PLASMA BIOCHEMISTRY, HEMATOLOGY, AND BLOOD PARASITES OF A TRANSLOCATED POPULATION OF GOPHER TORTOISES (GOPHERUS POLYPHEMUS) FROM GEORGIA

by

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(Under the Direction of Michael J Yabsley)

ABSTRACT

Gopher tortoises (*Gopherus polyphemus*) are a long-lived terrestrial tortoise endemic to the southeastern United States. Due to habitat loss and human intervention, they are one of the most translocated species. It is not clear as to the impact that disease or parasites have on populations and few long-term studies have been conducted to monitor the health status of gopher tortoises. The overall goal of this study was to contribute to the baseline health parameters of the species by establishing blood reference values (n = 145) and by evaluating the prevalence of haemogregarines (Apicomplexa: Adeleorina), an intraerythrocytic protozoan parasite, in a translocated population of tortoises on St Catherines Island, Georgia. Based on blood smears and ectoparasite data from 22 adults and 12 juveniles, 86% and 0%, respectively, which leads us to believe that tortoises hatched on the island have not been exposed to the potential vector, *Amblyomma turberculatum*.

INDEX WORDS: *Gopherus polyphemus*, Biochemistry, Hematology, Haemagregarine

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August 2014

DEDICATION

I dedicate this thesis to my husband, Stewart, and to my children, Ciaran, Rory, Honora, Aiden and Kellar. It is because of you that I have persevered and sacrificed. Your unconditional love and support have kept me going.

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I'd like to thank Michael Yabsley for taking a chance on me and giving me the opportunity to prove myself. He's been nothing but patient with me and has shown me all that I'm capable of. Some very valuable life lessons have been learned during my time in his lab.

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CHAPTER 1

INTRODUCTION and LITERATURE REVIEW

Ecology and threats to gopher tortoises

Gopher tortoise populations have declined throughout much of their historical range and are federally listed as threatened in the western portion of the species' range (west of the Mobile and Tombigbee Rivers in Alabama, Louisiana, and Mississippi). In Georgia, the tortoise is state-listed; however, it is now a candidate for federal listing in the eastern portion of its range (USFWS, 2011). This long-lived, charismatic species is considered a keystone in its habitat because over 300 other species, including the endangered indigo snake (*Drymarchon couperi*); utilize its burrows at some point in their life cycles (Eisenberg 1983, Jackson and Milstrey 1989). The gopher tortoise utilizes open, savanna-like habitats and longleaf pine (*Pinus palustris*) forests, a landscape that once dominated the southeastern region of the United States. Today, those habitats are threatened by construction and development activities and poor land management.

Disease is an often overlooked and poorly misunderstood threat to wild populations of gopher tortoises. Upper respiratory tract disease (URTD), which is primarily caused by *Mycoplasma agassizii* and *M. testudineum*, and to a lesser extent, viruses, has been reported in both captive and wild populations of gopher tortoises throughout its range (Smith 1998; Brown et al., 1999; Deimer Berish et al., 2000; Deimer Berish et al., 2010). The disease has contributed

to widespread morbidity and mortality of gopher tortoises (Brown et al., 1999; Deimer Berish et al., 2000; Gates et al., 2002; Seigel et al., 2003). Although other pathogens, such as ranavirus and herpesvirus, and several parasites have been reported from gopher tortoises, their effects on gopher tortoise health are largely unknown (Westhouse et al., 1996; Johnson et al., 2008; McGuire et al., 2013). In addition, stress from translocation or poor habitat, lack of food and water sources, extreme weather events and other underlying health conditions can exacerbate the effects of disease on individuals and populations (Wendlend et al., 2010). Tortoises that have been moved to a new site or habitat may be particularly vulnerable as they are potentially exposed to novel food sources, other established tortoises, predators, and potential burrowing sites. Unfortunately, sometimes individuals from more than one population are moved to a new single location, which may increase the risk of exposure of some tortoises to novel pathogens (Jacobson, 1993). There is currently a lack of information concerning what constitutes a healthy tortoise. Establishing baseline blood values for individuals and populations of wild gopher tortoise will aid veterinarians, biologists and land managers in recognizing potentially diseased tortoises and in making sound decisions concerning the re-establishment of viable populations of free-ranging tortoises.

Hematology and clinical chemistry analyses

Assessment of hematologic and biochemical parameters is part of a comprehensive group of parameters that can be used to evaluate the health of an individual, and populations of animals. These values allow veterinarians and biologists to evaluate an animal's physiologic response to its environment, particularly in free-ranging wildlife, and to assess pathologic changes in an individual. Typically, free-ranging animals are given cursory physical exams and

are screened for external parasites, but this rarely provides a comprehensive evaluation of the health of an animal.

Typical hematological data consists of a complete blood cell count (CBC) and a biochemical panel. The CBC is analyzed with whole blood and plasma and includes packed cell volume (PCV), total proteins (TP), total red blood cell count, total leukocyte count and differential leukocyte count. Biochemical assays are tested on either plasma or serum, with plasma being more commonly used in reptiles (Thrall et al., 2004), and include measuring concentrations of aspartate aminotransferase (AST), albumin/globulin ration (AG), albumin, pre-amylase, alkaline phosphatase (alk phos), bilirubin, blood urea nitrogen (BUN)/creatinine, calcium, creatine, CPK, chloride, cholesterol, glucose, gamma glutamyl transferase (GGT), globulin, lipase, lactic dehydrogenase (LDH), phosphorous, potassium, pre-albumin, sodium, triglycerides, total protein, and uric acid (Table 1).

Baseline blood values for a population are used to measure deviations from the normal value for each cellular concentration (Geffre et al., 2009; Nardini et al., 2013). Identifying normal blood values in a species are critical in distinguishing healthy animals from those that are impaired. However, analytes are highly variable amongst individuals and species, especially in reptiles, which in turn makes it difficult to elucidate hard and fast reference ranges for a given species. Factors that must be considered when interpreting blood data are whether an animal is captive or free-ranging and the conditions to which the animal was exposed to at the time of blood collection. Extrinsic factors that may affect values of blood analytes include species, season, temperature, nutrition, stress, disease, and blood collection site while intrinsic factors would include reproductive state, gender, and age (Musacchia and Sievers, 1956; Mader, 1996; Christopher et al., 1999; Ramon Lopez-Olvera et al., 2003; Hernandez et al., 2011; Scope et al.,

2013). Reference values are best established under controlled circumstances (e.g. clinically healthy animals maintained in a temperature-controlled, nutrition-controlled laboratory setting), utilizing at least 120 individuals to allow for expected variation. At the minimum, 40 individuals are suggested for statistically robust results (Thrall, 2004; Geffre et al., 2009). Establishing reference ranges of a wild population make the above requirements difficult to fulfill, at best.

The packed cell volume (PCV) estimates the percentage of erythrocytes that are circulating in the peripheral blood. Low PCV can indicate blood loss, various types of anemia, nutritional deficiencies, or dilution from over hydration, while an elevated PCV typically indicates concentration of plasma from dehydration. Erythrocytes in tortoises are larger than those in snakes and lizards and occupy more space in the blood; thereby total erythrocyte counts in tortoises tend to be lower than other reptiles (Mader 1996, Thrall 2004). Eastern Box Turtles (*Terrapene carolina carolina*) have been shown to exhibit seasonal variation in PCV, with the highest values occurring in spring and the lowest values in the fall (Kimble et al, 2012). A higher PCV has been found in males in desert tortoises and alligator snapping turtles, particularly in the fall which was attributed to a lower immune response (Dickinson et al, 2002; Chaffin et al., 2008). The proportions of leukocytes are measured to evaluate changes due to inflammation, diet, hydration, parasites and disease and are highly variable depending on species, sex, age, season, environmental conditions, and disease. Desert tortoises with URTD had elevated levels of monocytes, heterophils and lymphocytes (Christopher et al, 2003). Taylor and Jacobson (1982) found that total leukocyte and monocyte counts were significantly higher in the spring than samples from the fall.

In domestic animals, and for some parameters in reptiles, biochemical analytes are good indicators of physiological function in response to pathological processes (Table 1) and to

environmental conditions. Some tortoise-specific differences that have been documented include total protein and cholesterol, which were higher in female than in male gopher tortoises, while other studies have found that calcium, globulin, and albumin were significantly higher in reproductively active female free-ranging box turtles (*Terrapene carolina carolina*) (Kimble and Williams, 2012), and calcium, phosphorous, triglycerides and cholesterol were significantly higher in female Alligator Snapping Turtles (*Macrochelys temminckii*) from Georgia (Chaffin et al, 2008). All of these values are associated with egg production and display seasonal variability. Significant seasonal variation and differences between location and sex were reported in desert tortoises for AST, cholesterol, phosphorous, calcium, and triglycerides (Christopher et al, 1999, Dickinson et al, 2002). Both attributed seasonal differences to rainfall patterns and forage availability.

Response to parasite infestation is variable, but a study on *Psammodromus algirus* lizards reported a marked increase in monocytes in lizards with *Ixodes ricinus*. Interestingly, 43% (12/28) of lizards also had a positive haemagregarine parasitemia (Veiga et al, 1998). Elevated monocytes were also found in *Ameiva ameiva* lizards that were infected with Hemolivia (Bonadiman et al, 2010).

Limited data is available on the hematology and biochemical values of the gopher tortoise. Unlike the desert tortoises (*Gopherus agassizii* and *G. morafkai*), which have been the subject of several extensive studies (Christopher et al 1999, Christopher et al 2003, Dickinson et al 2002, Gottdenker et al 1995, Peterson 2002), only a few have attempted to establish baseline blood values for *G. polyphemus* (Diaz-Figueroa, 2005; Hernandez, 2011; Taylor and Jacobson, 1982). All of the studies were limited to a small number of individuals (n= 17 – 50) and sampling was done during a single time period (1- 12 months). Thus, it is unknown if variation

in values may occur seasonally, between age groups, between different habitats with variable resources or quality, or during periods of reproductive activity. No studies to date have evaluated blood values in juvenile tortoises.

Table 1. Major organs and the analytes used to assess function.

Organ	Analyte measured
Liver	Alk Phos, albumin, LDH, GGT, AST, ALT, amylase
Pancreas	Triglycerides, glucose, lipase, amylase
Kidney	Uric acid, total protein, glucose, BUN, creatinine, alk phos, ALT, sodium
Thyroid	Calcium, phosphorous
Small intestine	Amylase
Muscle	Creatinine kinase, LDH, AST, ALT

Blood parasites of gopher tortoises and other reptiles

Although reptiles are commonly infected with blood parasites, only a single study has reported any blood parasite in gopher tortoises, an uncharacterized haemagregarine from 71% of 14 tortoises from McIntosh County, Georgia (Hernandez et al., 2011). Haemogregarines (Apicomplexa: Adeleiorina) are the most common blood parasites of reptiles and this group of protozoan parasites contains four genera, *Haemogregarines, Hepatozoon, Karyolysus,* and *Hemolivia* (Wozniak, McLaughlin & Telford, 1994; Telford, 2009). These genera are distinguished primarily by different developmental patterns observed in the invertebrate (definitive) vector which is unknown for the vast majority of species (Telford, 2009). Thus, most

studies of these parasites only refer to them as ‘haemogregarines’. These parasites have a general host association, with the genus *Haemogregarina* being most common in aquatic turtles (leeches as vectors), *Hepatozoon* being common in snakes and lizards (mosquitoes and possibly other insects as vectors), the genus *Karyolysis* only been reported from lizards in the genera *Lacerta* and *Podarcis* (mites are vectors), and the two described reptile species of *Hemolivia* (which utilize ticks as vectors) infect a single species of lizard and tortoise. The general life cycle of haemogregarines consists of sporogony in the invertebrate host and merogony and gamogony in the vertebrate host (Wozniak et al., 1996). The transmission route varies by parasite genera with sporozoites of leeches likely entering aquatic turtles during a blood meal whereas there is some data that tick-borne *Hemolivia* are transmitted when the host ingests the tick containing oocysts (Paperna, 2006). In the natural vertebrate host, infections often become chronic with low parasitemias and no clinical signs, although rare, mild anemia has been reported (Wozniak et al., 1996). In unnatural hosts, haemogregarines can cause an inflammatory response which could cause more significant disease (Wozniak et al., 1994; Wozniak et al., 1996).

Historically, the classification of these parasites was based primarily on morphological differences between the gamont stage, the most common stage observed in peripheral blood (Telford, 2009). Although stages within the vectors are needed for definitive identification of genus, morphology of these stages are rarely available. However, Ball (1976) disputed the use of gamonts for genus and species distinction because of the significant morphologic similarity between gamonts of different parasite species. Thus, because of more stringent criteria for classification and the general lack of knowledge of these parasites within the intermediate (invertebrate) hosts, there has been a dramatic decrease in the number of species descriptions (Telford, 2009). However, recent genetic data have confirmed that the genera *Hemogregarina*,

Hemolivia and *Hepatozoon* are distinct, although only a few species of each genus have been included in these studies (Ujvari et al., 2004; Moco et al., 2011; Barta et al., 2012, Maia et al., 2012; Harris et al., 2013). Currently no genetic characterization work has been conducted on any species of *Karyolysus*.

Among the four genera, *Hemolivia* is likely the most relevant to the current study because we believe the parasite observed in gopher tortoises is a species of *Hemolivia.* Supporting data includes the host (a tortoise) and our preliminary data which suggests the parasite is transmitted by ticks. However, data on the developmental stages in ticks are needed to confirm this identity as some species of *Hepatozoon* can utilize ticks as vectors (although there are no reports of *Hepatozoon* in tortoises). Current knowledge of *Hemolivia* in reptiles and amphibians is shown in Table 2.

Table 2. Current knowledge of biology of the three species of *Hemolivia*.

Species	Host	Distribution	Vector (if known)	Prevalence in vertebrate	Reference
H. mauritanicum	*Testudo graeca, T. marginata*	Europe, Africa	*Hyalomma aegyptium*	2 of 14 (14%) *T. graeca* in Bulgaria 24 of 25 (92%) in Greece	Siroky et al., 2005
H. stellata	*Bufo marinus, Ameiva ameiva (*natural and experimental)	South America	*Ablyomma rotundatum*	3 of 20 (15%) *A. ameiva*, natural 5 of 5 (100%) *A. ameiva*, experimental	Lainson et al. 2007
H. mariae	*Tiliqua rugosa, Egernia stokesi*	Australia	*Amblyomma limbatum*	66 of 567 (12%) *T. rugosa* 23 of 39 (59%) *E. stokesi*	Smallridge and Bull, 2001

An unrelated protozoan genus, *Haemoproteus* (reptile species currently being transferred to genus *Haemocystidium*), has also been reported from aquatic turtles and tortoises; however, this genus is easily distinguished from the haemogregarines (Figure 1). Although relatively uncommon in reptiles, *Haemocystidium* species have been reported from *T. pardalis* (Leopard Tortoise) in Africa, *T. graeca* from Asia, and *Geochelone denticulata* (Yellow-footed Tortoise) from Brazil (Lainson and Naiff, 1998). Vectors are unknown but horse flies are believed to transmit a species of *Haemocystidium* among aquatic turtles (DeGiusti et al, 1973).

The primary goal of this thesis was to contribute to health data parameters for gopher tortoises in order to better evaluate health status. Specifically, the objectives were to 1) establish hematology and plasma biochemistry reference values and 2) evaluate prevalence and parasitemias of haemogregarines in gopher tortoises and 3) determine a relationship between gopher tortoise haemogregarines and the proposed vector, *Amblyomma tuberculatum*.

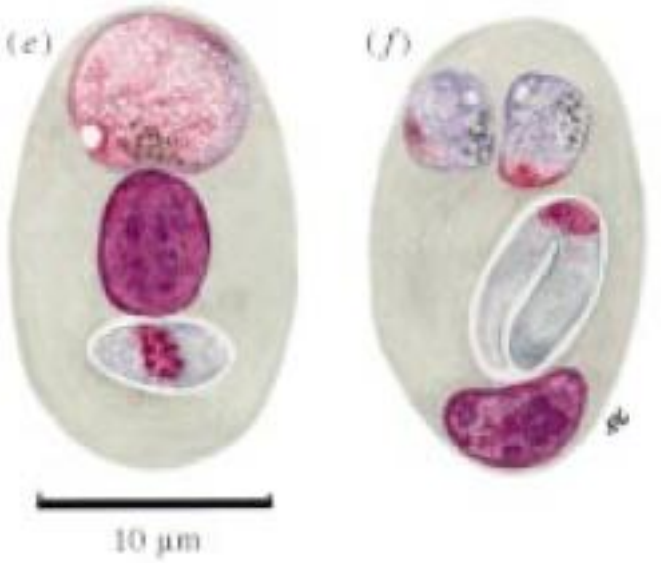

Figure 1. Illustration of a *Haemocystidium* gametocyte and an immature haemogregarine (under nucleus) (e) and two young *Haemocystidium* gametocytes (at top of cell) and a mature haemogregarine (f) in erthrocytes of *Geochlene denticulata*. (Lainson and Naiff, 1998)

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CHAPTER 2

PLASMA BIOCHEMISTRY AND HEMATOLOGY OF A TRANSLOCATED POPULATION OF GOPHER TORTOISES (*GOPHERUS POLYPHEMUS*)

ABSTRACT

Biochemistry and hematology are used to evaluate the health of individuals and populations. This can be problematic with wild, free-ranging species, in particular, threatened and endangered species, because of the number of samples that are available for analysis and because of the variation in blood values due to diet, habitat, stress and disease status. This study focused on a translocated population of gopher tortoises on St Catherines Island, GA (SCI). Data was available for 145 tortoises (adult = 91, juvenile = 50) and was collected from 1994 to 2011. Comparisons were made between age groups, gender, seasons and diagnostic labs (biochemistry only). Adults were higher in AST, albumin, cholesterol, total protein and glucose than juveniles. Males were higher in A/G, ALT and CO_2, while females had higher levels of calcium and phosphorous. Plasma biochemistry data from SCI was found to have significant variation as compared to a population of gopher tortoises from Moody Air Force Base (MAFB) in southern GA.

INTRODUCTION

The gopher tortoise (*Gopherus polyphemus)* is a terrestrial tortoise native to the southeastern United States. Currently, it is federally listed as threatened in the western portion of

its range and is state-listed in Georgia although it is a candidate for federal listing throughout the eastern portion of its range (USFWS, 2011). This long-lived, charismatic species is a keystone species in its habitat with > 350 other species, including the endangered indigo snake (*Drymarchon couperi*), inhabiting their burrows at some point in their life cycles (Eisenberg 1983, Jackson and Milstrey, 1989). The gopher tortoise utilizes open, savanna-like habitats and longleaf pine (*Pinus palustris*) forests, a landscape that once dominated the southeastern United States. Currently, these habitats are threatened by development activities and a lack of appropriate land management (Van Lear et al., 2005).

Hematologic and plasma biochemical parameters can be used to evaluate the health status of an individual, and, in general, a population of animals. Few data are available on the hematology and biochemistry of free-ranging gopher tortoises and due to the inherent difficulties assessing wild populations of gopher tortoises, these studies were limited in sample size and evaluation during a single time period (Diaz-Figueroa, 2005; Hernandez et al., 2011; Taylor and Jacobson, 1982). In addition, because these blood profiles can be influenced by many factors such as nutritional resources, temperature, species, gender, age and even location of blood collection (Musacchia and Sievers, 1956; Mader, 1996; Christopher et al., 1999; Ramon Lopez-Olvera et al., 2003; Hernandez et al., 2011; Scope et al., 2013), variations can occur seasonally and throughout the life of an individual tortoise.

Thus, there are many unknowns in regards to possible variation in certain values between seasons, age groups, reproductive status, or different habitats with variable resources or quality. In addition, no sampling of juveniles or repeated sampling of individual gopher tortoises has ever been conducted. This study was designed to provide data on hematologic and biochemical parameters for a population of gopher tortoises that has been under investigation since 1994.

These data should be of benefit to biologists, land managers and veterinarians who must make decisions regarding the general health of free-ranging gopher tortoises. Specifically, the objectives of this study were to 1) analyze hematologic and biochemical data for free-ranging gopher tortoises on St Catherines Island, Georgia, 2) investigate differences between sex, age, and season, and 3) compare serum biochemical values between tortoises on St. Catherines with those from a mainland site that varies considerably in habitat type and available resources.

METHODS

THE POPULATION ON ST. CATHERINE'S ISLAND

Study Site

In May 1994, 74 gopher tortoises were removed from a construction site in Bulloch County, GA and translocated to St. Catherines Island (SCI) in Liberty County, GA. This island is a privately owned barrier island that is 5670 hectares and is 16 km long x 4.8 km at the widest point. The northern portion of the island consists of savanna scrubland type habitat with low-lying shrubs and grasses and was the release site for the translocated tortoises. Since the initial release of the tortoises, recruitment and the release of rehabilitated healthy tortoises have increased the current population on SCI to approximately 200 tortoises (Tuberville, unpublished data).

Sample Collection and Processing

In May 1994, the tortoises at the Bulloch County development site were captured by utilizing several techniques including bucket traps, long wire hook by a professional gopher tortoise puller, hand capture and as a last resort digging out of the burrow with a shovel or heavy machinery. A complete physical exam and diagnostic health screen was performed on all of the tortoises. A thorough systematic physical examination included obtaining a body weight (kg) and morphologic measurements (straight carapace length and width, and straight plastron length) were performed. Gender was determined in adult tortoises based on plastron cavity, size of the gular scute, and mental glands. Inguinal palpation to determine the presence of eggs in the oviduct was performed in all tortoises. A dorso-ventral radiograph was taken on all adult females to confirm the presence and numbers of eggs and provided some measure of the sensitivity of inguinal palpation. Ticks, if present, were manually removed prior to transport (Norton T, personal communication). The ticks were preserved in ethanol for identification purposes and for further study (Sonderman, unpublished data). The tortoises were uniquely permanently marked by notching the marginal scutes and placing a passive integrated transponder (PIT) intramuscularly in the right shoulder area in subadults and adults utilizing sterile technique. Approximately 1-10 ml of whole blood (no more than 5% of the total blood volume or 0.5 ml per 100 grams) was collected from the jugular vein using a 23g sodium-heparinized butterfly catheter and syringe. Three blood smears were prepared within 10 min of collection, air dried and fixed in methanol. Slides were later stained with Diff Quick (Baxter Dickson), examined microscopically for parasites following a standard protocol, and used for differential white blood cell counts. The remaining blood was stored in a cooler until centrifuged within 4 hours of collection. Plasma was placed into 1.8-ml cryotubes and transferred to a -30 C freezer until

shipment to the diagnostic laboratory for processing. An aliquot of plasma was placed in a separate transport tube for determining antibodies against Mycoplasma spp (see below). Tortoises were kept in individual containers during transport from the mainland to SCI.

From 1994 to 1996, tortoises were captured and sampled twice a year. The first capture effort was in May, shortly after over-wintering and the second capture effort was in September and October, just prior to the tortoises entering the seasonal period of inactivity. The monitoring project on SCI is currently on-going and annual evaluations have been conducted every year since 1994, with the exception of 1999 and 2000 when no sampling was performed. Since 1997, tortoises were sampled throughout May and August. In 2010 and 2011, juvenile tortoises were targeted for capture and sampling.

Tortoises sampled after being introduced to the island were opportunistically captured or trapped in bucket traps or wire cage live traps (Burke and Cox, 1988). Individuals were held in large fenced enclosures for 24 hours prior to sampling. A complete systematic physical examination, weight, morphometrics, gender and age determination were obtained in a standardized format similar to the initial diagnostic evaluation. Individuals > 230mm were classified as adults (McRae et al., 1981). Tortoises were uniquely marked as described above. Blood samples were collected into a sodium heparinized syringe, using either a 22g (for adults) or 25g (for juveniles) needle. Blood was drawn from the brachial vein in tortoises > 500g due to the difficulties in retracting the head for sampling. The jugular vein was used in tortoises < 500g. Three blood smears were made within two hours of collection and fixed and stained in the same fashion as previously described. Samples were kept cool until processed. Plasma was refrigerated for a maximum of 48 hours prior to shipping to a commercial lab for processing.

Plasma for Mycoplasma serology was reported in Tuberville, 2008. The remainder of processing was the same as described above.

Sample Analysis

The samples were analyzed in a similar fashion throughout the study period. A small amount of heparinized whole blood was transferred to a microhematocrit tube and centrifuged (Becton Dickinson Autocrit Ultra 3, Franklin Lakes, NJ) for 5 minutes at 13000g to measure packed cell volume (PCV). The hematocrit was read on a micro-capillary reader (IEC, Needham Heights, MA). Plasma total solids (TS) were measured by refractometer. Manual methods were used to obtain total leukocyte estimates using previously established techniques. White blood cell counts were calculated in the field using the Eosinophil Unopette (Becton-Dickson, Rutherford, New Jersey, USA) hemocytometer technique (Cray and Zaias, 2004). Plasma samples were sent to one of two commercial labs for biochemistry analysis. Samples (n=39) from 1994 to 1996 were submitted to LabCorp (Burlington, NC) and 29 samples collected from 1998 to 2011 were submitted to the University of Miami Department of Pathology. At Miami, biochemistry profiles were performed using standard dry-slide determinations with a Kodak 700XR chemical analyzer by the Department of Pathology, University of Miami (Miami, Florida, USA). Vitros Performance Verifiers I and II (Ortho Diagnostics, Rochester, New York, USA) were used to test the chemistry analyzer. Results from the solutions, representing high and low controls, were compared to Vitros ranges. The analyzer was also calibrated with Ortho Vitros reagents. Any ‘‘flags’’ or errors were fully investigated. The following blood values were measured: glucose, sodium, potassium, carbon dioxide, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, phosphorus, calcium, uric acid, creatine

phosphokinase (CPK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), lipase, amylase, cholesterol, glucose, and gamma-glutamyl transferase (GGT).

THE POPULATION AT THE MOODY AIR FORCE BASE

Study Site

A second population of gopher tortoises was captured and sampled from Moody Air Force Base in Lowndes County, GA from 2001 to 2004. The installation maintains approximately 405 hectares of suitable gopher tortoise habitat consisting of scrubland and pine forests. Ninety-eight adult (n = 46 females, n= 52 males) tortoises were captured between March and September of each sampling year, except for 2002 when tortoises were captured only in August and September. Tortoises were opportunistically captured by hand or trapped using a wire cage live or bucket trap (Burke and Cox, 1988). Tortoises captured by hand were examined and sampled immediately, while those captured in traps were assessed within 15 minutes of discovery. All animals were immediately released. Blood collection was similar to what is described above for the adult tortoises sampled after 1994 and processed as described above for the plasma biochemical samples submitted to the University of Miami. Hematology was not done on MAFB tortoises.

Statistics

The mean, standard deviation, and distribution were determined for each hematology and biochemistry sample. Samples were eliminated from the study if there was any indication of

disease recorded in the individual animal records completed during the exam (e.g. sunken eyes, labored breathing, nasal discharge) or if the samples were of poor quality (diluted with hemolymph, clotted, hemolysed, or were lipemic). Because hematology samples are processed using standard protocols, hematology data were grouped, regardless of the processing lab. In order to test for reliability and comparability of biochemistry data between laboratories, data from SCI were analyzed by the commercial lab that performed the analysis (e.g. LabCorp or Miami).

Hematology and biochemistry data were compared across age, sex, season (for 1994 – 1996 only), and laboratory of analysis. Biochemistry data from SCI samples that were analyzed at the Miami laboratory were compared to data from MAFB by Tukey t-test. Descriptive statistics were analyzed using Microsoft Excel v. 2010. Tests for normality were done using Shapiro-Wilk ($p < 0.05$). If data were not from a Gaussian-distribution, they were log-transformed prior to being analyzed. Potential outliers were identified using Tukey test (Tukey, 1977). Following the American College of Veterinary Pathology (ACVP) reference interval guidelines (Freiderichs et al, 2012), reference values were not reported for analytes with fewer than 20 samples. Upper and lower limits of the 90% confidence interval were calculated for analytes with more than 20 samples. Potential differences between sex, age, season, and lab were detected using t-tests on normally distributed values. Non-Gaussian analytes were compared using Mann-Whitney U-tests. Reference values with 90% confidence intervals and comparisons between mean were analyzed using MedCalc for Windows v. 13.2.3 (MedCalc Software, Ostend, Belgium).

RESULTS

Tortoises from SCI

Biochemistry (n = 68) and hematology (n = 142) data was available for 145 gopher tortoises from SCI. When biochemistry data were analyzed by lab, one (adult) of 38 (34 adults, 4 juveniles) tortoises and three (all adults) of 26 (19 adults, 7 juveniles) tortoises analyzed at LabCorp and Miami, respectively, were identified as outliers and were excluded. Hematology data for 91 adults and 50 juveniles from both diagnostic labs were pooled. Data from an additional nine tortoises were not analyzed because tortoises were either clinically ill or samples were mishandled.

No significant differences were noted between adults and juvenile biochemistry analytes obtained from LabCorp with the exception of glucose ($p = 0.04$); therefore, glucose values for juvenile samples were removed before reference values were determined. The 95% reference values (90% confidence intervals) for combined adult and juvenile biochemistry analyzed at LabCorp are shown in Table 3. Juveniles were examined further, but due to the low sample size (<10), reference values could not be determined (Freiderichs et al, 2012). Summary statistics are presented in Table 4. Significant differences in the following plasma biochemistry analytes were noted between males and females: potassium ($p = 0.002$), cholesterol ($p = 0.0005$), calcium ($p = 0.0002$), TP ($p = < 0.0001$), glucose ($p = 0.01$), phosphorous ($p = 0.0013$), and triglycerides ($p = 0.0002$). In contrast to LabCorp, significant differences between adults and juveniles were noted for the following analytes tested at Miami: LDH ($p = 0.4$), TP ($p < 0.0001$), AG ($p <0.0001$), Glu ($p = 0.02$), ALT ($p = 0.02$), CO_2 ($p = 0.01$), lipase (p 0.01), chol ($p = 0.004$), AST ($p = 0.03$), albumin ($p = 0.002$), therefore they were evaluated separately. Following ACVP guidelines

(Freiderichs et al, 2012), parameters with fewer than 20 samples do not allow for determination of reference values. Table 5 reports summary statistics for adults and table 6 reports summary statistics for juveniles. Significant differences between males and females were noted for phosphorous ($p = 0.004$), ALT ($p = 0.03$), A/G ($p = 0.04$), calcium ($p = 0.01$), and CO_2 ($p = 0.02$). Several hematology values were significantly different between adults and juveniles including PCV ($p < 0.0001$), TS ($p < 0.0001$), heterophils ($p = 0.03$), and basophils ($p = 0.03$); therefore, these were analyzed separately. The 95% reference values (90% confidence intervals) for adults and juveniles are shown in Tables 7 and 8, respectively. The only hematology differences between males and females was PCV ($p = 0.006$).

Seasonal and annual comparisons

Seasonal data collected between May 1994 and May 1996 was only processed at the LabCorp diagnostic lab. Data from juveniles and adults were combined, except for glucose. Several significant differences were noted between plasma biochemistry values for samples collected in spring vs. fall of each year (Table 9). Significant differences in several plasma biochemistry values were also noted between years between 1994 and 1996 (Table 10). Among the hematology data, only PCV and TS were compared between seasons and years due to low sample sizes (Table 11). No data were available for fall 1995.

Tortoises from MAFB

Biochemical values were available for 54 adults. Biochemistry data for adults including 95% reference values (90% confidence intervals) for those analytes with ≥20 samples are shown in Table 12. Analyte values for males and females differed significantly for the following:

calcium ($p = 0.008$), lipase ($p = 0.0007$), cholesterol ($p = 0.0008$), and triglycerides ($p = 0.01$). When compared to gopher tortoises from SCI, biochemistry values were significantly different for all analytes except sodium, uric acid and triglycerides, regardless of diagnostic lab. Significant values for each diagnostic lab increased or decreased concurrently.

DISCUSSION AND CONCLUSION

Gopher tortoises on SCI appear healthy and did not exhibit clinical signs of any major diseases, including URTD. The blood analytes from SCI closely mirrored other published studies on gopher tortoises (Taylor et al, 1982; Hernandez, 2011). There were several significant differences between diagnostic labs for biochemical analytes and all but one (albumin) was higher for Miami. LDH was dramatically higher in tortoises analyzed at the Miami lab (1826.0 U/L v 482.92 U/L, $p < 0.0001$), while AST (244.2 U/L v 175.5 U/L, $p = 0.042$), and ALT (11.1U/L v 5.0 U/L, $p = 0.0008$) were slightly less so. LDH analyzed at Miami is also outside the normal range of values published for most reptiles (<1000 U/L) and gopher tortoises (272.8 mg/dL, Taylor and Jacobson, 1982; 428.4 U/L, Hernandez, et al 2011), but lower than levels found (5717 IU/L) in a study of Alligator Snapping Turtles (*Macrochelys temminckii*) (Chaffin et al, 2008). Increases in these enzymes are typically used to evaluate hepatic function, but can also be elevated due to muscle damage at time of blood collection. Glucose, as measured by Miami (122.3 mg/dL) was outside of normal reptile ranges (60 – 100 mg/dL) and significantly higher than samples analyzed by LabCorp (88.8 mg/dL, $p = 0.0004$). The differences could be due to either diet or handling and restraint of tortoises at the time of sample collection. Increased stress and physical activity can elevate glucose levels, but levels tend to decrease shortly after a period

of inactivity. Other significant differences between LabCorp and Miami include chloride (113.0 mEq/L v 99.0 mEq/L, p = 0.0002), total protein (4.98 g/dL v 4.02 g/dL, p = 0.0009), BUN/Creat (7.5 v 5.0, p = 0.0006), and UA (3.6 mg/dL v 2.8 mg/dL, p = 0.005), respectively. Chloride, as measured by Miami, was slightly higher than published terrestrial chelonian values (100 – 110 mEq/L) and could be evidence of dehydration. Albumin was the only value in which samples from Miami were significantly lower than those from LabCorp (1.0 g/dL v 1.15 g/dL, p = 0.015). Amylase, triglycerides, cholesterol, and CPK were substantially higher in the Hernandez study, while triglycerides were lower in the Taylor study. Differences in the above values may be attributed to nutritional resources, but another possible explanation, and more likely, for the variety of differences seen amongst tortoises on SCI is the use of two diagnostic labs, each using different chemical analyzers and reagents. When conducting a health study such as this, it is important to handle and analyze blood samples in a uniform and consistent manner. Biochemical results can exhibit tremendous variation depending on the analyzer machine, reagents, and calibration protocols and with personnel experience and therefore each lab has a set of internal reference intervals unique to the chemical analyzer in use (Geffre, 2009, Freiderichs et al, 2012). Maintaining consistency can be inherently difficult to achieve when the research spans a number of years and multiple personnel.

The tortoises on SCI were translocated to the island in May 1994, which might explain some of the seasonal differences. All of the significant analytes are influenced by diet. Blood sampling from spring 1994 was done before the animals were brought onto the island and before they were exposed to novel food items and habitat. Interestingly, there were no significant differences in PCV or TS between spring 1994 and spring 1996. This might be due to the length of time that the tortoises had been on the island and given the chance to acclimate to novel

nutritional resources. Males had higher PCV and lymphocyte counts than females. Females had elevated calcium and total protein levels as compared to males, but there were no seasonal differences for either, which was surprising as calcium and TP are used in egg production. Potassium was higher in males, as was seen in Louisiana gopher tortoises (Diaz-Figueroa, 2005) and exhibited seasonal differences. ALT and CO_2 were also higher in males, but the reasons are unknown. Another interesting observation is that the majority of the seasonal differences occurred when seasons were compared with fall 1994 or spring 1995, indicating that there might have been a significant weather event that could have contributed to environmental change. The southeastern United States experienced the worst ice storm in recorded history during January of 1994 (www.crh.noaa.gov), which might have depleted food resources to the tortoises going into the winter months and a period of inactivity. Another possibility is that there were fewer warm winter days and the tortoises spent more time in their burrows. Biochemical values were higher in the spring of 1995 than in the fall of 1994, and remained high until spring 1996.

Several biochemical differences between SCI and MAFB were detected. Sodium, uric acid and triglycerides were the only insignificant values between the two locations. MAFB tortoises were higher in phosphorous, potassium, GGT, ALT and BUN/Creat. These differences can likely be attributed to diet. Both locations experience similar climatic conditions in that they have long, prolonged periods of humidity with intermittent rainstorms in the summer followed by short mild winters. MAFB, however, does have large pine trees whereas the tortoise habitat on SCI consists of smaller shrubs. This would allow the tortoises on SCI much greater exposure to UV and warmer ambient temperatures. This study addresses the importance of evaluating populations at distinct geographical locations to determine seasonal and ecological differences

that may affect blood values and also emphasizes the need for further nutritional studies in gopher tortoises.

A recent study of URTD on SCI gopher tortoises indicates that 100% of tortoises are seropositive, but there is a low mortality rate and clinical signs of the disease are rare (Turberville et al, 2008). One tortoise in this study was eliminated from analysis due to raspy breathing and mucous covered nares, but it is unclear if these signs were due to URTD or stress at time of capture. Christopher et al, 2003 found that desert tortoises (*Gopherus agassizii*) in the Mojave Desert exhibited heterophilia and hyperglobulinemia in conjunction with clinical signs, which was not the case here. The authors also noted that detecting the disease was difficult with routine hematology and biochemical tests alone. URTD is a highly contagious disease transmitted through direct contact with infected tortoises and can be associated with high mortality rates (Christopher et al, 2003, Berish et al, 2010). For this reason, gopher tortoises on SCI should continue to be closely monitored for clinical signs of URTD.

This study is unique in that it spanned 18 years and is the first comprehensive review of gopher tortoise blood data. Further long-term evaluations of healthy populations of gopher tortoises are critical to ensure success of the species. Biochemical and hematological data are needed to assess viable populations and to aid management decisions in terms of translocating at-risk individuals and populations. This study was limited by the sample size available for review, specifically because we were working with a free-ranging population. Reference intervals are highly influenced by both intrinsic and extrinsic factors such as sex, season, nutritional resources, skill level and expertise of personnel, and diagnostic lab, and may even include asymptomatic individuals that were not detected before the study. Therefore, these results are not

meant to be definitive reference intervals and should be used as an aid when determining the health status of an individual or when considering the best course of treatment for a patient.

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Table 3. Reference values for gopher tortoises from SCI analyzed at the LabCorp diagnostic lab (n = 38). Adult and juvenile data were combined due to insignificance between values, except for glucose. Tortoises were sampled between 1994 and 1996. Not all analytes were analyzed for each tortoise.

Analyte[a] (n)	Mean	Min, Max	Std Dev	95% Ref Val (90% CI)
Calcium (38)	11.2[b]	8.8, 19.9	---	7.36 (6.59 - 8.12) 18.8 (16.0 - 20.86)
Phos (38)	3.05[b]	1.8, 11.3	---	1.19 (0.89 - 1.53) 9.28 (6.63 - 11.78)
Sodium (37)	138.24	127.0, 161.0	7.33	121.92 (118.55 - 126.15) 152.47 (147.9 - 156.95)
Potassium (38)	4.97	3.7, 6.2	0.58	3.74 (3.49 - 4.01) 6.15 (5.83 - 6.42)
Chloride (38)	99.82	80.0, 117.0	7.13	85.32 (81.68 - 89.81) 115.37 (111.3 - 118.68)
Cholesterol (38)	103.63	17.0, 193.0	48.36	1.71 (-17.27 - 23.02) 204.18 (179.16 - 223.2)
Triglyceride (38)	28.0[b]	10.0, 220.0	---	5.07 (3.57 - 7.19) 167.82 (106.57 - 249.82)
LDH (35)	482.92[b]	146.0, 1450.0	---	162.16 (125.06 - 215.99) 1479.55 (1103.9 - 1926.55)
AST (38)	175.5[b]	72.0, 1020.0	---	39.75 (31.72 - 56.67) 783.09 (529.31 - 1200.17)
GGT (38)	1.0[b]	1.0, 6.0	---	---
ALT (38)	5.0[b]	1.0, 25.0	---	0.96 (---) 28.71 (---)
AlkPhos (38)	51.0[b]	23.0, 126.0	---	25.66 (21.53 - 31.21) 96.63 (82.28 - 112.59)
TP (38)	4.02	2.5, 5.4	0.7209	2.54 (2.21 - 2.87) 5.5 (5.16 - 5.8)
Globulin (38)	2.86	1.5, 4.2	0.61	1.61 (1.34 - 1.91) 4.13 (3.84 - 4.38)
Albumin (38)	1.15[b]	0.6, 2.3	---	0.72 (---) 1.77 (---)
A/G (38)	0.41[b]	0.18, 0.79	---	0.23 (0.19 - 0.27) 0.72 (0.6 - 0.82)
BUN/Creat (38)	5.0[b]	3.0 to 15.0	---	2.04 (---) 11.86 (---)
Uric Acid (38)	2.84	1.7, 4.7	0.72	1.29 (0.95 - 1.63) 4.26 (3.85 - 4.64)
Glucose (34)*	88.82	53.0, 140.0	23.86	38.17 (27.52 - 48.24) 137.85 (124.37 - 148.83)

*juvenile data excluded.

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), CO_2 (mmol/L), amylase (U/L), lipase (U/L), cholesterol (mg/dL), LDH (U/L), CPK (mg/dL), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL).
b = Median is presented for analytes that are not Gaussian.

Table 4. Plasma biochemistry data[a] analyzed at LabCorp (n=4) for juvenile (SCL < 230mm) gopher tortoises on St Catherines Island, GA. Tortoises were captured between 1994 to 1996. The minimum and maximum values are bold within the raw data. Not all analytes were analyzed for each tortoise.

Tortoise	Ca	Phos	Na	K	Chlor	Chol	Trigly	LDH	AST	GGT	ALT	Alk Phos	TP	Glob	Alb	Bun/ Creat	UA	Glu
106	10.2	**3.7**	**138**	**4.1**	**92**	76	**21**	**161**	**75**	3	**3**	**23**	3.6	2.6	**1**	**5**	2.6	124
133	**9.8**	**2.5**	**143**	4.8	93	**44**	23	**551**	174	**1**	5	58	3.8	2.8	**1**	**3**	**3.6**	**147**
149	**13.1**	3.1	**138**	5	**107**	**116**	**79**	359	119	3	7	40	**4.6**	**3.2**	**1.4**	**5**	**1.8**	96
176	10.5	3.2	---	**6.2**	104	86	41	474	**272**	**6**	**13**	**66**	**3**	**2**	**1**	**5**	2.5	**94**
Mean	**10.9**	**3.13**	**138.0[b]**	**5.03**	**99.0**	**80.85[b]**	**41.0**	**386.25**	**160.0**	**3.25**	**7.0**	**46.75**	**3.75**	**2.65**	**1.0[b]**	**5.0[b]**	**2.62**	**115.25**
Std Dev	**1.49**	**0.49**	**---**	**0.87**	**7.62**	**---**	**26.88**	**169.63**	**84.94**	**2.06**	**4.32**	**19.21**	**0.66**	**0.5**	**---**	**---**	**0.74**	**25.21**

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), cholesterol (mg/dL), triglyceride (mg/dL), LDH (U/L), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL).

b = Median presented for analytes that are not Guassian.

Table 5: Reference values for adult (n = 22) gopher tortoises from SCI analyzed at the University of Miami diagnostic lab. Tortoises were sampled between 2002 and 2009. Not all analytes were analyzed for each tortoise.

Analyte[a] (n)	Mean	Min, Max	Std Dev
Calcium (19)	11.0[b]	10.0, 16.3	---
Phosphorous (18)	4.23	2.5, 7.2	1.38
Sodium (19)	142.95	113.0, 171.0	14.81
Potassium (19)	5.08	4.2, 5.9	0.49
CO_2 (19)	25.0[b]	16.0, 29.0	---
Amylase (19)	809.95	447.0, 1062.0	132.29
Lipase (13)	11.0[b]	3.0, 50.0	---
Chloride (6)	113.0	106.0, 122.0	6.03
Cholesterol (18)	124.74	55.0, 208.0	37.36
Triglycerides (10)	21.63[b]	9.0, 122.0	---
LDH (12)	1826.08	1208.0, 2502.0	372.71
CPK (12)	767.17	104.0, 1657.0	509.55
AST (18)	244.18[b]	150.0, 585.0	---
GGT (9)	7.11	5.0, 12.0	2.47
ALT (18)	11.06	6.0, 16.0	3.02
AlkPhos (6)	58.67	48.0, 71.0	10.25
TP (19)	4.98	2.0, 7.0	1.2
Albumin (19)	0.98	0.27, 1.86	0.36
A/G (19)	0.42	0.12, 0.63	0.13
Bun/Creat (19)	7.5[b]	5.0, 60.0	---
Uric Acid (13)	3.6[b]	2.2, 5.0	---
Glucose (19)	122.32	58.0, 197.0	41.12

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), CO_2 (mmol/L), amylase (U/L), lipase (U/L), cholesterol (mg/dL), LDH (U/L), CPK (mg/dL), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL).

b = Median is presented for analytes that are not Gaussian.

Table 6. Plasma biochemistry data$_a$ analyzed at Miami (n=7) for juvenile (SCL < 230mm) gopher tortoises on St Catherines Island, GA. Tortoises were captured between 2008 and 2010. The minimum and maximum values are bold within the raw data. Not all analytes were analyzed for each tortoise.

Tortoise	Ca	Phos	Na	K	CO_2	Amy	Lipase	Chol	LDH	CPK	AST	GGT	ALT	TP	Alb	A/G	Bun/ Creat	UA	Glu
309	11.1	**5.7**	**138**	7.1	**35**	**690**	**43**	93	1906	**53**	198	**5.0**	14	**2.7**	**0.53**	0.81	105	**4.8**	97
428	**9.2**	3.3	136	5.8	**21**	806	25	83	2867	71	**495**	---	**22**	2.4	---	---	**140**	4.7	82
434	10.3	3.6	125	**4.3**	30	**983**	27	**103**	**3317**	1419	176	**5.0**	13	2.2	0.43	**0.74**	10	1.0	**107**
732	10.2	4.0	**121**	4.3	31	938	25	66	**1692**	617	**132**	**5.0**	**9.0**	2.0	0.51	0.91	**6.7**	**0.7**	98
736	10.5	**3.1**	133	**9.5**	31	698	34	73	2135	1501	173	---	20	2.2	0.46	0.84	---	1.9	**55**
751	**12.1**	3.6	125	5	29	712	**21**	**57**	2161	**1605**	148	**5.0**	12	2.0	0.51	0.99	10	**4.8**	70
830	10.4	---	---	---	---	---	---	---	---	1315	210	---	---	**1.4**	**0.25**	**1.07**	---	2.1	66
Mean	**10.54**	**3.8$_b$**	**129.67**	**6.0**	**29.5**	**804.5**	**29.17**	**77.6$_b$**	**2346.3**	**940.1**	**198.73**	**5.0**	**15.0**	**2.13**	**0.44$_b$**	**0.89**	**24.04$_b$**	**2.86**	**82.14**
Std Dev	**0.89**	**---**	**6.92**	**2.01**	**4.64**	**128.6**	**8.01**	**---**	**618.8**	**679.86**	**---**	**0.0**	**4.98**	**0.4**	**---**	**0.12**	**---**	**1.85**	**19.3**

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), CO_2 (mmol/L), amylase (U/L), lipase (U/L), cholesterol (mg/dL), LDH (U/L), CPK (mg/dL), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL).

b = Median presented for analytes that are not Guassian.

Table 7. Hematology for 91 adult (SCL > 230mm) gopher tortoises from SCI. Reference values were not determined in analytes with n < 20 due to small sample size. Not all blood analytes were evaluated for each individual.

Analyte[a] (n)	Mean	Min, Max	Std Dev	95% Ref Val (90% CI)
PCV (81)	28.0[b]	17.0, 53.0	---	20.0 (18.74 – 21.33) 39.36 (36.81 – 41.82)
Total Solids (73)	4.11	2.0, 6.4	0.97	0.02 (-2.63 – 2.71) 8.23 (5.55 – 10.9)
Monocytes (46)	1.48	0.0, 11.0	2.40	---
Lymphocytes (46)	11.0[b]	1.0, 36.0	---	3.74 (2.78 – 5.09) 32.87 (23.71 – 44.46)
Heterophils (46)	69.17	28.0, 94.0	16.43	42.16 (35.41 – 50.03) 99.16 (92.87 – 104.81)
Eosinophils (46)	5.5	0.0, 34.0	7.32	-12.08 (---) 15.23 (---)
Basophils (46)	10.26	0.0, 38.0	9.65	-8.72 (-12.15 - -5.1) 24.92 (20.13 - 30.47)
RBC (18)	0.74[b]	0.55, 1.36	---	---
WBC (42)	6.35[b]	2.7, 17.3	---	2.93 (2.43 - 3.44) 15.38 (11.96 - 18.73)

a = Blood analyte units: PCV (%), Total Solids (%), monocytes (%), lymphocytes (%), heterophils (%), eosinophils (%), basophils (%), RBC (x 10^6), WBC (x 1000).

b = Median is presented for analytes that are not Gaussian.

Table 8. Hematology for 50 juvenile (SCL < 230mm) gopher tortoises from SCI. Analytes with n < 20 were not evaluated for reference values due to small sample size. Not all blood analytes were evaluated for each individual.

Analyte[a] (n)	Mean	Min, Max	Std Dev	95% Ref Val (90% CI)
PCV (48)	23.38	11.0, 32.0	4.31	16.34 (14.60 – 18.22) 30.99 (29.3 – 32.79)
Total Solids (35)	2.65	1.0, 5.6	0.99	0.85 (0.34 – 1.32) 4.29 (3.74 – 4.77)
Monocytes (21)	1.95	0.0, 8.0	2.52	-3.89 (---) 5.56 (---)
Lymphocytes (21)	14.9	0.0, 53.0	11.75	-7.69 (-16.36 – 1.93) 33.58 (24.32 – 42.05)
Heterophils (21)	58.52	15.0, 93.0	20.64	21.48 (---) 95.25 (---)
Eosinophils (21)	7.05	0.0, 30.0	8.41	-13.35 (---) 19.08 (---)
Basophils (21)	16.05	1.0, 34.0	10.81	-4.15 (-9.5 - 2.02) 35.84 (27.63 - 41.26)
RBC (12)	0.64[b]	0.52, 1.01	---	0.37 (---) 1.01 (---)
WBC (20)	6.2[b]	3.5, 15.9	---	0.19 (-1.92 - 2.27) 12.29 (9.37 - 15.09)

a = Blood analyte units: PCV (%), Total Solids (%), monocytes (%), lymphocytes (%), heterophils (%), eosinophils (%), basophils (%), RBC (x 10^6), WBC (x 1000).

b = Median is presented for analytes that are not Gaussian.

Table 9. Significant differences ($p < 0.05$) between spring and fall seasons from 1994 to 1996.

Analyte	Sp94 – F94	Sp94 – F95	Sp95 – F94	Sp95 – F95	Sp96 – F94	Sp96 – F95
Calcium	---	---	---	---	---	---
Phos	---	---	0.006	---	---	---
Sodium	---	---	0.006	0.002	---	---
Potassium	0.0001	< 0.0001	---	---	0.04	0.04
Chloride	---	---	---	0.0001	---	---
Cholesterol	0.04	---	0.02	---	---	---
Triglyceride	---	---	---	---	---	---
LDH	---	0.003	0.0001	0.004	0.003	---
AST	---	---	0.0003	0.003	0.0004	---
GGT	---	---	---	---	---	---
ALT	0.04	---	0.002	0.001	---	---
AlkPhos	0.002	< 0.0001	0.003	---	---	0.009
TP	---	---	---	---	---	0.03
Globulin	---	---	---	---	---	---
Albumin	0.05	---	0.03	---	---	0.02
A/G	---	---	---	---	---	---
BUN/Creat	0.02	0.002	---	---	---	---
Uric Acid	0.01	---	0.02	---	0.02	---
Glucose*	0.0007	---	< 0.0001	0.01	0.001	---

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), CO_2 (mmol/L), amylase (U/L), lipase (U/L), cholesterol (mg/dL), LDH (U/L), CPK (mg/dL), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL).

* = Juveniles not included in analysis.

Table 10. Significant differences ($p < 0.05$) between spring vs spring and fall vs fall seasons from 1994 to 1996. Data is available for Spring 1994 (n = 30), Fall 1994 (n = 7), Spring 1995 (n = 32), Fall 1995 (n = 16), and Spring 1996 (n = 41).

Analyte[a]	Sp94 – Sp95	Sp94 – Sp96	Sp95 – Sp96	F94 – F95
Calcium	---	---	---	---
Phos	---	---	0.04	---
Sodium	0.008	---	0.0002	---
Potassium	0.001	0.003	---	---
Chloride	0.006	---	0.002	---
Cholesterol	---	---	---	---
Triglyceride	---	---	---	---
LDH	< 0.0001	<0.0001	0.02	0.01
AST	0.0002	0.005	---	0.006
GGT	---	---	---	---
ALT	0.02	---	0.02	---
AlkPhos	< 0.0001	< 0.0001	0.0002	0.01
TP	---	0.001	0.008	---
Globulin	---	0.006	0.05	---
Albumin	---	0.003	0.0004	---
A/G	---	---	---	---
BUN/Creat	0.006	0.002	---	---
Uric Acid	---	---	---	---
Glucose*	0.0002	---	< 0.0001	0.0008

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), CO_2 (mmol/L), amylase (U/L), lipase (U/L), cholesterol (mg/dL), LDH (U/L), CPK (mg/dL), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL). * = juveniles not included in analysis.

Table 11. Significant differences ($p < 0.05$) for PCV (%) and TS between spring and fall seasons from 1994 to 1996. Data is available for Spring 1994 (n = 48), Fall 1994 (n = 15), Spring 1995 (n = 35), and Spring 1996 (n = 5). Fall 1995 is not reported due to low sample size (n = 2).

Analyte	Sp94 – F94	Sp94 – Sp95	Sp94 – Sp96	Sp95 – F94	Sp95 – Sp96
PCV	---	0.0004	---	0.002	0.03
TS	< 0.0001	---	---	0.008	---

Table 12. Reference values for gopher tortoises from MAFB analyzed at the Miami diagnostic lab (n = 54). Adult and juvenile data were not combined due to significance between values and few juvenile samples. Analytes with n < 20 were not evaluated for reference values due to small sample size. Tortoises were sampled between 2001 and 2004. Not all analytes were analyzed for each individual.

Analyte[a] (n)	Mean	Min, Max	Std Dev	95% Ref Val (90% CI)
Calcium (49)	7.79	1.2, 16.1	3.95	0.89 (-0.31 to 2.32) 14.36 (12.84 to 15.76)
Phos (42)	8.15[b]	3.5, 27.8	---	3.31 (2.64 to 4.13) 24.69 (19.04 to 30.04)
Sodium (20)	137.95	124.0, 164.0	10.33	116.33 (111.7 to 123.41) 153.47 (146.34 to 162.75)
Potassium (20)	6.65[b]	5.1, 13.2	---	4.11 (3.56 to 4.91) 10.77 (8.7 to 13.27)
CO_2 (20)	22.1	13.0, 31.0	5.12	12.95 (10.27 to 16.25) 31.26 (28.19 to 33.96)
ALT (19)	13.16	3.0, 25.0	6.48	---
LDH (42)	1088.13[b]	106.0, 7784.0	---	181.06 (109.78 to 286.0) 6934.46 (4501.09 to 9714.98)
CPK (43)	349.0[b]	20.0, 11522.0	---	19.38 (10.48 to 38.0) 6561.61 (3404.22 to 12278.64)
Amylase (17)	726.12	456.0, 1241.0	186.95	---
Lipase (17)	6.0[b]	1.0, 30.0	---	---
GGT (31)	10.0[b]	5.0, 44.0	---	4.57 (---) 21.04 (---)
Cholesterol (16)	58.97[b]	45.0, 298.0	---	---
Triglyceride (17)	39.0[b]	9.0, 521.0	---	25.66 (21.53 to 31.21) 96.63 (82.28 to 112.59)
TP (17)	3.36	1.0, 5.3	1.11	---
A/G (10)	0.21	0.15, 0.27	0.05	---
BUN/Creat (7)	10.0[b]	7.5 to 10.0	---	---
Uric Acid (51)	3.5[b]	0.2, 17.4	---	0.35 (0.17 to 0.61) 32.7 (17.85 to 45.75)
Glucose (47)	64.70	10.0, 146.0	30.61	10.95 (-0.2831 to 23.02) 116.16 (103.06 to 127.39)

a = Analyte units: calcium (mg/dL), phosphorous (mg/dL), sodium (mmol/L), potassium (mmol/L), CO_2 (mmol/L), amylase (U/L), lipase (U/L), cholesterol (mg/dL), LDH (U/L), CPK (mg/dL), AST (U/L), GGT (mg/dL), ALT (U/L), TP (g/dL), albumin (g/dL), uric acid (mg/dL), glucose (mg/dL). b = Median is presented for analytes that are not Gaussian.

CHAPTER 3

ASSOCIATION OF HEMOGREGARINE INFECTIONS IN *GOPHERUS POLYPHEMUS* WITH THE GOPHER TORTOISE TICK (*AMBLYOMMA TUBERCULATUM*)

ABSTRACT

Haemogregarines (genera *Haemogregarina, Hepatozoon,* and *Hemolivia*) are common intraerythrocytic parasites of reptiles, although they are relatively rare in tortoises. Because haemogregarines of African and European tortoises are transmitted by ticks, this study was conducted to investigate the potential role of the gopher tortoise tick (*Ambylomma tuberculatum)* as a vector of a recently detected haemogregarine in gopher tortoises (*Gopherus polyphemus*). Three tick-positive and two tick-negative sites were tested. One site, St Catherines Island (SCI) in Liberty County, GA, was included because tortoises were translocated from the mainland where they were infested with ticks to the island where ticks are rare. Tortoises were captured from 1994-2013 on St. Catherines Island and from 2009-2013 at other sites. Blood smears were prepared, stained with modified Giemsa, and examined for parasites. Positive samples were further analyzed to calculate a parasitemia. Tortoises from SCI had an 83% haemogregarine prevalence rate that decreased over time. Parasitemias ranged from 0.01% to 15.1%. Haemogregarine prevalence was found to correlate with tick positive sites. Results from this study suggest that haemogregarine infections in gopher tortoises are co-distributed with the tick

species *A. tuberculatum,* but further research is necessary to confirm that this haemogregarine parasite is transmitted by this tick species.

INTRODUCTION

Hemogregarines (Apicomplexa: Adeleorina) are intraerythrocytic protozoan parasites that infect a wide variety of vertebrates and are common hemoparasites of reptiles (Davies and Johnston, 2000; Telford, 2009). Currently, four genera (*Haemogregarina, Hepatozoon, Karyolysus,* and *Hemolivia*) infect reptile hosts and they are distinguished primarily by different developmental patterns observed in the invertebrate (definitive) vector (Telford, 2009). Clinical disease is typically not associated with infections, but anemia can occur with very high parasitemias (Jacobson, 2007, Telford, 2009) and inflammatory disease can occur in aberrant hosts (Wozniak et al., 1994). In addition, *Helmolivia*-infected Australia sleepy lizards (*Tiliqua rugosa*) have smaller home ranges compared to uninfected lizards (Bouma et al., 2007). Although hemogregarines are common parasites of tortoises (*Testudo graeca* and *T. marginata*) in Africa and Europe (Siroky et al., 2005, Harris, 2013), only a single study has documented hemogregarines in tortoises from North America (gopher tortoise, *Gopherus polyphemus)* (Hernandez et al., 2011). Currently, little is known about the ecology of the parasite including, generic status, prevalence, vector(s), or potential effects on the health of tortoises.

Gopher tortoise populations have declined throughout much of their historical range and are federally listed as threatened in the western portion of the species' range (west of the Mobile and Tombigbee Rivers in Alabama, Louisiana, and Mississippi). In Georgia, it is state-listed; however, it is now a candidate for federal listing in the eastern portion of its range (USFWS, 2011). The main threats to gopher tortoises include habitat loss and fragmentation, human

activity, and disease (e.g., upper respiratory tract disease). Translocation of tortoises into populations needing augmentation has increased, which could lead to risk of pathogen introductions.

This study was conducted to obtain data on the prevalence and potential role of the gopher tortoise tick, *Ambylomma tuberculatum*, as a vector of this previously reported hemogregarine (Hernandez et al., 2011). Because hemogregarines of some tortoises, lizards, and the tuatara (*Sphenodon punctatus*) are transmitted by hard ticks (Smallridge and Bull, 1999; Siroky et al., 2005; Paperna, 2006; Godfrey et al., 2011), we hypothesized that the gopher tortoise tick is a probable vector and thus would be associated with hemogregarine infections in the gopher tortoise.

METHODS

Sample sites

St Catherines Island

A long-term monitoring project was initiated on St Catherines Island (Liberty County, GA) in 1994 when 75 tortoises were translocated to the site from Bulloch County, GA. After capture and prior to being transported to St Catherines Island, each tortoise was subjected to a full physical exam and various metrics such as weight, height, and width were taken. Each tortoise was examined for ectoparasites, of which 100% were infested with *Amblyomma tuberculatum*, the gopher tortoise tick (Table 1). No other ectoparasites (e.g., *Ornithodorus turicatae* or mites) were observed. The ticks were removed by hand. Prior to 1994,

approximately 25 – 30 tortoises had been released on the island as waifs or rehabilitated animals. These tortoises were mostly from unknown sources and have an incomplete medical history. An attempt was made to capture as many of these tortoises as possible in 1994 (n=8) for health assessments and sampling. For a detailed description of the initial translocation project and the study site, see Tuberville et al., 2008.

From 1994-1998, tortoises were trapped seasonally that coincided with the time period just after the tortoises' inactive season (April and May) and the months just after the active season (August, September, and October). From 2001 to 2013, yearly captures were conducted between May and August. No sampling was conducted in 1999 or 2000. Prior to release on the island or after initial capture of established tortoises and hatchlings, each tortoise was uniquely marked with a permanent notch ID of the carapace and were pit-tagged in the inguinal region.

Jones Ecological Research Center (JERC)

Tortoises were sampled at the JERC in Baker County, GA. For the purposes of this study, sites were chosen within this site that were previously shown to have *A. turberculatum*-infested tortoises and other sites with little to no prevalence of ticks (McGuire, unpublished data). Samples were collected opportunistically from 2009 until 2012. Tortoises were examined and assessed as previously described.

Additional Sites

Two additional sites were surveyed during translocation projects, Reed Bingham State Park in Cook County, GA and a private development site in Chatham County, GA. Reed

Bingham was known to have a high tick prevalence while the Chatham County site was of unknown prevalence at the time of sampling. Tortoises and samples were handled as described above.

Sample collection

Blood was collected from rom the jugular, subcarapacial, or brachial veins using either manual restraint or chemical immobilization (McGuire et al, 2013). Thin blood smears were immediately prepared and allowed to air dry. Smears were fixed with methanol and stained with a modified Giemsa stain (Dif Quik; Jorgensen Laboratories, Loveland, CO). Any remaining blood was added to tubes lined with lithium heparin and frozen for future studies. Blood smears from the JERC were blinded as to site and tick status and were read by a single observer.

Sample Analysis

Slides were examined at 40x magnification to determine the presence of hemogregarine parasites. If parasites were found, the slide was examined at 100x magnification under oil immersion to determine parasitemia which was calculated based on the number of hemogregarines present after examining 1000 erythrocytes. If no parasites were detected in the first 1,000 erythrocytes, 10,000 erythrocytes were examined to confirm the negative result.

Data Analysis

Prevalence within established, translocated and hatchling tortoises on St Catherines Island was determined and compared to tick-positive and tick-negative sites at JERC using chi-

square analysis. Parasitemias amongst the same groups was analyzed using t-tests. Statistical analyses were done using GraphPad (GraphPad Software 2014, California).

RESULTS

Blood smears from 46 of the 75 tortoises that were translocated to St Catherines Island were examined and 83% were infected with hemogregarines on initial exam. Four negative tortoises remained negative throughout the study (sampled again in 1996, 2005 and 2010 (for two tortoises)) respectively, while another negative tortoise became infected with hemogregarines when sampled in 1995, 1998 and 2005. This tortoise was found to be infested with a single *A. turberculatum* when sampled in 1995 and 2005. Tortoises that were hatched on the island were captured beginning in 1994. Since that time, 33 hatchling tortoises have been sampled and none have been infected with hemogregarines. Among the tortoises that were established on the island before the translocation project, 4 of 23 (17%) were positive and parasitemias ranged between 0.01 to 0.2%. Unfortunately, these tortoises have unknown histories, but it is important to note that two of the four positives were first detected upon initial sampling in 1996 and 2004 and that all of the established tortoises sampled in 1994 were negative. Surprisingly, two of the positive established tortoises were negative at initial sampling, but then were positive at later sampling. One tortoise was found to be infested with a single tick when captured in 2004. The tortoise initially tested negative for haemagregarines in 1995 and in 1998. When captured in 2011 (the final sample period), the parasitemia was 0.01%. The other tortoise was negative in 2009, positive in 2010 (0.1% level of parasitemia), and then negative in 2013. Nineteen tortoises have been found to be infested with ticks (two tortoises were found to be infested at two different sampling periods) (Table 13) since the project began which leads us

to believe that *A. turberculatum* has not become established on the island and that the ticks that have been found were missed upon the pre-shipment exam prior to tortoises being translocated to the island.

Thirty-nine of the translocated tortoises were sampled at least twice, of which 77% were infected and their level of parasitemia declined between the two sampling periods (Figure 2). The highest parasitemia observed was 15% (in a 1998 sampling period) and the lowest was 0.01% (two tortoises in 1995, one tortoise in 2006 and one tortoise in 2010). Parasitemia values for three tortoises remained stable between the two sampling periods and five of the tortoises (described above) remained negative. One tortoise that was initially negative in 1994 was positive for haemogregines when it was sampled in May 1995, 1998 and 2005. This change in infection status coincided with the finding of a single tick in both 1995 and 2005. Similar to the translocated tortoises, the parasitemia of one of the hemogregarine-positive established tortoises decreased in a 5 yr period (0.19% in 2004 to 0.1% in 2009). Chi-square analysis of prevalence between newly released and established tortoises was statistically significant ($p = 0.0001$), as were comparisons between their levels of parasitemia ($p = 0.003$).

To better estimate the changes in parasitemia over time, samples from five tortoises that were recaptured annually seven or more times were analyzed more closely. Interestingly, for all five tortoises an increase in parasitemia was observed in the two to three years after translocation followed by a rapid decline (Figure 3). Four of the five tortoises were initially sampled in 1994, while the remaining tortoise was initially sampled in 1995.

Analysis of the JERC samples showed that ten were positive for hemogregarines (6%; 10/168), while 23/168 tortoises were positive for ticks (14%). Six tortoises were positive for both hemogregarines and ticks (4%) at the time of sampling. The highest parasitemia was 0.03%

and the lowest was 0.0001%. Chi-square analysis to determine the relationship between the low-prevalence versus high-prevalence sites was not significant ($p = 0.1$). Differences in the prevalence of haemagregarine parasite infection in JERC tortoises versus SCI tortoises were significant for the translocated tortoises ($X^2 = 117.6$, $df = 1$, $p = 0.0001$), but not for the established or hatchling tortoises ($X^2 = 2.4$, $df = 1$, $p = 0.12$; $X^2 = 1.0$, $df = 1$, $p = 0.32$ respectively).

Tortoises from the Reed Bingham and Chatham County sites were analyzed for prevalence of infection with haemogregarine parasites. Of the 17 tortoises examined at Reed Bingham, 15 (88%) were positive for ticks and 6 (35%) were positive for hemogregarines. For the Chatham County site, 52 tortoises were examined, all of which were negative for ticks. We analyzed 12 blood smears from this group of tortoises and all were negative for hemogregarines. The prevalence of haemagregarine parasite infections in Reed Bingham tortoises versus SCI tortoises were significantly different for the translocated tortoises ($X^2 = 11.0$, $df = 1$, $p = 0.0009$), and hatchlings ($X^2 = 10.1$, $df = 1$, $p = 0.002$) but not for the established tortoises. Chatham County was only significantly different to the established SCI tortoises ($X^2 = 18.5$, $df = 1$, $p = 0.0001$).

DISCUSSION

Our data shows that the tortoises on St Catherines Island are exhibiting a decrease in parasitemias over time. This indicates that the tortoises are not being reinfected with hemogregarines, and thus are not being exposed to the vector. At the time of translocation, gopher tortoises that were already established on the island tested negative for hemogregarines

and were believed to be tick-negative. In addition, tortoises that have hatched on the island are 100% negative for the parasite. The hatchlings are either not exposed to the vector and parasite or are being exposed at such low numbers that the haemogregarine parasite is not able to successfully infect the host. Previous studies in Africa have shown that ticks are a vector for the haemogregarine parasite (Cook, 2009, Harris 2013). The low abundance of ticks on St Catherines Island and the decrease in parasitemias over time, or in the case of hatchlings, negative prevalence, suggests that *A. turbuculatum* plays a role in the transmission of hemogregarines in gopher tortoises.

It is also worth mentioning that the parasitemias of five tortoises exhibited a spike in the first two to three years after the translocation, followed by a precipitous decrease and eventual stabilization at low parasitemia. It is not known whether these tortoises were infected prior to or shortly after being introduced to the island. However, it should be considered that the increase in parasitemias shortly after the introduction of this group of tortoises to the island is a response to stress induced by the move. The tortoises were exposed to novel nutritional resources and habitat, previously established tortoises, and possibly novel pathogens that could have influenced the rate of reproduction of the haemogregarine parasite. Although haemogregarines at normal parasitemias are not known to cause clinical disease in tortoises, a steep increase in parasitemias could lead to anemia and has been shown to result in elevated monocytes in other species? (Veiga et al, 1998, Bonadiman et al, 2010. The combination of stressors, and their effect on the individual tortoise, should be a consideration in future translocation projects.

Although ticks have been found on St Catherines Island, a presumed tick-free area, we do not feel that ticks have established on the island. *Amblyomma turbuculatum* is species specific and infests gopher tortoises in its adult stage of life. It is only in the nymph stage that *A.*

turbuculatum has been known to feed on mammalian species (Wilson and Durden, 2003). Prior to the translocation project on St Catherines Island in 1994, *A. turbuculatum* had not been reported on the resident tortoises or on other animal species residing on the island. On the occasions that the tick has been identified on the island, it was at a low abundance (1 – 2 ticks per tortoise), and occurred sporadically throughout the years. In fact, since 1994, *A. tuberculatum* has been found on the island tortoises only 21 times. This includes two tortoises that were found to be infested with an extremely low abundance during two separate sampling efforts. One tortoise that was found to be negative for hemogregarines on initial exam in 1994 was later found to have a parasitemia of 0.3% in 2005, indicating the tortoise was infected while residing on St Catherines. A single *A. turbuculatum* was located on this tortoise on physical exam in 2005. Two tortoises of unknown origin were also negative when first sampled in 1995 and 2009, respectively. Both tortoises had positive parasitemias on subsequent sampling in 2011 (0.01%) and 2010 (0.1%).

It is worth considering that the ticks that have been found during this time period could have been brought to the island by any of the rehabilitated or waif tortoises that have been released on St Catherines. Ticks are difficult to locate during a physical exam and have been known to escape detection because they attach in areas obscured by the head and limbs which are pulled toward the shell. However, the authors do not believe that this is a normal or commonplace occurrence. Another possibility is that larval ticks were brought on to the island by a mammal or bird. Although adult stage *A. tuberculatum* are species-specific, immature stages are known to parasitize a variety of small mammals and birds (Wilson and Durden, 2003). A study of host- parasite distribution in Mississippi reported that *A. tuberculatum* are more

sensitive to environmental and climatic factors than gopher tortoises and therefore, are not evenly distributed (Ennen and Qualls, 2011).

The Jones Research Center is a site of mixed tick abundance. The areas in which we found tortoises infested with ticks were areas that were previously known to have a robust tick population. It was in these areas that tortoises were detected with a parasite load (Table 14). Two other areas of the property with either a low or unknown tick prevalence due to minimum trapping efforts, produced only 2 tortoises infested with ticks and 2 other individuals infected with hemogregarines. The tortoises that tested positive for the parasite also had extremely low parasitemias (0.0059% and 0.0001%). Again, these findings indicate that the tortoises either were not coming into contact with the vector or were maintaining previous infections at a very low rate and were not being reinfected. One tortoise infested with ticks was found in a low tick abundance area and was found to also have Upper Respiratory Tract Disease (URTD). Tortoises with URTD are known to travel long distances outside of their natural home range (McGuire et al., 2013). Due to the prevalence of URTD in tortoise populations, this should be a special consideration when considering translocation projects. The ability to spread novel pathogens to a naïve population has the potential to cause devastating effects to an already threatened population.

The Reed Bingham and Chatham County sites also lend credence to a possible association of *A. turberculatum* and hemogregarines in gopher tortoises. Reed Bingham was a known tick abundant area prior to the study and the tortoises in that population tested positive for hemogregarines (Table 14). However, tortoises on the Chatham County site were found to be negative for both ticks and parasites. A fifth site, located on the mainland near St Catherines Island in McIntosh County, was highlighted in Hernandez, 2011. This study was the first

documented case of hemogregarines in gopher tortoises and they also found a high rate of parasites in the tortoises.

CONCLUSION

While we cannot say for certain at this time that *Amblyomma turbuculatum* is the definitive vector for hemogregarines in the gopher tortoise, it is plausible that based on our findings, there is an association between the two. Further study is warranted to determine the true relationship and if in fact, *A. turbuculatum* is the vector for gopher tortoise hemogregarines.

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Table 13. Infestation of gopher tortoises introduced to or hatched on St. Catherines Island with *Amblyomma tuberculatum*.

Group	No. tortoises infested/ No. tortoises examined (%)					
	1994	1995	1996	1997	1998	1999-2011
Translocated	75/75	4/31	7/33	1/30	0/39	3/129
Established	0/11	1/10	1/11	0/11	0/11	4/42
Hatchlings	0/4	0/3	0/2	0/1	0/1	0/405
Total	75/90 (83)	5/44 (11)	8/46 (17)	1/42 (2)	0/51	7/576 (1)

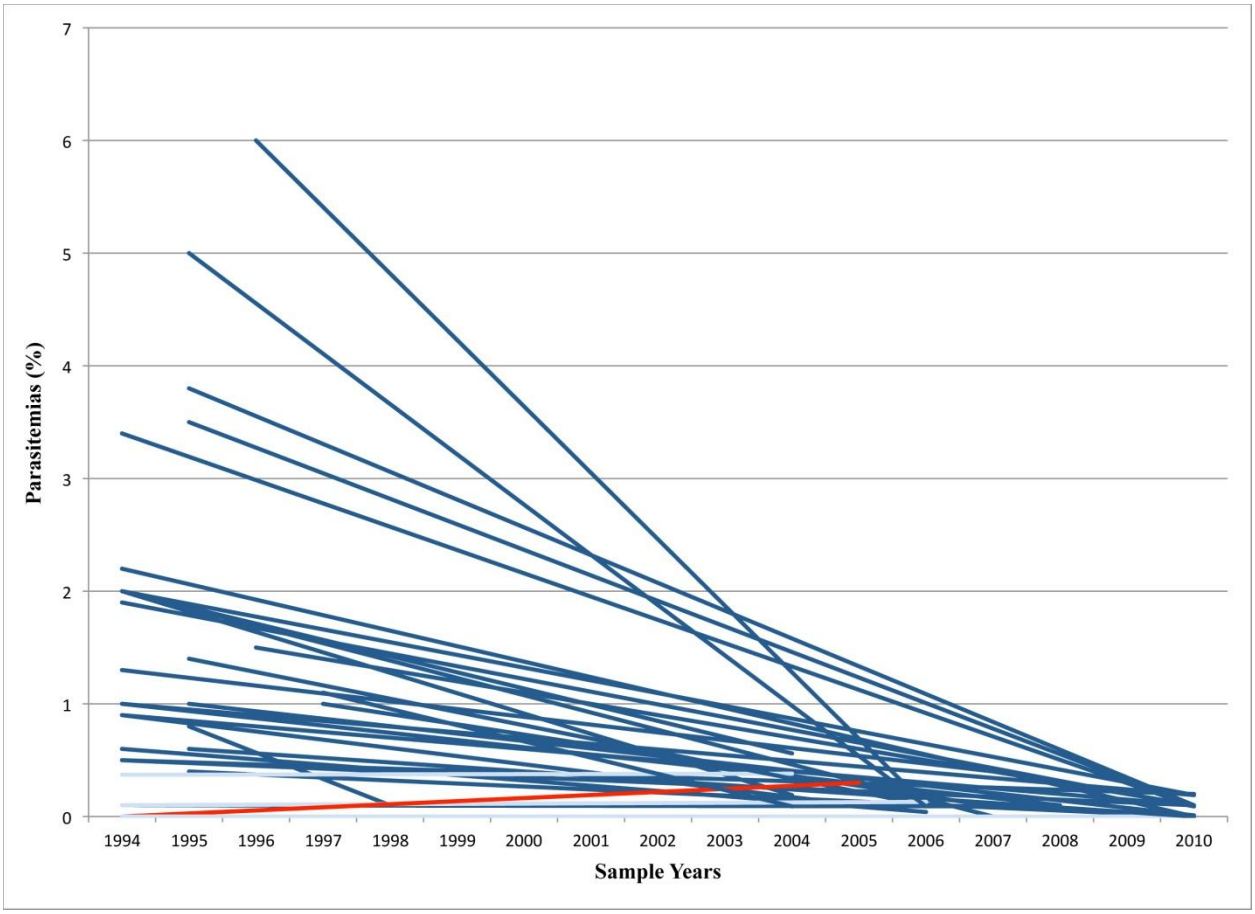

Figure 2. Parasitemia values at the first and last sampling time for 35 translocated tortoises from St. Catherines Island. Tortoises illustrated in dark blue had decreases in parasitemia while those in light blue had not changed (n = 6) between the two sampling times. A single translocated tortoise (in red) became infected during this study (see results). The parasitemia of 7% is not shown so as to better elucidate the findings of the other tortoises. However, the parasitemia for that tortoise also decreased from 7% in 1994 to 5.8% in 1998.

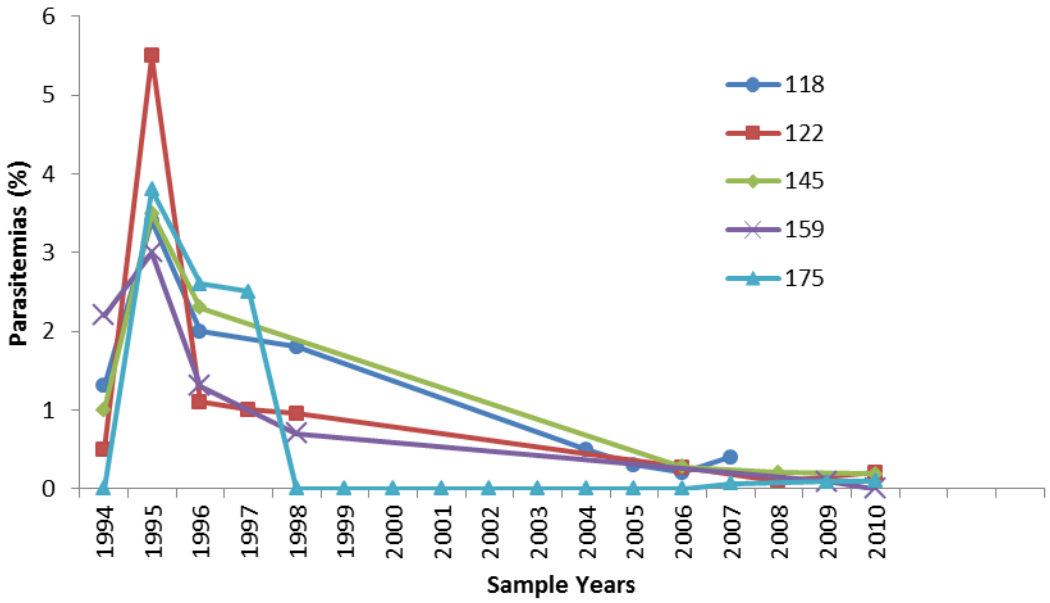

Figure 3. Parasitemias over time for five individual tortoises that were sampled at least seven times during the study period.

Table 14. Prevalence of tick infestation and infection with haemogregarines at four sites in Georgia.

Site	No. infested with ticks/No. examined (%)	No. infected with haemogregarines/ No. tested (%)
Ichuaway	23/168 (14)	10/168 (6)
Reed Bingham State Park	15/17 (88)	6/17 (35)
Private site in McIntosh County[1]	NR[2]	10/14 (71)
Private site in Chatham County	0/52	0/12
St. Catherine's Island[3]	0/18	0/18

[1] Data from Hernandez et al., 2011
[2] NR, not recorded. Ticks were noted and removed from tortoises but infestation rate was not available.
[3]Only tortoises hatched on the island are included

CHAPTER 4

CONCLUSIONS

The goal of this study was to better understand and contribute to the health parameter data for gopher tortoises in the southeastern United States. As a species in serious decline and one that is frequently translocated, it is imperative that researchers and veterinarians have a means of quantifying health through hematology and biochemical reference values. Identifying the effects of diseases such as URTD, ranavirus, and herpesvirus on various blood analytes will aid in management decisions, particularly in translocation. Blood data is variable among species, sex, season, age group, and location. Long-term monitoring of free-ranging populations will further identify the reference values that are unique to gopher tortoises.

It is not clear at this point, what role parasites play in the health of tortoise populations and how the transmission of parasites to naïve translocated populations will affect long-term survival. Further study into the definitive vector, mode of transmission, morphological characteristics and the affects that hemaparasites have on blood parameters are warranted.
It is our hope that with the baseline blood values that have been established through this research, and with the information gathered and presented on haemagregarines, that veterinarians and land managers will have the ability to greatly improve their efforts in conserving the gopher tortoise.