T.C. Glenn. 2011. Mating system in a gopher tortoise population established through multiple translocations: apparent advantage of prior residence. Biological Conservation 144: 175-183.

U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the gopher tortoise as threatened in the eastern portion of its range. Federal Register 76:45129-45162.

Walton, A.C. (1927). A revision of the nematodes of the Leidy Collection. Proc Acad Nat Sci Phil 79, 49-163.

Wendland, L. D. 2007. Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. PhD Dissertation in *Infectious Diseases and Pathology*. Gainesville, FL,University of Florida: 178 pages.

Wendland, L., P.A. Klein, E.R. Jacobson, and M.B. Brown. 2010. Strain variation in *Mycoplasma agassizii* and distinct host antibody responses explain differences between ELISA and Western blot assays. Clin. Vaccine Immunol. 17:1739-1745.

# CHAPTER 3

# SURVEILLANCE FOR UPPER RESPIRATORY TRACT DISEASE AND *MYCOPLASMA* IN FREE-RANGING GOPHER TORTOISES IN GEORGIA[1]

[1] Gonynor- McGuire JL, Smith LL, Guyer C, Lockhart JM, and Yabsley MJ. To be submitted to the Journal of Wildlife Diseases.

**Abstract**

Upper respiratory tract disease (URTD) in the gopher tortoise (*Gopherus polyphemus*) is highly contagious and has been implicated in the reduction of populations throughout their range. With the exception of a few limited studies, the prevalence of URTD in Georgia tortoise populations is poorly known. We found that exposure to both *Mycoplasma agassizii* and *M. testudineum*, pathogens associated with URTD, varied spatially among 10 tested Georgia tortoise populations. The prevalence of antibodies to *M. agassizii* in individual populations was either very low (0-3%, n=6 populations) or very high (96- 100%, n=4 populations), whereas there was variation in the prevalence of antibodies to *M. testudineum* among populations (38% - 61%), with only one site being negative. Five sites were seropositive for both pathogens, and these were the only sites where we observed tortoises with clinical signs consistent with URTD. Interestingly, sites that were only seropositive for *M. testudineum* also had no tortoises that exhibited clinical signs of URTD which supports evidence that this organism may be of limited pathogenicity for gopher tortoises. Collectively, these data indicate that both *M. agassizii* and *M. testudineum* are present in Georgia populations of gopher tortoises and that clinical disease is apparent in populations where both pathogens are present. Continued surveillance is needed to better understand the role of these two pathogens, as well as other potential pathogens, in the overall health of tortoise populations in Georgia, especially if future conservation efforts involve the translocation of tortoises.

**Introduction**

Gopher tortoise (*Gopherus polyphemus*) populations have declined throughout much of their historical range due to habitat loss and fragmentation, human activity, and disease (Auffenberg and Franz 1982; Diemer- Berish et al, 2010). In 1987, the gopher tortoise was

federally listed as threatened in the western portion of its range (west of the Mobile and Tombigbee Rivers in Alabama, Louisiana, and Mississippi) (USFWS, 1987). The tortoise is state-listed throughout the rest of its range and is currently a candidate for federal listing in the eastern portion of its range (USFWS, 2011).

Upper respiratory tract disease (URTD) in gopher tortoises is caused by several contagious pathogens including *Mycoplasma agassizii, M. testudineum,* herpesvirus, and ranavirus with the former two being the most commonly associated with URTD (Jacobson et al., 1991; Brown et al., 1995; Jacobson et al, 1995; Brown et al., 2002; Brown et al., 2004; Dickinson et al., 2005). URTD has been implicated in the reduction of some tortoise populations (Brown et al., 1994; McLaughlin, 1997; Brown et al., 1999; Diemer-Berish et al. 2000; Gates et al., 2002; Seigel et al., 2003). However, there is considerable debate about the ecology and impact of the URTD in free-ranging tortoise populations (Diemer et al., 2000; Seigel et al., 2003; McCoy et al., 2007; Sandmeier, 2009). URTD is characterized by mild to severe nasal and ocular discharge, conjunctivitis, and swelling of the eyes and nares. Currently it is unknown what factors predispose a tortoise to the development of clinical disease. For example, some gopher tortoise populations in Florida have a high prevalence of clinical URTD, while other populations with a high prevalence of *Mycoplasma* antibodies have low mortality (Diemer-Berish et al., 2010). Because *Mycoplasma* can cause persistent infections, it is possible that stressors such as habitat disturbance, translocation, or infections with other pathogens, could cause infected but clinically-normal tortoises to develop clinical signs. Also, because multiple species of *Mycoplasma* infect gopher tortoises, these species, or strains of a species, may vary in pathogenicity (Wendland, 2007).

To date, few studies have investigated the prevalence of *Mycoplasma* in gopher tortoise populations in Georgia and these studies were restricted to three isolated populations (Kahn, 2006; Tuberville et al., 2008; Hernandez et al., 2010). In 1994, 93 tortoises from Bullock County, Georgia were translocated to St. Catherines Island, in Lowndes County, Georgia. At the time of translocation, 80% of tortoises were positive for antibodies to *M. agassizii* (Tuberville et al., 2008). Currently, all tested adult tortoises in this population have been seropositive, yet clinical disease is very rare (T. Norton, personal communication). Screening of tortoises at Ft. Benning, in Muscogee County, Georgia for *Mycoplasma* revealed seasonal variation in exposure rates with only 27% of tortoises tested in the summer being positive and 44% of tortoises tested in the spring being positive for antibodies (Kahn, 2006). In a 2001 translocation of 106 tortoises from McIntosh County, Georgia to the Savannah River Site in Aiken County, South Carolina, one of 14 tortoises was positive for antibodies to *M. agassizii* and an additional four were suspect (Hernandez et al, 2010). None of these studies evaluated the prevalence of antibodies to *M. testudineum*. Molecular or culture evidence of *Mycoplasma* infections in Georgia are very limited with *M. agassizii* being identified from 10 of 21 (50%) tortoises from St. Catherines Island, in Liberty County (none had clinical signs; Tuberville et al., 2008) and *M. agassizii* being cultured from a single moribund tortoise from Baker County (Wendland 2007).

The current study was conducted to acquire knowledge of the distribution of *Mycoplasma* and URTD in gopher tortoise populations in Georgia. The specific goals were to determine the prevalence of URTD through health assessments and antibody and PCR prevalence of *M. agassizii* and *M. testudineum* in selected tortoise populations in Georgia.

## Methods

### Study Sites

Samples were collected from tortoises from 10 study sites in eight Georgia counties between 1995 and 2011 (Table 1 and Figure 1). Sites had varied land uses including public use (n=2), private lands (n=6), and military installations (n=2). All properties were originally reported by land managers to have >30 tortoises and tortoise population densities were available for four sites: Baker County (JERC), Telfair County A (OISP), Cook County (RBSP), and Richmond County (Fort Gordon). The JERC had approximately 7600 ha of suitable habitat with a tortoise population density of 0.64 ± 0.08 tortoises/ha (L. Smith, personal communication). The OISP had 95.4 ha of habitat and an estimated density of 1.23 ± 0.21 tortoises/ha (Ballou, 2013). RBSP has a high tortoise density of 3.08 ±0.67 tortoises/ha in 66 ha of habitat (Ballou, 2013). Ft. Gordon is at the northern extent of the range of the gopher tortoise and has approximately 13,000 ha of suitable habitat but a low tortoise density (0.02± 0.004 tortoises/ha) (J. Stober, unpublished data).

### Tortoise Capture and Sampling

Each site was trapped for up to two weeks in an attempt to capture 30 adult tortoises. Occupancy at adult gopher tortoise burrows (entrances ≥ 23cm wide) was determined using a Sandpiper Technologies® burrow camera (Sandpiper Technologies, Mantica, California) or sign of tortoise activity at the burrow. Wire-cage live traps (Tractor Supply, Brentwood, TN; Hav-a-hart, Lititz, PA) were set at the entrance of occupied tortoise burrows; traps were shaded with utility burlap. Trapping was continued until the tortoise was captured or two weeks had passed. In 2011 and 2012, juvenile tortoises were trapped at one site (JERC) with pit fall traps (Ballou, 2013). When possible, tortoises were also opportunistically hand captured. Upon capture,

animals were transported to a shaded area for sample collection. Each tortoise was physically examined and measured (straight-line carapace length, plastron length, gular length, anal notch and anal fork), weighed (McRae et al. 1981). At sites with long term monitoring objectives, tortoises were uniquely marked by notching the marginal scutes (Cagle, 1939) with a rotary tool (Dremel™, Racine, CA). Tortoises were examined for clinical signs suggestive of URTD (e.g., nasal exudates, conjunctivitis swollen eyes, lethargy, labored/wheezy breathing) and lesions suggestive of past URTD (e.g., nasal scarring, and asymmetrical nares). Gender was determined based on external morphology such as gular length, plastral concavity and the ratio of anal notch to anal fork width (McRae et al., 1981; Eubanks et al., 2003). Tortoises whose gender could not be determined were characterized as "unknown." It is estimated that gopher tortoises are sexually mature at >180mm CL for males and >220 mm CL for females (Iverson, 1980; Landers et al., 1982; Diemer and Moore, 1994). Due to difficulty in differentiating between males and females juveniles based on shell morphology, tortoises less than 230 mm CL were categorized collectively as "juveniles" (McRae et al., 1981). Adult tortoises that could not be sexed based on ambiguous characteristics were grouped as "unknown".

Between 0.5- 4ml of blood (<1% of body weight) was collected from either the caudal vein, brachial vein, or the subcarapacial venous sinus (Wendland, 2007). Blood was added to heparinized tubes and centrifuged. Plasma was removed and immediately frozen at -80°C.

In 2010 and 2011, nasal exudates were collected from tortoises exhibiting clinical signs of URTD (i.e., nasal discharge) using sterile rayon swabs (Puritan Medical Products Company LLC, Guilford, ME; Microbrush International, Grafton, WI). After collection, swabs were placed in tubes and frozen at -80 ºC until testing.

Tortoises were hydrated in warm water for 15-20 minutes (Wendland et al 2009) prior to release at the site of capture. All tortoises were sampled and released within 48-h of capture. Equipment was disinfected with a 10% bleach solution between tortoises. All methods were reviewed and approved by the University of Georgia's Animal Care and Use Committee (A2010 11-563).

Pathogen Testing

Plasma samples were submitted to the *Mycoplasma* research laboratory at the University of Florida College of Veterinary Medicine, Department of Infectious Diseases and Pathology (Gainesville, FL) for ELISA testing. All samples were tested for antibodies to *M. agassizii* and a subset were tested for antibodies to *M. testudineum*. Results for both *M. agasizzi* and *M. testudineum* were grouped into one of three classes based on antibody titers: positive (titer $\geq$ 64), negative (titer < 32) and suspect (titer = 32-63) (Wendland et al., 2007).

To detect shedding of *Mycoplasma* spp. in nasal exudates, polymerase chain reaction (PCR) testing for the 16S rRNA gene of *Mycoplasma* spp. was conducted. Genomic DNA was extracted from the swabs according to manufacturer's protocols (Qiagen DNA purification Kit, Germantown, MD, USA) and each sampled was tested in duplicate using primers GMF-1 (5'-ACACCATGGGAGCTGGTAAT), and GMR-1 (5'-CCTCATCGACTTTCAGACCCAAGGCAT) as described in Lauerman (1998) and Diemer-Berish et al. (2010).

Data Analysis

Chi-square analysis was used to test for differences in antibody prevalence and PCR results among sites and among age/gender classes. Linear regression was used to assess the relationship between carapace length and pathogen prevalence. Clinical signs (e.g., nasal

exudates, conjunctivitis, swollen eyes, lethargy, labored/wheezy breathing) were categorized as present or absent.

**Results**

Samples from at least 30 tortoises were obtained at five sites: JERC, Decatur Co., RBSP/Cook County, Telfair A, and Moody AFB. We were unable to capture 30 tortoises at other sites because population densities were lower than expected. In total, serum samples were collected from 580 tortoises at the 10 sites (Table 2). Additional tortoises were captured and examined for URTD signs at JERC; however, serum samples were not collected from these individuals (Table 2).

Prevalence of antibodies to *M. agassizii* and *M. testudineum* varied considerably among sites (Table 3). The prevalence of antibodies to *M. agassizii* was either very low (<3%) or very high (92%-100%), whereas prevalence of *M. testudineum* varied from 20-61%. A low prevalence of antibodies (≤3%) to *M. agassizii* was detected at six sites (Moody AFB, OISP, Telfair B, Camden, Lowndes, and Ft. Gordon) and a high prevalence (92-100%) at the remaining four sites (Baker, Mitchell, Decatur, and Cook). Tortoises (n=209) at nine sites were tested for both *M. agassizii* and *M. testudineum*. Tortoises seropositive for both *M. testudineum* and *M. agassizii* detected at five sites*:* JERC (n= 25/70), Mitchell Co. (n=2/5), Decatur Co. (n= 5/25), OSIP (n=1/30), and RBSP (n=19/33). Tortoises with acute and/or chronic clinical signs of URTD were only documented at sites with both *M. agassizii* and *M. testudineum* present in the population (Table 3). We found no relationship between *M. testudineum* and clinical signs ($\chi^2$= 3.190; P= 0.203). In general, higher prevalence of clinical disease was detected in populations with high prevalence of *M. agassizii* or presence of both *Mycoplasma*. Only two sites that were seronegative for *M. agassizii* had evidence of chronic URTD (tortoises with asymmetrical nares).

Nasal swabs were collected from 136 tortoises at three sites (JERC, n= 129; Mitchell, n= 1; and Decatur, n= 6) and 50 (37%) total swabs were PCR positive for *Mycoplasma* spp. (Table 3). Based on ELISA results, all three sites also had a high prevalence of *M. agassizii* antibodies (92-100%). Among the PCR positive tortoises (n=50), 39 (29%) had nasal discharge and 38 (28%) had past lesions suggestive of URTD. A total of 48 (37%) tortoises from JERC were PCR positive for *Mycoplasma* spp. and 26 (52%) of these positive tortoises had nasal discharge. The remaining 24 PCR positive samples were from asymptomatic tortoises (n=96, 25%). Of the six Decatur County swabs tested, two (33%) were PCR positive, both of which were seropositive for both *M. a*gassizii and *M. testudineum*. Both of the Decatur PCR positive tortoises were seropositive for *M. agassizii* and *M. testudineum*. The Mitchell County swab was PCR negative, but the tortoise was seropositive for *M. agassizii*. PCR and serology data are available for 39 tortoises across the three sites, 23 (59%) tortoises were seropositive for both *M. agassizii* and *M. testudineum* and 13 (33%) were PCR positive.

The antibody prevalence for *M. agassizii* among the 14 juvenile tortoises was significantly lower than that of adults (Fisher's exact test, $p < 0.0001$), but there was no difference in prevalence between adults and juveniles for *M. testudineum* (Table 4). A single juvenile from JERC was PCR positive. This tortoise (CL 20.4 cm) was seropositive for *M. testudineum*, but not *M. agassizii* (Table 4). A PCR negative juvenile (CL 21.2 cm) was suspect for *M. agassizii* and positive for *M. testudineum*. One tortoise of unknown gender with a CL of 23 cm (the juvenile/ adult cut-off) was seropositive for *M. agassizii*, suspect for *M. testudineum*, and PCR positive. When data from all sites were combined, there was no difference in prevalence for *M. agassizii* between males and females (Fisher's exact test, $p= 0.1480$), but the

prevalence of *M. testudineum* was significantly higher for females (43/88) compared with males (33/95) (Fisher's exact test, p= 0.0064) (Table 5).

Recapture data was available for two sites, Moody AFB and JERC. Thirty-seven tortoises from Moody AFB were recaptured and tested between 2000 and 2004 and 21 tortoises at JERC were tested in 1997 and then again between 2009 and 2011. At Moody AFB, serostatus changes were documented for three tortoises. One tortoise was a suspect for *M. agassizii* antibodies in August 2002 and August 2003, converted to a positive (titer 64) in September 2003, and was suspect again in September 2004. A second tortoise was seronegative for *M. agassizii* in August 2000, suspect in September 2001, and was negative in July 2002 and June 2003. The third tortoise was seronegative for *M. agassizii* in August 2000, suspect in September 2001 and seronegative in July 2003. The remaining 34 resampled tortoises at Moody AFB were negative at each sampling point. At JERC, all 21 resampled tortoises were positive in 1997 and remained seropositive for *M. agassizii* in 2009-2011. No differences were noted in seroprevalence between years at either site.

**Discussion**

Among the gopher tortoise populations tested in Georgia, the prevalence of *M. agassizii* antibodies was either very high or very low with a total of seven of 10 sites being positive. In contrast, antibodies to *M. testudineum* were detected at all but one site and the prevalence of this pathogen varied. Six of the 10 sites were seropositive for *M. agassizii* and eight sites were seropositive for *M. testudineum*. Five sites were seropositive for both pathogens and four of these sites had tortoises with clinical signs of URTD. *M. testudineum* can cause clinical disease in the absence of *M. agassizii*; however, there is some evidence that *M. testudineum* might be less pathogenic (Jacobson and Berry, 2012). Although limited surveillance has been completed,

Wendland (2007) documented *M. testudineum* at three out of eleven field sites, all of which were located in northeastern Florida and, to our knowledge, ours is the first report of *M. testudineum* in Georgia. In contrast to Wendland (2007) we detected *M. testudineum* on a greater proportion of sites than *M. agassizii*. Additionally, we only observed clinical signs in tortoises seropositive for *M. agassizii* alone or in tortoises seropositive for both *M. testudineum* and *M. agassizii*. Wendland (2007) also observed clinical signs (nasal discharge) in tortoises infected with *M. testudineum* (PCR positive nasal discharge) but it is unclear how many of those tortoises were coinfected. Similar to our study, they also documented clinical signs at sites with high seroprevalence of *M. agassizii*.

Contrary to other reports of fluctuating seroprevalence at sites (Kahn, 2006; Diemer-Berish et al., 2010), seroprevalence at two of our sites, Moody AFB and JERC, remained stable over the four and fifteen year periods, respectively. *M. testudineum* testing has not been done at Moody AFB, but it would be interesting to see if the pathogen is present in the population. Wendland et al. (2007) warned that a single positive in a population with low seroprevalence should be interpreted with caution as there are occasional false-positive results. Thus, we recommend that land managers continue health assessments and pathogen surveillance in concert with population monitoring efforts. Interestingly, the single positive tortoise from Moody AFB only developed a low titer and was “suspect” at two other testing time periods; therefore, the true infection status of this tortoise is unknown.

Prevalence rates at JERC were similar between two sampling periods (92% in 2009-2012 vs. 96% in 1997) even though the ELISA had been improved in regards to sensitivity and specificity between the two sampling periods (Wendland et al., 2007; Wendland et al., 2010). Diemer- Berish et al. (2010) observed potential for year to year variation in antibody-positive

tortoises for *M. agassizii*. Despite the high prevalence of antibodies to *M. agassizii* at JERC, the prevalence of current clinical signs and past URTD lesions was <30% suggesting that not all tortoises develop clinical signs or that past infections were mild and did not result in chronic lesions visible during this study. Future studies should be conducted to determine if antibody positive tortoises develop recrudescence of clinical signs.

Several immature tortoises from different sites had antibodies and/or were PCR positive for *Mycoplasma* sp. Previous studies have shown a correlation between body size (CL) and seroprevalence and failed to detect antibodies, in tortoises < 23 cm CL (Beyer, 1993; Wendland, 2007). The smallest animals seropositive for *M. agassizii* were from RBSP (CL 13.1 cm), and the smallest for *M. testudineum* was from JERC (CL 20.4 cm). In previous studies, juvenile tortoises that tested positive for exposure to *Mycoplasma* were from populations undergoing epizootic events (Wendland, 2010), otherwise most juveniles were negative for both *M. agassizii* and *M. testudineum* (Wendland, 2007). Even more interesting was the PCR positive tortoise (CL 20.4 cm) from JERC. This tortoise was seronegative for *M. agassizii* and positive for *M. testudineum*. At the Decatur site the only juvenile (CL 20.0 cm) with nare abnormalities was seropositive for *M. agassizii*, but negative for *M. testudineum*. Rostal et al. (2001) suggested that tortoises may have reduced clutch sizes, or may cease egg production during stages of acute infection. .

We observed lower than expected numbers of tortoises at a number of sites and as a result were unable to sample our target of 30. However, all tortoises at the Mitchell County site (n=7) were positive for exposure to *M. agassizzi* and two tortoises (40%) were also positive for *M. testudineum*. Two tortoises at the site had nasal lesions consistent with past URTD, but no clinical signs were observed. Similarly in Camden County and Ft. Gordon, sample sizes were

small. All tortoises trapped at the Camden County site and at Ft. Gordon tested negative for exposure to *M. agassizzi*. No tortoises at either site had clinical signs, but both had tortoises seropositive for *M. testudineum*. Two tortoises at Ft. Gordon had lesions consistent with previous URTD yet no antibodies to *M. agassizzi* were detected. These nasal lesions could have been due to *M. testudineum* infection or some other pathogen. Data from this site highlights that presence of URTD signs does not indicate that the tortoise or population is infected with *M. agassizii*. Two of our study site populations were subsequently translocated (Lowndes and Telfair B). Tortoises at both sites were negative for antibodies to *M. agassizii*, but positive for *M. testudineum*. Our data indicate *M. agassizzii* is endemic in several populations in Georgia. Ideally, no *Mycoplasma* positive tortoises should be relocated; however, at one site, a few native and/or waif tortoises (tortoise of unknown origin turned in to authorities such as state agencies) of unknown *Mycoplasma* status were already present. This is concerning because waif tortoises could be at an increased risk of infection themselves, or be a source of infection to naïve tortoises (McLaughlin, 2000).

The gopher tortoise is one of the most commonly translocated animals (Tuberville et al., 2011); therefore, it is important to understand the distribution of pathogens in populations. Multiple strains of *M. agassizii* have been documented (Wendland et al., 2010) and there is also a likelihood of geographic variation in virulence (Seigel et al., 2003). This study highlights the importance of continued monitoring of tortoise health because our results differed from previous studies in several important ways. In Florida, antibodies to *M. agassizii* were widespread and prevalence rates varied considerably (Diemer- Berish, 2000; Wendland, 2007; Berish, 2010), whereas in Georgia, the prevalence trend for antibodies to *M. agassizii* was either very high or very low/absent. *M. testudineum* is also a confirmed etiologic agent for URTD (Brown et al.,

2004). However, it has been hypothesized that *M. testudineum* could be less pathogenic than *M. agassizii* in desert tortoises (Jacobson and Berry, 2012). Upon necropsy in the Jacobson and Berry (2012) study, tortoises infected with *M. testudineum* had less severe lesions than those seen in *M. agassizii*. In our study, clinical signs were not seen at sites that were seropositive for only *M. testudineum.* The relationship between the two pathogens is unclear. However, we observed that sites with both pathogens had tortoises that exhibited clinical disease. Further surveillance for *Mycoplasma* spp., in addition to other pathogens, in more tortoise populations throughout the range is warranted. Long term studies need to be initiated, particularly in populations of management concern, in order to understand the consequences of disease and to fill in knowledge gaps concerning behavior and reproduction.

**Acknowledgments**

We thank Elizabeth Miller, Mallory Chronic, Will McGuire, and numerous personnel at the Jones Ecological Research Center for assistance in the field. We also thank landowners who provided access to their properties and sampling of tortoises. Funding for this project was provided by the Morris Animal Foundation (D13ZO-015), Gopher Tortoise Council, Sigma Xi, Sophie Danforth Conservation Biology Fund, Southeastern Cooperative Wildlife Disease Study through sponsorship with member states, Joseph W. Jones Ecological Research Center, and the D.B. Warnell School of Forestry and Natural Resources at The University of Georgia.

**Literature Cited**

Auffenberg W and Franz R. 1982. The status and distribution of the gopher tortoise *Gopherus polyphemus*. In North American tortoises: Conservation and ecology, ed. R.B. Bury. US Fish Wildl. Serv., Wildl. Res. Rep., 12: 95-126.

Ballou A. 2013. Aspects of gopher tortoise (*Gopherus* polyphemus) populations in Georgia: status, landscape predictors, and juvenile movements and burrow use. MSc. Thesis. University of Georgia, Athens, GA, 96pp.

Beyer S M. 1993. Habitat relations of juvenile gopher tortoises and a preliminary report of upper respiratory tract disease (URTD) in gopher tortoises. M.S. Thesis, Iowa State University, Ames, Iowa, 95 pp.

Brown DR. 2002. Mycoplasmosis and immunity of fish and reptiles. Frontiers in Bioscience 7: 1338-1346

Brown D R, Merritt JL, Jacobson ER, Klein PA, Tully JG, and Brown, MB. 2004. *Mycoplasma testudineum* sp. nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. Int J Syst Evol Microbiol 54: 1527–1529.

Brown DR, CrenshawBC, McLaughlin GS, Schumache IM, McKenn CE, Klein, PA, Jacobso ER, and Brown MB. 1995. Taxonomic analysis of the tortoise mycoplasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16S RNA gene sequence comparison. Int J Syst Bact 45: 348-350.

Brown, M. B., G. S. McLauglin, P. A. Klein, B. C. Crenshaw, I. M. Schumacher, D. R. Brown, and E. R. Jacobson, 1999. Upper Respiratory Tract Disease in the gopher tortoise is caused by *Mycoplasma agassizii*. J. of Clin. Micro. 37: 2262–2269.

Brown, M. B., I. M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994.

*Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62: 4580-4586.

Cagle, F. R. 1939. A system of marking turtles for future identification. Copeia 1939:170-173.

Dickinson, V.M. , I. M. Schumacher, J. L. Jarchow, T.Duck, and C.R. Schwalbe. 2005. Mycoplasmosis in free-ranging desert tortoises in Utah and Arizona. J. Wildl. Dis. 41: 839-842.

Diemer, J.E. 1992. Demography of the tortoise *Gopherus polyphemus* in northern Florida. J.Herp. 26:281-289.

Diemer, J. E., and C. T. Moore. 1994. Reproduction of gopher tortoises in north-central Florida. Pages 129-137 In R. B. Bury and D. J. Germano (eds.). Biology of North American tortoises. National Biological Survey, Fish and Wildlife Research 13.

Diemer, J.E., L. D. Wendland, C. A. Gates. 2000. Distribution and prevalence of upper respiratory tract disease in gopher tortoises in Florida. J. Herpetology, Vol. 34:1

Diemer-Berish, J.E., L.D. Wendland, R.A. Kiltie, E. P. Garrison and C.A. Gates. 2010. Effects of mycoplasmal upper respiratory tract disease on morbidity and mortality of gopher tortoises in northern and central Florida. Journal of Wildlife Diseases 46:695-705.

Eubanks, JO, Michener WK, and Guyer C.2003. Patterns of movement and burrow use in a population of gopher tortoise (*Gopherus polyphemus*). Herpetologica, 59 (2): 311-321.

Gates CA, Allen MJ, Diemer Berish JE, Stillwaugh DM, and Shattler SR. 2002. Characterization of a gopher tortoise mortality event in west-central Florida. – Fla Sci. 65: 159–184.

Hernandez, S.M., T. D. Tuberville, P. Frank, S.J. Stahl, M. M. McBride, K.A. Buhlmann, and S.J. Divers. 2010. Health and reproductive assessment of free-ranging gopher tortoise

(*Gopherus polyphemus*) population following translocation. J Herpetological Medicine and Surgery 20: 84- 93.

Iverson, J. B. 1980. The reproductive biology of *Gopherus polyphemus* (Chelonia: Testudinidae). Am Midl Nat 103:353-359.

Jacobson, E.R. and K.H. Berry. 2012. *Mycoplasma testudineum* in free-ranging desert tortoises, *Gopherus agassizii*. J Wildl Dis 48: 1063-1068.

Johnson, V. M., C. Guyer, S.M. Hermann, J. Eubanks, and W.K. Michener. 2009. Patterns of dispersion and burrow use support scramble competition polygyny in *Gopherus polyphemus*. Herpetologica 65: 214-218.

Kahn, P.F. 2006. The Physiological effects of relocation on gopher tortoises (*Gopherus polyphemus*). PhD Dissertation, Auburn University, Auburn, Alabama 192 pp.

Landers, J.L., W.A. McRae, and J.A. Garner. 1982. Growth and maturity of the gopher tortoise in southwestern Georgia. Bull Fl St Mus Bio Sci 28:81-110.

Lauerman, L.H., 1998. *Mycoplasma* PCR assays. In: Nucleic Acid Amplification Assays for Diagnosis of Animal Diseases. American Association of Veterinary Laboratory Diagnosticians, pp: 41-42.

Lockhart, J. M., G. Lee, J. Turco and L. Chamberlin, 2008. *Salmonella* from gopher tortoises (*Gopherus polyphemus*) in South Georgia. J Wildl Dis 44: 988-991

McCoy, E. D., H.R. Mushinsky, and J. Lindzey. 2007. Conservation strategies and emergent diseases: The case of upper respiratory tract disease in the gopher tortoise. Chel Conserv Biol 6:170-176.

McLaughlin, G. S. 1997. Upper respiratory tract disease in gopher tortoises, *Gopherus polyphemus*: Pathology, immune responses, transmission, and implications for

conservation and management. Ph.D. Dissertation, University of Florida, Gainesville, Florida, 110 pp.

McRae, W. A., J. L. Landers, and G. D. Cleveland. 1981. Sexual dimorphism in the gopher tortoise (*Gopherus polyphemus*). Herpetologica 37:46-52.

Rostal DC, Grumbles JS, and Lance VA. 2001. Reproduction and URTD in the desert tortoise (*Gopherus agassizii*): an eight year follow-up. In: *Proceedings of the 26th annual meeting and symposium of the Desert Tortoise Council*, Tucson, Arizona, 16-18 March, www.deserttortoise.org/abstracts/abstracts2001/2001abs34.html. Accessed April 2013.

Sandmeier F.C., C.R. Tracy CR, S. duPre, K. Hunter. 2009. Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: A reevaluation. Biol Conserv142: 1255-1268

Seigel, R. A., R.B. Smith, and N. A. Seigel. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. J Herp 37:137-144.

Tuberville, T.D., T.M. Norton, B.D. Todd and J.S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: A comparison of newly released and previously established animals. Biol Conserv 141:2690-2697.

Tuberville, T.D., T.M. Norton, B.J. Waffa, C. Hagen, T.C. Glenn. 2011. Mating system in a gopher tortoise population established though multiple translocations: apparent advantage of prior residence. Biol Conserv 144: 175-183.

Wendland, L. D. 2007. Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. PhD dissertation in *Infectious Diseases and Pathology*. Gainesville, FL, University of Florida**:** 178 pages.

Wendland, L.D., L.A. Zacher, P.A. Klein, D.R. Brown, D. Demcovitz, R. Littell, and M.R. Brown. 2007. Improved enzyme-linked immunosorbent assay to reveal *Mycoplasma agassizii* exposure: a valuable tool in the management of environmentally sensitive tortoise populations. Clin Vaccine Immunol 14: 1190-1195.

Wendland,L., H. Balbach, M. Brown, J. Diemer Berish, R. Littell, and M. Clark, 2009. Handbook on gopher tortoise (*Gopherus polyphemus*) gealth evaluation procedures for use by land managers and researchers. 82pp

Wendland, L., P.A. Klein, E.R. Jacobson, and M.B. Brown. 2010a. Strain variation in *Mycoplasma* agassizii and distinct host antibody responses explain differences between ELISA and Western blot assays. Clin Vaccine Immunol 17:1739-45.

Wendland, L.D., J. Wooding, C.L. White, D. Demcovitz, R. Littell, J. Berish, A. Ozgul, M.K. Oli, P.A. Klein, M.C. Christman, and M.B. Brown. 2010b. Social behavior drives the dynamics of respiratory disease in threatened tortoises. Ecology 91:1257-1262.

U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the gopher tortoise as threatened in the eastern portion of its range. Federal Register 76:45129-45162.

U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants; determination of threatened status for the gopher tortoise (*Gopherus polyphemus*). Federal Register 52(129):25376.

**Table 3.1. Characteristics of sites throughout Georgia in which gopher tortoises were sampled for prevalence of *Mycoplasma* spp.** Letters correspond to the county in which the sites are located (Figure 1).

| Site/County (County letter) | Ownership and land use | Approximate size and site characteristics |
|---|---|---|
| Jones Ecological Research Center (JERC)/Baker County (A) | Private, mixed research/hunting | 11,700 ha reserve, 22,000 of which ~6,880 ha of second growth longleaf pine/wiregrass forest and 2,020 ha of wildlife food plots. Surrounded by large-scale agriculture. |
| Forest Lodge Farms/ Mitchell County (B) | Private, silviculture | 607 ha pine plantation interspersed with hardwood stands and wildlife food plots. |
| Cedars Farm Plantation /Decatur County (C) | Private, hunting | 400 ha primarily longleaf pine/wiregrass forest. Surrounded by large-scale agriculture. |
| Orianne Indigo Snake Preserve (OISP)/ | Private, mixed research/hunting | 400 ha Indigo snake preserve. Sandhills with mixed loblolly pine/ hardwood. Longleaf pine restoration efforts underway. Surrounded by state-owned wildlife management areas. |
| Telfair County site B (D) | Private, agriculture | 200 ha mixed purpose agricultural land surrounded pine plantation and hardwoods. Tortoises were later relocated from this site. |
| Reed Bingham State Park (RBSP)/ | Public, state park | 650 ha Recreational area with a campground, 375 acre lake, and trails throughout primary tortoise habitat. |
| Ft. Gordon/ Richmond County (F) | Public, Department of Defense, military training | 13,118 ha open canopy pine forest with active artillery ranges/ artillery impact areas. |
| St. Mary's Airport/ Camden County (G) | Public, airport | 40 ha open grass habitat surrounded by dense pine/ mixed hardwoods, wetlands, and industrial development. |
| Moody Air Force Base/ Lowndes and Lanier Counties (H) | Public, Department of Defense, military training | 4,420 ha of which ~1,050 ha are mixed upland pine/ hardwood forest and ~2,225 ha are bottomland forest |
| Lowndes County (I) | Private, agricultural | 200 ha open field, mixed pine and mixed hardwoods. This was a waif site for rescued tortoises. Tortoises were later relocated from this site. |

**Figure 3.1. Map of Georgia showing counties where gopher tortoises were sampled.**

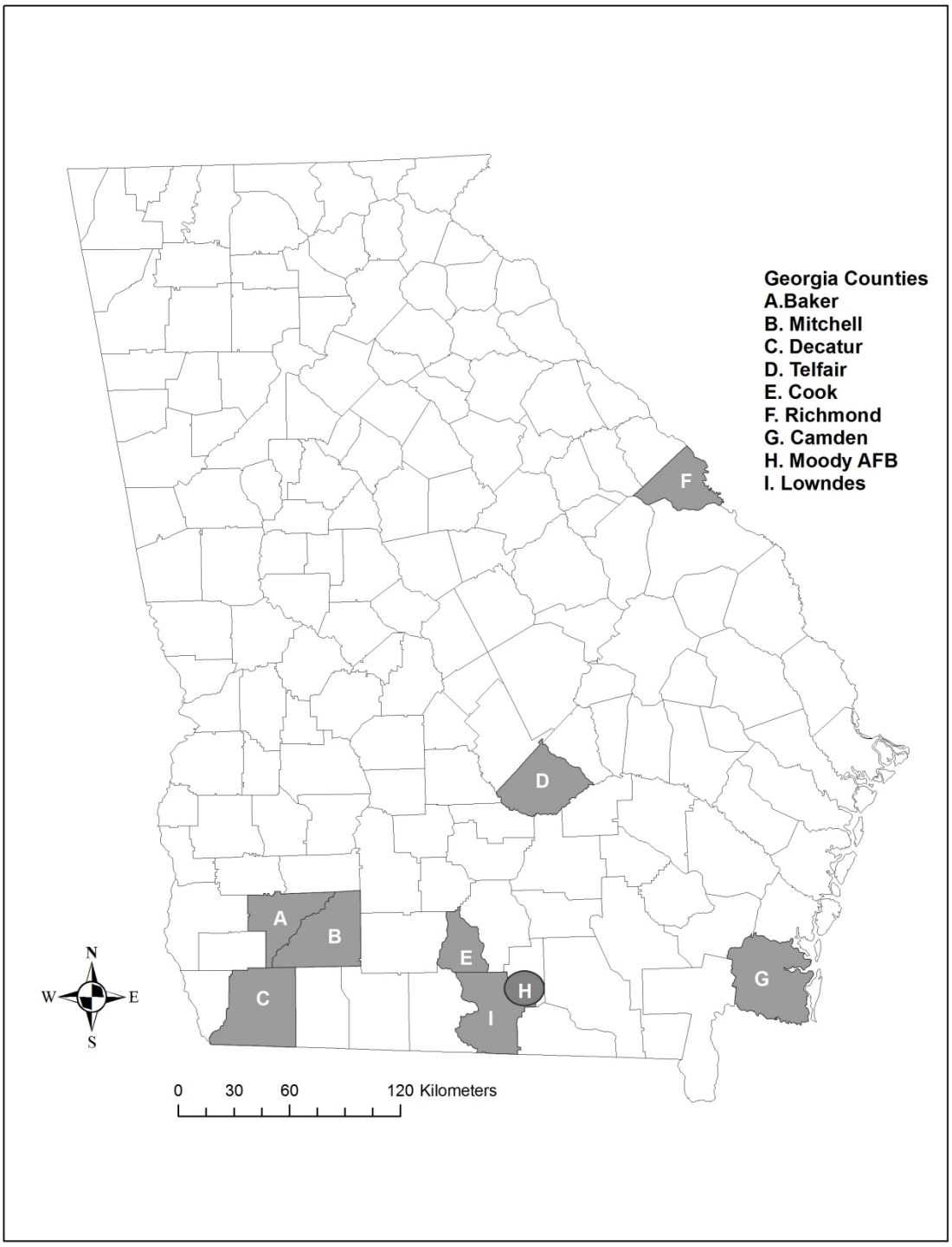

**Table 3.2. Demographic data for tortoises included in *Mycoplasma* surveillance. Baker 1997 data is not included.**

| Site/ County/ Base | Total | #Males | #Females | #Unknown Adults | Juvenile < 230 mm |
|---|---|---|---|---|---|
| JERC 2009-2012 | 191 | 94 | 73 | 13 | 11 |
| Mitchell | 7 | 2 | 5 | 0 | 0 |
| Decatur | 30 | 16 | 12 | 0 | 2 |
| OISP Telfair A | 35 | 10 | 21 | 3 | 1 |
| Telfair B | 27 | 15 | 11 | 1 | 0 |
| RBSP/ Cook | 35 | 13 | 15 | 6 | 1 |
| Ft. Gordon | 9 | 5 | 4 | 0 | 0 |
| Camden | 7 | 4 | 2 | 0 | 1 |
| Moody AFB | 100 | 86 | 71 | 0 | 0 |
| Lowndes | 11 | 5 | 6 | 0 | 0 |
| Total | 454 | 250 | 220 | 23 | 17 |

**Table 3.3. Serology and PCR (nasal exudates) results for *Mycoplasma* and prevalence of URTD in ten gopher tortoise populations in Georgia.**

| County/ Base | Map | Year | No. seropositive/No. tested (%) | | *Mycoplasma* No. PCR positive/No tested (%) | No. with clinical signs of URTD (%)* | No. with past URTD lesions (%)** |
|---|---|---|---|---|---|---|---|
| | | | *M. agassizii* | *M. testudineum* | | | |
| JERC | A | 1997 | 174/182 (96) | NT | NT | NT | NT |
| | | 2009-2012 | 125/136 (92) | 28/70 (40) | 48/129 (37 ) | 44/191 (23) | 101/366 (28) |
| Mitchell | B | 2010 | 7 /7(100) | 2/5 (40) | 0/1 | 1/7 (14) | 2/7 (29) |
| Decatur | C | 2010 | 29/30 (97) | 5/25 (20) | 2/6 (33) | 6/30 (20) | 4/30 (13) |
| OISP | Da | 2010 | 1/35 (3) | 14/30 (47) | NT | 0 | 3/36 (8) |
| Telfair B | Db | 2011 | 0/27 | 0/20 | NT | 0 | 0 |
| RBSP | E | 2010 | 34/35 (97) | 20/33(61) | NT | 3/35 (8) | 8/35 (23) |
| Ft. Gordon | F | 2010 | 0/9 | 3/8 (38) | NT | 0 | 2/9 (22) |
| Camden | G | 2010 | 0/7 | 4/7 (57) | NT | 0 | 0 |
| Moody AFB | H | 2000-2004 | 1/100 (1) | NT | NT | 2/100 (2%) | ND |
| Lowndes | I | 2012 | 0/11 | 5/11 (45) | NT | 0 | 2/11 (18) |
| TOTAL | | | 371/579 (64) | 81/209 (39) | 50/143 (35) | 56/162 (35) | 122/494 (25) |

*Clinical signs suggestive of URTD; however, no etiologic agent was determined as a cause for the clinical signs. Only JERC animals with at least one test, ELISA and/or PCR, were included.
**Past URTD lesions were either nasal scarring or nares abnormalities, again, no etiologic agent was identified for abnormalities. All JERC captures are included, but may not have been tested for *Mycoplasma*.
NT, not tested.

**Table 3.4. Serologic and molecular testing results and evidence for URTD for juvenile (CL< 23.0 cm) tortoises at four sites.**

| County/Base | *M. agassizii* ELISA | *M. testudineum* ELISA | *Mycoplasma* PCR | Clinical Signs | Scarring/ Lesions |
|---|---|---|---|---|---|
| Baker | 0/10* | 2/9 (22) | 1/2 (67) | 1/11 | 2/11 (18) |
| Decatur | 1/2 | 0/2 | NT | 0/2 | 1/2 (50) |
| Cook | 1/1 | NT | NT | 0/1 | 0/1 |
| Camden | 0/1 | 0/1 | NT | 0/1 | 0/1 |
| TOTAL | 2/14 (14) | 2/12 (17) | 2/3 (67) | 0/15 | 3/15 (20) |

NT, not tested.
*Number positive/Number tested (%)

**Table 3.5. Serologic and molecular testing results (number positive/percent tested) and presence of clinical signs or past lesions of URTD for gopher tortoises sampled at nine sites in Georgia.** Table is continued on next page.

| County/Base | *M. agassizii* | | *M. testudineum* | | PCR | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Baker | 79/80 *(98) | 59/60 (98) | 12/33 (36) | 12/25 (48) | 22/66 (33) | 21/49 (43) |
| Mitchell | 5/5 | 2/2 | 2/3 (67) | 0/2 | 0/1 | - |
| Decatur | 16/16 | 12/12 | 2/14 (14) | 3/9 (33) | 2/3 (67) | 0/3 |
| Telfair a | 0/10 | 0/21 | 3/9 (33) | 10/17 (59) | - | - |
| Telfair b | 0/15 | 0/11 | 0/10 | 0/9 | - | - |
| Cook | 13/13 | 14/15 (93) | 8/13 (62) | 10/14 (71) | - | - |
| Ft. Gordon | 0/4 | 0/4 | 1/4 | 1/4 | - | - |
| Camden | 0/4 | 0/2 | 2/4 | 2/2 | - | - |
| Lowndes | 0/5 | 0/6 | 1/5 | 4/6 | - | - |
| TOTAL | 113/152 (74) | 87/133 (65) | 31/95 (33) | 42/88 (47) | 24/70 (35) | 21/52 (40) |

**Table 3.5. continued. Serologic and molecular testing results and presence of clinical signs or past lesions of URTD for gopher tortoises sampled at nine sites in Georgia.**

| County/Base | Clinical Signs | | Scarring/ Lesions | |
|---|---|---|---|---|
| | M | F | M | F |
| Baker | 22/93 (24) | 19/72 (26) | 27/93 (29) | 23/72 (32) |
| Mitchell | 0/5 | 0/0 | 0/5 | 0/0 |
| Decatur | 0/16 | 3/12 (25) | 3/16 (19) | 0/12 |
| Telfair a | 0/10 | 0/21 | 0/10 | 1/21 (5) |
| Telfair b | 0/15 | 0/11 | 1/15 (7) | 1/11 (9) |
| Cook | 0/13 | 3/15(20) | 3/13 (23) | 2/15 (13) |
| Ft. Gordon | 0/5 | 0/4 | 1/5 (20) | 1/4 (25) |
| Camden | 0/4 | 0/2 | 0/4 | 0/2 |
| Lowndes | 0/5 | 0/6 | 0/5 | 0/6 |
| TOTAL | 22/166 (5) | 25/143 (17) | 35/166 (21) | 28/143 (20) |

# CHAPTER 4

# EFFECTS OF UPPER RESPIRATORY TRACT DISEASE ON MOVEMENT AND BEHAVIOR OF GOPHER TORTOISES (GOPHERUS POLYPHEMUS) IN A SOUTHWESTERN GEORGIA POPULATION[1]

[1] Gonynor- McGuire JL, Smith LL, Guyer C, and Yabsley MJ. To be submitted to the Journal of Wildlife Diseases.

**Abstract**

Impacts of disease on free-ranging wildlife populations are often poorly understood. We studied a gopher tortoise (*Gopherus polyphemus*) population in Georgia with a historically high prevalence of antibodies to *Mycoplasma agassizii* to assess the long-term effects of upper respiratory tract disease (URTD) on tortoise behavior. Health assessments, including serologic testing for antibiodies to two species of *Mycoplasma* spp., were performed on 136 tortoises at Ichauway, a private reserve in Baker County, Georgia. Of the 136 tortoises tested, 46 were from our core study area at Ichauway called Green Grove (GG). We radio-tracked thirty tortoises (16 adult males, 14 adult females) from GG to compare home range size to that of a previous study (1997) and monitored carapace temperature of these tortoises using data loggers to determine thermoregulatory behavior. An additional ten adult tortoises (6 males and 4 females) with severe clinical signs of URTD from elsewhere on the property were monitored for comparison with current data from tortoises from GG that were asymptomatic or had only mild symptoms of URTD. We found no significant difference in 95% minimum convex polygon (MCP) home range size between the GG 'asymptomatic' and 'mild' tortoises (mean 1.38ha). Home ranges of 'severe' tortoises were significantly larger (mean 166.75 ha) than asymptomatic and mild tortoises ($F= 5.60$, $df= 2$, $p= 0.0081$). Severe tortoises moved long distances over short periods of time, contradicting the hypothesis that chronically infected tortoises are less likely to emigrate. Prevalence of *M. agassizii* antibodies was similar among the three groups (98% overall), but prevalence of *M. testudineum* was lower in the asymptomatic (7%) and mild (14%) groups compared with the severe group (50%) ($p= 0.0019$). Variation in the average carapacial temperatures of severe tortoises varied significantly from temperatures of mild and asymptomatic tortoises ($H= 17.142$, $df= 2$, $p= 0.0002$), which suggests there were differences in

thermoregulatory behavior of severely ill tortoises. Our 15-year recapture data from GG suggests that, despite high seroprevalence, population density can remain constant over time. However, emigration of these animals, especially tortoises with clinical disease, may play an important role in dispersal and persistence of pathogens.

**Introduction**

The gopher tortoise (*Gopherus polyphemus*) is experiencing precipitous population declines throughout its range (Enge et al., 2006, Smith et al., 2006). Habitat loss is the greatest threat to tortoises. However, disease has been identified as an emerging threat (Berish, 2010; Wendland et al., 2010; Jacobson and Berry, 2012). Upper respiratory tract disease (URTD) in the gopher tortoise is a chronic disease that may impact population viability (Wendland, 2007; Ozgul et al., 2009; Perez-Heydrich et al., 2012). A number of pathogens have been associated with clinical signs of URTD including *Mycoplasma, Ranavirus*, and *Herpesvirus*, but among these, *M. agassizii* and *M. testudineum* are confirmed etiological agents that can cause mortality and are most often associated with disease (Jacobson, 1991; Brown et al., 1994; McLaughlin, 1997; Brown et al., 1999; Gates et al., 2002). To date, surveillance for these pathogens has been limited (Wendland, 2007; Johnson et al., 2010) with most studies focused on *Mycoplasma* spp.; however, much of this work has been restricted to *Mycoplasma* testing in Florida (Wendland, 2007; Berish et al., 2010).

*Mycoplasma* can be transmitted between tortoises through contact with nasal secretions (Brown et al., 1994, 1995, 1999; Wendland et al., 2007), which is important because tortoises frequently have direct, face-to-face contact during combat or courtship (Johnson et al., 2009). Based on seroprevalence data, it appears that transmission occurs as individuals reach sexual

maturity since antibodies to *Mycoplasma* are not frequently detected in juveniles (Wendland et al., 2010; J. McGuire et al., unpublished data, Chapter 3).

The long term impacts of URTD on gopher tortoise populations are unknown. Several factors such as how often a population experiences recurring epizootics, the length of time the population has been exposed, or other unknown factors may have long term negative ramifications (as summarized in Perez-Heydrich et al., 2012). Currently, there is considerable debate about the ecology and impact of the disease in free-ranging gopher tortoise populations (Seigel et al., 2003; McCoy et al., 2007; Sandmeier, 2009). Studies have shown that tortoises that might not be clinically ill, may maintain subclinical upper respiratory tract infection which results in extensive tissue damage over time (Jacobson et al., 1991; Shumacher et al., 1997; McLaughlin et al., 2000).

Cycles of convalescence and recrudescence of URTD have been confirmed in captive gopher tortoises (Brown et al., 1999b; Feldman, 2006), but to our knowledge, this phenomenon has not been evaluated in free ranging gopher tortoise populations. It is possible that gopher tortoises are only affected by URTD for a short period of time, after which they exhibit no clinical signs, but due to stress or other factors, can recrudesce at a later point and develop signs and shed bacteria (McLaughlin, 1997). Clinical signs can include nasal discharge, ocular discharge, nasal cavity obstruction, lethargy, emaciation, periocular and palpebral edema, and conjunctivitis (Jacobson et al., 1995; Shumacher et al., 1993; Shumacher et al., 1997; Seigel et al., 2003; Wendland, 2007). It is possible that populations with high seroprevalence for *Mycoplasma* will exhibit low incidence of clinical disease if they are experiencing low stress and occur on high quality habitats. For example, in Georgia, the seroprevelance of *Mycoplasma* in tortoises that were introduced to St Catherines Island in 1994 was 80%. Seroprevalence in this

population is currently 100%, but the incidence of clinical disease is low (Tuberville et al., 2008; T. Norton, Georgia Sea Turtle Center, personal communication). In contrast, a Florida population sampled in 1996 had low antibody prevalence, but high incidence of tortoises with clinical signs and high mortality (Epperson, 1997). At that time, *M. testudineum*, but not *M. agassizii*, was documented at the site; however, during follow-up sampling within the population, *M. agassizii* was also confirmed (Berish et al., 2010).

The social behavior of the gopher tortoise may facilitate transmission of pathogens such as *Mycoplasma* spp. (Johnson et al., 2009; Wendland et al., 2010). However, little is known about the manifestation of URTD or how it progresses within populations (Perez-Heydrich et al., 2012). In fact, clinical expression of disease may be intermittent, making it difficult to detect in a population without close monitoring (Brown, 2002; Wendland, 2007). Few studies have followed individuals long-term (Diemer-Berish et al., 2010; 2012) and even fewer studies have addressed the long-term impacts of disease persistence in a population. There is evidence that the majority of diseased tortoises die aboveground (Diemer-Berish et al., 2010). However, dead tortoises have been observed below ground in their burrows so mortality may go unnoticed (Seigel et al., 2003; DeGregorio et al., 2012; J. McGuire et al., unpublished data, Chapter 4). It has been hypothesized that chronically infected tortoises are less likely to emigrate than healthy animals (Ozgul et al., 2009) and thus, sick animals should be detectable in populations where they were exposed and are less likely to spread the pathogen to new populations.

To fully understand the effects of URTD on tortoise populations, it is crucial to monitor the behavior of individuals with the disease (Ozgul et al., 2009; Berish et al., 2010). To date, despite extensive testing of gopher tortoises in Florida for *Mycoplasma* and monitoring for URTD in tortoise populations, few evaluations of long-term survival of diseased tortoises have

been conducted (Berish et al., 2010). Importantly, URTD might not cause acute mortality in gopher tortoises, but rather, it may cause changes in movement patterns, foraging, basking, and hibernation, which could result in decreased fitness or fecundity. For example, data suggest that desert tortoises (*Gopherus agassizi*) with URTD bask during unusual times, remain above ground for abnormally long periods, and either fail to emerge or emerge late from hibernation (Berry and Christopher, 2001).

In the late-1990s, studies were initiated to investigate the home range size and behavior of gopher tortoises at Ichauway, the 11,600 ha research site of the Joseph W. Jones Ecological Research Center in Baker County, Georgia. Initial studies on movements and behavior of tortoises at Ichauway were focused in an approximately 49.5 ha area called Green Grove (GG; Figure 1) (Boglioli et al., 2000; Eubanks et al., 2003). Boglioli et al. (2000) reported a density of 1.3 tortoises/ha for the GG site and females had a mean home range size of 0.4 ± 0.08 ha, whereas mean home range size of males was 1.1 ± 0.13 ha (Eubanks et al., 2003). Eubanks et al. (2003) described very low dispersal out of the study area (only 3 of 75 males). In addition, blood samples from tortoises across Ichauway, including GG, were tested for the presence of antibodies for *M. agassizii*. At that time, 73 of 76 (96%) GG tortoises were positive for antibodies; overall prevalence across the site was equally high (J. McGuire et al., unpublished data, Chapter 3).

In 2011-2012, we were able to replicate methods used by Boglioli et al. (2000) and Eubanks et al. (2003) to compare seroprevalence of *Mycoplasma*, movements, and home range size of tortoises at GG 15-years after their initial evaluation. In addition, although severely ill tortoises have not been observed in the GG population, in the current study we were able to monitor movements of clinically ill tortoises from elsewhere on the property to look for

differences between these animals and those from GG. The specific goals of this study were to 1) assess seroprevalence and presence of clinical disease in a tortoise population with at least a 15 year exposure to *Mycoplasma* spp., 2) assess whether or not long-term exposure of tortoises to *Mycoplasma* spp. significantly altered movement patterns, such as home range size or fidelity of individual tortoises and 3) to compare movement and thermoregulatory behavior of apparently healthy (but seropositive) tortoises to those of tortoises with recrudescing clinical signs of URTD. We hypothesized that 1) a population with long term high seroprevalence would experience population changes such as decreased density, decreased site fidelity and smaller home range size, 2) tortoises with clinical disease would display limited movements across the landscape and 3) tortoises with clinical disease would experience significantly different carapacial temperatures than asymptomatic tortoises.

**Methods**

Study Area

This study was conducted at Ichauway, the 11,600 ha privately owned research site of the Joseph W. Jones Ecological Research Center in Baker County, Georgia (31º13′16.88″N and 84 º 28′37.81″W). Uplands at Ichauway are dominated by longleaf pine (*Pinus palustris)* and wiregrass (*Aristida stricta*) and are managed with prescribed fire on a two year return interval. Ichauway has a gopher tortoise population of greater than 4,800 individuals (L.L. Smith, unpublished data); however, our telemetry study and long term comparisons of movements and behavior was primarily focused within Green Grove (GG; Figure 1). Annual maximum air temperature at Ichauway is 33°C and the minimum air temperature is 2°C, with an annual average of 132.1 cm of rainfall (Georgia Automated Environmental Monitoring Network, 2012).

Green Grove Population Survey

In 2011, we resurveyed the gopher tortoise population at GG to determine the population density. We replicated the methods in the previous surveys (Boglioli et al., 2000; Eubanks et al., 2003), which included locating all burrows in the core GG site (Figure 1). Surveys were conducted with 1-3 observers who walked roughly parallel transects searching for burrows. At each burrow we searched for an ID tag from the previous surveys using a metal detector (Fisher Research Laboratory, Los Banos, CA) and we tagged any unmarked burrows with a unique ID number. Burrow location data were collected using a Nomad PDA Hand Held Computer (Trimble Navigation, Ltd., Sunnyvale, CA) with a Hemisphere Crescent GPS antenna (CSI Wireless, Calgary, AB, Canada) with sub-meter accuracy. Boglioli et al. (2000) and Eubanks et al. (2003) trapped all burrows to determine the number of tortoises; they also individually marked all tortoises by drilling marginal scutes of the carapace (Cagle, 1939). In our study, burrows were searched with a burrow camera (Sandpiper Technologies, Inc., Manteca, CA) to determine the number of tortoises. We calculated tortoise density (tortoises/ha) of the 49.5 ha core area of Green Grove to determine if the population density had changed over the 15-year period of record.

Pathogen Screening, Radio Telemetry and Temperature Activity Monitoring

Tortoises in the GG core area were trapped in May and June 2011 using wire cage traps (Tractor Supply, Brentwood, TN and Havahart®, Lititz, PA) covered with burlap for shade. Trapping continued until an approximately equal number of adult male (n=16) and adult female (n=14) tortoises (individuals >230 mm carapace length; Landers et al., 1980; McRae et al., 1981) were captured for radio-telemetry. Additionally, in 2011 and 2012 we opportunistically collected other tortoises encountered across the property. Ten tortoises that displayed clinical

signs of URTD (6 males and 4 females) were included in the radio-telemetry study. All tortoises were taken to the laboratory for processing, collection of biological samples, and transmitter attachment (J. McGuire et al., unpublished data, Chapter 3).

During processing, all tortoises were assessed for clinical signs suggestive of URTD (e.g., nasal exudates, swollen eyes, lethargy, labored/ wheezy breathing) as well as lesions suggestive of past URTD (e.g., nasal scarring and asymmetrical nares). For adult tortoises, sex was determined based on external morphology such as gular length, plastral concavity and anal notch to anal fork ratio (McRae et al., 1981; Eubanks et al., 2003). Blood (0.5- 4ml) was collected from either the caudal vein, brachial vein, or the subcarapacial venous sinus of tortoises (Wendland, 2007). The blood was centrifuged and the plasma was removed and immediately frozen at -80°C. Nasal exudates were collected from the nares of tortoises with nasal discharge using sterile rayon swabs (Puritan Medical Products Company LLC, Guilford, ME; Microbrush International, Grafton, WI) (McGuire, et.al., in press). After collection, swabs were placed in tubes and frozen at -80 ºC until testing. Equipment was disinfected with a 10% bleach solution between tortoises.

Tortoises unmarked at time of capture were marked by notching the marginal scutes (Cagle, 1939) with a Dremel™ Stylus hand tool (Dremel™, Racine, CA); identification numbers were also painted on the anterior and posterior portions of the carapace to facilitate recognition when using the burrow camera. Fourteen tortoises that were captured and radio-tagged in this study had been marked and radio-tracked previously by Boglioli et al. (2000) and Eubanks et al. (2003).

In the current study, tortoises were outfitted with radio-transmitters (American Wildlife Enterprises, Monticello, FL) attached to the right anterior marginal scutes using epoxy putty

(Rectorseal, EP-400, Houston, TX). To assess tortoise thermoregulatory behavior, Thermochron iButtons (Embedded Data Systems, DSL1922L-F5, Lawrenceburg, KY) were placed on the carapace of each tortoise using epoxy putty (DeGregorio, et al., 2012). Carapace temperature and internal body temperature are strongly correlated in turtles (Congdon, 1989; Grayson and Dorcas, 2004; Nussear, et al., 2007); thus, carapacial temperature readings are a good indication of thermoregulatory behavior of the tortoises. The iButtons used were capable of recording temperatures between -40°C and 85°C and were programmed to record temperature once every 60 minutes (which would allow approximately 8,000 readings). Combined weight of the transmitter and iButton was ~50 g, which was less than 5% of the tortoise's body mass. Animals were released within 48-h of capture at their original capture site.

Radio-tagged tortoises in GG were located once a week during the active season (June-September 2011 and April- June 2012) using a radio receiver (Communications Specialists Inc., Orange, CA) and a 3-element Yagi antenna (Wildlife Materials, Inc., Murphysboro, IL). In the inactive season (October 2011 – March 2012) tortoises were located at least once every two weeks. Tortoise locations were recorded using a Trimble Nomad GPS. For each location, we noted whether the tortoise was above ground or below ground in a burrow. Incidentally captured tortoises that exhibited severe clinical signs of URTD were tracked every 1-3 days until they settled in the same burrow for at least two weeks, after which they were located weekly. If the tortoises became moribund they were humanely euthanized by a veterinarian and submitted for necropsy to the Southeastern Cooperative Wildlife Disease Study (Athens, GA) or Auburn University diagnostic lab (Auburn, AL) for necropsy.

Pathogen Testing

Plasma samples were submitted to the *Mycoplasma* research laboratory at the University of Florida College of Veterinary Medicine, Department of Infectious Diseases and Pathology (Gainesville, FL) for *Mycoplasma* ELISA testing. Results for both *M. agassizii* and *M. testudineum* were grouped into one of three classes based on antibody titers: Positive (titer ≥ 64), Negative (titer < 32) and Suspect (titer = 32-63) (Wendland et al., 2007). To test for presence of *Mycoplasma* spp. in nasal exudates, polymerase chain reaction (PCR) targeting the 16S rRNA gene of *Mycoplasma* spp. was conducted; positive PCR results from nasal exudate is evidence of current infection and shedding of the pathogen. Genomic DNA was extracted from the swabs according to manufacturer's protocols (Qiagen DNA purification Kit, Germantown, MD). Each sample was tested in duplicate for *Mycoplasma* by PCR using primers GMF-1 (5'-ACACCATGGGAGCTGGTAAT) and GMR-1 (5'-CCTCATCGACTTTCAGACCCAAGGCAT) as described in Lauerman (1998).

All tortoise capture, handling, sample collection, and euthanasia methods used were evaluated and approved by The University of Georgia's Animal Care and Use Committee (AUP #A2009 6-112) and were conducted under scientific collection permits issued to the Jones Ecological Research Center (#29-WBH-09-151) and SCWDS, University of Georgia College of Veterinary Medicine (#29-WBH-10-46) by the Georgia Department of Natural Resources.

Data Analysis

All radio-tagged tortoises were categorized as either 'asymptomatic' (no clinical signs), 'mild' (clinical signs included clear nasal discharge, no eye swelling, no lethargy, normal retreating behavior), or 'severe' (clinical signs included cloudy nasal exudates, eye swelling and/or conjunctivitis, lethargy, labored open mouth breathing, or audible breathing; Figure 2,

McGuire et al., unpublished data, Chapter 2). Seroprevalence rates for *Mycoplasma* spp. among tortoises categorized as asymptomatic, mild, and severe were compared using a Fisher's exact test for independence.

Home range size (100 % MCP and 95% MCP; Hayne, 1949) was calculated for radio-tagged tortoises using Home Range Tools (HRT) for ArcGIS (Rodgers et al., 2007). We were unable to track tortoises with severe clinical disease for a full year, thus, our calculations of MCPs for these individuals was simply to allow a standard means of comparing among groups. Data from 1997-1998 for the 14 tortoises that were recaptured in our study were converted from North American Datum (NAD) 27 to NAD 83 and 95% MCPs were subsequently calculated using HRT. For each tortoise, the number of different burrows used and the minimum, maximum, and average distance moved between tracking locations was calculated in ArcGIS using XTools Pro 9 (ESRI). We tested for differences in mean home range size (95% MCP) and mean number of burrows used between GG males and females, and GG tortoises in 1997 and the current study, using ANOVA and a series of individual t-tests. We also compared mean MCP and distance moved among tortoises from GG in 2011-2012 (asymptomatic and mild) with those categorized as having severe clinical signs of URTD using ANOVA. We used ANOVA to determine if home range size (areas used) of tortoises in the current study varied by health status (asymptomatic, mild, or severe) and by sex (SAS version 9.3, SAS Institute, Inc., Cary, NC).

Temperature data from ibuttons were downloaded and imported into an Oracle database/SQL (Oracle Corporation, Redwood Shores, CA) for analyses. We calculated the mean temperature, standard deviation, and frequency of data points collected per hour for all time intervals for which we had temperature records for ≥10 individual tortoises. If the temperature reading for a tortoise fell outside one standard deviation of the overall mean temperature, we

considered this as “abnormal.” Frequency of abnormal temperatures was reported as a percentage of times each tortoise’s temperature fell outside one standard deviation. We compared burrow use and the frequency of abnormal temperatures among asymptomatic, mild, and severe URTD tortoises using a Kruskal- Wallis one-way ANOVA with Dunn’s multiple comparisons test using InStat (GraphPad Software, Inc., La Jolla, CA).

**Results**

Green Grove Population Survey

A total of 444 burrows (290 new/unmarked and 154 marked) were located during the Green Grove survey. We observed 103 tortoises resulting in an estimated density of 2.08 tortoises/ha. In 2011 and 2012, 79 tortoises (64 adults, 15 juveniles) were trapped in GG. Of the 64 adults trapped (36 males, 26 females, 2 unknown), 49 (76%) were recaptures from the previous study (Eubanks et al., 2003).

Pathogen Screening

Across Ichauway, the prevalence of *M. agassizii* was 92% (n=136) and 40% (n=70) for *M. testudineum* (J.L. McGuire, unpublished data, Chapter 3). All but one tortoise was seropositive for *M. agassizii* in GG (asymptomatic and mild group combined) and only two GG tortoises were seropositive for *M. testudineum*. All tortoises in the severe group were seropositive for *M. agassizii* and three were positive for *M. testudineum*. At the GG site, there was no significant difference in seroprevalence between asymptomatic and mild groups (Fisher’s Exact Test, p= 0.6044), but the *M. testudineum* seroprevalence was significantly higher in the mild group compared with the asymptomatic group (Fisher’s exact test, p= 0.0019) and the severe group compared with the asymptomatic group (p= 0.0276). The “suspect” results were not included because these individuals would need to be retested and we were not able to do that in

the current study. Based on unpublished data from 1997, only one GG recaptured tortoise had a change in *M. agassizii* exposure status from negative to positive (this tortoise was a juvenile in 1997). All other recaptured GG tortoises with prior serology (n=11) were positive in 1997 and in the current study.

All five (100%) nasal swabs from severe tortoises were PCR positive for *Mycoplasma*; this provides evidence of current infection and shedding of the pathogen. Five out of six (83%) nasals swab samples obtained from tortoises with mild clinical signs were PCR positive. Asymptomatic tortoises were not tested for shedding because nasal exudates were not present at the time of sampling. Sequencing data are pending for most, but tortoise 1149A was PCR positive for *Mycoplasma* spp. and sequencing confirmed that it was *M. agassizii*. Three severe tortoises were tracked for >300 days and each of these tortoises experienced recrudescence of clinical signs (Table 2) between tracking events. For example, 2036A displayed severe clinical signs at 74% of the observations (no clinical signs were observed 26% of the time), and was observed above ground a number of times at night (Table 2). Tortoise 1546A displayed severe clinical signs 67% of the above ground observations but no clinical signs during 33% of the observations. Tortoise 1149A had clinical signs 82% of the time, but none during 18% of the observations. Three of the severe tortoises (1149A, 1518A, and 2104A) were euthanized because their symptoms progressed to where they were considered morbidly ill. *Mycoplasma* related URTD was determined to be the cause of morbidity based on gross and histological lesions (McGuire et al., unpublished data, Chapter 3).

Radio Telemetry and Temperature Activity Monitoring

Radio-tagged tortoises in GG (16 males, 14 females) were tracked an average of 32 times from June 2011 through July 2012. We found no significant difference between 95% MCP and

100% MCPs (t= 1.140, df= 38, p= 0.2612); however, both estimates were calculated to allow comparison of our data to that of Eubanks et al. (2003). We found a significant difference in home range size (95% MCP) between GG males (n=16; 1.87ha ± 1.64) and GG females (n=14; 0.81ha ± 0.73) (t= 2.21, df= 28, p= 0.0.035; Table 3). Home range size differed among tortoises by health status (F=5.60, df= 2, p= 0.0081). Post-analysis Tukey test revealed a significant difference in the average MCP among severe tortoises and the other two groups. The mean MCP of the nine clinically ill tortoises (134.71 ha) was larger than that of asymptomatic and mild tortoises (Table 4, Figure 3) and there was considerable variation among individuals (range= 0.09- 489.69 ha, SD= 181.12).

The maximum distance GG males and females moved during the study was not significantly different (t= 0.40, df= 28, p= 0.68) with an average maximum distance for males and females being 152 ± 91.85 m and 140 ± 62.68 m, respectively. Also, the mean distance between locations was not significantly different between males (31.69 ± 23.78 m) and females (30.00 ±19.65) (t= 0.21, df= 28, p= 0.83). The overall range of distances moved between locations for GG tortoises was between 8.75 and 110.44 m. There was no significant difference in mean home range size (95% MCP) of 14 tortoises tracked between 1997-1998 and 2011-2012 (F= 0.24, df= 1, p > 0.628), either by time period or between sexes. All 14 tortoises recaptured were found in the same general area within GG as the 1997 study. The 30 telemetered GG tortoises were only observed above ground nine times (<1% of all observations) during the current study.

Ten tortoises (four females and six males) with severe clinical signs of URTD were radio-tracked between three and 334 days. The tortoise tracked for only 3 days was not included in the analyses. Four of the tortoises (1083A, 1149A, 1518A, 2104A) were ultimately

euthanized, three due to the severity of their clinical signs and a fourth as a result of injuries from being hit by a car. One tortoise (2033A) died in its burrow after 73 days of monitoring. Transmitters and iButtons were removed from the four surviving severe tortoises at the completion of the study and they were released at the site of capture (Table 2).

Severe tortoises made many long distance movements over short periods of time with distances moved ranging from 165 m to 3,498 m as compared to the GG group where the maximum distances moved ranged from 78.49 to 147.29 m. The longest documented movement in a day was a severe tortoise (Tortoise 1546A) that moved 755 m. On average asymptomatic and mild tortoises used 11 and 19 burrows, respectively. Severe tortoises used significantly fewer burrows (0-4) than asymptomatic and mild tortoises ($H= 20.298$, $df= 2$, $p$ 0.000039). Asymptomatic and mild tortoise burrow use did not differ significantly ($p> 0.05$, Dunn's Multiple Comparison). However, severe tortoise burrow use compared to both asymptomatic and mild tortoises was significantly different ($p < 0.001$, Dunn's Multiple Comparison). One tortoise, 1083A, was never observed using a burrow and two tortoises (2101A and 2033A) were observed using only one burrow each.

Tortoise temperatures

A total of 45 weeks of temperature data were obtained from 37 radio-tagged tortoises (29 asymptomatic and mild tortoises from GG and eight severe tortoises across the site). Average temperatures were calculated for the period from 13 June 2011- 29 January 2012 and 30 April – 22 July 2012). Temperatures of severe tortoises deviated from the average significantly more often than both the mild and asymptomatic groups ($H= 17.88$, $df= 2$, $p= 0.000131$; Figure 4). Using Dunn's multiple comparisons test, we found no difference between asymptomatic and mild tortoises ($p > 0.05$); however, severe tortoises had significantly higher temperature variation

when compared with the asymptomatic group ($p < 0.001$) and mild group ($p < 0.05$). The greatest temperature range experienced by a severe tortoise was from 9.56°C to 42.56°C over the 311 days it was tracked (tortoise 2036A; Table 2).

**Discussion**

We found that gopher tortoises from a population with consistently high seroprevalence for *Mycoplasma agassizii*, which were asymptomatic or had mild symptoms of URTD, exhibited stable home range sizes over a 15 year period. However, gopher tortoises from the same property that had severe clinical signs of URTD had significantly larger home ranges compared with asymptomatic and mildly symptomatic tortoises. Additionally, tortoises with severe clinical signs of URTD experienced greater deviation in carapacial temperatures than asymptomatic tortoises or mildly symptomatic tortoises, which suggests abnormal thermoregulatory behavior. Tortoises with clinical signs of URTD, especially when they were severe, spent more time out of their burrow, basking (i.e., fever; Kluger, 1986), than tortoises with no clinical signs, or conversely, spent more time in burrows (anapyrexia) than asymptomatic tortoises. Either behavioral change can lead to decreased condition and health. Nearly all of the tortoises in our study population tested positive for antibodies to *M. agassizii*, but the prevalence of antibodies to *M. testudineum* was higher in the severe URTD group which suggests that this species of *Mycoplasma*, or coinfection with the two pathogens, may play a role in the expression of clinical disease (McGuire et al., unpublished data, chapter 3) and alter behavior.

Our results were consistent with studies that show diseased chelonians can exhibit behavioral changes (Monagas and Gatten, 1983; Amaral et al., 2002; Swimmer, 2006). For example, box turtles (*Terrapene carolina*) infected with *Escherichia coli* selected higher temperature gradients at which to bask than uninfected control animals (Amaral et al., 2002).

Behavioral fever is thought to increase rates of survivorship in sea turtles affected by fibropapillomatosis (Swimmer, 2006). It is possible that clinically ill gopher tortoises use a similar physiological strategy. Other reptiles, such as snakes, have also shown a similar response to infection with *Aeromonas sobria* (Burns et al., 1996). Similar behavior has been noted in sick passerines (Adelman et al., 2010); infected birds moved to perches with more sun exposure.

One of our significant findings was that, on average, gopher tortoises with severe clinical signs had significantly larger home ranges than asymptomatic tortoises. However, it is important to note that there was a great deal of variation among individuals and a few severely ill tortoises that had very small home ranges (Figure 3). For tortoises, home range size is a function of resource requirements of individuals, and can vary seasonally or based on annual variation in weather parameters such as drought (Duda et al., 1999). It is not clear why the majority of our tracked severely ill gopher tortoises used such large areas; however, our findings refute the hypothesis that chronically URTD infected tortoises are less likely to emigrate (Ozgul et al, 2009). In fact, our data show that some sick tortoises may be more likely to emigrate or exhibit abnormal dispersal behavior as compared with asymptomatic tortoises which could result in lower detection probability of severely ill or dead tortoises at an individual site. Importantly, this increased area use and/or long distance movements would increase risk of contact between diseased tortoises and healthy animals. Tortoises that move over long distances may also be more likely to encounter roads, and thus, more susceptible to being hit by motor vehicles.

We also found that, despite high seroprevalence of *Mycoplasma* sp., tortoise density increased at the GG site. Some of this difference in tortoise density may be a reflection of the additional buffer incorporated into the 1997 study (total area of 100 ha versus 49.5 ha core area) or to differences in survey techniques (trapping burrows versus use of a camera scope). The

difference may also be a result of new individuals immigrating into the GG area and recruitment of juveniles into the population. Regardless, it does not appear that the GG area has experienced a population decline related to URTD. Impacts of URTD on the Ichauway population as a whole are unknown.

The 76% recapture rate at GG in our study demonstrates high site fidelity. Diemer-Berish et al. (2012) reported only an 8% recapture rate after 27 years at a site in Florida. We suspect that the difference in recapture rates between Diemer-Berish et al. (2012) and our study is related to differences in land use. Ichauway is intensively managed for conservation of the long leaf pine/ wiregrass ecosystem, with both frequent prescribed fire and occasional hardwood removal, whereas the Florida site was managed for timber production and consisted of slash-pine plantation and clear-cuts. However, tortoises may emigrate and immigrate more often than we previously suspected as there is a paucity of long term monitoring data on gopher tortoise populations (Diemer- Berish et al., 2012).

Our data supports previous reports (from captive animals) that clinical signs of URTD may recrudesce, which is likely to be more pronounced during stressful conditions such as drought, habitat loss, land use or increased density (McLaughlin, 1997; Diemer- Berish et al., 2000; Wendland et al., 2010; Cowled et al., 2012). It is important to understand the cyclic nature of the disease in gopher tortoise management. When planning for relocation managers need to understand that a single medical evaluation may indicate that tortoises are clinically normal but they may recrudesce. Also, as observed in this study, tortoises with severe clinical disease are capable of making long distance movements across the landscape and as sick tortoises are likely shedding bacteria, they could transmit the pathogen to other tortoises, and potentially other chelonians (Feldman, 2006). In our study, this cycle of disease coincided with the greatest

movement of tortoises. Care needs to be taken to consider the potential impact to the resident population of tortoises in areas to which they move. The results of this study highlight the potential risk of releasing sick tortoises in the wild and the importance of monitoring populations over time to understand the impacts of disease (Perez-Heydrich et al., 2012). Gopher tortoise populations with high seroprevalence of *Mycoplasma* can remain stable over time which is an important finding; however, emigration of clinically ill tortoises may play an important role in dispersal and persistence of pathogens.

**Acknowledgments**

We thank Will McGuire for refurbishing the transmitters and for assistance in the field, Jean Brock for her GIS expertise, and Micheal Simmons and Mike Conner for assistance with iButton data analysis, and staff at the Jones Ecological Research Center for being on the look-out for tortoises. Funding for this project was provided by the Morris Animal Foundation (D13ZO-015), Gopher Tortoise Council, Sigma Xi, Sophie Danforth Conservation Biology Fund, Southeastern Cooperative Wildlife Disease Study through sponsorship with member states, Joseph W. Jones Ecological Research Center, and the D.B. Warnell School of Forestry and Natural Resources at The University of Georgia.

**Literature Cited**

do Amaral, J.P.S., G.A. Martin, and V.H. Hutchison. 2002. The influence of baterical lipopolysaccharide on thermoregulation of the box turtle *Terrapene carolina*. Physiological and Biochemical Zoology 75: 273-282.

Berry, K.H., and M. M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Disease 37: 427–450.

Boglioli, M.D., W.K. Michener, and C. Guyer. 2000. Habitat selection and modification by the gopher tortoise, *Gopherus polyphemus*, in Georgia longleaf pine forest. Chelonian Conservation Biology 3:699-705.

Brown, D.R., Crenshaw, B.C., McLaughlin, G.S., Schumacher, I.M., McKenna, C.E., Klein, P.A., Jacobson, E.R., and Brown, M.B. 1995. Taxonomic analysis of the tortoise mycoplasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16S RNA gene sequence comparison. International Journal of Systematic Bacteriology 45: 348-350.

Brown, D.R. 2002. Mycoplasmosis and immunity of fish and reptiles. Frontiers in Bioscience 7: 1338-1346

Brown, M. B., G. S. McLauglin, P. A. Klein, B. C. Crenshaw, I. M. Schumacher, D. R. Brown, and E. R. Jacobson, 1999. Upper respiratory tract disease in the gopher tortoise is caused by *Mycoplasma agassizii*. Journal of Clinical Microbiology:2262–2269.

Brown, M. B., I. M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infection and Immunity 62: 4580-4586.

Burns, G., A. Ramos, and A. Muchlinski. 1996. Fever response in North American snakes. Journal of Herpetology. 30:133-139.

Cagle, F.R. 1939. A system of marking turtles for future identification. Copeia 1939: 155–162.

Congdon, J.D., R.E. Gatten, and S.J. Morreale. 1989. Overwintering activity of box turtles (*Terrapene carolina*) in South Carolina. Journal of Herpetology 23:179-181.

Cowled, B. D., Ward, M. P., Laffan, S. W., Galea, F., Garner, M., MacDonald, A. J., and Sarre, S. D. 2012. Integrating survey and molecular approaches to better understand wildlife disease ecology. Plos ONE, 7(10), 1-8.

DeGregorio, B.A., K.A. Buhlmann, and T.D. Tuberville. 2012. Overwintering of gopher tortoises (*Gopherus polyphemus*) translocated to the northern limit of their geographic range: temperatures, timing, and survival. Chelonian Conservation and Biology 11:84-90.

Diemer, J.E. 1992. Demography of the tortoise *Gopherus polyphemus* in northern Florida. Journal of Herpetology 26: 281-289.

Diemer- Berish, J.E., L. D. Wendland, C. A. Gates. 2000. Distribution and prevalence of upper respiratory tract disease in gopher tortoises in Florida. Journal of Herpetology Vol. 34:5-12

Diemer-Berish, J.E., L.D. Wendland, R.A. Kiltie, E. P. Garrison and C.A. Gates. 2010. Effects of mycoplasmal upper respiratory tract disease on morbidity and mortality of gopher tortoises in northern and central Florida. Journal of Wildlife Diseases 46:695-705.

Diemer-Berish, J.E., R.A. Kiltie, and T.M. Thomas. 2012. Long-term population dynamics of gopher tortoises (*Gopherus polyphemus*) in a pine plantation in northern Florida. Chelonian Conservation and Biology 11:50-58.

Duda, J.T., A.J. Krzysik, and J.E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. The Journal of Wildlife Management 63: 1181-1192.

Enge, K. M., J. E. Berish, R. Bolt, A. Dziergowski, and H. R. Mushinsky. 2006. Biological status report - gopher tortoise. Florida Fish and Wildlife Conservation Commission, Tallahassee.

Epperson, D. M. 1997. Gopher tortoise (*Gopherus polyphemus*) populations: Activity patterns, upper respiratory tract disease, and management on a military installation in northeast Florida. M.S. Thesis, University of Florida, Gainesville, Florida, 61 pp.

Eubanks, J. O., W. K. Michener, and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). Herpetologica 59:311-321.

Feldman, S.H., J. Wimsatt, R. E. Marchang, A. J. Johnson, W. Brown, J.C. Mitchell, and J.M. Sleeman. 2006. A novel *Mycoplasma* detected in association with upper respiratory disease syndrome in free-ranging eastern box turtles (*Terrapene carolina carolina*) in Virginia. Journal of Wildlife Diseases 42: 279-289.

Gates, C. A., M. J. Allen, J. E. D. Berish, D. M. Stillwaugh, AND S. R. Shattler. 2002. Characterization of a gopher tortoise mortality event in west-central Florida. Florida Scientist 65: 185–197.

Georgia Automated Environmental Monitoring Network, 2012. Accessed 3 December 2012, www.Georgiaweather.net.

Gibbons, J.W. 1986. Movement patterns among turtle populations: applicability to management of the desert tortoise. Herpetologica 42:104-113.

Grayson, K.L. and M.E. Dorcas. 2004. Seasonal temperature variation in the eastern painted turtle (*Chrysemys picta*). Herpetologica 60:325-336.

Guyer, C., V.M. Johnson, and S.M. Hermann. 2012. Effects of population density on patterns of movement and behavior of gopher tortoises (*Gopherus polyphemus*). Herpetological Monographs 26: 122-134.

Hayne, D.W. 1949. Calculation of size of home range. Journal of Mammalogy 30:1-18.

Jacobson, E.R. and K.H. Berry. 2012. *Mycoplasma testudineum* in free-ranging desert tortoises, *Gopherus agassizii*. Journal of Wildlife Diseases. 48: 1063-1068.

Jacobson, E.R., M. B. Brown, I. M. Schumacher, B. R.Collins, R. K. Harris, and P. A. Klein. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. Chelonian Conservation and Biology 1: 279–284.

Jacobson, E.R., J.M. Gaskin, M.B. Brown, R.K. Harris, C.H. Gardiner, J.L. LaPointe, H.P. Adams and C. Reggiardo. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). Journal of Wildlife Diseases 27: 296-316.

Johnson A.J., Wendland L., Norton T.M., Belzer W., Jacobson E.R., 2010. Development and use of an indirect enzyme-linked immunosorbent assay for detection of Iridovirus exposure in gopher tortoises (*Gopherus polyphemus*) and Eastern box turtles (*Terrapene carolina carolina*). Veterinary Microbiology. 142:160-167.

Johnson, V. M., C. Guyer, S.M. Hermann, J. Eubanks, and W.K. Michener. 2009. Patterns of dispersion and burrow use support scramble competition polygyny in *Gopherus polyphemus*. Herpetologica 65: 214-218.

Kluger, M. J. 1986. Is fever beneficial? The Yale Journal of Biology and Medicine. 59: 89-95.

Landers, J. L., J. A. Garner, and W. A. McRae. 1980. Reproduction of the gopher tortoise (*Gopherus polyphemus*) in southwestern Georgia. Herpetologica 36:353-361.

Lauerman, L.H., 1998. Mycoplasma PCR assays. In: Nucleic Acid Amplification Assays for Diagnosis of Animal Diseases. Am. Assoc. Vet. Laboratory Diagnosticians, pp: 41-42.

McCoy, E. D., H.R. Mushinsky, and J. Lindzey. 2007. Conservation strategies and emergent diseases: The case of upper respiratory tract disease in the gopher tortoise. Chelonian Conservation and Biology 6:170-176.

McGuire, J.L., S.M. Hernandez, L.L. Smith and M.J. Yabsley (accepted). Safety and utility of an anesthetic protocol for the collection of biological samples from gopher tortoises. Wildlife Society Bulletin.

McLaughlin, G. S. 1997. Upper respiratory tract disease in gopher tortoises, *Gopherus polyphemus*: Pathology, immune responses, transmission, and implications for conservation and management. Ph.D. Dissertation, University of Florida, Gainesville, Florida, 110 pp.

McLaughlin, G.S., E.R. Jacobson, D.R. Brown, C.E. McKenna, I.M. Schumacher, H.P. Adams, M.B. Brown, and P.A. Klein. 2000. Pathology of upper respiratory tract disease of gopher tortoises in Florida. Journal of Wildlife Diseases 36:272-283.

McRae, W.A., J.L. Landers and G.D. Cleveland. 1981. Sexual dimorphism in the gopher tortoise (*Gopherus polyphemus*). Herpetologica 37: 46-52

Monagas, W.R. and R.E. Gatten Jr. 1983. Behavioral fever in the turtles *Terrapene carolina* and *Chrysemys picta*. Journal of Thermal Biology. 3: 285-288.

Nussear, K.E., T.C. Esque, D.F. Haines, C.R.Tracy. 2007. Desert tortoise hibernation: temperatures, timing, and environment. Copeia 378-0386.

Ouedraogo, R.M., M.S. Goettel, and J. Brodeur. 2004. Behavioral thermoregulation in the migratory locust: a therapy to overcome fungal infection. Behavioral Ecology 138: 312-319.

Ozgul, A., M.D, Oli, B.B. Bolker and C. Perez-Heydrich. 2009. Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises. Ecological Applications 19:786-798.

Perez- Heydrich, C., M.K. Oli, and M.B. Brown. 2012. Population-level influence of a recurring diseases on a long-lived wildlife host. Oikos 121:377-388.

Rodgers, A.R., A.P. Carr, H.L. Beyer, L. Smith, and J.G. Kie. 2007. HRT: Home range toos for ArcGIS. Version 1.1. Ontario Ministry of Natural Resources, Centre for Northern Forest Ecosystem Research, Thunder Bay, Ontario, Canada.

Sandmeier F.C., C.R. Tracy CR, S. duPre, K. Hunter. 2009. Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: A reevaluation. Biological Conservation 142: 1255-1268

Seigel, R. A., R.B. Smith, and N. A. Seigel. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. Journal of Herpetology 37:137-144.

Schumacher, I.M., M.B. Brown, E.R. Jacobson, B.R. Collins, and P.A. Klein. 1993. Detection of antibodies to a pathogenic *Mycoplasma* in desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. Journal of Clinical Microbiology 31: 1454-1460

Schumacher, I.M., D. B. Hardenbrook, M.B. Brown, E.R. Jacobson, and P.A. Klein. 1997. Relationship between clinical signs of upper respiratory tract disease and antibodies to

*Mycoplasma agassizii* in desert tortoises from Nevada. Journal of Wildlife Diseases 33:261-266.

Smith, L.L. 1995. Nesting ecology, female home range and activity, and population sixe-class structureof the gopher tortoise, *Gopherus polyphemus*, on the Katharine Ordway Preserve, Putnam County, Florida. Bulletin of the Florida Museum of Natural History 73: 97-126.

Smith, L.L., T.D. Tuberville, and R.A. Seigel. 2006. Workshop on the ecology, status, and management of the gopher tortoise (*Gopherus polyphemus*), Joseph W. Jones Ecological Research Center, 16-17 January 2003: final results and recommendations. Chelonian Conservation and Biology 5:326-330

Swimmer, J.Y. 2006. Relationship between basking and fibropapillomatosis in captive green turtles (*Chelonia mydas*). Chelonian Conservation and Biology 5: 305-309.

Tuberville, T.D., T.M. Norton, B.D. Todd and J.S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: A comparison of newly released and previously established animals. Biological Conservation 141: 2690-2697

Wendland, L.D. 2007. Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. PhD Dissertation, University of Florida, Gainesville, Florida**.**

Wendland, L., P.A. Klein, E.R. Jacobson, and M.B. Brown. 2010. Strain variation in *Mycoplasma agassizii* and distinct host antibody responses explain differences between ELISA and Western blot assays. Clinical Vaccine Immunology 17:1739-1745.

**Table 4.1. *Mycoplasma testudineum* ELISA results for radio tracked Green Grove tortoises (asymptomatic and mild) and severe tortoises from across Ichauway, Baker County, Georgia in 2011-2012.** A positive result indicates that the tortoise had detectable antibodies and had been previously exposed (titer ≥64). A negative result means that there were no detectable antibodies to *Mycoplasma* at the time of the test (titer < 32). A suspect result is inconclusive and requires additional testing (titer=32).

| ELISA Result | Asymptomatic | Mild | Severe |
|---|---|---|---|
| Positive | 1 | 1 | 3 |
| Suspect | 10 | 2 | 2 |
| Negative | 3 | 4 | 3 |
| Total | 14 | 7 | 8 |

**Table 4.2. Individual radio-telemetry data for the Ichauway "severe" tortoise group with outcomes.** The range of temperatures are reported along with 1 standard deviation (SD) around the mean. Data were collected at Ichauway, in Baker County, Georgia in 2011 and 2012.

| Tortoise | Sex | Days Tracked | Days observed above ground (%) | Percent of time observed above ground with clinical signs | Home Range Size (ha) | Temperature °C Range ± SD | Tortoise Fate |
|---|---|---|---|---|---|---|---|
| 1083A | F | 3 | 3 (100) | 100% | 26.37 | * | Hit by car-euthanized |
| 1137A | M | 322 | 33 (10.2) | 9% | 0.15 | 22.09- 42.56 (3.56) | Released |
| 1149A | M | 334 | 23 (6.8) | 39% | 0.09 | 11.99- 38.16 (4.81) | Euthanized |
| 1518A | M | 122 | 7 (5.7) | 86% | 97.80 | 17.66- 45.60 (4.23) | Euthanized |
| 1546A | F | 43 | 33 (76.7) | 67% | 344.74 | 15.00-45.00 (4.85) | Released |
| 1670A | M | 53 | 2 (3.7) | 100% | 0.65 | 21.50- 44.51 (3.40) | Released |
| 2036A | F | 311 | 23 (7.4) | 74% | 250.24 | 9.56- 42.56 (5.9) | Released |
| 2101A | M | 80 | 3 (3.8) | 100% | 8.63 | * | Lost signal |
| 2104A | M | 79 | 5 | 80% | 483.69** | 20.7- 44.7 (3.75) | Euthanized |
| 2033A | F | 64 | 7 | 71% | - | 1.11- 30.19 (4.63) | Died in burrow |

* Data not available. **Tortoise was originally lost in June 2012 but the tortoise was relocated in July 2012 and the iButton was removed, but not the transmitter.

**Table 4.3. Mean home range size (HR: 95% MCP) and number of burrows used (BU) for gopher tortoises in Green Grove, at Ichauway, Baker County, GA in 1997-1998 [Eubanks et al. (2003) and 2011-2012 (current study)].** Means are reported ±1 SE; ranges are presented in parentheses. An asterisk indicates statistically significant differences (see text).

| Study | Female HR (ha) | Male HR (ha) | Female BU | Male BU |
|---|---|---|---|---|
| Eubanks et al. 2003<br>1997-1998 | 0.40 ± 0.08<br>(0.0-3.4)<br>n=53 | 1.10 ± 0.13<br>(0.0-4.8)<br>n=70 | 5.2* ± 0.3<br>(1.0-13.0)<br>n=53 | 10.0 ± 0.5<br>(2.0-22.0)<br>n=70 |
| This study<br>2011-2012 | 0.80 ± 0.73<br>(0.07- 2.54)<br>n=14 | 1.87 ± 1.64<br>(0.11- 5.47)<br>n=16 | 6.9* ± 0.7<br>(3.0-10.0)<br>n=14 | 8.4 ± 0.7<br>(5.0-15.0)<br>n=16 |

**Table 4.4. Mean deviation of average temperature and 95% MCP among asymptomatic, mild and severely URTD symptomatic gopher tortoises at Ichauway, Baker County, Georgia. SD= 1 standard deviation.**

| | **Asymptomatic Tortoises (n=19)** | | **Mild Tortoises (n=11)** | | **Severe Tortoises (n=8)** | |
|---|---|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range | Mean ± SD | Range |
| Deviation of temperature | 0.211 ± 0.06 | 0.10- 0.38 | 0.28 ± 0.10 | 0.12- 0.45 | 0.49 ± 0.17 | 0.28- 0.77 |
| 95% MCP (ha) | 1.16 ± 1.23 | 0.05 - 4.98 | 1.76± 1.61 | 0.10- 5.47 | 172.86± 275.42 | 0.09± 344.74 |

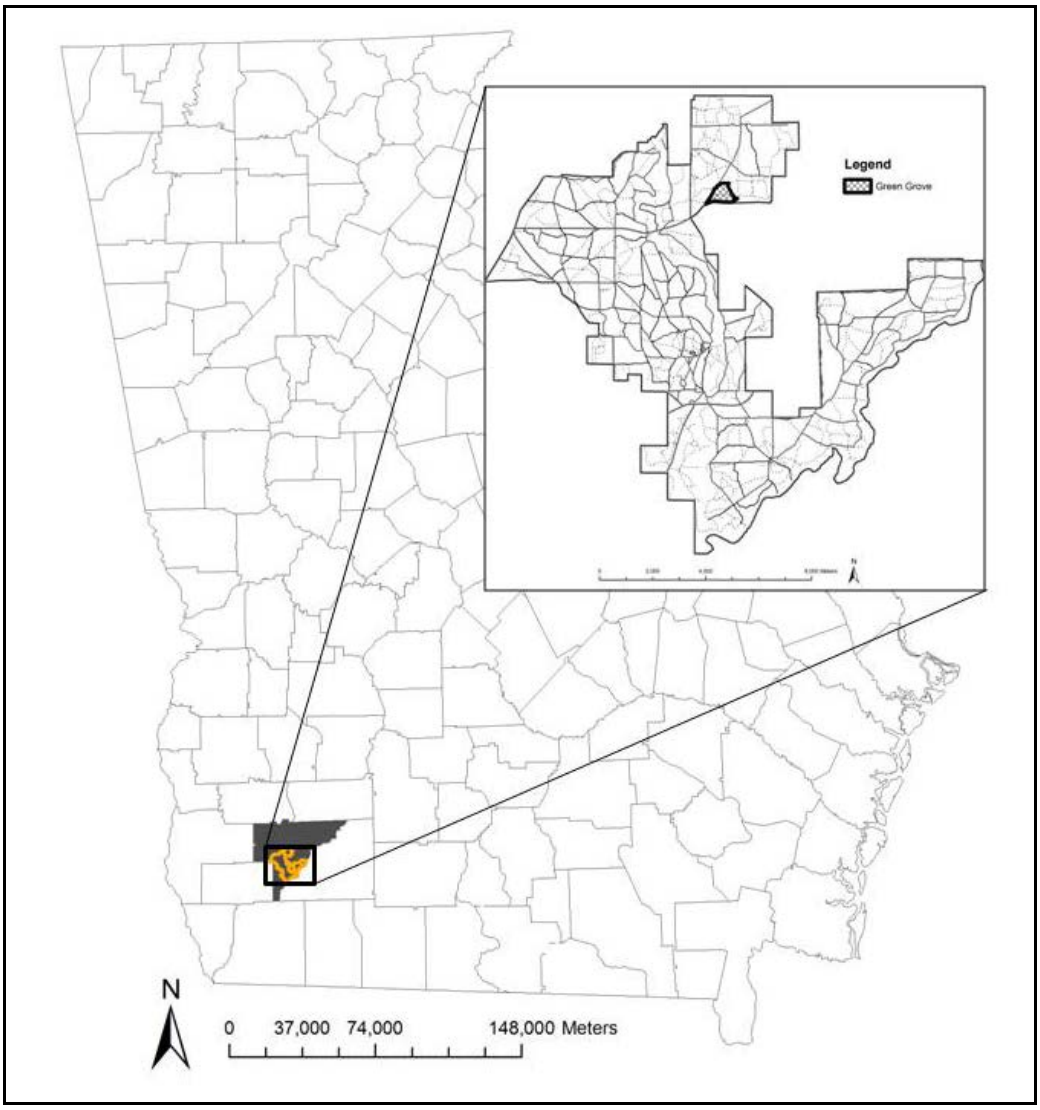


**Figure 4.1. Location of the Jones Ecological Research Center (JERC; yellow outline) at Ichauway in Baker County (inset box), Georgia.** The inset highlights JERC with the Green Grove study area hatched.

**Figure 4.2. Tortoises with severe clinical signs of upper respiratory tract disease (URTD).**

Visible clinical signs include cloudy nasal exudates and conjunctivitis.

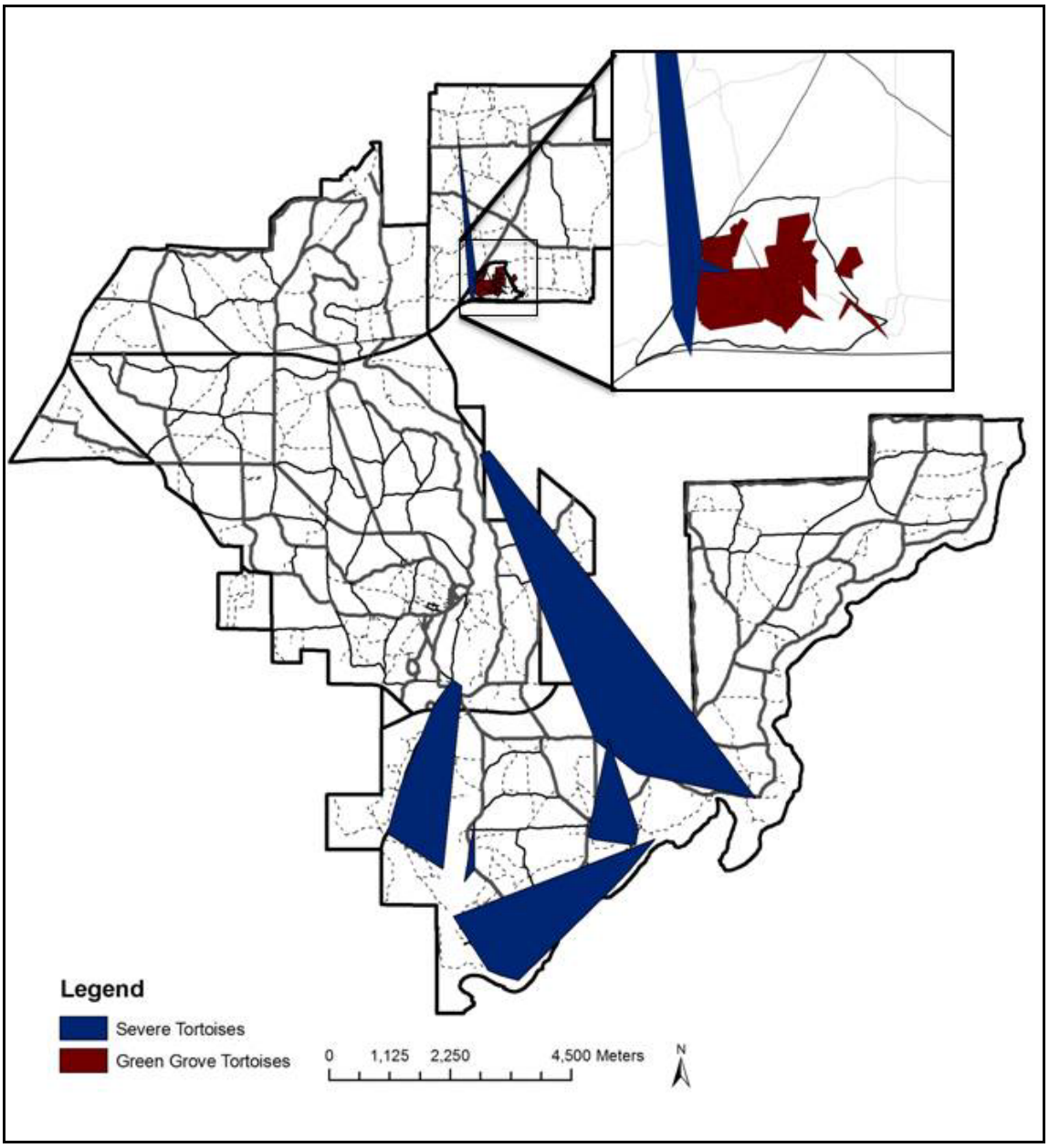


**Figure 4.3. Home ranges (95% MCP) of seven gopher tortoises with severe clinical signs of upper respiratory tract disease (URTD) (Blue) and 30 tortoises from the focal area, Green Grove (inset), that were asymptomatic or showed mild symptoms of URTD (Red).** Data were collected at Ichauway, in Baker County, Georgia in 2011 and 2012.

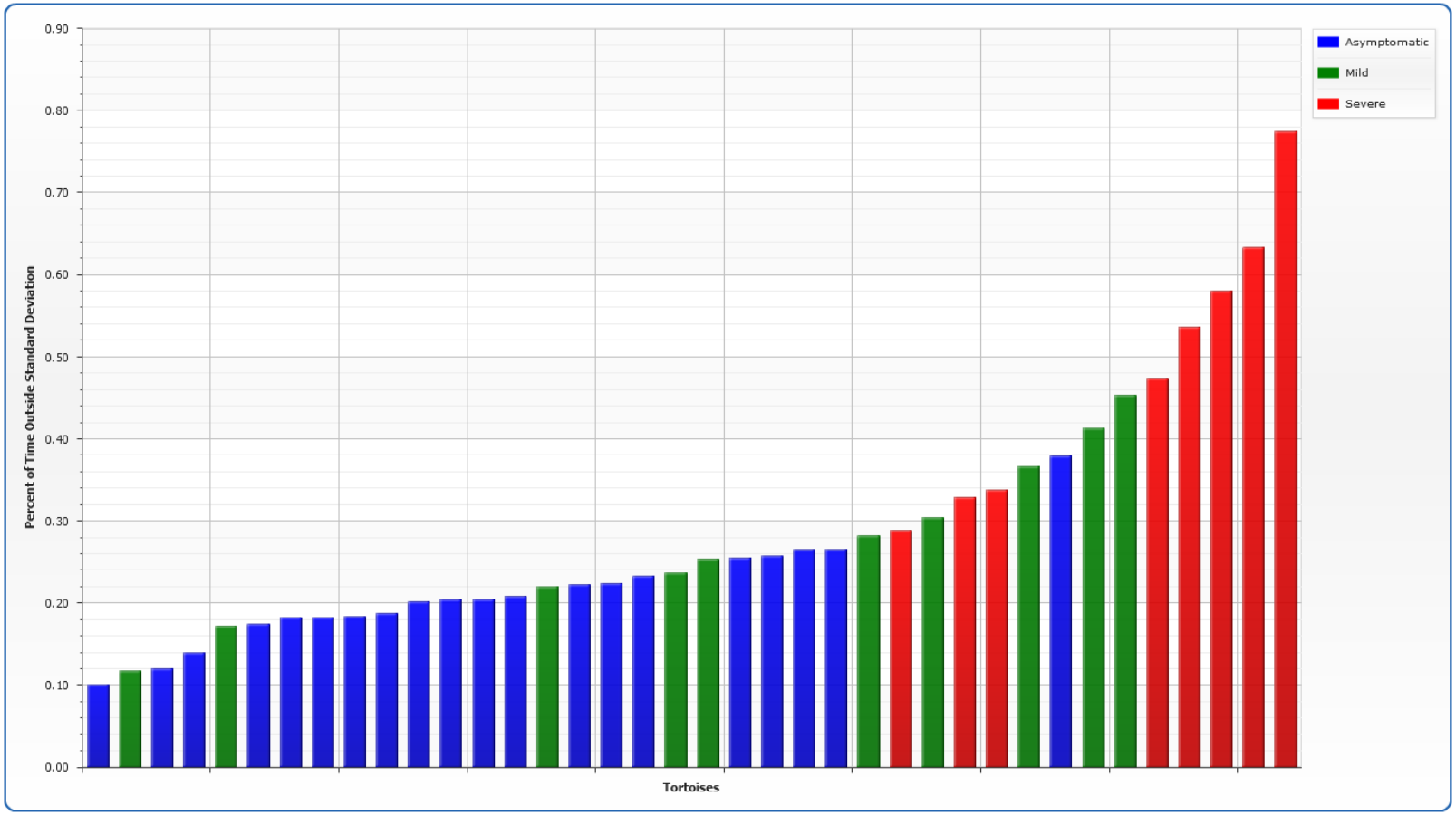


**Figure 4.4. Frequency with which individual gopher tortoises (n=38) at Ichauway in Baker County, Georgia, exhibited carapacial temperatures >1SD from the overall mean in 2011 and 2012.** Asymptomatic gopher tortoises are in blue (tortoises that did not exhibit clinical signs consistent with upper respiratory tract disease), mild clinical signs are in green, and severe clinical signs are in red.

# CHAPTER 5

# INTESTINAL PARASITES OF THE GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*) FROM EIGHT POPULATIONS IN GEORGIA[1]

[1]Gonynor- McGuire, J.L., E.A. Miller, T.M Norton, B.L. Raphael, J.S. Spratt, M.J. Yabsley. To be submitted to Parasitology Research.

**Abstract**

The gopher tortoise (*Gopherus polyphemus*), one of five tortoises endemic in the United States, was recently classified as a candidate for federal listing as a threatened species. Fecal samples collected from 117 tortoises from eight sites in Georgia were examined for endoparasites using a combination of sedimentation and flotation. Samples from an island population were examined for parasitic oocysts and ova only by flotation, protozoan cysts by trichrome stained direct smear and *Cryptosporidium* by direct immunofluorescense assay and ProSpecT rapid assay. A total of 99 tortoises (85%, range 0-100%) was infected with pinworms (*Alaeuris* spp.), 47 (40%, 0-86%) with cestodes (*Oochorstica* sp.), 34 (41%, 0-74%) with *Chapiniella* spp., two (3%, 0-33%) with *Eimeria paynei*, and a single tortoise each with a capillarid and ascarid (1%). On the island, *Entamoeba* were detected in one tortoise (2%) while *Cryptosporium* oocysts were detected in eight (17%). In conclusion, at least eight species of parasites were detected including *Cryptosporidium*, a possible pathogen of tortoises. Interestingly, we detected spatial variation in the distribution of several parasites among populations suggesting additional work should be conducted across a gradient of tortoise densities, land use and habitat characteristics.

**Introduction**

The gopher tortoise (*Gopherus polyphemus*), a terrestrial tortoise native to the southeastern United States, is listed as threatened under the U.S. Endangered Species Act in the western part of its range and the eastern populations (including those in Georgia) have recently been classified as a candidate for federal listing as a threatened species (USFWS, 2011). The gopher tortoise is considered to be a keystone species due to the number of other animals that utilize their burrows (citation). Nearly 350 vertebrate and invertebrate commensals reside with

the tortoise (Jackson and Milstrey, 1989). In Georgia, this includes the federally threatened eastern indigo snake (*Drymarchon couperi*), and rare gopher frog (*Rana capito*). Currently, major threats to the gopher tortoise include habitat loss and fragmentation, predation, and disease (Auffenberg and Franz, 1982; Hermann *et al.* 2002; McCoy *et al.* 2005; Wendland, 2007).

Limited work has been done to investigate parasites of gopher tortoises. During four studies conducted in Louisiana (one site) and Georgia (two sites), only 7-8 species of parasites in 3-4 taxonomic groups were reported (Walton, 1927; Ernst *et al.* 1971; Petter and Douglass 1976; Lichtenfels and Stewart 1981; Diaz-Figueroa 2005). As part of a concurrent study on the distribution of upper respiratory tract disease of gopher tortoises in Georgia, we conducted a survey for intestinal parasites by fecal examination from tortoises from eight populations across southern Georgia. These sites had a range of different habitats and land-use histories so we hypothesized that there would be differences in parasite prevalence.

**Methods**

Study Sites

Samples were collected from tortoises at eight sites in the coastal plain physiogeographic region of Georgia (Figure 1). The Jones Ecological Research Center in Baker County (Ichauway; Site A) is a privately owned 11,736 ha research property. Approximately 6,880 ha is suitable tortoise habitat which is primarily a longleaf pine (*Pinus palustris*) savanna fire-managed ecosystem with smaller areas of other pine and mixed pine forests. The property is surrounded by center-pivot agricultural fields. Forest Lodge Farms in Mitchell County (Site B) is a 607 ha privately owned family timber operation. The site is managed with prescribed fire in addition to mechanical and chemical removal of invasive species and hardwoods. Cedars Farm Plantation in Decatur County (Site C) is a 405 ha privately owned hunting plantation with intensively

managed longleaf pine-wiregrass habitat for quail. Orianne Indigo Snake Preserve in Telfair County (Site D) is a 405 ha preserve owned by a private conservation organization and is immediately surrounded by approximately 6,070 ha of conservation land (Wildlife Management Areas, a Nature Conservancy preserve, and other privately owned land in conservation easements). It is managed for rare and endangered species native to its sandhill habitat and research is conducted frequently on location. Reed Bingham State Park in Cook County (Site E) is a 526 ha state park in where there are many recreational attractions including campgrounds, walking trails, boating ramps, and picnic areas. There is high human traffic throughout the year and is known for illegal drop-off of waif tortoises (Chet Powell, personal communication). Ft. Gordon which spans multiple counties including Richmond, Jefferson, McDuffie and Columbia, (Site F) is a 22,662 ha military installation on Georgia's fall line which is one of the most northern population of tortoises in Georgia. Much of the land is undeveloped for training sites and firing ranges. Populations sampled from this installation were found in mixed pine and hardwood stands. St. Mary's Municipal Airport (Site G) is a small airport (116 ha) on the southern coast of the state. There are two paved runways, open grassy areas, and fencing that divides the property. Tortoises are primarily located on the periphery of these areas. St. Catherine's Island off the coast of Liberty County (site H) is a translocated population on a 5670 ha barrier island that includes a savanna-like grassland with longleaf, in addition to other habitats such as old fields and salt marsh (described in Tuberville, 2008). .

Sample Collection and Analysis

Samples from sites A-G were collected from May 2009 to August 2010 and samples from Site H were collected in 1996. Tortoises were captured using live animal traps (Hav-a-hart, Lititz, PA; Tractor Supply Company Inc., Brentwood, TN) set at the entrance of occupied

burrows, covered with burlap to protect from the sun, and checked twice daily at all sites except St. Catherine's Island, which were bucket trapped. Trapped tortoises were placed in individual plastic bins for transport and storage before sampling. Fecal samples were collected opportunistically from tortoises that defecated during sampling. Morphological measurements, including straight-line carapace length (to the nearest 1 mm) and mass (to the nearest 10 g) were collected for each tortoise. Tortoises >23 cm carapace length were considered adult tortoises, whereas those less than that were considered juveniles. Sex was determined based on shell morphology (McRae *et al.* 1981). Tortoises were released at site of capture following sample collection (AUP: A2010-11-563).

Fecal samples from sites A-G were stored in a 2% potassium dichromate solution at an approximately 10:1 ratio. For analysis, fecal samples were strained through two layers of cheesecloth and the liquid was centrifuged for 10 minutes at 2500 x g. The supernatant was decanted and a drop of the sediment was directly examined for parasitic ova. The remaining sediment was subjected to centrifugal flotation with Sheather's sugar solution. Samples from site H were submitted to the University of Florida College of Veterinary Medicine Diagnostic Lab for flotation and trichrome stained fecal smears and to Johns Hopkins for *Cryptosporidium* testing using by MERIFLUOR$^{TM}$ direct immunofluorescense assay (Meridian Bioscience, Inc., Cincinnati, OH) and ProSpecT rapid assay (Alexon-Trend, Inc., Ramsey, MN). Fisher's exact tests were used to determine if there were differences in prevalence of parasites between sites, type of site (public and private), gender, and size class (juvenile, adult).

**Results**

Fecal samples were analyzed from 117 tortoises at the eight sites (Table 1) The most common parasite detected was *Alaeuris* spp., (pinworms in Family Oxyuridae) with a total of 99

(85%) tortoises passing ova (Table 1). The only other helminth ova detected were cestode and *Chapiniella* eggs in 47 (40%) and 48 (41%) tortoises, respectively (Table 1). Two tortoises at Sites G and H were infected with *E. paynei* (Table 1). Unidentified capillarid and ascarid eggs (similar to *Sulcascaris*) were detected in single tortoises (2%) from Site H. Also at Site H, an *Entamoeba*, morphologically consistent with *E. invadens*, was detected on a fecal smear and *Cryptosporidium* oocysts were observed in eight (17%) samples. No trematode ova were detected in fecal sediments from Sites A-G.

No differences were noted in prevalence of any parasite species in regards to site classification (public vs. private), age of the tortoise (eight juvenile, 109 adults), or gender (52 male and 48 female). Pinworm prevalence was significantly higher at Sites A, E and H compared to Site D (p-values of 0.0351, 0.0112, and 0.0045, respectively). Site G had a significantly lower prevalence compared to other sites (p-values of <0.0001 to 0.0427) (Table 1). Considerable variation was noted in prevalence between sites for *Chapiniella* with sites D and F being negative and Site H having a significantly higher prevalence of 74% (Table 1). The prevalence of *E. paynei* was significantly higher at Site G compared to other sites, but the low prevalence of this parasite likely resulted in our inability to detect at some sites with low samples sizes.

**Discussion**

Only a few systematic studies have been conducted on endoparasites of gopher tortoises. Collectively, five studies conducted on approximately 42 tortoises have reported 10 species of parasites including two species of Strongylidae (*Chapiniella chitwoodae* and *C. gallatii)* from two sites in Georgia and single site in Louisiana, a Strongyloididae (*Strongyloides* spp. larvae) from Louisiana, an unidentified Trichostrongylidae in two sites in Georgia, four species of Oxyuridae (*Alaeuris gopher macrolabiata. A.* (=*Thelastomoides*) *previcolis, A. longicolils, and*

*A. paramazzottii)* from Georgia and Louisiana, an acanthocephalan (*Neoechinorhynchus pseudemydis*) (USPNC 82347) from Louisiana, and a coccidian (*E. paynei*) from a single tortoise in Georgia (Walton 1927, Ernst *et al.* 1971; Petter and Douglass 1976; Lichtenfels and Stewart 1981; Diaz-Figueroa 2005). In the current study of 117 tortoises from eight counties in Georgia, we found evidence of infection with four nematodes, a cestode, and three protozoans. This represents the first report of a cestode, believed to be an *Oochorstica* sp., a capillarid, an ascarid, and *Entamoeba* in gopher tortoises.

Pinworms are common parasites of reptiles, including tortoises. Similar to previous studies, pinworms were the most common parasite detected in most of our surveyed gopher tortoise populations. Unfortunately, we could not identify the pinworms to species as eggs of the four *Alaeuris* species reported from gopher tortoises are similar in size and shape (Bouamer *et al.* 2001). Interesting variations in *Alaeuris* and *Chapiniella* prevalence were noted at some of our sites. The majority of sites (A, B, C, E, F, and H) had high prevalence rates for *Alaeuris* as expected, but for an unknown reason, tortoises at Site G were all negative for *Alaeuris*. One possible reason for the lack of *Alaeuris* at this site is the habitat; site G is the airport site and thus lacks an overstory which combined with sandy well-drained soils might promote desiccation of parasitic ova, although *Chapiniella*, which also have direct life cycles, was present. Prevalence rates for *Chapiniella* were highly variable across sites. The only previous report of this genus was in 100% of eight tortoises from southern Georgia (Lichtenfels and Stewart, 1981). The two sites with no evidence of *Chapiniella* differed substantially, one being a conservation site and another being a military installation, but both populations had moderate-high prevalence rates for *Alaeuris*. However low sample sizes and low prevalence rates at other sites may have resulted in missed infections at Sites D and F. Although both *Alaeuris* and *Chapiniella* are directly

transmitted, the prevalence of *Alaeuris* was not associated with *Chapiniella* and no site was negative for both parasites. Transmission of these parasites are likely tortoise density dependent, thus spatial distribution of tortoise burrows at each site may influence transmission and this relationship should be examined in future studies.

Based on egg morphology, we believe it is an *Oochoristica* sp., a common genus of tapeworms from reptiles (currently reported from >86 species) including chelonians (e.g., *Oochorstica whitentoni* from the three-toed box turtle in Oklahoma (*Terrapene carolina triunguis*) (Steelman, 1939; Bursey *et al.* 1996; Arizmedi-Espinosa *et al.* 2005). In general, the prevalence of *Oochorstica* spp. in reptiles is low (<20%), but prevalence rates similar to our study have been reported for *O. islandensis* from the Island night lizard (*Xantusia riversiana*) and *O. leonregagnonae* in *Ctenosaura pectinata* (Bursey and Goldberg, 1992; Arizmendi-Espinosa *et al.* 2005). To accurately identify and classify the cestode detected in the current study, adult worms are needed for detailed morphologic analysis.

*Eimeria paynei* was only detected at two sites, but only at low prevalence rates. To date, infected tortoises have only been identified at three sites in Georgia (Ernst *et al.* 1971). In general, *Eimeria* would not cause significant disease in their hosts, although young animals may develop clinical disease if infection intensity is high. Our failure to find any Trichostrongylidae as reported by Lichtenfels and Stewart (1981) may have been due to eggs that are morphologically similar to *Chapiniella* (no adult worms available in this study), low prevalence of the parasite in surveyed populations, or low numbers of eggs being passed in feces.

We were only able to survey a single population for *Entamoeba* and *Cryptosporidium*, both of which were present in low prevalence at Site H. Both of these parasites have been reported from tortoises, including gopher tortoises (Raphael *et al.* 1997; Stedman *et al.* 2000).

*Entamoeba invadens* can cause clinical disease (colitis, diarrhea, liver abscesses) and death in numerous species of reptiles; a case of hepatic necrosis has been reported in a gopher tortoise (Stedman *et al.* 2000). *Cryptosporidium* spp. have been reported from a wide range of reptiles, amphibians, mammals, and birds. Clinical disease in reptiles, including other species of tortoises) is commonly associated with *Cryptosporidium* infections (Brownstein *et al.* 1977; Graczyk *et al.* 1998; Griffin *et al.* 2010). Genetic characterization of reptile samples indicate that numerous species infect tortoises, and due to similar morphology, specific identification of the *Cryptosporidium* detected in this study was not possible.

In conclusion, we detected at least eight species of parasites in Georgia populations of gopher tortoises. Unfortunately, we were unable to identify the helminths to species because we lacked adult worms. Future work should focus on the complete examination of tortoise carcasses (e.g., killed on road or natural deaths) to definitively identify adult parasites. Interestingly, we detected spatial variation in the distribution of several parasites in gopher tortoises in Georgia which may be due to habitat, soil, or land use (current/historical) variations among populations, or low samples sizes at certain locations. Additional work conducted across a gradient of these variables would be interesting to determine possible factors related to presence or prevalence. Importantly, there is currently no data available on endoparasites of gopher tortoises from Florida, South Carolina, Alabama, or Mississippi. Studies on any potential pathogens of gopher tortoises are warranted given that the species is listed as federally threatened west of the Mobile and Tombigbee Rivers in Alabama and tortoise populations in the eastern part of their range are classified as a candidate for federal listing as a threatened species (USFWS 2011).

**Acknowledgments**

We would like to thank Lora L. Smith for helpful comments on earlier versions of the manuscript. JLM was supported by the Warnell School of Forestry and Natural Resources at UGA and the J. W. Jones Ecological Research Center. Additional funding was provided by the Southeastern Cooperative Wildlife Disease Study through sponsorship with member states, The Gopher Tortoise Council, Sigma Xi, and the Sophie Danforth Conservation Biology Fund.

**Literature Cited**

Arizmendi-Espinosa, M.A., García-Prieto, L. and Guillén-Hernández, S. (2005). A new species of *Oochoristica* (Eucestoda: Cyclophyllidea) parasite of *Ctenosaura pectinata* (Reptilia: Iguanidae) from Oaxaca, Mexico. J. Parasitol 91, 99–101. doi:10.1645/GE-337R.

Auffenberg, W. and Franz, R. (1982). The status and distribution of the gopher tortoise (*Gopherus polyphemus*). In *North American Tortoises: Conservation and Ecology* (ed. Bury, R.B.), pp 95–126. Wildlife Research Report No. 12, Washington, D.C., USA.

Bouamer, S., Morand, S. and Bourgat, R. (2001). Oxyuroids of Palearctic Testudinidae: New definition for *Alaeuris* Seurat, 1918 (Nematoda: Pharyngodonidae) and redescription of *Alaeuris numidica* (Seurat, 1918). J. Parasitol 87, 128-133.

Brownstein, D.G., Strandberg, J.D., Montali, R.J., Bush, M., Fortner, J. (1977). *Cryptosporidium* in snakes with hypertrophic gastritis. Vet Pathol 14, 606-617. Doi:10.1177/030098587701400607

Bursey, C.R. and Goldberg, S.R. (1992). *Oochoristica islandensis* n. sp. (Cestoda: Linstowiidae) from the desert night lizard *Xantusia vigilis vigilis* (Xantusiidae). Trans Am Microsc Soc 111, 36–43.

Bursey, C.R., Goldberg, S.R. and Woolery, D.N. (1996). *Oochoristica piankai* sp. n. (Cestoda: Linstowiidae) and other helminths of *Moloch horridus* (Sauria: Agamidae) from Australia. J Helminthol Soc of Wash 63, 215–221.

Diaz-Figueroa, O. (2005). Characterizing the health status of the Louisiana gopher tortoise (*Gopherus polyphemus*). Thesis, Louisiana State University, Baton Rouge, USA. 109 pp.

Ernst, C.H. and Barbour, R.W. (1972). Turtles of the United States. University Press of Kentucky, Lexington. 347pp.

Ernst J.V., Fincher, G.T. and Stewart, T.B. (1971). *Eimeria paynei* sp. n. (Protozoa: Eimeriidae) from the gopher tortoise (*Gopherus polyphemus*). Proc Helminthol Soc Wash 38, 223-224.

Graczyk, T.K., Cranfield, M.R., Mann, J., and Strandberg, J.D. (1998). Intestinal *Cryptosporidium* sp. infection in the Egyptian tortoise, *Testudo kleinmanni*. Int J Parasitol 28, 1885-1888. doi:10.1016/S0020-7519(98)00143-X.

Griffin, C., Reavill, D.R., Stacy, B.A., Childress, A.L., and Wellehan J.F. Jr. (2010). Cryptosporidiosis caused by two distinct species in Russian tortoises and a pancake tortoise. Vet Parasitol 170, 14-19. doi:10.1016/j.vetpar.2010.01.039.

Hermann, S. M., Guyer, C., Waddle J. H., and Nelms M.G. (2002). Sampling on private property to evaluate population status and effects of land use practices on the gopher tortoise, *Gopherus polyphemus*. Biol Conserv 108, 289–298.

Jackson, D.R., Milstrey, E.G. (1989). The fauna of gopher tortoise burrows. In *Gopher Tortoise Relocation Symposium Proceedings* (ed. Diemer, J.E., Jackson, D.R., Landers, J.L., Layne, J.N., Wood, D.A.), pp. 86-98. Florida Game and Fresh Water Fish Commission, Nongame Wildlife Program Technical Report 5.

Lichtenfels, J.R. and Stewart, T.B. (1981). Three new species of *Chapiniella* Yamaguti, 1961 (Nematoda: Strongyloidea) from tortoises. Proc Helminthol Soc Wash 48, 137-147.

McCoy, E.D., Mushinsky, H.R., and Lindzey, J. (2005). Declines of the gopher tortoise on protected lands. Biol Conserv 128, 120-127. doi:10.1016/j.biocon.2005.09.021.

McRae, W.A., Landers, J.L. and Cleveland, G.D. (1981). Sexual dimorphism in the gopher tortoise (*Gopherus polyphemus*). Herpetologica 37, 46-52.

Petter, A.J. and Douglass, J.F. (1976). Etude des populations d'Oxyures du colon des *Gopherus* (Testudinidae). Bull Mus Natl Hist Natur, strie 3, no. 389, Zoologie 271, 731-768.

Raphael BL, Calle PP, Gottdenker N, James S, Linn WJ, McNamara T, Cook RA. (1997). Clinical significance of *Cryptosporidia* in captive and free-ranging chelonians. In *Proceedings of the Annual meeting of the American Association of Zoo Veterinarians*, Houston, Texas. AAZV; 1997, 19-20.

Stedman, N.L., Garcia, A., and Latimer, K.S. (2000). Severe hepatic necrosis associated with entamoebiasis in a gopher tortoise (*Gopherus polyphemus*). Proceedings of International Virtual Conferences in Veterinary Medicine: Diseases of Reptiles and Amphibians. http://www.vet.uga.edu/vpp/archives/ivcvm/2000/stedman/index.php

Steelman, G.M. (1939). *Oochoristica whitentoni*, a new anoplocephalid cestode from a land tortoise. J. Parasitol 25, 479-482.

U.S. Fish and Wildlife Service (USFWS). (2011). Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To List the Gopher Tortoise as Threatened in the Eastern Portion of Its Range; Final Rule. Accessed online 5 September 2012 http://www.fws.gov/northflorida/GopherTortoise/12-month_Finding/20110726_frn_Gopher-Tortoise_12month_finding.htm.

Walton, A.C. (1927). A revision of the nematodes of the Leidy Collection. Proc Acad Nat Sci Phil 79, 49-163.

Wendland, L.D. (2007). Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. Ph.D. Dissertation, University of Florida, Gainesville, Florida, 171 pp.

**Table 5.1. Parasitic ova and oocysts in fecal samples from eight populations of gopher tortoises (*Gopherus polyphemus*) from Georgia.**

| Site | Classification | n | No. Positive (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Alaeuris* spp. | *Oochorstica* sp. | *Chapiniella* spp. | *Eimeria paynei* | Capillarid | Ascarid |
| A | Private | 11 | 11 (100)[a*] | 5 (45)[a] | 2 (18)[a] | 0[a] | 0 | 0 |
| B | Private | 5 | 4 (80) [a,b] | 3 (60)[a] | 2 (40)[a] | 0[a] | 0 | 0 |
| C | Private | 10 | 8 (80) [a,b] | 7 (70)[a] | 1 (10)[b] | 0[a] | 0 | 0 |
| D | Private | 11 | 6 (55) [b] | 7 (64)[a] | 0[b] | 0[a] | 0 | 0 |
| E | Public | 21 | 20 (95) [a] | 16 (77)[a] | 7 (33)[a] | 0[a] | 0 | 0 |
| F | Public | 7 | 7 (100) [a,b] | 6 (86)[a] | 0[b] | 0[a] | 0 | 0 |
| G | Public | 6 | 0 [c] | 3 (50)[a] | 2 (33)[a] | 2 (33)[b] | 0 | 0 |
| H | Private | 46 | 43 (93)[a] | 0[b] | 34 (74)[a] | 2 (4)[**] | 1 (2) | 1 (2) |
| Total | | 117 | 99 (85) | 47 (40) | 48 (41) | 4 (3) | 1 (1) | 1 (1) |

* Different letters indicates significant difference in prevalence between individual sites.

**Oocysts were not sporulated so were not confirmed to be *E. paynei*.

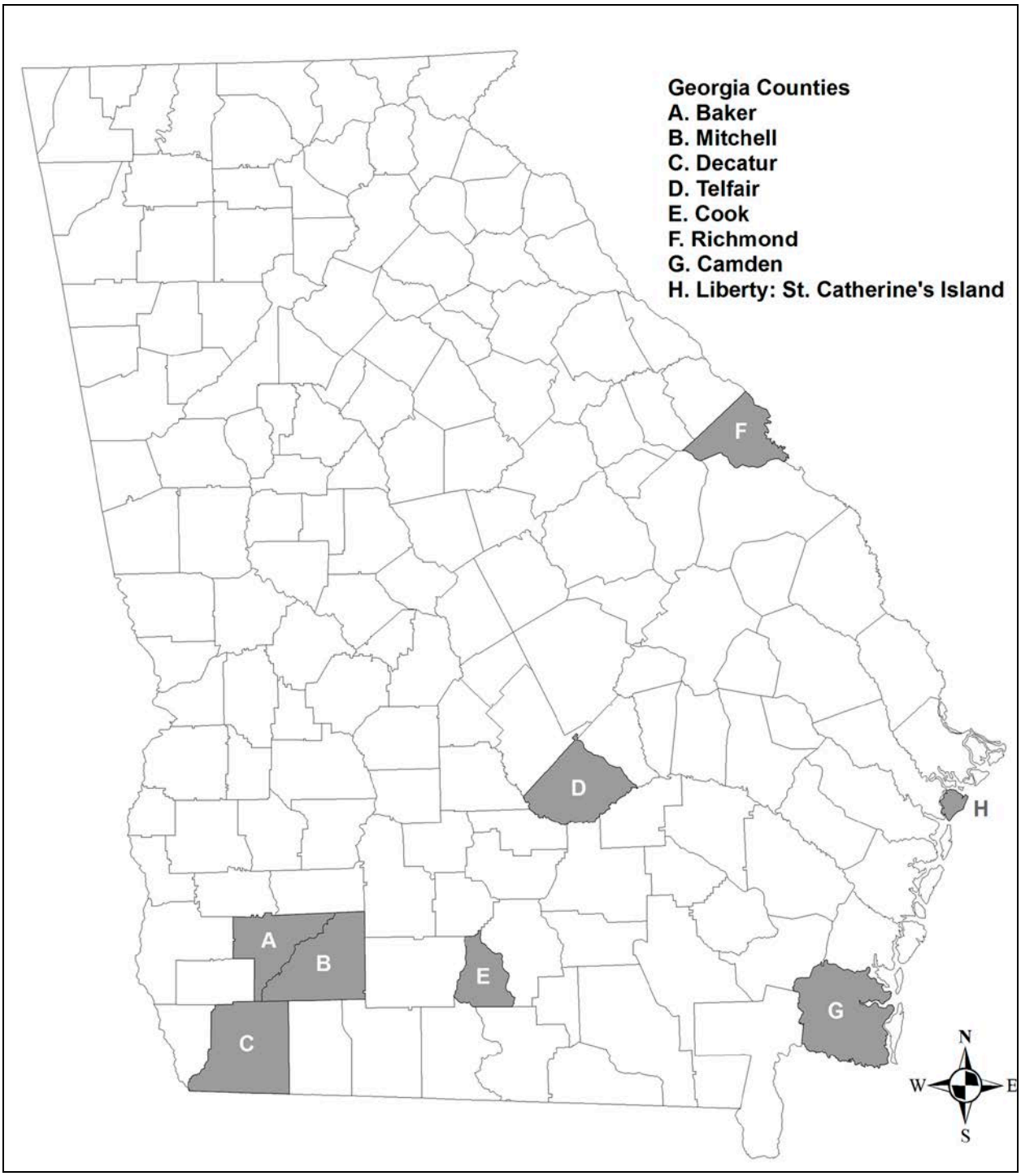


**Figure 5.1. Map of the eight study sites in Georgia, USA. Counties with populations sampled are shaded in dark grey.**

# CHAPTER 6

# SAFETY AND UTILITY OF AN ANESTHETIC PROTOCOL FOR THE COLLECTION OF BIOLOGICAL SAMPLES FROM GOPHER TORTOISES[1]

[1] McGuire, J.L., S.M. Hernandez, L.L. Smith, M.J. Yabsley. To be submitted to the *Wildlife Society Bulletin*.

**Abstract**

Adult gopher tortoises (*Gopherus polyphemus)* are difficult to physically restrain, particularly for examination of the upper respiratory tract and oral cavity, areas important for biological sample collection during disease surveillance studies. Collection of nasal lavage is required for determining the status *Mycoplasma* infection in tortoises. Anesthesia is often necessary to provide full relaxation in order to gain access to the head. Therefore, deep sedation or general anesthesia are required both for the welfare of the tortoise and to maximize the usefulness of biological samples collected. The objective of this study was to assess the utility and safety of a novel field anesthetic combination of dexmedetomidine-ketamine-morphine (75 mc/kg; 8 mg/kg; 1 mg/kg respectively), followed by reversal with atipamezole (0.02 mg/kg). Between May and October 2009, 126 tortoises were captured and anesthetized with this protocol. Average time to first effects was 9.7 min, to induction was 21.6 min, to total recovery after administration of reversal was 92.7 min and the mean time from administration of anesthesia through complete recovery for adult tortoises was 129 minutes but ranged from 40-300 minutes. Clinical signs of URTD significantly reduced the induction time such that tortoises with clinical signs loss consciousness faster than healthy tortoises. Study month significantly influenced the time to induction and time to recovery such that as the study progressed, both parameters decreased. This anesthetic protocol proved to be an effective, reliable, repeatable, and safe method to collect quality biological samples from gopher tortoises.

## Introduction

The gopher tortoise (*Gopherus polyphemus*) is 1 of 5 extant land tortoises in North America (Murphy et al., 2011) and is considered a keystone species (Eisenberg, 1983) because the burrows it excavates provides habitat for over 300 species (Jackson and Milstrey, 1989; Lips, 1991; Witz et al., 1991). Threats to gopher tortoise populations include loss of habitat, fire suppression and upper respiratory tract disease (Smith et al., 2006). In the western U.S., high mortality rates in desert tortoise (*Gopherus agassizzii*) populations have been attributed to upper respiratory tract disease (URTD; Jacobson et al., 1991; Berry, 1997) associated with *Mycoplasma* spp infections, which may also be contributing to significant population-level effects for gopher tortoises (Berish et al., 2010; Wendland, 2010). Therefore, disease surveillance investigations to determine the exposure status of gopher tortoise populations are ongoing and paramount for making management decisions, particularly those involving translocation of animals, and especially because translocation is frequently used as a conservation tool for gopher tortoises threatened by development activities (Tuberville et al. 2008).

In light of disease concerns, efficient and minimally-invasive handling protocols are necessary to collect biological samples from gopher tortoises. Adult gopher tortoises are difficult to physically restrain for complete physical exams and particularly to expose the head, neck and legs for biological sample collection (Bennett, 1998). A thorough examination of the upper respiratory tract and oral cavity is often necessary for detecting clinical signs associated with URTD and other syndromes described in gopher tortoises (Harper et al, 1982; Pettan-Brewer et al., 1996). The collection of nasal lavage is particularly difficult for field researchers (Berish et al., 2010). Thus, anesthesia may be required to minimize stress on the tortoise

(Dickens et al., 2010), to achieve a complete physical exam, and to maximize the quality and usefulness of the biological samples collected.

The physiology of chelonians creates challenges in monitoring and maintaining adequate anesthetic depth and quality. Problems typically encountered are 1) unpredictable absorption and metabolism of anesthetic drugs, 2) inconsistent depth of anesthesia and muscle relaxation, 3) prolonged recovery periods, and 4) anesthetic drug distribution variability associated with the renal portal system (Maxwell, 1979; Rooney et al., 1999; Mosley, 2005; Schumacher, 2007; Ziolo, 2009). Therefore, despite the fact that a number of anesthetic agents are routinely utilized in chelonians, they each present with challenges for field studies. Inhalants have been used successfully in turtles and tortoises to maintain anesthesia (Moon and Stabenau, 1996; Rooney et al., 1999; Mosley, 2005; Norton, 2005) yet field researchers do not have access to vaporizers (Oppenheim, 1995; MacLean et al., 2008), and the use of inhalants for induction of anesthesia in chelonians is inefficient because the typical breath-holding behavior of reptiles during physical restraint makes absorption unreliable (Maxwell, 1979; Bennett, 1991; McArthur, 2004; Mosley, 2005; Schumacher and Yelen, 2006). Modern intravenous anesthetics, such as propofol, offer quick induction and appropriate relaxation for short procedures in reptiles, but reliable access of an intravenous site in a conscious gopher tortoise is a challenge. Therefore, field studies typically rely on intramuscular agents. The ideal injectable drug for field studies should be 1) water soluble for rapid induction, 1) provide a rapid and smooth induction and recovery, 2) result in little cardiovascular or respiratory system depression, 3) provide adequate muscle relaxation for the procedure in question, 4) have a wide safety margin and 5) be inexpensive (Shumacher and Yelen, 2006; Grimm and Lamont, 2007; Kreeger, 2007; Amass, 2011). In reality such a drug does not exist. Specifically, adequate muscle relaxation is integral to the physical manipulation

needed to collect nasal lavages to conduct disease surveillance investigations for URTD in tortoises. To date, combinations of medetomidine-ketamine have shown to be most useful for terrestrial chelonians (Mosley, 2005). This combination provides a fast induction, adequate relaxation, and minimizes the adverse effects typical of using ketamine or medetomidine alone (Carpenter, 2004). Lastly, the alpha-agonist component is easily reversible (Chittick et al, 2002). Dennis and Heard (2002) evaluated the intravenous administration of a medetomidine-ketamine combination in a small sample of gopher tortoises and found no significant adverse effects to the heart rate or body temperature, concluding that the combination was effective for short-term anesthesia, yet warned hypoventilation might necessitate the use of supplemental oxygen. Sleeman and Gaynor (2000) also concluded medetomidine could be used to safely induce sedation in desert tortoises, but recommended supplemental oxygen if procedures lasted longer than 120 min, and suggested that future studies should address the utility of medetomidine and ketamine in combination with analgesic agents such as butorphanol. A major consideration when handling wild tortoises for disease surveillance projects is that the procedures do not induce painful stimuli which have been shown to interfere with the animal's metabolism and immune function (Gaynor and Muir, 2008). The addition of an opioid, such as butorphanol, has long been viewed by veterinary practitioners as beneficial to provide analgesia and reduce stress associated with pain in a variety of animals (Grimm and Lamont, 2007). Recent studies, however, have shown that commonly used mixed opioid κ-receptor agonist–μ-receptor antagonists, such as butorphanol, may not provide adequate pain reduction for invasive procedures in reptiles (Sladky et al., 2007). In contrast, morphine, a pure μ-receptor agonist, apparently provided adequate pain control in red-eared sliders (*Trachemys scripta*; Sladky et al., 2007).

We were interested in a field anesthetic protocol that produced a fast, smooth induction, minimal side effects, was analgesic and reversible, allowing for rapid and smooth recoveries that facilitated the return of an animal to its environment in a short period of time. Thus, the objective of this study was to assess the utility and safety of a novel field anesthetic combination of dexmedetomidine-ketamine-morphine for the handling and collection of biological samples from gopher tortoises. We additionally predicted that the presence of clinical signs of URTD would significantly impact the quality of anesthesia and time to recovery.

**Methods**

Study Area

This study was conducted at the Joseph W. Jones Ecological Research Center (JWJERC) at Ichauway in Baker County, Georgia, USA. The JWJERC is an 11,735 hectare ecological research facility and the property has greater than 5000 tortoises (L.Smith, unpubl.data). Approximately 4000 acres of open longleaf pine (*Pinus palustris*) and wiregrass (*Aristida stricta*) understory provides optimal gopher tortoise habitat (Drew et al., 1998). The property is managed as a research site in addition to being maintained as a traditional quail (northern bobwhite, *Colinus virginianus*) hunting reserve.

Capture of Tortoises

During the summer of 2009 (May- October), gopher tortoises were captured using wire cage traps (Tomahawk Live Traps, Hazelhurst, WI) placed at the burrow entrance. To reduce stress to tortoises and provide protection from the elements, the traps were covered with burlap and checked each morning and afternoon. Captured tortoises were individually transported in plastic bins (64.77 cm x 44.45 cm x 39.37 cm) and were maintained in a temperature-controlled room at 26.7˚C.

Tortoises were processed within 24 hrs of capture and were never held for more than 48 hours. Morphological measurements and sex determination data was collected at this time. Sex was determined based on carapace length (CL), gular length, plastral concavity, and the ratio of anal notch to anal fork (Eubanks et al., 2003; McRae et al., 1981); however, for some individuals, generally those <23 cm in CL, sex could not be determined. Only individuals of known sex were used in the analysis to determine effects of sex on level of anesthesia and recovery. Individuals were classified as adults based on a CL >23 cm (McRae et al., 1981; Landers et al., 1980). To identify individuals, tortoises were marked as described in Cagle (1939). Tortoises were then weighed to the nearest 1 g and manually restrained by 1 person.

Anesthesia and Sample Collection

A combination of ketamine (8 mg/kg), dexmedetomodine (75mc/kg), and morphine (1 mg/kg) was delivered intramuscularly (IM) with a 25-gauge needle deep into the triceps muscle of the right front leg. The time of injection (Time 0) was recorded. After injection, tortoises were placed in a plastic bin on top of a heating pad to ensure that the body temperature was maintained at or near 26.7˚C (Shumacher and Yelen, 2006). The time (minutes) it took for the tortoise to show the first effects of anesthesia (henceforth “first effects”) was recorded. The first effects of anesthesia were defined as relaxation of the head or limbs, ataxia, and/or loss of normal ambulatory movement. We also recorded the induction time (henceforth “induction”) or length of time it took the tortoise to become completely anesthetized such that the limbs were out and easily manipulated, the head could be extended, the oral cavity could be opened without resistance and the tortoise was ready for sample collection (Schumacher and Yelen, 2006);. Tortoises were provided ventilatory assistance throughout the procedure by manually pumping the front legs approximately 2-3 times per minute (Dennis and Heard, 2002; Shumacher and

Yelen, 2006). The total time it took to collect a nasal lavage and blood was called ”sampling time”. At the end of the sampling time, we administered the alpha-2 antagonist, atipamezole (0.02 mg/kg) and the opioid antagonist, nalaxone (0.2 mg/kg), IM on the left front leg and recorded the time of injection (henceforth “time reversed”). We also recorded the time to spontaneous movement, which was the first moment the gopher tortoise began to move its head or limbs without external stimuli after delivery of reversal drugs, and the interval between time reversed and total recovery. Total recovery was determined when the animal had returned to its pre-anesthetic condition and was considered safe to release. Lastly, we calculated the total anesthetic time, which was the total time from time 0 until release (Table 1). The same person subjectively judged the quality of anesthesia each time by scoring each anesthetic event as “light” (the animal moved prematurely during manipulation or flinched during sampling), “appropriate” (no premature arousal occurred), or “deep” (the tortoise was deemed excessively anesthetized and required prolonged assisted ventilation). We also categorized the total anesthetic time as “appropriate” (if the animal returned to its pre-anesthetic in under 120 minutes) or “prolonged” (greater than 120 minutes).

During anesthesia, the eyes were moistened with sterile petroleum ophthalmic ointment (Paralube® Ointment, E. Fougera & Company, Melville, NY). Biological samples, including blood and nasal flush, were collected when the tortoise was anesthetized (sampling time). A complete physical exam was performed, paying particular attention to clinical signs associated with URTD [discharge from the eyes/nares, congested or noisy breathing, conjunctivitis or oral lesions (Jacobson et al., 1991)]. Tortoises were then placed on dorsal recumbency and a nasal lavage was performed by flushing approximately 3 ml of sterile saline through the choana using an open-ended 3 ½ Fr Tom Cat™ Catheter (Tyco Healthcare, Kendall Sovereign™) attached to a

syringe. Saline was collected from the nares in a sterile tube (Wendland, 2007). Tortoises were then placed on sternal recumbency and blood was collected from the dorsal tail vein, brachial vein, or the subcarapacial venous sinus. Once sampling was completed and reversal was administered, tortoises were returned to their holding bin and observed until complete recovery. The hematocrit (HCT%; percent cell volume) was determined by collecting a small amount of blood into a capillary tube, and centrifuging it at 14 rpm for 5 minutes.

All capture, handling, anesthesia and sample collection methods used were evaluated and approved by the University of Georgia's Animal Care and Use Committee (AUP #A2009 6-112). The study was conducted under Scientific Collection permits issued to the Jones Ecological Research Center by the Georgia Department of Natural Resources (Scientific Collecting Permit #29-WBH-09-151).

Statistical Analyses

Linear regression was used to determine whether Hct% or study month influenced time to induction or time to spontaneous movement. Logistic regression was used to determine if the quality of anesthesia, time to recovery and quality of recovery were related to Hct% and study month.

Paired t-tests were used to determine whether the mean time to induction and mean time to spontaneous movement differed depending on presence or absence of clinical signs. Paired t-tests were also used to determine whether the mean time from administration of reversal to recovery differed depending on presence or absence of clinical signs. Analysis of Variance (ANOVA) was used to determine if sex was a significant variable in determining time to induction or time to spontaneous movement. We used Chi-square tests and Fisher's exact tests

to determine if quality of anesthesia, time or recovery, or probability of a long recovery was dependant on variables such as the presence (or absence) of clinical signs and sex.

**Results**

Between the months of May and October, 126 tortoises ((119 adults; 62 male (23.1-31.6 cm CL), 51 female (2.69-33.6 cm CL) and 10 unknown (22.6-31.8 cm CL) due to ambiguous characteristics) and 3 juveniles (13.3-20.4 cm CL)), were captured and anesthetized. Due to the small number of juveniles sampled, we only analyzed results from adults. However, of the 3 juveniles sampled, the quality of anesthesia was judged to be light throughout the procedure, which included flinching in 1 tortoise and leg movement the others. None of the juveniles took longer than 120 minutes to recover, with a minimum recovery time of 43 minutes. Of these 3 tortoises, 1 displayed clinical signs of UTRD.

No significant differences were found in any variables measured (ie. quality of anesthesia, time to recovery) between males and females, therefore, analyses include all adults. Average time to first effects was 9.7 minutes, to induction was 21.6 min, to total recovery after administration of reversal was 92.7 min and the mean time from administration of anesthesia through complete recovery for adult tortoises was 129 minutes but ranged from 40-300 minutes. The mean time from administration of anesthesia through complete recovery for adult tortoises was 129 minutes but ranged from 40-300 minutes (Table 2).

None of the variables we examined (Hct%, presence or absence of clinical signs, sex, or study month) significantly influenced whether or not quality of anesthesia was light (n= 28) or appropriate (n= 95) (Table 3). Tortoises exhibiting “deep” anesthesia were not included in this analysis due to the small sample size (n=3). Interestingly, 2 of these 3 tortoises had URTD clinical signs.

Time from reversal administration to recovery averaged 92.7 minutes for all tortoises. The time to complete recovery was appropriate for the majority of the tortoises (75%) with only 26% exhibiting a prolonged recovery (Table 4). Long recoveries ranged from 180-300 minutes. No tortoises required additional doses of reversal drugs or further supportive care to achieve total recovery prior to release

Twenty-two tortoises (17.6%) were displaying clinical signs of UTRD at time of sampling (Table 4). Tortoises with clinical signs had a shorter mean time to induction than those without ($t= 2.61$, $df= 123$, $p= 0.0102$). We found no relationship between clinical signs and quality of anesthesia (light or appropriate: Fisher's exact test $p= 0.1580$), time to spontaneous movement ($t= -0.18$, $df= 117$, $p = 0.8580$), quality of recovery (Fisher's exact test $p= 0.5901$), or the total recovery for tortoises ($t= 0.8432$, $df= 126$, $p= 0.4007$).

The number of tortoises sampled each month varied (Figure 1). Despite this, we found that capture month was associated with a significant decrease in induction time ($p= 0.0443$ $R^2=0.0325$) by approximately 0.93 minutes per month as the study progressed. Study month did not significantly influence the quality of anesthesia ($p= 0.5360$). Time to spontaneous movement post anesthesia significantly varied by month ($p=0.0135$, $R^2= 0.0510$) with a decreasing average of 1.65 minutes. A "long" time to recovery also significantly varied by month ($p= 0.0274$) such that a tortoise was more likely to experience a "long" recovery in the first month of the study in comparison to the sixth month.

The significance of study month led us to perform a post-hoc analysis focusing on the researcher. The total sampling time differed among study months ($p <0.0001$), such that total sampling time decreased approximately 5 minutes as the study progressed, suggesting that the researcher became more efficient as the sampling season progressed (Figure 1).

**Discussion**

The combination of dexmedetomidine, ketamine, and morphine proved to be an effective protocol for producing an appropriate level of anesthesia to collect biological samples from gopher tortoises. We were able to safely handle and collect samples from 126 tortoises without morbidity or mortality. In fact, 5 tortoises have been opportunistically recaptured since the initial health assessment and they did not show any signs of adverse effects from this procedure. Twenty nine percent of tortoises were categorized as experiencing "light" anesthesia because they "flinched" slightly during the nasal lavage; however, this level of anesthesia never prevented collection of samples and no animal required supplemental anesthetics. Tortoises that were categorized as having experienced "deep" anesthesia took longer than the average to recover; yet they all recovered without complications. The recovery of the tortoises was shorter, as their percent hematocrit (Hct%) increased. Percent cell volume, or hematocrit % is an indication of the relative number of circulating red blood cells. A decrease in time to recovery may be due to faster delivery of oxygen to the brain by blood more heavily oxygenated, but further work would be needed to confirm this.

Two specific anomalies are worth noting. In June, 2 female tortoise deposited eggs during the anesthetic period. Both also urinated excessively and recovered quickly from anesthesia (both less than 95 minutes from induction to recovery and less than 46 minutes from time of reversal to recovery). It is unknown if the anesthetics used are associated with premature egg laying, or if was a result of the capture and handling procedure. Alpha-agonists are associated with abortion in cattle, as a consequence of premature uterine contractions (Branson, 2007). It is possible that the same mechanism occurs in reptiles, but further research would be needed to prove this. Dexmedetomidine and related compounds are routinely used to anesthetize

both sick and healthy tortoises and this effect has not been previously reported (Posner and Burns, 2009). To avoid any further losses of eggs, we did not anesthetize female tortoises suspected to be gravid after June. Two male tortoises extruded their penis after administration of atipamezole; however, male tortoises have been observed to do this during handling (Douglass and Layne, 1978) and there is no evidence that this response was due to any of the drugs.

The study month significantly impacted induction time, total sampling time and recovery time, such that as the sampling season progressed, these periods were shortened. The induction and recovery time is likely due to the body temperature and metabolism of tortoises at the time of initial drug administration, which was higher at the time of processing, due to warmer ambient temperatures, as the season progressed. Tortoises earlier in the year have not been active as long, are experiencing shorter days of thermoregulation and are assumed to have slower metabolisms (Douglass and Layne, 1978; Schumacher, 2007) which directly affect drug absorption and distribution. The decrease in sampling time is likely a result from the handler becoming more efficient as sampling season progressed. Efficiency in sampling is important in chelonians, particularly in procedures requiring prolonged dorsal recumbency as this position impairs gas exchange and circulation because gravity pulls the abdominal organs on top of the lungs and major vessels, impairing ventilation and blood flow (Sleeman and Gaynor, 2000).

The drug combination in this study was chosen to minimize serious adverse side effects typically reported when these drugs are used, especially when used alone, or at higher doses than our study. For example, medetomidine alone can cause bradycardia, hypotension, low oxygen saturation, and hypoventilation (desert tortoises; Sleeman and Gaynor, 2000). Specifically in gopher tortoises, a medatomidine and ketamine combination provided effective immobilization for clinical procedures but resulted in hypoventilation and increased mean arterial pressure

(Dennis and Heard 2002). Additionally, opioid drugs, specifically morphine, caused respiratory depression in red-eared slider turtles (Sladky et al., 2007). The cardiopulmonary effects noted when using medetomidine are typically reversed when atipamezole is administered. However, Dennis and Heard (2002) noted that atipamezole induced severe hypotension when administered IVand Sleeman and Gaynor (2000) did not see improvement in respiratory rate. Sladky et al (2007) concluded that morphine, alone, caused respiratory depression and lengthly respiratory depression, but appeared to provide adequate pain relief for procedures. A number of tortoises in this population were exhibiting clinical signs of URTD at the time of this study (McGuire, unpublished data). Upper respiratory tract disease can influence both ventilation and gas exchange through both inflammation and mechanical obstruction of airways (Brown et al, 1999). This may explain the significantly faster time to induction and loss of consciousness that tortoises with clinical signs exhibited. Although tortoises with URTD recovered well, these tortoises are not healthy and without specifically monitoring the cardiopulmonary effects of this protocol (e.g. measuring oxygen saturation or blood pressure), we cannot comment further on the effects of this protocol. This should be considered in the future studies.

This procedure, as described, was successful for examination and sample collection of gopher tortoises, however, the absorption, distribution and metabolism (and consequent excretion) of all anesthetics in reptiles is dominated by body temperature (Rossi, 2006; Schumacher and Yelen, 2006). Therefore, this protocol will produce similar results only if the core body temperature of tortoises is maintained at at least 27C, generally believed to be an optimal temperature for drug metabolism (Schumacher and Yelen, 2006). This method was uniquely useful in allowing thorough examination of the oral cavity and collection of a nasal lavage in the gopher tortoise.

## Management Implications

Minimizing stress during the collection of biological samples is of most importance with wildlife. Succesful disease surveillance relies upon appropriate sample collection in the field. Studies that attempt to determine infection status with *Mycoplasma* rely on a suite of tests, including nasal lavage for culture and PCR, and blood collection for serology. *Mycoplasma* is a bacterium that lacks cell wall and is known to tightly adhere to epithelial cells (Brown et al., 1999). When nasal lavage is required to dislodge as many bacteria as possible and influences the success of testing (McLaughlin et al., 2000; Wendland et al, 2007; duPre et al, 2011). Collection of appropriate volume of blood and nasal lavage neccessitate anesthesia levels that provide full relaxation in tortoises. This anesthetic protocol proved to be an effective, reliable and safe method to collect quality biological samples from gopher tortoises in the field.

## Acknowledgments

Funding was provided by the Southeastern Cooperative Wildlife Disease Study, D.B. Warnell School of Forestry and Natural Resources, The J.W. Jones Ecological Research Center and The Gopher Tortoise Council. We would like to thank M. A. Wilkins (Cronic) for her effort in the field.

**Literature Cited**

Amass, K. 2011. Chemical Immobilization of Animals, Technical Field Notes: 2011. Safe Capture International. Mt. Horeb, Wisconsin, USA.

Bienzle, D., and C.J. Boyd. 1992. Sedative effects of ketamine and midazolam in snapping turtles (*Chelydra serpentina*). Journal of Zoo and Wildlife Medicine 23:201-204.

Bennett, R.A. 1998. Reptile Anesthesia. Seminars in Avian and Exotic Pet Medicine 7:30-40.

Bennett, R.A., 1991. A review of anesthesia and chemical restraint in reptiles. Journal of Zoo and Wildlife Medicine 22:282–303.

Berish, J.E.D., L.D. Wendland, R.A. Kiltie, E.P. Garrison, and C. A. Gates. 2010. Effects of Mycoplasmal Upper Respiratory Tract Disease on morbidity and mortality of gopher tortoises in Northern and Central Florida. Journal of Wildlife Diseases 46: 695- 705.

Berry, K.H. 1997. Demographic consequences of disease in two desert tortoise populations in California, USA. Pages 91-97 *in* Proceedings of Conservation, restoration, and management of tortoises and turtles- an international conference, New York Turtle and Tortoise Society, New York, New York.

Branson, K.R. 2007. Injectable and alternative anesthetic techniques. Pages 273-300 *in* W. J. Tranquilli and J.C. Thurmon (ed.). Lumb & Jones Veterinary Anesthesia and Analgesia. Blackwell Publishing, Ames, Iowa.

Brown, M. B., G. S. McLauglin, P. A. Klein, B. C. Crenshaw, I. M. Schumacher, D. R. Brown, and E. R. Jacobson, 1999. Upper Respiratory Tract Disease in the Gopher Tortoise Is Caused by *Mycoplasma agassizii*. Journal of Clinical Microbiology 37: 2262–2269.

Cagle, F.R. 1939. A system of marking turtles for future identification. Copeia 1939: 155–162.

Carpenter, J.W. 2004. Exotic animal formulary, 3e. Elsevier Saunders, Philadelphia, Pennsylvania.

Chittick, A.J., M.A. Stamper, J.F. Beasley, G.A. Lewbart, and W.A. Horne. 2002. Medatomidine, ketamine, and sevoflurane for anesthesia of injured loggerhead sea turtles: 13 cases (1996-2000). Journal of American Veterinary Medical Association 221:1019- 1025.

Dennis, P.M. and D.J. Heard. 2002. Cardiopulmonary effects to a medetomidine- ketamine combination administered intravenously in gopher tortoises. Journal of American Veterinary Medical Association 220:1516-1519.

Dickens, M.J., D.J. Delehanty, and L.M. Romero. 2010. Stress: An inevitable component of animal translocation. Biological Conservation 143: 1329-1341.

Douglas, J.F. and J.N. Layne. 1978. Activity and thermoregulation of the gopher tortoise (*Gopherus polyphemus*) in southern Florida. Herpetologica 34:359-374.

Drew, M. B., L. K. Kirkman, and A. K. Gholson, Jr. 1998. The vascular flora of Ichauway, Baker County, Georgia: A remnant longleaf pine/wiregrass ecosystem. Castanea 63:1-24.

duPre, S.A., C.R. Tracy, and K.W.Hunter. 2011. Quantitative PCR method for detection of *mycoplasma* spp. DNA in nasal lavage samples from the desert tortoise (*Gopherus agassizii*). Journal of Microbiological Methods 86: 160-165.

Eisenberg, J.F. 1983. The gopher tortoise as a keystone species. *In* Bryant,R.J., Franz, R., Eds, Proceedings of the 4th Annual Meeting of the Gopher Tortoise Council, Florida State Museum, Gainesville, Florida.

Eubanks, J.O., W.M. Michener and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). Herpetologica 59: 311-321.

Gaynor, J.S., and W.W. Muir III. 2008. Handbook of Veterinary Pain Management. Second edition. Mosby, Maryland Heights, Missouri, USA.

Grimm, K.A. and L. A. Lamont. 2007. Clinical pharmacology. Pages 3- 36 *in* G.D. West, Heard and N. Caulkett, editors. Zoo Animal & wildlife immobilization and anesthesia. Blackwell Publishing, Ames, Iowa, USA.

Harper, P.A.W., Hammond D.C., Heuschele W.P. 1982. A herpesvirus-like agent associated with a pharyngeal abscess in a desert tortoise. Journal of Wildlife Diseases 18:491-494.

Heaton-Jones, T.G., C. Jeff., H. Ko. And D.L. Heaton-Jones. 2002. Evaluation of medetomidine-ketamine anesthesia with atipamezole reversal in American Alligators (*Alligator mississipiensis*). Journal of Zoo and Wildlife Medicine 33:36-44.

Jackson, D. R. and E. G. Milstrey. 1989. The fauna of gopher tortoise burrows. Pages 86-98 in J. E. Deimer, D. R. Jackson, J. L. Landers, J. N. Layne, and D. A. Woods, editors. Proceedings of the gopher tortoise relocation symposium, Florida Game and Fresh Water Fish Commission Nongame Wildlife Program Technical Report 5. Tallahassee, Florida, USA.

Jacobson, E.R., J.M. Gaskin, M.B. Brown, R.K. Harris, C.H. Gardiner, J.L. LaPointe, H.P. Adams and C. Reggiardo. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). J. Wild. Dis. 27: 296-316.

Kreeger, T.J. 2007. Handbook of Wildlife Chemical Immobilization. International Wildlife Veterinary Services. Laramie, WY.

Landers, J. L., J. A. Garner, and W. A. McRae. 1980. Reproduction of the gopher tortoise (*Gopherus polyphemus*) in southwestern Georgia. Herpetologica 36:353-361.

Lips, K. R. 1991. Vertebrates associated with tortoise (Gopherus polyphemus) burrows in four habitats in south-central Florida. Journal of Herpetology 25:477–481.

MacLean, R.A., C.A. Harms, and J.Braun-McNeill. 2008. Propofol anesthesia in Loggerhead (*Caretta caretta*) sea turtles. Journal of Wildlife Diseases 44: 143-150.

Maxwell, J.H. 1979. Anesthesia and Surgery. Pages 127-152 *in* M. Harless and H. Morlock, editors. Turtles Perspectives and Research. Robert E. Krieger Publishing, Malabar, Florida, USA.

McArthur, S. 2004. Anesthesia, analgesia and euthanasia. Pages 379-401 *in* S. McArthur, J. Meyer, and R. Wilkinson, editors. Medicine and surgery of turtles and tortoises. Blackwell Publishing Ltd, Oxford, England.

McLaughlin, G.S., E.R. Jacobson, D.R. Brown, C.E. McKenna, I.M. Schumacher, H.P. Adams, M.B. Brown, and P.A. Klein. 2000. Pathology of upper respiratory tract disease of gopher tortoises in Florida. Journal of Wildlife Diseases 36:272-283.

McRae, W.A., J.L. Landers and G.D. Cleveland. 1981. Sexual dimorphism in the gopher tortoise (*Gopherus polyphemus*). Herpetologica 37: 46-52

Moon, P.F., and Stabenau E.K. 1996. Anesthetic and postanesthetic managementof sea turtles. Journal of the American Veterinary Medical Association 208:720–726.

Mosley C.A.E. 2005. Anesthesia and analgesia in reptiles. Seminars in Avian and Exotic Pet Medicine 14: 243-262.

Murphy, R.W., K.H. Berry, T. Edwards, A.E. Leviton, A. Lathrop, and J.D. Riedle. 2011. The dazed and confused identity of Agassiz's land tortoise *Gopherus agassizii* (Testudines, Testudinidae) with the description of a new species, and its consequences for conservation. Zookeys 113:39-71.

Norton, T.M., J. Spratt, J. Behler, and K. Hernandez. 1998. Medetomidine and ketamine anesthesia with atipamezole reversal in private free-ranging gopher tortoises, *Gopherus polyphemus.* Pages 25-27 *in* Proceedings of the Association of Reptile and Amphibiam Veterinarians, ARAV product website. < http://www.arav.org/products/>. Accessed 4 May 2012.

Norton, T.M. 2005. Chelonian Emergency Care. Seminars in Avian and Exotic Pet Medicine 14: 106–130.

Oppenheim, Y.C., and P.F. Moon. 1995. Sedative effects of Midazolam in red-eared slider turtles (*Trachemys scripta elegans*). Journal of Zoo and Wildlife Medicine 26:409-413.

Pettan-Brewer K.C.B., Drew M.L., Ramsay E., Mohr R.C., Lowenstine L.J. 1996. Herpesvirus particles associated with oral and respiratory lesions in a California desert tortoise (*Gopherus agassizii*). Journal of Wildlife Disease 32:521-526.

Posner, L. P. and P. Burns. 2009. Sedative agents: tranquilizers, Alpha-2 agonists, and related agents. Pages 337- 380 *in* Veterinary Pharmacology and Therapeutics, 9th Edition. Wiley-Blackwell, Ames, Iowa.

Rooney, M.B., G. Levine., J. Gaynor, E. Macdonald., and J. Wimsatt. 1999. Sevoflurane anesthesia in desert tortoises (*Gopherus agassizzi*). Journal of Zoo and Wildlife Medicine 30: 64-69.

Rossi, J.V., 2006. General Husbandry and Management, Pages 25-41 In Mader D.R. (ed.): Reptile Medicine and Surgery. Saunders Elsevier, St. Louis.

Schumacher J., and T. Yelen. 2006. Anaesthesia and analgesia. Pages 442-452 *in* Mader D.R. (ed.): Reptile Medicine and Surgery. Saunders Elsevier, St. Louis. 442–452.

Schumacher, J. 2007. West, G., D. Heard. and N. Caulkett. Chelonians (Turtles, Tortoises, and Terrapins). Pages 259- 266 *in* Zoo Animal and Wildlife Immobilization and Anesthesia. Blackwell Publishing Ltd, Oxford, UK.

Smith, L.L., T.D. Tuberville, and R.A. Seigel. 2006. Workshop on the ecology, status, and management of the gopher tortoise (*Gopherus polyphemus*), Joseph W. Jones Ecological Research Center, 16-17 January 2003: final results and recommendations. Chelonian Conservation and Biology 5:326-330.

Sladky, K.K., V.Miletic, J. Paul-Murphy, M.E. Kinney, Rebecca K. Dallwig., and S. M. Johnson. 2007. Analgesic efficacy and respiratory effects of butorphanol and morphine in turtles. Journal of American Veterinary Medical Association 230:1356-1362.

Sleeman, J.M. and J. Gaynor. 2000. Sedative and cardiopulmonary effects of medetomidine and reversal with atipamezole in desert tortoises (*Gopherus agassizii*). Journal of Zoo and Wildlife Medicine 31:28-35.

Tuberville, T.D., T.M. Norton, B.D. Todd and J.S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: A comparison of newly released and previously established animals. Bio. Con. 141: 2690-2697

Tuberville T.D., Clark E..E, Buhlmann K.A., Gibbons J.W. 2005. Translocation as a conservation tool: site fidelity and movement of repatriated gopher tortoises (Gopherus polyphemus). Animal Conservation, 8:349-358

Wambugu, S.N., P.K. Towett, S.G. Kiama, K.S.P. Abelson, and T.I. Kanui. 2010. Effects of opioids in the formalin test in the Speke's hinged tortoises (*Kinixy's spekii*). Journal of Veterinary Pharmacology and Therapeutics. 33: 347-351.

Wendland, L.D. 2007. Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. PhD Dissertation, University of Florida, Gainesville, Florida.

Wendland, L.D., P.A. Klein., E.R. Jacobson, and M.R. Brown. 2010. *Mycoplasma agassizii* strain variation and disting host antibody responses explain differences between enzyme-linked immunosorbent assays and Western Blot Assays. Clinical Vaccine Immunology 17:1739-1745.

Witz, B.W., D. S. Wilson, and M.D. Palmer. 1991. Distribution of *Gopherus polyphemus* and its vertebrate symbionts in three burrow categories. American Midland Naturalist 126:152–158.

Ziolo, M.S., and M.F. Berelsen. 2009. Effects of propofol administered via the supravertebral sinus in red-eared sliders. Journal of the American Veterinary Medical Association 3:390- 393.

**Figure 6.1. Summary of average times of all continuous variables by month.** Variables include anaesthetic first effects (1st effect), induction time (Induct T), sample start, total time it took the researcher to obtain samples (Total T Samp), time reversal was give (T Rev), time to spontaneous movement (T Spont) and total recovery time (Recov T).

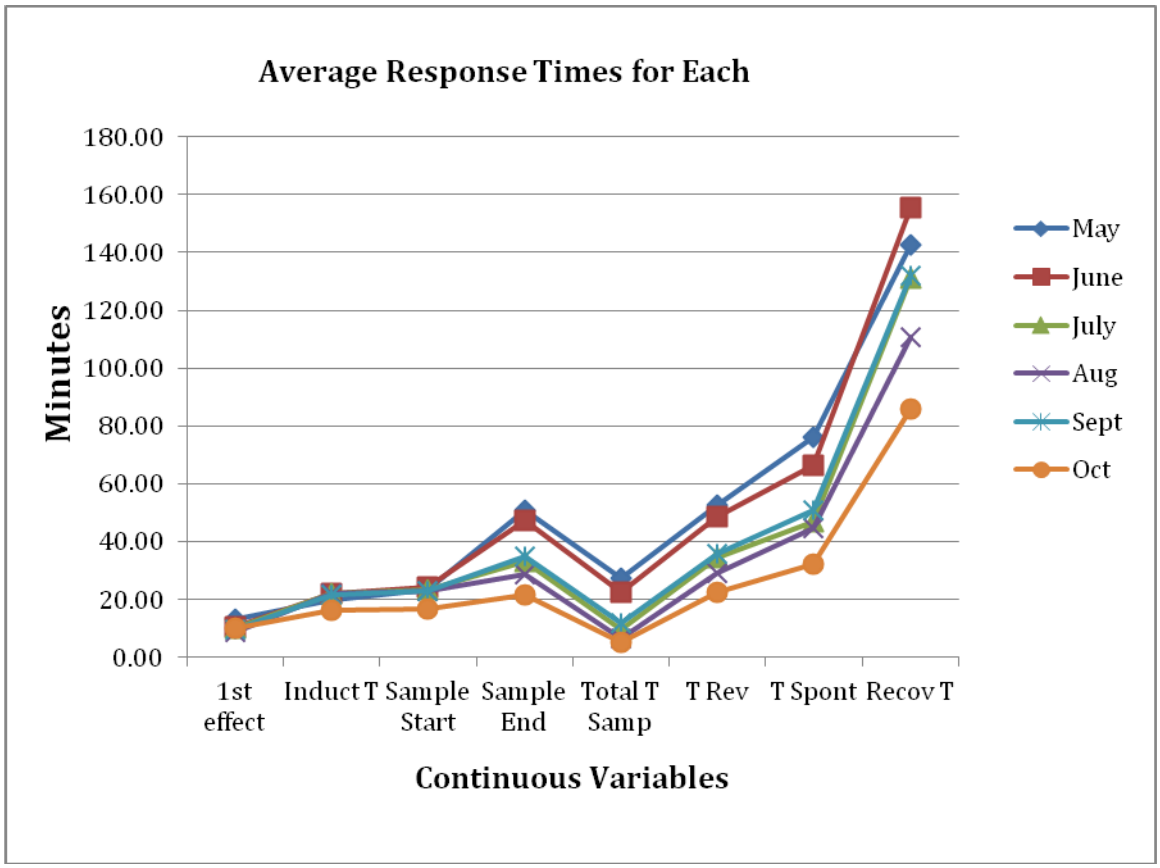

**Table 6.1. Time periods recorded to evaluate the effectiveness of a medetomidine-ketamine-morphine anesthetic protocol for the collection of health assessment data, blood and nasal lavage samples from gopher tortoises in Georgia.**

| Time stage | Definition |
|---|---|
| First effects | Time from injection of anesthesia drugs until we observed relaxation of head or limbs, ataxia, and/or loss of normal ambulatory movements. Some resistance noted. |
| Total time of induction | Time that the tortoise was completely anesthetized; i.e., the limbs were easily manipulated and head could be extended without resistance. |
| Sampling time | The total time it took to collect biological samples (nasal lavage and blood) |
| Reversal | Time at which reversal drugs were injected. |
| Time to spontaneous movement | Time from injection of reversal drugs until a gopher tortoise began to move its head or limbs without external stimuli |
| Reversal to recovery | Time from injection of reversal drugs until total recovery, the animal had returned to its pre-anesthetic condition and was determined to be safe to release. |
| Time to complete recovery | Time from injection of anesthesia until the animal had returned to its pre-anesthetic condition and was determined to be safe to release. |

**Table 6.2. Mean time (m) to different effect stages for an anesthesia protocol for gopher tortoises (N= 128) using a combination of medetomidine-ketamine-morphine.**

| Stage | Mean (± SD) | Range |
|---|---|---|
| Time to first effects | 9.69 ± 2.79 | 4-17 |
| Time to induction | 21.62 ± 5.48 | 10-36 |
| Total sampling time | 11.49 ± 9.17 | 2-40 |
| Time to spontaneous movement | 50.37 ± 14.67 | 27-100 |
| Reversal to recovery | 92.74 ± 6.42 | 10-266 |
| Time to complete recovery | 129.42 ± 65.20 | 40-300 |

**Table 6.3. Mean time (in minutes) to different effect stages for gopher tortoises that achieved appropriate, light and deep anesthesia.** *

| | Appropriate (n=95) | | Light (n=28) | | Deep (n=3) | |
|---|---|---|---|---|---|---|
| **Stage** | **Mean (± SD)** | **Range** | **Mean (± SD)** | **Range** | **Mean (± SD)** | **Range** |
| Time to first effects | 10.11 ± 2.78 | 4-17 | 8.80 ± 2.49 | 4-13 | * | * |
| Time to induction | 21.33 ± 5.45 | 10-36 | 23.04 ± 6.23 | 13-36 | 17.67 ± 3.02 | 14-20 |
| Total sampling time | 11.44 ± 8.89 | 2-40 | 11.68 ± 10.89 | 2-38 | 6.33 ± 3.50 | 3-10 |
| Time to spontaneous movement | 51.14 ± 15.08 | 27-100 | 48.81 ± 14.35 | 32-79 | 42 ± 4.62 | 34-50 |
| Reversal to recovery | 98.16 ± 60.73 | 18-266 | 73.83 ± 74.47 | 10-263 | * | * |
| Time to complete recovery | 134.51 ± 63.78 | 45-300 | 110.17 ± 78.02 | 40-300 | * | * |

**Table 6.4. Mean time (in minutes) to different effect stages for gopher tortoises that experienced appropriate or prolonged recovery from anesthesia.**

| | Appropriate (n=96) | | Long (n=32) | |
|---|---|---|---|---|
| **Stage** | **Mean (± SD)** | **Range** | **Mean (± SD)** | **Range** |
| Time to first effects | 9.42 ± 2.80 | 4-17 | 10.60 ± 2.50 | 5.5-16 |
| Time to induction | 21.78 ± 5.70 | 11-36 | 21.25 ± 4.95 | 10-32 |
| Total sampling time | 9.46 ± 7.53 | 2-35 | 17.11 ± 10.34 | 3-40 |
| Time to spontaneous movement | 47.17 ± 13.02 | 27-85 | 58.94 ± 14.38 | 27-100 |
| Reversal to recovery | 59.49 ± 30.06 | 10-123 | 171.81 ± 42.72 | 112-266 |
| Time to complete recovery | 93.13 ± 31.15 | 40-170 | 213.75 ± 41.29 | 180-300 |

**Table 6.5. Mean time (in minutes) to different effect stages for adult gopher tortoises with clinical signs of *Mycoplasma* related upper respiratory tract disease as compared to tortoises where no clinical signs were detected.**

| | Clinical signs(n=22) | | No clinical signs (n=106) | |
|---|---|---|---|---|
| **Stage** | **Mean (± SD)** | **Range** | **Mean (± SD)** | **Range** |
| Time to first effects | 7.78 ± 2.53 | 4-12 | 10.06 ± 2.70 | 5-17 |
| Time to induction | 18.91 ± 5.24 | 11-36 | 22.20 ± 5.36 | 10-36 |
| Total sampling time | 8.10 ± 3.02 | 3-24 | 12.13 ± 9.51 | 2-40 |
| Time to spontaneous movement | 43.90 ± 10.18 | 27-80 | 51.79 ± 14.72 | 27-100 |
| Reversal to recovery | 83.31 ± 70.66 | 10-263 | 95.59 ± 60.32 | 10-266 |
| Time to complete recovery | 112.25 ± 71.99 | 40-300 | 132.06 ± 64.52 | 20-300 |

# CHAPTER 7

# CONCLUSIONS

The primary objective of this dissertation was to better understand the distribution and impacts of upper respiratory tract disease (URTD) in gopher tortoise (*Gopherus polyphemus*) populations in Georgia. Knowledge of disease threats to tortoise populations throughout their range is critical given that the gopher tortoise (including Georgia populations) was recently classified as a candidate for federal listing as a threatened species (USFWS, 2011). With tortoises being relocated due to human activities (both planned translocations in response to development and relocation of individuals by the lay public) it is imperative that researchers and managers understand, and plan for, disease outbreaks in gopher tortoise populations. Health assessments are crucial in these decisions and the future management of gopher tortoises. To obtain baseline data on *Mycoplasma* prevalence in Georgia, I sampled a number of sites in addition to collecting historical data from other researchers. In an effort to better understand the potential impacts of disease on a free-ranging gopher tortoise population, I focused on one site (Jones Ecological Research Center) for various aspects of this dissertation. Conclusions from the individual chapters of my dissertation are summarized below.

**Study 1 (Chapter 3)**

The goal of this study was to investigate the prevalence of *Mycoplasma* spp. at a range of sites in Georgia. I conducted health assessments of gopher tortoises at nine sites and incorporated data from a previous study. I found that exposure to both *Mycoplasma agassizii* and *M. testudineum* varied spatially among Georgia tortoise populations. Among the populations

sampled the prevalence of antibodies to *M. agassizii* was either very low (0%) or very high (100%), whereas there was more variation in prevalence of *M. testudineum* (38% to 61%, with only one site negative). Five sites were seropositive for both pathogens and had tortoises with clinical signs consistent with URTD. Sites where no clinical signs were observed were positive for only *M. testudineum*. These data contradict work that has been done in Florida, where *M. agassizii* was widely-distributed and *M. testudineum* was more restricted (Brown et al., 2004; Wendland, 2007). Additionally, a number of juvenile gopher tortoises (CL <230mm) in our study were positive for both species of *Mycoplasma*. One juvenile from JERC was PCR positive, which combined with serology demonstrates that tortoises can become infected by both pathogens at a younger age than has been previously reported (Beyer, 1993; Wendland, 2007).

The all or none prevalence of *M. agassizii* suggests that some sites have a history with the pathogen, where others may not. It is currently unclear what the consequence of having both pathogens in a population is. However, despite small sample sizes at some sites, it appears that tortoises at sites with both pathogens were more likely to exhibit clinical disease. Gopher tortoises are one of the most commonly translocated animals (Tuberville et al., 2008); therefore, it is important to understand the distribution of pathogens in populations and to consider the potential to expose tortoises to novel pathogens. Multiple strains of *M. agassizii* have been documented (Wendland et al., 2010) and there is also a likelihood of geographic variation in virulence (Seigal et al., 2003). These issues warrant further study in Georgia and elsewhere.

**Study 2 (Chapter 4)**

I found that gopher tortoises on a long-term study site (Green Grove), located within the Joseph W. Jones Ecological Research Center (JERC) with consistently high seroprevalence for

*Mycoplasma agassizii*, exhibited stable home range sizes and showed 76% site fidelity after 15 years. In contrast, gopher tortoises (site-wide at JERC) with severe clinical signs of URTD used significantly larger home ranges than asymptomatic and mildly symptomatic tortoises. Additionally, tortoises with severe clinical signs selected a broad range of temperatures, in comparison to the asympomatic and mild groups, which suggests abnormal thermoregulatory behavior. These results were consistent with studies that show diseased chelonians can exhibit behavioral change to combat infection of a pathogen (Monagas and Gatten, 1983; Amaral et al., 2002; Swimmer, 2006). My findings refute the previously proposed hypothesis that chronically URTD infected tortoises are less likely to emigrate (Ozgul et al, 2009). In fact, my data show that clinically ill tortoises may be more likely to emigrate resulting in lower detection of deceased tortoises at an individual site. It is important to understand the cyclic nature of URTD when planning for relocation, and to prepare for the potential subsequent long distance dispersal. The results of this study highlight the risk of translocation of gopher tortoises and the importance of monitoring populations over time to understand the impacts of disease (Perez-Heydrich et al. 2012).

**Study 3 (Chapter 5)**

Limited work has been done on gopher tortoise endoparasites in the gopher tortoises (Walton 1927, Ernst *et al.* 1971; Petter and Douglass 1976; Lichtenfels and Stewart 1981; Diaz-Figueroa 2005). In the current study of 117 tortoises from eight counties in Georgia, I found evidence of infection with eight parasites including four nematodes, a cestode, and three protozoans. This represents the first report of a cestode, believed to be an *Oochorstica* sp., a capillarid, an ascarid, and *Entamoeba* in gopher tortoises. I found highly variable distribution of

many parasites. Similar to previous studies, pinworms were the most common parasite detected in most of the surveyed gopher tortoise populations (Bouamer *et al.* 2001). Considerable variation was noted in prevalence between sites for *Chapiniella* with two sites negative and on site with a significantly higher prevalence than all other sites with 74% (Table 1). *Entamoeba* and *Cryptosporidium*, were both present in low prevalence at one site. Both of these parasites have been reported from tortoises, including gopher tortoises (Raphael *et al.* 1997; Stedman *et al.* 2000). *Entamoeba invadens* can cause clinical disease (colitis, diarrhea, liver abscesses) and death in numerous species of reptiles; a case of hepatic necrosis has been reported in a gopher tortoise (Stedman *et al.* 2000). *Cryptosporidium* spp. have been reported from a wide range of reptiles, amphibians, mammals, and birds. Clinical disease in reptiles, including other species of tortoises) is commonly associated with *Cryptosporidium* infections (Brownstein *et al.* 1977; Graczyk *et al.* 1998; Griffin *et al.* 2010). Therefore, endoparasites need to be considered when evaluating the health of gopher tortoises, particularly tortoises being relocated. It would be interesting to investigate the parasite communities of commensal organisms as well.

**Study 4 (Chapter 6)**

The objective of this study was to assess the utility and safety of a novel field anesthetic combination of dexmedetomidine-ketamine-morphine (75 mc/kg; 8 mg/kg; 1 mg/kg respectively), followed by reversal with atipamezole (0.02 mg/kg). I also wanted to assess whether or not the presence of clinical signs of URTD would impact the quality of anesthesia or recover. The purpose anesthetic was to aid in handling of tortoises for the purpose of getting sterile biological samples. Adult gopher tortoises are difficult to physically restrain for complete physical exams and particularly to expose the head, neck and legs for biological sample

collection (Bennett, 1998). Additionally, nasal lavage is required to dislodge as many bacteria as possible and quality of these samples influence the success of testing (McLaughlin et al., 2000; Wendland et al, 2007; duPre et al, 2011). Average time to first effects was 9.7 min, to induction was 21.6 min, to total recovery after administration of reversal was 92.7 min and the mean time from administration of anesthesia through complete recovery for adult tortoises was 129 minutes but ranged from 40-300 minutes. Clinical signs of URTD significantly reduced the induction time such that tortoises with clinical signs loss consciousness faster than healthy tortoises. Study month significantly influenced the time to induction and time to recovery such that as the study progressed, both parameters decreased. This anesthetic protocol proved to be an effective, reliable, repeatable, and safe method to collect quality biological samples from gopher tortoises.

**Management Concerns**

Documenting the presence of disease in populations, particularly those of management concern, requires the establishment of long term monitoring programs. The work presented in this dissertation not only highlights the importance of long-term data sets, the data also illustrates the benefits of focusing on individuals in a population. Previous studies have failed to monitor individuals over time and, as a result, they may miss important aspects of disease ecology. Once a tortoise is seropositive for *Mycoplasma* it will potentially remain a source of infection for its lifetime (Wendland, 2007). This is concerning because I found clinically ill travelled very long distances, despite their moribund condition. Therefore, all tortoises marked for relocation should be tested for *Mycoplasma* spp. and monitored for URTD. There are currently no regulatiosn in place requiring testing for gopher tortoises slated for translocation or for those at recipient sites, although, parties involved voluntarily have the tortoises tested. One time visual inspections of

individuals are not adequte to rule out infectious diseases as clinical signs can recrudesce and tortoise health can unexpectedly decline. The population level effects of URTD on tortoises are unknown and this topic warrants further investigation. The collective data in this dissertation should serve as a baseline dataset for Georgia, but additional data are needed.

Chronic persistent disease has the potential to significantly impact populations (Ozgul et al., 2009; Perez-Heydrich et al., 2012). It is concerning that URTD was found in a number of populations in Georgia. The recrudescence of clinical disease could potentially shorten the lifespan of the tortoise, thereby impacting potential reproductive contributions. Models presented by Perez-Heydrich et al., (2012) showed that potential impact on a population is based on how long the disease persists and how often the disease recurs. Data necessary to confirm their hypothesis will only be obtained through the establishment of monitoring programs. Ozgul et al., (2009) recommend monitoring populations over a 10-20 year period. Unless a population is a long term research site, such as with the JERC, it may be prudent to carry out population surveys more frequently, perhaps 5-10 year periods. Additionally, pathogen screen should also occur in concert with population surveys.

**Literature Cited**

do Amaral, J.P.S., G.A. Martin, and V.H. Hutchison. 2002. The influence of baterical lipopolysaccharide on thermoregulation of the box turtle *Terrapene carolina*. Physiological and Biochemical Zoology 75: 273-282.

Beyer, S. M. 1993. Habitat relations of juvenile gopher tortoises and a preliminary report of upper respiratory tract disease (URTD) in gopher tortoises. M.S. Thesis, Iowa State University, Ames, Iowa, 95 pp.

Brown, D. R., J. L. Merritt, E. R. Jacobson, P. A. Klein, J. G. Tully and M. B. Brown. 2004. *Mycoplasma testudineum* sp. nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. Int J Syst Evol Microbio 54: 1527–1529.

Brownstein, D.G., Strandberg, J.D., Montali, R.J., Bush, M., Fortner, J. (1977). *Cryptosporidium* in snakes with hypertrophic gastritis. Vet Pathol 14, 606-617. Doi:10.1177/030098587701400607

Diaz-Figueroa, O. (2005). Characterizing the health status of the Louisiana gopher tortoise (*Gopherus polyphemus*). Thesis, Louisiana State University, Baton Rouge, USA. 109 pp.

duPre, S.A., C.R. Tracy, and K.W.Hunter. 2011. Quantitative PCR method for detection of *mycoplasma* spp. DNA in nasal lavage samples from the desert tortoise (*Gopherus agassizii*). Journal of Microbiological Methods 86: 160-165.

Ernst J.V., Fincher, G.T. and Stewart, T.B. (1971). *Eimeria paynei* sp. n. (Protozoa: Eimeriidae) from the gopher tortoise (*Gopherus polyphemus*). Proc Helminthol Soc Wash 38, 223-224.

Eubanks, J. O., W. K. Michener, and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). Herpetologica 59:311-321.

Graczyk, T.K., Cranfield, M.R., Mann, J., and Strandberg, J.D. (1998). Intestinal *Cryptosporidium* sp. infection in the Egyptian tortoise, *Testudo kleinmanni*. Int J Parasitol 28, 1885-1888. doi:10.1016/S0020-7519(98)00143-X.

Griffin, C., Reavill, D.R., Stacy, B.A., Childress, A.L., and Wellehan J.F. Jr. (2010). Cryptosporidiosis caused by two distinct species in Russian tortoises and a pancake tortoise. Vet Parasitol 170, 14-19. doi:10.1016/j.vetpar.2010.01.039.

Lichtenfels, J.R. and Stewart, T.B. (1981). Three new species of *Chapiniella* Yamaguti, 1961 (Nematoda: Strongyloidea) from tortoises. Proceedings of the Helminthological Society Washington 48: 137-147.

McLaughlin, G. S., E.R. Jacobson, D.R. Brown, C.E. McKenna, I.M. Schumacher, H.P. Adams, M.B. Brown and P.A. Klein. 2000. Pathology of upper respiratory tract disease of gopher tortoises in Florida. 36: 272-283.

Monagas, W.R. and R.E. Gatten. 1983. Behavioural fever in the turtles *Terrapene carolina* and *Chrysemys picta*. Journal of Thermal Biology 8:285-288.

Ozgul, A., M.D, B.B, and C. Perez-Heydrich. 2009. Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises. Ecological Applications, 19:786-798.

Perez-Heydrich C., M.K. Oli, and M. Brown. 2012. Population- level impacts of a recurring disease on a long-lived wildlife host. Oikos 121:377-388.

Petter, A.J. and Douglass, J.F. (1976). Etude des populations d'Oxyures du colon des *Gopherus* (Testudinidae). Bull Mus Natl Hist Natur, strie 3, no. 389, Zoologie 271, 731-768.

Raphael BL, Calle PP, Gottdenker N, James S, Linn WJ, McNamara T, Cook RA. (1997). Clinical significance of *Cryptosporidia* in captive and free-ranging chelonians. In

*Proceedings of the Annual meeting of the American Association of Zoo Veterinarians*, Houston, Texas. AAZV; 1997, 19-20.

Seigel, R. A., R.B. Smith, and N. A. Seigel. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. Journal of Herpetology 37:137-144.

Stedman, N.L., Garcia, A., and Latimer, K.S. (2000). Severe hepatic necrosis associated with entamoebiasis in a gopher tortoise (*Gopherus polyphemus*). Proceedings of International Virtual Conferences in Veterinary Medicine: Diseases of Reptiles and Amphibians. http://www.vet.uga.edu/vpp/archives/ivcvm/2000/stedman/index.php

Swimmer, J.Y. 2006. Relationship between basking and fibropapillomatosis in captive green turtles (*Chelonia mydas*). Chelonian Conservation and Biology 5: 305-309.

Tuberville, T.D., T.M. Norton, B.D. Todd and J.S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: A comparison of newly released and previously established animals. Biol Conserv 141:2690-2697.

U.S. Fish and Wildlife Service (USFWS). 2011. Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition To List the Gopher Tortoise as Threatened in the Eastern Portion of Its Range; Final Rule. Accessed online 5 September 2012 http://www.fws.gov/northflorida/GopherTortoise/12-month_Finding/20110726_frn_Gopher-Tortoise_12month_finding.htm.

Walton, A.C. (1927). A revision of the nematodes of the Leidy Collection. Proc Acad Nat Sci Phil 79, 49-163.

Wendland, L.D. 2007. Epidemiology of mycoplasmal upper respiratory tract disease in tortoises. PhD Dissertation, University of Florida, Gainesville, Florida**.**

Wendland, L., P.A. Klein, E.R. Jacobson, and M.B. Brown. 2010. Strain variation in *Mycoplasma agassizii* and distinct host antibody responses explain differences between ELISA and Western blot assays. Clin. Vaccine Immunol. 17:1739-1745.