

ARIZONA GAME AND FISH DEPARTMENT

RESEARCH BRANCH  
TECHNICAL REPORT #21

HEALTH STUDIES OF  
FREE-RANGING MOJAVE  
DESERT TORTOISES IN  
UTAH AND ARIZONA  
*A Final Report*

VANESSA M. DICKINSON  
TIMOTHY DUCK  
CECIL R. SCHWALBE  
JAMES L. JARCHOW, D.V.M.  
December 1995

ARIZONA GAME AND FISH  
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Arizona Game and Fish Department  
Research Branch

Technical Report Number 21

Health Studies of Free-Ranging Mojave Desert Tortoises in Utah and Arizona

*A Final Report*

Vanessa M. Dickinson  
Research Branch, Arizona Game and Fish Department

Timothy Duck  
Arizona Strip District, Bureau of Land Management

Cecil R. Schwalbe  
National Biological Service, University of Arizona

James L. Jarchow, D.V.M.  
Sonora Animal Hospital, Tucson, Arizona

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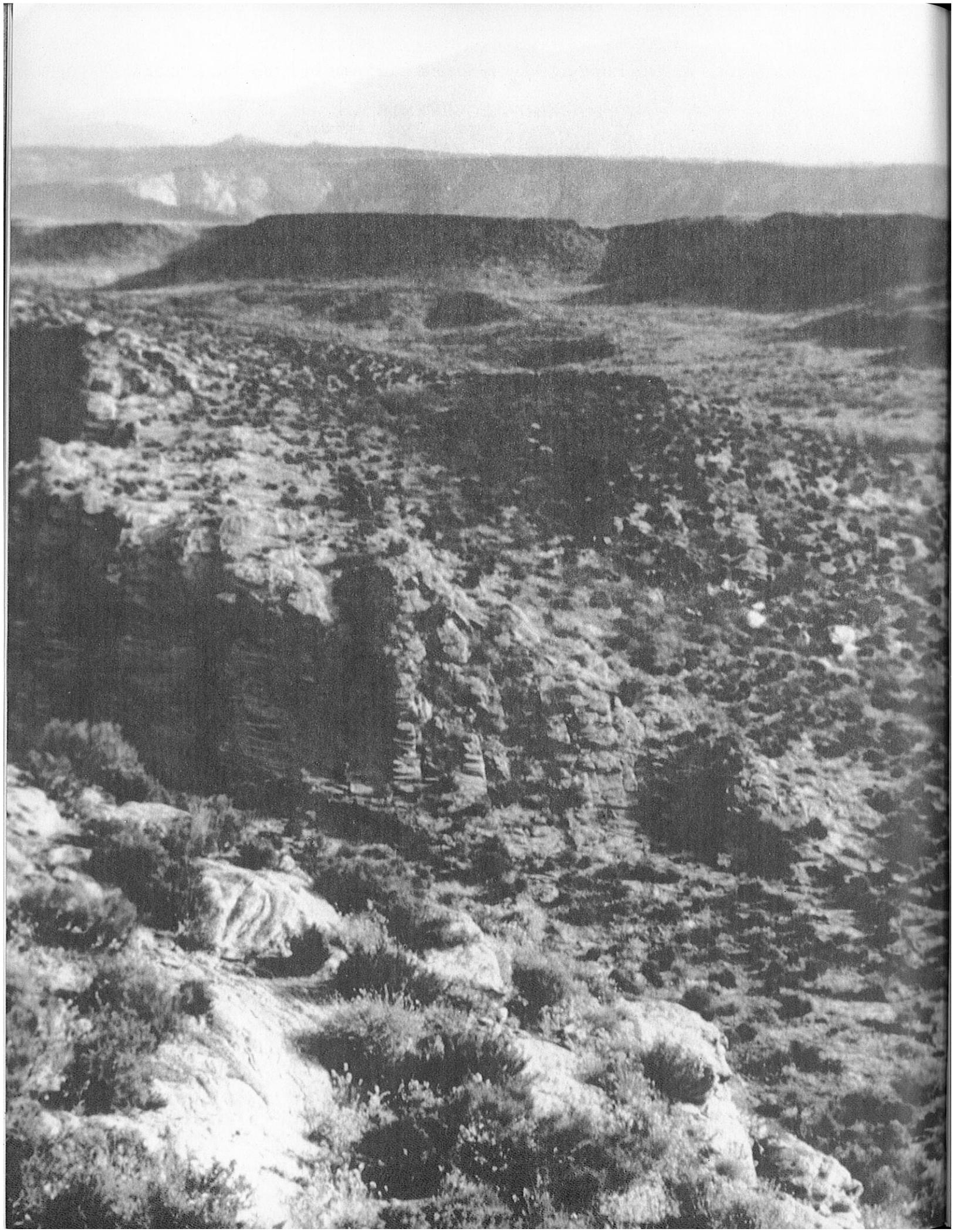
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# Health Studies of Free-Ranging Mojave Desert Tortoises in Utah and Arizona

Vanessa M. Dickinson  
Research Branch, Arizona Game and Fish Department

Timothy Duck  
Bureau of Land Management, Arizona Strip District

Cecil R. Schwalbe  
National Biological Service, University of Arizona

James L. Jarchow, D.V.M.  
Sonora Animal Hospital, Tucson, Arizona

*Abstract:* Desert tortoises (*Gopherus agassizii*) are long-lived reptiles found in the deserts of the southwestern United States. Concerns in 1989 over declines in the Mojave desert tortoise population prompted a 5-year health study of 2 free-ranging populations in the eastern Mojave Desert: the first from City Creek, Washington County, Utah, and the second from Littlefield, Mohave County, Arizona. We captured and radio-tagged 92 tortoises from 1989-93, then attempted to recapture them 3 times a year. To examine blood chemistry, bacteria, and for upper respiratory tract disease evaluation, we immobilized each tortoise and collected blood; nasal, choanal, and cloacal swabs; nasal aspirate; and fecal matter. Tortoise blood chemistry parameter values differed ( $P < 0.001$ ) between sites and sexes, and among seasons and years. Females had higher ( $P < 0.05$ ) levels of cholesterol, triglycerides, calcium, phosphorus, and vitamin E than did males. Seasonal and annual differences related to rainfall patterns, forage availability, and presence of disease. Ten tortoises had positive titers for *Mycoplasma agassizii* and 11 had clinical signs of upper respiratory tract disease. Two of 17 cloacal bacteria we isolated were pathogenic. Ninety-one percent of the tortoises had nonpathogenic pinworm ova in their feces, the only intestinal parasite we found. Our baseline data of healthy tortoises facilitates monitoring populations for evidence of dehydration and disease.

*Key words:* Arizona, blood chemistry, desert tortoise, *Gopherus agassizii*, hematology, *Mycoplasma agassizii*, normal ranges, parasites, upper respiratory tract disease, Utah.

## INTRODUCTION

Concern for population declines led to the emergency listing of the Mojave desert tortoise population on August 4, 1989, as an endangered species (U.S. Fish and Wildl. Serv. 1989), then its subsequent listing as threatened on April 2, 1990 (U.S. Fish and Wildl. Serv. 1990). Habitat loss and epidemic disease were suspected reasons for the population decline.

Understanding the health of free-ranging desert tortoises is important for assessing and managing their populations (Berry 1984). Of special concern are desert tortoises in small, isolated populations at the northern limits of their range (Bury et al. 1994). Blood parameters (see glossary) used to diagnose chelonian diseases can assess the physiological status of a population (Roskopf and Woerpel 1982, Jacobson et al. 1991). Epidemiology studies provide an estimate of past exposures of a population to infectious

diseases (Brown et al. 1994a). Brown et al. (1994a) recommended conducting long-term tortoise studies to understand the dynamics of diseases in populations.

An important tortoise disease is upper respiratory tract disease (URTD). Most tortoises affected with URTD are reproductive adults, which can have disastrous effects for small, isolated populations (Jacobson et al. 1991). Jacobson et al. (1991) hypothesized habitat degradation and reductions in forage quality may be factors in the severity and spread of URTD. Furthermore, malnutrition is known to cause immunosuppression in turtles and increased susceptibility to disease (Borysenko and Lewis 1979) and may affect tortoises the same way.

No physiological information exists for free-ranging desert tortoises in Utah and Arizona that indicates the influence of health on population status. Some physiological information exists for free-ranging Mojave tortoises in eastern California

(Christopher et al. 1992, 1993) and in southern Nevada (O'Connor et al. 1994). Most hematological and clinical chemistry (Minnich 1977, Roskopf 1982, Nagy and Medica 1986, Jacobson et al. 1991, O'Connor et al. 1994, Rostal et al. 1994), bacteriological (Fowler 1977, Snipes and Biberstein 1982, Jackson and Needham 1983, Jarchow and May 1989, Knowles 1989, Jacobson et al. 1991), and parasitic (Jarchow and May 1989) information has been collected from captive Mojave tortoises.

Another problem in assessing the status of tortoise populations is that normal reference ranges of hematological and biochemical parameters for free-ranging tortoises have only been reported for eastern California (Christopher et al. 1992, 1993). Deviations from expected values for "healthy tortoises" are important in assessing impacts of stresses such as habitat loss, competition with domestic livestock for available forage, off-road-vehicle use, and drought on free-ranging populations.

Because tortoise populations have declined, Mojave desert tortoises were federally listed, and because only limited information existed on the health status of free-ranging tortoises, we began a 5-year health investigation on free-ranging tortoises. Our objectives were to:

- Collect baseline data on blood chemistry, bacteriology, and parasitology of free-ranging desert tortoises in the Mojave Desert
- Determine if physiological differences in site, sex, season, and year occurred between 2 populations of free-ranging tortoises
- Infer seasonal tortoise activities from physiological parameter values and ongoing Mojave Desert nutrition studies
- Establish normal reference ranges of healthy desert tortoises
- Attempt to differentiate between ill and healthy tortoises

## STUDY AREA

We selected 2 study sites in the northeastern Mojave Desert: (1) City Creek in Washington County, Utah (37°10' N, 113°35' W); and (2) Littlefield in Mohave County, Arizona (37°4' N, 113°55' W) (Fig. 1). Both sites were 1.6 km<sup>2</sup> in size. These sites were 47.3 km apart, geographically separated by the Beaver Dam Mountains, Santa Clara Valley, and several major roadways. We added incidental information from Paradise Canyon in Washington County, Utah (37°9' N, 113°36' W) following discovery in July

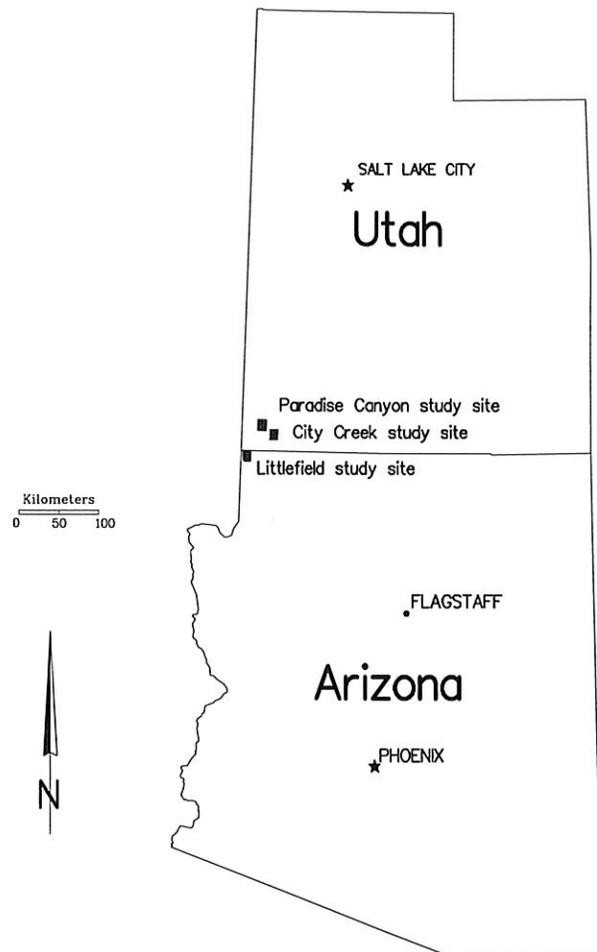


Figure 1. Locations of City Creek, Utah; Littlefield, Arizona; and Paradise Canyon, Utah, study sites.

1992 of an adult male tortoise with signs of URTD (Fig. 1). This site was 2.4 km west of City Creek.

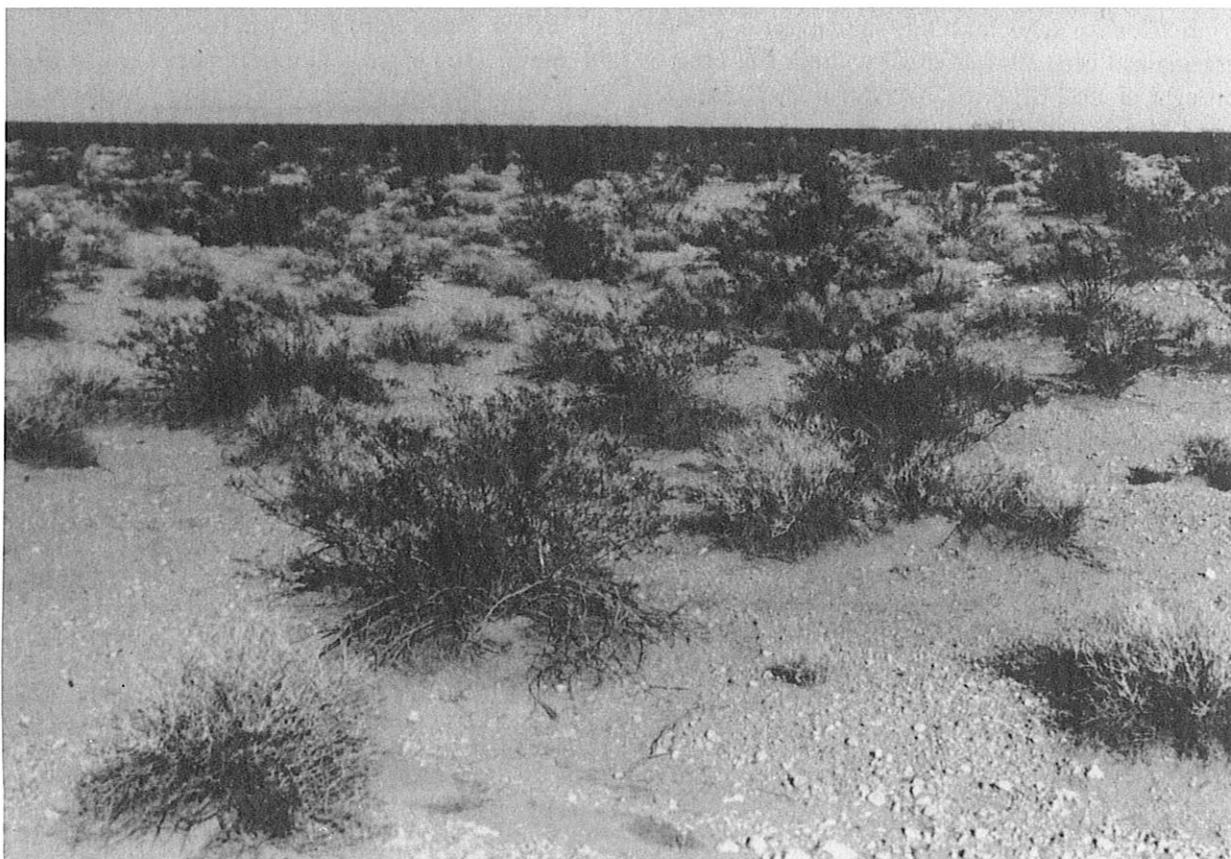
The City Creek site was 2 km north of St. George, Utah (Fig. 2). This site was first inventoried for desert tortoises in 1988, which indicated a thriving population with high densities and recruitment of smaller size classes (Bezette et al. 1989). The vegetation was Mojave desertscrub with creosotebush (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), sagebrush (*Artemisia filifolia*), and squirreltail (*Elymus elymoides*) dominant (Esque 1994). The elevation ranged from 975 m to 1,067 m. Only limited cattle grazing had been allowed in City Creek since the drought of 1988 (L. Price, Ariz. Strip Dist., Bur. Land Manage., St. George, Ut., pers. commun.). The vegetation and terrain of Paradise Canyon

were similar to those reported for City Creek.

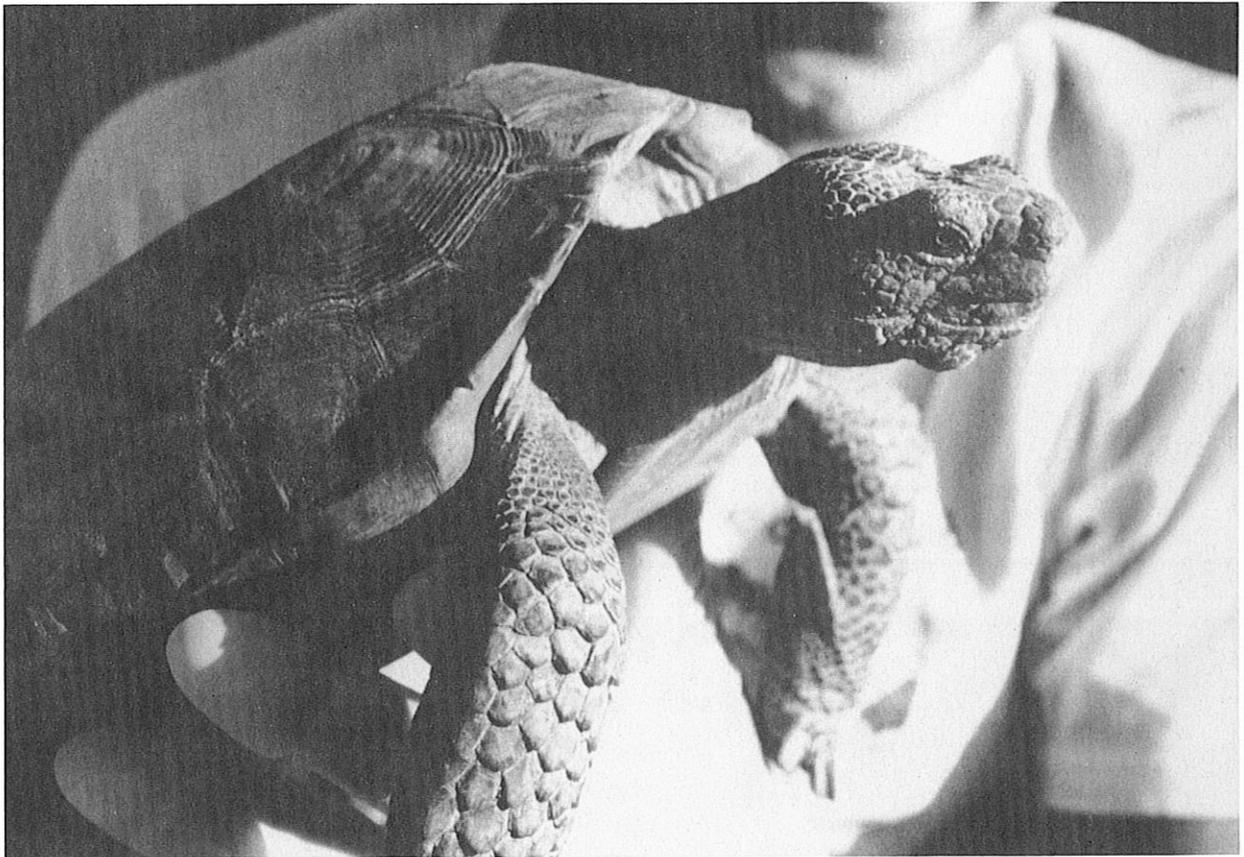
The Littlefield site was 3.5 km north of Littlefield, Arizona, near the border separating Arizona and Utah (Fig. 3). This site had been monitored for tortoise population trends since 1977. Vegetation was Mojave desertscrub dominated by creosotebush, white bursage, and Joshua tree (*Yucca brevifolia*) (Turner 1994). The elevation ranged from 597 m to 622 m. Previous research indicated the population density of tortoises declined on the Beaver Dam Slope, Utah, in the mid-1970s, with higher than expected mortality on the Littlefield plot (Coffeen and Welker 1989). Littlefield had been part of the Beaver Dam grazing allotment since the late 1970s, which only permitted cattle grazing 1 year out of 6.



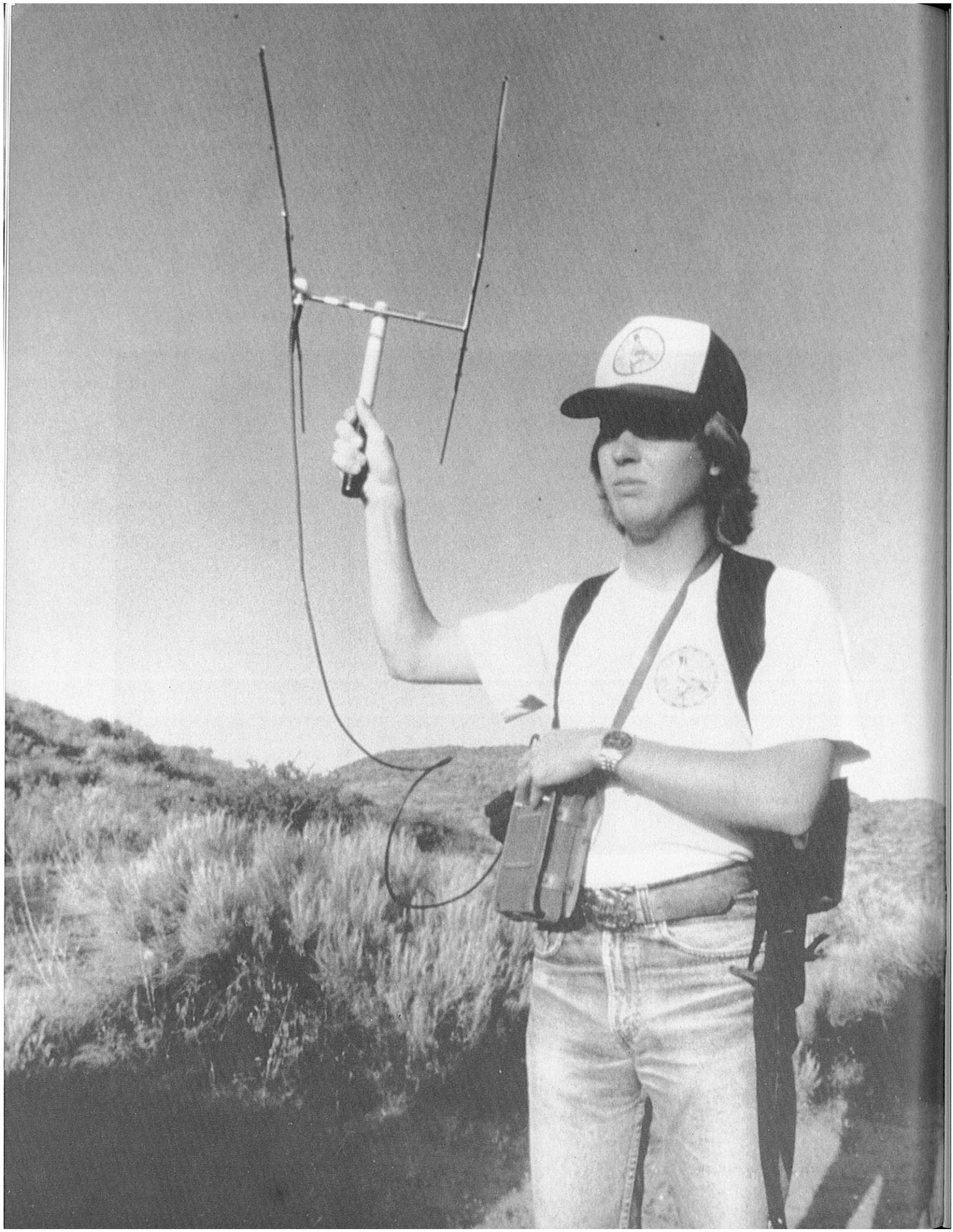
Figure 2. City Creek study site showing typical terrain and white bursage.



*Figure 3.* Littlefield study site showing typical creosotebush and white bursage community.



Examining adult desert tortoise prior to sampling.



## METHODS

### Capture and Telemetry

We made 3 trips in 1989 (May, July, August) to both sites to capture tortoises in their sheltersites or out in the open. We located tortoises by shining a light into all underground shelters. Once we found an adult tortoise (>208 mm median carapace length [MCL]), we used 5-min gel epoxy to affix radio transmitters (Model SB2, AVM Instrument Co., Ltd., Livermore, Calif., or Model 125, Telonics, Mesa, Ariz.) to its anterior marginal scutes (Fig. 4). We collected both male and female tortoises. For further identification, marginal scutes were notched following the Utah and Arizona Strip notching protocol (Berry 1988). We stopped using AVM transmitters in 1991 because the 35-mm film canister used to house the transmitter was too large. The canister size and shape may have contributed to the failure of several units as the canisters became stuck or caught in limestone caves.

We sexed tortoises by plastron indentation, tail morphology, and gular size. Tortoises that could not be accurately sexed were classified as unknown. We recaptured a minimum of 5 and a maximum of 20 adult tortoises at each site 3 times a year, in spring (May), summer (July), and fall (September). We made 1 sampling trip to City Creek and Littlefield in 1990, then sampled each site 3 times a year (May, July, September) in 1991, 1992, and 1993. Paradise Canyon was added to the sampling schedule in July 1992; we sampled Paradise Canyon twice in 1992 and 3 times in 1993.

### Health Assessment

Health assessment of tortoises evolved rapidly during our study. This was the direct result of federal listing and an evaluation of health concerns after the original study was initiated. Because of these issues, our methods of data collection were not consistent throughout our study period, but design changes were implemented in a parallel fashion between study sites (Table 1).

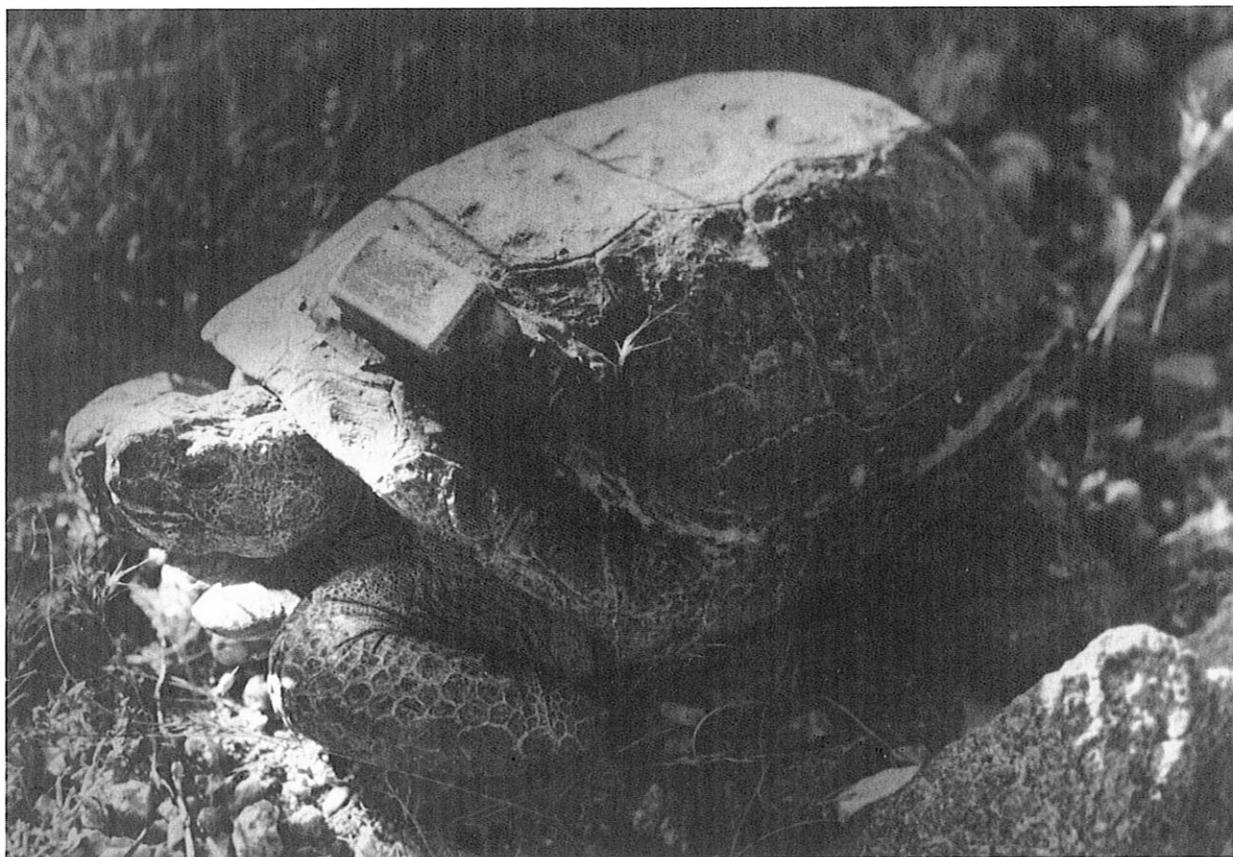


Figure 4. Adult desert tortoise with radio transmitter glued to its anterior marginal scutes.

Table 1. Summary of methods used in blood sampling by year at 3 sites in the Mojave Desert, 1989-93.

Year	Parameter	Methodology
1989	Collection	6.0 ml jugular venipuncture, 22 gauge needle
	Anesthetic	25 mg/kg body weight ketamine hydrochloride
	Whole Blood	0.6 ml whole blood in 3.0 ml ethylenediaminetetraacetic (EDTA) acid vacutainer; field packed cell volume (PCV) ( $\bar{x}$ of 2 hematocrit tubes centrifuged in the field)
	Blood Smears	None
	Serum	Blood collected in 5.0 ml vacutainers (no additives), allowed to clot for 30 min; 2 aliquots on dry ice: (1) 0.25 ml of serum for corticosterone analysis, and (2) remaining serum for determination of blood urea nitrogen (BUN) total protein, albumin, aspartate aminotransferase (AST) cholesterol, triglycerides, calcium, sodium, and potassium.
1990	Collection	Same
	Anesthetic	15 mg/kg body weight ketamine hydrochloride
	Whole Blood	0.6 ml whole blood in lithium heparin microtainer; field PCV and hemoglobin
	Blood Smears	None
	Serum	Blood (3.0 ml) collected in 5.0 ml vacutainers, allowed to clot for 5 min; 2 aliquots on dry ice: (1) same variables as 1989, except corticosterone, and (2) vitamins A and E, copper, selenium, iron, and zinc.
Plasma	Blood (3.0 ml) collected in 5.0 ml lithium heparin vacutainers, mixed for 5 min, centrifuged for 5 min; 2 aliquots on dry ice identical to those analyzed for serum.	
1991	Collection	Same
	Anesthetic	Same
	Whole Blood	Same
	Blood Smears	Two air dried blood smears examined for white blood cells (WBC) estimate, differential WBCs, platelet estimate, red blood cell morphology, hemoparasites, and evidence of blood diseases.
	Plasma	Same; 3 aliquots in liquid nitrogen: (1) same variables as 1990 except 3 additions (creatinine, phosphorus, osmolality) and 1 deletion (AST), (2) same, and (3) corticosterone, estradiol, and testosterone.

Table 1. (continued) Summary of methods used in blood sampling by year at 3 sites in the Mojave Desert, 1989-93.

Year	Parameter	Methodology
1992	Collection	Same
	Anesthetic	Same
	Whole Blood	Same
	Blood Smears	Same
	Plasma	Same; 4 aliquots in liquid nitrogen: (1) same as 1991 except 4 additions (glucose, uric acid, AST, alanine aminotransferase [ALT]), (2) same, (3) same, and (4) ELISA for detection of <i>Mycoplasma agassizii</i> .
1993	Collection	Same
	Anesthetic	Same
	Whole Blood	additional test; fibrinogen
	Blood Smears	Same
	Plasma	Same; 4 aliquots in liquid nitrogen the same as those in 1992 except 9 additions to the first aliquot (total globulins, bile acid, alkaline phosphatase (ALP), total bilirubin, direct bilirubin, indirect bilirubin, chloride, carbon dioxide, anion gap).

Hematology and blood chemistry parameters are organized in natural clinical groupings.

*Physical examination.* At each recapture, we physically examined each tortoise for disease, weighed tortoises with a 5-kg Pesola scale (Pesola, Switzerland), and measured tortoises with a caliper and 24-cm ruler (Fig. 5). We specifically looked for evidence of URTD by examining the nose and eyes. Tortoise breathing and behavior were also noted. Tortoises were considered to have clinical signs of URTD if they showed nasal discharge, conjunctivitis, and wheezing. We handled all tortoises with surgical gloves and kept them in clean, individual cardboard boxes to minimize the probability of disease transfer among animals.

*Immobilization.* In 1989, we immobilized tortoises for blood and dermal bone collection with 25 mg/kg of ketamine hydrochloride (Ketaset; Fort Dodge Lab., Fort Dodge, Ind.) injected intramuscularly into the rear leg using a 25-gauge needle. From 1990 to 1993, we immobilized tortoises with a smaller dose

(15mg/kg) of ketamine hydrochloride as only blood was collected.

*Blood.* After immobilization, we collected 6.0 ml of whole blood by jugular venipuncture using a 22-gauge needle (Fig. 6). In 1989, we placed 0.6 ml of whole blood in a 3.0 ml-vacutainer containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) for hemoglobin and fibrinogen determination and sent the tube to the laboratory within 24 hrs. From 1990 to 1993, we substituted a lithium heparin microtainer (Becton Dickinson, Rutherford, N.J.) for the EDTA vacutainer; EDTA was discovered to lysis tortoise red blood cells (Jacobson 1987).

From 1989 to 1990, we placed the remaining whole blood into a vacutainer containing no additives (Becton Dickinson, Rutherford, N.J.) to obtain serum, allowed it to clot for 30 min, and then centrifuged for 5 min. From 1990 to 1993, we placed the remaining whole blood in a lithium heparin vacutainer (Becton Dickinson, Rutherford, N.J.) to obtain plasma, mixed for 5 min, and then centrifuged for 5 min. We replaced serum with

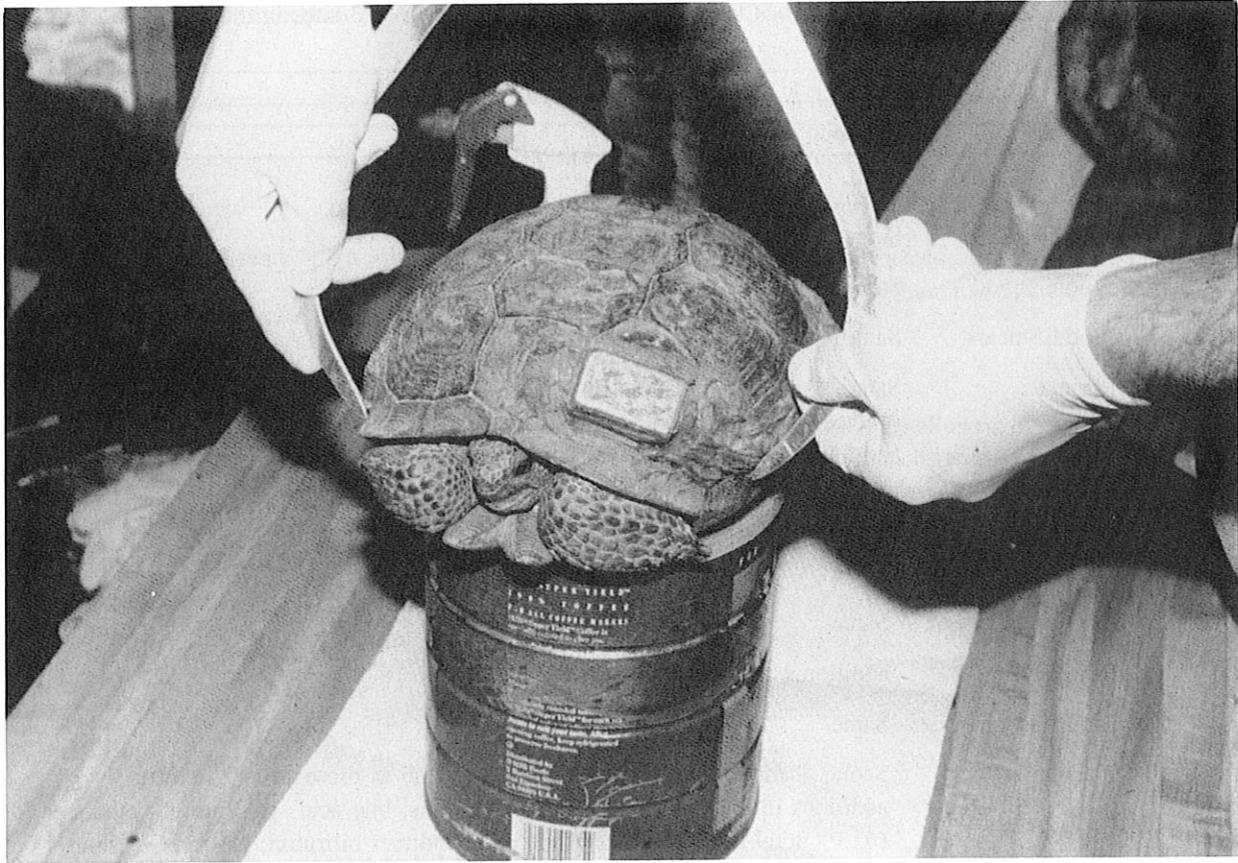


Figure 5. Measuring the width of a desert tortoise with calipers.

plasma as plasma became the accepted standard for desert tortoise health studies (E. Jacobson, Dep. Small Anim. Clin. Sci., Univ. Fla., Gainesville, Fla., pers. commun.). We pipetted off the serum and plasma and then divided it into aliquots. We placed the serum on dry ice, whereas plasma was placed in cryogenic vials (Whatman LabSales, Hillsboro, Oreg.) and immediately frozen in liquid nitrogen. We sent serum samples on dry ice to the laboratories within 24 hrs of collection and plasma samples in liquid nitrogen on dry ice within 2 days of collection.

Serum was divided into 3 aliquots. The first aliquot (0.25 ml) was analyzed for corticosterone in 1989 to evaluate stress due to prolonged handling. We compared serum corticosterone levels from animals bled within 5 min at the capture site (defined as unstressed), to tortoises transported to a central site and bled within 2 hrs of capture (defined as stressed). The second aliquot (1.5 ml) was analyzed for blood chemistry determinations with a 550 Express Analyzer (Ciba-Corning, Oberlin, Oh.). Serum was analyzed for

9 blood variables: blood urea nitrogen (BUN), total protein, albumin, aspartate aminotransferase (AST), cholesterol, triglycerides, calcium, sodium, and potassium. The third aliquot (2.0 ml) was analyzed for vitamins A and E, copper, selenium, iron, and zinc. Vitamin levels were measured by high-pressure chromatography (Model 110A, Beckman, Fullerton, Calif.), and selenium levels by gas chromatography (Model 5880, Hewlett Packard, Avondale, Pa.). Copper, iron, and zinc were measured by atomic absorption (Model Video 12, Instrumentation Lab, Waltham, Md.).

Plasma was divided into 4 aliquots. The first aliquot (1.0 ml) was analyzed for 24 blood variables: glucose, blood urea nitrogen (BUN), creatinine, uric acid, total protein, albumin, total globulins, bile acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus, cholesterol, triglycerides, total bilirubin, direct bilirubin, indirect bilirubin, sodium, potassium, chloride, total carbon dioxide, anion gap, and osmolality. Total globulins and anion gap values

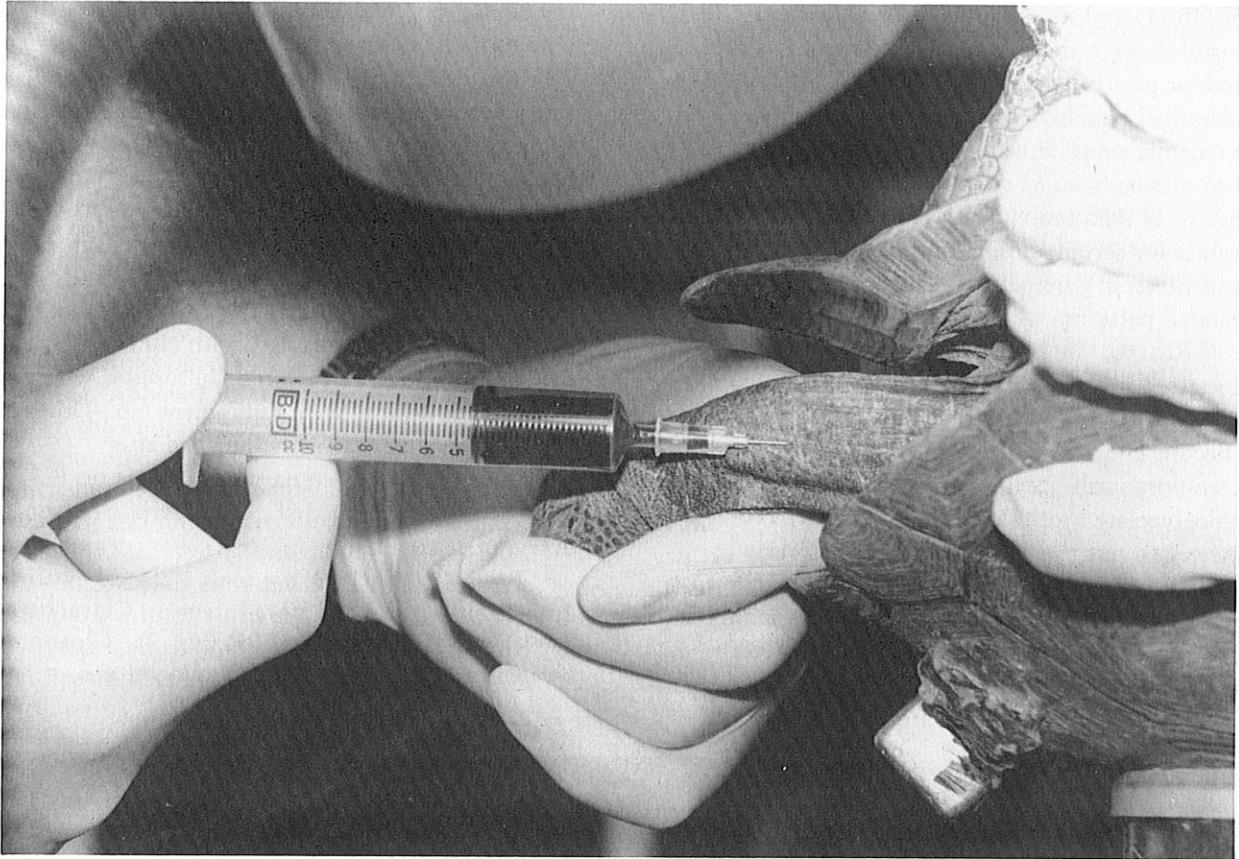


Figure 6. Collecting whole blood from the jugular vein of a desert tortoise.

were calculated using the following formulas:

Total globulins = total protein - albumin

Anion gap = (sodium + potassium) -  
(chloride + total carbon dioxide)

The second aliquot (1.5 ml) was analyzed for vitamins A and E, copper, selenium, iron, and zinc. The third aliquot (1.0 ml) was analyzed for corticosterone, estradiol, and testosterone by radioimmunoassay (Lance et al. 1985). The fourth aliquot (0.5 ml) was analyzed by an enzyme-linked immunosorbent assay (ELISA) for *M. agassizii*, as described by Schumacher et al. (1993).

We determined packed cell volume (PCV) in the field by calculating the mean of 2 capillary tube values. We made 2 air-dried blood smears in the field and sent them to the laboratory within 2 days. Smears were stained with modified Wright's stain and examined for white blood cell (WBC) estimate, differential WBCs (heterophils, lymphocytes, monocytes, azurophils, eosinophils, basophils), platelet estimate, red blood cell (RBC) morphology, hemoparasites, and evidence of

anisocytosis, polychromasia, and anemia. Anemia was subjectively determined based on smear thickness, evidence of polychromasia, and RBC spacing and density. The WBC estimate was calculated by counting the number of WBCs in 10 fields under a 50-x microscope and then multiplying the count by 2,000. We calculated the number of each WBC type (e.g., heterophils) by multiplying the percentage of each type by the WBC estimate.

We compiled normal reference ranges as the mean  $\pm 2$  standard deviations (SD) of blood parameters for healthy tortoises. We separated the data by sex to compile gender reference ranges for serum and plasma. Outliers were values  $> 2$  SD from the mean (Hoffman 1971). We considered tortoises whose values were  $> 2$  SD from the mean as possibly abnormal, and those with values  $> 3$  SD from the mean as probably abnormal. Each tortoise with abnormal parameter values ( $> 2$  SD) was evaluated individually. We defined healthy tortoises as animals with no clinical signs

of URTD and negative titers for *M. agassizii*. Ill tortoises were those with clinical signs of URTD and/or positive titers for *M. agassizii*. We identified possibly abnormal and probably abnormal values in tortoise hematology, serum, and plasma from 61 scatterplots. We evaluated the health of each individual tortoise with abnormal values for serum and plasma by examining blood and bacterial parameters, changes in weight, and rainfall patterns.

**Bacteria.** In 1989, we swabbed 9 City Creek tortoises at 2 sampling sites (nares, choana) and placed them in 3 transport media (Cultorettes [Becton Dickinson, Cockeysville, Md.], Stuart transport swabs [Starplex, Etokicoke, Ont.] thioglycolate broth [Microbio Products, Tempe, Ariz.]) to test the effectiveness of bacteria collection. We kept nasal and choanal swabs on wet ice and sent them to the laboratory within 24 hrs. Swabs were cultured for *Chlamydia*-like organisms, *Flavobacterium* spp., *Mycoplasma* spp.,

*Pasteurella* spp., and *Staphylococcus* spp. The *Chlamydia*-like organisms were defined as *Chlamydia* elementary body-like structures that could not be sequentially transferred from embryo chicken egg yolk (C. A. Reggiardo, Vet. Diagnostic Lab, Univ. of Ariz., Tucson, Ariz., pers. commun.).

In 1989 and 1990, we took 2 swabs from each tortoise nares and choana and stored them in Cultorettes. From 1991 to 1993, we only took choanal swabs from tortoises with clinical signs of URTD. In 1992, we replaced Cultorettes with Transtube (Medical Wire Equipment Co., Dover, N.J.). Transtube was recommended after problems isolating bacteria with Cultorettes (C. Reggiardo, Dep. Vet. Med., Univ. Ariz., Tucson, Ariz., pers. commun.).

From 1991 to 1993, we took 1 cloacal swab from each tortoise and stored them in Cultorettes (1991) and Transtube (1992-93) (Fig. 7). Cloacal swabs were used for gram stain and culture.

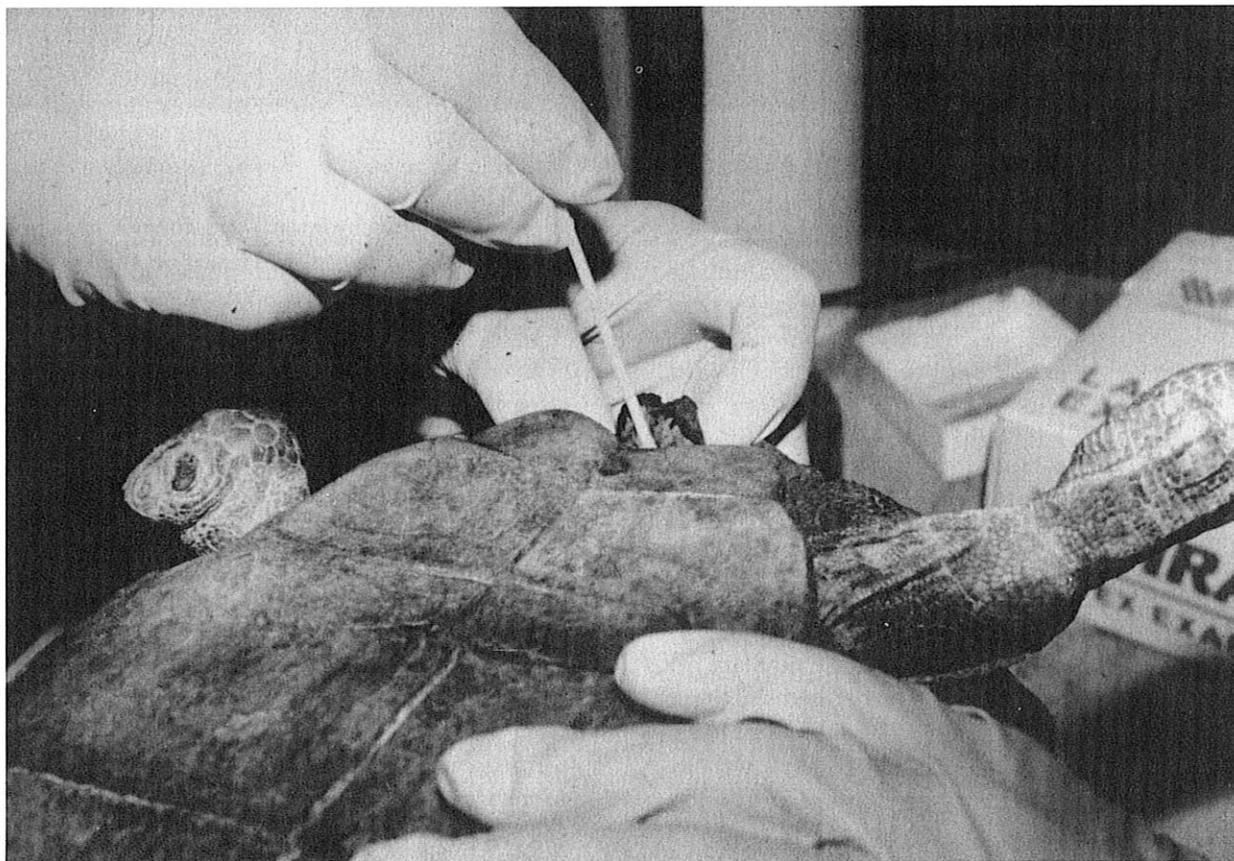


Figure 7. Collecting cloacal bacteria from a desert tortoise with a swab.

Plates were incubated at room temperature (23-25 C) and at 37 C. Bacterial cultures were grown using MacConkey agar (gram negative bacteria), Selenite agar (*Salmonella*, *Shigella*), Hektoen agar (*Salmonella*, *Shigella*), and Campylobacter agar (*Campylobacter*). When possible, bacteria were classified as gram positive or gram negative and identified to species.

From 1990 to 1991, we flushed each tortoise naris with 1 open-end 3.5 Fr. Tom Cat catheter (Sherwood Medical, St. Louis, Mo.) attached to a 3.0-ml syringe filled with 0.5 ml of 0.9% sodium chloride (Abbott Lab., Chicago, Ill.) (Fig. 8). We placed the aspirate from both nares in a cryogenic vial containing 1.0 ml of tryptic soy broth (MicroBio Products, Tempe, Ariz.), mixed the vial contents, and immediately froze in liquid nitrogen. From 1992 to 1994, we used 50% less saline (0.25 ml) to flush each naris and 50% less tryptic soy broth (0.5 ml) for culturing aspirate; less solution was suspected to promote *Mycoplasma* spp. growth (M. Brown, Dep. Infect. Dis., Univ. Fla.,

Gainesville, Fla., pers. commun.). Nasal aspirate was cultured for *Mycoplasma* spp., *Pasteurella* spp., and *Chlamydia*-like organisms.

*Fecal.* From 1990 to 1993, we collected fresh fecal samples from each tortoise and placed each sample in a separate glass vial. We sent the fecal samples on wet ice to the laboratory within 24 hrs. Fecal samples were analyzed for internal parasites by direct microscopic examination and fecal flotation.

*Rehydration and release.* We rehydrated tortoises after sampling to replace any fluids voided during handling (Fig. 9). We injected 1-2% body mass of equal parts Normosol (Abbott Laboratories, Chicago, Ill.) and 2.5% dextrose in 0.45% sodium chloride (Abbott Laboratories, Chicago, Ill.) into the body cavity of each tortoise with a 20-gauge needle. Tortoises were released at the point of capture during early morning of the day following health assessment, >10 hrs after injection of ketamine hydrochloride (Fig. 10).



Figure 8. Collecting nasal bacteria from a desert tortoise by flushing the nares with saline.

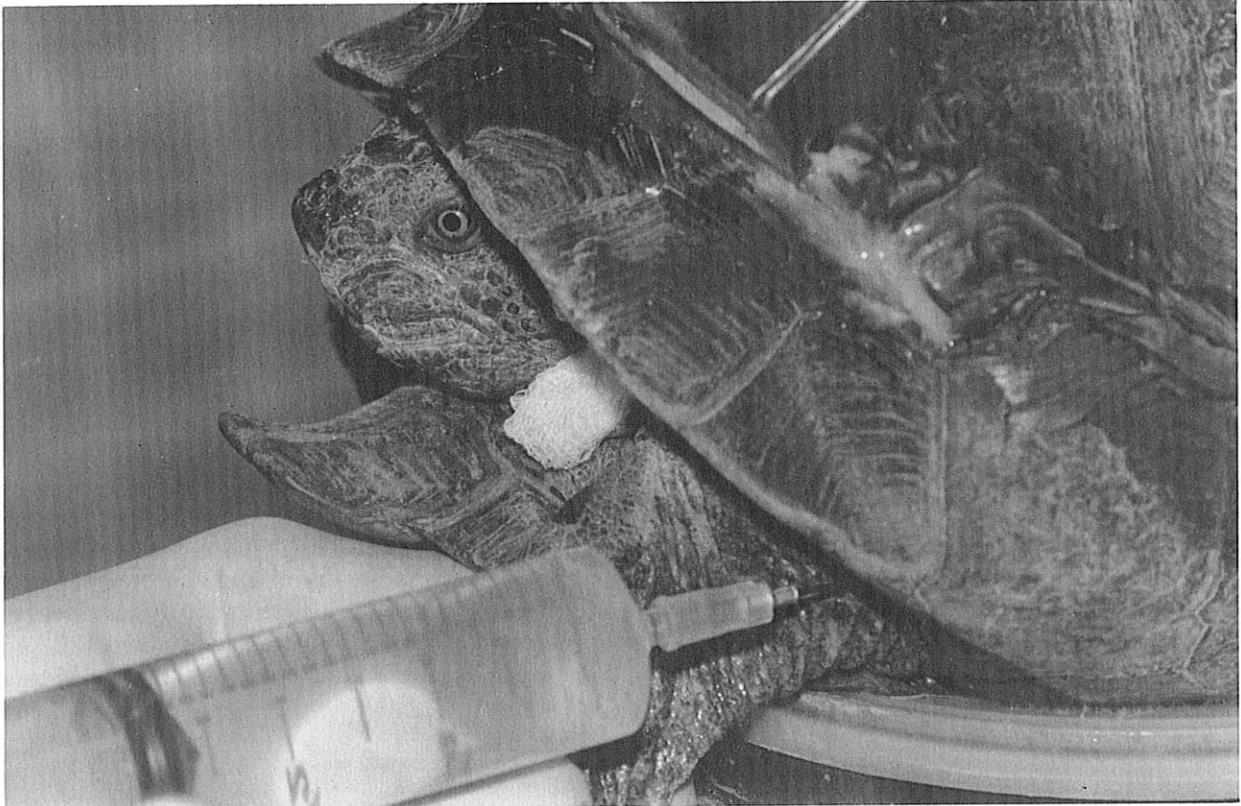


Figure 9. Rehydrating a desert tortoise by injecting equal parts Normosol and dextrose into its body cavity.



Figure 10. Releasing a desert tortoise at the point of capture in the morning after health assessment.

## Weather

Permanent U.S. National Oceanic Atmosphere Administration weather stations recorded ambient temperature (maximum, minimum) and rainfall, while temporary automatic stations recorded ambient temperature, relative humidity, rainfall, soil temperature, soil moisture, and wind speed. Permanent weather data were collected daily at St. George, Utah, and Beaver Dam, Arizona (Natl. Oceanic Atmos. Adm. 1988-93). The St. George weather station was 6.1 km south southwest of City Creek. The Beaver Dam weather station was 3.4 km southwest of Littlefield. Permanent weather stations were in habitats similar to the study sites.

We collected weather data from automatic weather stations (Model System 10, Rainwise, Inc., Bar Harbor, Me.) located randomly in the City Creek and Littlefield study sites. We installed the automatic weather stations at both sites in July 1992. The automatic stations collected the following data every hour: date, time, ambient temperature (1.4 m height), relative humidity (1.4 m height), rainfall (2.2 m height), soil temperature (10 cm depth), soil moisture (10 cm depth), and average wind speed (1.9 m height). Rainfall was recorded as the total for the hour. Soil moisture probes (Irrometer Co., Inc., Riverside, Calif.) recorded soil moisture on a scale of 0-200 centibars, where 0-10 indicated saturated conditions and 100-200 indicated dry conditions.

## Statistical Analyses

We analyzed data with tests only when statistical assumptions were met. We considered all differences to be significant at  $P < 0.05$ . We tested the data for normality with probability plots (SPSS, Inc. 1990) and used parametric statistics for all the data except bacteria and internal parasites. We analyzed data from ill and healthy tortoises separately.

We analyzed body mass and MCL, hematology, serum and plasma biochemistry, and weather data using multiple analysis of variance (MANOVA; StatSoft, Inc. 1994). Rao's  $R$  was used as our multivariate  $F$  value. We used Tukey's honest significant difference (HSD) test to identify differences between means. Except for serum corticosterone results, we simultaneously analyzed all parameters for the effect of site, sex, season, and year. Because of unequal sample sizes we only focused on effects that were statistically

significant. Serum corticosterone results were analyzed by 2-tailed Student's  $t$ -tests for the effect of handling stress.

Body mass and MCL were analyzed for the effects of site, sex, season, and year. Six hematology parameters (heterophils, lymphocytes, monocytes, eosinophils, basophils, azurophils) were analyzed for the effects of site, season, and year. Seven serum biochemical parameters (BUN, total protein, albumin, AST, cholesterol, triglycerides, calcium) were analyzed for the effects of site and year. We analyzed plasma (1991-93) for 8 biochemical parameters (creatinine, total protein, albumin, cholesterol, triglycerides, calcium, phosphorus, vitamin E) for the effects of site, sex, season, and year. We separately analyzed plasma from 1993 for 12 biochemical parameters (glucose, BUN, uric acid, AST, ALT, ALP, total bilirubin, indirect bilirubin, vitamin A, chloride, total carbon dioxide, osmolality) for the effects of site and season. We analyzed weather data for 6 parameters (ambient temperature, relative humidity, rainfall, soil temperature, soil moisture, wind speed) for the effects of site, season, and year.

We analyzed nasal and cloacal bacteria (1992-93) and internal parasites (1990-93) presence or absence using Kruskal-Wallis analysis of variance (K-W ANOVA; StatSoft, Inc. 1994) for the effects of site, season, and year.



## RESULTS

We captured a total of 92 tortoises; 42 tortoises from City Creek, 49 tortoises from Littlefield, and 1 tortoise from Paradise Canyon. Forty-seven (51%) tortoises were not radio-tagged and were only sampled once. We lost 17 (18%) tortoise radio signals, either from radio transmitter failure or animals leaving the study sites. We recorded 2 mortalities (1 in City Creek, 1 in Littlefield) and 1 mortally-injured tortoise (Littlefield). We found this injured tortoise with a crushed carapace in its burrow on May 18, 1991 (Fig. 11). Cattle tracks were observed leading to and away from the burrow with 1 hoof mark entering the burrow directly over the tortoise. We euthanized this tortoise on May 19, 1991, because of its extensive internal injuries.

### Body Mass and Median Carapace Length

Tortoise body mass and MCL (Appendix 1) differed ( $P < 0.001$ ), as we found effects of site

and sex, but no other effects or interactions (Table 2). City Creek tortoises were heavier compared to Littlefield tortoises ( $P = 0.001$ ). Male tortoises were heavier and longer than female tortoises ( $P < 0.001$ ).

### Hematology

Hematology values (Appendix 2) differed ( $P < 0.001$ ) among seasons and to a lesser degree between sites, while blood abnormalities differed ( $P < 0.001$ ) between sites and among years. We found site, season, and year interactions in hematology values (Table 3).

Excluding ill tortoises from the analyses, we found healthy male and female Littlefield tortoises had higher levels of heterophils in July and September 1993, higher lymphocytes in July and September 1991, and higher azurophils in September 1993 than any other season and year ( $P < 0.001$ ) (Fig. 12). In May 1993, healthy male tortoises had higher ( $P < 0.001$ ) PCV levels compared to healthy females. We found no

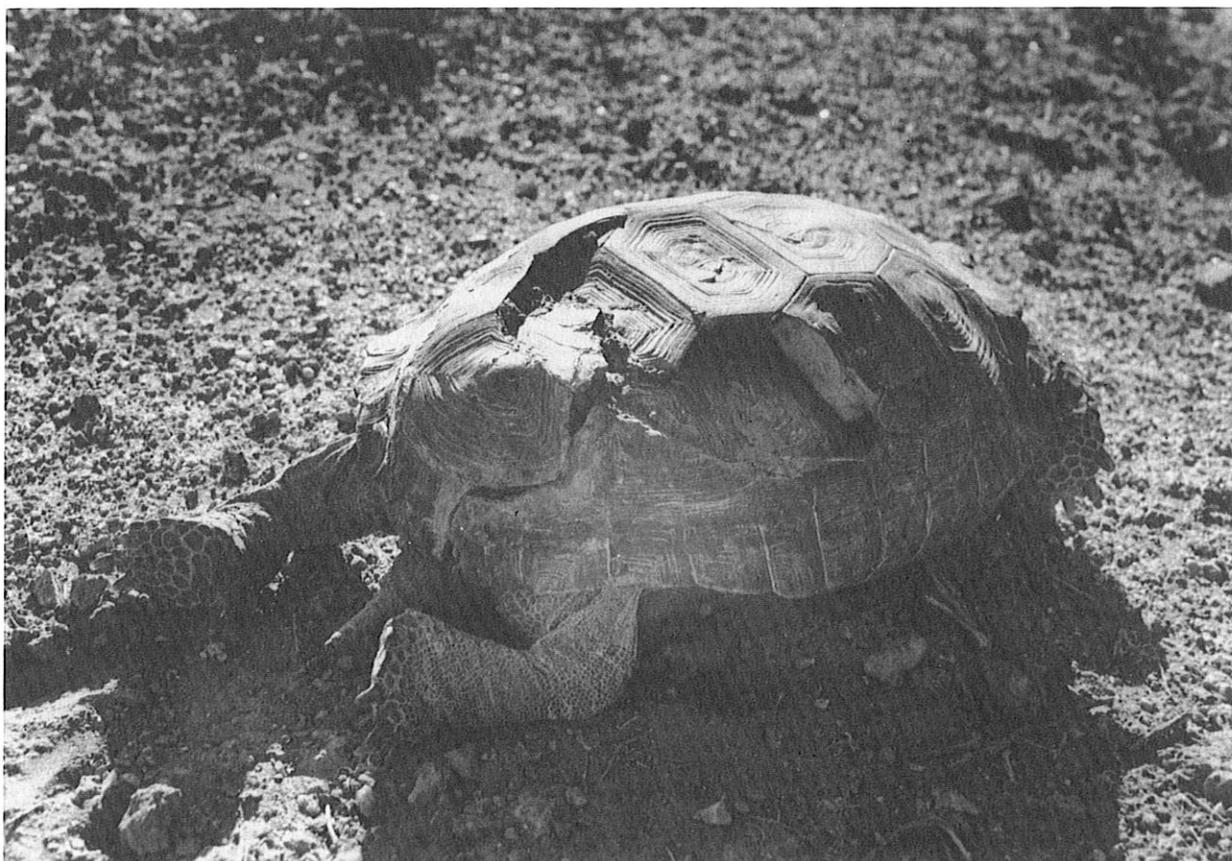


Figure 11. Desert tortoise found in its burrow with a crushed carapace. Cattle tracks were observed leading to and away from the burrow with 1 hoof mark entering the burrow directly over the tortoise.

Table 2. Main effects of 2 sites (City Creek, Ut.; Littlefield, Ariz.), 3 seasons (May, July, September), and 3 years (1991-93) upon the dependent variables body mass and median carapace length using a 4-way MANOVA ( $2 \times 2 \times 3 \times 3$ ).  $n = 167$ .

	Rao's <i>R</i>	df 1	df 2	<i>P</i>
Site	7.07	2	129	0.001
Sex	22.40	2	129	<0.001
Season	2.07	4	258	0.08
Year	1.32	4	258	0.3
Site x sex	0.31	2	129	0.7
Site x season	1.31	4	258	0.3
Sex x season	0.81	4	258	0.5
Site x year	0.51	4	258	0.7
Sex x year	0.67	4	258	0.6
Season x year	0.78	8	258	0.6
Site x sex x season	0.47	4	258	0.7
Site x sex x year	0.37	4	258	0.8
Site x season x year	1.18	8	258	0.3
Sex x season x year	0.95	8	258	0.5
Site x sex x season x year	0.44	8	258	0.9

Table 3. Main effects of 2 sites (City Creek, Ut.; Littlefield, Ariz.), 3 seasons (May, July, September), and 3 years (1991-93) upon the dependent variables heterophils, lymphocytes, monocytes, azurophils, eosinophils, and basophils using a 3-way MANOVA ( $2 \times 3 \times 3$ ).  $n = 163$ .

	Rao's <i>R</i>	df 1	df 2	<i>P</i>
Site	2.54	6	134	0.02
Season	4.95	12	268	<0.001
Year	5.38	12	268	<0.001
Site x season	1.69	12	268	0.07
Site x year	2.73	12	268	0.002
Season x year	4.01	24	468	<0.001
Site x season x year	1.48	24	468	0.07

difference ( $P > 0.05$ ) for other hematology parameters (hemoglobin, monocytes, eosinophils, basophils) and all other site, season, and year combinations.

We observed 2 types of blood abnormalities, polychromasia and anisocytosis. We observed polychromasia in 14 tortoises, 13 in 1991 and 1 in 1992. Twelve tortoises had slight polychromasia, while only 2 tortoises had mild polychromasia. Seventy-seven percent of polychromasia occurred in 1991 in Littlefield tortoises. We observed 3 tortoises with anisocytosis in May 1991, 2 from City Creek and 1 from Littlefield.

#### Clinical Chemistry

*Serum.* Serum clinical chemistry (Appendix 3), but not serum electrolytes (Appendix 4) varied between sites and among years; we found effects of site and year, but no interactions in serum values (Table 4). We found higher ( $P < 0.001$ ) levels of BUN, total protein, and albumin in Littlefield tortoises in 1990 compared to any other site and year. Results were similar when the effects of 1 ill Littlefield tortoise were removed. We found no difference ( $P > 0.05$ ) for other serum parameters (cholesterol, triglycerides, calcium, AST) and all other site and year combinations. We found no difference ( $t = -0.44$ ,

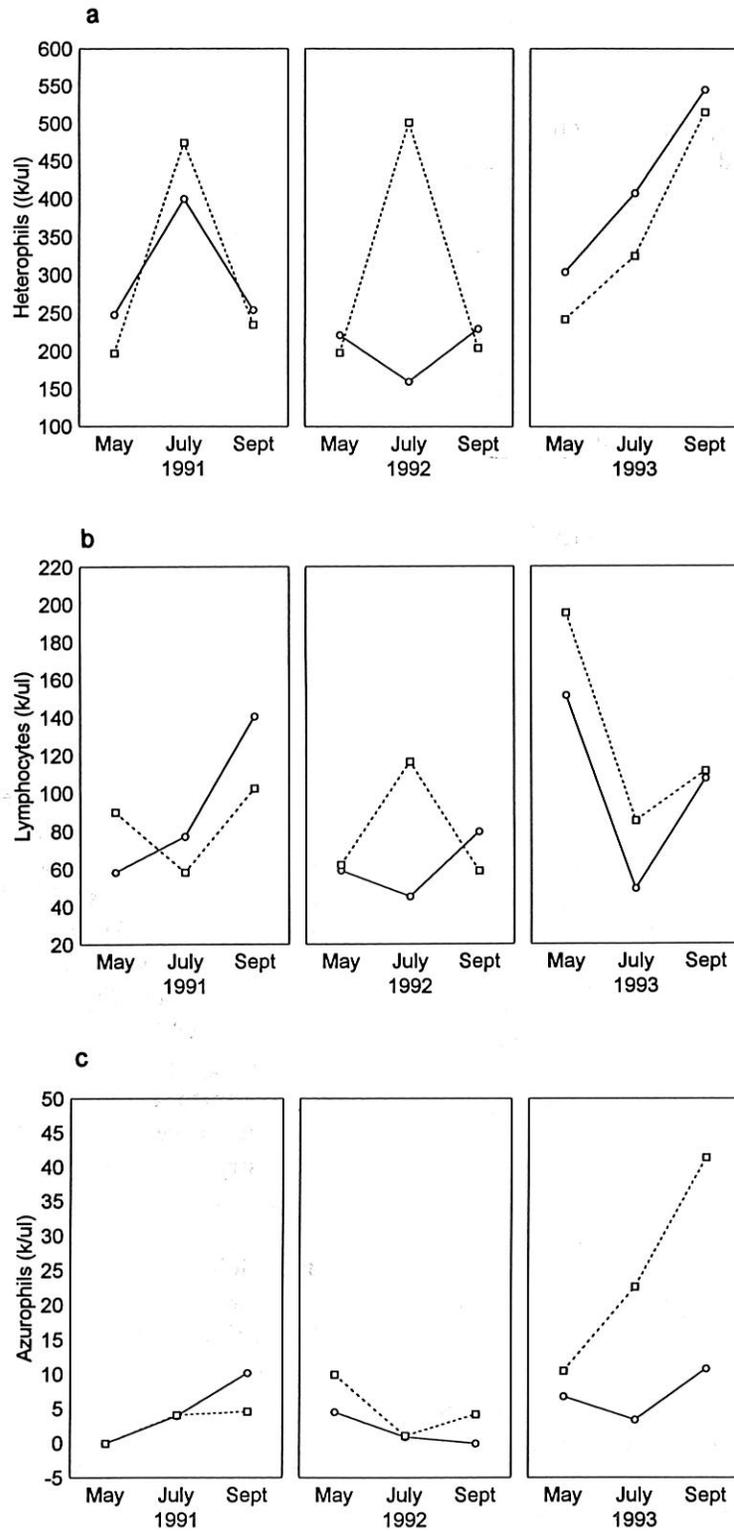


Figure 12. Line graphs of seasonal changes in (a) heterophils, (b) lymphocytes, and (c) azurophils for healthy male and female Mojave desert tortoises ( $n = 81$ ) at 2 sites (City Creek, Ut; Littlefield, Ariz.), 1991-93. Solid line = City Creek; dotted line = Littlefield.

Table 4. Main effects of 2 sites (City Creek, Ut.; Littlefield, Ariz.), and 2 years (serum; 1989-90) upon the dependent variables blood urea nitrogen, total protein, albumin, aspartate aminotransferase, calcium, cholesterol, and triglycerides using a 2-way MANOVA (2 x 2).  $n = 27$ .

	Rao's R	df 1	df 2	P
Site	3.55	7	45	0.004
Year	3.69	7	45	0.003
Site x year	0.63	7	45	0.7

$P > 0.05$ ,  $n = 36$ ) in corticosterone levels between stressed and unstressed tortoises in 1989.

**Plasma.** Plasma biochemistry (Appendix 5), but not plasma electrolytes and osmolality (Appendix 6), varied with health status, site, sex, season, and year. We found site, sex, season, and year interactions in plasma values (1991-93) (Table 5).

When we removed ill and female tortoises from the analyses, we found higher ( $P < 0.03$ ) levels of albumin and cholesterol in healthy male Littlefield tortoises in May 1991 and 1992 compared to any other season and year (Fig. 13). City Creek and Littlefield male tortoises had lower ( $P < 0.001$ ) calcium levels in September 1992, and higher ( $P < 0.001$ ) levels of vitamin E in 1993 (Fig. 13). We found no difference ( $P > 0.05$ ) for other plasma parameters (creatinine, total protein, triglycerides, phosphorus) and all other site, sex, season, and year combinations.

We found effects of site and season, but no interactions, when we analyzed the plasma 1993 data alone (Table 6). We found higher ( $P < 0.001$ ) levels of uric acid, AST, and ALT at both City Creek and Littlefield in May 1993 compared to any other season and year. We found no difference ( $P > 0.05$ ) in other plasma parameters (glucose, BUN, ALP, total bilirubin, indirect bilirubin, chloride, carbon dioxide, vitamin A, osmolality) and all other site and season combinations.

Compared with healthy males, healthy female tortoises had higher ( $P < 0.02$ ) levels of cholesterol, triglycerides, calcium, phosphorus, and vitamin E (Fig. 14). Higher levels of all these parameters, except calcium, occurred in May. We also found healthy female tortoises had May and

September peaks in cholesterol, triglycerides, and estradiol.

### Health Profiles

We found few ill tortoises at either study site. Ill tortoises had different blood chemistries and nasal bacteria than did healthy animals. We found 11 (14%) ill tortoises; 8 (73%) at Littlefield, 2 at City Creek, and 1 at Paradise Canyon. All but 1 ill tortoise had positive titers for *M. agassizii*. One City Creek tortoise showed signs of URTD (wheezing) in September 1993, but tested negative for *M. agassizii*. We observed 2 tortoises (1 in Littlefield, 1 in Paradise Canyon) for 2 years that had clinical signs of URTD in every season. We observed 1 Littlefield tortoise (H036) for 5 years; during the first 4 years this tortoise was healthy, but in the fifth year it showed signs of URTD, lost weight in each subsequent season, and showed signs of dehydration in the last sampling period.

Compared with healthy tortoises, ill tortoises had higher ( $P < 0.05$ ) levels of heterophils, azurophils, BUN, sodium, and *P. testudinis* in their nasal cavities, and lower levels of phosphorus (Table 7). Seventy-three percent of ill tortoises had abnormal blood values. We evaluated the health of all tortoises with abnormal values for serum (Appendix 7) and plasma (Appendix 8). We found tortoises with abnormal values from Littlefield (51%), City Creek (47%), and Paradise Canyon (100%). We compiled reference ranges of hematology and serum blood chemistry for healthy tortoises (Appendix 9). We also compiled reference ranges of hematology and plasma blood chemistry for healthy tortoises and ranges for ill tortoises (Appendix 10).

### Bacteriology

**Nasal.** Some tortoises had positive titers for *M. agassizii* and pathogenic bacteria in their nasal cavities. Five tortoises (17%) had positive titers for *M. agassizii* (Fig. 15). Two of the 4 tortoises (Littlefield 213, Paradise Canyon 12) had positive titers in all 4 sampling periods (September 1992, May 1993, July 1993, September 1993). Littlefield tortoise 002 and 213 and Paradise Canyon tortoise 12 had clinical signs of URTD and positive titers in all 4 ELISA sampling periods. Another Littlefield tortoise (H036), was seronegative in September 1992, and seropositive in May, July, and September 1993. Tortoises with positive titers for *M. agassizii* showed clinical signs of URTD,

Table 5. Main effects of 2 sites (City Creek, Ut.; Littlefield, Ariz.), sex, 3 seasons (May, July, September), and 3 years (plasma; 1991-93) upon the dependent variables packed cell volume, hemoglobin, creatinine, total protein, albumin, cholesterol, triglycerides, calcium, phosphorus, and vitamin E using a 4-way MANOVA (2 x 2 x 3 x 3).  $n = 167$ .

	Rao's <i>R</i>	df 1	df 2	<i>P</i>
Site	9.31	10	115	<0.001
Sex	38.04	10	115	<0.001
Season	11.83	20	230	<0.001
Year	27.12	20	230	<0.001
Site x sex	5.71	10	115	<0.001
Site x season	3.91	20	230	<0.001
Sex x season	5.83	20	230	<0.001
Site x year	3.14	20	230	<0.001
Sex x year	4.44	20	230	<0.001
Season x year	4.47	40	437	<0.001
Site x sex x season	1.59	20	230	0.06
Site x sex x year	1.18	20	230	0.3
Site x season x year	2.76	40	437	<0.001
Sex x season x year	1.97	40	437	<0.001
Site x sex x season x year	1.28	40	437	0.1

Table 6. Main effects of 2 sites (City Creek, Ut.; Littlefield, Ariz.), and 3 seasons (plasma; May, July, September, 1993 only) upon the dependent variables glucose, blood urea nitrogen, uric acid, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, indirect bilirubin, vitamins A, chloride, total carbon dioxide and osmolality using a 2-way MANOVA (2 x 3).  $n = 47$ .

	Rao's <i>R</i>	df 1	df 2	<i>P</i>
Site	3.35	12	28	0.004
Season	9.13	24	56	<0.001
Sex x season	1.48	24	56	0.1

Table 7. Mean hematological, biochemical (plasma), and bacterial values for ill<sup>a</sup> ( $n = 11$ ) and healthy<sup>b</sup> ( $n = 81$ ) Mojave desert tortoises, 1991-93.

Value	Ill tortoises <sup>a</sup>	Healthy tortoises <sup>b</sup>	<i>P</i>	<i>n</i>
Heterophils (k/ul)	452.0	294.4	<0.001	163
Azurophils (k/ul)	14.6	7.2	0.04	163
Blood urea nitrogen (mg/dl)	13.0	0.7	<0.001	197
Phosphorus (mEq/l)	1.8	2.7	<0.001	197
Sodium (mEq/l)	139.8	133.5	0.003	197
<i>Pastuerella testudinis</i> (nasal cavity)	65.0%	8.0%	0.03	105

<sup>a</sup> Ill tortoises defined as animals with clinical signs of upper respiratory tract disease and/or positive titers for *Mycoplasma agassizii*.

<sup>b</sup> Healthy tortoises defined as animals with no clinical signs of upper respiratory tract disease and negative titers for *M. agassizii*.

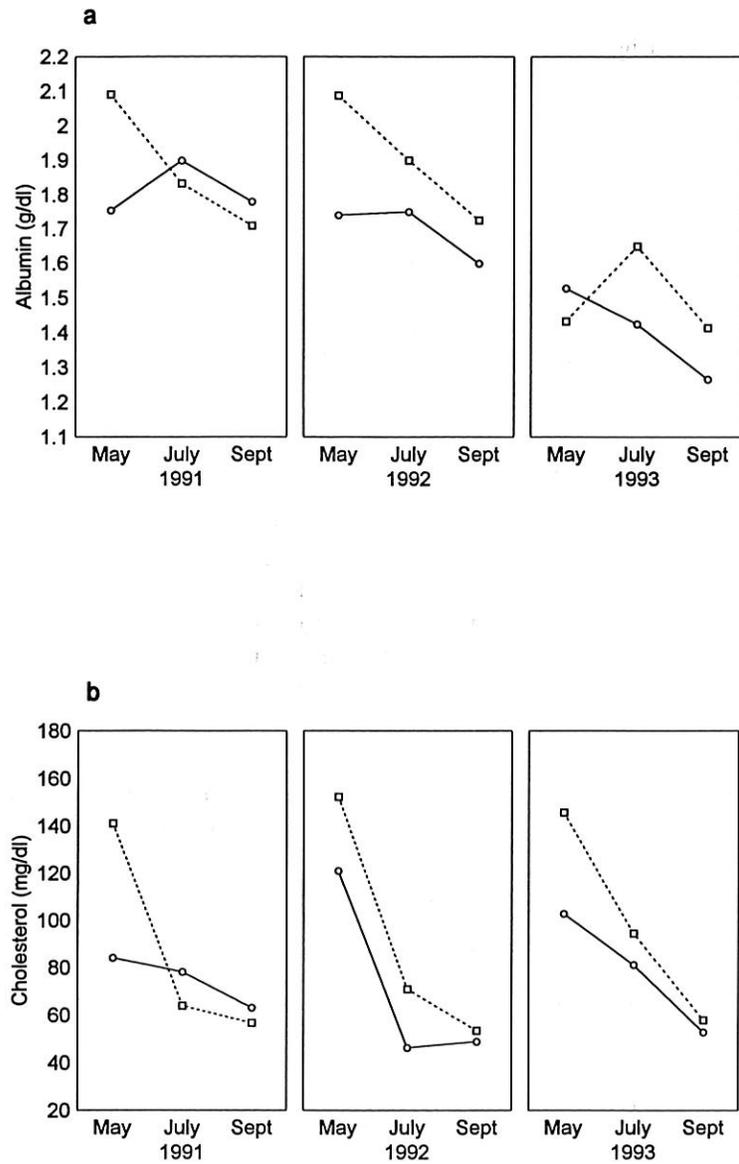


Figure 13. Line graphs of seasonal changes in (a) albumin, (b) calcium, (c) cholesterol, and (d) vitamin E for healthy male Mojave desert tortoises ( $n = 51$ ) at 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1991-93. Solid line = City Creek; dotted line = Littlefield.

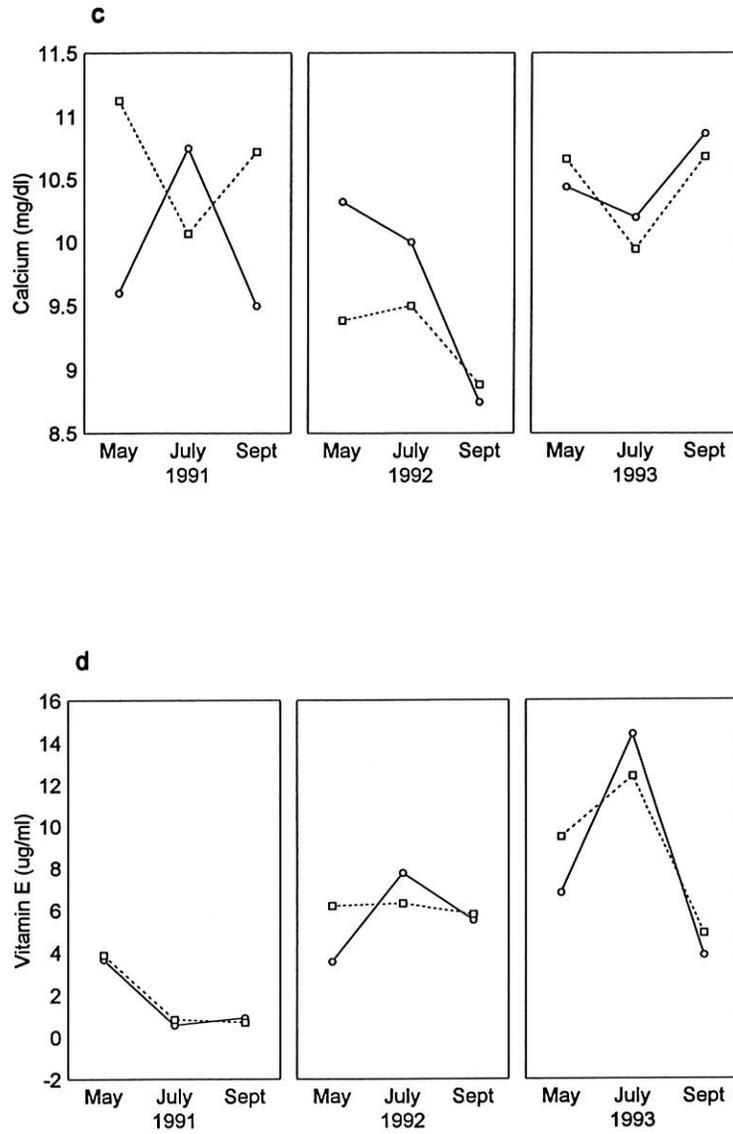


Figure 13. (continued) Line graphs of seasonal changes in (a) albumin, (b) calcium, (c) cholesterol, and (d) vitamin E for healthy male Mojave desert tortoises ( $n = 51$ ) at 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1991-93. Solid line = City Creek; dotted line = Littlefield.

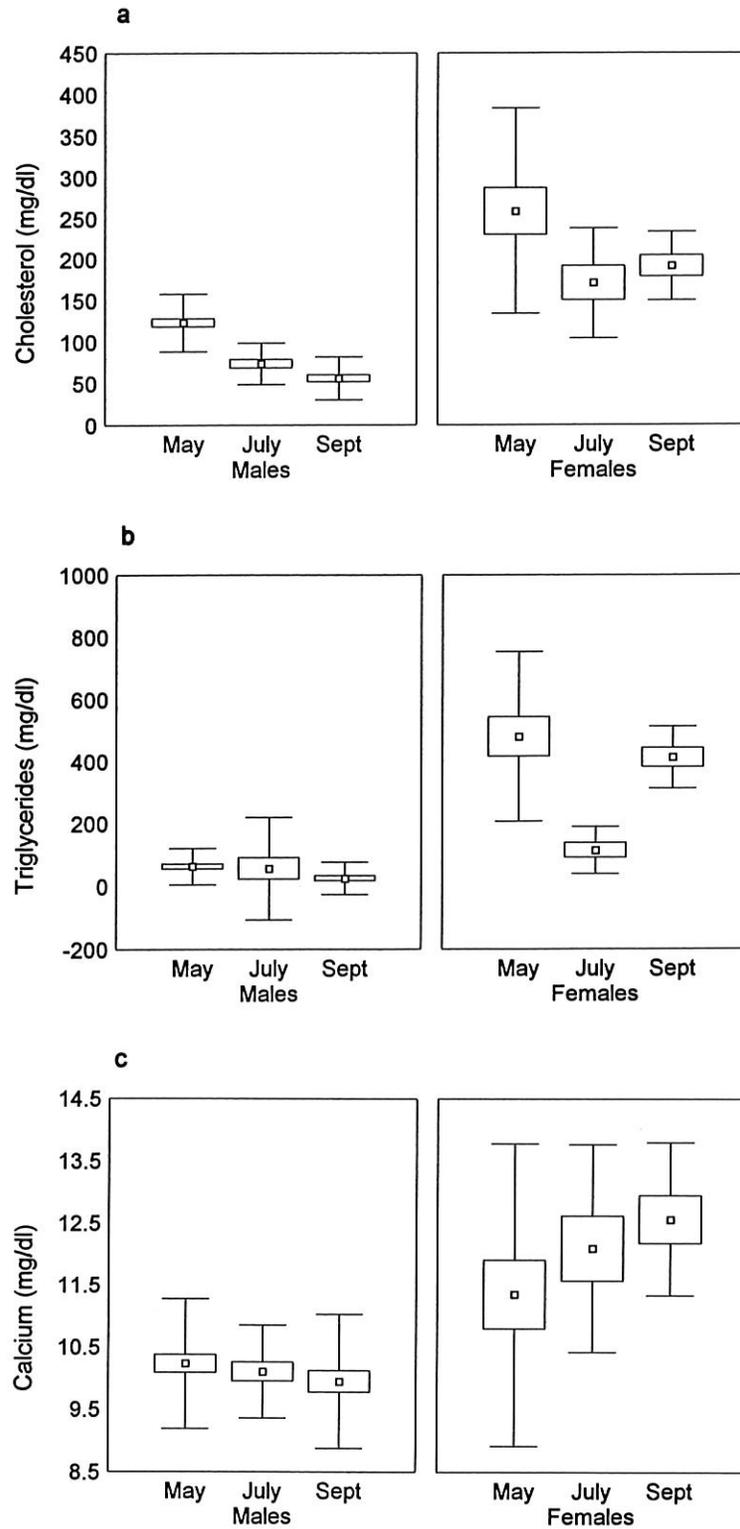


Figure 14. Box and whisker plots of seasonal changes in (a) cholesterol, (b) triglycerides, (c) calcium, (d) phosphorus, and (e) vitamin E for healthy male and female Mojave desert tortoises ( $n = 81$ ) for 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1991-93.

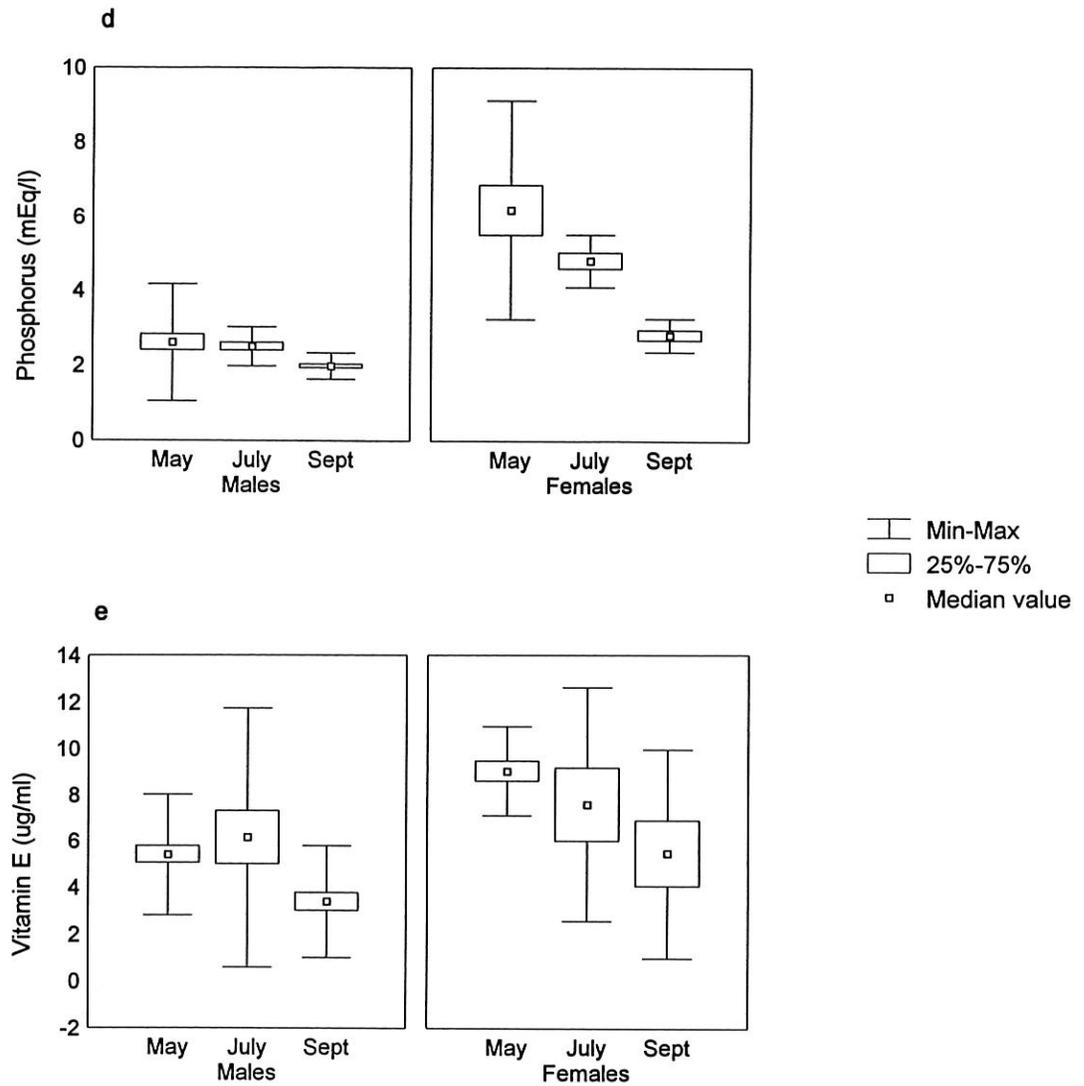


Figure 14. (continued) Box and whisker plots of seasonal changes in (a) cholesterol, (b) triglycerides, (c) calcium, (d) phosphorus, and (e) vitamin E for healthy male and female Mojave desert tortoises ( $n = 81$ ) for 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1991-93.

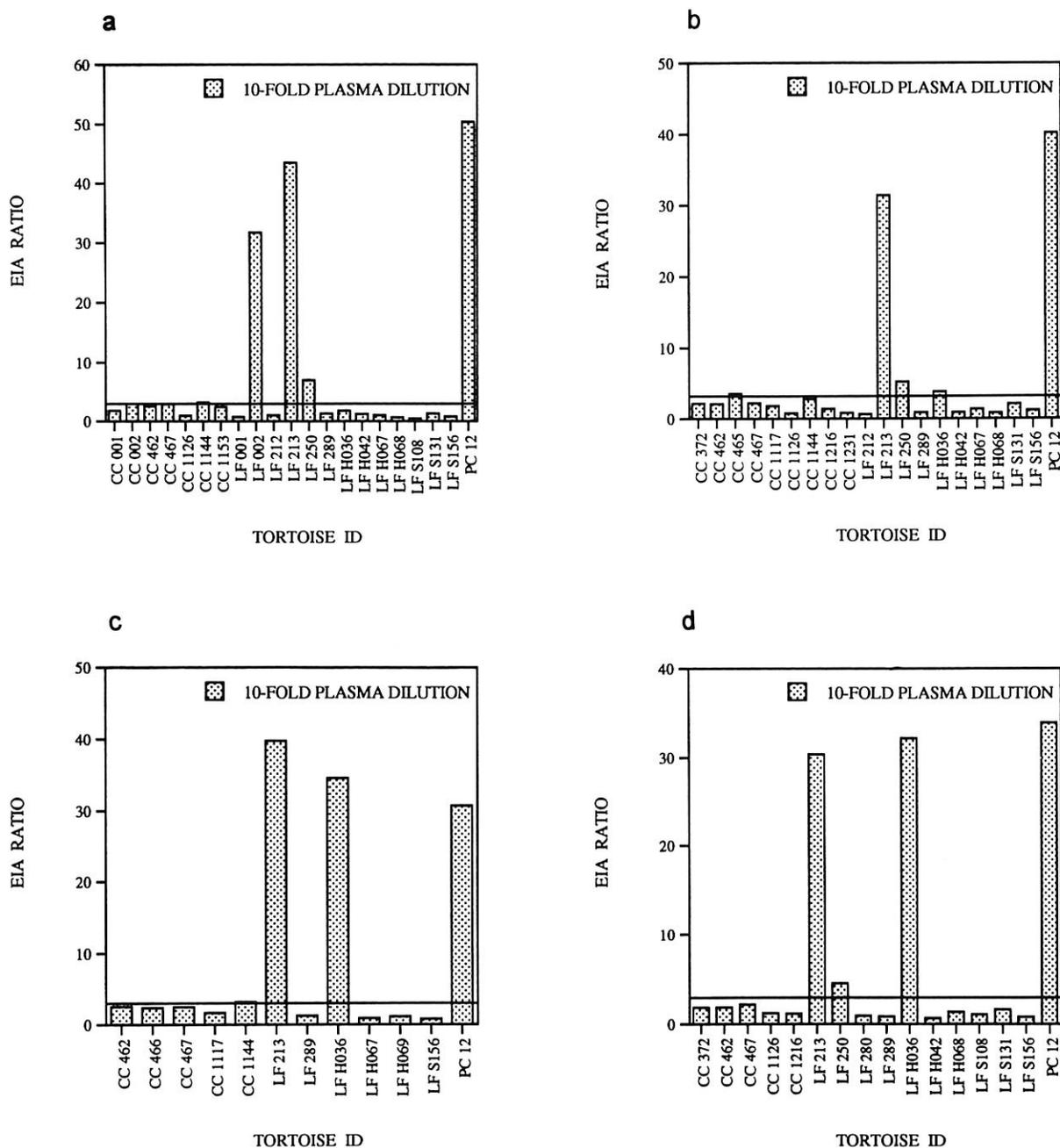


Figure 15. Plasma antibody titers for *Mycoplasma agassizii* for Mojave desert tortoises from City Creek (CC), Ut.; Littlefield (LF), Ariz.; and Paradise Canyon (PC), Ut., from (a) September 1992, (b) May 1993, (c) July 1993, and (d) September 1993. A positive titer has an EIA ratio of sample > 3.

except 1. This Littlefield tortoise (250) had positive titers each time it was captured, but showed no clinical signs of URTD.

From 221 samples, we found 5 species of bacteria in the nasal cavity (Appendix 11), with 2 documented pathogens (*Mycoplasma* spp., *Pasteurella testudinis*). Four nasal bacteria were isolated with nasal and choanal swabs. We found *Chlamydia*-like organisms with nasal and choanal swabs in Cultorettes ( $n = 2$ ) and *Flavobacterium* spp. with nasal swabs stored in thioglycolate broth ( $n = 1$ ). We found *P. testudinis* using choanal swabs stored in Cultorettes ( $n = 4$ ), Stuart transport tubes ( $n = 2$ ), and thioglycolate broth ( $n = 3$ ). We found *Staphylococcus* spp. using choanal swabs stored in Cultorettes ( $n = 1$ ) and thioglycolate broth ( $n = 1$ ), and nasal swabs in Stuart transport tubes ( $n = 1$ ) and thioglycolate broth ( $n = 1$ ). We did not isolate *Mycoplasma* spp. from nasal or choanal swabs. We isolated 3 nasal bacteria from nasal flushes. The majority of bacteria were *Mycoplasma* spp. (38%), followed by *P. testudinis* (22%), and a *Chlamydia*-like organism (5%).

Compared to healthy tortoises, we found higher ( $X^2 = 31.9$ ,  $df = 1$ ,  $P < 0.001$ ) levels of *P. testudinis* in the nares of ill tortoises. We also found higher ( $X^2 = 7.5$ ,  $df = 1$ ,  $P < 0.001$ ) levels of *P. testudinis* in tortoise nares in September than other times of the year.

**Cloacal.** From 213 samples, we found 17 species of bacteria in the cloaca (Appendix 12), 2 of which were opportunistic pathogens (*Pseudomonas* spp., *Salmonella* spp.). The majority (61%) of cloacal bacteria were nonpathogenic *Staphylococcus* spp.

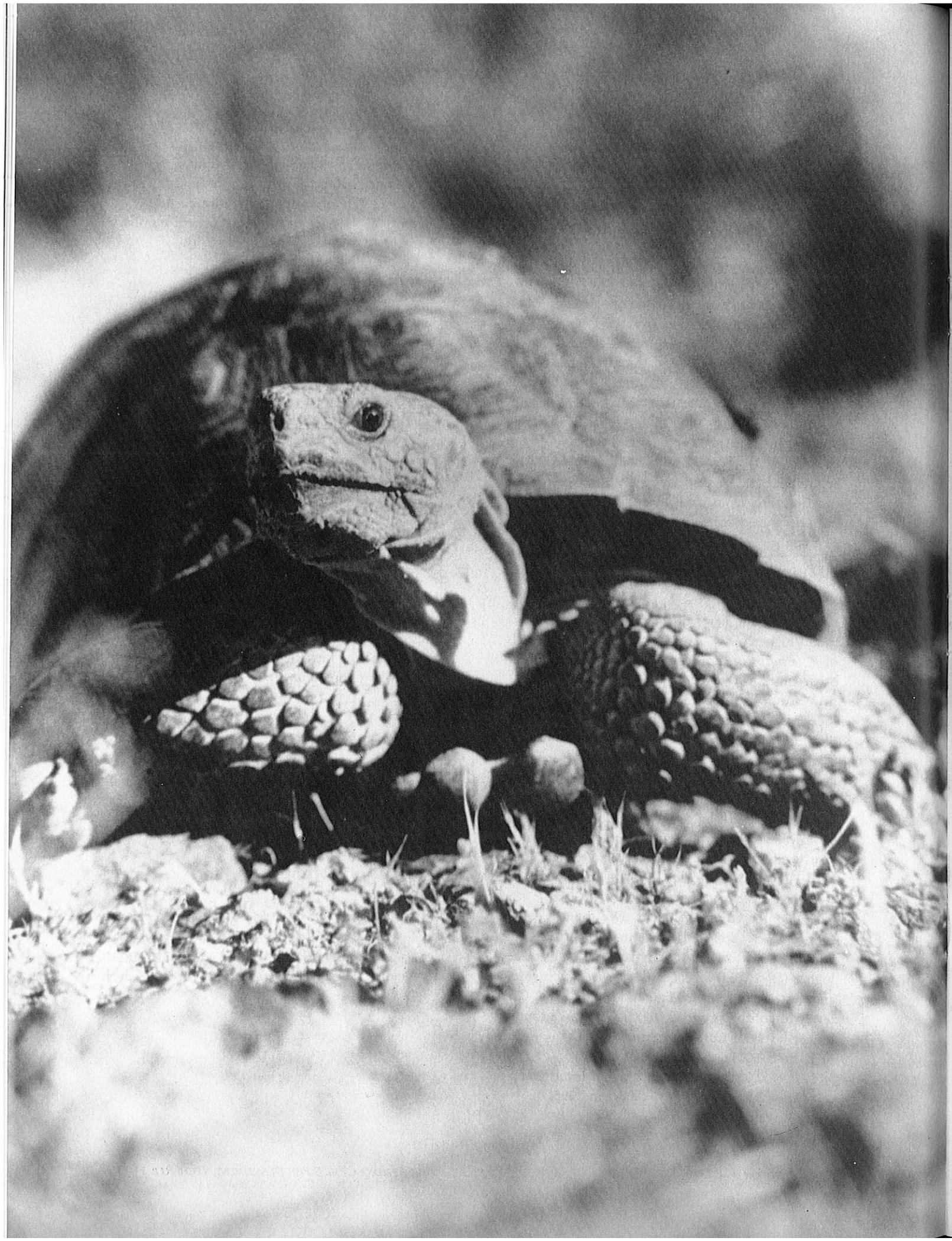
Three cloacal bacteria showed site differences, 4 showed seasonal differences, and 3 showed yearly differences. City Creek tortoises had higher levels of coliforms ( $X^2 = 4.1$ ,  $df = 1$ ,  $P < 0.02$ ) and *Escherichia coli* ( $X^2 = 4.9$ ,  $df = 1$ ,  $P < 0.001$ ) compared to Littlefield. We found *Campylobacter* spp. only once in 1 ill tortoise from Paradise Canyon. In healthy tortoises, we found higher ( $P < 0.03$ ) levels of *Corynebacterium* spp. and *Pasteurella* spp. in May, and higher ( $P < 0.03$ ) levels of coliforms and *E. coli* in July. We found higher levels of *Corynebacterium* spp. ( $X^2 = 6.8$ ,  $df = 1$ ,  $P < 0.001$ ) and *Pasteurella* spp. ( $X^2 = 4.1$ ,  $df = 1$ ,  $P < 0.001$ ) in 1992, and higher levels of *E. coli* ( $X^2 = 7.5$ ,  $df = 1$ ,  $P < 0.001$ ) in 1993, irrespective of health status.

### Parasitology

Pinworms (*Trachygonetria* spp.) were the only parasites found; infection rate did not differ ( $P > 0.05$ ) between sexes and among seasons or years. Ninety-nine percent of tortoise feces had pinworm eggs ( $n = 137$  fecal samples). We found no difference ( $P > 0.05$ ) in mean eosinophil numbers for tortoises with and without pinworm.

### Weather

Rainfall at St. George, Utah, was below average during 1989-90 and 1990-91, and below average at Beaver Dam, Arizona during 1988-89, 1989-90, and 1991-92. Mean annual rainfall ( $\bar{x} \pm SD$ ) at the St. George weather station was  $20.3 \pm 6.2$  cm over a 63-year period (1928-91). Mean annual rainfall at Beaver Dam was  $18.5 \pm 5.9$  cm over a 36-year period (1956-92) (Appendix 13). The lowest rainfall was recorded in June in all years at both St. George and Beaver Dam. Analysis of the permanent weather station data (1988-93) and automatic station data (1992-93) for both sites showed higher ( $P < 0.001$ ) rainfall in February than other months. The automatic weather stations indicated lower ambient temperature in December; higher relative humidity in January, February and March; higher soil moisture in May, June, and July; and mean wind speed greater at Littlefield in 1993 than any other site or year (Appendix 14).



## DISCUSSION

### Physiological Variations in Hematology, Clinical Chemistry, and Bacteria

*Environmental Conditions.* Seasonal and yearly changes in rainfall and forage availability were major environmental conditions affecting tortoise health. Winter is the season of highest rainfall in the Mojave Desert (Turner 1994). Tortoise foraging activity primarily occurs in the spring and early summer (Berry 1975) and appears related to rainfall patterns (Ruby et al. 1994). In our study, healthy male tortoises had higher levels of albumin, cholesterol, and vitamin E in May, which may suggest spring foraging.

During our study, there were above and below average years in terms of rainfall and forage availability. In 1992 and 1993, rainfall was above average which probably resulted in more available forage. In support of this, Esque (1994) found City Creek and Littlefield had greater vegetative biomass production in 1992 compared to 1990 and 1991. In 1992 and 1993, plasma vitamin E levels suggested more foraging by tortoises than the previous 3 years.

Ill tortoises were associated with years of below average rainfall (1989, 1990, 1991). Male tortoises with signs of URTD (May 1991) were probably catabolizing body reserves as indicated by their high levels of albumin and cholesterol. Jacobson et al. (1991) hypothesized higher levels of cholesterol in the blood were a response to starvation; tortoises met energy needs through lipid catabolism.

*Sex Differences.* Tortoise gender influenced seasonal changes in hematology and clinical chemistry. Other tortoise species have similar changes. For example, Hart et al. (1991) reported healthy female Aldabra giant tortoises (*Geochelone gigantea*) had lower levels of PCV compared to males.

Most seasonal changes in clinical chemistry occurred when female tortoises were in vitellogenesis. Recent studies reported female desert tortoises had higher levels of albumin, calcium, phosphorus (O'Connor et al. 1994), and cholesterol (Christopher et al. 1994) compared to males. Rostal et al. (1994) reported calcium levels increased from May to September in captive female desert tortoises. Elevated calcium levels have been associated with vitellogenesis (Ho 1987). Taylor and Jacobson (1982) associated

vitellogenesis with higher levels of cholesterol in female gopher tortoises (*G. polyphemus*). Higher levels of cholesterol and lipids were observed in gravid female Mediterranean tortoises (*Testudo graeca* and *T. hermanni*) in August (time of oviposition) compared to males (Lawrence 1987).

The observed peaks in cholesterol and triglycerides in May and September suggests females were preparing for vitellogenesis in the spring and in the fall. Rostal et al. (1994) reported vitellogenesis in the spring and fall in captive desert tortoises as indicated by increased calcium levels and follicular growth. We also saw increased levels of estradiol during vitellogenesis, which had been reported in free-ranging gopher tortoises (Palmer and Guillette 1990).

Higher levels of AST and ALT in May possibly result from male aggression during mating in healthy male tortoises. For instance, high levels of AST are associated with tissue damage since muscle cells of most species contain a large amount of this enzyme (Coles 1986). Rostal et al. (1994) observed male-to-male combat in April and May. O'Connor et al. (1994) suggested that an increase in AST levels in male desert tortoises was associated with mating or fighting in the spring. High levels of AST could also be a result of male tortoises ingesting hepatotoxins with their spring forage (O'Connor et al. 1994). Another possibility is male and female tortoises have different basal levels of enzyme activity.

*Disease. Upper Respiratory Tract Disease.* As expected, tortoises with signs of URTD had higher levels of heterophils, azurophils, BUN, sodium, and *P. testudinis*, and lower levels of phosphorus. Jacobson et al. (1991) found higher levels of hemoglobin, BUN and sodium, and lower levels of phosphorus in tortoises with signs of URTD, whereas Christopher et al. (1992) found higher levels of heterophils, cholesterol, and potassium. Hart et al. (1991) reported an increase in heterophils in response to inflammatory disease in free-ranging Aldabra giant tortoises.

The duration of URTD in free-ranging tortoises is unclear. Jacobson et al. (1991) reported URTD in free-ranging tortoises was chronic in nature and lasted up to 1 year before the tortoises eventually died. They reported ill tortoises in captivity "may survive for several years before succumbing to systemic disease." However, we found free-ranging tortoises had

signs of URTD for 2 years out of 5. M. Christopher (Dep. Vet. Path., Univ. Calif. Davis, Calif., unpubl. data) found free-ranging desert tortoises with signs of URTD for at least 3 years. More long-term health studies may find tortoises living longer with URTD.

Our ELISA results confirmed clinical signs of URTD in 4 of 5 tortoises. In contrast, Christopher et al. (1992, 1993) found no relationship between antibody status and signs of URTD. We had problems with the ELISA. In 1 case, the test appeared too sensitive, as Littlefield tortoise 250 had positive titers for *M. agassizii* and no signs of URTD. This tortoise could appear asymptomatic if the tortoise was: (1) immunologically competent enough to ward off URTD; (2) another organism caused the ELISA to appear positive; or (3) the physical exam did not reveal obscure signs of URTD. In support of the second point, recent research at the University of Florida detected a new species of mycoplasma in seropositive Mojave desert tortoises (D. Brown, Dep. Infect. Dis., Univ. Fla., Gainesville, Fla., unpubl. data).

Detailed physical examination for evidence of URTD in conjunction with an ELISA will likely detect URTD in a tortoise. Another way to confirm the presence of *M. agassizii* is to use a polymerase chain amplification (PCR) test to isolate a specific gene of *M. agassizii* (D. Brown, Dep. Infect. Dis., Univ. Fla., Gainesville, Fla., pers. commun.).

Interestingly, there was no correlation between *Mycoplasma* spp. found in the nasal cavity and URTD. Recent transmission studies showed that a newly recognized mycoplasma, *M. agassizii*, was the causative agent of URTD (Brown et al. 1994b). Previously, Christopher et al. (1993) had found cultures of *Mycoplasma* spp. in tortoises with no signs of URTD or positive titers. Another species of mycoplasma may exist in Mojave desert tortoises, as there is a discrepancy between the numbers of tortoises with positive titers and cultured *Mycoplasma* spp.

**Bacteria.** Bacterial diseases may cause seasonal changes in hematology. For example, high numbers of heterophils and azurophils were found in Littlefield tortoises with chronic inflammation, possibly caused by *Pseudomonas* spp.

Healthy desert tortoises have many species of nasal and cloacal bacteria. Jacobson et al. (1991) found 11 species of bacteria in the respiratory tract

(choanae, nasal cavity, trachea, lung) of ill (URTD) and healthy desert tortoises. They found microorganisms resembling *Mycoplasma* spp. in 27% of ill tortoises. *P. testudinis* has been found in both ill and healthy desert tortoises (Snipes and Biberstein 1982, Jacobson et al. 1991, Christopher et al. 1993) and may be commensal in healthy free-living tortoises (Snipes and Biberstein 1982). Unlike the apparently commensal *P. testudinis*, the cloacal bacterium, *Salmonella* spp., has been frequently cultured from reptile abscesses (Frye 1981, Marcus 1981).

**Parasites.** Pinworms are among the most common and numerous intestinal worms of lizards and turtles (Marcus 1981). Earlier, Jarchow and May (1989) found moderate to large numbers of pinworm in 12 possibly debilitated free-ranging tortoises from the Beaver Dam Slope in Utah and Arizona. The host can reinfect itself by breathing or ingesting the eggs (Noble et al. 1989). Tortoises share burrows and have been documented eating soil and scat (Esque and Peters 1994), activities which contribute to continued reinfection. Though the majority of tortoises had pinworms, this condition is not life threatening (Marcus 1981).

Glassman et al. (1979) found higher numbers of eosinophils in alligators (*Alligator mississippiensis*) with intestinal leeches. We found that tortoises with pinworms did not have increased numbers of eosinophils. We suspect there is no eosinophilia associated with pinworms, as we observed eosinophilia in 5 tortoises that did not result from pinworm infestation. Another type of intestinal parasite may be responsible for the high numbers of eosinophils. In Aldabra giant tortoises, high eosinophil counts were associated with evidence of fecal parasites (Hart et al. 1991).

### Health Profiles

Christopher et al. (1992, 1993) developed normal reference ranges for free-ranging desert tortoises in eastern California. Our ranges were comparable to those, except for 3 blood parameters. We found 3 times less BUN, twice as much ALP, and 3 times as much total bilirubin than reported by Christopher et al. (1992, 1993). Differences in reference ranges were probably a function of methods and/or tortoise age. Our normal reference ranges for fibrinogen, creatinine, bile acid, triglycerides, copper, selenium, iron, zinc, and vitamins A and E are the first reported.

Tortoises with signs of URTD have more WBCs (e.g., lymphocytes, heterophils, basophils), indicating an inflammatory process. Higher levels of bile acids, AST, ALT, and bilirubin could indicate liver disease, whereas lower PCV and higher phosphorus could indicate renal disease (Tietz 1987).

Dehydration in tortoises was evident from high levels of PCV, BUN, and uric acid. High levels of BUN and osmolality have been reported in starved and dehydrated tortoises (Dantzler and Schmidt-Nielsen 1966, Balinsky et al. 1967, Baze and Horne 1970). Christopher et al. (1993) found BUN was the best indicator of hydration status. High levels of BUN and electrolytes were found in water-stressed desert tortoises (O'Connor et al. 1994). We believe high levels of glucose and corticosterone indicated handling stress.

Some female tortoises exhibited high levels of cholesterol and triglycerides in the spring and may have laid large clutches of eggs. We can only hypothesize this, since neither ultrasonography nor radiography were used to verify reproductive status.

Elevated levels of total protein and vitamins A and E levels were associated with foraging tortoises (T. Esque, Dixie Resour. Area, Bur. Land Manage., St. George, Ut., pers. commun.).

### Sampling Techniques

Using an immobilizing drug and different autoanalyzers did not seem to confound comparisons between tortoise health studies. Lower dosages of ketamine hydrochloride were just as effective as higher doses at immobilizing tortoises for blood collection. Despite using ketamine hydrochloride and a monochromatic autoanalyzer, our blood chemistry values were similar to those of Christopher et al. (1993); Christopher et al. (1993) did not use anesthesia to immobilize tortoises and used a bichromatic autoanalyzer. However, using anesthesia, such as ketamine hydrochloride, can result in differences in blood chemistry values (Jacobson 1983, Bennett et al. 1992). Furthermore, Bolten et al. (1992) cautioned that the type of autoanalyzer may confound comparisons between clinical chemistry studies; they found different chemistry results using monochromatic and bichromatic autoanalyzers.

In tortoises, blood placed in EDTA-coated tubes will hemolyze and result in inaccurate blood

counts (Jacobson 1987). Serum is not recommended for reptile studies because clot formation is unpredictable, clotting time may allow the respiring blood cells to change the chemical composition of the sample, and less fluid volume results (Bolten et al. 1992).

We did not find positive cultures of *Mycoplasma* spp. until May 1992. We changed our field protocol to use 50% less saline to flush the nares and 50% less tryptic soy broth to culture the aspirate. Changes in laboratory procedure included filtering the aspirate and using twice as much culture medium. We believe the discovery of *Mycoplasma* spp., was likely due to a change in field and laboratory procedures rather than the introduction of infected animals to the study sites.

The 1989 handling stress study may have been designed incorrectly. We found no difference in corticosterone levels between stressed and unstressed tortoises. In contrast, Morris and Owens (1992) found bleeding sea turtles (*Caretta caretta*, *Lepidochelys kempi*) accounted for an upward trend in corticosterone levels. We may have had 2 problems in analyzing handling stress in desert tortoises: (1) we do not know what factors stress tortoises, and (2) corticosterone may not be a good indicator of stress. For example, male Kemp's ridley sea turtles (*Lepidochelys kempi*) had elevated corticosterone levels when stressed by netting, whereas turning the turtles over on their backs did not result in elevated levels (R. Valverde, Dep. Bio., Texas A&M Univ., College Station, Tex., pers. commun.). Interestingly, we found higher levels of corticosterone in tortoises with abnormal blood values. These data may suggest ill tortoises are more easily stressed than healthy tortoises.



## MANAGEMENT IMPLICATIONS

Assessing the physiological status of threatened populations is critical for development of appropriate management plans. Considering Littlefield had more tortoises with signs of URTD and more abnormal blood values, this population may be more susceptible to ill health during periods of below average rainfall, when food resources may be limited. During these periods, competition for food resources with domestic livestock should be diminished. In addition, the Littlefield tortoise population should be monitored for URTD and ill tortoises isolated from healthy tortoise populations. The larger number of seropositive Littlefield tortoises may mean more Littlefield tortoises will develop URTD.

A combination of physical exams, laboratory tests, and microbiology best identified ill tortoises. We recommend the following to assess the health of desert tortoise populations: (1) use quality sample collection, handling, and analysis protocol; (2) use long-term monitoring of tortoise populations; and (3) use physical exams, laboratory tests, and microbiology to identify ill tortoises.

Following are specific recommendations for quality sample collection, handling, and analysis.

1. Detailed physical examination with emphasis on signs of URTD.
2. Collect whole blood for complete blood counts (CBC) in lithium heparin microtainers. Analyze whole blood for PCV.
3. Prepare blood smears, and analyze for differential WBCs.
4. Collect plasma in lithium heparin vacutainers for clinical chemistry analyses. Mix and centrifuge heparinized blood for a consistent time period. Samples should be mixed and centrifuged for 5 minutes each. Analyze plasma for BUN, uric acid, total protein, albumin, AST, ALT, calcium, phosphorus, cholesterol, triglycerides, potassium, osmolality, and vitamins A and E.
5. Collect plasma and analyze for *M. agassizii* antibodies with an ELISA.
6. Collect cloacal bacteria with swabs and analyze for *Pseudomonas* spp. and *Salmonella* spp.
7. Compare hematology and clinical chemistry to normal reference ranges, and compare ELISA and bacterial results with past studies.

Designing and implementing appropriate health monitoring programs is also important in assessing the physiological status of tortoise populations. If the objective of the monitoring program is to compare tortoise health between 2 sites with different land management practices, factors such as soil type, vegetation, rainfall patterns, and incidence of disease should be similar. Otherwise, such factors must be accounted for in any analysis. We recommend collecting health data at the same time and frequency at each study site for ensuring proper statistical comparison. Data interpretation should follow a multivariate analysis approach and take into account the interaction of hematology and blood chemistry parameters.

## LITERATURE CITED

- Balinsky, J. B., I. L. Choritz, C. G. Coe, and G. S. Van Der Schans. 1967. Amino acid metabolism and urea synthesis in naturally aestivating *Xenopus laevis*. *Comp. Biochem. Physiol.* 22:59-68.
- Baze, W. B., and F. R. Horne. 1970. Ureogenesis in chelonia. *Comp. Biochem. Physiol.* 34:91-100.
- Bennett, J. S., K. A. Gossett, M. P. McCarthy, and E. D. Simpson. 1992. Effects of ketamine hydrochloride on serum biochemical and hematologic variables in rhesus monkeys (*Macaca mulatta*). *Vet. Clin. Pathol.* 21: 15-18.
- Berry, K. H. 1975. The desert tortoise relocation project: Status report for 1974. Contract F-9353, Dep. Transp., State Calif. 26pp.
- \_\_\_\_\_, editor. 1984. The status of the desert tortoise (*Gopherus agassizii*) in the United States. Contract No. 11310-0083-81, Long Beach, Calif. 30pp.
- \_\_\_\_\_. 1988. Bureau of Land Management's techniques manual for collecting and analyzing data on desert tortoise populations and habitat. Bur. Land Manage., Riverside, Calif. 30pp.
- Bezette, R. J., A. N. Bashor, J. A. Bashor, and M. Coffeen. 1989. Population analysis of the desert tortoise on the City Creek Study Plot, Washington County, Utah. Utah Div. Wildl. Resour., Salt Lake City, Ut. 30pp.
- Bolten, A. B., E. R. Jacobson, and K. A. Bjorndal. 1992. Effects of anticoagulant and autoanalyzer on blood biochemical values of loggerhead sea turtles (*Caretta caretta*). *Am. J. Vet. Res.* 53:2224-2227.
- Borysenko, M., and S. Lewis. 1979. The effect of malnutrition on immunocompetence and whole body resistance to infection in *Chelydra serpentina*. *Dev. Comp. Immunol.* 3:89-100.
- Brown, M. B., P. A. Klein, I. M. Schumacher, and K. H. Berry. 1994a. Health profiles of free-ranging desert tortoises in California: Results of a two year study of serological testing for antibody to *Mycoplasma agassizii*. Contract No. B950-C2-0046, Bur. Land Manage., Riverside, Calif. 54pp.
- \_\_\_\_\_, I. M. Schumacher, P. A. Klein, K. Harris, T. Correll, and E. R. Jacobson. 1994b. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infect. Immun.* 62:4580-4586.
- Bury, R. B., T. C. Esque, L. A. DeFalco, and P. A. Medica. 1994. Distribution, habitat use, and protection of the desert tortoise in the eastern Mojave desert. Pages 57-72 in R. B. Bury and D. J. Germano, eds. *Biology of North American Tortoises*, Res. 13, Natl. Biol. Surv., Washington, D.C.
- Christopher, M. M., R. Brigmon, and E. R. Jacobson. 1994. Seasonal alterations in plasma B-hydroxybutyrate and related biochemical parameters in the desert tortoise (*Gopherus agassizii*). *Comp. Biochem. Physiol.* 108:303-310.
- \_\_\_\_\_, I. Wallis, K. A. Nagy, B. T. Henen, C. C. Peterson, C. Meienberger, I. Girard, and J. K. Klaassen. 1992. Laboratory health profiles of free-ranging desert tortoises in California: Interpretation of physiological and pathological alterations, October 1990 - October 1991. Final Rep. No. 2, Contract No. B950-C1-0060, Bur. Land Manage., Riverside, Calif. 102pp.
- \_\_\_\_\_, I. Wallis, K. A. Nagy, B. T. Henen, C. C. Peterson, B. Wilson, C. Meienberger, and I. Girard. 1993. Laboratory health profiles of free-ranging desert tortoises in California: Interpretation of physiological and pathological alterations, March 1992 - October 1992. Final Rep. No. 3, Contract No. B950-C1-0060, Bur. Land Manage., Riverside, Calif. 133pp.
- Coffeen, M., and H. Welker. 1989. Population changes on the Woodbury-Hardy study area between 1981 and 1986, Beaver Dam Slope, Washington County, Utah. Utah Div. Wildl. Resour., Salt Lake City, Ut. 44pp.

- Coles, E. M. 1986. Veterinary clinical pathology. Fourth ed. W.B. Saunders Co., Philadelphia, Pa. 486pp.
- Dantzler, W. H., and B. Schmidt-Nielsen. 1966. Excretion in fresh-water turtle (*Pseudemys scripta*) and desert tortoise (*Gopherus agassizii*). Am. J. Physiol. 210:198-210.
- Esque, T. C. 1994. Diet and diet selection of the desert tortoise. M.S. thesis. Colo. State Univ., Fort Collins, Colo. 243pp.
- \_\_\_\_\_, and E. L. Peters. 1994. Ingestion of bones, stones, and soil by desert tortoises. Pages 105-111 in R. B. Bury and D. J. Germano, eds. Biology of North American Tortoises, Res. 13., Natl. Biol. Surv., Washington, D.C.
- Fowler, M. E. 1977. Respiratory diseases in desert tortoises. Pages 79-99 in Proc. Am. Assoc. Zoo Vet., Davis, Calif.
- Frye, F. L. 1981. Biomedical and surgical aspects of captive reptile husbandry. Vet. Med. Publ. Co., Edwardsville, Kans. 456pp.
- Glassman, A. B., T. W. Holbrook, and C. E. Bennett. 1979. Correlation of leech infestation and eosinophilia in alligators. J. Parasit. 65:323-324.
- Hart, M. G., H. J. Samour, D. M. J. Spratt, B. Savage, and C. M. Hawkey. 1991. An analysis of haematological findings on a feral population of Aldabra giant tortoises (*Geochelone gigantea*). Comp. Haematol. Int. 1:145-149.
- Ho, S. 1987. Endocrinology of vitellogenesis. Pages 145-169 in D. Norris and R. Jones, eds. Hormones and reproduction in fishes, amphibians, and reptiles. Plenum Press, New York, N.Y.
- Hoffman, R. G. 1971. Establishing quality control and normal ranges in the clinical laboratory. Exposition Press, New York, N.Y. 30pp.
- Jackson, O. F., and J. R. Needham. 1983. Rhinitis and virus antibody titers in chelonians. J. Small Anim. Practitioner 24:31-36.
- Jacobson, E. R. 1987. Reptiles. Vet. Clin. North Am. 17:1203-1225.
- \_\_\_\_\_. 1983. Hematologic and serum chemical effects of a ketamine/xylazine combination when used for immobilizing springbok. J. Am. Med. Assoc. 183:1260-1262.
- \_\_\_\_\_, J. M. Gaskin, M. B. Brown, R. K. Harris, C. H. Gardiner, J. L. LaPointe, H. P. Adams, and C. Reggiardo. 1991. Chronic upper respiratory disease of free-ranging desert tortoises (*Xerobates agassizii*). J. Wildl. Dis. 27:296-316.
- Jarchow, J. L., and C. J. May. 1989. Report on investigation of desert tortoise mortality on the Beaver Dam Slope, Arizona and Utah. Ariz. Game and Fish Dep., Phoenix, Ariz. 23pp.
- Knowles, C. 1989. A survey for diseased desert tortoises in and near the Desert Tortoise Natural Area, spring 1989. Bur. Land Manage., Riverside, Calif. 30pp.
- Lance, V., K. A. Vliet, and J. L. Bolaffi. 1985. Effect of mammalian luteinizing hormone-releasing hormone on plasma testosterone in male alligators, with observations on the nature of alligator hypothalamic gonadotropin releasing hormone. Gen. Comp. Endocrinol. 60:138-143.
- Lawrence, K. 1987. Seasonal variation in blood biochemistry of long term captive Mediterranean tortoises (*Testudo graeca* and *T. hermanni*). Res. Vet. Sci. 43:379-383.
- Marcus, L. C. 1981. Veterinary biology and medicine of captive amphibians and reptiles. Lea and Febiger, Philadelphia, Pa. 239pp.
- Minnich, J. E. 1977. Adaptive response in the water and electrolyte budgets of native and captive desert tortoises, *Gopherus agassizii*, to chronic drought. Pages 102-129 in M.

- Trotter, ed. Desert Tortoise Counc. Symp. Proc., Las Vegas, Nev.
- Morris, Y. A., and D. W. Owens. 1992. Corticosterone and stress in sea turtles. *Am. Zool.* 22:956.
- Nagy, K. A., and P. A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. *Herpetol.* 42:73-92.
- National Oceanic Atmospheric Administration. 1989-1993. Climatological data for Arizona. U.S. Dep. Commer. Natl. Climate Data Center, Asheville, N.C.
- Noble, E. R., G. A. Noble, G. A. Schad, and A. J. MacInnes. 1989. The biology of animal parasites. Sixth ed. Lea and Febiger, Philadelphia, Pa. 574pp.
- O'Connor, M. P., J. S. Grumbles, R. H. George, L. C. Zimmerman, and J. R. Spotila. 1994. Potential hematological and biochemical indicators of stress in free-ranging desert tortoises and captive tortoises exposed to a hydric stress gradient. *Herpetol. Monogr.* 8:5-26.
- Palmer, B. D., and L. J. Guillette, Jr. 1990. Morphological changes in the oviductal endometrium during the reproductive cycle of the tortoise, *Gopherus polyphemus*. *J. Morphol.* 204:323-333.
- Roskopf, W. J. 1982. Normal hemogram and blood chemistry values for California desert tortoises. *Vet. Med./Small Anim. Clin.* 1:85-87.
- \_\_\_\_\_, and R. W. Woerpel. 1982. The use of hematologic testing in diagnostic medicine: An introduction. *Chelonian Documentation Center Newsl.* 1:30-34.
- Rostal, D. D., V. A. Lance, J. S. Grumbles, and A. C. Alberts. 1994. Seasonal reproductive cycle of the desert tortoise (*Gopherus agassizii*) in the eastern Mojave desert. *Herpetol. Monogr.* 8:72-82.
- Ruby, D. E., L. C. Zimmerman, S. J. Bulova, C. J. Salice, M. P. O'Connor, and J. R. Spotila. 1994. Behavioral responses and time allocation differences in desert tortoises exposed to environmental stress in semi-natural enclosures. *Herpetol. Monogr.* 8:27-44.
- Schumacher, I. M., M. B. Brown, E. R. Jacobson, B. R. Collins, and P. A. Klein. 1993. Detection of antibodies to a pathogenic mycoplasma in desert tortoises (*Gopherus agassizii*) with upper respiratory tract disease. *J. Clin. Microbiol.* 31:1454-1460.
- Snipes, K. P. and E. L. Biberstein. 1982. *Pasteurella testudinis* sp. nov.: a parasite of desert tortoises. *Int. J. Syst. Bacteriol.* 32:201-210.
- SPSS, Inc. 1990. Advanced Statistics Student Guide. SPSS Inc., Chicago, Ill. 506pp.
- StatSoft, Inc. 1994. Statistica, Vols. 1-3. StatSoft Inc., Tulsa, Okla. 3,959pp.
- Taylor, R. W., and E. R. Jacobson. 1982. Hematology and serum chemistry of the gopher tortoise, *Gopherus polyphemus*. *Comp. Biochem. Physiol.* 72:425-428.
- Turner, R. M. 1994. Mohave Desertscrub. Pages 157-168 in D. E. Brown, ed., Biotic communities of the American Southwest, Desert Plants 4, Univ. Ariz., Tucson, Ariz.
- Tietz, N. W., editor. 1987. Fundamentals of clinical chemistry. Third ed. W.B. Saunders Co., Philadelphia, Pa. 1,010pp.
- U.S. Fish and Wildlife Service. 1989. Endangered and threatened wildlife and plants; desert tortoise. *Fed. Reg.* 54:42270-42277.
- \_\_\_\_\_. 1990. Endangered and threatened wildlife and plants: determination of threatened status of the Mojave population of the desert tortoise. *Fed. Reg.* 55:12178-12191.

**GLOSSARY**

- Alanine aminotransferase (ALT; SGPT) - A liver specific enzyme. High levels are usually associated with liver damage.
- Albumin - A protein, synthesized by the liver, found in blood. Albumin affects osmotic pressure and may act as the primary source of reserve amino acids for tissue proteins. A decrease in total albumin may result from deficient intake of protein, deficient synthesis of albumin, excessive protein breakdown, or direct loss of albumin.
- Alkaline phosphatase (ALP) - An enzyme present in many body tissues and induced by growth, bone calcification, hyperadrenocorticism, and cholestatic liver disease.
- Anemia (anemic) - Decreased mass of red blood cells, as determined by reduced levels of hemoglobin, packed cell volume, or red blood cell count.
- Anisocytosis - Blood abnormality prominent in severe anemias where red blood cell size varies.
- Aspartate aminotransferase (AST; SGOT) - Enzyme found in significant quantities in many tissues including liver, muscle, and red blood cells. AST is released with cell damage or necrosis.
- Azurophils - Type of white blood cell with round or monocytoid cell with a central nucleus and fine azurophilic granules.
- Basophils - Type of white blood cell with a central nucleus and dark purple cytoplasmic granules. Increase in response to chronic disease.
- Bile acids - Organic compounds synthesized and excreted by the liver into the bile. Elevated bile acids are indicative of reduced liver function.
- Bilirubin - Orange-yellow bile pigment produced from the breakdown of hemoglobin released from senescent red blood cells. Liver disorders can be assessed by studying the changes in bilirubin levels. Total bilirubin refers to all bilirubin in the serum. Direct bilirubin refers to the fraction that is conjugated with amino acids. Indirect bilirubin is the difference between total bilirubin and direct bilirubin, and represents unconjugated bilirubin.
- Blood urea nitrogen (BUN) - Elevated BUN is due to a variety of causes such as dehydration, high protein diet, starvation, increased protein catabolism, blockage of urine flow, and kidney disease.
- Calcium - Important in bone formation, egg production, nerve function, muscle contraction, blood clotting, and cell permeability. Almost all blood calcium is found in the plasma. Changes in total protein affect protein-bound calcium and the total serum calcium concentration.
- Carbon dioxide - Changes in dissolved carbon dioxide concentrations and bicarbonate levels in the blood occur with respiratory illnesses and kidney disease.
- Cachexia - State of advanced malnutrition.
- Choana - The opening of the nasal cavity into the roof of the mouth.
- Chloride - Major extracellular anion. Changes in serum chloride can indicate dehydration, kidney disease, and severe vomiting/diarrhea.

Cholesterol - A steroid synthesized in all tissues. Cholesterol levels may be used as indicators of liver and thyroid function. Low cholesterol levels are associated with liver disease, hyperthyroidism, low fat diet, and starvation.

Cloaca - Common opening for the digestive, urinary, and reproductive tracts.

Copper - An important mineral necessary for hemoglobin formation, enzyme systems, bone development, and reproduction. Its deficiency is associated with anemia, infertility, nervous system disorders, hair pigmentation problems, and lack of resistance to disease. Copper uptake is influenced by the presence of molybdenum, iron, calcium, and zinc.

Corticosterone - A hormone, produced in the adrenal cortex, which is synthesized in response to stress.

Creatinine - A nitrogenous compound excreted by the kidney. An increase in plasma creatinine results from decreased kidney function or dehydration.

Enzyme-linked immunosorbent assay (ELISA) - Test that detects the antibody response of desert tortoises to *M. agassizii*, a mycoplasma which causes upper respiratory tract disease (URTD). Results of the test are reported as positive, suspect, and negative and are based upon an enzyme immunoassay (EIA) ratio (EIA ratio =  $A_{405}$  of sample/ $A_{405}$  of negative control where  $A_{405}$  = spectrophotometer absorbance at 405 nm). A positive test result has an EIA ratio of sample  $>3$ , a suspect test result has an EIA ratio of sample  $>2$  and  $\leq 3$ , and a negative sample has an EIA ratio of sample  $\leq 2$ . Only tortoise plasma without *M. agassizii* antibodies is used as negative control.

Eosinophilia - An increase in the number of eosinophils in the blood.

Eosinophils - Type of white blood cell with round cells with an oval or round nucleus containing large red cytoplasmic granules. Elevated levels in endotherms associated with parasitism and hypersensitivity.

Glucose - The end product of the metabolism of starch and glycogen. Its serum level is maintained in a narrow range by the coordinated effects of hormones such as insulin and glucagon.

Hemoparasites - Parasites found in the blood or blood cells.

Hemoglobin - Oxygen-carrying protein of red blood cells.

Hepatic - Pertaining to the liver.

Heterophils - Type of white blood cell with large round cells with round or oval eccentric nucleus. Increase in response to acute disease.

Heterophilia - Increased numbers of circulating heterophils.

Hyperparathyroidism - Increased function of the parathyroid gland resulting in elevated levels of parathyroid hormone. Parathyroid hormone stimulates resorption of calcium and phosphorus from bone.

Leukocytosis - Increased numbers of circulating white blood cells.

Lymphocytes - Type of white blood cell with round or irregular shaped cells with a variable amount of cytoplasm. Nucleus round or indented and centrally placed.

Lymphocytosis - Increased numbers of circulating lymphocytes.

Monocytes - Type of white blood cell with large round or oval cells with an oval nucleus. Cytoplasm with several small vacuoles.

Monocytosis - Increased numbers of circulating monocytes.

Osmolality - Concentration of solute in solution. In dehydration, where water loss exceeds solute loss, there is an increase in osmolality levels.

Osteomalacia - Softening of the bones; a condition characterized by bone demineralization in response to vitamin D, calcium, and/or phosphorus deficiency.

Osteoporosis (Osteopenia) - A condition characterized by a decreased bone volume.

Packed cell volume (PCV) - The measurement of the ratio of the volume occupied by red blood cells to the volume of whole blood. High PCV levels may indicate dehydration while low levels indicate anemia.

Phosphorus - A mineral important in bone formation. Elevated levels are associated with bone diseases such as osteomalacia. Phosphorus also influences cell membrane structure and function, protein synthesis, and several enzyme systems. An excess of calcium and magnesium can cause a decrease in phosphorus absorption.

Polychromasia - Immature red blood cells in the peripheral blood. May be a response to anemia.

Potassium - A mineral that functions in nerve and muscle excitability, carbohydrate metabolism, and enzyme activation. Deficiencies in herbivores are rare as high potassium levels are found in growing plants.

Reference range - Range of values representing 95% of the population. Range is calculated from an entire data set of values from a group of individuals. These resulting normal values are used to determine if a test result is abnormal.

Selenium - A mineral that closely interacts with vitamin E in the prevention of lipid peroxidations in the cell. Its deficiency produces diseases similar to those observed in vitamin E deficiency. The concentration of selenium in plants depends on the plant species and soil characteristics of a given area.

Sodium - The major extracellular cation. Loss of sodium is associated with diarrhea, vomiting, or renal disease.

Total protein - Plasma proteins occur as a wide variety of chemical compounds such as albumin, globulins, fibrinogen, glycoproteins, and lipoproteins. Excessive loss of proteins resulting from renal disease, draining wounds, or starvation are reflected in reduced total protein values. Low total protein levels are often due to decreases in albumin. Elevated total protein may occur with dehydration or increased globulins.

Triglycerides - Released when lipid tissue (fat) is catabolized.

Uric acid - Major excretory product resulting from protein catabolism in reptiles. Accumulations of uric acids may result from severe dehydration or renal disease, and can lead to visceral gout and death.

Vitamin A - A fat-soluble vitamin present as provitamin A carotenoids, mainly B-carotene, in all green parts of growing plants. It is stored in large concentrations in the liver and used during dietary inadequacy. Deficiencies result in vision problems, loss of integrity of the protective lining of mucosal surfaces, reproductive failure, reduced growth, and impaired immunity with an increase in frequency and severity of infections.

Vitamin E - A fat-soluble vitamin and a significant biological antioxidant. It protects cell membranes and preserves cell integrity by avoiding lipid peroxidation. Deficiencies are associated with immunosuppression, reproductive failure, myopathies, neuropathies, liver necrosis, and vascular alterations. Vitamin E is found in green plants and grain, and quickly decreases in concentration as plants mature, or when oxidized by heat.

Vitellogenesis - Process of yolk accumulation in the ovarian follicles.

White Blood Cells (WBC) - Include granulocytes (heterophils, eosinophils, basophils) and mononuclear cells (lymphocytes, monocytes, azurophils). Increased levels of white blood cells often indicate infection.

Zinc - A mineral which functions in bone development, several enzyme systems, and is required for normal protein synthesis, and metabolism. Excess calcium impairs zinc absorption, and excess zinc interferes with copper metabolism and may cause anemia.

Appendix 1. Body mass and median carapace length measurements for Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1990-93. Data presented as  $\bar{x} \pm SD$  ( $n$ ).

Parameter	May	July	September
Body mass (kg)	3.28 $\pm$ 0.56 (55) <sup>1</sup>	3.18 $\pm$ 0.56 (30)	3.14 $\pm$ 0.58 (58)
	2.65 $\pm$ 0.39 (20) <sup>2</sup>	2.24 $\pm$ 0.52 (11)	2.52 $\pm$ 0.34 (16)
Median carapace length (mm)	254.3 $\pm$ 25.9 (55)	254.7 $\pm$ 19.2 (30)	256.7 $\pm$ 18.6 (58)
	237.6 $\pm$ 13.9 (20)	230.1 $\pm$ 18.0 (11)	239.7 $\pm$ 10.0 (16)

<sup>1</sup> Males.

<sup>2</sup> Females.

Appendix 2. Hematologic values for Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1991-93. Data presented as  $\bar{x} \pm SD$  ( $n$ ).

Parameter	May	July	September
Packed cell volume field (%)	28.0 $\pm$ 4.7 (54) <sup>1</sup>	26.6 $\pm$ 4.3 (30)	26.1 $\pm$ 3.9 (58)
	24.5 $\pm$ 4.3 (20) <sup>2</sup>	24.5 $\pm$ 3.5 (11)	23.3 $\pm$ 3.5 (16)
Hemoglobin (g/dl)	11.5 $\pm$ 1.6 (53)	11.2 $\pm$ 1.6 (30)	10.9 $\pm$ 1.1 (43)
	10.6 $\pm$ 2.1 (20)	10.8 $\pm$ 1.7 (11)	10.0 $\pm$ 1.6 (11)
Fibrinogen (mg/dl)	187.7 $\pm$ 24.1 (15)	150.0 $\pm$ 10.0 (9)	134.6 $\pm$ 10.0 (14)
	170.0 $\pm$ 26.5 (3)	146.7 $\pm$ 28.9 (3)	130.5 $\pm$ 7.8 (2)
White blood cell estimate (k/ $\mu$ l)	5.0 $\pm$ 2.3 (49)	7.3 $\pm$ 2.8 (29)	6.1 $\pm$ 2.6 (45)
	4.7 $\pm$ 2.5 (18)	5.0 $\pm$ 2.6 (10)	6.2 $\pm$ 3.0 (11)
Heterophils (k/ $\mu$ l)	216.4 $\pm$ 132.0 (49)	451.3 $\pm$ 247.6 (29)	337.5 $\pm$ 192.4 (45)
	297.2 $\pm$ 151.5 (18)	304.5 $\pm$ 235.7 (10)	325.2 $\pm$ 221.1 (11)
Lymphocytes (k/ $\mu$ l)	121.0 $\pm$ 91.6 (49)	81.3 $\pm$ 52.7 (29)	105.4 $\pm$ 54.1 (45)
	52.8 $\pm$ 36.1 (18)	64.4 $\pm$ 35.7 (10)	101.9 $\pm$ 59.6 (11)
Monocytes (k/ $\mu$ l)	9.7 $\pm$ 13.4 (49)	9.7 $\pm$ 17.3 (29)	17.1 $\pm$ 14.7 (45)
	6.8 $\pm$ 7.9 (18)	5.6 $\pm$ 6.9 (10)	27.0 $\pm$ 23.0 (11)
Azurophils (k/ $\mu$ l)	5.8 $\pm$ 9.6 (49)	8.0 $\pm$ 14.2 (29)	12.5 $\pm$ 19.5 (45)
	1.9 $\pm$ 5.2 (18)	4.7 $\pm$ 7.9 (10)	13.9 $\pm$ 24.2 (11)
Eosinophils (k/ $\mu$ l)	10.1 $\pm$ 16.2 (49)	7.8 $\pm$ 13.3 (29)	7.9 $\pm$ 11.3 (45)
	16.7 $\pm$ 31.5 (18)	19.1 $\pm$ 26.9 (10)	42.0 $\pm$ 57.1 (11)
Basophils (k/ $\mu$ l)	130.0 $\pm$ 73.9 (49)	178.4 $\pm$ 86.1 (29)	130.9 $\pm$ 64.2 (45)
	95.7 $\pm$ 86.0 (18)	98.8 $\pm$ 38.3 (10)	108.0 $\pm$ 65.2 (11)

<sup>1</sup> Males.

<sup>2</sup> Females.

Appendix 3. Serum biochemical parameter values for Mojave desert tortoises from 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1989-90. Data presented as  $\bar{x} \pm SD$  (*n*).

Parameter	September
Blood urea nitrogen (md/g)	6.8 $\pm$ 8.1 (50) <sup>1</sup>
	2.6 $\pm$ 4.2 (7) <sup>2</sup>
Total protein (g/dl)	2.8 $\pm$ 0.6 (52)
	2.8 $\pm$ 0.5 (8)
Albumin (g/dl)	1.6 $\pm$ 0.2 (53)
	1.7 $\pm$ 0.3 (8)
Total globulins (g/dl)	1.2 $\pm$ 0.5 (52)
	1.1 $\pm$ 0.3 (8)
Aspartate aminotransferase (IU/l)	39.3 $\pm$ 13.9 (53)
	42.4 $\pm$ 13.6 (8)
Calcium (mg/dl)	9.3 $\pm$ 1.5 (53)
	11.3 $\pm$ 2.1 (8)
Cholesterol (mg/dl)	58.0 $\pm$ 29.7 (51)
	135.1 $\pm$ 67.6 (8)
Triglycerides (mg/dl)	51.7 $\pm$ 124.3 (52)
	294.1 $\pm$ 188.3 (8)
Copper (ppm)	0.38 $\pm$ 0.09 (13)
	0.43 (1)
Selenium (ppm)	0.02 (12)
	0.01 (1)
Zinc (ppm)	2.7 $\pm$ 0.4 (17)
	2.1 (1)
Vitamin A ( $\mu$ g/ml)	0.3 $\pm$ 0.1 (22)
	0.3 $\pm$ 0.02 (2)
Vitamin E ( $\mu$ g/ml)	2.4 $\pm$ 0.7 (22)
	3.1 $\pm$ 0.5 (2)
Corticosterone (ng/ml)	5.1 $\pm$ 2.7 (24)
	2.6 $\pm$ 1.5 (6)

<sup>1</sup> Males.

<sup>2</sup> Females.

Appendix 4. Serum electrolyte parameter values in Mojave desert tortoises from 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1989-90. Data presented as  $\bar{x} \pm SD$  (*n*).

Parameter	September
Sodium (mEq/l)	134.4 $\pm$ 3.7 (36) <sup>1</sup> 132.0 $\pm$ 7.2 (5) <sup>2</sup>
Potassium (mEq/l)	4.9 $\pm$ 0.9 (37) 5.1 $\pm$ 0.4 (5)

<sup>1</sup> Males.

<sup>2</sup> Females.

Appendix 5. Plasma biochemical parameter values for Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1991-93. Data presented as  $\bar{x} \pm SD$  (*n*).

Parameter	May	July	September
Glucose (mg/dl)	105.4 $\pm$ 21.0 (17) <sup>1</sup> 106.7 $\pm$ 5.7 (3) <sup>2</sup>	126.6 $\pm$ 30.3 (19) 98.5 $\pm$ 13.9 (8)	101.2 $\pm$ 16.1 (29) 87.6 $\pm$ 13.5 (5)
Blood urea nitrogen (mg/dl)	2.6 $\pm$ 9.0 (45) 0.2 $\pm$ 0.4 (17)	2.6 $\pm$ 5.3 (27) 1.4 $\pm$ 2.2 (10)	4.2 $\pm$ 10.7 (55) 0.4 $\pm$ 0.7 (10)
Creatinine (mg/dl)	0.2 $\pm$ 0.07 (54) 0.2 $\pm$ 0.09 (20)	0.3 $\pm$ 0.07 (30) 0.3 $\pm$ 0.1 (11)	0.2 $\pm$ 0.09 (44) 0.2 $\pm$ 0.1 (11)
Uric acid (mg/dl)	3.9 $\pm$ 1.7 (17) 3.5 $\pm$ 1.3 (3)	3.2 $\pm$ 1.3 (19) 4.4 $\pm$ 1.9 (8)	3.7 $\pm$ 1.4 (29) 2.8 $\pm$ 1.0 (5)
Total protein (g/dl)	3.6 $\pm$ 0.6 (55) 3.5 $\pm$ 0.5 (20)	3.8 $\pm$ 0.5 (30) 3.7 $\pm$ 0.5 (11)	3.3 $\pm$ 0.5 (58) 3.2 $\pm$ 0.4 (16)
Albumin (g/dl)	1.8 $\pm$ 0.3 (55) 2.0 $\pm$ 0.5 (20)	1.7 $\pm$ 0.2 (30) 1.8 $\pm$ 0.4 (11)	1.6 $\pm$ 0.2 (58) 1.8 $\pm$ 0.4 (16)
Total globulins (g/dl)	1.8 $\pm$ 0.5 (55) 1.5 $\pm$ 0.4 (20)	2.0 $\pm$ 0.4 (30) 1.9 $\pm$ 0.5 (11)	1.6 $\pm$ 0.4 (58) 1.4 $\pm$ 0.4 (16)
Bile acid ( $\mu$ mol/l)	3.6 $\pm$ 6.8 (15) 17.7 $\pm$ 15.9 (3)	2.0 $\pm$ 1.9 (9) 0 (3)	3.2 $\pm$ 2.1 (13) 0.2 $\pm$ 0.3 (2)
Aspartate aminotransferase (IU/l)	149.8 $\pm$ 52.7 (17) 165.0 $\pm$ 110.7 (3)	50.2 $\pm$ 18.7 (19) 43.7 $\pm$ 21.6 (8)	47.3 $\pm$ 22.1 (42) 35.5 $\pm$ 15.3 (10)
Alanine aminotransferase (IU/l)	6.8 $\pm$ 3.8 (17) 8.7 $\pm$ 5.5 (3)	2.5 $\pm$ 2.0 (19) 1.2 $\pm$ 1.9 (8)	2.7 $\pm$ 2.3 (29) 3.2 $\pm$ 2.8 (5)
Alkaline phosphatase (IU/l)	62.2 $\pm$ 33.5 (17) 74.0 $\pm$ 12.5 (3)	85.3 $\pm$ 47.9 (9) 113.3 $\pm$ 27.1 (3)	78.6 $\pm$ 23.3 (14) 79.5 $\pm$ 20.5 (2)
Calcium (mg/dl)	10.2 $\pm$ 1.0 (55) 11.3 $\pm$ 2.4 (20)	10.1 $\pm$ 0.8 (30) 12.2 $\pm$ 1.6 (11)	9.8 $\pm$ 1.0 (58) 12.9 $\pm$ 1.2 (16)
Phosphorus (mEq/l)	2.6 $\pm$ 1.5 (55) 6.2 $\pm$ 2.9 (20)	2.4 $\pm$ 0.6 (30) 4.7 $\pm$ 0.7 (11)	1.9 $\pm$ 0.4 (44) 2.8 $\pm$ 0.4 (11)

<sup>1</sup> Males.

<sup>2</sup> Females.

## Appendix 5. (continued)

Parameter	May	July	September
Cholesterol (mg/dl)	124.1 ± 34.6 (55)	81.9 ± 32.4 (30)	61.7 ± 41.5 (58)
	255.6 ± 122.4 (20)	169.7 ± 64.0 (11)	187.6 ± 38.6 (16)
Triglycerides (mg/dl)	62.0 ± 55.9 (55)	48.5 ± 143.6 (30)	22.4 ± 41.5 (58)
	464.0 ± 275.4 (19)	117.6 ± 72.3 (11)	412.8 ± 112.3 (16)
Total bilirubin (mg/dl)	0.5 ± 0.5 (17)	0.2 ± 0.08 (9)	0.2 ± 0.07 (14)
	0.5 ± 0.4 (3)	0.08 ± 0.01 (3)	0.2 ± 0.01 (2)
Direct bilirubin (mg/dl)	0.04 ± 0.008 (17)	0.01 ± 0.005 (8)	0.02 ± 0.008 (14)
	0.04 ± 0.03 (3)	0.01 ± 0.006 (3)	0.01 (2)
Indirect bilirubin (mg/dl)	0.4 ± 0.5 (17)	0.1 ± 0.09 (9)	0.2 ± 0.07 (14)
	0.5 ± 0.4 (3)	0.06 ± 0.01 (3)	0.2 ± 0.01 (2)
Copper (ppm)	0.4 ± 0.1 (16)	0.4 ± 0.09 (21)	0.4 ± 0.08 (41)
	0.4 ± 0.1 (8)	0.3 ± 0.1 (8)	0.4 ± 0.07 (11)
Selenium (ppm)	0.06 ± 0.02 (23)	0.08 ± 0.04 (20)	0.07 ± 0.03 (40)
	0.06 ± 0.008 (8)	0.07 ± 0.02 (8)	0.06 ± 0.03 (11)
Iron (ppm)	--	0.9 ± 0.5 (7)	0.9 ± 0.5 (7)
	--	0.7 ± 0.2 (4)	0.6 ± 0.08 (3)
Zinc (ppm)	2.3 ± 0.5 (12)	2.3 ± 0.6 (9)	2.8 ± 0.5 (23)
	2.5 ± 0.06 (3)	2.6 ± 0.3 (4)	2.7 ± 0.4 (6)
Vitamin A (µg/ml)	0.4 ± 0.2 (55)	0.5 ± 0.1 (24)	0.4 ± 0.1 (56)
	0.5 ± 0.3 (20)	0.5 ± 0.1 (9)	0.3 ± 0.1 (14)
Vitamin E (µg/ml)	6.3 ± 2.9 (55)	5.5 ± 5.3 (30)	3.4 ± 2.2 (56)
	8.7 ± 2.3 (20)	7.0 ± 5.2 (11)	5.5 ± 3.8 (14)
Testosterone (ng/ml)	--	--	--
	2.1 (1)	--	--
Estradiol (pg/ml)	--	--	--
	214.8 ± 121.9 (7)	115.4 ± 109.5 (5)	477.4 ± 187.6 (5)
Corticosterone (ng/ml)	2.5 ± 1.2 (15)	3.8 ± 2.0 (9)	8.1 ± 2.2 (14)
	0.7 ± 0.4 (5)	2.5 ± 2.5 (4)	2.1 ± 0.2 (3)

Appendix 6. Plasma electrolytes and osmolality parameter values in Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1991-93. Data presented as  $\bar{x} \pm SD$  (*n*).

Parameter	May	July	September
Sodium (mEq/l)	135.3 $\pm$ 9.0 (46) <sup>1</sup>	135.3 $\pm$ 6.0 (30)	135.8 $\pm$ 6.9 (58)
	130.9 $\pm$ 7.0 (19) <sup>2</sup>	131.6 $\pm$ 9.7 (11)	134.2 $\pm$ 7.4 (15)
Potassium (mEq/l)	3.6 $\pm$ 0.5 (46)	4.0 $\pm$ 0.6 (30)	3.8 $\pm$ 0.5 (58)
	3.5 $\pm$ 0.4 (19)	4.1 $\pm$ 0.4 (11)	4.2 $\pm$ 1.2 (15)
Chloride (mEq/l)	116.4 $\pm$ 16.3 (17)	115.6 $\pm$ 28.1 (9)	114.7 $\pm$ 5.5 (14)
	117.3 $\pm$ 3.1 (3)	89.3 $\pm$ 70.4 (3)	111.5 $\pm$ 24.7 (2)
Total carbon dioxide (mEq/l)	31.2 $\pm$ 4.8 (17)	30.4 $\pm$ 3.6 (9)	38.4 $\pm$ 3.9 (14)
	23.0 $\pm$ 7.1 (3)	26.1 $\pm$ 2.7 (3)	31.7 $\pm$ 0.9 (2)
Anion gap (mEq/l)	-11.9 $\pm$ 6.5 (8)	-2.9 $\pm$ 29.3 (9)	-10.7 $\pm$ 4.3 (14)
	-4.0 $\pm$ 0.8 (2)	29.6 $\pm$ 65.2 (3)	-4.1 $\pm$ 3.5 (2)
Osmolality (mOs/kg)	291.4 $\pm$ 41.3 (55)	298.3 $\pm$ 36.3 (19)	290.7 $\pm$ 37.9 (44)
	270.7 $\pm$ 31.4 (20)	299.0 $\pm$ 45.9 (8)	269.1 $\pm$ 25.8 (11)

<sup>1</sup> Males.

<sup>2</sup> Females.

Appendix 7. Summary and evaluation of abnormal blood parameter values for serum from Mojave desert tortoises at 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1989-90. Possibly abnormal values  $<2$  SD from the mean are designated by an arrow sign pointing down, and values  $>2$  SD by an arrow pointing up. Probably abnormal values  $>3$  SD from the mean are identified by an asterisk (\*). A slash mark (-) means the tortoise was not sampled that period. CC = City Creek, LF = Littlefield.

Tort # - Sex	9/89	9/90
CC463 - M		
Corticosterone (ng/ml)	↑ 12.2	

Evaluation: High corticosterone levels in September 1989 unexplained.

Tort # - Sex	9/89	9/90
CC469 - F		
Cholesterol (mg/dl)	↑ 170.0	--

Evaluation: Female in vitellogenesis in September 1989 as cholesterol levels were high.

Tort # - Sex	9/89	9/90
CC1144 - M		
Asparate aminotransferase (IU/l)		↑ 70.0

Evaluation: Elevated asparate aminotransferase levels in September 1990 possible indication of liver or muscle damage. This tortoise did not have a high asparate aminotransferase level again until May 1993 (plasma).

Tort # - Sex	9/89	9/90
CC1153 - F		
Albumin (g/dl)	--	↑ 2.2
Cholesterol (mg/dl)	--	↑ 160.0

Evaluation: Female in vitellogenesis in September 1990 as indicated by elevated albumin and cholesterol levels.

Tort # - Sex	9/89	9/90
CC1245 - M		
Asparate aminotransferase (IU/l)	--	↑ 68.0

Evaluation: Elevated asparate aminotransferase levels possible indication of liver or muscle damage.

Tort # - Sex	9/89	9/90
LFH036 - M		
Vitamin A ( $\mu$ g/ml)		↑ 0.5

Evaluation: High levels of vitamin A in September 1990 possibly a result of increased foraging. This is supported by an increase in body mass in May 1991.

## Appendix 7. (continued)

Tort # - Sex	9/89	9/90
<b>LFH067 - F</b>		
Albumin (g/dl)		↑ 2.1
Calcium (mg/dl)		↑ 14.4
Cholesterol (mg/dl)	↑ 204.0	
Triglycerides (mg/dl)	↑ 409.3	↑ 488.2

Evaluation: Female exhibiting vitellogenesis in September 1989 and September 1990 as indicated by increased albumin, calcium, cholesterol, and triglyceride levels. This female showed a massive reproductive effort in May 1991, May 1993, and May 1993 (plasma).

Tort # - Sex	9/89	9/90
<b>LFH068 - M</b>		
Total protein (g/dl)		↑ 4.6
Albumin (g/dl)		↑ 2.1
Calcium (mg/dl)		↑ 13.8

Evaluation: This tortoise appears to be well-fed in September 1990 as total protein, albumin, and calcium levels were high and there was a body mass gain of 0.05 kg.

Tort # - Sex	9/89	9/90
<b>LFS108 - F</b>		
Cholesterol (mg/dl)	--	↑ 223.0
Triglycerides (mg/dl)	--	↑ 483.6

Evaluation: This female was probably in vitellogenesis in September 1990 as high triglycerides and cholesterol levels were evident. This tortoise showed similar increases of triglycerides in September 1991 and September 1993 (plasma).

Tort # - Sex	9/89	9/90
<b>LFS139 - M</b>		
Albumin (g/dl)	--	↑ 1.0

Evaluation: Undetermined significance of albumin level in September 1990.

Tort # - Sex	9/89	9/90
<b>LFS156 - M</b>		
Blood urea nitrogen (mg/dl)		↑ 53.0*
Total protein (g/dl)		↑ 5.7*
Albumin (g/dl)		↑ 2.4*
Calcium (mg/dl)		↑ 16.9*

Evaluation: This tortoise was probably dehydrated (elevated blood urea nitrogen, total protein, albumin) and may have not foraged for some time prior to sampling. This tortoise lost body mass in 1990 (0.35 kg). Elevated blood urea nitrogen may indicate tissue catabolism.

## Appendix 7. (continued)

Tort # - Sex	9/89	9/90
LF209 - M		
Asparate aminotransferase (IU/l)	↑ 71.0	

Evaluation: Elevated asparate aminotransferase levels in September 1989 possible indication of liver or muscle damage.

Tort # - Sex	9/89	9/90
LF210 - M		
Cholesterol (mg/dl)		↑ 169.0

Evaluation: High cholesterol levels in September 1990 may be due to fat catabolism. September 1990 body mass (2.1 kg) is lower than the body mass reported in May 1991 (2.3 kg).

Tort # - Sex	9/89	9/90
LF280 - M		
Triglycerides (mg/dl)		↑ 809.5*

Evaluation: High triglycerides in September 1990 may be due to fat catabolism. Body mass dropped from 4.2 kg in September 1989 to 3.4 kg in September 1990.

Tort # - Sex	9/89	9/90
LF289 - M		
Asparate aminotransferase (IU/l)		↑ 85.0*

Evaluation: Elevated asparate aminotransferase levels in September 1990 possible indication of liver or muscle damage.

Appendix 8. Summary and evaluation of abnormal blood parameter values for plasma from Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1990-93. Possibly abnormal values <2 SD from the mean are designated by an arrow sign pointing down, and values >2 SD by an arrow pointing up. Probably abnormal values >3 SD from the mean are identified by an asterisk (\*). A slash mark (/) means the tortoise was not sampled that period. NA = test not available. Incorporated into the evaluation of the laboratory data are results of rainfall, hematology (begun 5/91), enzyme-linked immunosorbent assay (ELISA) (begun 9/92), nasal bacterial cultures (*P. testudinis*, begun 5/91; *Mycoplasma* spp., begun 5/92), and cloacal bacteria cultures (*Pseudomonas* spp., *Salmonella* spp., begun 7/91). CC = City Creek, LF = Littlefield, PC = Paradise Canyon.

Tort # - Sex	9/90
CC30 - M	
Copper (ppm)	↑0.6

Evaluation: Undetermined significance of increased copper in September 1990.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC372 - F										
Packed cell volume field (%)			--	--	--	--	--		--	↓15.0
Hemoglobin (g/dl)			--	--	--	--	--		--	↓6.8
Eosinophils (k/μl)	NA		--	--	--	--	--	↑75.6	--	
Albumin (g/dl)			--	--	--	--	--		--	↓0.9
Bile acid (μmol/l)	NA	NA	--	--	--	--	--	↑22.0	--	
Calcium (mg/dl)			--	--	--	--	--	↓7.3	--	
Vitamin A (μg/ml)		↑1.0	--	--	--	--	--		--	

Evaluation: Increased vitamin A levels in May 1991 suggestive of vitellogenesis and/or spring foraging. Increased bile acids in May 1993 may indicate liver disease; cause of eosinophilia is uncertain. Anemia (low packed cell volume, low hemoglobin) and lower albumin levels in September 1993 may be a result of reduced liver function or starvation. *Mycoplasma* spp. was collected from the nasal cavity in both May and September 1993. *Pseudomonas* spp. was found in the tortoise cloaca in September 1993.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC461 - M										
Packed cell volume field (%)	↑35.5			--	--	--	--	--	--	--
White blood cell estimate (k/μl)	NA		↑12.0	--	--	--	--	--	--	--
Heterophils (k/μl)	NA		↑960.0*	--	--	--	--	--	--	--
Triglycerides (mg/dl)			↑805.0	--	--	--	--	--	--	--

Evaluation: Increased packed cell volume levels in September 1990 probably indicate dehydration. According to weather data 1990 was a drought year. Heterophilia and leucocytosis may indicate an inflammatory process in July 1991 but there was no physical evidence of disease. High triglycerides in July 1991 were probably a result of fat catabolism. Weather data shows 1991 as a drought year so inadequate forage was probably present at this time.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC462 - M										
Packed cell volume field (%)			--		--	--	↓15.0			
Monocytes (k/ $\mu$ l)	NA		--	↑49.0	--	--				
Eosinophils (k/ $\mu$ l)	NA		--	--	--	--		↑63.0		
Total bilirubin (mg/dl)	NA	NA	--	NA	--	--	NA	↑1.1		
Indirect bilirubin (mg/dl)	NA	NA	--	NA	--	--	NA	↑1.1		
Vitamin E ( $\mu$ g/ml)			--	--	--	--			↑14.7	
Corticosterone (ng/ml)			--	--	--	--		↑11.8		
Potassium (mEq/l)			--	--	--	--				↑5.6

Evaluation: Undetermined significance of monocytosis in September 1991. Tortoise appeared anemic (low packed cell volume) in September 1992. High levels of plasma corticosterone during the same period were probably due to handling stress. Increased bilirubin levels in May 1993 may indicate liver disease. The significance of eosinophilia is uncertain. High levels of vitamin E and potassium in September 1993 were probably due to foraging. Opportunistic pathogens cultured from the nasal cavity included *Mycoplasma* spp. (9/92, 5/93, 7/93), and from the cloaca included *Pseudomonas* spp. (9/92, 5/93, 7/93).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC463 - M										
Packed cell volume field (%)		--		↑36.0	--	--	--	--	--	--
Azurophils (k/ $\mu$ l)	NA	--		↑42.0	--	--	--	--	--	--

Evaluation: Tortoise possibly suffering from dehydration in September 1991 as packed cell volume levels were high. Associated azurophilia and body mass loss (0.5 kg) may be related to dehydration.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC465 - M										
Eosinophils (k/ $\mu$ l)	--		--	--	--	--	--	↑67.5	--	--
Asparate aminotransferase (IU/l)	--	NA	--	--	--	--	--	↑234.0*	--	--
Alanine aminotransferase (IU/l)	--	NA	--	--	--	--	--	↑13.0	--	--

Evaluation: Tortoise appeared suffering from liver disease in May 1993 as increased levels of asparate aminotransferase and alanine aminotransferase were evident. Undetermined significance of high levels of eosinophils at the same time. This tortoise also had *Mycoplasma* spp. cultured from its nasal cavity in May 1993.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC466 - M										
Packed cell volume field (%)			--		↓16.0	--	--	--		--
Vitamin E ( $\mu$ g/ml)			--		--	--	--	--	↑14.2	--

Evaluation: Tortoise was possibly anemic in May 1992 as packed cell volume and red blood cells estimate (blood smears) were low. Tortoise appeared to be foraging well in July 1993 as indicated by increased vitamin E levels. Opportunistic pathogens cultured from the nasal cavity included *Mycoplasma* spp. (5/92, 7/93) and *P. testudinis* (5/91, 9/91).

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC467 - M										
Packed cell volume field (%)					↓15.0					
Basophils (k/μl)	NA							↑300.0		
Glucose (mg/dl)	NA	NA	NA	NA	NA				↑158.0	
Creatinine (mg/dl)	NA						↓0.0			
Selenium (ppm)						↑0.2*				
Vitamin E (μg/ml)									↑16.0	

Evaluation: Tortoise suffering from anemia and polychromasia in May 1992. Anemia indicated by low packed cell volume and red blood cell estimate (blood smears). Increased glucose levels in July 1993 may be stress induced, while increased vitamin E levels at the same time indicate the tortoise was foraging. *Mycoplasma* spp. was continuously cultured since May 1992. Other opportunistic bacteria included *P. testudinis* (nasal; 7/91, 7/92) and *Pseudomonas* spp. (cloacal; 7/92).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC592 - F										
Packed cell volume field (%)			--		↓14.0	--	--	--	--	--
Eosinophils (k/μl)	NA	↑115.5*	--			--	--	--	--	--
Albumin (g/dl)		↑2.5	--			--	--	--	--	--
Triglycerides (mg/dl)	↑543.1		--			--	--	--	--	--
Phosphorus (mEq/l)	NA		--		↑10.4	--	--	--	--	--

Evaluation: Female tortoise was probably in vitellogenesis in September 1990. Increased albumin in May 1991 also probably associated with vitellogenesis. Undetermined significance of eosinophilia in May 1991. This tortoise showed evidence of anisocytosis in May 1991. Decreased packed cell volume and increased phosphorus levels in May 1992 may indicate renal disease which may have been fatal for this female. This female showed no sign of apparent vitellogenesis in May 1992. Bacteria found in the nasal cavity bacteria included *P. testudinis* (5/91, 9/91) and *Mycoplasma* spp. (5/92).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC593 - F										
Creatinine (mg/dl)	NA		--	--	↓0.0	--	--	--	--	--
Vitamin A (μg/ml)		↑0.8	--	--		--	--	--	--	--

Evaluation: Increased levels of vitamin A in May 1991 probably a result of foraging. Tortoise had anisocytosis and polychromasia also in May 1991. Undetermined significance of low creatinine levels. Only bacteria cultured was *Mycoplasma* spp. (nasal) in May 1992.

Tort # - Sex	5/91
CC595 - M	
Creatinine (mg/dl)	↓0.0

Evaluation: Undetermined significance of low creatinine levels.

## Appendix 8. (continued)

Tort # - Sex	7/91
CC660 - M	
Basophils (k/ $\mu$ l)	†456.0*

Evaluation: Basophilia in July 1991 may have been an artifact due to laboratory staining technique. *P. testudinis* was found in the nasal cavity.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC1117 - F										
Hemoglobin (g/dl)							--		†14.3	--
Basophils (k/ $\mu$ l)	NA	†294.0					--			--
Creatinine (mg/dl)	NA					†0.5	--			--
Albumin (g/dl)		†2.5					--	↓1.0		--
Calcium (mg/dl)	†14.4				†14.5		--			--
Triglycerides (mg/dl)		†535.7					--			--
Vitamin E ( $\mu$ g/ml)							--		†16.6*	--
Chloride (mEq/l)	NA	NA	NA	NA	NA	NA	--		↓8.0*	--

Evaluation: This female tortoise was probably in vitellogenesis in May 1991. Basophilia, possible anemia, and polychromasia may have been associated with vitellogenesis. Vitellogenesis probably occurred in May 1992 and May 1993 but not of the same magnitude as May 1991. Vitellogenesis magnitude was determined from a review of triglycerides. Increased calcium in September 1990 and May 1992 probably related to vitellogenesis. High vitamin E and chloride levels in July 1993 indicate this tortoise was foraging. Field observations confirm that this tortoise was feeding in late June (June 16, 21, 28) and early July (July 8). Undetermined significance of high hemoglobin level in July 1993. This tortoise had *Mycoplasma* spp. (5/92, 7/92, 5/93, 7/93) and *P. testudinis* (5/91) cultured from its nasal cavity.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC1126 - M										
Lymphocytes (k/ $\mu$ l)	NA		--	--		--		†247.5	--	
Aspartate aminotransferase (IU/l)		NA	--	--		--		†194.0	--	
Alanine aminotransferase (IU/l)	NA	NA	--	--		--		†12.0	--	
Copper (ppm)			--	--	†0.7*	--			--	
Selenium (ppm)			--	--		--	†0.1		--	
Corticosterone (ng/ml)			--	--		--	†11.1		--	

Evaluation: This tortoise had no physical evidence of disease but had polychromasia in May 1991. Likely hepatic inflammation with necrosis and associated lymphocytosis in May 1993. High levels of plasma corticosterone in September 1992 were probably due to handling stress. Potentially pathogenic bacteria include *Pseudomonas* spp. cultured from the cloaca in May 1992 and September 1993. *Mycoplasma* spp. was cultured from the nasal cavity in May 1992 and May 1993.

Tort # - Sex	5/91
CC1131 - F	
Creatinine (mg/dl)	↓0.0
Triglycerides (mg/dl)	†941.0*

Evaluation: This female was probably in vitellogenesis in May 1991. Undetermined significance of low creatinine levels.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC1144 - M										
Packed cell volume field (%)							↓17.0			--
Monocytes (k/μl)	NA			↑48.0						--
Total protein (g/dl)							↓2.3			--
Asparate aminotransferase (IU/l)		NA						↑188.0		--
Alanine aminotransferase (IU/l)	NA	NA	NA	NA	NA			↑11.0		--
Alkaline phosphatase (IU/l)	NA	NA	NA	NA	NA	NA	NA		↑183.0*	--
Vitamin A (μg/ml)				↑1.0						--
Potassium (mEq/l)									↑5.7	--
Chloride (mEq/l)	NA	NA	NA	NA	NA	NA	NA		↓48.0	--

Evaluation: Tortoise appeared healthy until September 1991. Possible infection indicated by monocytosis in September 1991. Tortoise metabolized fat reserves and lost body mass in September 1991 (0.7 kg). Tortoise appeared mildly anemic in September 1992 with low packed cell volume and decreased numbers of red blood cells (blood smears). Elevated asparate aminotransferase and alanine aminotransferase levels in May 1993 were possible indicators of hepatic inflammation and possible necrosis. This apparently debilitated tortoise had reduced body mass, low chloride, high alkaline phosphatase, and high potassium levels in July 1993. High levels of potassium was probably a result of decreased fluid intake and decreased urination. Low chloride levels were probably a result of reduced foraging. A reduction of bile flow may have caused high alkaline phosphatase levels. Opportunistic bacteria from the nasal cavity included *Mycoplasma* spp. (7/92, 9/92, 5/93, 7/93, 9/93) and *P. testudinis* (9/91, 5/92, 7/92). Bacteria from the cloaca included *Salmonella* spp. (9/91, 5/92) and *Pseudomonas* spp. ((7/92, 9/92, 5/93, 7/93, 9/93).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC1153 - F										
Eosinophils (k/μl)	NA		↑84.0	↑70.0		--	--	--	--	--
Creatinine (mg/dl)	NA					--	↓0.0	--	--	--
Selenium (ppm)						--	↑0.1	--	--	--
Potassium (mEq/l)				↑8.2*		--	--	--	--	--

Evaluation: Significance of eosinophilia undetermined in July and September 1991. This tortoise had clinical signs of upper respiratory tract disease in July 1991, and *Pseudomonas* spp., a potential pathogen, was cultured from its cloaca. *Pseudomonas* spp. was also cultured from the cloaca in September 1992. Bacteria from the nasal cavity included *Mycoplasma* spp. (9/92) and *P. testudinis* (5/91, 9/91). Below average rainfall in 1991 contributed to lack of water and possibly resulted in increased potassium levels in September 1991. Undetermined significance of decreased creatinine and increased selenium in September 1992.

Tort # - Sex	7/92
CC1171 - F	
Copper (ppm)	↓0.1

Evaluation: Undetermined significance of decreased copper levels. Opportunistic bacteria included *Mycoplasma* spp. (nasal) and *Pseudomonas* spp. (cloacal).

## Appendix 8. (continued)

Tort # - Sex	5/92
CC1173 -M	
Creatinine (mg/dl)	↓0.0
Copper (ppm)	↑0.7

Evaluation: Undetermined significance of high copper and low creatinine levels.

Tort # - Sex	5/92
CC1201 - F	
White blood cell estimate (K/ $\mu$ l)	↑11.2
Calcium (mg/dl)	↑14.2
Copper (ppm)	↓0.2

Evaluation: Female tortoise was possibly in vitellogenesis in May 1992 as indicated by increased calcium levels. Unknown significance of decreased copper. Leucocytosis may be associated with vitellogenesis. Tortoise was anemic as indicated by reduced red red blood cells (blood smears).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC1216 - M										
Packed cell volume field (%)	--	--	↓13.0*	--	--	--	--	--	--	--
Hemoglobin (g/dl)	--	--	↓5.1*	--	--	--	--	--	--	--
Lymphocytes (k/ $\mu$ l)	--	--	↑237.5	--	--	--	--	--	--	--
Monocytes (k/ $\mu$ l)	--	--	↑76.0*	--	--	--	--	--	--	--
Creatinine (mg/dl)	--	--	↑0.5	--	--	--	--	--	--	--
Asparate aminotransferase (IU/l)	--	--	--	--	--	--	--	↑193.0	--	--
Alanine aminotransferase (IU/l)	--	--	NA	--	--	--	--	↑11.0	--	--
Total bilirubin (mg/dl)	--	--	NA	--	--	--	--	↑1.0	--	--
Indirect bilirubin (mg/dl)	--	--	NA	--	--	--	--	↑1.0	--	--

Evaluation: In July 1991 this tortoise was anemic as indicated by reduced packed cell volume, hemoglobin, and reduced red blood cells (blood smears). Elevated creatinine may reflect muscle catabolism or reduced renal output; lymphocytosis and monocytosis indicate an inflammatory response. Elevated asparate aminotransferase and alanine aminotransferase associated with indirect bilirubin in May 1993 may indicate liver damage. Anemia and liver damage may have made this tortoise more susceptible to disease. This tortoise had clinical signs of upper respiratory tract disease in September 1993 but was negative for *M. agassizii* during the same period. Opportunistic bacteria cultured included *Mycoplasma* spp. (nasal; 5/93) and *Pseudomonas* spp. (cloacal; 5/93, 9/93).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
CC1231 - M										
Blood urea nitrogen (mg/dl)	--	↑21.0	--	--	--	--	--	--	--	--
Total protein (g/dl)	--	↓2.1	--	--	--	--	--	--	--	--
Asparate aminotransferase (IU/l)	--	NA	--	--	--	--	--	↑267.0*	--	--
Alanine aminotransferase (IU/l)	--	NA	--	--	--	--	--	↑13.0	--	--
Calcium (mg/dl)	--	↓6.9	--	--	--	--	--	--	--	--

Evaluation: Tortoise possibly suffering from cachexia in May 1991 as indicated by high blood urea nitrogen levels and low total protein. Elevated asparate aminotransferase and alanine aminotransferase may indicate hepatic disease in May 1993. *Pseudomonas* spp. was cultured from the cloaca in May 1993.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
<b>LFH036 - M</b>										
White blood cell estimate (k/ $\mu$ l)	NA				↑12.0					
Heterophils (k/ $\mu$ l)	NA		↑12.8		↑756.0					
Lymphocytes (k/ $\mu$ l)	NA	↑308.0*	↑998.4*					↑250.0		
Azurophils (k/ $\mu$ l)	NA								↑45.0	
Blood urea nitrogen (mg/dl)									↑25.0	↑74.0*
Total protein (g/dl)									↑4.7	↑4.6
Total globulins (g/dl)	NA	NA		NA	NA	NA	NA		↑2.8	↑2.8
Selenium (ppm)			NA	↑0.2*						
Vitamin A ( $\mu$ g/ml)		↑0.8								
Osmolality (mOs/kg)	NA									↑401.0

Evaluation: Reoccurring lymphocytosis, and heterophilia and later development of azurophilia indicate inflammation. High white blood cell estimate levels in July 1991 and July 1992 were probably a result of high heterophil counts. Tortoise tested negative in September 1992 for *M. agassizii*, and then tested positive in the next 3 sampling periods (5/93, 7/93, 9/93). This tortoise first showed signs of upper respiratory tract disease in May 1993 (ELISA; suspect test) and then continued to show signs in July 1993 and September 1993 with positive ELISA titers. Mild anemia (reduced red blood cells [blood smears]) in July 1991 and polychromasia in September 1991 may have been associated with inflammation. High blood urea nitrogen levels in July and September 1993 and high osmolality in September 1993 may indicate dehydration. Rainfall was nonexistent in July and September 1993. Increased total globulins in the same period possibly indicate an antibody response to upper respiratory tract disease. Opportunistic bacteria included *Mycoplasma* spp. (5/92, 7/92, 9/92, 5/93, 7/93, 9/93), *P. testudinis* (9/91, 7/93, 9/93) from the nasal cavity, and *Pseudomonas* spp. (7/92, 9/92, 7/93) from the cloaca.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
<b>LFH042 - M</b>										
Total protein (g/dl)		↑5.2*	--			--			--	
Asparate aminotransferase (IU/l)		NA	--			--		↑177.0	--	
Alkaline phosphatase (IU/l)	NA		--	NA	NA	--	NA	↑164.0	--	
Osmolality (mOs/kg)	NA	↑439.0*	--			--			--	

Evaluation: Relative dehydration indicated by high osmolality and total protein levels in May 1991. During the same period this tortoise was anemic (low red blood cells estimate [blood smears]) and had polychromasia. Elevated asparate aminotransferase and alkaline phosphatase in May 1993 possibly indicate hepatic inflammation and liver necrosis, or may be the result of musculo-skeletal injury although no physical evidence of this was apparent. Opportunistic bacteria include *Mycoplasma* spp. (5/92, 9/92, 5/93, 9/93) and *P. testudinis* (9/92, 9/93) from the nasal cavity, and *Pseudomonas* spp. (5/92, 5/93, 9/93) from the cloaca.

Tort # - Sex	9/90
<b>LFH053 - M</b>	
Zinc (ppm)	↑4.1

Evaluation: Undetermined significance of increased zinc in September 1990.

## Appendix 8. (continued)

Tort # - Sex	5/91
LF351 - F	
Hemoglobin (g/dl)	↑15.8

Evaluation: Tortoise had polychromasia, increased hemoglobin, and clinical signs of upper respiratory tract disease in May 1991. Increased hemoglobin resulted in polychromasia. Polychromasia may be associated with upper respiratory tract disease as a compensatory mechanism to increase oxygen carrying capacity of red blood cells.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LFH067 - F										
Creatinine (mg/dl)	NA			--			↑0.5			--
Uric acid (mg/dl)	NA	NA	NA	--	NA	↑7.9				--
Albumin (g/dl)				--	↑2.8*					--
Bile acid (μmol/l)	NA	NA	NA	--	NA	NA	NA	↑31.0*		--
Aspartate aminotransferase (IU/l)		NA		--				↑272.0*		--
Alanine aminotransferase (IU/l)	NA	NA	NA	--	NA			↑14.0*		--
Calcium (mg/dl)				--	↑15.0			↑14.2		--
Cholesterol (mg/dl)		↑361.0		--	↑621.0*			↑553.0*	↑285.0	--
Triglycerides (mg/dl)		↑815.6*		--	↑1009.0*			↑1000.0*		--
Direct bilirubin (mg/dl)	NA	NA	NA	--	NA	NA	NA	↑0.07		--
Phosphorus (mEq/l)	NA			--	↑10.0					--

Evaluation: This female had a massive reproductive output every May as indicated by the high levels of cholesterol and triglycerides; May 1992 was extremely productive as levels of calcium, albumin, and phosphorus were elevated. Increased levels of bile acids, aspartate aminotransferase, alanine aminotransferase, and direct bilirubin in May 1993 indicate some hepatic damage possibly associated with this reproductive output. In July 1993 cholesterol remained elevated possibly indicating continued catabolism of fat stores. Elevated uric acid in July 1992 may reflect post-laying dehydration. Undetermined significance of elevated creatinine in September 1992. *Mycoplasma* spp. (7/92, 9/92, 5/93, 7/93) was cultured from the nasal cavity, and *Pseudomonas* spp. (7/92, 9/92, 7/93) from the cloaca.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LFH068 - M										
Lymphocytes (k/μl)			--					↑297.5	--	
Monocytes (k/μl)		↑74.8*	--						--	
Creatinine (mg/dl)			--				↓0.0		--	

Evaluation: Early monocytosis and later lymphocytosis were likely responses to infection. This tortoise had anisocytosis in May 1991. Undetermined significance of low creatinine levels in September 1992. Opportunistic bacteria included *Mycoplasma* spp. (9/92, 5/93, 9/93) from the nasal cavity, and *Pseudomonas* spp. (7/92, 9/92, 9/93) from the cloaca.

## Appendix 8. (continued)

Tort # - Sex	7/93
LFH069 - F	
Hemoglobin (g/dl)	↑7.6
Calcium (mg/dl)	↑13.7
Vitamin E (μg/ml)	↑15.6

Evaluation: Undetermined significance of high levels of hemoglobin, calcium, and vitamin E. Opportunistic bacteria cultured in July 1993 include *Mycoplasma* spp. (nasal) and *Pseudomonas* spp. (cloacal).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LFS108 - F										
Hemoglobin (g/dl)		↑14.6	--					--	--	
White blood cell estimate (k/μl)	NA		--			↑11.8	--	--		↑11.8
Heterophils (k/μl)	NA		--			↑920.0	--	--		↑814.2
Monocytes (k/μl)	NA		--	↑80.0*			--	--		↑47.2
Azurophils (k/μl)	NA		--				--	--		↑82.6*
Eosinophils (k/μl)	NA		--	↑88.0			--	--		
Albumin (g/dl)			--				--	--		↓1.0
Calcium (mg/dl)	↑14.6		--				--	--		↑13.9
Phosphorus (mEq/l)	NA	↑13.9*	--				--	--		
Triglycerides (mg/dl)	↑562.6		--	↑508.0			--	--		↑604.0*
Vitamin E (μg/ml)			--				--	--		↑13.7

Evaluation: Monocytosis, heterophilia, and later development of azurophilia suggest inflammation. Female was probably in vitellogenesis in September 1990, 1991, and 1993 as indicated by elevated triglyceride levels. Elevated levels of calcium (9/90, 9/93) and vitamin E (9/93) were likely associated with vitellogenesis. High white blood cell estimate levels in July 1992 and September 1993 a result of high heterophil counts. High phosphorus in May 1991 may be associated with vitellogenesis or decreased urine output. Undetermined significance of high hemoglobin levels in May 1991 and eosinophilia in September 1991. Lower albumin levels in September 1993 may have been associated with chronic infection, possibly by *Pseudomonas* spp. Opportunistic bacteria in the nasal cavity included *Mycoplasma* spp. (5/92, 7/92, 9/92, 9/93) and *P. testudinis* (9/91, 9/92). Bacteria in the cloaca included *Pseudomonas* spp. (7/92, 9/92, 9/93).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LFS131 - M										
Monocytes (k/μl)	NA		--						--	↑57.0
Azurophils (k/μl)	NA		--						--	↑47.5
Uric acid (mg/dl)	NA		--					↑7.9	--	
Bile acid (μmol/l)	NA	NA	--	NA	NA	NA	NA	↑20.5	--	
Total bilirubin (mg/dl)	NA	NA	--	NA	NA	NA	NA	↑1.0	--	
Indirect bilirubin (mg/dl)		NA	--	NA	NA	NA	NA	↑0.9	--	
Vitamin E (μg/ml)			--					↑12.8	--	

Evaluation: Tortoise had polychromasia in May 1991 with no abnormal laboratory values. Elevated levels of bile acid, and bilirubin in May 1993 suggest hepatic injury or bile stasis. Elevated uric acid in May 1993 may have resulted from dehydration or renal disease. The monocytosis and azurophilia seen in September 1993 may be associated with *Pseudomonas* spp. *Mycoplasma* spp. (5/92, 7/92, 9/92, 5/93) and *P. testudinis* (9/92) were cultured from the nasal cavity. *Pseudomonas* spp. (cloaca) was only cultured in September 1993.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LFS153 - F										
Eosinophils (k/ $\mu$ l)	NA	--	--	↑187.5*	--	--	--	--	--	--
Calcium (mg/dl)	--	--	--	↑14.0	--	--	--	--	--	--

Evaluation: Female undergoing vitellogenesis in September 1991 as indicated by elevated calcium levels. Undetermined significance of eosinophilia in the same period. *Mycoplasma* spp. (nasal) and *Pseudomonas* spp. (cloaca) were cultured in September 1992. No abnormal laboratory values were associated with these bacteria.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LFS156 - M										
Lymphocytes (k/ $\mu$ l)	NA	--	--	--	--	--	--	↑300.0	--	--
Azurophils (k/ $\mu$ l)	NA	--	--	--	--	--	--	↑45.0	↑48.0	--
Basophils (k/ $\mu$ l)	NA	--	--	--	--	--	--	--	↑304.0	--
Glucose (mg/dl)	NA	NA	NA	NA	NA	--	--	--	↑175.0	--
Creatinine (mg/dl)	NA	--	--	--	↑0.0	--	--	--	--	--
Albumin (g/dl)	NA	--	--	--	↑3.0*	--	--	--	--	--
Bile acid ( $\mu$ mol/l)	NA	NA	NA	NA	NA	NA	NA	↑20.0	--	--
Total bilirubin (mg/dl)	NA	NA	NA	NA	NA	NA	NA	↑1.7*	--	--
Indirect bilirubin (mg/dl)	NA	NA	NA	NA	NA	NA	NA	↑1.6*	--	--
Vitamin E ( $\mu$ g/ml)	--	--	--	--	--	--	--	--	--	↑16.4

Evaluation: This male had polychromasia in July 1991 and September 1991. Undetermined significance of high albumin and low creatinine levels in May 1992. Early lymphocytosis, persistent azurophilia, and later development of basophilia may indicate some inflammation in the early part of 1993. Inflammation possibly a result of *Pseudomonas* infection. High levels of bile acid and bilirubin in May 1993 suggest hepatic injury and/or bile stasis. High level of vitamin E in July 1993 likely a result of foraging as there was a 0.1 kg body mass gain. Elevated glucose levels in July 1993 possible due to handling stress. The inflammation may have disappeared by September 1993 as no abnormal laboratory values nor opportunistic bacteria were found at that time. Nasal cavity bacteria included *Mycoplasma* spp. (7/92, 9/92, 5/93, 7/93) and *P. testudinis* (9/91). Only *Pseudomonas* spp. (9/92, 5/93, 7/93) was found in the cloaca.

Tort # - Sex	9/92
LF002 - M	
Potassium (mEq/l)	↑5.2

Evaluation: Increased potassium levels in September 1992 may indicate lack of water and decreased urination. Rainfall was below average during this time. Another possibility is disease contributed to this tortoise not drinking. This tortoise had clinical signs of upper respiratory tract disease and was positive for *M. agassizii* in September 1992. Other bacteria collected at the same time included *Mycoplasma* spp. (nasal), *P. testudinis* (nasal), and *Pseudomonas* (cloacal).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LF204 - M										
Packed cell volume field (%)	--	↑39.0	--	--	--	--	--	--	--	--
Total protein (g/dl)	--	↑5.1	--	--	--	--	--	--	--	--

Evaluation: Elevated packed cell volume and total protein levels in May 1991 may indicate dehydration though average rainfall occurred at this time. Tortoise may have been actively foraging in May 1991. Littlefield received average rainfall in May 1991 which contributed to abundant vegetation. *P. testudinis* was found in the nasal cavity in September 1991.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LF210										
Packed cell volume field (%)	↓15.0				--	--	--	--	--	--
Phosphorus (mEq/l)	NA	↑12.5			--	--	--	--	--	--

Evaluation: Tortoise was anemic in September 1990 as packed cell volume levels were low. Increased phosphorus levels in May 1991 may have been associated with hyperparathyroidism and osteomalacia. This tortoise showed clinical signs of upper respiratory tract disease in July 1991. In a 1989 dermal bone study (Wronski et al. 1992) this tortoise had evidence of mild osteomalacia. *P. testudinis* was found in the nasal cavity in September 1991, the last time this tortoise was sampled.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LF213 - M										
White blood cell estimate (k/ $\mu$ l)	--	--	--	--						↑12.4
Heterophils (k/ $\mu$ l)	--	--	--	--			↑785.4			↑731.6
Lymphocytes (k/ $\mu$ l)	--	--	--	--				↑344.0*		↑285.2
Azurophils (k/ $\mu$ l)	--	--	--	--						↑86.8*
Glucose (mg/dl)	--	--	--	--	NA	↑181.0			↑164.0	
Blood urea nitrogen (mg/dl)	--	--	--	--	↑57.0*		↑29.0*			
Copper (ppm)	--	--	--	--			↑0.6			
Vitamin E ( $\mu$ g/ml)	--	--	--	--					↑12.9	

Evaluation: Lymphocytosis with heterophilia, and later azurophilia probably an inflammatory response to upper respiratory tract disease. Tortoise had clinical signs of upper respiratory tract disease in every season since capture in May 1992. This tortoise also consistently tested positive for *M. agassizii* since the ELISA sampling was initiated in September 1992. Tortoise probably in catabolic state in May 1992 and September 1992 as indicated by elevated blood urea nitrogen levels. Handling stress occurred in July 1992 and July 1993 as glucose levels were abnormally high. Bacteria cultured from the nasal cavity included *Mycoplasma* spp. (9/92, 5/93, 7/93, 9/93), and *P. testudinis* (9/92, 5/93, 7/93, 9/93). *Pseudomonas* spp. (7/92, 9/92, 5/93, 9/93) was found in the cloaca.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
LF224 - F										
Monocytes (k/ $\mu$ l)	--		--	↑45.0	--	--	--	--	--	--
Vitamin A ( $\mu$ g/ml)	--	↑0.9	--		--	--	--	--	--	--

Evaluation: Increased vitamin A levels in May 1991 may indicate vitellogenesis and/or foraging in this female. This tortoise had clinical signs of upper respiratory tract disease in September 1991 but tested negative for *M. agassizii*. Monocytosis in September 1991 a possible response to upper respiratory tract disease. *P. testudinis* was found in the nasal cavity in September 1991.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
<b>LF250 - M</b>										
Hemoglobin (g/dl)		↑15.5	--		--				--	
Lymphocytes (k/ $\mu$ l)	NA		--		--			↑352.5*	--	
Monocytes (k/ $\mu$ l)	NA		--		--				--	↑51.0
Azurophils (k/ $\mu$ l)	NA		--		--				--	↑71.4*
Creatinine (mg/dl)	NA		--		--		↑0.0		--	
Uric acid (mg/dl)	NA	NA	--	NA	--			↑7.4	--	
Total protein (g/dl)			--		--	↑4.7			--	

Evaluation: Lymphocytosis and later monocytosis and azurophilia may be a response to inflammation. Tortoise had polychromasia, anemia (low red blood cells [blood smears]), and high hemoglobin in May 1991. Hemoglobin was again elevated in May 1993. This tortoise was positive for *M. agassizii* in September 1992, May 1993, and September 1993 but showed no clinical signs of upper respiratory tract disease. Increased total protein levels in July 1992 probably a result of foraging. Undetermined significance of low creatinine levels in September 1992. High levels of uric acid in May 1993 may be due to decreased urine production. Bacteria cultured from nasal cavity included *Mycoplasma* spp. (7/92, 9/92, 5/93). Cloacal bacteria included *Pseudomonas* spp. (9/92, 5/93, 9/93) and *Salmonella* spp. (9/92).

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
<b>LF280 - M</b>										
Total protein (g/dl)		↑4.7			--	--	--	--	--	

Evaluation: Tortoise appeared well-fed in May 1991 as total protein levels and body mass increased. Tortoise had polychromasia in May 1991 and July 1991. *Mycoplasma* spp. was found in the nasal cavity in September 1993.

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
<b>LF289 - M</b>										
Basophils (k/ $\mu$ l)			--					↑325.0	↑345.0	
Vitamin A ( $\mu$ g/ml)		↑1.0	--							

Evaluation: High levels of vitamin A in May 1991 suggest increased foraging. Average rainfall for May 1991 probably contributed to abundant vegetation at Littlefield. Basophilia in May 1993 and July 1993 may be related to *Pseudomonas* spp. infection. Opportunistic bacteria included *Mycoplasma* spp. (5/92, 9/92, 5/93, 7/93, 9/93) and *P. testudinis* (9/92) from the nasal cavity, and *Pseudomonas* spp. (7/92, 9/92, 5/93, 7/93, 9/93) from the cloaca.

## Appendix 8. (continued)

Tort # - Sex	9/90	5/91	7/91	9/91	5/92	7/92	9/92	5/93	7/93	9/93
PC12 - M										
White blood cell estimate (k/ $\mu$ l)	--	--	--	--	--					↑ 12.5
Heterophils (k/ $\mu$ l)	--	--	--	--	--					↑ 1000.0*
Monocytes (k/ $\mu$ l)	--	--	--	--	--					
Creatinine (mg/dl)	--	--	--	--	--					
Albumin (g/dl)	--	--	--	--	--					↓ 1.0
Selenium (ppm)	--	--	--	--	--	↑ 0.1				
Iron (ppm)	--	--	--	--	--	↑ 1.9	↑ 1.9			
Vitamin E ( $\mu$ g/ml)	--	--	--	--	--		↑ 0.0		↑ 14.9	
Corticosterone (ng/ml)	--	--	--	--	--		↑ 11.5			

Evaluation: Tortoise had clinical signs of upper respiratory tract disease in every season since capture (7/92). This male also consistently tested positive for *M. agassizii* since the ELISA sampling was initiated in September 1992. High levels of iron in July 1992 and September 1992 may be an early sign of iron storage disease. Upper respiratory tract disease possibly responsible for heterophilia and monocytosis in July 1993. High heterophil count in July 1993 probably caused the increase in white blood cell estimate. High vitamin E levels in July 1993 likely due to fat catabolism. No rainfall was reported in nearby City Creek during July 1993. High corticosterone levels in September 1992 probably related to handling stress. Undetermined significance of high selenium levels in July 1992, and low creatinine levels in September 1992. Low albumin levels in September 1993 probably resulted from chronic infection. Opportunistic bacteria in the nasal cavity included *Mycoplasma* spp. (7/92, 9/92, 5/93, 7/93, 9/93) and *P. testudinis* (7/92, 9/92, 7/93, 9/93). Bacteria in the cloaca included *Pseudomonas* spp. (7/92, 9/92, 5/93, 9/93).

Appendix 9. Overall means, standard deviations, and reference ranges for body mass, median carapace length, hematological, and serum biochemical parameters in healthy Mojave desert tortoises from 2 sites (City Creek, Ut.; Littlefield, Ariz.), 1989-90. -- = not sampled.

Parameter	Reference Range $\bar{x} \pm 2 \text{ SD } (n)$
Body mass (kg)	3.1 $\pm$ 0.56 (51) <sup>1</sup> 2.7 $\pm$ 0.60 (9) <sup>2</sup>
Median carapace length (mm)	256.0 $\pm$ 16.0 (26) 237.7 $\pm$ 19.7 (3)
Packed cell volume field (%)	26.9 $\pm$ 3.4 (50) 23.6 $\pm$ 2.5 (8)
Hemoglobin (g/dl)	11.2 $\pm$ 1.0 (9) --
Blood urea nitrogen (mg/dl)	5.8 $\pm$ 4.7 (49) 1.0 $\pm$ 0.9 (6)
Total protein (g/dl)	2.7 $\pm$ 0.4 (50) 2.8 $\pm$ 0.5 (8)
Albumin (g/dl)	1.6 $\pm$ 0.1 (50) 1.7 $\pm$ 0.3 (8)
Total globulins (g/dl)	1.1 $\pm$ 0.4 (50) 1.1 $\pm$ 0.3 (8)
Aspartate aminotransferase (IU/l)	37.8 $\pm$ 10.3 (49) 38.3 $\pm$ 7.7 (7)
Calcium (mg/dl)	9.1 $\pm$ 0.9 (51) 11.3 $\pm$ 2.1 (8)
Cholesterol (mg/dl)	52.4 $\pm$ 19.7 (48) 135.1 $\pm$ 67.6 (8)
Triglycerides (mg/dl)	36.8 $\pm$ 63.6 (51) 294.1 $\pm$ 188.3 (8)
Copper (ppm)	0.4 $\pm$ 0.09 (13) --
Selenium (ppm)	0.02 (11) --
Zinc (ppm)	2.7 $\pm$ 0.4 (17) --

<sup>1</sup> Males.

<sup>2</sup> Females.

## Appendix 9. (continued)

Parameter	Reference Range $\bar{x} \pm 2$ SD ( <i>n</i> )
Vitamin A ( $\mu\text{g/ml}$ )	0.2 $\pm$ 0.09 (21) 0.3 $\pm$ 0.02 (2)
Vitamin E ( $\mu\text{g/ml}$ )	2.3 $\pm$ 0.6 (21) 3.1 $\pm$ 0.5 (2)
Corticosterone (ng/ml)	4.8 $\pm$ 2.2 (23) 2.6 $\pm$ 1.5 (6)
Sodium (mEq/l)	134.4 $\pm$ 2.8 (34) 132.0 $\pm$ 7.2 (5)
Potassium (mEq/l)	4.9 $\pm$ 0.9 (37) 5.1 $\pm$ 0.4 (5)

Appendix 10. Reference ranges for body mass, median carapace length, hematological, and plasma biochemical parameters in healthy Mojave desert tortoises from 2 sites (City Creek, Ut.; Littlefield, Ariz.), and ranges for the same parameters in ill Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1991-93. -- = not sampled.

Parameter	Healthy Reference Range $\bar{x} \pm 2 \text{ SD}$ ( <i>n</i> )	Ill Range ( <i>n</i> )
Body mass (kg)	2.3 - 4.2 (119) <sup>1</sup> 1.8 - 3.2 (44) <sup>2</sup>	1.9 - 4.4 (19) <sup>1</sup> 1.9 - 3.0 (3) <sup>2</sup>
Median carapace length (mm)	227 - 290 (118) 215 - 256 (43)	114 - 294 (19) 224 - 250 (3)
Packed cell volume field (%)	19 - 36 (116) 18 - 31 (42)	19 - 32.5 (19) 23.5 - 29 (3)
Hemoglobin (g/dl)	8.3 - 14.2 (105) 7.6 - 12 (36)	8.3 - 12.8 (19) 9.5 - 15.8 (3)
Fibrinogen (mg/dl)	110 - 220 (28) 125 - 200 (8)	130 - 200 (10) --
White blood cell estimate (k/ $\mu$ l)	1.5 - 10.2 (96) 1.7 - 8.5 (33)	2.6 - 12.5 (17) 3.0 - 7.5 (3)
Heterophils (k/ $\mu$ l)	16.5 - 640.2 (103) 70.2 - 694.4 (34)	135.2 - 1000 (17) 184.8 - 375 (3)
Lymphocytes	7.2 - 237.5 (101) 0 - 145.6 (34)	2.6 - 344 (17) 42 - 172.5 (3)
Monocytes	0 - 40.8 (100) 0 - 30 (34)	0 - 50 (17) 0 - 45 (3)
Azurophils	0 - 30 (98) 0 - 24 (35)	0 - 86.8 (17) 0 - 22.5 (3)
Eosinophils	0 - 36 (101) 0 - 88 (34)	0 - 37.5 (17) 6 - 84 (3)
Basophils	0 - 270 (101) 0 - 225 (34)	26.4 - 280 (17) 60 - 112.5 (3)
Glucose (mg/dl)	75 - 140 (47) 69 - 117 (16)	63 - 181 (15) --
Blood urea nitrogen (mg/dl)	0 - 8 (104) 0 - 1 (33)	0 - 74 (18) --
Creatinine (mg/dl)	0.1 - 0.4 (102) 0 - 0.4 (37)	0 - 0.4 (19) 0.1 - 0.3 (3)

<sup>1</sup> Males.

<sup>2</sup> Females.

## Appendix 10. (continued)

Parameter	Healthy Reference Range $\bar{x} \pm 2$ SD ( <i>n</i> )	Ill Range ( <i>n</i> )
Uric acid (mg/dl)	1.5 - 6.1 (47) 1.5 - 5.6 (15)	1.9 - 6 (15) --
Total protein (g/dl)	2.4 - 4.5 (118) 2.5 - 4.4 (43)	2.5 - 4.7 (19) 3.2 - 3.8 (3)
Albumin (g/dl)	1.3 - 2.2 (117) 1 - 2.5 (42)	1 - 1.9 (19) 1.8 - 2.1 (3)
Total globulins (g/dl)	1.5 - 2.5 (26) 1.5 - 2.6 (8)	1.3 - 3.0 (19) 1.1 - 1.7 (4)
Bile acid ( $\mu$ mol/l)	0 - 6 (25) 0 - 31 (8)	0 - 8 (10) --
Aspartate aminotransferase (IU/l)	20 - 177 (58) 20 - 172 (20)	18 - 143 (15) --
Alanine aminotransferase (IU/l)	0 - 11 (47) 0 - 9 (15)	0 - 5 (15) --
Alkaline phosphatase (IU/l)	34 - 139 (28) 60 - 142 (8)	30 - 123 (10) --
Calcium (mg/dl)	8.1 - 12 (118) 8.2 - 15 (42)	8.8 - 11.6 (19) 9.4 - 13.5 (3)
Phosphorus (mEq/l)	1 - 3.7 (109) 2.2 - 9 (36)	1.2 - 3.7 (19) 2.8 - 7.2 (3)
Cholesterol (mg/dl)	20 - 171 (123) 109 - 361 (42)	7 - 271 (19) 151 - 191 (93)
Triglycerides (mg/dl)	4 - 160 (121) 18.2 - 815.6 (40)	5 - 102 (19) 154.4 - 180.7 (3)
Total bilirubin (mg/dl)	0 - 1 (28) 0.1 - 0.9 (8)	0.1 - 0.8 (9) (10) --
Direct bilirubin (mg/dl)	0 - 0.1 (29) 0 (7)	0.01 - 0.04 (10) --
Indirect bilirubin (mg/gl)	0 - 1 (28) 0 - 0.8 (8)	0.1 - 0.8 (10) --

## Appendix 10. (continued)

Parameter	Healthy Reference Range	Ill Range ( <i>n</i> )
	$\bar{x} \pm 2$ SD ( <i>n</i> )	
Copper (ppm)	0.2 - 0.6 (64)	0.3 - 0.6 (11)
	0.3 - 0.5 (22)	0.3 - 0.5 (3)
Selenium (ppm)	0 - 0.1 (70)	0.03 - 0.1 (9)
	0 - 0.1 (23)	0.03 - 0.07 (3)
Iron (ppm)	0.4 - 1.2 (12)	1.8 - 1.9 (2)
	0.4 - 0.9 (7)	--
Zinc (ppm)	1.6 - 3.6 (40)	1.5 - 2.3 (5)
	2.3 - 3 (12)	--
Vitamin A ( $\mu$ g/ml)	0.05 - 0.8 (11)	0.05 - 0.7 (19)
	0.05 - 0.9 (40)	0.4 - 0.7 (2)
Vitamin E ( $\mu$ g/ml)	0.5 - 10.1 (116)	0.8 - 14.9 (19)
	0.5 - 13.7 (40)	0.5 - 4.9 (3)
Corticosterone (ng/ml)	0.6 - 9.6 (32)	2.6 - 11.6 (4)
	0.3 - 2.4 (11)	--
Sodium (mEq/l)	118 - 158 (117)	112 - 157 (17)
	121 - 152 (42)	113 - 134 (3)
Potassium (mEq/l)	2.7 - 4.8 (115)	2.9 - 5.2 (17)
	2.6 - 5 (41)	3.3 - 4.3 (3)
Chloride (mEq/l)	92 - 130 (28)	109 - 130 (10)
	94 - 130 (7)	--
Total carbon dioxide (mEq/l)	23.5 - 43.1 (30)	28.7 - 44.4 (10)
	17.1 - 32.4 (8)	--
Anion gap (mEq/l)	-10.8 - 34.1 (22)	-23.8 - -1.9 (8)
	0 - 10.3 (6)	--
Osmolality (mOs/kg)	240 - 354 (98)	237 - 401 (17)
	235 - 333 (34)	230 - 263 (2)

Appendix 11. Microbial isolates from the nasal cavity of Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1989-93. Microbes collected with nasal flushes unless indicated.

Organism	Number positive	<i>n</i>
<i>Chlamydia</i> -like <sup>a</sup>	10	221
<i>Flavobacterium</i> spp. <sup>b</sup>	1	10
<i>Mycoplasma</i> spp.	83	226
<i>Pasteurella testudinis</i> <sup>c</sup>	49	221
<i>Staphylococcus</i> spp. <sup>d</sup>	5	12

<sup>a</sup> Isolated from choanal and nasal swabs in Culturettes and nasal flushes.

<sup>b</sup> Isolated from nasal swab in thioglycolate broth.

<sup>c</sup> Isolated from choanal swabs in Culturettes, Stuart transport tubes, and thioglycolate broth.

<sup>d</sup> Isolated from choanal and nasal swabs in Culturettes, Stuart transport tubes, and thioglycolate broth.

Appendix 12. Microbial isolates from the cloacal cavity of Mojave desert tortoises from 3 sites (City Creek, Ut.; Littlefield, Ariz.; Paradise Canyon, Ut.), 1989-93.

Organism	Number positive	<i>n</i>
<i>Campylobacter</i> spp.	1	5
<i>Citrobacter amolonaticus</i>	2	77
<i>Citrobacter</i> spp.	1	77
Coliforms	3	56
<i>Corynebacterium</i> spp.	10	138
Diphtheroids	6	138
<i>Enterobacter-Klebsiella</i>	75	138
<i>Escherichia coli</i>	37	138
<i>Lactobacillus</i> spp.	7	77
<i>Pasteurella testudinis</i>	12	77
<i>Pasteurella</i> spp.	5	133
<i>Pseudomonas</i> spp.	59	138
<i>Salmonella</i> spp.	3	212
<i>Shigella</i> spp.	25	172
<i>Staphylococcus</i> spp.	130	138
<i>Streptococcus</i> spp.	15	133
Yeast	2	77

Appendix 13. Monthly rainfall (cm) and ambient temperature (maximum, minimum; C) data from permanent weather stations from 2 sites (City Creek, Ut.; Littlefield, Ariz.) in the Mojave Desert, 1988-93. -- = not available.

## City Creek, Utah

Location: St. George, Washington Co., Utah

Elevation: 940 m

	1988			1989			1990		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	3.4	11.3	-1.8	2.54	10	3.6	1.04	12.2	-2.2
February	0.8	17.7	-0.4	11.66	14.3	-0.7	3.17	14.5	0.4
March	0.7	20.9	2.5	1.09	23.1	5.1	0.63	21.3	6.3
April	7.3	24.1	8.2	0	29.3	10.4	1.42	27.0	10.4
May	0.2	29.9	11.6	1.24	30.3	12.9	0.41	30.2	12.7
June	2.0	36.9	18.2	0.03	35.9	16.7	0.84	37.1	18.3
July	1.0	40.2	21.9	0.48	40.3	21.5	0.25	39.2	22.1
August	4.1	37.1	19.0	3.84	35.9	18.7	1.57	36.7	20.1
September	0.4	33.6	13.3	0	33.9	13.9	2.01	34.0	16.5
October	0.1	30.8	10.8	0.74	27.3	6.7	0.51	26.7	7.9
November	1.5	18.4	1.9	0.05	19.7	0.6	0.28	17.8	1.3
December	2.5	11.8	-1.9	0	13.7	-4.0	0.51	8.5	-6.6
Total Annual Rainfall	24.0			21.67			12.64		

	1991			1992			1993		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	1.9	11.8	-3.1	1.45	11.1	-2.1	12.04	10.4	1.5
February	1.37	19.5	1.3	5.89	15.5	3.2	6.83	13.2	2.3
March	3.99	16.1	3.1	9.68	17.3	6.6	2.11	20.8	5.9
April	0.1	23.1	6.7	0	28.3	10.4	0.23	25.3	8.3
May	0.36	26.7	10.4	1.90	31.5	15.1	0.25	31.6	14.0
June	0.56	33.6	16.1	0	35.8	18.3	0.23	34.7	17.1
July	0.25	39.5	20.9	0.86	37.7	20.9	0	37.8	20.4
August	0.36	37.4	21.1	0.79	37.5	22.0	1.93	37.0	20.8
September	0.71	34.0	16.6	0	35.1	16.5	0	34.2	14.8
October	2.11	23.7	6.9	3.81	28.3	11.0	2.31	27.2	8.7
November	1.17	16.3	1.5	0	15.6	0.7	1.4	16.7	0.4
December	1.63	11.5	-2.6	4.47	8.6	2.0	0.9	13.4	-2.7
Total Annual Rainfall	14.51			28.85			29.23		

## Appendix 13. (continued)

Littlefield, Arizona

Location: Beaver Dam, Mohave Co., Arizona

Elevation: 573 m

	1988			1989			1990		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	3.4	11.3	-1.8	3.96	12.7	0.4	1.8	14.3	0.2
February	0.8	17.1	-0.4	1.52	16.4	2.0	6.07	16.1	1.8
March	0.7	20.9	2.5	1.5	25.2	6.7	0.33	23.6	6.9
April	7.3	24.1	8.2	0	30.7	11.6	1.12	27.9	10.7
May	0.2	29.9	11.6	1.4	31.7	14.2	1.04	30.7	12.9
June	2.0	36.9	18.2	0.05	37.0	17.6	0.84	37.8	18.2
July	1.0	40.2	21.9	2.54	40.8	23.1	2.24	39.4	22.6
August	4.1	37.1	19.0	4.42	37.3	20.1	2.16	37.3	20.3
September	0.4	33.6	13.3	0.3	34.8	16.7	3.43	34.2	18.2
October	0.1	30.8	10.8	2.64	27.2	10.0	0.71	27.4	9.1
November	1.5	18.4	1.9	0	21.9	4.7	2.44	18.8	3.2
December	2.5	11.8	-1.9	0	16.8	-0.8	0.25	9.2	-3.9
Total Annual Rainfall	24.0			18.33			22.43		

	1991			1992			1993		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	1.57	11.6	-1.9	2.46	13.4	1.6	8.05	12.5	2.0
February	0.71	19.4	2.3	3.81	18.0	5.4	7.09	14.6	3.8
March	4.72	15.1	1.7	8.20	19.5	7.1	--	--	--
April	0	22.7	4.7	0	28.4	9.7	0.33	26.6	7.8
May	0.99	27.1	9.4	2.79	31.9	15.1	0.43	32.5	14.3
June	0.28	32.5	14.3	0.08	36.4	16.4	0.79	34.9	16.1
July <sup>1</sup>	4.84	40.4	20.3	0.30	38.4	20.5	--	--	--
August	0.3	38.4	21.2	0.74	39.4	22.9	--	--	--
September	0.69	34.8	16.6	0.13	35.6	17.3	--	--	--
October	1.73	30.3	11.2	3.63	29.4	11.5	--	--	--
November	1.02	18.5	4.7	0.03	17.7	1.9	--	--	--
December	1.75	13.5	2.1	3.25	11.0	-0.6	--	--	--
Total Annual Rainfall	18.2			25.42			16.69		

<sup>1</sup> Only six reporting days in July 1991.

Appendix 14. Monthly rainfall (cm), ambient temperature (C), soil temperature (C), soil moisture (%), relative humidity (%), and wind speed (km/hr) from automatic weather stations from 2 sites (City Creek, Ut.; Littlefield, Ariz.) in the Mojave Desert, 1992-93. -- = not available. Weather stations erected in July 1992 and dismantled in September 1993.

## City Creek, Utah

Location - Lat: 37° 10' N Long: 113° 35' W

	Rainfall		Temp		Soil Temp		Soil Moisture		Rel Hum		Wind	
	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993
January	--	2.82	--	4.6	--	7.7	--	51.6	--	87.2	--	1.3
February	--	7.42	--	5.6	--	8.9	--	45.9	--	87.5	--	2.3
March	--	2.26	--	10.7	--	15.3	--	65.6	--	73.6	--	2.3
April	--	0.41	--	13.6	--	19.5	--	70.4	--	52.1	--	4.0
May	--	0.03	--	16.6	--	24.6	--	167.5	--	43.1	--	5.2
June	--	0.3	--	22.5	--	30.7	--	224.4	--	38.3	--	4.7
July	0.8	0	27.1	26.0	32.6	34.4	215.5	233.7	32.5	22.3	2.6	3.9
August	3.6	0.33	27.3	30.3	32.7	35.3	168.1	233.5	36.6	16.0	2.2	2.6
September	0.3	--	22.9	--	27.9	--	129.3	--	36.1	--	1.7	--
October	3.8	--	16.7	--	21.6	--	226.4	--	49.4	--	1.3	--
November	0.0	--	7.8	--	11.9	--	159.7	--	72.8	--	1.3	--
December	--	--	--	--	--	--	--	--	--	--	--	--
Total Annual Rainfall	8.5	13.57										

## Littlefield, Arizona

Location - Lat: 37° 4' N Long: 113° 55' W

	Rainfall		Temp		Soil Temp		Soil Moisture		Rel Hum		Wind	
	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993
January	--	2.51	--	8.8	--	9.5	--	63.1	--	70.1	--	5.3
February	--	5.82	--	9.2	--	10.4	--	65.0	--	69.9	--	6.6
March	--	2.67	--	13.7	--	15.4	--	88.1	--	63.2	--	3.9
April	--	0.23	--	17.1	--	21.6	--	134.4	--	44.6	--	5.0
May	--	0.13	--	23.5	--	28.5	--	207.5	--	34.7	--	7.6
June	--	0.61	--	26.8	--	32.3	--	233.1	--	27.0	--	7.4
July	0.3	0	31.6	29.9	34.9	35.7	233.5	233.6	26.2	21.7	3.7	6.8
August	1.0	0	31.8	32.6	35.8	36.4	233.6	233.6	28.3	17.2	4.0	6.3
September	0.3	--	26.8	--	30.3	--	233.6	--	26.5	--	3.7	--
October	2.0	--	20.4	--	23.6	--	224.0	--	40.5	--	2.7	--
November	0	--	9.9	--	12.3	--	151.5	--	51.1	--	2.9	--
December	0.3	--	5.2	--	7.4	--	200.1	--	60.7	--	3.2	--
Total Annual Rainfall	3.9	11.97										