

1. Project Description

Objective – The purpose of this field study is to investigate the long-term effects of Upper Respiratory Tract Disease (URTD) on a population of wild Gopher tortoises (*Gopherus polyphemus*) in South Florida. The data collected during the course of this study will be analyzed in conjunction with test-retest data taken on the same population of Gopher tortoises over the past decade. Specific target individuals from this population that have tested positive for *M. agassizii* and/or *M. testudineum* antibodies at least once in the past will be retested and the levels of antibodies present will be analyzed in comparison to past results of the same individuals. The knowledge this analysis will provide will greatly contribute to the greater understanding of the progression of URTD in Gopher tortoises and its effect on the survival and quality of life of the species. The present number of studies that have retested URTD-positive tortoises over long periods of time is minuscule and such studies are crucial to completely understanding the impact of URTD on Gopher tortoise demographics. Moreover, such knowledge could potentially contribute to the revision of current policies on conservation and relocation of URTD-positive Gopher tortoises by the Florida Fish and Wildlife Conservation Commission.

Background – Population declines have been observed in three North American *Gopherus spp.* including *Gopherus polyphemus*, the Gopher tortoise. Gopher tortoises have experienced significant population decline throughout their geographic range of the Southeastern United States (McCoy et al. 2007; Ozgul et al. 2009; McGuire et al. 2014) and were classified as threatened in Florida as of 2007 (Karlin 2008; Wendland et al. 2010). The Gopher tortoise is a burrowing species with a relatively long lifespan, and constructs subterranean burrows for shelter (Wendland et al. 2010). Although the majority of its recent population decline is attributed to habitat loss and fragmentation due to anthropogenic activity, infectious disease-related mortality is also a significant contributing factor (Ozgul et al. 2009).

Upper Respiratory Tract Disease (URTD) is a highly contagious infectious disease whose effect on Gopher tortoise populations is not fully understood and severely under-researched (McCoy et al. 2007; McGuire et al. 2014). The emergence of URTD as a major threat to Gopher tortoise populations began in the early 1990s (Wendland et al. 2010) and has proven to cause adult mortality (McCoy et al. 2007; Ozgul et al. 2009). URTD is caused by several pathogens, but *Mycoplasma agassizii* and *Mycoplasma testudineum* are the most common causative agents of the disease (McCoy et al. 2007; Jacobson et al. 2014; McGuire et al. 2014). The most common symptoms of URTD observed in Gopher tortoises are palpebral edema, or eyelid swelling, conjunctivitis, congestion, sneezing/coughing, and nasal and ocular discharges due to lesions (Karlin 2008; Jacobson et al. 2014). However, subclinical infections are also possible where no physical symptoms are present (Jacobson et al. 2014). Transmission most likely occurs via direct contact between tortoises, generally during combat or reproductive behaviors (Karlin 2008; Ozgul et al. 2009; Jacobson et al. 2014). In experimental studies, clinical signs of the disease can be seen as soon as two weeks post infection, whereas seroconversion can take approximately 8 weeks (Karlin 2008; Jacobson et al. 2014).

An enzyme-linked immunosorbent assay (ELISA) test is used to detect URTD caused by *M. agassizii* and *M. testudineum* by measuring the presence of antibodies for said pathogens in plasma samples from targeted individuals (Karlin 2008; Wendland et al. 2010; Jacobson et al. 2014). This test can reveal the presence of *M. agassizii* and *M. testudineum* in tortoises not

showing clinical signs of URTD, which could imply the individual being tested is a carrier for the disease or recovering from a previous infection (Karlin 2008; Jacobson et al. 2014). However, the ELISA test cannot definitively diagnose URTD in Gopher tortoises alone. More invasive investigation of the upper respiratory tract is necessary to make such diagnoses. However, the results of the ELISA test have proven to be highly correlated with the presence of the infection in conjunction with the display of physical symptoms such as nasal discharge (Karlin 2008).

The presence of URTD and its associated pathogens have been documented in several South Florida populations, including the population targeted in this study (Karlin 2008). The long-term effects of URTD on Gopher tortoise individuals and populations are unknown due to lack of long-term monitoring of infected individuals (Karlin 2008; Ozgul et al. 2009; Wendland et al. 2010; Jacobson et al. 2014). However, URTD in tandem with the threat of habitat loss due to human activity put the Gopher tortoise at risk of further severe population decline (Karlin 2008; Wendland et al. 2010). Under current regulations of the Florida Fish and Wildlife Conservation Commission, relocation of URTD-positive Gopher tortoises is prohibited and said individuals are often euthanized (FFWCC 2006b). The course of URTD in Gopher tortoises includes several phases of varying expression of clinical symptoms and seroprevalence. It is assumed that death is the ultimate end result, however no research to date supports this (Ozgul et al. 2009). More research is necessary to accurately and completely define the horizontal transmission of URTD and its true effects on Gopher tortoise populations (Ozgul et al. 2009; Jacobson et al. 2014).

Methodology – This field study will focus on the wild Gopher tortoise population in Range IV of the Abacoa Greenway in Jupiter, FL. More specifically, we will track down and retest about 25 specific target tortoises who have tested positive for *M. agassizii* and/or *M. testudineum* at least once before. The majority of these tortoises are micro-chipped or their location within the study area is documented. The targets will be located and we will take a blood sample as well as routine size measurements. My PI and I will externally examine each target for external symptoms of URTD, all of which will be documented, if any.

The collected blood samples will be shipped to the Mycoplasma Research Laboratory of the University of Florida to undergo an ELISA serology for both *M. agassizii* and *M. testudineum*. The ELISA serology test will determine the presence (if any) of *M. agassizii* and/or *M. testudineum* antibodies in the plasma sample separated from the blood samples taken by my PI and myself, and at what levels the antibodies are present. The results will be mailed from the University of Florida to my PI for statistical analysis in comparison to past results of the same tortoises.

Anticipated Outcomes – We anticipate the results of the ELISA serology and subsequent comparison of these results to past results from the same tortoises will reveal the true nature of the progression of URTD in Gopher tortoises. The compilation of the results of this study with the results from the same tortoises from up to the past decade (depending on the tortoise) will provide significant insight into how levels of *M. agassizii* and/or *M. testudineum* antibodies fluctuate over time. Ideally, the data will reveal that contraction of URTD does not imply mortality, which could be a major factor determining the appropriate management and conservation of Gopher tortoise populations.

2. Timeline

Date(s)	Objective
October 1, 2015	Project Start Date
October 1 – December 10 2014	Locate target individuals and collect blood samples/data
December 2014 – January 2015	ELISA Serology at the University of Florida Mycoplasma Research Lab
February 1 – March 9, 2015	Data analysis
March 10, 2015	Project End Date
March 19-21, 2015	Present Research at the Florida Academy of Science Annual Meeting
April 3, 2015	Present Research at the Florida Atlantic University Undergraduate Research Symposium
April 10, 2015	Present Research at the Harriet L. Wilkes Honors College Undergraduate Research Symposium
May 1, 2015	Anticipated Graduation Date

3. Budget and Budget Justification

Item Description	Supplier	Price	Quantity Needed	Total Price
ELISA Serology for <i>M. agassizii</i> and <i>M. testudineum</i>	Mycoplasma Research Laboratory, University of Florida	\$56	25	\$1400
FedEx Shipment of Samples	FedEx	\$40	1	\$40
Total				1440

Budget Justification

1. The ELISA Serology for *M. agassizii* and *M. testudineum* is necessary for determining the presence and levels of *M. agassizii* and *M. testudineum* antibodies in the targeted Gopher tortoises. The Mycoplasma Research Laboratory of the University of Florida is the closest facility to our study site that performs the necessary ELISA Serology tests and the price listed is the current price the laboratory charges for the ELISA Serology test for both *M. agassizii* and *M. testudineum*.
2. Shipping the collected samples to the University of Florida in Gainesville, FL is necessary to receive the test results for this study. FedEx is the only carrier that delivers directly to the Mycoplasma Research Laboratory of the University of Florida, which is in the best interest for the preservation of the samples during shipment. Though the total cost of the tests and shipping is \$1,440 exceeds the \$500 offered by this grants, the remaining cost will be covered by separate funds provided by my PI, Dr. Jon Moore of Florida Atlantic University.

References

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