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Characterizing the health status of the Louisiana gopher tortoise (Gopherus polyphemus)

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CHARACTERIZING THE HEALTH STATUS OF THE LOUISIANA GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*)

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DEDICATION

This thesis is dedicated to my wife, Melissa, and my parents who have encouraged and supported me as I continued with my academic endeavors.

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TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Gopher tortoise (*Gopherus polyphemus*) populations have experienced precipitous declines from habitat loss, and human and disease related mortality. The goal of this study was to characterize the health status of free-ranging Louisiana gopher tortoises. Gopher tortoises were collected during two distinct trapping seasons: fall (August-October 2002) and spring (April-June 2003). Captured tortoises were given a physical exam and the carapace and plastron length and width, weight, and body temperature were recorded. Blood was collected from the subcarapacial vein and submitted for the following testing procedures: complete blood count, plasma chemistry, infectious disease serology (*Mycoplasma*), and toxicologic screen (copper, mercury, zinc, and lead). Fifty-nine tortoises were captured during the study. Fifty-seven (97%) of the tortoises were adult animals and two (3%) were juveniles. Twenty (34%) of the tortoises were captured in the fall and thirty nine (66%) were captured in the spring. There were thirty male (53%) and twenty-seven female (47%) tortoises captured between the two trapping periods. The gender of the hatchling tortoises could not be determined. Complete blood counts, plasma biochemistry analyses and toxin screens were performed on fifty seven adult tortoises. There were several differences detected in the white blood cell count between trapping season. The white blood cell count and heterophil count were significantly higher in the spring compared to the fall. Significant gender differences were observed for potassium, calcium, and phosphorus. Marked seasonal variation was observed with glucose, uric acid, and GGT. Only creatinine kinase levels differed

significantly between sites. There was a difference in the mercury and copper levels between gender. Only copper differed significantly between season. Overall, 26% of the tortoises were serologically suspect or positive for *Mycoplasma agassizii*. There was no difference in parasite shedding between gender, site of capture, or season. The parasites identified in these tortoises were consistent with findings in gopher tortoises throughout their range. In general, our findings suggest that these tortoises are in good health.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

The gopher tortoise, *Gopherus polyphemus*, is a large burrowing chelonian from the southeastern United States. The primary range of the gopher tortoise extends from southern South Carolina through Georgia, Florida, Alabama, Mississippi, and into southeastern Louisiana. Once abundant throughout its range, the gopher tortoise has been extirpated through much of its range as a result of negative anthropogenic impacts, including urban and agricultural development, habitat loss and fragmentation, road mortality, and the introduction of domestic predators. Although research concerning habitat utilization, reproductive biology, and the disease status of the gopher tortoise have been pursued in Florida and Georgia (Auffenberg and Franz, 1982; Lander et al., 1982; Diemer et al., 1989; Smith, 1995), there is limited published information about these tortoises from the remainder of their range (Smith et al., 1997).

Louisiana has the smallest and most threatened population of gopher tortoises. Extreme population decline has led to the classification of this species as federally Threatened by the Fish and Wildlife Service (U.S. Fish and Wildlife Service, 1990). The gopher tortoise is legally protected in all states within its range and is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, which requires permits for the exportation of the species from the United States to any signatory nation (Levell, 1995).

The family Testudinidae is comprised of twelve genera and approximately 50 living species of tortoises (Ernst and barbour, 1989). These reptiles are found in the Americas, Europe, Africa, Asia, and certain oceanic islands. At least four species of tortoises are known to have existed in North America. Three of the living species, the desert tortoise (*Gopherus agassizii*), Texas tortoise (*Gopherus berlandieri*), and the Bolson tortoise (*Gopherus flavomarginatus*) are found in the southwestern United States and northern Mexico. The gopher tortoise (*Gopherus polyphemus*) is the only chelonian species living in the southeastern United States. *Gopherus* is derived from the word gopher, and was in common use at the time in North America to describe burrowing animals. The species was first described in 1802 by the French scientist Francois-Marie Daudin, who named the species after Polyphemus, the legendary cave-dwelling giant in the Odyssey (Daudin, 1802) (Figure 1).

The range of the gopher tortoise once encompassed much of the longleaf pine (*Pinus palustris*) forests of the southeastern United States (Auffenberg and Franz, 1982). Unfortunately, much of the original longleaf pine habitat has been reduced by over 85% because of habitat loss and degradation, leaving forests that are small and highly fragmented (Noss, 1989). As a consequence, gopher tortoise population densities have declined by over 80% in the best remaining habitat (Herman et al., 2002). In addition to the negative anthropogenic influences on the tortoise's habitat, other negative activities, such as hunting, have also affected tortoise densities. Hunting for gopher tortoises and other species that use their burrows, such as rattlesnakes, cause the tortoise burrows to be disturbed.

Figure 1. It is interesting to note that the gopher tortoise was probably named after Polyphemus, the cave-dwelling giant in the *Odyssey*, for its burrowing habitat and great strength. Pictured here is an adult Louisiana gopher tortoise. Note the characteristic shovel-like flattened forefeet for burrowing.

G. polyphemus was originally hunted as a food source during the Great Depression and was sold by landowners and collectors. The effects of these population reductions are magnified by the status of the gopher tortoise as a keystone species (Paine, 1966) since the burrows that tortoise excavate provide habitat or refuge for over 360 species (Jackson and Milstrey, 1989). Additionally, the mound of sand deposited at the mouth of a burrow during its construction disrupts ground cover vegetation and affects the understory community in a way that may promote high plant species richness (Kaczor and Hartnett, 1990; Hermann, 1993). Because of these factors, gopher tortoises are thought to be keystone members of the longleaf pine ecosystem (Guyer and Bailey, 1993).

Gopher tortoises are susceptible to a wide variety of health problems. Infectious and non-infectious diseases are known to cause illness and death in captive reptiles (Clark et al., 1981; Frye, 1991; Jacobson, 1980), and recently several diseases have been identified in wild reptile populations. More diseases have been reported in wild chelonians compared to the other major groups of reptiles (Jacobson, 1992).

An upper respiratory tract disease (URTD) associated with *Mycoplasma agassizii* has been reported in both captive and wild populations of tortoises (Jacobson et al., 1991 and 1995; Lawrence and Needham, 1985). The disease was first documented in 1989 in wild gopher tortoises from Sanibel Island, Florida (McLaughlin, 2000). *Mycoplasma agassizii* can infect the mucosal membranes of the nasal cavity and respiratory tract, stimulating a significant immune mediated inflammatory response. The disease is characterized clinically by edema of the

eyelids, ocular discharge, conjunctivitis, mucoid or purulent nasal discharged, and in advanced cases, pneumonia-like symptoms. Mycoplasmosis is one of the more common illnesses diagnosed in wild chelonians. Because the clinical signs attributed to mycoplasmosis may overlap with other infectious agents, such as herpesvirus (Jacobson and Origgi, 2002; Origgi, 2001), iridovirus (Westhouse et al., 1996), *Pasteurella sp*., *Serratia sp*., *Pseudomonas sp*., and noninfectious agents such as hypovitaminosis A, it is important to pursue appropriate diagnostics tests to confirm the status of a tortoise. Despite published reports of broad exposure to the causative agent of URTD among gopher tortoises throughout much of their range (Smith et al., 1998; Berish et al., 2000), the effects of this disease on the viability of the gopher tortoise population are just beginning to receive attention.

Basic data on the status of the gopher tortoise in Louisiana has been extremely limited. For example, Auffenberg and Franz (1982) reported finding only six populations in the state, and considered tortoises to be endangered in Louisiana. However, surveys by R. Martin of the Louisiana Natural Areas Inventory have suggested that tortoises are more widespread and numerous than previously believed (Seigel and Hurley, 1992). In addition to numerous scattered colonies and individuals, at least one large population is located at the Ben's Creek Wildlife Management Area, a privately owned forest managed by the state. Virtually no ecological data are available for these animals.

Louisiana gopher tortoise survival is challenged by many variables, including habitat destruction, environmental modification, road mortality, illegal

collection, and disease. Despite state laws in Louisiana, and the Endangered Species Act (1990), habitat destruction and illegal collection continue to be a major threat to gopher tortoise survival. Gopher tortoises and many other reptiles depended on this habitat for their survival. Although the fate of the gopher tortoise in Louisiana is unknown, measures are being taken to gather information for recommendations to governmental agencies to set aside protected habitat.

1.2 Objectives and Hypotheses

Field evaluation of free-ranging wildlife requires the systematic documentation of a variety of environmental conditions and individual parameters of health and disease, particularly in the case of endangered or threatened species. Studies that intend to characterize the plight of a particular species should follow a systemic approach.

Shrinking and fragmented habitat have led to an increased likelihood of wildlife becoming more isolated. This isolation has led to a number of problems with regards to the long term management of a species. The health status of wild tortoises in the U.S. has increasingly becoming a discussion point for conservation programs. Currently, there is a dearth of baseline health parameters data on free-ranging tortoises. This lack of baseline information is especially critical, as there continues to be a misplaced emphasis in chelonian conservation on translocation, reintroduction, and other interventionist management.

The purpose of this project is to characterize the health status of free-ranging Louisiana gopher tortoises (*Gopherus polyphemus*). Because these animals serve as sentinels for their respective ecosystems, information pertaining

to emerging infectious diseases and toxins can be used to assess both the overall health of the Louisiana population of the gopher tortoise and their ecosystem. This information can then be used to plan management strategies to sustain these animals and their environment into the future. Our objectives were to: (1) collect blood samples to perform complete blood counts and plasma biochemistry analyses and assess the general physiologic status of the tortoises (2) collect blood samples to perform screens for potential environmental toxins, (3) determine the seroprevalence of *Mycoplasma agassizii*, and (4) determine the prevalence of endoparasite shedding in the Louisiana gopher tortoise.

The specific hypotheses to be tested in this study include: a) gopher tortoises from Louisiana will be seropositive for mycoplasmosis, b) gopher tortoises from Louisiana will be positive for nematodes, c) white blood cell counts will be lower in the fall than the spring, d) lymphocyte counts from gopher tortoises will be lower in the fall than the spring, e) female gopher tortoises will have higher calcium and phosphorus levels than males, f) gopher tortoises will have lower glucose levels in the fall than the spring, and g) male gopher tortoises will have higher levels of copper, zinc, lead and mercury than the females.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Biology of the Gopher Tortoise

This large fossorial tortoise from the southeastern United States has well adapted shovel-like flattened forefeet for burrowing, elephantine hind feet, and a gular projection on the plastron (Ernst et al., 1994). Both sets of limbs lack webbing and have at most two digits. The vertebrae of the gopher tortoise are fused to its carapace, and this upper shell is comprised of dermal bony elements called the osteoderms. The shell is oval in shape, and the plastron and carapace are connected laterally between the legs by a portion of the shell termed a bridge. The oblong carapace is widest in front of the bridge and commonly highest in the sacral region. The shell has openings that allow the tortoise to completely hide their head and legs. This behavior, combined with the high domed shells aid in deterring predators.

The growth rings (annuli) of gopher tortoises are located in the scutes and are usually well delineated in juveniles and young adults, giving the dark-brown to grayish-black carapace a somewhat rough, ridged appearance (Ernst et al., 1994). The scutes of adult gopher tortoises are generally smooth so the rings are indistinguishable. The gular osteoderm of the large, hingeless plastron are elongated, project anteriorly, and may curve upward. The skin of the head and limbs is grayish black, while that of the limb sockets is yellowish in color (Ernst et al., 1994). Well developed mental glands lie beneath the chin, and four to six rostral pores lie on the internarial region (Winokur and Legler, 1975).

Ernst et al. (2001) described the anapsid skull of the gopher tortoise as lacking temporal fenestrae. Chelonians have a keratinized beak in place of teeth. Muscles of the jaw are modified from other tetrapods by passing over the trochlear process, which allows for greater force when closing the mouth. The adductor muscle performs a double function where one component draws the mandible dorsally and the other component pulls the lower jaw posteriorly. These movements allow the gopher tortoise to tear the tough plant material that it feeds on.

Morphological characteristics such as the length of the gular projection, plastron/carapace length, size of the mental glands, degree of plastral concavity, and thickness of the anal scutes have been reported to be sexually dimorphic in gopher tortoise (Goin and Goff, 1941; Mount, 1975); however, these characteristics have not been evaluated using a sample of individuals of confirmed sex. McRae et al. (1981) reported that shell dimensions and the anal notch of adult female gopher tortoise were significantly larger than those of adult males in Decatur County, Georgia. In the same population, the degree of plastral concavity, width between the posterior tips of the anal scutes, thickness of the anal scutes, larger mental glands and length of gular projection were significantly greater in males. Sexual dimorphism in these characters is independent of sex related body size, and apparently is a result of selection to aid in breeding and successful reproduction. These dimorphic characters were best observed in older individuals.

Few studies have focused attention on ways to improve gopher tortoise reproductive success because little is known about the basic reproductive biology

of this species, including the mating system and timing of the mating season (Landers, et al., 1980; Taylor, 1982; Diemer and Moore, 1994; Smith, 1995; Butler and Hull, 1996). An understanding of the reproductive biology of the gopher tortoise is necessary to determine how or when one should concentrate recovery efforts for this species.

Gopher tortoises are long lived, and produce one relatively small clutch of eggs annually or biannually (Diemer and Moore, 1994). Sexual maturity is achieved at carapacial lengths (CL) of 23-24 cm in males and 25-26.5 cm (CL) in females (Auffenberg and Iverson, 1979; Iverson, 1980; Landers et al., 1982; Diemer and Moore; 1994; Mushinsky and Esman; 1994). In the Conecuh National Forest in southwestern Georgia, tortoises reach sexual maturity at approximately 20 years of age (Aresco and Guyer, 1999). Tortoises in Mississippi and Louisiana mature between 15-20 years of age (Smith et al., 1997). In Florida, tortoises mature between 9-18 (males) and 10-21 (females) years of age (Diemer and Moore, 1994). Because gopher tortoises may not reach reproductive maturity until 10-20 years of age, and because young tortoises are inconspicuous, a reduction in recruitment and survival of young individuals caused by land management techniques may not be detected until the number of older individuals has declined significantly (Iverson, 1980; Landers et al., 1980).

The mating season has not been clearly defined for this species. Spring observations of copulation have been recorded in southern Florida (Kenefick, 1954), Georgia (Landers, et al., 1980), and South Carolina (Wright, 1982). The only report of fall copulation is from Sanibel Island, southwestern Florida

(McLaughlin, 1990). These periods of copulation coincide with high levels of sexual hormones in both males and females (Taylor, 1982). Landers et al. (1980) observed from late March through October several cases in Georgia and Florida in which the burrows of known females seemed to be the object of male courtship behavior. In Georgia, males and females were observed copulating or in pairs only from April through June. In three cases from Georgia (July to August), females turned sideways in the burrow entrance and remained there for periods of 1-3.5 hours for courtship by males. Auffenberg (1969) observed that just before the male's behavior changed from head bobbing to biting, the female rubbed the side of her face and the mental glands across her outstretched front legs. In addition, Auffenberg speculated that the scent from the glands is possibly transferred by the female from her chin to her front legs by means of the scales near the elbow. Landers et al. (1980) noted that these glands were quite enlarged and contained copious fluid during spring and summer. The glands of adult females appear active during the spring, but regress after mid-June.

Taylor (1982) reported that fall breeding will coincide with high levels of 17-beta-estradiol, progesterone, the onset of vitellogenesis, and high testosterone levels that follow spermiation and increased testicular weight. This suggests that spermatogenesis may be androgen dependent (Mendonca and Licht, 1986). Ott et al. (2000) reported that testosterone levels in males increase in June and peak in August but remain high into October. In addition, corticosterone levels, which are positively correlated with stress levels, in male tortoises decreases as body size increases. The increase in testosterone levels also correspond to peak movement

activity, with males moving significantly greater distances, occupying significantly more burrows, and sharing burrows with females significantly more often in August and September than in other months (Ott, 1999).

In females, blood estrogen levels rise in August and remain high through October. Female testosterone levels peak around May, decrease in June and remain low into July. Testosterone levels then increase in August and September (Ott et al., 2000). Ott et al. (2000) observed corticosterone levels decreased in females as the number of burrows they used increased. This suggested that the availability of more burrows was associated with a decrease in stress levels in females.

The impacts of stress on reproduction should be examined more closely in free-ranging gopher tortoise. Progesterone, estrogen, and testosterone levels have been found to be higher in wild tortoises than captive tortoises (Ott et al., 2000; Taylor, 1982). It is not known whether the lower hormonal levels negatively impair reproductive function. However, Taylor's (1982) study did indicate that stress may negatively impact reproductive behavior. If free-ranging gopher tortoises have a negative response to capture stress, it may be possible that natural stressors, such as decrease food availability, increased canopy cover, or low quality habitat, can impact levels of sex steroid hormones. Future studies on the influence of habitat stress on reproduction should be pursued.

In gopher tortoises, vitellogenesis begins around September and is nearly completed by November. During the winter, the follicles enlarge little, if any, and then grow to ovulatory size in March and April (Iverson, 1980). According to

Landers et al. (1980), follicles ≤ 25 mm in diameter in the fall will not be ovulated the following spring. Follicles significantly smaller than those in the group of larger follicles are probably not ovulated and are likely resorbed during the summer. Since follicular development occurs during the driest season (fall), diversion of energy for excessive production of follicles would likely be disadvantageous for this tortoise as both quality and quantity of food are limited during the fall and winter. Because ovulation of follicles occurs during the spring season, females most likely store sperm through the winter months and fertilize the ova after they ovulate in the spring (Saint-Girons, 1973; Gist and Jones, 1987).

Relatively little has been published on the nesting and hatchling biology of gopher tortoises in the western portion (Alabama, Mississippi, and Louisiana) of their range (Marshall, 1987; Hurley, 1993; Smith et al., 1997). Most information on nesting periods has been reported from Florida. In Florida, egg deposition was noted from late May to early June (Landers et al., 1980). In contrast, in southern Mississippi, egg deposition occurred from approximately mid-May through mid-July with a peak activity between May and June (Smith et al., 1997; Epperson and Heise, 2003). In eastern Louisiana, reproductive frequency reached a maximum of about 90% in late May, declined to 25% by early June and was absent in July. Seigel and Hurley (1992) observed that tortoises from Louisiana lay eggs at approximately the same time as do tortoises in southwestern Georgia, where the egg deposition apparently reaches a maximum in late May and early June.

Nesting sites for egg deposition occur most frequently around the burrow apron; however, tortoises can deposit eggs in other suitable sites (Butler and Hull,

1996; Landers et al., 1980). Clutch size is positively related to carapace length in both western and eastern populations of tortoises (Diemer and Moore, 1994). In general, the clutch size of *G. polyphemus* from Louisiana and Mississippi were similar to the clutch sizes from northern and north-central Florida, but lower than southwestern Georgia and central and southern Florida (Smith et al., 1997). Gopher tortoise clutches average 5-8 eggs (Godley, 1989; Butler and Hull, 1996; Smith et al., 1997). The lowest reported mean clutch size (3.9) is from South Carolina (Wright, 1982), while the highest reported mean clutch sizes (7.8, 8.9) are from central and southern Florida. The record size for a gopher tortoise clutch was 25 eggs, and was recorded in southwest Florida (Godley, 1989). Clutch size can be highly variable within the same season. Hatching and emergence occurs from August through October (Ernst et al., 1994). In Mississippi, emergence peaks from August to September, and the incubation period ranges from 52-109 days (Epperson and Heise, 2003). In Florida, incubation is approximately 80-90 days (Iverson, 1980), Georgia 97-106 days (Lander et al., 1980), and in South Carolina it may take as long as 110 days (Wright, 1982). Overall, the incubation period between the eastern and western populations average 88 days (Iverson, 1980; Smith, 1995). After emergence, hatchlings disperse to find burrows for their winter dormancy. At hatching, tortoises average 32 grams in weight and have a mean carapace length of 4.79 ± 0.380 cm (U.S. Fish and Wildlife Service, 1990).

Nest and hatchling predation rates have been well documented in gopher tortoise populations (Alford, 1980; Wilson, 1991; Landers et al., 1980; Butler and Hull, 1996). Estimates of greater than 94% mortality of both nests and hatchlings

have been recorded in Florida (Alford, 1980). Based on these numbers, an adult female only produces a successful clutch once every 9-10 years (Landers et al., 1980). There are a number of predators that prey on nests, hatchlings, and juveniles, including raccoons (*Procyon lotor*), nine-banded armadillo (*Dasypus novemcinctus*), red-tailed hawks (*Buteo jamaicensis*), eastern indigo snakes (*Drymarchon corais*), eastern diamondback rattlesnakes (*Crotalus adamanteus*), Florida cottonmouths (*Agikstrodon piscivorous*), gray foxes (*Urocyon cinereoargenteus*), striped skunks (*Mephitis mephitis*), and imported re fire ants (*Solenopsis invicta*) (Fitzpatrick and Woolfenden, 1978; Marshall, 1987; Smith, 1995; Butler and Sowell, 1996; Mount, 1981; Douglass and Winegarner, 1977; Vetter, 1970; Landers et al., 1980).

Predation rates for both eggs and hatchlings have been well documented in Alabama, Georgia, South Carolina, and Florida (Marshall, 1987; Landers et al., 1980, Wright, 1982; Butler and Hull, 1996). The majority of the nest predation occurred within two weeks of oviposition. At Camp Shelby, Mississippi, a hatching success of 28% was considered lower than the reports of hatching success at other sites (Epperson and Heise, 2003), including 86% in southeastern Georgia (Landers et al., 1980), 67%-97% in north Florida (Smith, 1995), 73% in northeastern Florida (Butler and Hull, 1996), 65% in Louisiana (Hurley, 1993), and 50% in southern Mississippi (Brode, 1959). The low hatching rate observed at Camp Shelby may have been attributed to environmental and reproductive factors. Environmental factors that may explain low hatching success include egg viability, soil temperature or moisture, soil type, and soil compaction. Compaction of soil,

soil nutrients and overall productivity can occur as result of the continued use of heavy equipment in forestry management (Powers et al., 1990).

In Ben's Creek Wildlife Management Area, Louisiana, it was noted that a high proportion (60%) of tortoise nests died sometime relatively early in development (Siegel and Hurley, 1992). Hurley (1993) postulated that such developmental mortality may be related to the soil composition in Ben's Creek Wildlife Management Area. Soil composition may reduce oxygen availability, especially in the heavy clay soil areas, or lead to an increase in moisture retention, leading to the death of the embryos from drowning (Hurley, 1993). Two samples of soil from the burrow apron where complete nest death occurred were composed of 48.7% sand, 30.5% slit, and 20.8% clay and 33.4% sand, 47.8% slit, and 18.8% clay respectively. However, soil composition from the successful nest site burrow apron was composed of 72.6% sand, 11.8% silt, and 15.6% Clay. According to Hurley (1993), with this percent of sand composition, oxygen and water should be able to enter and pass through the nest site easily, causing no damage to the eggs. Hurley (1993) also noted that heavy rainfall during the months of May, June, and July coincided with the timing of early development of eggs at the nest site, and may have contributed to the mortalities. Brode (1959) studied 40 nests in Mississippi and found that most of the eggs in each nest hatched, but that only about half of the hatchlings emerged. At Camp Shelby, Mississippi, 39% of the eggs were considered nonviable. However, Epperson and Heise (2003) postulated that the poor egg viability at the site was influenced by the density of

the tortoises at Camp Shelby, the demography of tortoise population or the age of individuals.

Although a majority of our knowledge regarding gopher tortoise biology has resulted from extensive studies conducted primarily in Florida and Georgia, the information has applications in Louisiana. As population densities of tortoises in both Louisiana and Mississippi continue to decline (Auffenberg and Franz, 1982), mating opportunities for adult females will also decline. Measures to protect this and similar species are certainly warranted.

2.2 Ecology and Movement Patterns of the Gopher Tortoise Population

Again, the majority of the ecological data on gopher tortoises are based on study populations from Florida and Georgia (Auffenberg and Franz, 1982; Diemer et al., 1989; McRae et al., 1981), and data from the western portion of the range are almost completely absent. Presence of sandy soils and low in height plant forage are the two major ecological conditions that influence local distribution of the tortoise (Franz and Auffenberg, 1978). In Georgia and Florida, tortoises inhabit xeric uplands or coastal dunes with sandy, well drained soils dominated by longleaf pine-turkey oak (*Quercus laevis*) (Smith et al., 1997). In contrast, most of the tortoise habitats in Louisiana and Mississippi occur in areas where the soils have a substantial clay component and a low sand content. The flora can also be quite different in the western portion of their range, as wiregrasses (*Aristida stricta*) are replaced by bluestem grasses (*Andropogon* sp.), and the predominant trees are typically loblolly pine (*Pinus taeda*) and slash pine (*Pinus elliottii*), which are

planted for pine production, in place of the original longleaf pine (*Pinus palustris*) (Wahlenberg, 1946; Ware et al., 1993).

Many herbaceous plants are readily eaten by the gopher tortoise. The gastrointestinal tract of wild individuals examined by Carr (1952) contained large amounts of grass and leaves, with occasional fruits (blackberries, saw palmetto berries, and gopher apple), bones, charcoal, and insect chitin. Macdonald and Mushinsky (1988) found that the following plants comprised the majority of the tortoises diet, including *Andropogon* sp. (bluestem grasses), *Panicum* sp. (panicum grasses), *Aristida* sp. (wiregrass), *Stenotaphrum* sp. (St. Augustine grass), and *Heterotheca* sp. (silk grass). Wiregrass (*Aristida stricta*) was the most commonly ingested grass. Mushinsky (2003) documented that plant diversity and quality were positively correlated to growth rate for juvenile gopher tortoises. Juvenile tortoises tend to eat plants that have a higher nitrogen content than adults. However, adult tortoises are able to consume plants with lower protein and nutrient contents and less crude fiber because they have finished the majority of their growth.

High levels of plant diversity are connected with the frequency of fire in an area. Periodic natural fires play an important role in maintaining tortoise habitat by opening up the canopy and promoting the growth of herbaceous food plants. If natural fires are suppressed, habitats may become unsuitable for tortoises. Among other things, fire stimulates flowering and seed production of herbs and pyrophytic shrubs, deters invasion by fire intolerant woody vegetation, and exposes mineral soil for seedlings of indigenous herbs and longleaf pine (*Pinus*

palustris) to become established. Gopher tortoises and many other species depend on this habitat for their survival.

Longleaf pine is one of the best fire-adapted species in North America. They are critically imperiled in the southeastern United States, with less than 10% of the acreage remaining that was present just 100 years ago (Wahlenberg, 1946). Longleaf forests and savannahs in Louisiana continue to be threatened at an accelerating rate as a result of human intervention. The principal perils are expanding commercial and residential development, conversion for agricultural pursuits, altered hydrology in flatwoods via drainage canals and ditches, interruption of the natural fire regime, and prevailing forest management systems that favor primarily loblolly pine (*Pinus taeda*), shortleaf pine (*Pinus echinata*), and slash pine (*Pinus elliottii*). Remnant longleaf pine forests and savannahs are critical centers of biotic diversity, provide significant habitat for numerous rare species and many kinds of wildlife, and are important relics of our natural heritage.

Gopher tortoises are the most fossorial of the four North American species of tortoises, digging burrows that may extend 5 meters down from the surface and 15 meters in length (Diemer, 1986; Hansen, 1963). The burrows provide a microclimatic stable environment not only for the tortoises but also for numerous commensal species. Among the vertebrates living in the burrows are opossums (*Didelphis virginiana*), mice and rats (*Neotoma* sp, *Podomys* sp), cottontail rabbits (*Sylvilagus floridanus*), weasels (*Mustela vison*), skunks (*Mephitis putorius*), river otter (*Lutra provocax*), red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), burrowing owls (*Speotyto* sp.), quail (*Colinus* sp.), snakes (*Agkistrodon* sp.,

Cemophora sp., *Coluber* sp., *Crotalus* sp., *Drymarchon* sp., *Elaphe* sp., *Heterodon* sp., *Masticophis* sp., *Micrurus* sp., *Pituophis* sp., *Sistrurus* sp., *Thamnophis* sp.), amphisbaenians (*Rhineura* sp.), lizards (*Anolis*, *Cnemidophorus* sp., *Eumeces* sp., *Ophisaurus* sp., *Sceloporus* sp., *Scincella* sp.), frogs, toads, and treefrogs (*Acris* sp., *Bufo* sp., *Eleutherodactylus* sp., *Gastrophryne* sp., *Rana* sp., *Scaphiopus* sp.) (Carr, 1952; Lawler, 1977; Franz, 1986; Jackson and Milstrey, 1989; Jones and Franz, 1990; Ernst et al., 1994). Many arthropods also share these burrows (Lago, 1991). It is this creation of the burrow refuge that has acknowledged the gopher tortoise by ecologists as the keystone species for its habitat.

Movement patterns and home ranges of most reptilian species has not been well defined (Auffenberg and Iverson, 1979). However, there are few studies on social systems and movement ecology of tortoises of the genus *Gopherus*. Woodbury and Hardy (1948) identified seasonal movement patterns and estimated home range size in a population of desert tortoise. Auffenberg and Weaver (1969) and Rose and Judd (1975) studied the movement of the Texas tortoise. Major factors and behavior known to influence movements in some *Gopherus* species include habitat density and composition, mate seeking and dependence on burrows for escape and from protection from extreme environmental temperatures (McRae et al., 1981; Boglioli, 1999; Diemer, 1992; Douglass, 1990).

McRae et al. (1981) reported that movement patterns and home ranges of gopher tortoises from southwestern Georgia varied with season and were determined by social interaction. Females were more sedentary during spring and summer than males. Burrow preference and movement of males (up to 250 m)

were determined by hierarchial behaviors, feeding activity, and mate selection. The mean feeding radius for adults was only 13 m from the burrow. Further, individuals dispersed from breeding colonies by late summer and half of them moved to their autumn-winter range. Adults used a minimum of three burrows during an activity season ($\bar{x}$ = 4 for females, 7 for males). In contrast, juveniles typically moved only a short distance (15 m) from the nest during their first year of life and used only one or two burrows.

In 1992, eight male gopher tortoises from Louisiana were outfitted with radio-telemetry to record their movement patterns. All movements occurred during September-October and May-June. All individuals used more than one burrow during the study. The largest distance traveled by one individual was 750 m. However, during the mark recapture study, the distanced traveled by these individuals ranged from 350 m to 1.4 km, which was almost double from that observed during the telemetry studies (Seigel and Hurley, 1993).

In another study, Eubanks et al. (2003) reported that telemetered females in Georgia moved more frequently (54.0 ± 3.36 m) during the summer months (June-October) but traveled longer distances in September. Males exhibited a peak movement (85.2 ± 1.73 m) between August and September, and it was attributed to mating activity. The longest distance between subsequent tracking locations was 592 m by a female tortoise and 638 m by a male tortoise. In addition, mean distance per move, number of burrows used, and the annual home range size were greater in males than in females. Further, no relationship was found between body size and annual home range area for adult females and males. These

results, in addition to the published patterns of reproductive behaviors (Diemer, 1992; McRae et al., 1981; Douglass, 1990) and hormonal cycle (Ott et al., 2000), suggest that reproduction is an important stimulus for tortoise movement.

When comparing movement patterns and home ranges between populations, it was found that gopher tortoises from protected lands in Florida (Diemer, 1992; Smith, 1995; Smith et al., 1997) have similar average annual home ranges to those tortoises found in Green Grove, southwestern Georgia (Eubanks et al., 2003). The home range sizes from Green Grove were much larger than those reported for tortoises in private lands managed for timber production (McRae et al., 1981). In addition, the mean annual home range size of males (1.1 ha – average distance of 118 m (388ft)) from Green Grove was greater than gopher tortoises colonies in Mississippi (McRae et al., 1981).

An understanding of movement patterns within the gopher tortoise ecosystem can offer valuable insights into the reproductive behavior and potential for the transmission of infectious diseases. Future studies to better understand habitat requirements necessary to sustain tortoises in Louisiana population are warranted.

2.3 Characterizing the Health Status

2.3.1 Hematology

Characterizing the health status of a reptile is often based on physical examination and evaluation of hematologic and clinical biochemical values. Such values are available for only a few reptiles, and even less for free-ranging chelonian species. Reference values are determined and evaluated statistically to

produce reference intervals, which, in turn, are used to evaluate clinical laboratory data (Dybkaer, 1981; Solberg, 1981). Reference intervals in ectothermic vertebrates exhibit extensive variation between and within species. Many factors, both intrinsic and extrinsic, make it difficult to establish reference blood intervals for a species. Some of the most important intrinsic factors are species, sex, age, and physiologic status. Among the extrinsic factors are season, temperature, habitat, diet, and captivity (Campbell, 1996; Dessauer, 1970; Frye, 1991; Lawrence and Hawkey, 1986; Gottdenker and Jacobson, 1995). The site and method of blood sample collection are also extrinsic factors that may influence hematologic and biochemical values. Deviations from expected values for healthy tortoises can be used to assess the impact of stresses such as habitat loss, infectious diseases, forage competition with domestic livestock, off-road-vehicle use, and drought on free-ranging tortoise populations.

Hematologic and biochemical analyses have been described to a limited extent for captive desert tortoises and free-ranging tortoises in the western Mojave Desert, northeastern Mojave Desert, Sonoran Desert in Arizona, and Alachua and Lake Counties in Florida (Rosskopf, 1982; Jacobson et al., 1991; Christopher et al., 1997; Dickinson et al., 1995; Dickinson et al., 2002; Dickinson et al., 1996; Christopher et al., 1994; Taylor and Jacobson, 1982).

Data for the gopher tortoise have included red and white blood cell counts (Ryerson, 1943), erythrocyte size measurements (Hartman and Lessler, 1964), hematocrit, specific gravity, blood volume, plasma volume, and total body water (Thorson, 1968), percent protein (Friar, 1964), total protein, sialic acid (Seal,

1964), and calcium, sodium, magnesium, and cholesterol (Jackson et al., 1974). No previous study has presented values for a wide range of blood variables for the gopher tortoise or attempted to assess the possible effects of age, gender, site, and season.

In chelonian species, the blood collection sites used to collect samples have included the jugular vein, dorsal coccygeal vein, brachial vein, lateral coccygeal vein, postoccipital venous plexus, femoral venous plexus, orbital sinus, heart, and nail clipping (Samour et al., 1984; Marks and Citino, 1990; Gottdenker and Jacobson, 1995; Murray, 2000; Divers, 2002). The jugular vein has been reported as the preferred site for venipuncture in some tortoise species (Gottdenker and Jacobson, 1995; Jenkins, 1996; Lloyd and Morris, 1999), but according to other authors the dorsal coccygeal vein, postoccipital venous plexus, and the brachial vein are preferred (Samour et al., 1984; Marks and Citino, 1990; Jackson, 1991; Murray, 2000; Divers, 2002). Overall, it is easier to collect a sample from the brachial vein, postoccipital venous plexus, and dorsal coccygeal vein than from the carotid artery or the jugular vein.

In desert tortoises (*Gopherus agassizii*), venipuncture site can have an affect on the hematologic and biochemistry results. Lymph contamination of the blood samples collected from the postoccipital venous plexus, in contrast to samples obtained from the jugular vein, was found to be a casual factor (Gottdenker and Jacobson, 1995). Blood sampling in a vessel other than the jugular vein or the carotid artery is always blind and may result in lymph contamination (Jacobson et al., 1992; Lloyd and Morris, 1999). Lymph

composition is different from blood and serum, so contamination of blood samples with lymph may affect the hematologic and biochemical results. Volume percentages of cellular components are generally lower in lymph than in blood. Enzyme activities and total protein are lower in lymph than in serum, whereas chloride levels are higher (Jacobson et al., 1992; Gottdenker and Jacobson, 1995). Standardization of blood collection techniques will improve the accuracy of blood data measurements and comparison (Gottdenker and Jacobson, 1995).

Descriptions of the morphologic characteristics of blood cells in chelonians are limited. As from mammals, circulating blood cells in reptiles can be broadly grouped as red blood cells (RBC), granulocytes, and mononuclear cell. Red blood cells are morphologically similar among various species of reptiles. They are nucleated and oval in shape, and represent the majority of the circulating blood cells. Thrombocytes, which are next in abundance, are also nucleated, but morphologic variability does exist in these cells between reptiles. In 1970, Saint Girons identified 9 different types of cells in reptiles using light microscopy, including erythrocytes, eosinophils, basophils, azurophils, heterophils, lymphocytes, monocytes, thrombocytes, and plasma cells. In blood smears, thrombocytes may be confused with lymphocytes because they appear morphologically similar. Sypek and Borysenko (1988) reported erythrocytes, eosinophils, heterophils, basophils, monocytes, lymphocytes, and thrombocytes, which were identified on the basis of light microscopy, and cytochemical and ultrastructural morphologic characteristics.

Morphologic and cytochemical characteristics of blood cells have been described in the desert tortoise (*Gopherus agassizii*) (Allman et al., 1992). Allman et al. (1992) reported that the morphologic features of the white blood cells (WBC) in the desert tortoise were similar to those of other species of tortoises, with minor differences observed in the numbers and shape of the granules observed in heterophils and eosinophils. The remaining blood cells resembled those from other species of tortoise (Hawkey and Dennett, 1989). Currently, there are no reports on the morphologic or cytochemical characteristics of blood cells from gopher tortoise. Clarification of the morphologic characteristics and classification of blood cells, especially WBC, from gopher tortoises is important because this species information could be used to assist those attempting to interpret WBC counts for clinical and research specimens in the wild.

Hibernation has been associated with a number of hematologic and biochemical changes. A hibernation lymphopenia has been reported in several species of tortoises (Lawrence and Hawkey, 1986; Christopher et al., 1999). The lymphopenia was considered to be associated with suppressed immune function and antibody production, secondary to low ambient temperature, changes in photoperiod, and an elevation in plasma cortisol levels (Saad, 1988; Montali, 1988; Lance, 1994). Because lymphocytes synthesize immunoglobulins, globulin concentration is subsequently affected. Immunoglobulins and antibody responses are greatest in the spring and summer (Dessauer, 1974; Lance, 1994). In contrast, seasonal lymphocyte peaks were noted in years with above average rainfall (Christopher et al., 1999).

Although the exact function of basophils is unknown for reptiles, reduced numbers during hibernation may also be indicative of decreased immune function or an absence of antigenic response. In contrast, monocytes and azurophils increased during hibernation. Taylor and Jacobson (1982) observed the same increase in the monocyte count in the spring while working with the Florida gopher tortoises. Monocytes differentiate into macrophages and contribute to the inflammatory response. Although commonly observed in chelonian and squamata, the function of azurophils remains unknown (Alleman et al., 1992).

Christopher et al. (1999) noted that eosinophil numbers in desert tortoises did not change during hibernation, but did decrease in the fall. This finding in desert tortoises was different from that observed in captive *Testudo* sp. in the fall (Lawrence and Hawkey, 1986). Although an eosinophil response has been reported with parasitism and a Type I hypersensitiviry reaction (Rosskopf, 1982), it is not a consistent finding in reptiles (Montali, 1988).

Heterophil numbers did not differ significantly on the basis of sex or season during hibernation (Christopher et al., 1999). Because the heterophil is not affected by corticosterone (stress), cell numbers would not be expected to decrease during hibernation. The altered WBC observed in tortoises during hibernation suggests a shift in the way tortoises process antigen, or changes in WBC production, circulation or sequestration (Christopher et al., 1999).

Some of the parameters measured during biochemical analyses include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase (CK), total protein (TP), albumin, globulin,

creatinine, calcium, phosphorus, sodium, potassium, chloride, TCO2, anion gap, and uric acid. Urea and creatinine are not included because they are unreliable indicators of renal disease in reptiles. Unlike ureotelic mammals, some chelonian species are uricotelic and produce highly variable quantities of urea, depending upon their hydration status. Indeed, reptiles with endstage renal diseases and visceral gout often have normal urea and creatinine values. Calcium and phosphorus ratio is the most reliable indicator of renal disease. The activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) can all be raised in liver disease in mammals. In reptiles, only AST has been found to be sensitive and hence clinically useful.

Dickinson et al (2002) reported that desert tortoise biochemistries differed between sites and sexes and among season and year. However, seasonal and annual differences in hematology and biochemistry parameters were related to rainfall patterns, forage availability, and physiological conditions. Christopher et al. (1999) found that desert tortoises in the Mojave Desert had significant differences in packed cell volume, hemoglobin concentration, AST, cholesterol, triglyceride, calcium, and phosphorus concentrations between genders. Marked seasonal variation was observed for most of the parameters in conjunction with the reproductive cycle or seasonal rainfall. Site differences were minimal, and largely reflected geographic differences in precipitation patterns. In contrast, Taylor and Jacobson (1982) found that the total leukocyte count, monocyte count, sodium, and chloride for gopher tortoises from Florida exhibited significant difference between fall and spring determinations. Further, total protein and cholesterol

levels were significantly greater in females than in males. By developing a better understanding for what are "normal" hematologic and biochemical values, researchers and veterinarians will be able to better characterize the health status of the gopher tortoises under their supervision.

This research was the first, to the author's knowledge, to collect baseline hematologic and biochemistry data for free-ranging Louisiana gopher tortoises. The reference intervals reported in this study are important for establishing a database for comparative studies with gopher tortoises in other locations and under different management conditions. In addition, further investigation into gopher tortoises WBC and immune function is essential for better understanding seasonal variations and their potential impact on response to disease.

2.3.2 Infectious Diseases

Considering the great diversity of reptile species and environments in the world, a correspondingly large number of microbial pathogens can be expected to produce disease. Many infectious diseases in reptiles have been described and many others undoubtedly await characterization. In each order of reptiles, examples of viral, bacterial, and fungal microbial pathogens have been reported in the scientific literature. Several microbial pathogens unique to specific reptiles and others that produce disease in a wide array of animals, including man, have been studied at the comparative, evolutionary, and epidemiologic levels. The study of reptile infectious diseases and the diseases they produce has afforded rich opportunities and unique insight in primary host-pathogen interaction.

Infectious diseases are a significant factor in chelonian conservation programs, especially for release and translocation projects (Jacobson et al., 1995). Herpesvirus, Iridovirus, and *Mycoplasma agassizzii* are known to cause respiratory disease and widespread morbidity in captive and free-ranging chelonians, particularly gopher tortoises (Jacobson, 1994; Pettan-Brewer et al., 1996; Cooper et al., 1988; Drury et al., 1999; Drury et al., 1998; Heldstab and Bestetti, 1989; Jacobson et al., 1985; Muro et al., 1998; Johnson et al., 2003; Heldstab and Bestetti, 1982; Marschang et al., 1999; Mao et al., 1997; Westhouse et al., 1996; Johnston and Jacobson, 2004; De Voe et al., 2004; Johnston et al., 2004; Brown et al., 2001; Willette et al., 2001; Brown et al., 1999a and 1999b; Brown et al., 1994; Jacobson et al., 1991; Schumacher et al., 1997; Schumacher et al.,1999; McLaughlin et al., 2000; Christopher et al., 2003; Schumacher et al., 1993; Lederle et al., 1997; Homer et al., 1998).

Herpesviruses are considered one of the most important viruses to infect tortoises. Herpesviral infections in chelonians have been associated with several diseases in different species including Greek (*Testudo graeca*) and Hermann's (*Testudo hermanni*) tortoises in Europe, Argentine tortoises (*Geochelone chilensis*) imported to the United States from South America, desert tortoises (*Gopherus agassizzii*), and several species of tortoises imported into Japan for the pet trade (Jacobson, 1994; Pettan-Brewer et al., 1996; Cooper and Bone, 1988; Drury et al., 1999; Drury et al., 1998; Heldstab and Bestetti, 1989; Jacobson et al., 1985; Muro et al., 1998; Johnson et al., 2003). Herpesvirus infections also have been reported in captive desert tortoises (*Gopherus agassizii*) (Harper et al., 1982; Martinez-

Silvestre et al., 1999; Origgi et al., 2001). A variety of clinical signs, including stomatitis, rhinitis, conjunctivitis, pneumonia and neurologic disorders have been observed in tortoises (Heldstab and Bestetti, 1989). Glossitis can be severe, and diphteritic plaques on the tongue and hard palate of the tortoises in the advanced stages of the disease are a common finding. The disease appears to target the upper respiratory tract of the tortoise, but involvement of the lower respiratory tract has also been reported. The transmission route of herpesvirus is totally unknown, however, aerosalization and direct contact with contaminated discharged are considered most likely.

The diagnosis of herpesvirus infection in tortoises can be carried out using serologic tests or tests that detect the virus, viral antigen or viral nucleic acid. Viral neutralization and ELISA assays have been used to detect the presence of antibodies to herpesviruses in tortoises (Marschang et al., 2001; Johnston et al., 2003; Origgi and Jacobson, 1999). Of the serologic tests, serum-neutralization is considered the gold standard for the detection of exposure to herpesvirus in tortoises. This test can detect the presence of serum-neutralizing antibodies, and requires approximately 11-14 days to confirm the presence of the antibodies (Frost and Schmidt, 1997). However, experience has shown that the detection of these antibodies depends not only on the time post infection but also on the species involved (Marschang et al., 2003). Some tortoise species appear to develop antibodies at a greater rate than others (Origgi, et al., 2000; Marschang et al., 2001). Established latent infections in infected animals can be a challenge for the diagnosis of herpesvirus. Another factor affecting the diagnosis of a herpesviral

infection in the tortoise is the existence of at least two different herpesvirus serotypes (tHV1= tortoise herpesvirus serotype 1 and tHV2= tortoise herpesvirus serotype 2) and genotypes (Marschang et al., 2001). Further, many of the tests that have been described for the diagnosis of herpesvirus infection are sero or genotype specific and can lead to false negatives (Marschang et al., 2003).

No specific therapy is currently available. An in vitro study with acyclovir and gancyclovir has shown the ability of these chemotherapeutics in reducing viral replication (Marschang et al., 1997). Origgi (2004) found that acyclovir suppressed the virus in a group of infected tortoises, for at least three months.

Iridoviridae are double stranded DNA viruses capable of infecting invertebrates and poikilothermic vertebrates (Murphy and Kingsbury, 1990). Five genera are recognized: *Iridovirus*, *Chloriridovirus*, *Lymphocystivirus*, *Ranavirus*, and "goldfish virus-1-like viruses" (Essbauer and Ahne, 2001). Of these, members of the genus *Ranavirus*, originally identified in frogs (frog virus-3 (FV3)), are emerging as important infectious agent in other amphibians, fish, and reptiles.

Several accounts of Iridoviral infections have been documented in chelonians (Heldstab and Bestetti, 1982; Marschang et al., 1999). In the United States, only five cases have been reported; a Russian tortoise (*Testudo horsfieldii*), a box turtle (*Terrapene carolina carolina*), a free-ranging gopher tortoise (*Gopherus polyphemus*) from Florida, a group of free-ranging eastern box turtle (*Terrapene carolina carolina*) from North Carolina, and three captive Burmese star tortoises (*Geochelone platynota*) in a collection at St. Catherine's

Island Wildlife Survival Center, Georgia (Mao et al., 1997; Westhouse et al., 1996; Johnston and Jacobson, 2004; De Voe et al., 2004; Johnston et al., 2004). Iridoviral infections have also been described in green tree pythons (*Morelia viridis*) illegally imported into Australia from New Guinea (Hyatt et al., 2002) and African chameleons (*Chamaeleo* sp.) (Telford and Jacobson, 1993).

Clinical signs are similar to those described with herpesvirus infections, and include conjunctivitis, rhinitis, stomatitis, and edema throughout the cervical region. The distribution of the postmortem lesions in a free-ranging gopher tortoise were limited to the respiratory tract and pharyngo-esophageal region. Inhalation or ingestion of the virus may have served as the portal of infection (Westhouse et al., 1996). In contrast, De Voe et al. (2004) reported clinical signs in a group of eastern box turtles as having cutaneous abscessation, oral ulceration or abscessation, respiratory distress, anorexia, and lethargy. The predominant postmortem lesion was a fibrinoid vasculitis of the skin, mucous membranes, lungs, and liver. This was the first report of integumentary lesions associated with an iridoviral infection in chelonians.

Diagnosis of iridovirus infection is supported by the isolation of the virions, the identification of basophilic intracytoplasmic viral particles from affected tissues, and systemic lesions consistent with the infection (Westhouse et al., 1996; Johnson and Jacobson, 2004; De Voe et al., 2004). No specific therapy is currently available.

Because only one gopher tortoise has been found to be infected with this

virus, the impact of this disease on populations of free-ranging gopher tortoises does not appear to be significant at this time.

Mycoplasma sp. is responsible for causing respiratory disease in a number of different vertebrate hosts, including humans, swine, cattle, laboratory rodents, poultry, and reptiles. In most animals, respiratory mycoplasmosis is a slowly progressing, chronic, and clinically silent infection that can be exacerbated by environmental factors, stress, or other microbial agents (Cassell et al., 1985; Schoeb et al., 1985; Simecka et al., 1992; Weinach et al., 1985). It is thought that most hosts have difficulty in eliminating the *Mycoplasma* sp., even in the presence of a strong immune response, and as a general rule, the animal is presumed to be infected for life. In fact, the host immune response is critical for the development of lesions (Simecka et al., 1992). Respiratory mycoplasmosis in most species is characterized by increased numbers of inflammatory cells, particularly in foci, and lymphoid hyperplasia (Brown et al., 1994; Brown et al., 1999b; McLaughlin et al., 2000; Simecka et al., 1992).

The pathogenic mechanisms of *Mycoplasma* sp. are dependent upon the attachment to the host surface provoking an immune response, which may be either detrimental or beneficial to the host. Once attached to the host, *Mycoplasma* sp. can cause cell injury by a variety of methods, including ciliostasis, hydrogen peroxide production, and toxin production (Razin et al., 1998). *Mycoplasma* sp. may be synergistic with other infectious agents (Cassell et al., 1985; Schoeb et al., 1985). It is presumed that the *Mycoplasma* sp. is the initial colonizer of the

respiratory tract, and that changes caused by the infection predispose the host to subsequent secondary infections.

Mycoplasma agassizii is the etiologic agent of upper respiratory tract disease (URTD) in tortoises. This disease has been observed in number of wild and captive tortoise species, including the desert tortoise (*Gopherus agassizii*), gopher tortoise (*Gopherus polyphemus*), Texas tortoise (*Gopherus berlandieri*), Chaco tortoise (*Geochelone chilensis*), leopard tortoise (*Geochelone pardalis*), Indian star tortoise (*Geochelone elegans*), forsten tortoise (*Indotestudo forstenii*), radiated tortoise (*Geochelone radiata*), flat-tailed tortoise (*Pyxis planicauda*), spider tortoise (*Pyxis arachnoides*), African spurred tortoise (*Geochelone sulcata*), Florida box turtle (*Terrapene Carolina bauri*), Greek tortoise (*Testudo graeca graeca*), and spur-thighed tortoise (*Testudo graeca ibera*) (Brown et al., 2001; Willette et al., 2001; Brown et al., 1999a and 1999b; Brown et al., 1994; Jacobson et al., 1991; Schumacher et al., 1997; Schumacher et al.,1999; McLaughlin et al., 2000; Christopher et al., 2003; Schumacher et al., 1993; Lederle et al., 1997; Homer et al., 1998).

Clinical signs of URTD can overlap with other infectious agents, including *Mycoplasma cheloniae*, *Mycoplama testudinis*, herpesvirus, iridovirus, *Pasteurella testudinis* sp. nov., *Serratia* sp., *Pseudomonas* sp., and other noninfectious agents such as hypovitaminosis A (Origgi and Jacobson, 1999; Westhouse et al., 1996; Brown et al., 1994). The disease is manifested clinically as rhinitis, and is accompanied by various levels of mucopurulent discharge from the nares, and conjunctiviis with varying levels of ocular discharge and palpebral edema.

Additional symptoms may include the recession of the eye into the orbit, and dullness to the skin and scutes (Jacobson et al., 1991; Brown et al., 1994). Further, clinical signs may be present intermittently, thus an infected tortoise may appear clinically ill or healthy at any given time. Microscopic lesions may include the infiltration of inflammatory cells into the nasal cavity mucosa and submucosa and hyperplasia and degeneration of upper respiratory tract epithelium (Jacobson et al., 1991; Brown et al., 1994; Jacobson et al., 1985).

The route of infection is most likely through direct contact with infected tortoises or aersolization. *Mycoplasma* sp. lack a cell wall and are susceptible to desiccation; therefore, they do not persist in the environment for any appreciable length of time. Current evidence supports a theory that contaminated fomites such as field equipment might contribute to the spread of the disease, especially when proper disinfection techniques are not used. McLaughlin (1997) reported that seronegative gopher tortoises did not seroconvert or develop URTD after being housed in outdoor enclosures that once housed seropositive tortoises. This suggested that environment transmission does not occur routinely in gopher tortoises (McLaughlin, 1997). Vertical transmission was described in a very limited number of infected gopher tortoises; however, no egg transmission studies were performed (McLaughlin, 1997). Further, Schumacher et al. (1999) described *Mycoplasma agassizii* specific maternal antibodies in desert tortoise hatchlings. It was found that desert tortoise females transfer specific IgG and IgM antibodies to their offspring that are still detectable after 1 year.

Brown et al. (1999b) reported that experimentally infected gopher tortoises (0.25 ml/nostril; 10^8 color changing units (CCU) of *M. agassizii* 723) showed clinical signs of URTD compatible with those observed in tortoises with natural infections. All experimentally infected tortoises (*n* = 9) seroconverted, and levels of antibody were statistically higher in infected animals than in control animals (*P*< 0.0001). Control tortoises (*n* = 10) did not show clinical signs, did not seroconvert, and did not have detectable *M. agassizii* by either culture or PCR. Further, *M. agassizii* 723 did not influence the clinical expression of URTD or the antibody response, suggesting that the strain chosen for the study was highly virulent. On the basis of the results of the transmission, the authors concluded that *M. agassizii* is an etiologic agent of URTD in gopher tortoise.

Diagnosis of *Mycoplasma* sp. infection can be carried out using: 1) direct mycoplasmal culture; 2) detection of mycoplasmal chromosomal DNA by polymerase chain reaction (PCR); or 3) detection of anti-Mycoplasma antibodies in tortoise plasma by enzyme-linked immunosorbent assay (ELISA). Each test provides different information that collectively can be used to define a tortoise's mycoplasmal status. *Mycoplasma* sp. isolates can be identified on the basis of their 16SrRNA gene restriction fragment length polymorphism patterns. Currently, five different patterns of *Mycoplasma* sp. have been described in tortoises. Three of them are recognized as distinct species including *Mycoplama testudinis* originally isolated from the cloaca, *Mycoplasma agassizii*, and *Mycoplasma cheloniae* (Hill, 1995; Brown et al., 1995; Brown et al., 2001; McLaughlin, 1997). Thus, the chance of serologic cross reactivity is more than likely (Brown et al.,

1995; Brown et al., 2001). Individual strains of a given species may vary with respect to their virulence potential, and some species may be commensals rather than pathogens.

The principle of the ELISA is to detect the presence of anti-*Mycoplasma agassizii* antibodies in tortoise plasma (Schumacher, 1993). The results are analyzed quantitatively but interpreted categorically (negative, suspect, positive). Under the current system, a negative titer is < 32, a suspect titer is 32, and a positive titer is ≥ 64. The monoclonal antibody used in the ELISA (HL673), is from a mouse antibody against the light chain of both IgM and IgY (the equivalent of IgG in mammalian species) tortoise immunoglobulins. The advantages of the ELISA include its high assay sensitivity and its ability to detect the stage of infection by using different antibodies (IgM-acute vs IgY-Chronic) (Jacobson and Origgi, 2002). A positive test proves past exposure, but not current infection. The major disadvantage associated with this assay is the potential for false positive results caused by cross-reaction of tortoise antibodies to other bacteria with similar antigens. At the same time, there is the possibility for false negative results due to a lack of cross-reactivity with other *Mycoplasma* sp. that cause URTD (Schumacher, 1993; Brown et al., 1995; Brown et al., 2001). Specificity of the ELISA depends on the affinity and availability of antibodies produced by the tortoise in response to *M. agassizii* antigens (Brown et al., 1995).

Since PCR has a high specific affinity for DNA primers, synthesized DNA can be further analyzed to confirm species-specificity (Brown et al., 1995).

The advantages by PCR include direct proof of infection at the time the sample was taken, no inhibition by sample contamination with other microbes, and accurate identification. Disadvantages include specialized laboratory equipment, the high cost of special reagents, the potential for cross-contamination leading to false positive results, and a portion of the sample is consumed in the reaction (Brown et al., 1995).

These infectious diseases are an ever present risk to wildlife, particularly during situations in which animals are removed from their natural habitat for captive breeding programs or during conditions of stress such as release into new habitats, translocation, and encroachment on their habitats by urbanization. Research on the impact of mycoplasmosis on wildlife has been limited, but reports of recent disease outbreaks in different wildlife species are provoking interest in mycoplasmosis as a newly emerging disease threat.

URTD is an excellent example of the importance of wildlife diseases in population biology. Coupled with anthropogenic changes, this disease is believed to be a major factor in declines of gopher tortoise population in the eastern and western portions of their range. Increasing awareness of the role of infection diseases in Louisiana population will result in inclusion of disease monitoring and health assessment in management decisions.

2.3.3 Toxicology

The degradation of the environment due to environmental pollutants has been occurring for centuries. Over the past four decades, various wildlife species have been used as sentinels to monitor the health of their respective

environments. Examples include the accumulation of heavy metals in large game fish, elevated radioactive isotope levels in tissues of Lapland reindeer, and organochlorine poisoning in fish-eating birds that resulted in eggshell thinning and reduced survival in their young (Peakall, 1970). We are just now beginning to understand how the numbers and diversity of wildlife and plant communities reflect the health of their environment. Perhaps even more importantly, that diverse and healthy plant and animals communities may determine the long-term health of their environment.

Assessing ecosystem health is a daunting task, requiring the selection of indicator species that are representative of the system (Burger and Gochfeld, 1997). In general, concentrations of elements should be higher in organisms that are higher on the food chain, particularly top carnivores. Although there has been considerable attention devoted to determining the levels of contaminants in birds, mammals, and even fish (Eisler, 1987; Burger, 1993), there has been little attention devoted to reptiles (Hall, 1980; Delaney et al., 1988; Yanochko et al., 1997). This is remarkable given that some chelonians, snakes, and crocodilians are preferentially carnivores, and much longer lived than their avian and mammalian counterpart.

There is limited data regarding heavy metal and trace element levels in reptiles. Mercury bears special comment because it is considered the most serious environmental threat to the well-being of fish and wildlife in the southeastern United States (Facemire et al., 1995). Due to mercury contamination, the entire Everglades watershed in southern Florida has been

closed to the hunting of alligators (*Alligator mississippiensis*), and health advisories have been issued to curtail consumption of largemouth bass (*Micropterus salmoides*) (Fleming et al., 1995). Chronic exposure to mercury, from raccoons and alligators, is probably responsible for lower than expected population densities of Florida panthers in large portions of their range (Roelke et al., 1991).

Birds can eliminate heavy metals both in their eggs and in their egg shells (Burger et al., 1994). Many researchers have examined the same hypothesis for reptiles. Concentrations of mercury in snakes (*Agkistrodon piscivorus*) ranged as high as 1600 ppb (Facemire et al., 1995). Bonin et al. (1995) reported only mercury levels for snapping turtle (*Chelydra serpentina*) egg contents from the St. Lawrence River in Canada; levels for six pooled samples ranged from 50 to 180 ppb. These mercury levels were similar to the egg contents of red-eared slider turtles (*Trachemys scripta*) from the Savannah River site, South Carolina (Burger and Gibbons, 1998). However, Burger and Gibbons (1998) also noted that concentrations were higher in egg contents than in shell for lead and selenium, while chromium was higher in the shell.

Witowski and Frazier (1992) examined metal levels in bone from sea turtles (*Lepidochelys olivacea*) and indicated the need for further work. Metal levels in sea turtles (*Caretta caretta*) eggs varied by nesting beaches, providing evidence that these sea turtle populations are composed of demes (Stoneburner, 1980). Heinz et al. (1991) evaluated metal levels in wild alligator eggs, and found that levels were quite low for most metals. Burger (1992) examined metal levels in the tissue and skin of pine snake (*Pituophis melanoleucus*) hatchlings from New

Jersey, and found higher levels of lead, mercury, and chromium in the skin compared to the whole body tissue, suggesting that they rid their bodies of these metals through the skin. In addition, shed skins of cobras and scales of wall lizards from heavily polluted areas in India are shown to contain significantly higher levels of lead than those of the same animals from rural areas (Kaur, 1988). The effects of the elevated levels of lead on the health of snakes and wall lizards are unknown, but studies on mice and rats suggest that high levels of lead may be detrimental to the health and longevity of animals (Kaur, 1988).

In Storelli and Marcotrigiano's (2003) study on marine turtles, high levels of mercury and cadmium were found in both the liver and kidney. The majority of the lead burden existed in the bones and carapace, while arsenic was present mainly in muscle tissue. Metals in the eggs were mainly present in the yolk. Significantly higher concentrations of mercury, copper, zinc, and iron were found in the yolk than albumen, while the shell contained the highest levels of manganese and copper.

In comparison with cetaceans and sea birds (Honda et al., 1990; Thompson, 1990), marine turtles showed higher hepatic copper and iron concentrations, while the brain and salt gland showed relatively high copper levels (Caurant et al., 1999; Sakai et al., 2000). The fat tissue, bone, and carapace contained considerable levels of zinc (Caurant et al., 1999; Sakai et al., 2000). High zinc levels in fat tissue might be associated with pigment proteins which seem to play a role in zinc storage in the body (Sakai et al., 2000).

Burger (2002) reported the levels of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in the eggs, liver, and muscle of diamondback terrapins (*Malaclemys terrapin*). Levels of most metals were higher in the liver than in muscle, except for chromium and arsenic, where levels were similar. Selenium, chromium, and manganese were sequestered in the eggs of diamondback terrapin at higher levels than were other heavy metals. Overall, the levels of metals were not sufficient to cause problems for the terrapin or for consumers eating their muscle, although the levels of mercury in liver (21 – 53 ppb) may be sufficiently high to cause some sublethal effects.

Arsenic is widely distributed in the biosphere. The toxicity of arsenic depends on its oxidation states and molecular form. Saeki et al. (2000), Kubota et al. (2002), and Storelli and Marcotrigiao (2000) reported that the inorganic arsenic concentrations in marine turtles livers were present about three times higher than in the muscle tissue. It may be reasonable to suppose that because inorganic arsenic carries out its toxic action at the level of the sulfhydrylic groups of some enzymes, many metabolic processes of vital importance, which occur in liver, might be altered.

Post-mortem examinations of several desert tortoises have revealed suspected elevated concentrations of heavy metals, including cadmium, mercury, lead, molybdenum, arsenic, selenium, chromium, and nickel in the livers and kidneys of ill tortoises (Homer et al., 1996). Free-ranging tortoises that showed evidence of liver and kidney degeneration have contained elevated mercury,

cadmium, or lead concentrations (Homer et al., 1996). Elevated concentrations of these metals may contribute to tortoise morbidity and mortality.

Owing to the lack of studies on the toxic effects of these environmental pollutants on free-ranging chelonian species, conclusive data cannot be placed in a proper context. There is clearly a need to evaluate environmental pollutants and their physiologic effects in Louisiana gopher tortoises in an effort to determine if they have any negative impacts on these animals.

2.3.4 Parasitology

While there may be thousands of parasites identified in reptiles, the significance of most of these parasites to their reptilian host is unknown. Parasites normally encountered in reptiles range from single celled forms (coccidians, hemogragarines, malarial parasites, hemoflagellates, gut flagellates, and ciliates) to multicellular organisms (flukes, tapeworms, spiny headed worms, round worms, pentastomes, mite, and ticks). Their presence, however, is not necessarily associated with disease. Determining the significance of a parasite can be difficult. Susceptibility to disease caused by parasitism is related to stress, environmental temperature, hygiene, concurrent disease, the number of parasites, availability of intermediate host, nutritional status and age.

In the wild, where an animal is not confined within a small space, the environmental concentration of a parasite is not high. As a result, the parasite burden to any given reptile host is usually low. A parasite that is found in low numbers may not pose a health problem for the host, but if present in high numbers may be harmful. Anthropogenic impacts, particularly the release of

captive animals, have been hypothesized to play a role in the transmission dynamic of infectious disease in the free-ranging gopher tortoise population. Introduction of parasites from captive tortoise into naïve population may result in a disease outbreak.

Over a 1000 species of nematodes have been described in reptiles (Anderson, 1992). They may vary in size and are most commonly found in the intestinal tract. In some cases, they are also found in blood vessels and visceral organs. They are capable of causing severe pathological changes in the host. They may have a direct (pinworms and strongyloid nematodes) or indirect life cycle (ascarids, spirurids, and filaroids) (Anderson, 1992). The clinical signs associated with nematodiasis often are vague, but can include anorexia, anemia, regurgitation, bloat, signs of obstruction, and wasting disease.

Lichtenfels and Stewart (1981) described strongyloid nematodes from the small and large intestines of eight gopher tortoises from Georgia. The large intestines of all eight gopher tortoises were parasitized by Strongyloidea and Oxyuroidea, and the small intestine of two tortoises by Trichostrongyloidea. Two new species from the family Strongyloidea were described in the same study, *Chapiniella chitwoodae* and *Chapiniella gallatii*.

Oxyurids are common parasites of lizards, chelonians, and some snakes. Mature oxyurids are small and often pin shaped, hence the common terminology "pinworms." Telford (1981) suggested that in their natural environment the worms live as commensals, and may help prevent constipation by breaking up fecal material. Baker et al. (1998) evaluated eleven fecal samples from wild Sonoran

Desert tortoises. *Tachygonetria* sp (pinworm) ova were identified in 11 of the fecal samples. *Entamoeba* sp. were also found in these tortoises.

Many species of amoebae can be found in the gastrointestinal tract of reptiles. Some of these protozoa are commensals, while others are considered pathogens. Most cases of reptilian amebiasis are usually attributed to, *Entamoeba invadens*, although there are multiples species of reptilian *Entamoeba* sp. that can cause disease (Lane and Mader, 1996; Ratcliffe and Geiman, 1934). Although chelonians, crocodilians, and reptile eating snakes and lizards are considered to be resistant to the disease, it is also possible for them to develop clinical disease. Severe hepatic necrosis associated with *Entamoeba invadens* in a captive gopher tortoise has been described (Jacobson et al., 1983).

The hard ticks of the genera *Ixodes*, *Hyalomma*, *Haemaphysalis*, *Amblyomma*, and *Aponomma* are reported in a variety of reptiles, but many are host specific. Soft bodied ticks of the genera *Argasidae* and *Ornithodoros* may infest snake, lizards, and chelonians. Some species are host specific, while others are not. Ticks are important parasite because they can damage the host's skin and transmit certain bacteria, blood parasites and viruses. A survey of arthropods associated with gopher tortoise burrows in Mississippi revealed the presence of seven burrow commensals, including the large gopher tortoise tick *Amblyomma tuberculatum* (Lago, 1991).

Arthropods comprise a major component of the gopher tortoise burrow fauna, and this group has received considerable attention in the past. The burrow commensals are generally coprophagous, parasitic on the tortoises, or predators

on other burrow arthropods (Lago, 1991). As is true for any organism with a narrow habitat requirement as seen with the gopher tortoise, any major change in habitat availability could have devastating effects on the species involved.

Ornithodoros sp. ticks are the preeminent vector of the *Borrelia* sp. spirochaetes that cause relapsing fever (Harwood and James, 1979). *Ornithodoros* sp. ticks also are highly efficient reservoirs and the only proven biological vector of the African swine fever virus (ASFV) (Botija, 1963; Hess, 1981). *Ornithodoros turicata* is a potential vector of ASFV in northern central Florida (Hess, 1981). This species of tick is not host specific, but it is found in the burrows of gopher tortoises from Florida (Young and Goff, 1939). In 1989 and 1991, Adeyeye and Butler sampled gopher tortoise burrows for *O. turicata* using carbon dioxide baits and vacuum extraction. There were approximately 47 - 1,338 ticks found per burrow. The tick population consisted predominantly of nymphal stages, with a significant increase in the larval population during the months of June and July. Ticks exhibited low interburrow movement, but depth preferences fluctuated with season; more ticks occur in the apron of the burrow during the hot, humid months.

Further studies to investigate the role of parasites as a potential source for infectious diseases in gopher tortoise population are warranted. Our study is the first to attempt to characterize parasite burdens in the Louisiana population.

2.4 Conservation

Louisiana gopher tortoise survival faces many challenges, including habitat destruction, environmental modification, road mortality, illegal poaching, and

disease. The gopher tortoise serves an important role in its ecosystems and is known as a keystone species because it excavates burrows that are used as permanent homes or temporary shelters by over 360 species of invertebrates and vertebrates.

The range of the gopher tortoise once encompassed much of the longleaf pine (*Pinus palustris*) forests of the southeastern United States (Auffenberg and Franz, 1982). The original longleaf pine habitat has been reduced by over 85% due to urban development, agricultural development, and harvesting by paper industry. The remaining forests are small and highly fragmented (Noss, 1989). As a consequence, gopher tortoise population densities have declined by over 80% in the remaining habitat (Herman et al., 2002).

Because gopher tortoises do not reach reproductive maturity until 15-20 years of age, the effect of losing adult tortoises from Louisiana can have a significant effect on the survival of this population. Suitable habitat is at a premium with very few habitats remaining in Louisiana. It is interesting to note that Louisiana gopher tortoises lay smaller clutches (5-8 eggs) compared to their Floridian counterparts (15-20 eggs). Even if the eggs are layed, Alford (1980) has estimated that from the time of oviposition through the first year of life, 94% of the young are killed by predators. Nearly all the original longleaf pine (*Pinus palustris*) forests that once dominated much of the southeastern United States have been converted to other pine species; primarily loblolly pine (*Pinus taeda*), shortleaf pine (*Pinus echinata*), and slash pine (*Pinus elliottii*). Gopher tortoises and many other reptiles depended on this habitat for their survival. Different species of trees can

effect the soil pH. Soil acidity may have a negative effect on the general health of the gopher tortoises by increasing the likelihood of disease. It can be concluded that Louisiana gopher tortoises have a tenuous hold on the limited habitats that remain. The long term success of the Louisiana gopher tortoise is dependent upon developing a conservation program that can sustain this population in the face habitat loss and the introduction of infectious diseases. The purpose of this project was to collect baseline health data that can be used to assess the status of the population and design long term conservation programs.

CHAPTER THREE

CHARACTERIZING THE HEALTH STATUS OF THE LOUISIANA GOPHER TORTOISE

3.1 Introduction

Gopher tortoises (*Gopherus polyphemus*) are large, fossorial reptiles with a geographic range that extends from extreme southern South Carolina south along the Atlantic Coastal Plain through Florida and west along the Gulf Coastal Plain to extreme southeastern Louisiana (Ernst and Barbour, 1989; Ernst et al., 1994). The longleaf pine ecosystem that covered this range has been reduced as a result of anthropogenic influences, including habitat destruction, fragmentation, and degradation (Noss, 1989). These changes have led to an 80% reduction in gopher tortoise population densities range-wide (Auffenberg and Franz, 1982). High predation rates on both eggs and hatchlings have been well documented on gopher tortoise populations in the eastern and western portion of their range (Alford, 1980; Landers et al., 1980; Wilson, 1991; Butler and Hull, 1996; Smith, 1997; Epperson and Heise, 2003). Furthermore, the gopher tortoise has been considered a keystone species because over 360 species seek refuge in burrows that tortoises excavate (Eisenberg, 1983; Jackson and Milstrey, 1987). Thus, the loss of gopher tortoise would have far reaching effects to the survival of other species. For this reason, this species has been listed as protected in all states within its range (Ernst et al., 1994).

These unnatural influences on the tortoises can lead to changes in their behavior, and may lead to increased exposure to infectious diseases, toxins, and

parasites. While the effects of these unnatural influences have been studied extensively in the eastern populations of the tortoises (Auffenberg and Franz, 1982; Landers et al., 1982; Diemer et al., 1989; Smith, 1995), there has been no investigation in the Louisiana population. The general lack of information regarding this species, and its importance to the ecosystem, suggests that there is a need to develop a research program to characterize the health of the Louisiana gopher tortoise.

The purpose of this study was to gather, analyze, and critically interpret accurate baseline laboratory data from eight sites of free-ranging gopher tortoise from Louisiana over a one year period. Our specific objectives were to: (1) collect blood samples to perform complete blood counts and plasma biochemistry analyses and assess the general physiologic status of the tortoises (2) collect blood samples to perform screens for potential environmental toxins, (3) determine the seroprevalence of *Mycoplasma agassizii*, and (4) determine the prevalence of endoparasite shedding in the Louisiana gopher tortoise.

3.2 Materials and Methods

3.2.1 Study Area

The study was conducted from August 2002 through June 2003 in southeastern Louisiana. Gopher tortoises were collected during two distinct trapping seasons: fall (August-October 2002) and spring (April-June 2003). During the fall, gopher tortoises were collected from two wildlife management areas in Louisiana, Ben's Creek Wildlife Management Area, Washington Parish and Sandy Hollow Wildlife Management Area, Tangipahoa Parish. During the

spring, gopher tortoises were collected from seven different locations, including Ben's Creek Wildlife Management Area (Washington Parish), Sheridan (Washington Parish), Lee Memorial Forest (Washington Parish), Varnados (Washington Parish), Sun (Washington Parish), Florida Gas Pipeline (Washington Parish), and Folsom (St. Tammany Parish).

3.2.2 Data Collection

Tortoises were captured by hand or using a Havahart[a] one door live trap placed in front of a burrow entrance (Figures 2). To prevent any chance of mortality, all traps were sheltered from direct sun and were checked at least twice daily. Tortoises were also collected when found moving across roads or while foraging near active burrows. Hand held global positioning system (GPS) receivers were used to determine the location of each tortoise (burrow). Data sheets were used to record basic ecological data (Appendix A). Captured tortoises were given a unique identifying mark by notching the posterior marginal scutes with a small triangular file (Cagle, 1939) (Figures 3a, 3b). Once the tortoise was assigned a number, a physical exam was performed. The carapace and plastron length and width were recorded for each tortoise using a standard metric measuring tape. Body temperature and environmental temperature were determined immediately after capture using a digital thermometer. A portable metric scale[b] was used to determine body weight for each tortoise.

The gender of each tortoise was determined using secondary sex characteristics, including the size of the mental glands, size and curvature

[a] Tomahawk Live Trap Co., WI 54487
[b] Acculab[R] SV-30, Acculab Service, Edgewood, NY 11717

Figure 2. An example of a Havahart trap set in front of a gopher tortoise burrow.

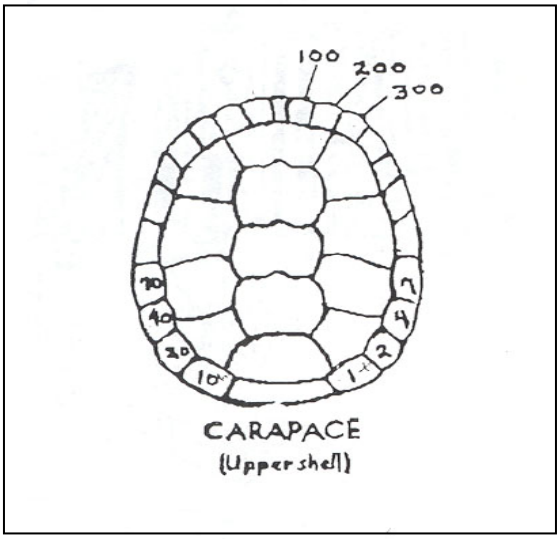

Figure 3a. Numbering system use to identify gopher tortoises (Cagle, 1939).

Figure 3b. Posterior marginal scutes showing the file marks. This gopher tortoise was identified as GT No. 12.

of the gular horn, and the presence or absence of a concavity on the posterior plastron. Male gopher tortoises have prominent mental glands on the mandible. As male tortoises age, gular horns become more pronounced and the posterior plastron becomes more concave. In contrast, female tortoise mental glands are much smaller and the posterior plastron is almost flat.

The tortoises were manually restrained for blood collection. Blood was collected from the subcarapacial vein using a 22-gauge needle fastened to a 6-ml syringe. Blood samples were stored in microtainers containing lithium heparin, ethylene diamine tetraacetic acid (EDTA), or no anticoagulant. A complete blood count, plasma biochemistry panel, *Mycoplasma agassizii* serology, and heavy metal and trace element analyses (arsenic, copper, mercury, zinc, lead, and selenium) were performed from the blood collected from each tortoise. Blood samples were stored on wet ice and transported to the Louisiana State University School of Veterinary Medicine for processing. A nasal flush was collected for

Mycoplasma agassizii testing by infusing 12ml of saline through the right nare and collecting the saline as it drained from the oral cavity. Fecal samples were collected using an appropriate fecal loop. The sample were placed into 10% buffered formalin, and submitted for parasitological examination. The tortoises were released at the site of capture.

Upon returning to the LSU-SVM, blood smears were made after mixing five drops of blood with one drop of 22% bovine albumin[c]. After being air-dried, the blood smears were stained using a modified Wright's stain[d]. Packed cell volumes were measured using micro-centrifugation. Plasma samples were processed using an Olympus AU 600 chemistry machine[e] within 24 hours of collection. The following values were measured: glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase (CK), total protein (TP), albumin, globulin, creatinine, calcium, phosphorus, sodium, potassium, chloride, TCO2, anion gap, and uric acid. The Louisiana Veterinary Medical Diagnostic Laboratory processed the plasma samples for the heavy metal and trace element analyses using the furnace atomic absorption (mercury and lead) and flame atomic absorption spectrophotometry (copper and zinc).

Serum samples and nasal flushes for *Mycoplasma agassizii* testing were transported on frozen gel packs to the *Mycoplasma* Research Laboratory at the University of Florida, Gainesville, Florida. The serologic assay was performed

[c] Gamma Biological Inc., Houston, TX 77092
[d] Hema Tek Stain Pak, Bayer Corporation, Elkhart, Indiana, USA
[e] Olympus, Melville, NY, USA

using an ELISA to assess exposure, while a PCR based assay was used to determine if any of the tortoises were *Mycoplasma* positive on the nasal flush.

Fecal samples were processed using a formalin/ethyl acetate sedimentation technique. Three milliliters of the formalized fecal sample was combined with 10-ml of sterile saline and centrifuged at 2,000 rpm for 2 minutes. The supernatant was decanted and the pellet re-suspended in 9-ml of 10% formalin and 3-ml ethylene acetate. The sample was shaken vigorously for 15 seconds, and then centrifuged at 2,000 rpm for 2 minutes. Again, the supernatant was decanted. The fecal pellet was re-suspended in 10% formalin and several drops of the sample pipetted onto a microscope slide and covered with a glass cover slip. The samples were evaluated for parasites using a light microscope. Five replicates were performed on each fecal sample.

3.3 Statistical Analyses

The distribution of each physical measurement, hematologic and plasma chemistry parameters, and heavy metal and trace element was determined. The Shapiro-Wilk test was used to evaluate the distribution of the data. Data that was not normally distributed was Winzorized. However, no more than 10% of the data was removed to normalize the data. The mean, standard deviation, median, 25% and 75% quartiles, and minimum/maximum were determined for each parameter. For normally distributed measures, a 95% confidence interval was calculated. In cases were the prevalence estimate was 0, the 95% confidence interval were calculated with the technique described by van Belle and Millard (1998). Levene's test for equality of variances was used to determine if the data were

homogeneous. Comparisons were made between sexes, season, and trapping locations. A one-way ANOVA was used to assess between group differences for normally distributed data. Specific between grouped differences were evaluated using a Tukey's test. For data that were not normally distributed, a Kruskal-Wallis one-way ANOVA and Dunn's test were used to assess differences between and within groups, respectively. After completion of the crude analysis, a univariate general linear model was used to identify interactions between sex, season, and trapping location. Values of $P<0.05$ were considered to reflect statistical differences.

Mantel-Haenszel chi-square tests and odds ratios (OR) were used to evaluate gender, season, and site for the *Mycoplasma* and parasite outcomes. Adjusted OR were used to evaluate gender and season while controlling for site for each outcome. Adjusted OR were also calculated for site while controlling for season. The Breslow-Day test (B-D) for homogeneity was used to evaluate homogeneity of OR's across strata. A B-D statistic with a $p < 0.05$ was considered to be statistically significant and the strata were considered heterogeneous. A summary odds ratio (Cochran-Mantel-Haenszel) was calculated for each (adjusted) comparison with homogeneous strata. Adjusted and crude odds ratios were compared to assess confounding. A10% change in OR was selected as the criteria for detecting confounding. Variables with $p < 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS 8.0[f].

[f] SPSS Inc., Chicago, IL

3.4 Results

3.4.1 Trapping season

The trapping seasons were comprised of 68 trapping days and were conducted from 08/12/02-10/9/02 (fall) and 04/16/03-06/12/03 (spring). Fifty-nine tortoises were captured during the study. Fifty-seven (97%) of the tortoises were adult animals and two (3%) were juveniles. Twenty (34%) of the tortoises were captured in the fall trapping season and 39 (66%) were captured in the spring. There were thirty male (53%) and twenty-seven (47%) female tortoises captured between the two trapping periods. The gender of the hatchling tortoises could not be determined. Tortoises were collected from eight different trapping locations, including 24 (41%) from Florida Gas Pipeline, 16 (27%) from Ben's Creek WMA, 8 (14%) from Sandy Hollow, 4 (7%) from Varnados, 3 (5%) from the Lee Memorial Forest, three (5%) from Sheridan, one (2%) from Folson, and one (2%) from Sun. The juvenile tortoise captured in the fall was from Sandy Hollow, and the spring juvenile capture was from Florida Gas Pipeline.

3.4.2 Physical parameters

Carapace and plastron measurements were collected from all 57 adult tortoises. The mean, standard deviation, and 95% confidence intervals for the carapace and plastron lengths and widths are reported in Appendix B. All of the measurements for the shell parameters were normally distributed (carapace length: Shapiro-Wilk statistic (W): 0.1, p=0.2; carapace width: W: 0.1, p=0.2; plastron length: W: 0.1, p=0.2; plastron width: W: 0.1, p=0.2). Female tortoises had a longer carapace length (females: 32.4 cm, males: 30.6; p=0.001), carapace

width (females: 33.2 cm, males: 31.2; p=0.003), plastron length (females: 25.7 cm, males: 24.3; p=0.006), and plastron width (females: 22.3 cm, males: 21.0; p=0.007) than males. There was no significant difference in the shell measurements between tortoises captured from the different locations (CL: p=0.7; CW: p=0.6; PL: p=0.4; PW: p=0.11) or between seasons (CL: p=0.7; CW: p=0.8; PL: p=0.5; PW: p=0.2).

The distribution of body weights for this Louisiana population followed a Gaussian distribution (K-S: 0.08, p=0.2) (Appendix B). Female tortoises were significantly (p=0.001) heavier (4.2 kg, 95% CI: 3.8-4.5) than male tortoises (3.3, 95% FD: 3.1-3.5). There was no significant difference in body weight between the eight study areas (F=8.0, p=0.32) or seasons (F=0.2, p=0.7).

3.4.3 Hematological testing

Packed cell volumes (PCV) and total solids (TS) were determined in 50 of the gopher tortoises. Seven samples were removed because of lymph dilution. Descriptive statistics for the PCV and TS are reported in Appendix C. The PCV and TS values were normally distributed (PCV: W- 0.06, p=0.2; TS: W- 0.06, p=0.2). There was no difference in the PCV or TS between sites (PCV: p=0.5; TS: p=0.4), gender (PCV: p=0.1; TP: p=0.7), or seasons (PCV: p=0.5; TP: p=0.1).

Complete blood counts were performed on 50 adult tortoises. Seven samples were removed from the analysis because of lymph dilution. The complete blood count (W: 0.8, p=0.01), heterophils (W: 0.8, p=0.00), lymphocytes (W: 0.00, p=0.00), monocytes(W: 0.8, p=0.00), eosinophils (W: 0.17, p=0.00), and basophils (W: 0.7, p=0.00) were not normally distributed. Tortoises captured in the fall were

more likely (p=0.045) to have a lower WBC (median: 4.0 x 10^3) than those captured in the spring (median: 7.1 x 10^3) (Appendix D). In addition, tortoises captured in the fall were more likely (p=0.002) to have a lower heterophil count (median: 1.5 x 10^3) than those captured in the spring (median: 2.3 x 10^3) (Appendix D). Because there was no difference in the other granulocytes between genders, the site of capture, or seasons, the date was pooled (Appendix E). Figures 4-6 represents morphologic features of the various circulating blood cells observed in the gopher tortoise.

Plasma biochemistry analyses were performed on 50 adult tortoises during the two trapping seasons. Calcium (p=0.001), phosphorus (p=0.02), and potassium (p=0.03) differed between genders (Appendix F). Calcium was significantly higher in females (median: 14.7, 25 - 75%: 12.0 - 17.7) than males (median: 10.7, 25 - 75%: 9.8 - 11.4). Phosphorus was also significantly higher in females (median: 3.9, 25 - 75%: 2.1 - 7.7) than males (median: 2.3, 25 - 75%: 1.8 - 3.3). However, potassium was higher in males (median: 5.6, 95% CI: 5.3 - 6.0) than females (median: 5.0, 95% CI: 4.6 - 5.2). Seasonal differences were noted for glucose (p=0.001), GGT (0.001), and uric acid (p=0.01). GGT was significantly higher in the fall than spring trapping seasons, whereas glucose and uric acid levels were higher in the spring trapping season (Appendix G). Differences in CK (p=0.005) was detected between the two primary field sites and are presented in Appendix H. Because there were no differences noted in AST, creatinine, ALP, total protein, albumin, globulin, sodium and chloride between gender, site of capture, or season, the data were pooled (Appendix I).

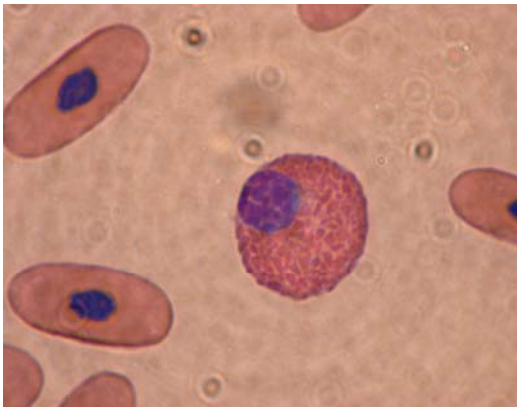

Figure 4. Heterophil and normal mature erythrocytes from a gopher tortoise.

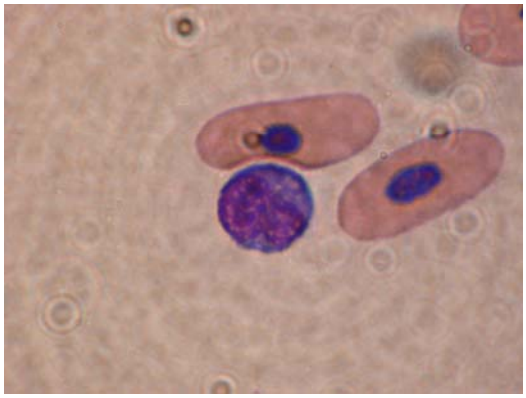

Figure 5. Normal lymphocyte from a gopher tortoise.

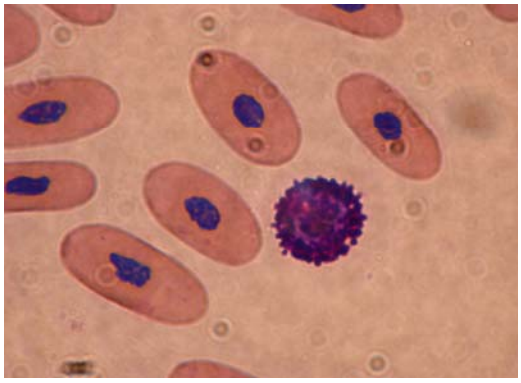

Figure 6. Basophil from a gopher tortoise.

3.4.4 Heavy metal testing

Descriptive statistics and the distribution analysis for heavy metals and trace element parameters were analyzed. Copper (p=0.001) and mercury (p=0.04) differed between genders. Copper was significantly higher in males (mean: 0.73, 95% CI: 0.68 - 0.79) than in females (mean: 0.52, 95% CI: 0.46 - 0.57). Mercury was also significant higher in males (median: 0.02, 25 - 75%: 0.017 - 0.035) than females (Appendix J). The highest mercury level was from GT#8 (0.82 ppm). In addition, tortoises captured in the fall were more likely (p=0.02) to have lower copper levels (mean: 0.56, 95% CI: 0.47 - 0.64) than those captured in the spring (mean: 0.66, 95% CI: 0.65 - 0.72) (Appendix K). Because there was no difference in zinc or lead levels between gender, site of capture, and season, the data were pooled (Appendix L). The highest lead level was from GT #4 (0.51 ppm).

3.4.5 Serologic testing

In the fall trapping season, none (0/19; 95% CI: 0-16) of the gopher tortoises produced an antibody titer (<1:32) to *Mycoplasma agassizii*. In the spring trapping season, thirty-nine tortoises were tested, and 24 (61%, 95% CI: 46-76) were negative, 7 (18%; 95% CI: 6-30) were suspect, and 8 (21%; 95% CI: 8-34) were positive. The highest titer was 1:128 (GT #29), and the remaining seven positive titers were 1:64. Overall, 26% (15/58, 95% CI: 15-37) of the tortoises were serologically suspect or positive for *Mycoplasma agassizii*. Differences in *Mycoplasma agassizii* was detected between Florida gas pipeline (50%, 11/22) and Ben's Creek (6.2%, 1/16) (OR: 15.0, 95% CI: 1.7 - 134.0). The seasonal

findings were confounded by site. Further, there was no significant differences between gender (p=0.8) during the study.

3.4.6 Parasitological testing

In the fall trapping season, fourteen (68%) fecal samples were collected from the 19 tortoises captured in this study. Eleven additional samples were collected from burrow entrances or traps. Parasites were identified in 8 (61%; 95% CI: 34-80) of the fecal samples collected from the gopher tortoises. Hookworms (62.5%, 5/8; 95% CI: 29-96), oxyurids (62.5%, 5/8; 95% CI: 29-96), *Strongyloides* sp. larvae (50%, 4/8; 95% CI: 15-85), and *Strongyle* sp. larvae (25%, 2/8; 95% CI:0-55) were the only parasites identified in the fecal samples. We could not characterize the parasites to the generic level because identification requires an adult worm, which could not be collected ante-mortem. Nine (82%; 95% CI: 60-100) of the fecal samples collected from the burrow entrances or traps contained endoparasites. Figures 7-10 represent microscopic examples of the various parasites observed in this study.

In the spring trapping season, 39 tortoises were handled and feces were collected from 26 (67%, 95% CI: 49-85) of the tortoises. Oxyurids (61%, 16/26; 95% CI: 44-80), hookworms (56%, 9/26; 95% CI: 37-75), and strongyloides (27%, 7/26; 95% CI: 10-44) were identified in the fecal samples.

Overall, 80% (28/35, 95% CI: 67 - 93) of the fecals examined during the fall and spring trapping seasons were positive for parasites. Oxyurids (65.7%, 23/35; 95% CI: 50.0 – 81.4) were the most common parasite found. In addition to oxyurids, strongyloides larvae (34.3%, 12/35; 95% CI: 18.6 – 49.0), hookworms

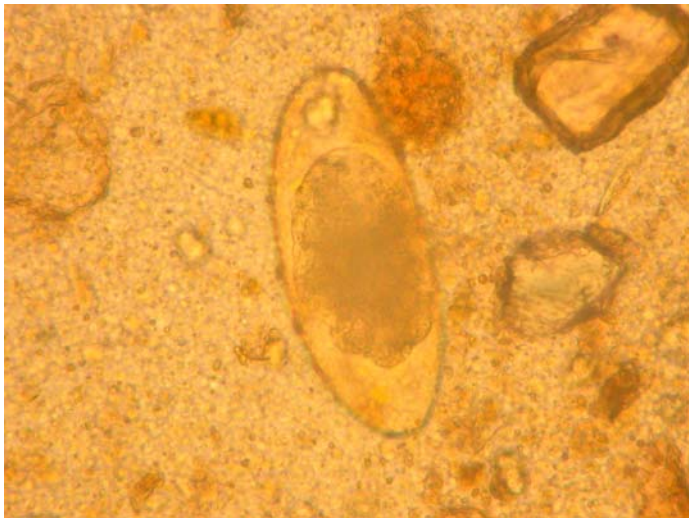

Figure 7. Oxyurid nematode from a gopher tortoise fecal sample.

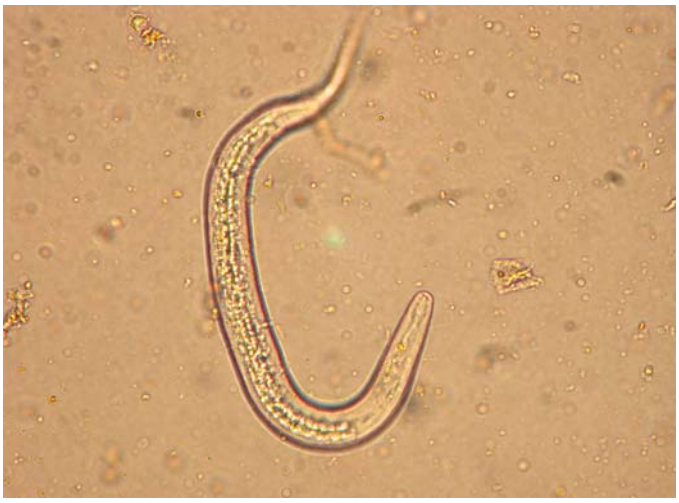

Figure 8. Strongyloides larvae from a gopher tortoise fecal sample.

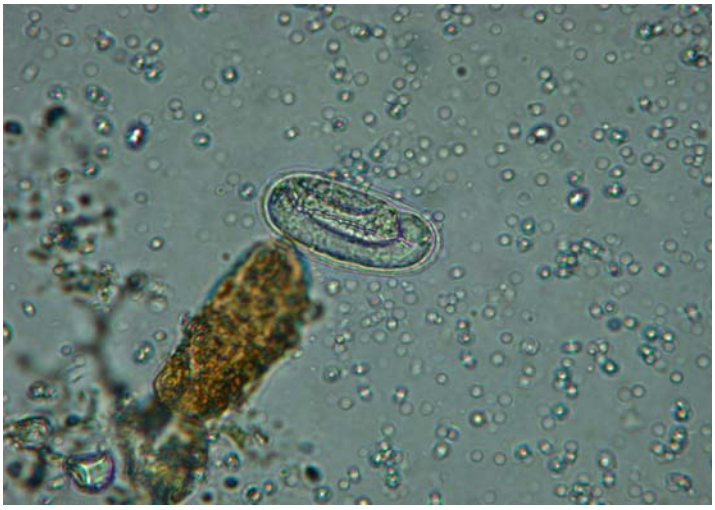

Figure 9. Hookworm egg from a gopher tortoise fecal sample.

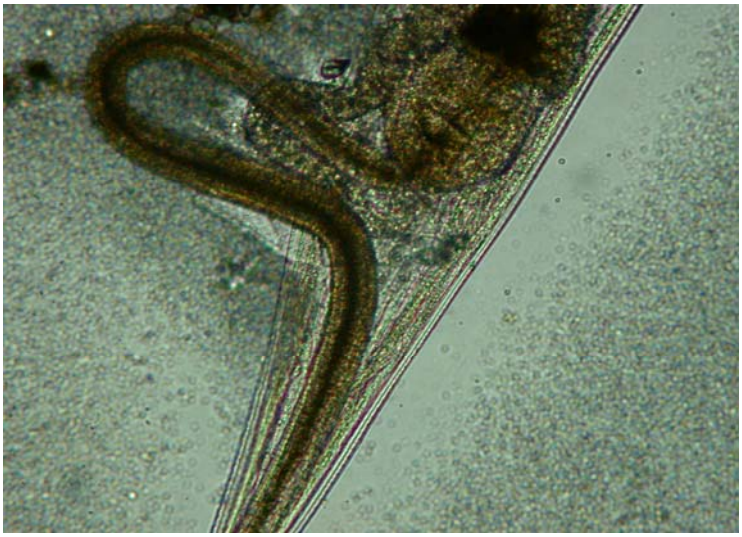

Figure 10. Oxyurid nematode larvae from a gopher tortoise fecal sample showing the esophageal bulb – characteristic of female larvae.

(28.6%, 10/35; 95% CI: 13.6 – 43.6), and strongyles (20.0%, 12/35; 95% CI: 6.8 – 33.2) were found in moderate amounts. There was no difference in the parasite burdens between the gender, site of capture, or season ($p<0.05$).

3.5 Discussion

This study represents the first attempt to develop baseline health parameters for Louisiana gopher tortoises. The original goal for this study was to collect 75 gopher tortoises (35 fall, 40 spring/summer) between July 2002-June 2003. Unfortunately, the tortoises were more difficult to capture than was expected. A total of 59 tortoises were collected between the two seasons.

Our findings related to carapace and plastron measurements suggest that there is minimal variation in the size of the tortoises from different sites in Louisiana. It was interesting to note that the female tortoises were larger and heavier than the male tortoises in this study. McRae et al. (1981) reported the same observations with gopher tortoise from Georgia. In general, male tortoises are larger than female tortoises (Landers et al., 1982; Smith, 1995). This difference may be the result of older and larger male tortoises being removed from the population. Because male tortoises generally have larger home ranges than females, it is possible that males are more likely to encounter human-associated trauma (e.g., hit by car and deforestation injuries) or be affected by infectious diseases or toxins (endocrine disruptors) within their natural range. The difference in size between genders was significant and should be evaluated further.

Hematology and blood biochemical parameters are essential to assess the health and physiologic status of stoic animals like the gopher tortoise. Although

blood samples are routinely collected from tortoises to perform complete blood counts, morphologic criteria for distinguishing the various circulating blood cells have not been established in the gopher tortoise.

Alleman (1996) described the morphologic features and cytochemical staining characteristics of circulating blood cells for the desert tortoise (Alleman, et al., 1996); however, these same features also need to be described for the gopher tortoise. The hematological parameters measured in this study were considered to be within reference limits for captive chelonian species. The findings suggest that these tortoises are, in general, healthy. Several of the blood samples appeared to represent lymph-diluted samples (n = 7), and were not included in the analysis. The complete blood counts in tortoises captured during the fall were significantly lower than in the spring. The same seasonal variation was noted in gopher tortoises from Florida (Taylor and Jacobson, 1982). Lower total white blood cell counts are not an uncommon finding in reptiles during the fall. As the environmental temperature decreases, the reptile's metabolic rate and immune function also decrease. Lymphocytes were the predominant white cell found in these tortoises. The lymphocytes play several important roles in the reptile, including producing antibodies and attacking foreign material (natural killer cells). A hibernation lymphopenia has been observed in wild reptile populations previously (Saad, 1988; Lawrence and Hawkey, 1986). Based on these previous studies (Taylor and Jacobson, 1982), we hypothesized that these cells would be lower in the fall than spring. However, this effect was not found, and suggests that a hibernation lymphopenia does not occur in Louisiana gopher tortoises.

There were several differences detected in the plasma biochemistries. The elevated calcium and phosphorus levels in female tortoises were not unexpected. Female tortoises routinely mobilize calcium and phosphorus during their reproductive cycle for egg production and vitellogenesis. The elevated potassium levels detected in male tortoises was statistically significant, but there is no apparent biological relevance for the difference. Elevated CK was found to be different within site of capture. Increased levels of CK suggest that there is some degree of skeletal muscle necrosis and overexertion occurring in these animals. The elevation of this enzyme could be attributed to sample collection, as it is not uncommon for these enzymes to be elevated after making multiple attempts at blood collection. Because the fall was the first collection period and I was more inexperienced at sample collection, it is possible that this difference could be attributed to sample collection. Although glucose and uric acid were elevated in the spring sampling period, there is no apparent biological relevance for the differences detected in this study. In contrast, GGT was found to be higher in the fall than the spring. Although little is known about this enzyme in reptilian species, in our study we found that the GGT levels were higher than those found in captive vertebrates. Wagner and Wetzel (1999) reported that plasma and tissue activities of GGT in iguanas were low or undetectable, which was consistent with findings in mammalian and avian species. However, elevation from this enzyme has been correlated with hepatobiliary disease in mammalian species.

Animals exposed to environmental toxicants often suffer health effects similar to those described in humans. Post-mortem examinations of desert

tortoises have revealed elevated concentrations of heavy metals, including cadmium, mercury, lead, molybdenum, arsenic, selenium, chromium, and nickel in the livers and kidneys of ill tortoises (Homer et al., 1996). Free-ranging tortoises that showed evidence of liver and kidney degeneration have contained elevated mercury, cadmium, and lead concentrations (Homer et al., 1996). Elevated concentrations of these metals may contribute to tortoise morbidity and mortality.

The heavy metal and trace element blood values reported in this study were consistent with blood levels described in higher vertebrates, with few exceptions. Almost 30% of the tortoises (17/57) had plasma zinc levels higher (>2.0 ppm) than those considered safe for birds. Copper levels were significantly higher in male tortoises compared to female tortoises. Again, the increased range of the male tortoise could place this gender at a higher risk of exposure. Zinc and copper are frequently used in fungicides and other commercial pesticide products. Whether these animals were exposed to these products naturally or from interactions with humans is unknown. In contrast, Caurant et al. 1999 reported that concerning essential metals in marine turtles the highest copper concentration were found in the liver, while the brain and salt gland showed relatively high copper levels. Fat tissue, bone, and carapace contained considerable levels of zinc (Caurant et al., 1999; Sakai et al., 2000). High zinc levels in fat tissue might be associated with pigment proteins which seem to play a role in zinc storage in the body (Sakai et al., 2000). The load of heavy metals in these marine turtles strictly correlated with the species seems to depend on their different food behavior.

A couple of interesting cases were identified throughout the study. One Gopher tortoise (GT#8) had mercury levels of 0.82 ppm and another tortoise (GT#4) had lead levels of 0.78 ppm. In comparison to other vertebrates, these values are considered elevated. Chitty (2003) reported lead toxicosis in a Greek tortoise (*Testudo graeca*) with blood levels of 0.39 ppm. Since these animals were considered clinically normal based on their complete blood count, plasma chemistry analyses, and *Mycoplasma* serology, we don't know if this values will have a negative effect on the tortoise survival within their natural ecosystem. Further study to evaluate tissue levels in these tortoises and their environment are warranted.

The *Mycoplasma* serologic results from the fall were encouraging. However, the seroprevalence after the spring season suggested that as many as 26% of the tortoises were suspect or positive for exposure to *Mycoplasma*. We found that *Mycoplasma* results for season were confounded by site. The wide difference in seasonal prevalence was likely associated with the fact that tortoises from Florida gas pipeline (FGP) were not captured during the fall. Tortoises from the FGP area were more likely to be seropositive than tortoise from Ben's Creek. The tortoises found in FGP are in a more confined space and are at higher densities than Ben's Creek. In addition, the vegetation is less sparse, suggesting that these animals may have to travel further to feed. The combination of these factors likely explains the higher degree of exposure in the Florida gas pipeline population.

The results of this study suggest that the tortoises were exposed to *Mycoplasma*. Possible explanations for the existence of seropositive gopher

tortoises are speculative. The disease may be indigenous in some tortoise populations and may flare up at times of anthropogenic or environmental stress. Alternatively, the disease could have been introduced by release of infected captive or wild tortoises. Jacobson et al. (1995) noted that the origin of *M. agassizii* in desert tortoise has not been determined, but speculated that a pathogenic strain may have been introduced into wild populations in California and Nevada by release of captive desert tortoises. The possible introduction of URTD by release of infected gopher tortoises does explain the wide distribution of the disease between different sites during this study. We believed that transmission of the disease may have been exacerbated by stress associated with relocations. Additional samples from seropositive tortoises must be performed to confirm infection vs. exposure. None of the tortoises in this study had clinical signs consistent with URTD. Because the majority of the Louisiana gopher tortoises are restricted to defined habitats, the introduction of *Mycoplasma* could be devastating. Further study to characterize the epidemiology of *Mycoplasma* in Louisiana gopher tortoises is warranted.

Little is known about the progression of URTD in free-ranging tortoises once they have been exposed to *Mycoplasma agassizii*, or what biotic or abiotic factors contribute to manifestation of URTD. Whereas some individuals exhibit severe signs of URTD following exposure, some may never show clinical signs (Brown et al., 1994). It has been suggested that URTD is clinically manifested during times of stress and may be caused by factors such as drought, habitat degradation, or overcrowding (Jacobson et al., 1991). In addition, the manifestation of clinical

signs of the disease may also depend upon the physiological condition of tortoises which, in turn, is related to environmental conditions (Lederle et al., 1997).

Continuation of long-term monitoring for signs of URTD and other diseases with similar signs in Louisiana populations are necessary to clarify environmental factors which contribute to the manifestation of the disease. Remote populations should be tested and compared to populations that have been subject to urban expansion and development to assess how disturbances are influencing the disease status of individuals.

Endoparasites are a constant threat to the health and well being of reptiles. Parasite burdens in wild individuals may vary depending on the time of the year (Ferguson, 1995). The relationship between parasites and season has been attributed to stress factors on the animals during drier times without food and water. There was no difference in parasite shedding between gender, site of capture, or season. The parasites identified in these tortoises were consistent with findings in gopher tortoises throughout their range. However, future work to classify the parasites to the species level should be pursued. Although there were eighteen tortoises that were characterized as parasite-free, this is likely an underestimate of the true prevalence of endoparasites as only a single sample was collected from each tortoise for this study.

Interestingly, there were no ectoparasites found in any of the tortoises. Ectoparasites have been associated with anemia and the transmission of diseases in reptiles. The absence of these parasites may be associated with the changes in the tortoise habitat as a result of the anthropogenic influences within their

ecosystem. Further, the larvae and nymph usually requires a blood meal to molt to the next stage (Khali and hoogstraal, 1981), and in the absence of suitable host and inadequate relative humidity, the tick life cycle will become prolonged. Adeyeye and Butler (1989) reported that the reason for few adult ticks (*Ornithodoros turicata*) in nature was attributed to a long life cycle under field conditions. Future work to classify the parasites to the species level should be pursued.

This study is the first attempt to characterize the health status of the Louisiana gopher tortoise. In general, our findings suggest that these tortoises are in good health. However, some of our findings suggest that these keystone species should continue to be monitored to protect them into the future.

CHAPTER FOUR

SUMMARY AND CONCLUSIONS

4.1 Summary

Louisiana gopher tortoise (*Gopherus polyphemus*) are critically threatened and tortoise numbers in the wild are low enough to be severely affected by factors which would normally have little effect on a reptilian population. The population size of these tortoises may have been relatively small for an extended period of time resulting in a reduced genetic diversity that could further adversely affect population growth. While human related factors have probably had the greatest effect on the population, the current numbers of tortoises from Louisiana are at low ebb allowing additional factors such as infectious diseases to have significant effect on population size.

4.2 Conclusions

The health status of wild tortoises is increasingly becoming a discussion point in conservation programs. There is a dearth of baseline health parameter data on free-ranging tortoises. This lack of baseline information is especially critical, as there continues to be an emphasis in chelonian conservation on translocation, reintroduction, and other interventionist management, as the primary conservation response to dwindling tortoise population. Without a basic knowledge of the health status of a population, translocation and reintroduction programs could be more detrimental than beneficial.

Although the tortoises sampled in this study were apparently healthy, at least one major chelonian disease was not investigated, herpesvirus. Because

of the small size of this population and the stress associated with habitat changes, this infectious disease could become an important pathogen. Fortunately, research is being pursued in 2005 to assess the prevalence of this disease in the tortoises.

Although the fate of the gopher tortoise in Louisiana is unknown, measures are being taken to gather information for recommendations to governmental agencies to set aside protected habitat. Despite state laws in Louisiana, and the Endangered Species Act (1990), habitat destruction, illegal poaching, and automobile accidents will continue to be a major threat to gopher tortoise survival.

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APPENDIX A

Data collection sheet.

DATE_____ **TIME** _____ **HRS**

TORTOISE NUMBER_____ **TRAP #** _____

LOCATION: Ben's Creek Sandy Hollow Other: _____

GPS Coordinates _____
_____

AGE JUVENILE or ADULT

GENDER MALE or FEMALE

WEIGHT _____

Carapace length_____
Carapace width _____
Plastron length _____
Plastron width _____

Body temperature _____ **Air temperature** _____

SAMPLE COLLECTION

1. Feces YES or NO

2. Ectoparasites YES or NO

3. Blood
6 Lithium heparin **1** EDTA **1** Red top

4. Rectal Culture YES or NO

5. Mycoplasma flush YES or NO

COMMENTS:
_____
_____
_____
_____
_____

APPENDIX B

Descriptive statistics for the carapace, plastron and weight measurements for all tortoises and by gender.

	Mean	St. Dev	95% CI	Min/Max
Carapace Length (cm)	31.4	2.4	30.8-32.0	24.0-37.5
Male	30.6	1.9	29.9-31.3	27.0-35.5
Female	32.4	2.6	31.3-33.4	24.0-37.5
Carapace Width (cm)	32.1	2.8	31.4-33.0	24.5-38.0
Male	31.2	2.1	30.5-32.0	26.5-36.1
Female	33.2	3.1	31.9-34.4	24.5-38.0
Plastron Length (cm)	25.0	2.1	24.3-25.5	19.0-29.8
Male	24.3	1.9	23.6-35.0	21.9-29.8
Female	25.7	2.2	24.8-26.5	19.0-29.5
Plastron Width (cm)	21.6	2.0	21.0-22.0	17.0-26.0
Male	21.0	1.8	20.3-21.6	17.0-25.0
Female	22.3	2.1	21.5-23.2	17.0-26.0
Body Weight (kg)	3.7	0.8	3.5-3.9	1.6-5.8
Male	3.3	0.5	3.1-3.5	2.6-4.5
Female	4.2	0.8	3.8-4.5	1.6-5.8

APPENDIX C

Descriptive statistics for the PCV and TS values for gopher tortoises (*Gopherus polyphemus*).

	Mean	St. Dev	95% CI	Min/Max
PCV (%)	26.8	7.3	25.0-29.0	14-50
Total solids (g/dl)	3.8	1.6	3.4-4.2	1.0-7.0

APPENDIX D

Differences in white blood cell count parameters (# cells) and heterophil count between trapping season.

White Blood Cell Count	Median	25-75%	Min/Max
Fall	4,000	4,700	1,200-18,500
Spring	7,100	5,200	2,000-28,000

Heterophil Count	Median	25-75%	Min/Max
Fall	1,530	300-2,855	108-7000
Spring	2,305	1,353-4,581	776-14,000

APPENDIX E

Descriptive statistics for granulocyte count for gopher tortoises from Louisiana.

	Median	25-75%	Min/Max
Lymphocytes	1,680	880-3,538	120-12,025
Monocytes	920	474-2,359	24-5,700
Eosinophils	0	0-0	0-68
Basophils	272	85-470	0-2,240

APPENDIX F

Descriptive statistics for plasma chemistry analysis for gopher tortoises. Differences in plasma chemistry parameters between gender.

	Median	25-75%	Min/Max
Calcium			
Male	10.7	9.8-11.4	7.7-14.7
Female	14.7	12.0-17.7	9.8-35.8

	Median	25-75%	Min/Max
Phosphorus			
Male	2.3	1.8-3.3	1.2-5.4
Female	3.9	2.1-7.7	1.5-13.1

	Mean	95% CI	St. Dev.	Min/Max
Potassium				
Male	5.6	5.3-6.0	0.9	3.8-8.3
Female	5.0	4.6-5.2	0.7	3.9-6.8

APPENDIX G

Descriptive statistics for plasma chemistry analysis for gopher tortoises. Differences in plasma chemistry parameters between trapping seasons.

	Median	25-75%	Min/Max
GGT			
Fall	5.0	3.0-7.0	1.0-8.0
Spring	0.6	0.0-1.0	0.0-3.0

	Median	25-75%	Min/Max
Uric Acid			
Fall	0.8	0.2-2.2	0.0-2.8
Spring	2.2	1.2-3.2	0.1-7.0

	Mean	95% CI	St. Dev.	Min/Max
Glucose				
Fall	78.1	68.3-87.9	19.0	53-115
Spring	100.2	93.5-106.9	20.3	50-149

APPENDIX H

Descriptive statistics for plasma chemistry analysis for gopher tortoises. Differences in plasma chemistry parameters between trapping location.

	Median	25-75%	Min/Max
Creatinine Kinase			
Ben's Creek	**338.5**	**68.5-1294.7**	**24.0-2580**
FGP	**80.5**	**35.0-174.2**	**24-434.0**

APPENDIX I

Descriptive statistics for plasma chemistry analysis for gopher tortoises. No differences between gender, location, or season.

	Median	25-75%	Min/Max
AST	141.0	91.0-168.0	55-1407
Creatinine	0.09	0.0-0.1	0.0-0.7

	Mean	95% CI	St. Dev.	Min/Max
ALP	37.5	33.4-41.5	14.6	15-74
Total Protein	3.96	3.6-4.3	1.2	1.5-6.3
Albumin	1.25	1.12-1.36	0.42	0.5-2.3
Globulin	2.7	2.5-2.9	0.9	1.0-5.3
Sodium	129.8	128.6-131.1	4.8	119.0-142.0
Chloride	101.4	99.7-103	6.2	88.0-119

APPENDIX J

Descriptive statistics for heavy metal and trace element parameters for gopher tortoises. Copper and mercury levels for male and female gopher tortoises from Louisiana.

	Median	25-75%	Min/Max
Mercury			
Male	**0.02**	**0.017-0.035**	**0.0-0.82**
Female	**0.01**	**0.0-0.02**	**0.0-0.05**

	Mean	95% CI	St. Dev.	Min/Max
Cooper				
Male	**0.73**	**0.68-0.79**	**0.14**	**0.47-1.0**
Female	**0.52**	**0.46-0.57**	**0.14**	**0.27-0.90**

APPENDIX K

Differences in copper levels between trapping season.

	Mean	95% CI	St. Dev.	Min/Max
Cooper				
Fall	**0.56**	**0.47-0.64**	**0.16**	**0.27-0.80**
Spring	**0.66**	**0.65-0.72**	**0.18**	**0.32-1.0**

APPENDIX L

Descriptive statistics for zinc and lead levels for gopher tortoises from Louisiana. No differences between gender, location, or season.

	Median	25-75%	Min/Max
Lead	0.0	0.0-0.02	0.0-0.51

	Mean	95% CI	St. Dev.	Min/Max
Zinc	1.6	1.5-1.8	0.6	0.4-3.2

VITA

Orlando Diaz-Figueroa was born on December 27, 1974 in Hato Rey, Puerto Rico to Carmen M. Figueroa and Orlando Diaz-Quirindongo. As a child, Orlando was surrounded by many animals including fish and reptiles. This led to develop a keen interest in exotic animals. He graduated from Colegio San Jose in Rio Piedras, Puerto Rico in 1992 and continues his education at Louisiana State University. In Louisiana, he developed an interest in Veterinary Medicine and research by working at the Laboratory Animal Medicine Research Center at Louisiana State University. He was inducted to the Dean's list while pursuing his Bachelor of Science. He graduated from Louisiana State University in 1997 with a Bachelor of Science degree in Animal Science. He was admitted to the School of Veterinary Medicine at Tuskegee University in the fall of 1997 and was awarded his Doctor of Veterinary Medicine degree in May 2001. During his last year at the School of Veterinary Medicine, he was named All-American Collegiate Scholar, by the United States Achievement Academy. At the same time, he was inducted to the Phi Zeta National Veterinary Honor Society – Rho Chapter at Tuskegge University. In the fall of 2001, he accepted the position of an internship in companion avian/exotic and small animal medicine at Animal Care Unlimited and the Ohio Wildlife Center which he successfully completed in the summer of 2002. He is in the process of completing a Zoological Medicine Residency at Louisiana State University, and is pursuing eligibility for the American Board of Veterinary Practitioners specialty, avian emphasis. While completing his Master of Science, the Louisiana State University had the honor to induct him to the National Society

of collegiate Scholars for his scholarship and leadership. He will receive the degree of Master of Science at the May 2005 commencement. His current hobbies are keeping and breeding snakes, tortoises, and cichlids from South America and Africa.