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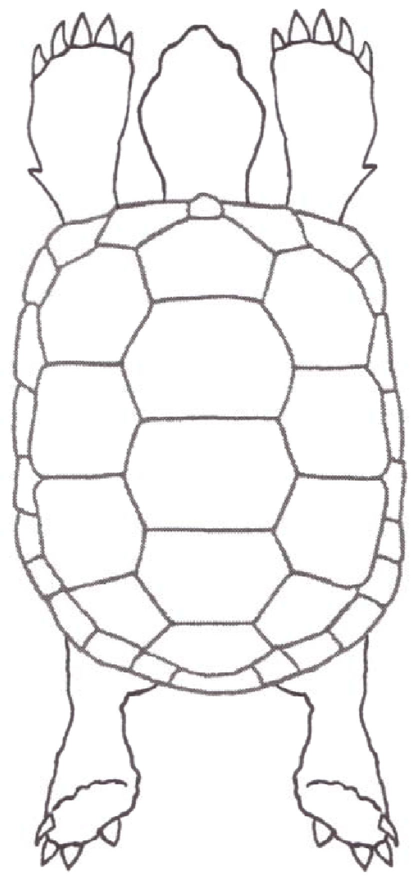
Handbook on Gopher Tortoise (*Gopherus polyphemus*)

Health Evaluation Procedures for Use by Land Managers and Researchers

Lori Wendland, Harold Balbach, Mary Brown, Joan Diemer Berish, Ramon Littell, and Melissa Clark

January 2009



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Health Evaluation Procedures for Use by Land Managers and Researchers

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Final Report

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Abstract: The gopher tortoise is a widespread species, but one at risk. Recently, greater interest in the survival of the species has led to a series of programs and proposals for a region-wide program of cooperative management. Relocating the animals when their habitat is threatened by human disturbance is a common management practice on all lands. However, the health of the tortoises may influence the success of these relocations. A process to better incorporate health and disease related information into management decisionmaking was identified as an important missing element. The newly developed handbook contains decision trees, charts and other aids, including a special section identifying warning signs of serious health problems. The handbook thus facilitates decisionmaking regarding the health status of gopher tortoises by land managers, military and otherwise, when developing management plans involving relocation or augmentation of tortoise populations on their lands. The primary emphasis is on basic physical examinations of gopher tortoises because the manual is designed for use by land management personnel. This handbook is part of a larger project initiated within the U.S. Army environmental research program to address specific gaps in information regarding gopher tortoise population ecology and health.

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Preface

This study was conducted for the Army Environmental Quality research program (PE 62720A896) under project number G9K2FL, “Gopher Tortoise Candidate Conservation Agreement Support” The technical monitor was Scott Belfit, DAIM-ED.

The work was completed under the direction of the Ecological Process Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). The CERL Principal Investigator and contract monitor was Dr. Harold Balbach. The handbook development was initially performed by the University of Florida, Gainesville, FL. Dr. Lori Wendland was the UFL Principal Investigator, Dr. Mary Brown is a Professor in the College of Veterinary Medicine, Dr. Ramon Littell was Professor and Chair of the Department of Statistics, and Melissa Clark was a research assistant in the College of Veterinary Medicine. Joan Diemer Berish is a biologist with the Florida Fish and Wildlife Commission, Gainesville, FL. The work was completed under Cooperative Ecosystem Studies Unit agreement No.W9132T-07-2-0005. Alan B. Anderson is Chief, CN-N, and Dr. John T. Bandy is Chief, CN. The associated Technical Director was Dr. William D. Severinghaus. The Director of CERL is Dr. Ilker Adiguzel.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Commander and Executive Director of ERDC is COL Gary E. Johnston, and the Director of ERDC is Dr. James R. Houston.

Unit Conversion Factors

Multiply	By	To Obtain
acres	4,046.873	square meters
degrees Fahrenheit	(F-32)/1.8	degrees Celsius
feet	0.3048	meters
inches	0.0254	meters

1 Introduction

Background

The Department of Defense manages nearly 25 million acres of land, and approximately 10 percent of these lands represent installations believed to contain gopher tortoises. This manual is part of a larger project initiated by the U.S. Army Engineering Research and Development Center (ERDC) to address specific gaps in information regarding gopher tortoise population ecology and health. Regional management approaches towards gopher tortoises on DoD lands may eventually include the development of a Candidate Conservation Agreement (CCA) to ensure the survival and recovery of the species on their lands. Such an agreement will have to address a number of issues pertaining to the viability of existing populations, and the health of animals within the populations is a vital component. Often, management actions conducted on both public and private lands involve the relocation of gopher tortoises. Although upper respiratory tract disease (URTD) has received significant attention over the past decade, there are numerous other diseases and conditions which have the potential to affect the success of these management activities. A mechanism for incorporating health and disease-related information into decisionmaking is an important missing element.

The gopher tortoise (*Gopherus polyphemus*) is considered to be declining throughout its range (Smith et al. 2006) and is Federally listed in the western portion. The U.S. Fish and Wildlife Service has also been petitioned to list the remaining populations (Save Our Wild Scrub et al. 2006). The Florida Fish and Game Commission estimates that—in that state alone—74,000 gopher tortoises have been impacted by incidental take permits issued to developers in the past 14 years (J. Berish, pers. comm.).

The inherent impacts of infectious diseases on wildlife conservation and biodiversity are evident; however until recently, were not often considered. Lack of knowledge with respect to the potential infectious diseases present within wild populations, the impact of disease status on relocation or reproduction of species, and disease impacts to long-term population viability creates a major dilemma for wildlife biologists, conservationists, and public policy makers. This is even more critical when the species concerned is a keystone species such as the gopher tortoise, which is critical to the health of its ecosystem and the survival of many additional species.

Objective

This manual was specifically designed for use by land management personnel, including those from Army and other services installations, to facilitate decisionmaking regarding the health status of gopher tortoises when developing plans for management, relocation or augmentation of tortoise populations on their lands. The specific data presented are intended to be used in the preparation of biological assessments (BAs) and biological opinions (BOs) related to Army training activities potentially impacting gopher tortoise populations, and for endangered species management plans (ESMPs), integrated natural resources management plans (INRMPs), and in the preparation of ecological risk assessments involving training or equipment testing where the tortoise is present.

Scope

The current study is intended to apply to all gopher tortoise populations in the Southeastern states, to be used by land managers of both public and private lands.

Approach

A basic overview of the importance of health and disease information is presented to introduce these concepts. Because the manual is designed primarily for land managers, who are not likely to conduct large-scale studies on their own, the primary emphasis is on physical examinations rather than detailed health assessments. However, additional information and resources are provided in the appendixes for instances when more comprehensive health assessments are needed. Relevant decision trees and charts are included, and a special section on warning signs of potential health related problems and recommended responses has been provided. Many of the more technical terms specific to the tortoise biology and anatomy, and many elements of veterinary medical terminology are highlighted when they first appear. Each of these is defined in the Glossary.

Mode of Technology Transfer

The information included in this report is one portion of the materials prepared by the Engineer Research and Development Center to assist installation natural resources and threatened and endangered species program managers. The primary means of communicating the gopher tortoise health manual information will be through publication in the scientific literature, as well as through the availability of this report.

This report will be made accessible through the World Wide Web (WWW) at URL:

http://www.cecer.army.mil

2 Preparatory Information

Permits and approvals to work with tortoises

It is important to follow all relevant permitting and regulatory rules when working with wildlife. Any research being conducted on vertebrate animals generally requires an approval from the Institutional Animal Use and Care Committee (IACUC) for academic institutions and industry, or from other similar review panels in order to meet the Animal Welfare Act regulations. Additionally, permits are required from state and sometimes Federal wildlife agencies to conduct work with gopher tortoises.

Gopher tortoise health

Health is vital to the success and well-being of any population. Management activities may improve the health of a population by increasing the quality or availability of suitable habitat and food resources. Alternatively, certain management activities may negatively impact the health of a population by imposing additional stressors to which the animals may have difficulty adapting. In the case of gopher tortoises, relocation is a management tool that has become increasingly popular. When done correctly, relocation projects may result in healthy, viable gopher tortoise populations. However, as with any management activity, there is the potential for negative effects on the population, including introduction of disease with potential subsequent die-off events. Because tortoise health can substantially influence the success of any management activity, baseline information should be collected before implementing the management actions and then again afterwards to measure the results. See Appendixes A and B for a discussion of health monitoring or in-situ gopher tortoise populations and for tortoise population relocation.

How health is measured

Health is measured in all species using parameters such as food/water intake and fecal/urine output (physiological balance), body weight in relation to a known standard, reproductive performance, blood biochemical parameters, social and environmental factors, amount of physical activity, availability of suitable habitat, absence of disease, and several other variables. Many of these variables are easy to measure in captive animals; however, most are extremely difficult to measure in wild species. The most common components of a comprehensive health assessment of animals are:

- Physical examination
- Blood hematology and biochemistry
- Tests for nutritional status
- Tests for internal and external parasites
- Tests for infectious diseases
- Tests to assess reproductive capacity
- Tests for exposure to environmental toxins

Unfortunately, normal reference ranges for some of these variables are simply not available for free-ranging gopher tortoises. Increasing concern about tortoise health has lead to several recent studies conducted at multiple institutions that ultimately will provide much health-related data in the near future.

Importance of wildlife health and disease

The use of health assessments is a relatively new concept in wildlife sciences. In the past, infectious diseases were not considered when conducting wildlife management activities because it was generally believed that wild animal populations were large enough to adapt to, or deal with, any potential impacts from disease (Spalding and Forrester 1993). However, substantial increases in human-induced impacts to the natural environment have occurred over the past 50 years. The primary issues facing all species of wildlife, including gopher tortoises, are habitat loss and habitat degradation due to human development activities (Mitchell and Klemens 2000). Urbanization of the southeastern Coastal Plain is increasing at an alarming rate; this makes public lands critical refuges for the conservation of tortoises. However, given the loss of suitable habitat, reduced fire management in some areas due to urban perimeters, reduced available home ranges with resultant increases in tortoise densities, and increased unauthorized relocations by well-intentioned people, disease represents a real threat to gopher tortoise populations (Hutchins et al. 1991; Nettles 1992; Viggers et al. 1993; Woodford 1993; Nettles 1996). Lack of knowledge regarding the effect of diseases on the long-term viability of gopher tortoise populations creates a major dilemma for land managers, wildlife biologists, conservationists, and public policy makers. Because gopher tortoises are long-lived and do not reach reproductive maturity for 10-20 years, a disease outbreak that causes the death of a large number of tortoises may result in devastating population losses, making it difficult for the population to recover.

Diseases of tortoises

Most of the available information regarding diseases in tortoises originated from captive animals (Jacobson 1994; Origgi and Jacobson 2000). Little is known about diseases in wild tortoises; however, respiratory and shell diseases have been the most commonly reported problems in wild chelonians (Jacobson and Gaskin 1990; Jacobson et al. 1991; Beyer 1993; Jacobson 1994; Jacobson et al. 1994; Berry 1996; Lovich et al. 1996; Garner et al. 1997; Lederle et al. 1997; McLaughlin 1997; Homer et al. 1998; Smith et al. 1998; Berish et al. 2000; McLaughlin et al. 2000; Origgi and Jacobson 2000; Rose et al. 2001; Johnson 2006). Most disease research in wild gopher tortoises has focused on URTD caused by *Mycoplasma agassizii*, a bacterium that can infect the respiratory tract of tortoises (Brown et al. 2001). The primary reason for emphasis on mycoplasmal URTD is because it is the only disease for which controlled experimental studies have been done in *Gopherus* spp. (Brown et al. 1994, 1999). Additionally, diagnostic tests specifically validated for gopher tortoises are available (Schumacher et al. 1993; Brown et al. 1995; Wendland et al. 2007). However, a number of other pathogens can cause similar clinical signs (i.e., symptoms) (Jacobson 1994; Pettan-Brewer et al. 1996; Westhouse et al. 1996; Origgi and Jacobson 2000; Origgi et al. 2004; Johnson 2006), but because diagnostic tests are not readily available, little is known about the importance and prevalence of these microorganisms in wild tortoises. Table 1 provides a list of clinical signs that may be encountered when examining tortoises, and some of the potential causes of these signs. Although it is not an exhaustive list of every potential problem that may be encountered when conducting physical examinations of tortoises, it covers the most common signs of illness.

In some cases, a disease may become apparent and get very severe soon after an animal has been exposed to the pathogen. Such diseases are generally called ‘acute diseases’ because the onset and progression of the disease is rapid and severe, sometimes resulting in death. The direct impacts of an acute disease to a population often become apparent quickly since these diseases usually spread through a population of animals very rapidly. In other cases, the disease may be more insidious, and exposure to the pathogen results in a chronic illness that progresses slowly over time. Animals with a chronic disease may have reduced reproductive capacity, may not grow normally, may be more susceptible to secondary infections, and in some cases, may have a reduced life span. Identifying the impacts of such diseases can be a difficult task. There is an abundance of literature describing the potentially severe, long-term consequences of chronic disease on animal populations (Spalding and Forrester 1993; Hess 1996; Heffernan et al. 2005). However, the full effect of chronic disease on a long-lived species such as the gopher tortoise may take decades to be seen. Therefore, it is important that populations are monitored using standardized techniques so that any changes associated with health problems may be detected over time.

Table 1. Clinical signs that may be observed when conducting physical examinations on gopher tortoises in the field.

Clinical Signs	Possible Conditions/Causes
Behavioral	
Extending neck, difficulty breathing, mouth gaped open	Respiratory disease (e.g., pneumonia; many potential causes)
Weakness, non-responsive, outside burrow during cold weather	Severe debilitation (many potential causes)
Head	
Eye discharge (watery or discolored), swollen eyelids, red and/or swollen third eyelids	Eye infection, URTD*, abrasion/irritation (often induced by bucket trapping)
Nasal discharge (watery or discolored)	URTD*, frothy oral discharge from stressed tortoise mistaken for nasal discharge, clear nasal discharge sometimes seen after a tortoise has been drinking
Erosion or depigmentation of skin around nares	Trauma, chronic nasal discharge associated with URTD*, skin infection
Abnormal breath sounds (wet/crackling sounds)	URTD*, pneumonia (many potential causes)
Swollen tympanum (membrane over ear)	Abscess (bacterial infection), vitamin deficiency, trauma
Oral cavity	
Pale mucous membranes	Anemia, severe debilitation, shock
Ulcers, crusts or scabs (e.g., plaques) on tongue or inside mouth	Herpesvirus, iridovirus, bacterial or fungal infection, embedded foreign body
Limbs/body	
Skin swelling	Abscess/infection, parasite, tumor, trauma
Skin discoloration	Incomplete shed, infection, scar from prior injury

Clinical Signs	Possible Conditions/Causes
Emaciation/reduced muscle mass	Starvation, severe debilitation (many potential causes)
Lameness, swollen joints	Trauma/injury, nutritional disease, metablic disease
Shell	
White or yellow discoloration, flaking	Healing traumatic injury, bacterial or fungal infection, dyskeratosis (vitamin deficiency or toxicity)
Soft spots, especially at mid-carapace	Nutritional disorder, toxicity, trauma
Red blotches	Trauma (if localized), severe systemic infection (if diffuse across shell)
Malformed shell	Trauma, nutritional disorder
* URTD: Upper respiratory tract disease caused by *Mycoplasma agassizii*, iridovirus, herpesvirus or other pathogen.	

3 Health Assessment Methods

Establishing goals

The previous chapter listed the components of a comprehensive health assessment (on p 5). When initiating a project that involves the assessment of tortoise health, it is necessary to develop overall goals and specific objectives for the project and the tortoise population of interest. Establishing clear, practical, and measurable objectives will greatly facilitate the development of management or research plans and decisionmaking processes. The goals for the project (cf. Figure 1) , available financial resources, and importance of the tortoise population will help determine the specific objectives for the project and consequently, which components of the health assessment will be utilized. Whether the health-related objectives are to assess health within a population, to conduct a survey for exposure to a particular pathogen, or to evaluate individual tortoises prior to relocation, it is important to develop a plan of action that will adequately meet the established goals.

Surveying the population

This topic has been addressed in a separate manual (Smith et al. 2008, in editing); however, different methods may be more suitable when surveying to estimate population densities versus assessing the health of a population. If a systematic health screen or more detailed study is to be performed, see Appendix C for further discussion of surveying, sampling schemes and necessary sample size calculations.

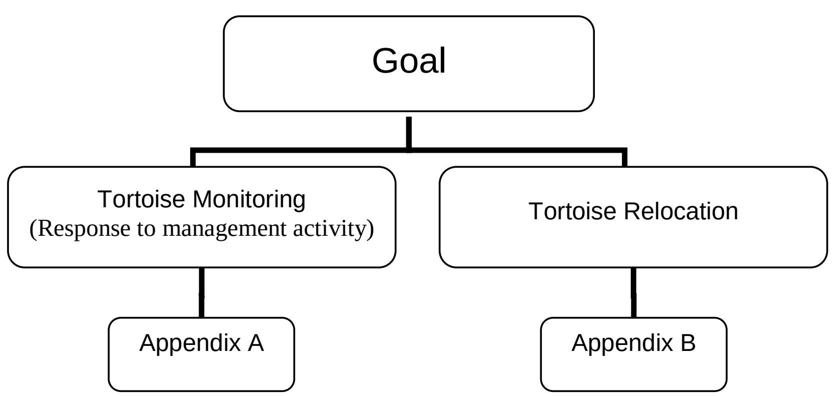

Figure 1. Establishment of health-related objectives based on overall project goals.

Disinfection/sanitation protocols

Caution must be taken whenever handling or sampling gopher tortoises to ensure that field personnel do not aid in the spread of infectious microorganisms. Cleaning refers to the physical removal of organic debris (dirt, feces, urine, blood, etc.) from objects or living tissue. Disinfection refers to the elimination or inhibition of the growth of microorganisms (except bacterial spores) on non-living objects, whereas antisepsis involves the same process for living tissue. In contrast, sterilization is the complete elimination or destruction of all forms of microorganisms (including bacterial spores) on non-living objects, and is generally not possible in the field.

Field personnel must follow a standard protocol that includes the disinfection of all equipment and the use of an antiseptic on their hands between tortoises. Field personnel should wear disposable latex or nitrile gloves whenever handling tortoises, and change gloves between individual tortoises. Since gloves are often torn when handling tortoises, the use of an antiseptic on the hands between animals is strongly recommended. A sample disinfection protocol is provided (Appendix D); however, other protocols may be developed to meet the needs of the specific project or management goal.

Tortoise capture and handling

Gopher tortoises are usually captured opportunistically as encountered, or in traps set directly in front of burrow openings. All traps, regardless of the type, need to be provided with a cover to shade the tortoise in order to prevent heat stress. Traps must be checked at least once daily to reduce the amount of time that tortoises are in the trap. After capture, the tortoise is held individually in a clean plastic bin until it can be assessed and returned to its burrow. The plastic bin should be large enough to allow the tortoise to turn around inside the container. For adult tortoises, we recommend minimum dimensions of L- 1’9”, W- 1’3”, H-1’. Traps and bins must be cleaned and disinfected between every tortoise to reduce the chance of pathogen transmission.

Physical examinations.

Health studies in wildlife typically include physical examinations and the collection of biological samples for a number of diagnostic tests (Christopher et al. 1999; Karesh et al. 1999; Berry and Christopher 2001; Hanni et al. 2003; Kilbourn et al. 2003; Deem et al. 2005; Deem et al. 2006; Uhart et al. 2006). Excellent guidelines have been published describing techniques for the evaluation of health in chelonians (Jacobson et al. 1999; Berry and Christopher 2001). Berry and Christopher (2001) provide sample data sheets and have helpful line drawings that show, in detail, how to examine the eyes and periocular area of tortoises. The manuscript also describes normal behaviors observed in desert tortoises (*Gopherus agassizii*), many of which also are observed in gopher tortoises. Anyone planning to conduct a health study in wild tortoises should first read that journal article (available online at http://www.jwildlifedis.org/cgi/reprint/37/3/427).

Performing a physical examination requires, at minimum, knowledge about normal tortoise behavior and physical appearance. If biological samples are going to be collected, training or assistance from an individual with experience working with and collecting specimens from reptiles is required. The basic components of the physical exam include an overall assessment of the posture/behavior of the tortoise, an examination of the eyes, nares (i.e., nostrils), beak, oral cavity, skin, muscle mass, and shell. Morphometric (i.e., body) measurements are also important for identifying and determining the maturity of individual tortoises, and specific parameters can be used to evaluate the body condition of tortoises (Nagy et al. 2002). A sample data sheet has been attached (see Appendix E, form adapted from Berry and Christopher 2001 and McRae et al. 1981) and the reader is referred to Berry and Christopher (2001) for more detailed descriptions. A brief explanation of the physical exam, skills required and step by step procedure for completing the physical exam data sheet are described in Appendices F and G. Appendix H provides photographic examples of numerous clinical signs that may be encountered when conducting exams.

Diagnostic tests

Diagnostic assays (i.e., tests) greatly complement the physical examination because many diseases have an incubation or subclinical period when tortoises may not exhibit any outward signs of illness. Specific assays, therefore, may actually detect a disease process before clinical signs develop. Hands-on training is necessary to learn appropriate sample collection and handling techniques, and even more expertise is often required to interpret test results. However, diagnostic tests provide extremely valuable information regarding the immunological, nutritional, and physiological status of the animal, and can indicate specific organ dysfunction or disease. In some cases, the presence of a specific set of clinical signs observed in tortoises may prompt one to run diagnostic tests to try to determine the underlying cause of the signs.

Diagnostic tests vary in expense (Figure 2), and the specific tests chosen will depend on the project or management goals, available funding, and expertise of the personnel involved. See Appendix I for a more detailed description of the benefit and value of specific diagnostic tests.

- Comprehensive necropsy
- Biopsies
- Cytology
- Culture
- Hemotologic tests (CBC
- Serum biochemisty panels
- Serology
- Total protein levels
- Packed cell volume
- Fecal exams

↑ *Increasing expense*

Figure 2. Most common diagnostic tests used to assess animal health.

Biological sample collection and storage

Proper sample collection and handling is essential for meaningful, reliable, and repeatable test results. Samples that have not been collected, processed, or stored correctly may provide inaccurate results and lead to inappropriate conclusions or decisions. For example, lymphatic vessels lie directly adjacent to the most commonly used blood vessels for sample collection in tortoises. Often blood samples are contaminated with clear lymphatic fluid. Lymph contamination of blood samples may alter diagnostic test results dramatically, and therefore, when it occurs, it should always be noted on the sample and on data sheets, and results should be interpreted cautiously. Additionally, because reptilian body temperatures and blood cells are different from mammals, laboratories should be selected that have experience working with reptilian samples. Some organisms cultured from the tortoise respiratory tract do not grow at 37 ^{o}C, the human body temperature standard used for most diagnostic laboratory incubators. Recommendations for sample handling and storage, proper supplies, and several suggested laboratories are included in Appendix J.

Banking plasma

Even when diagnostic tests are not being performed at the onset of the project, blood samples may be collected, processed and stored for future use in the event that a problem develops in the population. These stored samples can be used for retrospective studies in the event that a disease epizootic occurs, if new diagnostic tests become available, or if additional resources are made available in the future. The red blood cell (RBC) pellet that results from centrifugation of a blood sample to separate the serum should be saved because it may be used for DNA-based studies and therefore, may be of value in the future. It is absolutely critical that blood products be stored appropriately to prevent degradation of the samples. Recommendations for plasma and RBC banking are included in Appendix J.

Post-handling care of tortoises

Tortoises should be processed and returned to their burrows as quickly as possible to minimize stress for the tortoise. Any animal found in a dehydrated condition should be soaked in its individual bin with a small amount of luke-warm water for 10 to 15 min prior to release. The water should only cover the bottom of the bin to a depth that allows the tortoise to easily drink, and the depth should never exceed the tortoise's shoulders. Tortoises placed in a tub of water will often drink to rehydrate themselves. Soaking is recommended even if the hydration of the tortoise is unknown, particularly when animals are held for more than 15-20 minutes, or become agitated and urinate excessively. A standard protocol of soaking tortoises after handling will not hurt the animals, and may actually help them. One should NEVER leave soaking animals unattended as they could flip themselves over in the bin and drown, even in shallow water. Furthermore, the water must be changed and the bin thoroughly disinfected between animals.

4 Recognizing Potential Health Problems

Sometimes field workers come across circumstances that provide warning signs of potential health problems within a tortoise population. Many of these warning signs may be detected while land managers are conducting other work, particularly during standard forestry practices. However, others require more detailed, systematic surveys to detect because the signs are subtle and less likely to be noticed when not specifically looking for them. Several potential warning signs have been discussed below. All of these findings should prompt further investigation, as described under each section. **Importantly, land managers and/or field staff must coordinate with and obtain appropriate authorizations from state and Federal wildlife agencies before initiating gopher tortoise projects and especially before removing gopher tortoises from their habitat. Whether on private or public lands, permissions to handle, hold, and relocate any wildlife are the prerogative of the state wildlife agency. This is in addition to any permissions required from the USFWS for a listed species.**

Potential problem A: Presence of dead tortoises

Dead tortoises/shells are only occasionally seen in the environment under normal circumstances. The published annual mortality rate for adult gopher tortoises is very low, and has been estimated at approximately 3 percent (Landers 1980). Therefore, field staff should only see approximately three shell remains for every 100 adult tortoises estimated to be present within a population. If field workers see shells at a greater frequency, a more thorough investigation is warranted.

Response to potential problem A

Any time dead tortoises are found in the environment, it is recommended that field workers geo-reference the position of the remains and examine the remains for evidence of predation, trauma, and other lesions. The approximate time since death can be estimated using established methods (Dodd 1995). Shells should either be removed or marked with paint or flagging so they are not counted twice. Some form of recordkeeping should also be used because what may initially appear to be only a few animals may in fact be a warning sign of a bigger problem. If only a few dead tortoises are encountered annually, for example, this may represent normal mortality within the population and no further action may be needed. However, if five or more carcasses are found within a small area, then more action may be necessary. In such cases, a systematic survey of the population is recommended to determine the size and geographical extent of the possible mortality event. When conducting these surveys, field workers should document and examine the dead tortoises as described above. Determining the significance of the mortality event will require general knowledge regarding the size of the tortoise population. The proportion of the habitat to survey will depend on the size of the habitat, the density of tortoises, and resources of the manager. Consultation with a professional that has infectious disease experience will be important to obtain appropriate guidance. Survey findings may prompt the manager to trap and assess the health of tortoises within the area.

Potential problem B: Tortoises exhibiting atypical behaviors

Gopher tortoises spend the majority of their time inside burrows. Behaviors that may indicate a health problem include tortoises found outside of their burrows at night or during cold winter days, and tortoises found sitting in the mouth of the burrow for many hours and not retreating into the burrow when you advance. Another atypical behavior includes the construction of a shallow, superficial burrow (called a pallet) and staying in that burrow for several consecutive days during the summer. All of these behaviors are suggestive of a weak or lethargic tortoise, one that may be very ill. There have been isolated reports of gopher tortoises leaving their burrows at night in the summer months (Radzio et al. 2007); however, a quick evaluation should allow the field worker to determine if the tortoise is weak. An additional possible exception to these behavioral warning signs may be when tortoises are initially relocated into a new habitat. Disorientation and stress associated with relocation may result in a number of atypical behaviors for weeks to months after the relocation, and therefore, in these cases, behavioral changes must be interpreted cautiously and coupled with physical examinations when possible.

Response to potential problem B

When a tortoise with atypical behavior is encountered, at minimum, a physical examination should be performed. If the animal is found to be weak, exhibiting severe clinical signs of disease, or in poor body condition, the animal should be held in appropriate conditions until professional assistance can be obtained (e.g., plastic bin in a secure location at an appropriate temperature (approximately 75–85 °F) and with adequate ventilation). A more extensive health assessment should be performed as soon as possible.

Potential problem C: Presence of clinical signs in a number of tortoises

Clinical signs to be particularly aware of when working with tortoises include nasal, eye, or oral discharge (other than the frothy oral discharge commonly seen in stressed tortoises), lethargy, low body weight, reduced muscle mass and weakness, severely sunken eyes, abnormal respiratory sounds, and severe shell or skin lesions. It is important to note, however, that severe clinical signs of disease are uncommonly observed when conducting annual or sporadic physical examinations of gopher tortoises (Wendland 2007). Therefore, the presence of any clinical sign in a significant proportion of the tortoises evaluated (e.g., ≥ 20 percent) provides evidence that a health problem may be occurring within the population.

The method of tortoise capture (e.g., opportunistic or trapped) is an important consideration when determining the significance of clinical signs. When tortoises are trapped in buckets, swollen eyelids are commonly observed and are not a good indicator of URTD, for example (Wendland 2007). Further, when tortoises are stressed, field workers may observe a frothy oral discharge that can be expelled through the nose as well. This situation is usually obvious, as the tortoise exerts itself excessively attempting to escape from the trap or bin.

Response to potential problem C

Table 1 provides a list of potential causes of clinical signs observed in tortoises. If a particular clinical sign is observed in a number of tortoises, then animals should be trapped and health assessments performed, including diagnostic tests, to try to determine the underlying cause. A systematic sampling scheme that includes adequate sample sizes should be used to ensure reliable results. Consultation with a researcher that has experience with disease studies is recommended (see Appendix K).

The severity of clinical disease observed may prompt action even if only one or two sick animals are encountered. Animals that have substantially reduced muscle mass and significant weakness or lethargy probably will not live for very long. If such animals are encountered in the environment, they should be collected and held for examination by someone with extensive experience assessing reptile health. A decision may be made to humanely euthanize the animal so a comprehensive necropsy can be performed to determine the cause of the animal's illness. Appendix K also includes a list of veterinary pathologists with reptile experience. **It must be emphasized that it is extremely uncommon to find tortoises in this condition, and such animals may provide critical information regarding disease within the population.**

Warning signs found during gopher tortoise studies.

Skewed size class distributions

An important consideration when attempting to assess the health and viability of a gopher tortoise population is whether all size classes of tortoises are present in appropriate proportions (Alford 1980). Burrows provide a convenient mechanism to assess this issue because generally, the burrow size closely matches the size of the tortoise inhabiting it (Martin and Layne 1987; Doonan and Stout 1994). The presence of a wide variety of burrow sizes provides evidence that all life stages are present, including immature, young adults, and large, older adults. Potential signs of a problem include a lack of burrows for immature size classes, few burrows for large adult tortoises, or if most of burrows are the same size (within 3 or 4 cm). However, to detect such a shift in size class distributions, a systematic burrow survey must be conducted. Additionally, the experience of the observers, the density of the vegetation, and the proportion of habitat sampled will all influence the likelihood that juvenile burrows in particular will be detected. Therefore, if the juvenile size class appears to be missing from the population, the expected sensitivity of the techniques used must be considered. If juveniles really are missing from the population, then the land manager needs to consider all of the potential reasons they could be missing (e.g., poor substrate/soils, dense vegetation, increased predators, very low population density, poor adult health, etc.).

Low population densities

Gopher tortoise population densities vary substantially throughout the species geographic range (see the Draft *Gopher Tortoise Survey Handbook*, Meyer et al. 2008). A number of factors may contribute to low density, including poor habitat quality (e.g., due to habitat degradation), the site having marginal habitat for gopher tortoises (e.g., not the right type of habitat for tortoises), past human predation, or die-off events associated with a disease process. Land managers should attempt to identify potential causes of the observed low population density. Possible actions include a search for historic records on the tortoise population, an evaluation of historical aerial photographs to determine if there have been changes in land use or habitat quality over time, and importantly, an evaluation of soil maps to determine if appropriate soils occur in the area. Local biologists, naturalists, land caretakers or even hunters may be excellent sources of information. On site, field personnel should look for the presence of a large proportion of old, abandoned burrows or evidence of dead tortoises (e.g., shells or bone fragments). Restocking such sites may be desirable if the factors that are believed to have contributed to the low densities are no longer a problem or can be corrected by land management practices.

Investigating Mortality Events or Disease Epizootics

The investigation of mortality events and disease epizootics requires more extensive training in infectious diseases and epidemiology. Most investigations focus on determining the cause of the outbreak, the extent of the outbreak, and sometimes identifying possible interventional strategies to minimize adverse impacts and spread of disease. This is labor intensive and requires extensive financial resources. At minimum, pathological examinations on sick and dying tortoises by a qualified pathologist, and health studies on normal animals within the population are needed. If such an event occurs, professional consultation with qualified personnel is recommended to determine the best approach given the available resources and status of the population at risk. Appendix K contains a list of researchers with expertise in reptile disease and pathology for reference.

5 Interpreting Results and Management Decisionmaking

Interpreting results

Data interpretation must be placed within the context of the overall goals, the questions asked, the limitations of the data collected, and the experience level of the individuals conducting the study. Although the collection of health related data from tortoises requires some level of basic training, interpretation and decisionmaking based on the results requires a thorough understanding of normal tortoise biology and the health assessment tools used. For example, if clinical signs were observed in tortoises when conducting physical examinations, to interpret the findings one must be aware of the severity of the clinical signs (relative to normal), potential causes for the signs, and their biological significance. Alternatively, if clinical signs are not observed, managers must recognize that many diseases have subclinical periods (e.g. times when clinical signs are not exhibited) or have clinical signs that are intermittently expressed. Thus, although physical examinations are extremely useful, they do not provide conclusive evidence about the overall health of the individual tortoise.

There are a number of important considerations when interpreting results of diagnostic tests. For serological tests that detect antibodies, a positive result indicates that the tortoise has been exposed to a given pathogen, but it does not tell you if the tortoise is currently infected. The use of assays that have been validated properly and have had positive results correlated with pathological lesions is important to help make decisions regarding the significance of a positive test. If hematology or biochemistry data are being used, one must be able to discern normal values from abnormal, and additionally must understand the influence of season, reproductive status and age on the test results. In particular, awareness of the limitations of the established sampling schemes, sample sizes and methods selected for the project, as well as limits of the diagnostic tests are all very important considerations.

Management Decisions

A variety of management strategies are available to improve tortoise health and limit disease spread, but are specific to given situations and are beyond the scope of this manual. Most management decisions, whether based on the results of diagnostic assays or other scientific endeavors, will have some degree of uncertainty associated with them. Management decisions must be made with a clear understanding of these limits. The establishment of clear objectives will assist greatly in weighing the importance of the limitations and facilitate better management decisions based on findings of the study. It is important to note that definitive or clear cut answers may not be available, and therefore judgments must be made based on the best available scientific information at the time. A strong commitment to follow-up monitoring with well-designed research programs will be critical to document results from management decisions, and adapt conservation strategies as deemed necessary.

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Glossary and Acronyms

Anemia:
A reduction in the number or volume of red blood cells in the blood.

Antisepsis:
The elimination or inhibition of the growth of microorganisms (except bacterial spores) on living tissue (i.e., skin).

Beak:
An external anatomical structure with a keratinized, horny covering which serves as the mouth in some animals.

Biochemistry panels:
A number of tests relating to the chemistry of a living organism and vital processes that occur in living cells. Tests provide information regarding the function of major organ systems and metabolic processes.

Biological samples:
Samples collected from living organisms or their products (i.e., blood, discharges, etc.).

Catastrophic event:
A sudden, short-lived, violent event that has a profound impact on a population (i.e., hurricane or other natural disaster, substantial population mortality associated with a disease, etc.).

Chelonians:
Belonging to the order Chelonia, which includes the turtles and tortoises.

Complete Blood Count (CBC):
A series of tests that assess the quantity of each type of blood cell in a sample of blood, often including the amount of hemoglobin and the proportions of red blood cells and various white blood cells. Tests provide information regarding the immunological status of the animal and function of specific organ systems.

Chronic disease:
A disease with slow progression and a long duration.

Cleaning:
The physical removal of organic debris (dirt, feces, urine, blood, etc.) from an inanimate object or living tissue.

Clinical sign:
An abnormality observed during an examination of an animal; similar to a symptom, which is an abnormality observed in a human.

Cluster sampling:
A method of selecting groups of individuals to sample. When the population to be sampled occurs in clusters, random clusters are selected for study.

Debilitation:
Being in a state of severe weakness; lack or loss of strength.

Disease:
An abnormality in structure or function of a living organism. May be identifiable with clinical signs or may be subclinical (i.e., with no outward visible signs).

Disinfection:
The elimination or inhibition of the growth of microorganisms (except bacterial spores) on inanimate objects.

Donor population:
Population of tortoises supplying individuals for relocation.

Dyskeratosis:
Abnormal development of keratin cells in the shell and skin of tortoises. Condition is believed to be caused by exposure to toxins or due to a nutritional deficiency.

ELISA (Enzyme-Linked ImmunoSorbent Assay):
A biochemical technique used to detect the presence of specific antibody or antigen in a blood sample.

Epidemiology:
The frequency and distribution of disease in populations and the detection of the source and cause of disease.

Epizootic:
A disease that affects a large number of animals at the same time within a particular region or geographic area.

Etiologic agent:
The cause or origin of a disease.

False positive:
A test result that is read as positive but actually is negative; a test that shows evidence of a disease when it not present.

Goal:
A broad statement of a condition or accomplishment to be achieved in the future; goals may be unattainable, but provide direction and inspiration. A goal is normally expressed in broad, general terms and is timeless in that it has no specific date by which it is to be completed.

Hematology:
The study of the nature, function, and diseases of the blood and of blood-forming organs.

Immunological:
Pertaining to immunology, or the study of all aspects of the immune system, immunity from disease, the immune response, and immunologic techniques of analysis.

Incubation:
The maintenance of control over temperature, humidity, and oxygen concentration in order to provide optimal conditions for growth and development of an organism.

Incubation period:
The time period between invasion of the body by a pathogen and development of the initial signs of disease. This period may range from days to years, depending on the type of disease.

Keystone species:
A species whose existence and function within an ecosystem affects other species within the system.

Lymph:
A yellowish, transparent fluid that is composed of water, plasma proteins, chemical substances and lymphocytes (a type of white blood cell).

Lymphatic vessels:
The vessels that collect lymph from the tissues and transport it to the bloodstream.

Metabolic disease:
A disease in which normal body metabolism is altered resulting in an excess, absence or shortage of specific enzymes or substances needed for energy balance.

Microorganisms:
An organism of microscopic or submicroscopic size, including bacteria, viruses, fungi and protozoa.

Morphometric measurements:
The measurement of forms and shapes (i.e., for these purposes, the measurement of tortoise body shapes, including plastron /carapace length, body thickness, body width, plastral concavity, and other body measurements).

Naïve populations:
A population that has not be subjected to a pathogen of interest.

Nares:
Term for the openings of the nasal cavity in reptiles and birds; nostrils.

Necropsy:
The examination of an animal’s body after death; similar to an autopsy, which is an examination of a human’s body after death.

Objective:
A measurable, time-specific statement of results that responds to pre-established goals. An objective forms the basis for further planning to define the precise steps to be taken and resources to be used.

Off site:
Referring to populations not on the site of interest or study.

On site:
Referring to a population on the site of interest or study.

Packed cell volume (PCV):
The percentage of red blood cells relative to the total volume of blood in the sample.

Pathogen:
Any disease producing agent, includes viruses, bacteria and other microorganisms.

Pathology:
The study and diagnosis of disease through examination of organs, tissues, cells and bodily fluids.

Periocular:
Area around the eye.

Physiological:
Pertaining to physiology, or the study of the physical and chemical factors and processes associated with the normal functioning of a living organism.

Prevalence:
A ratio of the number of cases of a disease to the number of individuals at risk in the population for a given time period.

Random sampling scheme:
Randomly selecting portions of the population to sample, such that each individual has an equal chance of being selected.

Recipient population:
The population receiving individuals.

Rescue relocation:
Relocation of individuals/populations for a humane purpose.

Restocking:
Relocation for the purpose of replenishing a population.

Scute:
A bony external plate or scale, as on the shell of a turtle.

Serology:
Dealing with the immunological properties and actions of serum (i.e. blood); evaluation of antigen-antibody reactions in a laboratory setting to determine if an animal has been exposed to a specific pathogen.

Shock:
A life-threatening medical condition where blood flow to the body tissues is inadequate, often resulting in reduced oxygen and nutrient delivery to the tissues, and sometimes cardiac arrest (the heart stops beating).

Sterilization:
The complete elimination or destruction of all forms of microorganisms (including spores).

Stratified random sampling:
A method of sampling where the population is first divided into distinct subsets based on specific criteria, and then each subset is sampled randomly. The purpose is to ensure that there is proportional representation of each subset in the final sample based on a prearranged schedule.

Subclinical:
Not showing characteristic clinical signs or symptoms.

Total Protein (TP):
A rough measure of all the proteins found in the plasma portion of the blood.

Total surveys:
A complete census survey of the entire study area.

Variable Strain Virulence:
Differences in disease severity caused by different strains of a pathogenic microorganism.

Upper Respiratory Tract Disease (URTD):
An illness caused by an infection which involves the upper respiratory tract (nasal passages, nasopharynx, pharynx, larynx, and extrathoracic trachea). Can be caused by several bacteria, viruses, or fungi.

Appendix A: Health Monitoring for in-Situ Populations

When initiating a project that involves the assessment of tortoise health, it is necessary to develop overall goals and specific objectives for the project and the tortoise population of interest. The goals for the project, available financial resources, and importance of the tortoise population will help determine the specific objectives and consequently, which components of the health assessment will be utilized. If the objective is to perform an initial, cursory assessment of the tortoises within a given population, then physical examinations may be all that is necessary. Additionally, health assessment needs may be minimal if regular prescribed burning is the management activity being done. However, if the health monitoring is being done because dead tortoises were found, or if a management activity will occur that may have negative consequences, then more detailed health assessments will be necessary to provide meaningful data for decisionmaking. Table A1 provides example objectives, suggested health assessment components, skills necessary to perform different tasks, expected results from the different types of studies, and examples of how the data may be applied. The reader is referred to Appendix I for a description of specific diagnostic tests and definitions of acronyms.

Table A1. Gopher tortoise health monitoring objectives.

Goals	Resource Needs	Health Assessment Components*	Skill level needed	Expected Results	Application of Data
Comprehensive Health Study	Greatest	• Physical Exams • CBC/Chemistry • Serologic Tests • Parasite Exams • Other diagnostics for infectious diseases	• Sample collection-basic training • Interpretation of results – veterinary training	• Detailed health and disease information • Extensive baseline data for monitoring long-term population health.	• Long-term monitoring • Predict disease impacts • Develop responsive management strategies
Health Screen	Moderate	• Physical Exams • PCV/TP • ± Disease diagnostics	• Sample collection-basic training • Interpretation of results – veterinary consultation	• Basic/preliminary health and disease information.	• Long-term monitoring • Direct future research
Disease/Pathogen Survey	Moderate	• Physical Exams • Specific disease diagnostics	• Sample collection basic training • Interpretation of results – understanding of normal tortoise exam findings, diagnostic test results, test limitations, and basic statistics	• Baseline information on physical exam parameters. • Presence/absence data or pathogen/disease prevalence within population (depends on extent of study)	• Long-term monitoring • Direct future research
Cursory physical examination	Minimal	• Physical Exams	• Data collection-basic training • Interpretation of results-understanding of normal tortoise exam findings	• Baseline information on physical exam parameters. • May detect outward signs of disease.	• Long-term monitoring

Appendix B: Health Monitoring for Gopher Tortoise Relocations

Although the preservation of gopher tortoise populations *in situ* (i.e., within their original habitat) is always preferred, there is a substantial drive to relocate displaced individuals for restocking or humane purposes. In many urbanizing regions, removing tortoises from the path of development is necessary or even mandated. Relocation carries with it the inherent risk of exposure to infectious diseases for both recipient and donor populations. The conditions, goals, disease concerns, and suggested health assessment needs must be determined before the project is initiated. Again, establishing clear goals for the relocation will facilitate the planning and subsequent decisionmaking process. The purpose of this document is to provide a potential framework for dealing with the threat of infectious diseases for tortoise relocation. Mycoplasmal upper respiratory tract disease (URTD, or mycoplasmosis) is one of only a few diseases for which substantive data exist regarding wild populations and is used here as a model. However, this approach may be applicable for any infectious disease where diagnostic capabilities exist. The tables that follow are conceptual matrices, and any relocations performed using such an approach ideally should be coupled with monitoring protocols to evaluate the outcome of relocation. Further, such plans should be dynamic and easily modified as new scientific information becomes available.

Several categories of recipient populations are defined for this discussion (Table B1). It is implied that the existing tortoise populations on these sites are below a target density or the site would not be a candidate for relocation.

1. Highest conservation value: Critical Populations
   a. established population at low densities
   b. established population at minimal densities or non-existent
2. Conservation value with no access to established tortoise populations
3. Conservation value with access to established tortoise populations on or off-site
4. Minimal conservation value with access to established tortoise populations on or off-site
5. Minimal conservation value with no access to established tortoise populations (humane or rescue relocation)

Table B1. Recipient population conditions, goals, disease issues, and suggested health assessment needs.

Recipient Population	Established or adjacent populations	Goals	Disease an issue?	Health Assessment Needs
Highest conservation value	Yes	Healthy populations; minimize risks to adjacent/ existing populations	Yes — can impact both recipient and donor populations	Maximum on both donor and recipient populations. Monitor for success.
Highest conservation value	No	Healthy populations	Yes — due to established conservation goal	Maximum. Monitor for success.
Moderate conservation value	Yes	Healthy populations; minimize risks to adjacent/ existing populations	Yes — can impact both recipient and donor populations	Moderate, or based on land manager's guidelines and risk to adjacent populations.
Moderate conservation value	No	Site specific	Questionable — depends on goals and site specifics	Based on land manager's guidelines. Monitor for success.
Minimal conservation value	Yes	Humane or rescue relocation. Minimize risks to adjacent/ existing populations	Yes — can impact recipient and/or adjacent populations	Moderate or based on land manager's guidelines and risk to adjacent populations.
Minimal conservation value	No	Humane or rescue relocation.	No	Low. Based on land managers guidelines.

Table B2 below provides a disease risk matrix for the relocation of tortoises on the basis of disease prevalence. A significant limitation of this approach is that it does not address the possibility that pathogens may differ in their ability to cause disease (i.e., pathogens with variable strain virulence), which can occur with any infectious disease. There is presently inadequate scientific information to incorporate strain differences into such a matrix, however. Further, this approach only considers one infectious disease, and, clearly, there are other pathogens that can impact tortoise populations. Very little is known about infectious diseases in free-ranging gopher tortoise populations. Die-off events are likely multi-factorial (i.e., due to many causes) in nature and, therefore, more comprehensive investigations incorporating detailed pathological studies are required to better understand these complex processes. For these reasons, it is critical that a strong commitment to continued research and monitoring be incorporated into gopher tortoise management plans to increase our knowledge base regarding infectious diseases and also to follow the outcome of relocations made on the basis of specific disease prevalence.

Table B2. Disease risk for the relocation of tortoise populations.

	Recipient Population (RP)		
Donor Population (DP)	Negative or Low Prevalence (RP)	Moderate Prev. (RP)	High Prev. (RP)
Negative or Low Prevalence (DP)	Least Risk	Mod: DP Low: RP	High: DP Low: RP
Moderate Prevalence (DP)	Mod: RP Low: DP	UNKNOWN RISK (presumed low)	High: DP Low: RP
High Prevalence (DP)	High: RP Low: DP	High: RP Low: DP	UNKNOWN RISK (presumed low)

Appendix C: Population Surveys, Sampling Schemes and Sample Size Concerns

This appendix provides a brief overview of important considerations for surveying gopher tortoise populations, the use of sampling schemes to ensure that a representative sample is collected, and a coarse guideline for estimating sample sizes to detect the presence of a disease within a population. The methods used to collect these data will strongly influence your results, and therefore, it is important to have a clear understanding of the strengths and limitations of your approach. Detailed discussions of each topic are beyond the scope of this manual and therefore, the appendix only provides introductory information. The reader is referred to specific references to obtain more detailed information when needed. Consultations with biologists and perhaps even statisticians and veterinarians are necessary for large projects to ensure that you obtain meaningful results.

Surveying the population

This topic has been addressed in a separate manual (see the Draft *Gopher Tortoise Survey Handbook*, Meyer et al. 2008); however, different methods may be more suitable when surveying to estimate population densities versus assessing the health of a population. The distance method recommended in the survey handbook does not provide the location of burrows within the site, except along transect lines. If a systematic health screen or more detailed study is to be performed, specific plans will need to be developed for population surveys, appropriate tortoise sampling schemes and sample sizes needed to meet your goals. For example, if the sampling objectives are to detect simple presence/absence of a pathogen (or exposure to a pathogen) within a population, a stratified random or random sampling scheme using burrows identified during line transects will usually meet the needs of the project. However, if the goals are to determine the distribution of a disease throughout a site, to identify a high risk region for exposure within the site, or to investigate the epidemiology of a disease within a given population, substantially more detailed survey techniques are required, such as total surveys. For large tortoise populations, it is usually not practical to conduct total surveys across the entire population and, consequently, smaller areas are selected for more intensive study.

Surveys for dead tortoises should be conducted at the same time that field crews are searching for burrows. Conducting dead tortoise surveys simultaneously not only maximizes the use of staff and field time, but also ensures a systematic and consistent approach towards these surveys. Field staff should geo-reference the position of tortoise remains and examine the remains for evidence of predation, trauma, and other lesions. The approximate time since death can be estimated using established methods (Dodd 1995). Shells should either be removed or marked with paint or flagging so they are not counted twice. Adult tortoise mortality rates are quite low; therefore, visual observation of fresh shells on a site provides direct mortality information and may provide early warning signs of disease. Similarly, the presence of older shell remains and bone fragments may indicate prior disease or even past human predation on the site. This type of information is very useful, particularly if tortoise population densities are lower than anticipated.

Sampling schemes

If tortoises are being relocated, then presumably all tortoises will be removed from an area, and sampling schemes will not be an issue. But if tortoises are being studied as part of a monitoring program, it is important to establish an appropriate sampling scheme to ensure that a representative sample is selected from the population of interest. The most common sampling schemes include simple random sampling, stratified random sampling, cluster sampling, and systematic sampling (Thompson 1992). Each scheme has advantages in different situations. For example, when investigating a disease outbreak, it may be most productive to use cluster sampling in the area where the majority of clinically ill or dead animals were observed. Then, work outward concentrically to determine the extent of the disease. The reader is referred to a basic statistics textbook (Lohr 1999; Scheaffer et al. 1995; Thompson 1992) for descriptions of each type of sampling scheme to determine which technique will best suit the project objectives.

Sample size calculations

A critical component of any project is ensuring that enough subjects can be studied to satisfactorily address the project objectives. Establishing appropriate sample sizes for the study will include an evaluation of what practically can be accomplished given the study limitations (time, funding, etc.), and decisions regarding the level of statistical confidence needed for the study (i.e., the probability that conclusions from the data are true).

Table C1 shows sample sizes needed to detect a single diseased animal in a population with a specified prevalence of disease. For example, suppose 25 percent of the animals in a population are infected with a given pathogen (i.e., 25 percent prevalence). Then a sample of eight animals will have a 90 percent chance (i.e., 90 percent power) of containing at least one diseased animal. Calculation of this probability assumes that: (1) the population is large (say, 1000 or more animals), and (2) that the sample is randomly drawn from the population. The detection probability is greater with smaller populations.

In most cases, the true prevalence of disease within a population will be unknown at the onset of a study. Such tables are valuable if the goal is to determine that a disease is present at or below a certain target level. However, if the study objective is to determine the prevalence of disease within a population, then larger sample sizes will be required. Consultation with a statistician or a biologist with statistical experience is recommended in those circumstances.

Table C1. Estimated sample size required to detect a single positive animal (i.e., using a diagnostic test) for a given prevalence of disease and statistical power.

		Prevalence		
Power	35%	25%	15%	5%
0.8	4	6	10	31
0.9	5	8	14	45
0.95	7	10	18	58
0.99	11	16	28	90

Appendix D: Disinfection and Sanitation.

Caution must be used whenever handling or sampling gopher tortoises to ensure that pathogens (i.e., disease causing microorganisms) are not introduced to the site through contaminated equipment. Further, field personnel must take necessary precautions to ensure that they do not aid in the unintended transmission of pathogens among the individual tortoises sampled. Therefore, development and implementation of a step-by-step disinfection protocol is essential for field studies. An effective disinfection protocol must address the microorganisms being targeted, the characteristics of the disinfectant, and the impact a disinfectant may have on the environment. Furthermore, the health and safety of field personnel and gopher tortoises is also of vital importance.

A number of products are available for disinfection/sanitation purposes. The most common antimicrobial products fall within one of the following classes: alcohols (i.e., hand gels), chlorine (i.e., bleach), iodine/iodophors (i.e., povidone iodine), chlorhexidine (i.e., VikronS), oxidizers (i.e., Nolvasan), phenols (i.e., Lysol), quarternary ammonia (i.e., Roccal), and aldehydes (i.e., Wavicide). Each has varying effectiveness for different classes of microorganisms and the reader is referred to the University of Nebraska — Lincoln Extension website entitled, 'Selection and Use of Disinfectants' for more information to aid in the selection of an appropriate product to meet the needs of his/her project.* The effectiveness of any disinfectant or antiseptic is determined by the concentration of the product used, the organic load (amount of dirt/debris), the level of microorganism contamination, the condition of the object being cleaned (cracks, crevices, wood vs. plastic surfaces), the amount of time that the cleaning agent is allowed to contact the surface, ambient temperature, and sometimes the environmental pH. In field conditions where high organic loads are almost always present, effective antisepsis and disinfection are not possible without first cleaning to remove excessive debris. Therefore, regardless of the agent used, an initial cleaning is required.

The sample protocol listed below describes methods used for a gopher tortoise health study conducted in Florida. Certainly, other protocols may be

* Available through URL: http://www.ianrpubs.unl.edu/epublic/live/g1410/build/g1410.pdf

developed to meet the needs of the specific project or management goal. Users should follow local and state regulations for transport and disposal of disinfectant solutions.

Sample Protocol for Disinfection Solutions*

Standard solution: 1:20 dilution of 5 percent household bleach in water.

Stronger solution: 1:10 dilution of 5 percent household bleach in water. This solution is used to disinfect traps/equipment between sites and equipment contaminated with high organic loads (i.e., dirt, feces, etc.).

Protocol for Equipment

All equipment and work surfaces must be cleaned before and after handling each tortoise. Efforts should be made to first remove organic debris (i.e., dirt, feces, etc.) by rinsing the area with water, brushing the area off with paper towels, or cleaning the equipment/work surface with the standard bleach solution and wiping with paper towels. The equipment should then be soaked with the standard disinfection solution and allowed to air dry. The exception is metal equipment, which can be rinsed with water after approximately 5 minutes of contact time with the cleaning solution.

Alternatively, drill bits and small metal equipment can be placed in a bath containing a non-corrosive instrument disinfecting solution (e.g., Nolvasan) prepared according to the manufacturer's recommendations. Between tortoises, instruments should stay in the bath for at least 10 minutes before being used on another individual. Having duplicate equipment available for use will minimize the inconvenience of this procedure. Instrument baths should be changed regularly and disposed of according to the label recommendations. Drills and any other large metal equipment that cannot be submerged in a bath should first be cleaned to remove organic debris (i.e., by rinsing with water or wiping with paper towels) and then drenched with an appropriate disinfectant (i.e., Nolvasan mixed at an appropriate dilution and stored in a spray bottle) and allowed to air dry.

Buckets and metal traps should also be disinfected between tortoises, even when working on a single site, so researchers do not aid in the transmission of pathogens. If traps will be immediately reused in the field at the

* Solutions should be stored in dark bins or in opaque bottles and should be made fresh regularly (i.e., daily or weekly depending on storage conditions). Bleach should be purchased in small bottles or dispensed into small bottles to minimize deterioration from opening/closing the lid.

same site, disinfectant solutions can be mixed in spray bottles or garden sprayers. Most gopher tortoise researchers carry tortoises back to a vehicle for collecting research data, and water/scrub brushes can easily be carried for cleaning equipment.

Between study sites, equipment, especially traps, are scrubbed using a dish soap and mild bleach solution (i.e., 1:20 dilution). All organic material must be removed using scrub brushes to ensure that the equipment is thoroughly cleaned. After rinsing with water, the 1:10 bleach disinfectant solution is sprayed on the equipment and allowed to air dry. This will reduce the chance of cross-contamination between study sites. For metal traps and equipment, a final rinse with water after 15-20 minutes of contact time with the bleach solution is recommended to minimize rust.

Protocol for Personnel

Gloves are to be worn, and changed between handling individual tortoises to prevent the transfer of pathogens from one tortoise to another. Wearing gloves additionally will protect field personnel from potentially irritating disinfectants. Hands must also be sanitized between every tortoise because often the tortoise's toenails tear the gloves. Acceptable sanitizing solutions include soap & water (if available) and a hospital grade ethyl alcohol hand wash (minimum alcohol concentration of 60 percent). Example product: Alcare Foamed Alcohol Scrub (Steris Co.). Removal of organic debris is essential for proper sanitation, and, therefore, a water rinse before using the product will be more effective if hands are extremely dirty. Alcohol hand washes should be allowed to air dry while rubbing hands vigorously to appropriately distribute the product.

Appendix E: Sample Data Sheet

Gopher Tortoise Data Sheet

Date: ______________________ Study Site: ______________________________________

Time Sampled: ______________ Tort. ID: ____

Soil: ☐ Wet ☐ Damp ☐ Dry

Captured: ☐ Trap Trap/Burrow #: ______________________________________

☐ Opportunistic

Description: Activity/Location (Walking, Basking, Eating, etc.):

____

Position (in trap): ☐ Face Down ☐ Face Up ☐ Flat ☐ Carapace Down (upside down)

Urine in trap (Yes/ No/ Unknown) Feces in trap (Yes/ No/ Unknown)

HEALTH ASSESSMENT

Behavior: ☐ Alert-responsive ☐ Quiet-responsive ☐ Depressed or Lethargic

Posture: ☐ Can withdraw tightly into shell ☐ Weak limbs ☐ Head drooping or limp

Breathing:

Normal ☐

Abnormal (Wet respiratory sounds / Difficulty breathing (forelimb pumping, head bobbing). ☐

Comments: ____

Site	Characteristic	Severity*	Description
Beak:	Dry / Damp / Wet / Sandy	______	Frothy saliva, food, other__________
Left Nare:	Normal / Nasal discharge	______	Clear/ Cloudy/ White/ Yellow/ Green; Sandy/ Occluded
Right Nare:	Normal / Nasal discharge	______	Clear/ Cloudy/ White/ Yellow/ Green; Sandy/ Occluded
Left Nare Shape:	Normal / Abnormal	______	Eroded/ Oblong/ Tear-drop shaped
Right Nare Shape:	Normal / Abnormal	______	Eroded/ Oblong/ Tear-drop shaped
Left Eye:	Normal / Abnormal	______	Discharge / Dull / Cloudy / Sunken / Sandy / Bulging
Right Eye:	Normal / Abnormal	______	Discharge / Dull / Cloudy / Sunken / Sandy / Bulging
Left Eyelid:	Normal / Swollen	______	Upper / Lower
Right Eyelid:	Normal / Swollen	______	Upper / Lower
Left Periocular:	Normal / Swollen	______	Upper / Lower
Right Periocular:	Normal / Swollen	______	Upper / Lower
Left Conjunctiva:	Normal/ Abnormal	______	Enlarged/ Red
Right Conjunctiva:	Normal/ Abnormal	______	Enlarged/ Red

***(severity: 1=mild; 2=moderate; 3=severe)**

Lesions (cuts, scrapes, etc.) on beak, eyes, lids or periocular area: ______________________________

Mouth: ☐ Observed ☐ Not observed

☐ Mouth/tongue pink ☐ Mouth pale pink ☐ Mouth white ☐ Crusts & Plaques ☐ Discharge

Describe any abnormalities: ____

Ticks: Hard bodied #_________ (or) ☐ more than 10

Soft bodied #_________ (or) ☐ more than 10

Shell/ Body Injuries: ☐ Yes (Draw lesions on diagram) ☐ No

Source: ☐ Trauma ☐ Disease ☐ Unknown ____________________

Time Span: ☐ New Injury ☐ Apparently old, present for long time (chronic)

Location: ☐ Plastron ☐ Carapace ☐ Front limb ☐ Hind limb ☐ Head ☐ Neck ☐ Other

Lesion description (limbs/head/other): ____________________

Shell lesion distribution: **C**=carapace; **P**=plastron

C P ☐ **Mild** (only at seams, covers < 10%)

C P ☐ **Moderate** (covers 11-40 %)

C P ☐ **Severe** (covers > 40%)

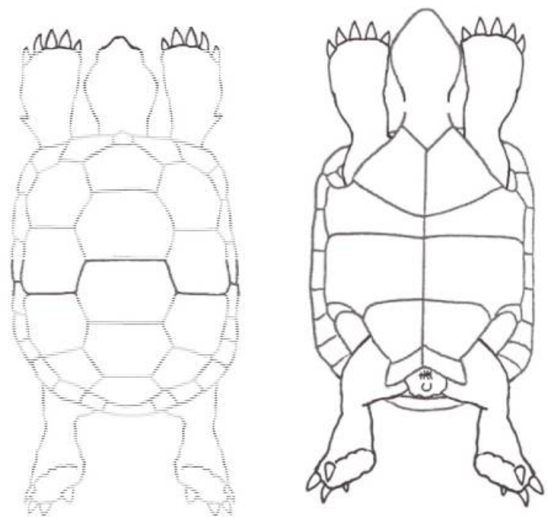
Shell lesion severity:

C P ☐ **Mild** (lightly discolored at edges, little flaking)

C P ☐ **Moderate** (several layers of laminae discolored, more flaking, small superficial holes)

C P ☐ **Severe** (scutes eroded or missing, bone exposed)

Other comments: ____________________



Carapace View Plastron View

BODY MEASUREMENTS (in mm)

Total Body Length: ____________________

Carapace Length: ____________________

Plastron Length: ____________________

Max. Body Width: ____________________

Max. Thickness: ____________________

Anal Width: ____________________

Anal Notch: ____________________

Plastron Concavity: ____________________

Plastron Rings: ____________________

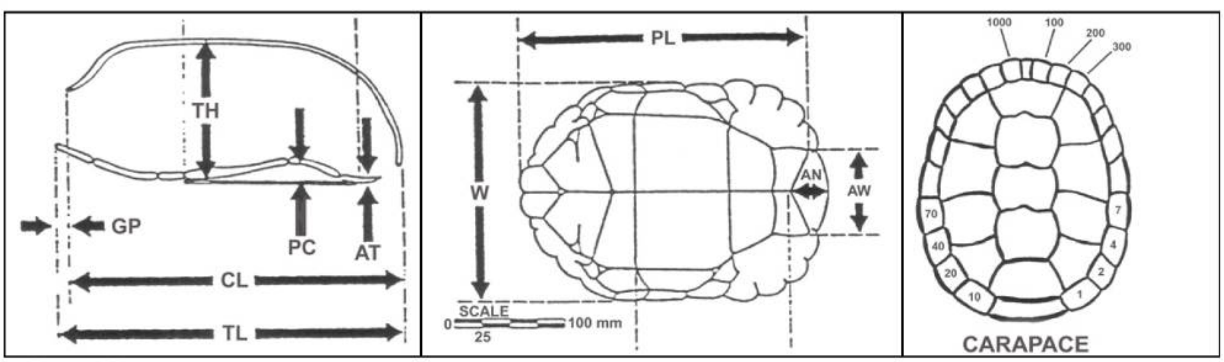
Sex: Male/ Female/ Adult Unknown/ Juvenile

Body Weight (kg): ____________

☐ Urinated (Before/After weight measured)

☐ Defecated (Before/After weight measured)

☐ Too worn to tell



Checklist:

☐ **Blood sample/smears** ☐ **Photos** ☐ **Ticks Collected**

☐ **Nasal flush** ☐ **Radiographs** ☐ **Data sheet complete**

☐ **Tortoise Marked** ☐ **Fecal Sample** ☐ **Reviewed by Sup.** _____

Appendix F: Physical Examinations

Physical examinations provide valuable information about the health of individual tortoises. Because gopher tortoises are a long-lived species, it is extremely important to use standardized techniques for data collection so that information can be compared over time. This appendix provides a description of how to conduct a basic physical examination of a gopher tortoise and follows the order of the sample data sheet. We have found that it is important to complete certain portions of the physical exam first so potential clinical signs are not affected by handling of the animal. For example, turning a tortoise on its back for measurement of plastron length is stressful to the animal, and this should be done after the tortoise is examined for clinical signs of disease. Therefore, we use the order described below for a systematic evaluation of every animal. This approach also helps field staff remember to consistently collect all of the data, because they fill out the form as they proceed with the exam.

A thorough and accurate physical examination can only be performed if the individual performing the exam has knowledge of normal tortoise physical appearance and behavior. Prior training by someone with experience assessing tortoise health is required. Otherwise, the collected data will have less value and will have to be interpreted with caution. This appendix provides an overview for conducting examinations but is no substitute for appropriate training and hands-on experience. Photographs in Appendix G (p 51) show many of the clinical signs described below for additional reference.

1. **Overall posture/behavior:** (Knowledge of tortoise behavior is required to discern between normal/abnormal).
    a. *Alert and responsive or quiet but responsive.* These two categories identify behavioral characteristics of normal tortoises. Alert/responsive tortoises paddle their forelimbs when held, attempt to escape, and repeatedly retract into their shell when handled. Quiet/responsive tortoises are shy and tend to remain retracted into their shell when being handled, but they have normal strength.
    b. *Depressed and lethargic.* These animals may hang forelimbs limp when lifted, may have poor muscle mass, are weak, and do not resist gentle tugging on their limbs.
    c. *Ambulate (walk/move)* — normally/abnormally.

d. *Breathing sounds (normal, congestion, distress)*. Tortoises may normally create a very faint, high-pitched whistle when expelling air out of their nares. Wet, crackling, or gurgling sounds associated with congestion are abnormal, and pumping forelimbs up and down symmetrically when breathing may indicate pneumonia or other causes of respiratory distress. Open-mouthed breathing may also be a sign of respiratory distress, although this must be distinguished from occasional 'gaping' that stressed tortoises may do.

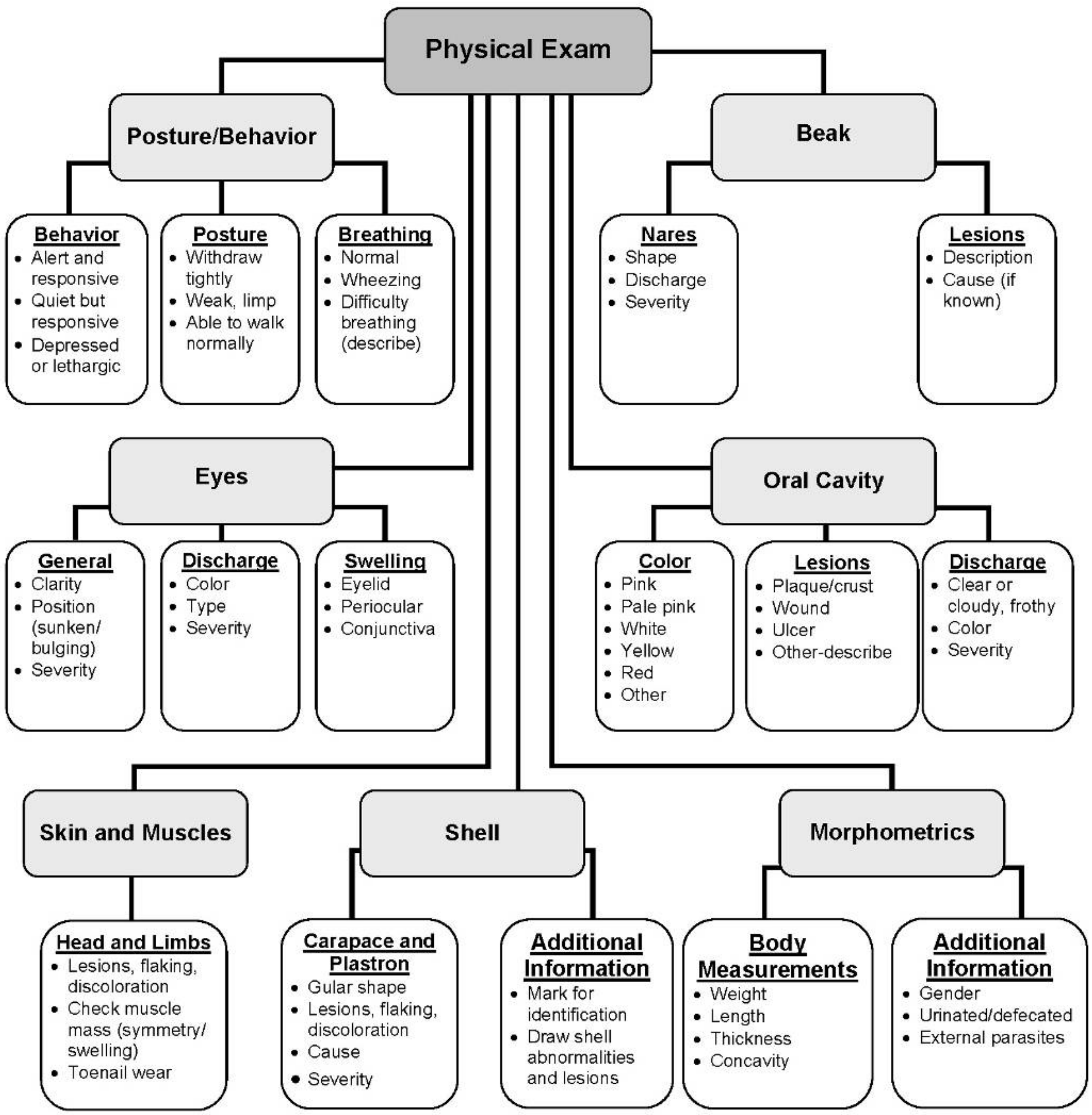
2. ***Examine beak***
   a. Evaluate the nares (nostrils)
      (1) Discharges — clear/watery or cloudy (purulent); describe color of discharge and characterize the amount as mild, moderate, or severe. Note if dirt or debris is adhered to or obstructing nares. Check the inside edge of forelimbs for the presence of nasal discharge that the tortoise has wiped off from its nares.
      (2) Erosion or irregular shape of the nares (provides evidence of long term discharges).
   b. Evaluate beak for fractures or malocclusion (jaw misaligned).
3. **Examine eyes** (see Berry and Christopher 2001 for diagrams). May need a flashlight or, in some cases, magnification to examine.
   a. Clarity of eye (cornea and lens), position of eye within orbit (i.e., is eye bulging or sunken into orbit?).
   b. Discharges — clear/watery or cloudy (purulent); characterize as mild, moderate, or severe and describe color.
   c. Examine eyelids, conjunctiva (third eyelid), and periocular area (area around the eye) — look for swelling, redness, or traumatic wounds (i.e., lacerations). Characterize severity as mild, moderate, or severe.
4. **Examine oral cavity** — Tortoises will often open their mouths with gentle but consistent downward pressure on the lower jaw. Do not force the mouth open as injury could occur. If collecting biological samples, tortoises will sometimes open their mouths during the process, and quick exams of the mouth can be done then.
   a. Presence of food
   b. Color of tongue and mucous membranes on the inside of the mouth (Note color: pink, pale pink, white, pale yellowish, deep red).
   c. Discharges (clear, frothy, yellow) — tortoises often have a frothy oral discharge when stressed and this should not be interpreted as abnormal.
   d. Lesions

(1) Ulcers (raw areas), plaques (white or tan crusts adhered to tongue or mucous membranes), lacerations, or foreign bodies (usually plant material).

5. **Examine skin and musculature**
   a. Excessive flaking, discoloration of the skin, wounds, scars or evidence of prior injuries.
   b. Evaluate muscle mass on head and limbs to look for muscle loss or atrophy.
   c. Check to make sure the limbs are symmetric, look for swollen areas or malformations (especially around joints), and check toenails for symmetrical wear patterns.
   d. Note the presence of external parasites
      (1) Ticks, mites, fly larvae (maggots).
      (2) Number, species of ectoparasites (i.e., < or >10).
6. **Examine shell** (seams and scutes) — see Berry and Christopher (2001) for examples.
   a. Look for flaking, discoloration, defects/erosions, soft areas, fractures, and chew marks.
   b. Note the distribution and severity of lesions; describe the lesions.
   c. Photographs and drawings are extremely useful.
7. **Body measurements (morphometrics) and tortoise identification**
   a. Standard body measurements — recommend following protocol by McRae et al. (1981) and taking body weight.
   b. Note whether the tortoise has urinated/defecated or if you can palpate eggs in females as these may significantly affect body weight. Measurements may be used to assess body condition (Nagy et al. 2002).
   c. Depending on the goals, tortoises may be marked for identification by drilling holes or filing notches in the marginal scutes of the shell (Cagle 1939). Use of a standard numbering scheme is recommended, and detailed records should be kept to ensure that numbers are not duplicated. Alternative methods for identification are available (i.e., pit tags, tattoos, etc.), but have not been used extensively in wild gopher tortoise populations.

Appendix G: Physical Exam Flow Chart

Flow chart provides a quick field reference for conducting physical examinations on gopher tortoises. See Appendix F for a more detailed description of a physical examination, and Appendix H for photographs showing examples of clinical signs.



Appendix H: Photographic Examples of Clinical Signs Observed in Gopher Tortoises.

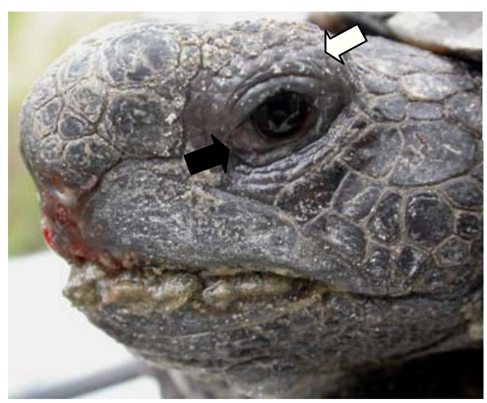
Photographs included in this appendix were taken as part of work supported by grants from the National Institutes of Health-National Science Foundation Ecology of Infectious Diseases program (DEB-0224953) and National Institutes of Health K08 award (5K08AI57722)

Figure H1. Frothy oral discharge in stressed tortoises. Such animals need to be assessed and released as quickly as possible to minimize their stress.

Figure H2. Lethargic tortoise that is weak. This tortoise let its legs and head hang limp and did not resist gentle tugging on its legs or manipulation of its head.

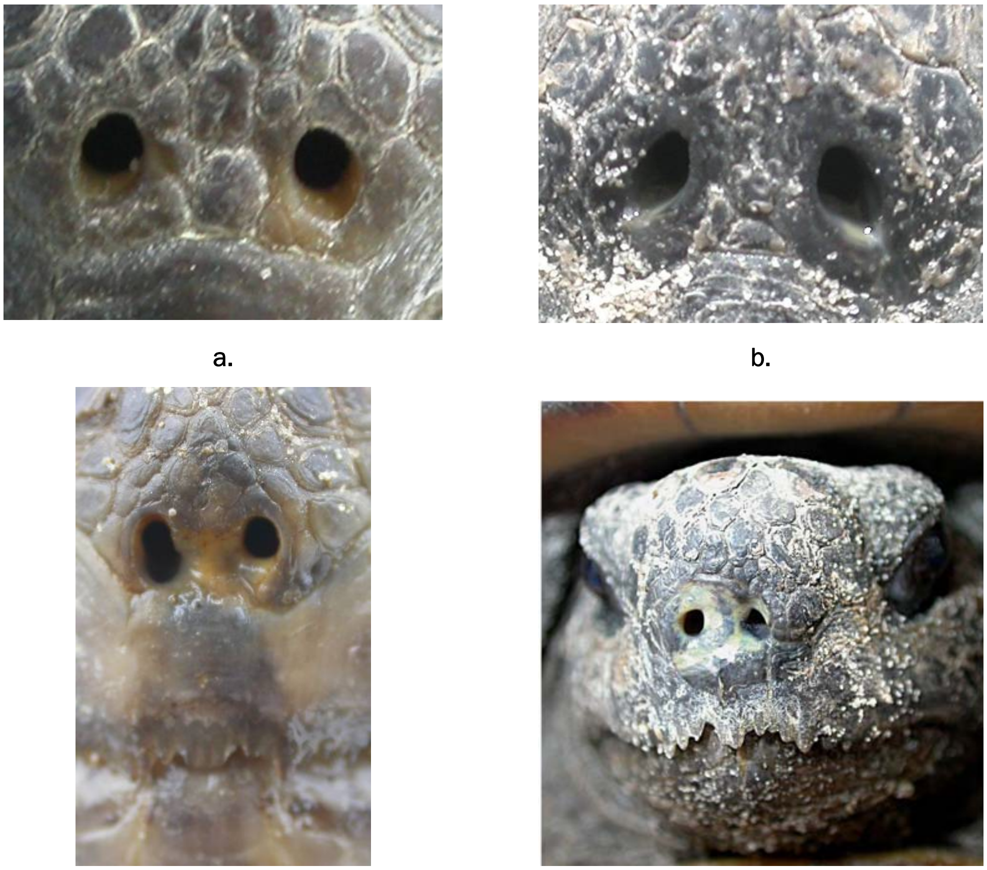
Figure H3. Tortoise with severely sunken eyes. This clinical sign may be an indication of dehydration or poor health.

a. b.



c. d.

Figure H4. Clinical signs consistent with upper respiratory tract disease, including nasal discharge, eye discharge, and conjunctivitis. Mild to severe nasal discharge is present in all four of these tortoises. Tortoises a, d, and d also have an ocular (eye) discharge. Tortoises c and d also have a condition called conjunctivitis (red/swollen third eyelid, solid black arrow shown on Tortoise d). Tortoise d has a severe, bloody nasal discharge and periocular swelling (white arrow). All four tortoises were positive by ELISA for exposure to *Mycoplasma agassizii* and Tortoises c and d were culture and PCR positive for the bacterium.



a. b.

c. d.

Figure H5. Close-up photographs of tortoise nares. This evaluation is important because tortoises with mycoplasmal URTD may only intermittently exhibit a nasal discharge. Irregularly shaped or eroded nares suggest chronic and/or recurrent nasal discharge. Tortoise a shows the appearance of normal gopher tortoise nares. Tortoise b shows mildly eroded or tear-drop shaped nares; a mild nasal discharge is also visible. Tortoise c shows a tortoise with moderately eroded nares and a mild nasal discharge. In some cases, grooves may be visible extending from the nares down the front of the beak or the skin around the nares may be depigmented. Tortoise d has severe erosion of the nares with secondary infection of the skin around the nares. In some cases, trauma to may contribute to the severity of these lesions.

a.

b.

c.

d.

e

Figure H6. Assessment of the eyelids. Trapping method can significantly affect eyelid swelling, and must be taken into consideration when interpreting these signs. Tortoise (a) is normal; (b) shows moderate swelling of the eyelids, and (c) and (d) have severe swelling of the eyelids and also has conjunctivitis; (e) has had a traumatic injury to its eyelid and beak (which are not clinical signs of disease).

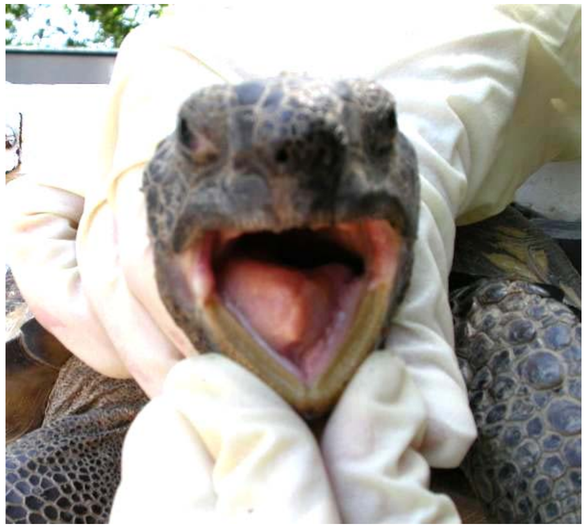


Figure H7. Gopher tortoises often will open their mouths with steady but gentle downward pressure on their mandible (lower jaw). This tortoise has normal oral mucous membranes that are pink and moist.

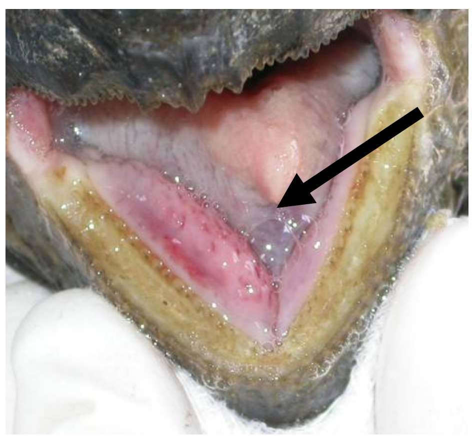
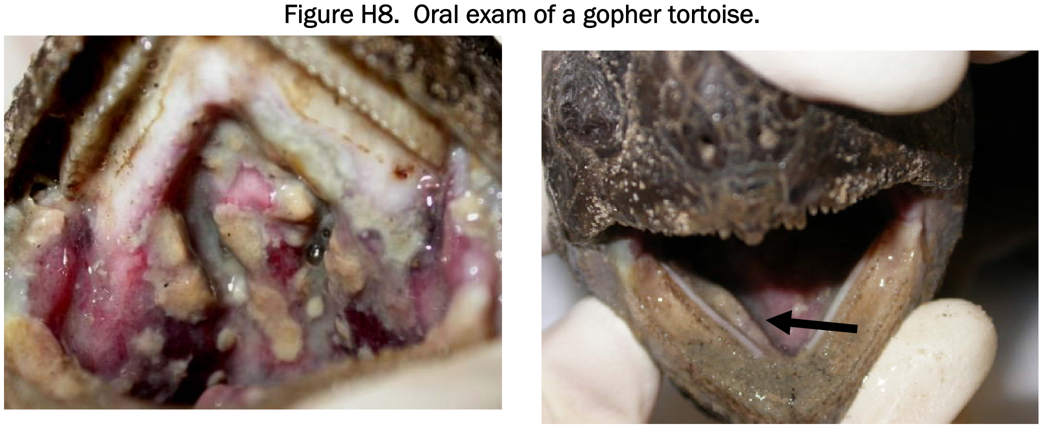


Figure H8. Oral exam of a gopher tortoise.



a. b.

Figure H9. Tan colored plaques on the roof of the mouth (left) and on the lower jaw over the salivary tissue (right).

Figure H10. When examining tortoises, the presence of extra or abnormal scutes should be noted (black arrow). Such notes may aid in future identification of the animal. This tortoise also had an old, healing fracture (white arrow).

a. b.

Figure H11. Shell lesions commonly observed in gopher tortoises. Although the specific cause of these lesions is unconfirmed, it is important to characterize the abnormality as much as possible. The discoloration and flakiness on Tortoise A starts at the scute margins and extend inwards, whereas the lesions on Tortoise B start in the center of the scute and extend outwards. Such information may become important later in diagnosing the problem. See Table 1 in the manual for potential causes.

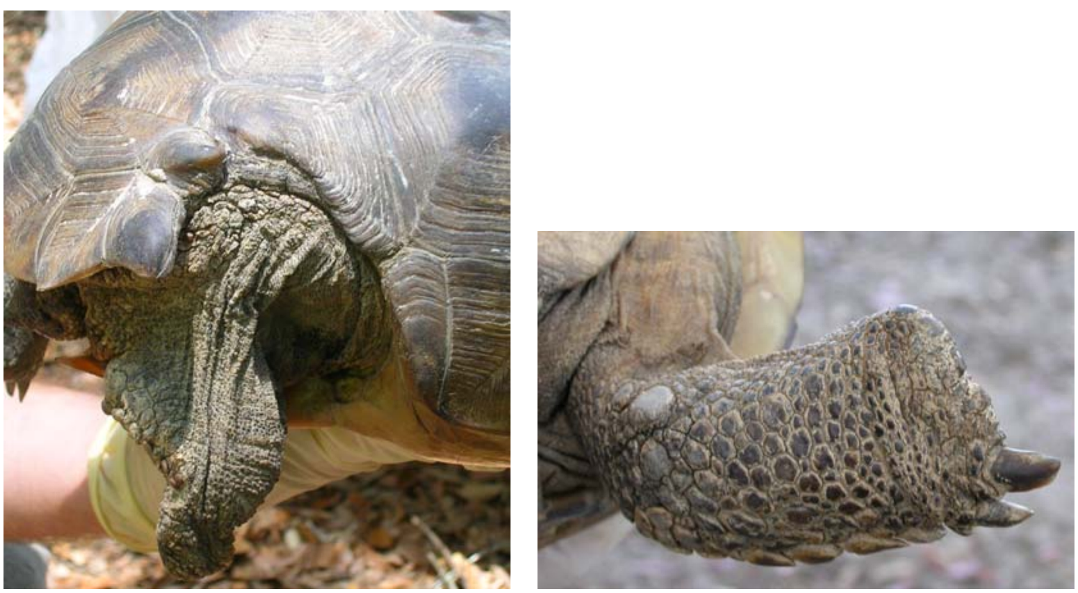
a. b.

Figure H12. Swollen forelimb (a) and hind limb (b). Both of these lesions were determined to be abscesses.

Figure H13. Predator related injuries – chewed gular scutes.

a. b.

Figure H14. Traumatic injuries to the shell. The fractures shown below are old, healing injuries, however, detailed exams should always be performed on these tortoises to evaluate for signs of internal infections (e.g., poor body condition, lethargy, open or draining wounds, presence of maggots).



a. b.

Figure H15. Severe injury that resulted in loss of part of the rear carapace and hind limb. Tortoises are occasionally found in the wild with missing limbs and appear to survive well; (b) shows a tortoise with broken toenails. Irregular toenail wear patterns may indicate that the tortoise has an injury to that limb.

a. b.

Figure H16. The most common tick species found on gopher tortoises is the gopher tortoise tick (Amblyomma tuberculatum). Tortoise (a) has an engorged tick on its nose. This species of tick is most commonly found attached to soft skin, e.g., Tortoise (b).

a. b.

Figure H17. Soft-bodied ticks (*Ornithodoros turicata*) are generally found in cracks in the shell or between scutes. Often dozens of ticks can be found in such crevices when examined closely.

Appendix I: Diagnostic Tests

Many diseases have an incubation or subclinical periods when tortoises may not exhibit any outward signs of illness. Such diseases may not be detected during regular physical examinations. Therefore the use of diagnostic tests will greatly complement physical exam data. Specific assays may actually detect a disease process before clinical signs develop. In some cases, the observation of a specific set of clinical signs in tortoises may prompt one to run diagnostic tests to try to determine the underlying cause of the signs.

Diagnostic tests vary in expense, and the specific tests chosen will depend on the project or management goals, available funding, and expertise of the personnel involved. It is important to note that hands-on training is necessary to learn appropriate sample collection and handling techniques, and even more expertise is required to interpret the results. Given the complexity of this topic, this appendix contains more technical information and medical terminology than the other appendixes.

This appendix is meant to provide introductory information about the most commonly used diagnostic tests, including brief descriptions of the benefits, value and limitations of the tests. It is not intended for use by land managers to specifically select diagnostic tests based on these brief descriptions. If the reader is considering the use of diagnostic tests, consultation with a veterinarian or researcher that has experience with reptiles is recommended to select the best tests to meet the needs of the project.

1. Diagnostic tests performed on blood/ blood products
   (**NOTE:** Lymph contamination of blood samples may greatly alter the results of ALL of these tests. Also, sample clotting and hemolysis (rupture of the red blood cells resulting in red colored plasma) may alter certain tests. Samples with lymph contamination or hemolysis should be clearly marked and interpreted cautiously).
   a. Packed Cell Volume (PCV)
      (1) Within a blood sample, this test provides an approximation of the percentage of red blood cells present in relation to the whole, unclotted blood sample.
      (2) Easy to perform; minimal expense and training required.

(3) Provides information about tortoise hydration and may help to detect an animal with anemia (a reduction in the number or volume of red blood cells in the blood).

(4) Limitations: provides valuable basic information but will not provide a cause for abnormal results.

b. Total Protein levels (TP)

(1) Usually done with PCV.

(2) Easy to perform; minimal expense and training required.

(3) Provides information about tortoise hydration and blood protein levels.

(a) Abnormal values may be due to a wide range of problems, including inflammation, parasitism, malnutrition, intestinal disease, and liver/kidney disease.

(b) Limitations: provides valuable basic information but will not provide a cause for abnormal results.

c. Hematologic tests (Complete blood count, CBC)

(1) Usually done with biochemistry panel; moderate expense.

(2) Sample collection easy, but sample analysis must be done by a qualified laboratory with experience analyzing reptilian blood.

(3) Proper sample collection and handling is critical (see Appendix I).

(4) Provides much more detailed information about physiological and immunological status of tortoise (i.e., organ function, hydration, presence of inflammation indicative of infection or some cancers). Underlying cause of abnormality sometimes may be determined.

(5) See discussion under biochemistry below for limitations.

d. Biochemistry panel

(1) Usually done with CBC (moderate expense).

(2) Sample collection easy, but sample analysis must be done by a qualified laboratory with experience analyzing reptilian blood.

(3) Provides much more detailed information about physiological and immunological status of tortoise (i.e., organ function, electrolyte balance, hydration, calcium/phosphorus balance). Underlying cause of abnormality sometimes may be determined.

(4) Limitations with CBC/Biochemistry:

(a) Reference ranges for gopher tortoises not well established.

i. Taylor and Jacobson, 1981. Comp Biochem Physiol, 72A: 425-428.

ii. Alleman et al., 1992. Am J Vet Res, 53:1645-1651.

iii. Christopher et al., 2003, J Wildl Dis, 39: 35-56. (desert tortoise)

iv. Christopher et al., 1999, J Wildl Dis, 35: 212-238 (desert tortoise)

(b) Wide ranges and a high degree of variability with results.

(c) Influenced by age, season, and gender; therefore, can be difficult to interpret without significant training.

e. Serological tests

(1) Sample collection easy, but sample analysis must be done by a qualified laboratory; moderate expense.

(2) For antibody tests, results indicate if the tortoise has been exposed to a pathogen; useful for pathogen survey studies.

(3) Limitations:

(a) Does not indicate if tortoise is presently infected with the pathogen.

(b) Few validated diagnostic tests available.

(c) Predictive values of assays are important for interpretation of results, but they are not widely available.

(d) *Mycoplasma agassizii* ELISA — validated for gopher tortoises, predictive values published (Wendland et al. 2007).

i. Limitations: Poor cross-reactivity with other mycoplasmal species that may cause URTD (*M. testudineum* ELISA is being developed and validated).

(e) Herpesvirus ELISA

i. Limitation: Developed/validated with Mediterranean Tortoises → unknown significance in gopher tortoises (Origgi et al. 2001).

(f) Iridovirus ELISA

i. Limitation: Developed/validated with red-eared sliders as a model. Controlled studies not done in gopher tortoises but several accounts of disease in gopher tortoises (Johnson 2006).

2. Diagnostic tests performed on discharges or lavage (flush) specimens

a. Aerobic/Fungal cultures (moderate expense)

(1) Swabs easy to perform in field (i.e., using culturette), but training required for proper sample collection techniques.

(2) Can culture any open lesion to identify microorganisms present.

(3) Cultures must be done by a qualified laboratory with experience analyzing reptilian samples.

(4) Limitations:

(a) Appropriate samples can be difficult to collect.

(b) May or may not identify the primary causative agent for the lesion, may only identify secondary bacterial/fungal infections.

b. Mycoplasma culture/PCR (moderate expense)
   (1) Improved results when tortoises have a nasal discharge.
   (2) Performed on a nasal flush sample.
   (3) Cultures must be done by a qualified laboratory with experience analyzing reptilian samples AND growing mycoplasmas.
   (4) Positive result indicates current infection.
   (5) Limitations: Problem with false negative results due to difficulty in growing the organism and obtaining good samples; few qualified diagnostic laboratories.

c. *Virus cultures/PCR* (moderate expense)
   (1) Performed on swabs of oral crusts/plaques.
   (2) Specific media/swabs required.
   (3) Cultures must be done by a qualified laboratory with experience analyzing reptilian samples AND with virus isolation.
   (4) Limitations: Problem with false negative results for some viruses due to intermittent shedding; few qualified diagnostic laboratories.

d. *Cytology* (moderate expense)
   (1) Discharge placed on glass slides for staining and microscopic evaluation.
   (2) Limitations: Provides description of discharge/inflammatory process but often cannot identify underlying cause.

3. Diagnostic techniques for parasitic infections

a. Enteric (Intestinal) Parasites
   (1) Samples easy to collect (tortoises often defecate when handled), and tests inexpensive to run.
   (2) Fecal flotation, sedimentation, and direct smears require training and experience in identifying parasites and ova (parasite eggs).
   (3) Provide information regarding parasite prevalence and diversity.
   (4) References
      (a) Hendrix, C.M. 1998. Diagnostic Veterinary Parasitology. Mosby, St. Louis.
      (b) Klingenberg, R.J. 1993. Understanding Reptile Parasites. Advanced Vivarium Systems, Lakeside, CA.
   (5) Limitations: Many parasite species are difficult to identify conclusively by ova, and many species are not described.

b. *Ectoparasites* (external parasites)
   (1) Ticks, mites or fly larvae (maggots).
   (2) Parasites can easily be collected in a tube containing 70 percent ethanol for identification using guides, or submission to an entomologist (most common species of ticks include *Amblyomma turbiculatum* and *Ornithodores turicata*).

4. Diagnostic tests that require a veterinarian for sample collection
    a. Biopsies and needle aspirates
        (1) Performed on swollen areas or skin/shell lesions.
        (2) Provide a description of the lesion and sometimes the underlying cause.
        (3) Expensive to collect and analyze.
    b. Euthanasia and necropsy
        (1) Provide critical information about the cause of severe illness in a sick tortoise.
        (2) Expensive and requires assistance from a pathologist with reptile experience.

Appendix J: Sample Processing, Diagnostic Laboratories, and Supplies

This appendix describes how to calculate an appropriate volume of blood that may be collected from an individual tortoise based on body weight, proper sample handling and storage to ensure high quality samples, and a list of laboratories that have experience analyzing reptilian samples. For specific diagnostic tests, such as aerobic/anaerobic bacterial cultures or virus isolation, the reader is referred directly to the laboratory performing the analyses for more detailed information. Additionally, the appendix includes sample supply lists for materials that may be used when conducting tortoise health monitoring. Supply lists have been broken down into specific tasks so that land managers wishing to implement a monitoring plan can develop a draft budget based on this information.

Volume of blood that can be safely drawn

Calculations should be performed for all tortoises under 500 g in body weight to ensure that the sample volume collected is appropriate. The blood volume of a tortoise ranges from 5 – 8 percent of the animal's body weight in grams. The maximum amount of blood that can be collected at one time is up to 10 percent of the tortoise's blood volume (Mader 2006). For example, the amount of blood that can be safely collected from a 3.0 kg tortoise is calculated as follows:

3.0 kg X 1000g/kg= 3000 g body weight
3000 g X 0.05= 150 ml approximate blood volume
150 ml X 0.10 = 15 ml MAXIMUM blood draw

However, 15 ml is well beyond the volume normally collected from adult tortoises. Generally 1.5 – 3 ml of whole blood will provide adequate volume for a wide range of analyses. This calculation becomes of critical importance when hatchling or juvenile tortoises are sampled.

Quality control for blood samples

After collection of the sample, tortoise blood should immediately be placed into a tube containing lithium heparin as the anticoagulant (green top tube, see supplies listed below). Certain blood parameters can be substantially affected by contamination of the sample with lymph, and also by sample clotting or hemolysis (rupture of the red blood cells resulting in red or pink plasma). Such samples must be clearly labeled and notes should be written on the data sheets so that the results can be interpreted appropriately. While in the field, samples should be stored on wet ice or ice packs and transferred to a refrigerator as soon as possible. Certain diagnostic tests require unspun, whole blood, and must be submitted to the laboratory within 24 hours of sample collection. For those that require plasma, the samples should be centrifuged as soon as possible to separate the red and white blood cells from the clear, plasma component of the blood. If a centrifuge is not available, samples to be submitted for serology can be left sitting upright in a refrigerator overnight to separate the blood components. Plasma (the clear component of blood) is transferred into an appropriate, screw-capped polypropylene tube for storage. The plastic that the tube is made of is an important consideration because certain plastics may bind antibodies and affect your results. Polypropylene is strongly recommended for this reason. Screw-capped tubes are also strongly recommended because plasma may evaporate if stored in snap-topped containers.

If plasma is to be stored for 1 week, it can be kept in a refrigerator. However, if samples will be stored for >1 week, the samples should be frozen in a standard, non-defrosting freezer (set at -20 °C) or a deep freezer (set at -80 °C). Importantly, samples stored in a freezer with an automatic defroster may degrade over time as the temperature will vary to prevent frost accumulation.

Diagnostic laboratories

The laboratories listed below provide a variety of diagnostic tests for reptiles. If diagnostic tests will be performed as part of your study, please contact the laboratories well in advance to determine if they have expertise performing the specific diagnostic tests you need, if they can accommodate your samples, and how they would like the samples to be collected, stored, and shipped.

General full-service laboratories

Antech Diagnostics
Eastern Region
Phone: (800) 872-1001

Auburn University, College of Veterinary Medicine
Clinical Pathology Service
166 Greene Hall

Auburn University, AL 36849-5519
Phone: (334) 844-2653

<u>Louisiana State University</u>
Veterinary Teaching Hospital and Clinics
Baton Rouge, Louisiana 70803
Contact: Dr. Javier G. Nevarez, Director of the Wildlife Hospital of Louisiana
Phone: (225) 578-9600

<u>Mississippi State University, College of Veterinary Medicine</u>
Diagnostic Laboratory Services
Mississippi State, MS 39762
Phone: (662) 325-1375

<u>University of Florida Veterinary Medical Center</u>
Clinical Pathology Service
2015 SW 16th Avenue, Rm VS-50
Gainesville, FL 32608
Phone: (352) 392 - 2235 ext. 4400
Fax: (352) 392 - 2938

<u>University of Georgia, College of Veterinary Medicine</u>
Southeastern Cooperative Wildlife Disease Study
589 D.W. Brooks Drive
Wildlife Health Building
Athens, GA 30602-7393
Phone: (706) 542-1741
Fax: 706-542-5865

<u>University of Miami, Avian and Wildlife Laboratory</u>
Comparative Pathology
1600 NW 10 Avenue, RMSB 7101A
Miami, FL 33136
Phone: (800) 596-7390 or (305) 243-6700
Fax: (305) 243-5662

Specific tests

<u>Herpesvirus and iridovirus serology; PCR: Herpesvirus, iridovirus, papillomavirus, adenovirus, chlamydiales, coccidia, and cryptosporidium</u>
Dr. Elliott Jacobson
University of Florida, College of Veterinary Medicine
2015 SW 16th Ave, Rm V2-238
Gainesville, FL 32608
352-392-2226 x 5775

<u>Mycoplasma serology, culture, and PCR</u>
Dr. Mary Brown
University of Florida, College of Veterinary Medicine
1600 SW Archer Rd, BSB3-50
Gainesville, FL 32611
Phone: (352) 392-2239 ext. 3986

Supply lists

Tortoise handling supplies

- *Plastic storage boxes*
  Use to hold individual tortoises temporarily to prevent co-mingling of tortoises and cross-contamination between tortoises. Bin should be large enough for the tortoise to turn around in. Rec. minimum dimensions for adults: L- 1 ft 9 in., W- 1 ft 3 in., H-1 in.
- *Regular bleach (e.g., Clorox®)*
  Use 1 part bleach/19 parts water solution to disinfect all equipment and surfaces after each tortoise. See disinfection guidelines.
- *Other non-corrosive disinfectant*
  Use to clean metal equipment and surfaces as per label recommendations. Example product: Nolvasan®, Fort Dodge. See disinfection guidelines.
- *Hand sanitizer*
  Use to sanitize hands between every tortoise. Example product: Alcare® foamed alcohol hand scrub, Steris Corp. See disinfection guidelines.
- *Disposable gloves (*latex or nitrile)

Blood collection supplies

- *Gauze sponges*
  2 x 2; Use to prepare blood collection site.
- *Skin cleanser*
  Use to prepare blood collection site (before alcohol) to remove dirt/debris. Example product: ChlorhexiDerm® Scrub, DVM Pharmaceuticals, Inc.
- *Isopropyl alcohol*
  Use to prepare blood collection site after skin cleanser.
- *1 CC and/or 3 CC syringes*
  Smaller sized syringes used for juvenile tortoises.
- *Poly hub needles*
  25 X 5/8 GAUGE; 22 X 1 GA.; 22 X 2 GA.
- *Heparin*
  (hep. sodium, 10,000 U/ml); Coat syringes to prevent clotting, if needed.
- *Microtainer plasma separator tubes with lithium heparin (green top)*
  Tubes have a clay substance that separates the red blood cells from the plasma when the tube is spun. Convenient for removing the plasma.

- *Microtainer plasma tubes with lithium heparin (green top/ no separator)*
  Tubes lack the clay separator. Best for small sample volumes or when whole blood is being submitted for a complete blood count.
- *Microhematocrit tubes (nonheparinized) and tube sealant*
  (If PCV/TP being performed)
- *Microscope slides*
  Best to use cytology grade for blood smears, and lower quality slides for fecal analyses.
- *Five-slide microscope slide mailer*
  Use when slides need to be mailed to a laboratory
- *Transfer pipets, sterile/individually wrapped*
  Use to transfer plasma to polypropylene tubes.
- *Polypropylene screw-capped tubes*
  Example product: Sarstedt screw cap micro tubes.

Nasal flush supplies

- *Disposable gloves (*latex or nitrile)
- *Gauze sponges* (2 X 2 [see above])
- *Isopropyl alcohol*
- *10 CC syringes*
- *Poly hub needles* (20 X 1 GA. [see above])
- *IV catheter*
  Example product: Terumo Surflo 22 GA. X 1 in.
- *Sterile 0.9% NaCl* (saline) IV Fluid (250 or 500 ml bag)
- *Sterile 100 ml urine collection container*
- *SP-4 media aliquots* (1 ml)
  (to add to flush) Obtain from Remel Laboratories (te. 800-255-6730)

Tick collection supplies

- *Plastic screw-capped tubes (*any plastic material)
- *70% ethyl alcohol*
- *Tweezers or hemostats to remove ticks* (as needed)

Fecal sample collection supplies

- *Twirl packs or zip-lock bags*

Appendix K: Reptile Health and Disease Researchers

Before initiating a detailed or complex health monitoring program for gopher tortoises, professional consultation with veterinarians or researchers with experience in reptilian health is strongly recommended. Professionals with this expertise may help land management personnel determine the best health monitoring approach given the available resources and status of the population of concern. Sometimes field workers come across circumstances that provide warning signs of potential health problems within a tortoise population. Seeking appropriate guidance is imperative in those cases.

A number of sources for veterinary or wildlife disease assistance are available. Private veterinary practitioners and research veterinarians with reptile experience may be found by visiting the Association for Reptilian and Amphibian Veterinarians (ARAV) website (http://www.arav.org/USMembers.htm; members listed by state). Universities with veterinary colleges usually have a wildlife or zoological medicine service and affiliated faculty with this expertise. The nearest university with a veterinary school may be found by visiting the website for the Association of American Veterinary Medical Colleges (AAVMC; http://www.aavmc.org/students_admissions/vet_schools.htm). The website provides links to all of the veterinary colleges, and from there, further contact information may be obtained. An alternate approach is to contact a local zoological park to determine if the veterinary staff is available to conduct collaborative research or help investigate tortoise health in a nearby gopher tortoise population. Some zoos have conservation programs and may be interested in assisting with such projects. Contact information for local zoological parks may be found at the Association of Zoos and Aquariums website (http://www.aza.org/AboutAZA/).

This appendix provides a list of several researchers or research veterinarians that occur within the range of the gopher tortoise and have expertise in reptile health, disease and/or pathology. Most of the professionals listed are affiliated with research institutions. The reader is referred to the ARAV website listed above for more information about private veterinary practitioners that may be available to assist with tortoise health studies.

Reptile health and disease researchers.

<u>Auburn University, College of Veterinary Medicine</u>
Dr. Marie Rush
Department of Wildlife and Zoological Medicine
Auburn University, AL 36849-5519
Phone: (334) 844-4690 or (334) 844-6347

<u>Louisiana State University, College of Veterinary Medicine</u>
Dr. Javier G. Nevarez, Director of the Wildlife Hospital of Louisiana
Veterinary Teaching Hospital and Clinics
Baton Rouge, Louisiana 70803
Phone: (225) 578-9600

<u>Mississippi State University, College of Veterinary Medicine</u>
Dr. Frank Austin, Extension Reptile Veterinarian
P.O. Box 6100
Mississippi State, MS 39762-6100
Phone: (662) 325-1272

<u>University of Florida, College of Veterinary Medicine</u>
Dr. Darryl Heard, Dr. Ramiro Isaza, Dr. Elliott Jacobson, Dr. Jim Wellehan
Zoological Medicine Service
Gainesville, FL 32611
Phone: (352) 392-2226

<u>University of Florida, College of Veterinary Medicine</u>
Dr. Mary Brown; Dr. Francesco Origgi; Dr. Lori Wendland
Department of Infectious Diseases and Pathology
Gainesville, FL 32611
Phone: (352) 392-2239

<u>University of Florida, College of Veterinary Medicine</u>
Dr. Ellis Greiner (parasitology)
Department of Infectious Diseases and Pathology
Gainesville, FL 32611
Phone: (352) 392-2239

<u>University of Georgia, College of Veterinary Medicine</u>
Southeastern Cooperative Wildlife Disease Study
589 D.W. Brooks Drive
Wildlife Health Building
Athens, GA 30602-7393
Phone: (706) 542-1741

<u>University of Georgia, College of Veterinary Medicine</u>
Dr. Stephen Hernandez-Divers; Dr. Sonia Hernandez-Divers
Exotic Animal, Wildlife & Zoological Medicine
Department of Small Animal Medicine and Surgery
Athens, GA 30602-7390
Phone: (706) 542-3221

The Georgia Sea Turtle Center
Dr. Terry M. Norton, Director of Veterinary Services
214 Stable Road
Jekyll Island, Georgia 31527
Phone: (912) 635-4070

Veterinary Pathologists.

Note: Most veterinary schools will have a pathology service and may have pathologists on staff with reptile experience. The reader should contact the school directly for more information.

Alabama Dept of Agriculture and Industries
Dr. John Roberts
Thompson-Bishop-Sparks State Diagnostic Laboratory
890 Simms Road
Auburn, AL 36831-2209
Phone: (334) 844-7267

Antech Diagnostics
Eastern Region — Multiple pathologists on staff.
Phone: (800) 872-1001

Northwest ZooPath
Dr. Michael Garner; Dr. John Trupkiewicz
654 W. Main Street
Monroe, WA 98272
Phone: (360) 794-0630

University of Florida, College of Veterinary Medicine
Dr. Lisa Farina, Dr. Francesco Origgi, and Dr. Scott Terrell
Department of Infectious Diseases and Pathology
Phone: (352) 392-2239

University of Georgia, College of Veterinary Medicine
Southeastern Cooperative Wildlife Disease Study
589 D.W. Brooks Drive
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14. ABSTRACT

The gopher tortoise is a widespread species, but one at risk. Recently, greater interest in the survival of the species has led to a series of programs and proposals for a region-wide program of cooperative management. Relocating the animals when their habitat is threatened by human disturbance is a common management practice on all lands. However, the health of the tortoises may influence the success of these relocations. A process to better incorporate health and disease related information into management decisionmaking was identified as an important missing element. The newly developed handbook contains decision trees, charts and other aids, including a special section identifying warning signs of serious health problems. The handbook thus facilitates decisionmaking regarding the health status of gopher tortoises by land managers, military and otherwise, when developing management plans involving relocation or augmentation of tortoise populations on their lands. The primary emphasis is on basic physical examinations of gopher tortoises because the manual is designed for use by land management personnel. This handbook is part of a larger project initiated within the U.S. Army environmental research program to address specific gaps in information regarding gopher tortoise population ecology and health.

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