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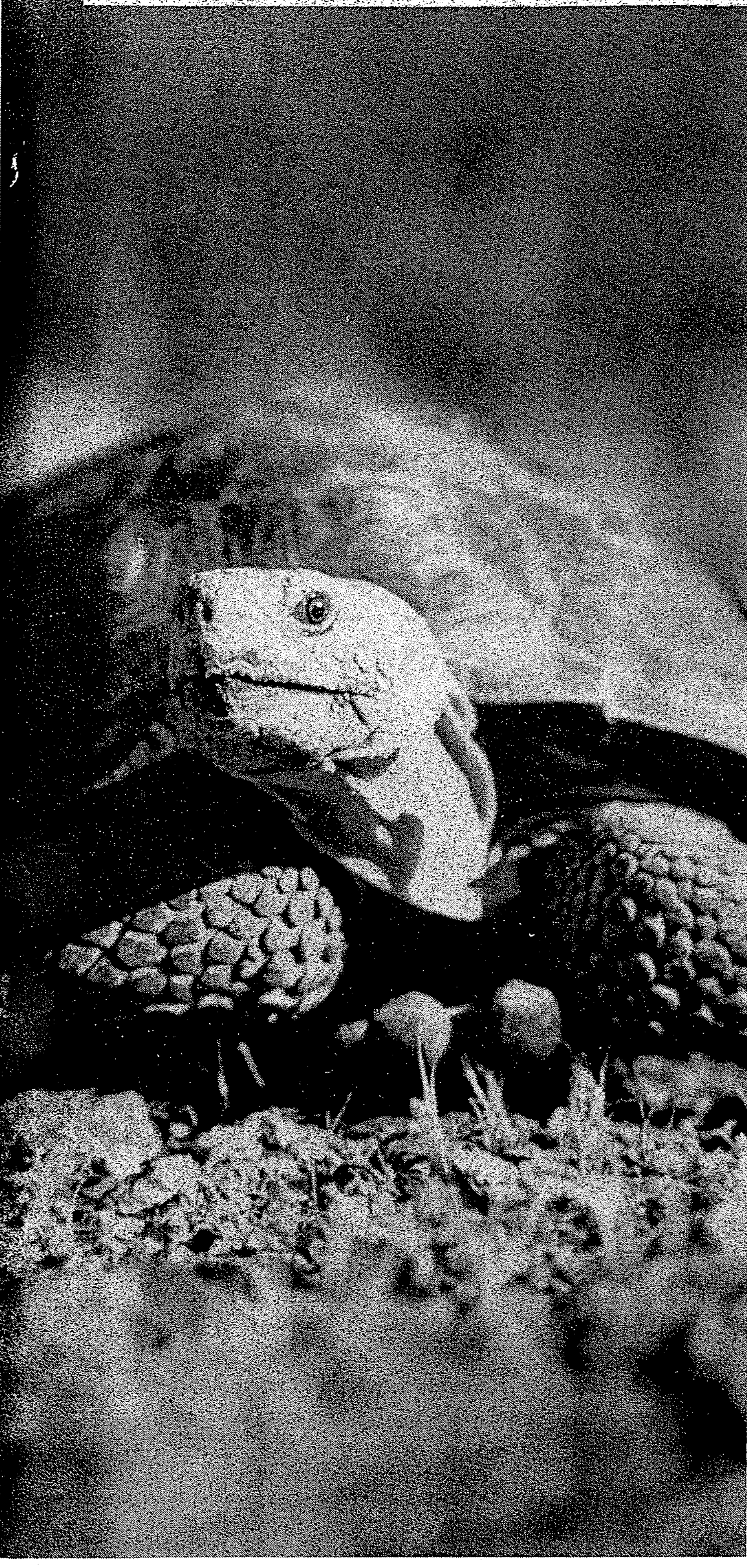

RESEARCH BRANCH
TECHNICAL REPORT #24

HEALTH STUDIES OF FREE-RANGING SONORAN DESERT TORTOISES IN ARIZONA

A Final Report

VANESSA M. DICKINSON
JAMES L. JARCHOW, D.V.M.
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October 1996

ARIZONA GAME AND FISH
DEPARTMENT HERITAGE FUND

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To conserve, enhance, and restore Arizona's diverse wildlife resources and habitats through aggressive protection and management programs, and to provide wildlife resources and safe watercraft and off-highway vehicle recreation for the enjoyment, appreciation, and use by present and future generations.

Arizona Game and Fish Department
Research Branch

Technical Report Number 24

Health Studies of Free-ranging Sonoran Desert Tortoises in Arizona

A Final Report

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Health Studies of Free-ranging Desert Tortoises in the Sonoran Desert of Arizona

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Abstract: Desert tortoises (*Gopherus agassizii*) are long-lived reptiles found in the deserts of the southwestern United States. Concerns over population declines in the Mojave Desert population due to drought, predation, and disease prompted a comparative study in the Sonoran Desert. We began a 5-year health study in 1990 of 2 free-ranging desert tortoise populations in the Sonoran Desert; the first from Little Shipp Wash, Yavapai County, Arizona, and the second from the Harcuvar Mountains, La Paz County, Arizona. We captured and radio-tagged 36 tortoises from 1990-94, then attempted to recapture them 3 times a year. We weighed, measured, and evaluated each tortoise for clinical signs of upper respiratory tract disease (URTD). We chemically immobilized each tortoise and collected blood, nasal aspirate, choana and cloacal swabs, and fecal matter to perform hematology, blood chemistry, microbiological, and parasitology assessments. 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 males. Seasonal and annual differences in hematology and blood chemistry related to rainfall patterns, forage availability, and disease presence. We found 2 species of pathogenic bacteria in tortoise cloacae (*Pseudomonas* spp., *Salmonella* spp.), and 1 pathogenic bacteria in the nasal cavity (*Pasteurella testudinis*). Three tortoises had *Mycoplasma agassizii*, the causative agent of URTD (2 Harcuvar Mtns., 1 Little Shipp). Using an enzyme-linked immunosorbent assay (ELISA) we found 1 Harcuvar tortoise with a suspect titer for *M. agassizii* in 1993, and 1 Harcuvar tortoise with a positive titer in 1994. Results from a polymerase chain amplification (PCR) test in 1994 confirmed the presence of *M. agassizii* in 1 Little Shipp tortoise. Ninety-six percent of the tortoises sampled had nonpathogenic pinworm (*Trachygonetria* spp.) ova in their feces, the only intestinal parasite found in the study. Baseline laboratory data will enable future monitoring of tortoise populations for evidence of dehydration and disease.

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

INTRODUCTION

The Mojave population includes tortoises north and west of the Colorado River, while the Sonoran population includes tortoises south and east of the Colorado River. Concern for population declines led to the emergency listing of the Mojave desert tortoise population in 1989 as endangered (U.S. Fish and Wildl. Serv. 1989), then subsequent listing as threatened in 1990 (U.S. Fish and Wildl. Serv. 1990). Habitat loss and epidemic disease were suspected reasons for the Mojave population decline.

The Sonoran population was a category 2 candidate for Federal listing in 1989 (U.S. Fish and Wildl. Serv. 1989). In response to a petition to list the desert tortoise throughout its range, the U.S. Fish and Wildlife Service (Service) found in 1991 that the Sonoran population did not warrant listing under the Endangered Species Act (U.S. Fish and Wildlife Serv. 1991). In 1995, the Service discontinued the category 2 list (U.S. Fish and Wildlife Serv. 1996), thus, the Sonoran population currently has no status under the Endangered Species Act.

Understanding the health of free-ranging desert tortoises is important for assessing and managing their populations (Berry 1984). Blood parameters (see glossary) used to diagnose chelonian diseases can be used to assess the physiological status of a population (Rosskopf and Woerpel 1982, Jacobson et al. 1991). Epidemiology studies provide an estimate of past exposures of a population to infectious diseases (Brown et al. 1994*a*). Brown et al. (1994*a*) recommended conducting long-term tortoise studies to understand the dynamics of diseases in populations.

URTD is an important tortoise disease. 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 that 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 from the Sonoran Desert. Some physiological information exists for free-ranging Mojave tortoises in northwestern Arizona (Jarchow and May 1989), eastern California (Knowles 1989; Christopher et al. 1992, 1993), and southern Nevada (O'Connor et al. 1994). Most hematological and clinical chemistry (Minnich 1977, Rosskopf 1982, Nagy and Medica 1986, Jacobson et al. 1991, O'Connor et al. 1994, Rostal et al. 1994), and microbiological (Fowler 1977, Snipes and Biberstein 1982, Jackson and Needham 1983, Jacobson et al. 1991) 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, forage competition with domestic livestock, off-road-vehicle use, and drought on free-ranging tortoise populations (Berry and Nicholson 1984).

To better understand the health status and because only limited information existed on the health status of free-ranging Sonoran tortoises, we began a 5-year health investigation on free-ranging Sonoran tortoises. Our objectives were to:

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

STUDY AREA

We selected 2 study sites in the Sonoran Desert: Little Shipp Wash in Yavapai County, Arizona (34°31′ N, 113°5′ W), and the Harcuvar Mountains in La Paz County, Arizona (34°6′ N, 113°17′ W) (Fig. 1). Both study sites were 1 km from permanent desert tortoise study plots used for population monitoring. These sites were 65 km apart, geographically separated by mountains and major roadways.

Little Shipp Wash was 9.6 km southeast of Bagdad, Arizona (Fig. 2). Elevations ranged from 788-975 m. Vegetation was upland Sonoran Desert characterized by little-leaf palo verde (*Cercidium microphyllum*), saguaro (*Carnegia gigantea*), ocotillo (*Fouquieria splendens*), mesquite (*Prosopis juliflora*), cat-claw acacia (*Acacia greggii*), fairy duster (*Calliandra eriophylla*), flat-topped buckwheat (*Eriogonum fasciculatum*), and Engelmann's prickly pear (*Opuntia engelmannii*). Grasses and forbs included red brome (*Bromus rubens*), Indian wheat (*Plantago insularis*), purple three-awn (*Aristida purpurea*), big galleta grass (*Hilaria rigida*), and slender janusia (*Janusia gracilis*).

The Harcuvar Mountain site was 24.1 km northwest of Aguila, Arizona (Fig. 3). Elevations ranged from 792-1,006 m. Vegetation was upland Sonoran Desert characterized by saguaro, ocotillo, little-leaf palo verde, cholla (*Opuntia* sp.), fairy duster, flat-topped buckwheat, red brome, and Indian wheat as well as a sparse population of Joshua trees (*Yucca brevifolia*).

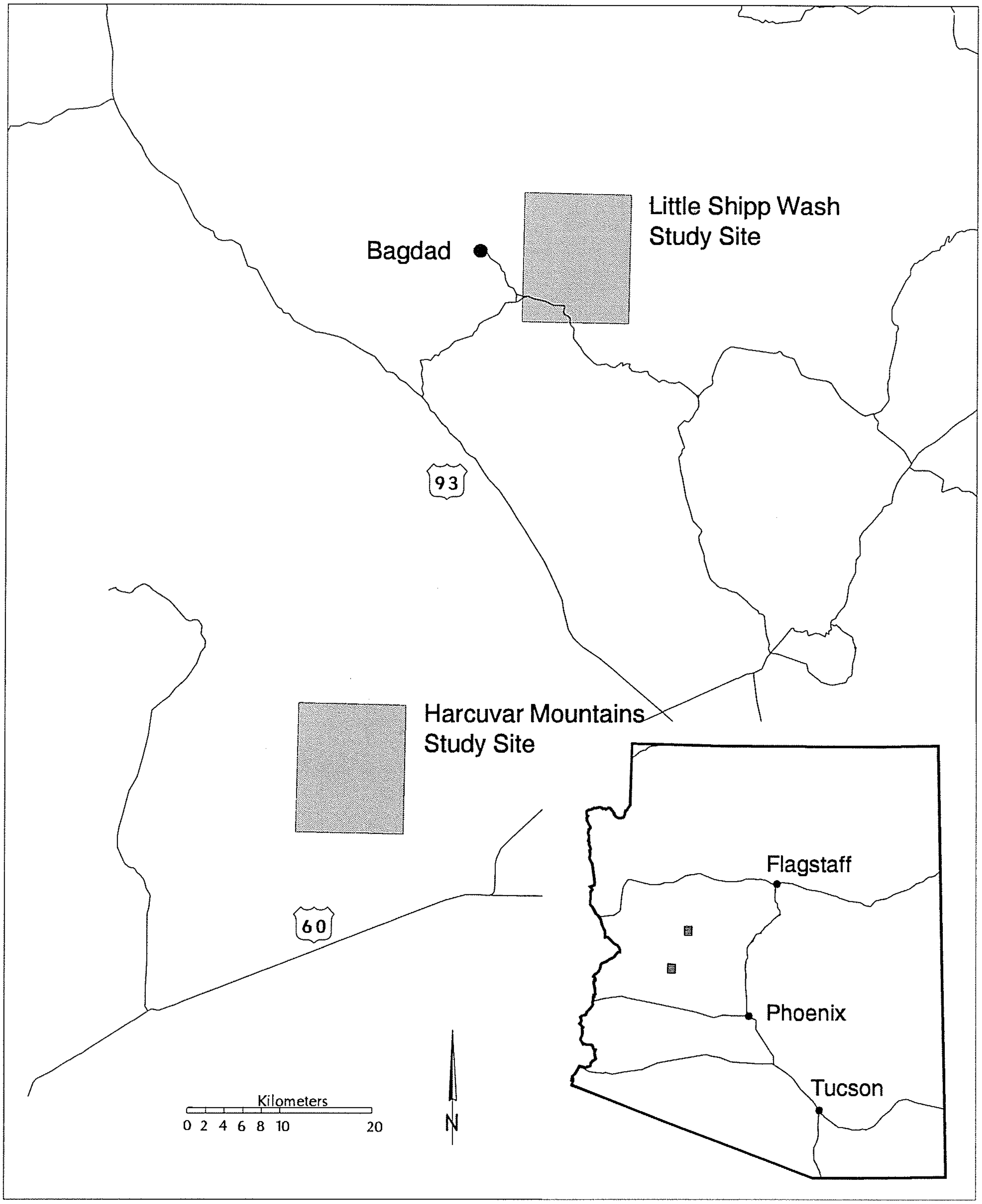


Figure 1. Locations of Little Shipp Wash and Harcuvar Mountains, Arizona, study sites.

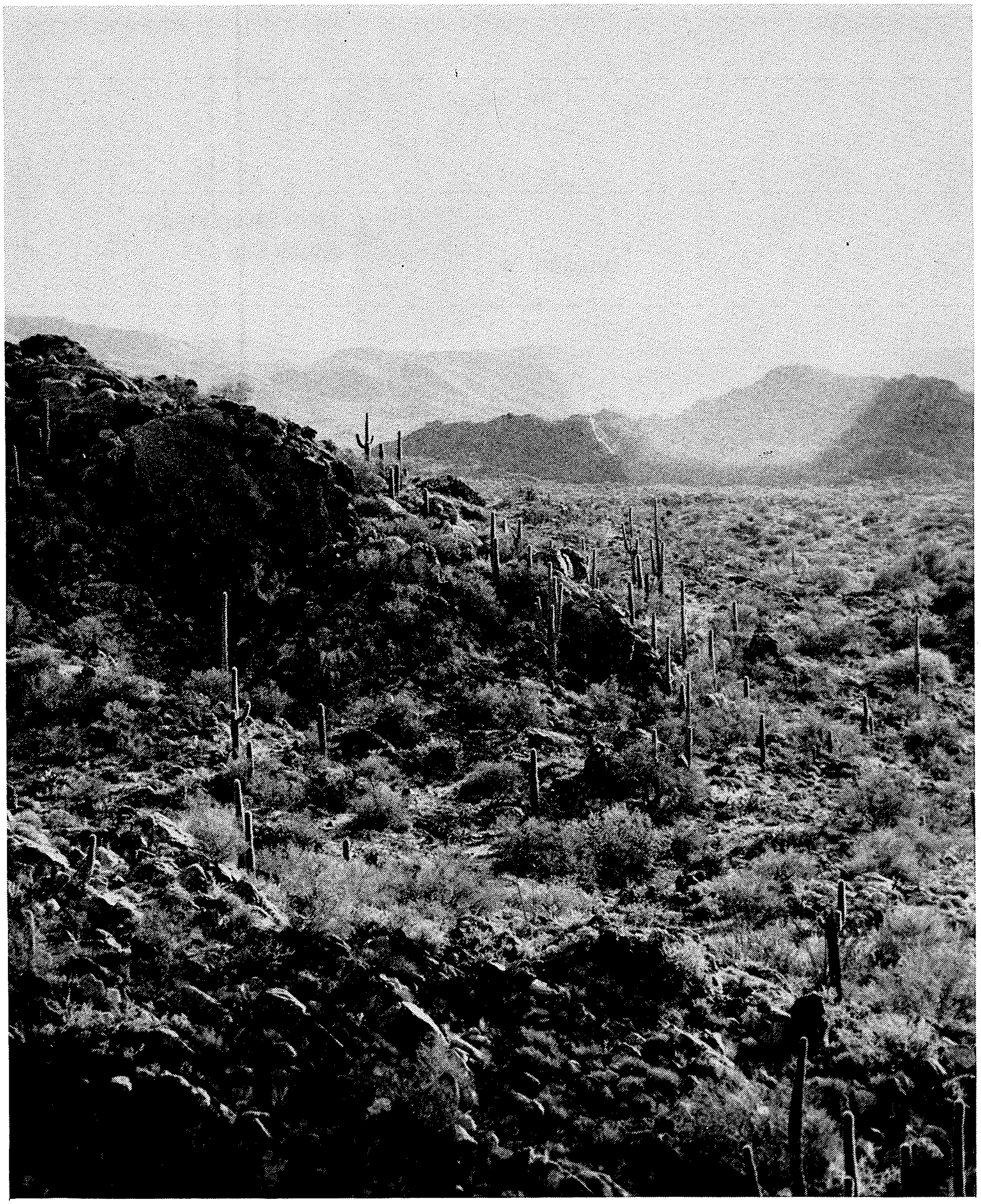


Figure 2. Little Shipp Wash study site showing typical terrain and saguaros.

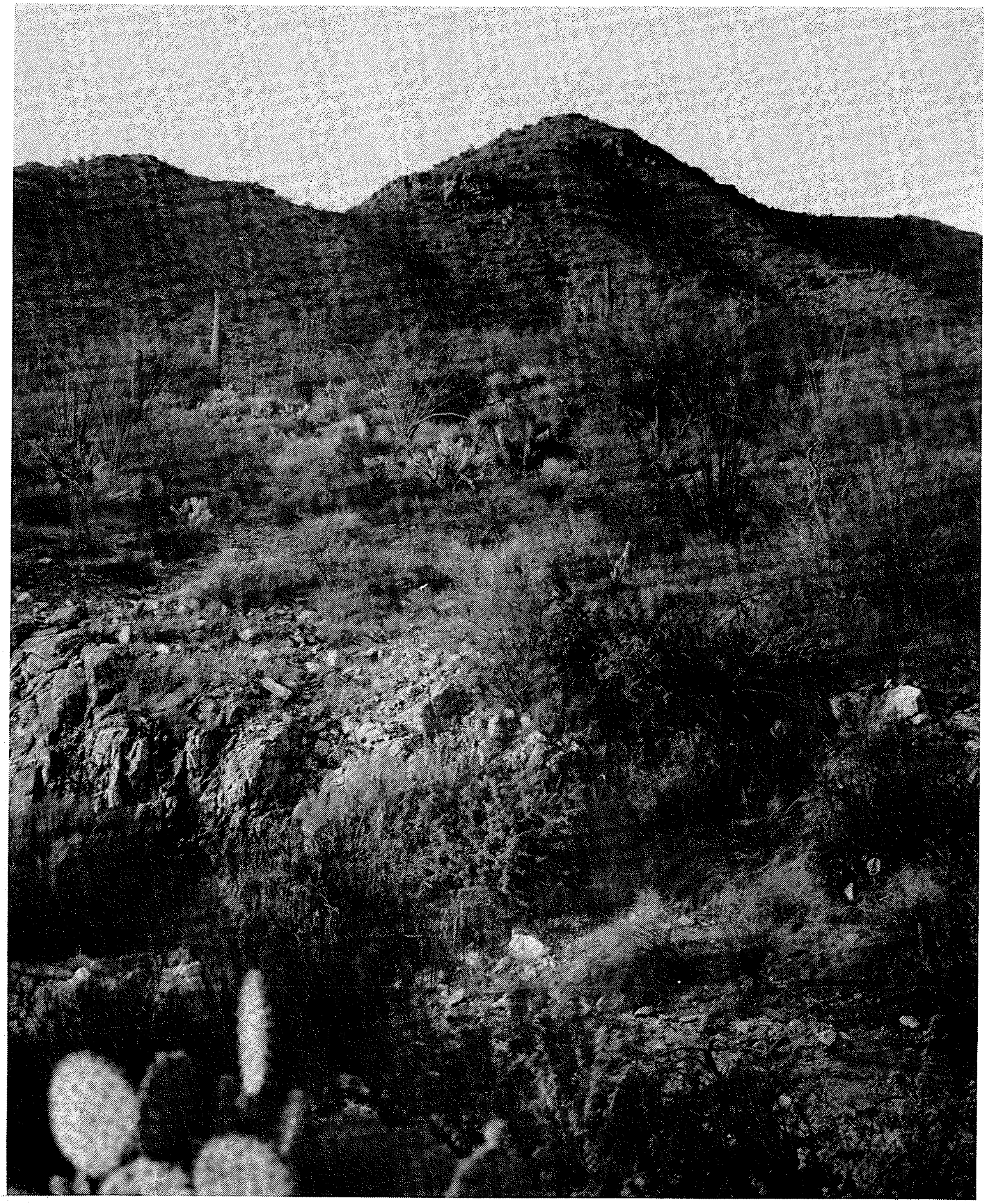


Figure 3. Harcuvar Mountain study site showing typical terrain and saguaros, ocotillos, and Joshua trees.

METHODS

Capture and Telemetry

Most study animals were captured in 1990 in their sheltersites or out in the open. We located many tortoises by shining a light into 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 125, Telonics, Mesa, Ariz.) to its anterior marginal scutes (Fig. 4). The transmitters weighed 47-53 g, measured 4.1 cm x 2.4 cm x 2.0 cm, and had an active life of 9-18 months. We collected both male and female tortoises. For further identification, marginal scutes were notched following the system used on BLM tortoise monitoring plots in Arizona, California, and Nevada (Berry 1988).

We determined the sex of tortoises based on plastron indentation, tail morphology, and gular size (Woodbury and Hardy 1948, Patterson 1972, Coombs 1973). Tortoises that could not be accurately sexed were classified as unknown. We recaptured a minimum of 5 and a maximum of 20 adult tortoises during each sampling trip. We made 2 sampling trips to Little Shipp and the Harcuvars in 1990 (September, November), then sampled each site 3 times a year (April, June, September) in 1991 through 1994. April represented the time of peak tortoise activity, June the time of peak stress due to increased temperatures and decreased rainfall, and September the time of decreased activity.

Health Assessment

Health assessment protocols evolved rapidly during our study. This was a result of an evaluation of health concerns after the original study was initiated. Consequently our methods of data collection changed during the study period, but were similar between study sites (Appendix 1).

Physical Examination. At each recapture, we physically examined each tortoise for disease, weighed tortoises with a 5-kg Pesola scale, and measured tortoises with a caliper and 24-cm ruler (Fig. 5). We specifically looked for evidence of

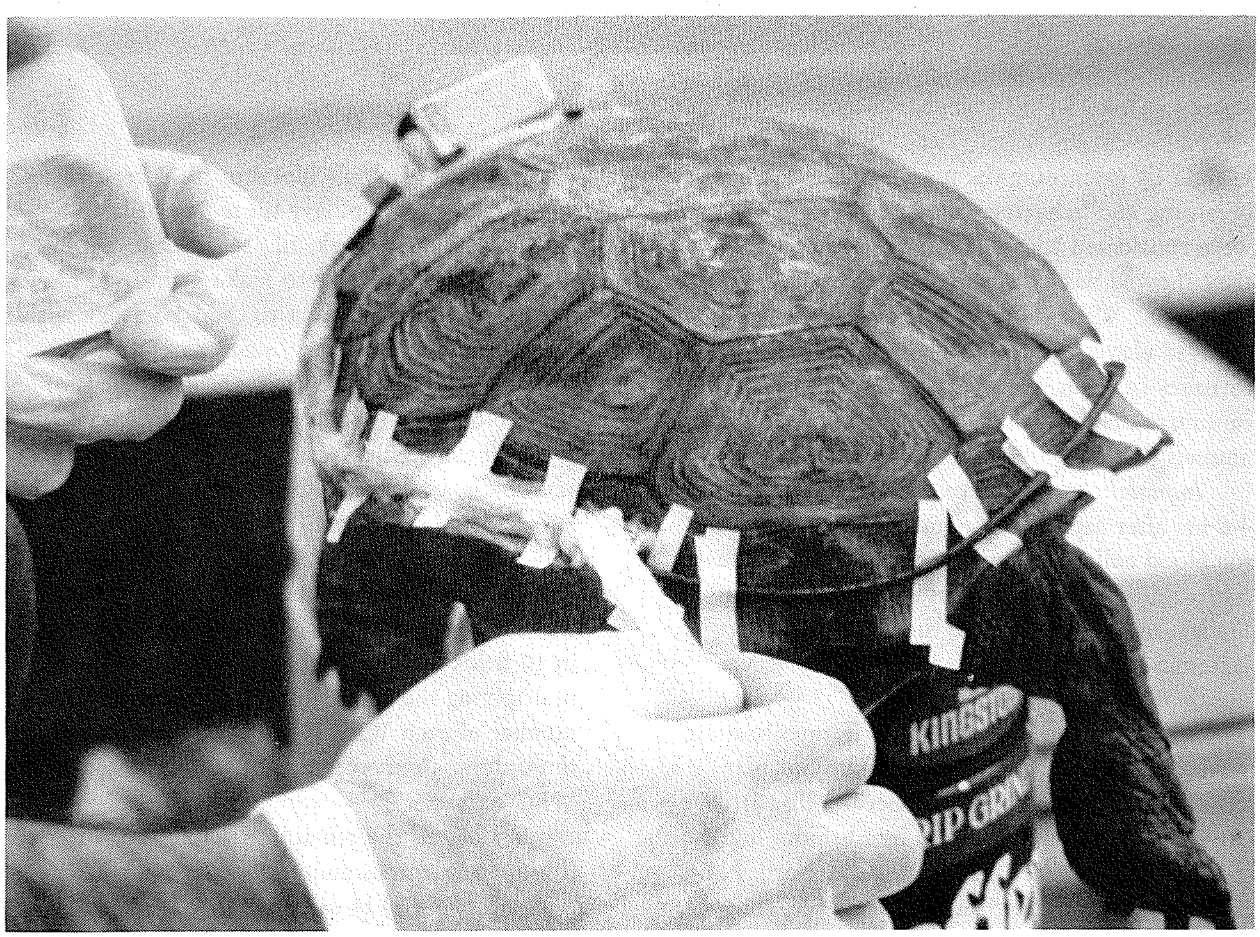


Figure 4. Adult desert tortoise with radio transmitter being glued to its carapace.

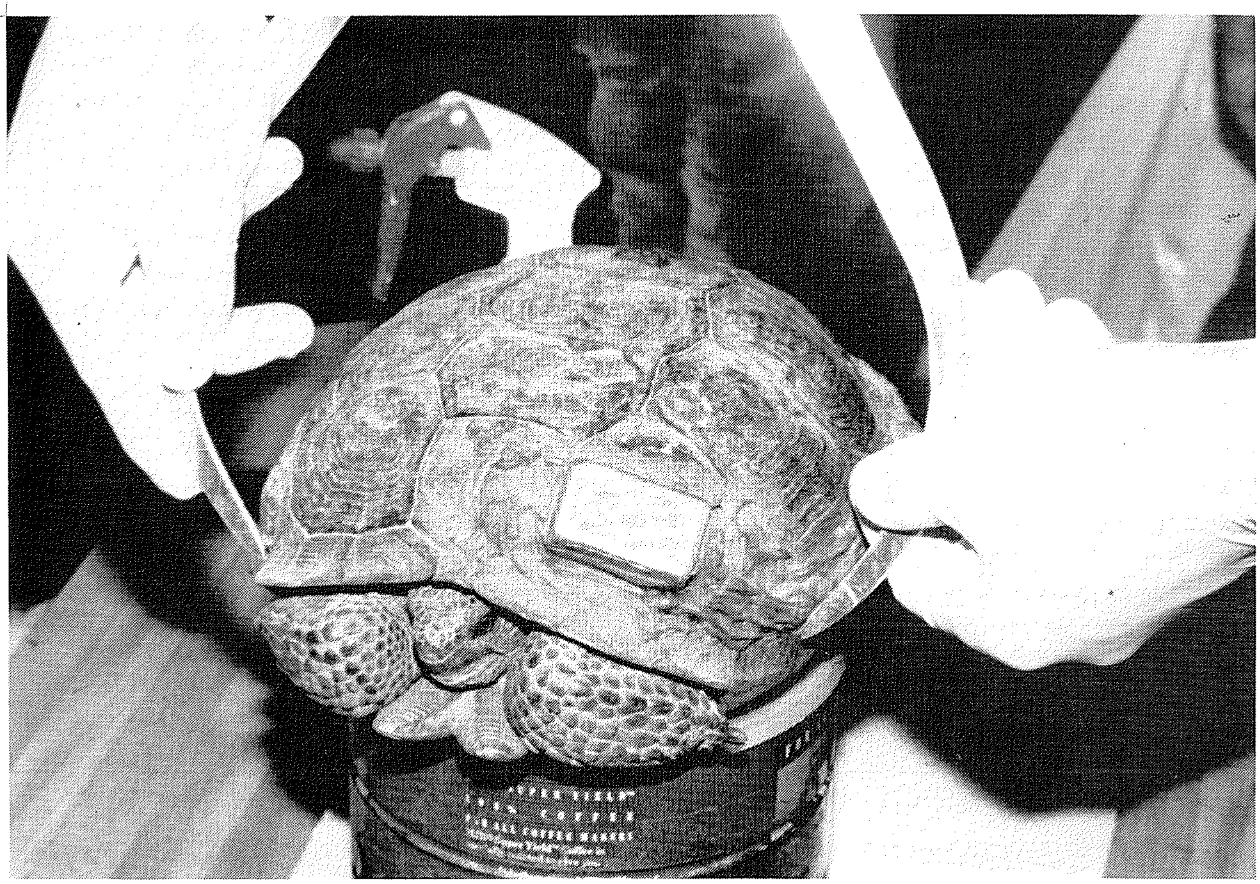


Figure 5. Measuring the width of a desert tortoise with calipers.

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 gloves, changed gloves between tortoises, and kept tortoises in clean, individual cardboard boxes to minimize the probability of disease transfer among animals (Fig 6).

Immobilization. Tortoises were immobilized 4-6 hrs after capture. We immobilized tortoises for blood collection with 15 mg/kg of ketamine hydrochloride (Ketaset; Fort Dodge Lab., Fort Dodge, Ind.). We injected the Ketaset intramuscularly into a rear leg using a 25-gauge needle (Fig. 7).

Blood. Twenty minutes after immobilization, we collected 6.0 ml of whole blood by jugular venipuncture using a 22-gauge needle (Fig. 8). We placed 0.6 ml of whole blood in a lithium heparin microtainer (Becton Dickinson, Rutherford, N.J.), which was mixed for 5 min, kept on ice, and mailed to the laboratory within 24 hrs. We used several drops of whole blood to fill 2 heparinized microhematocrit capillary tubes (Scientific Products, McGaw Park, Ill.) to determine packed cell volume (PCV), and to make 2 air-dried blood smears. Smears were sent to the laboratory within 2 days. In the laboratory, 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. 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 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 pipetted

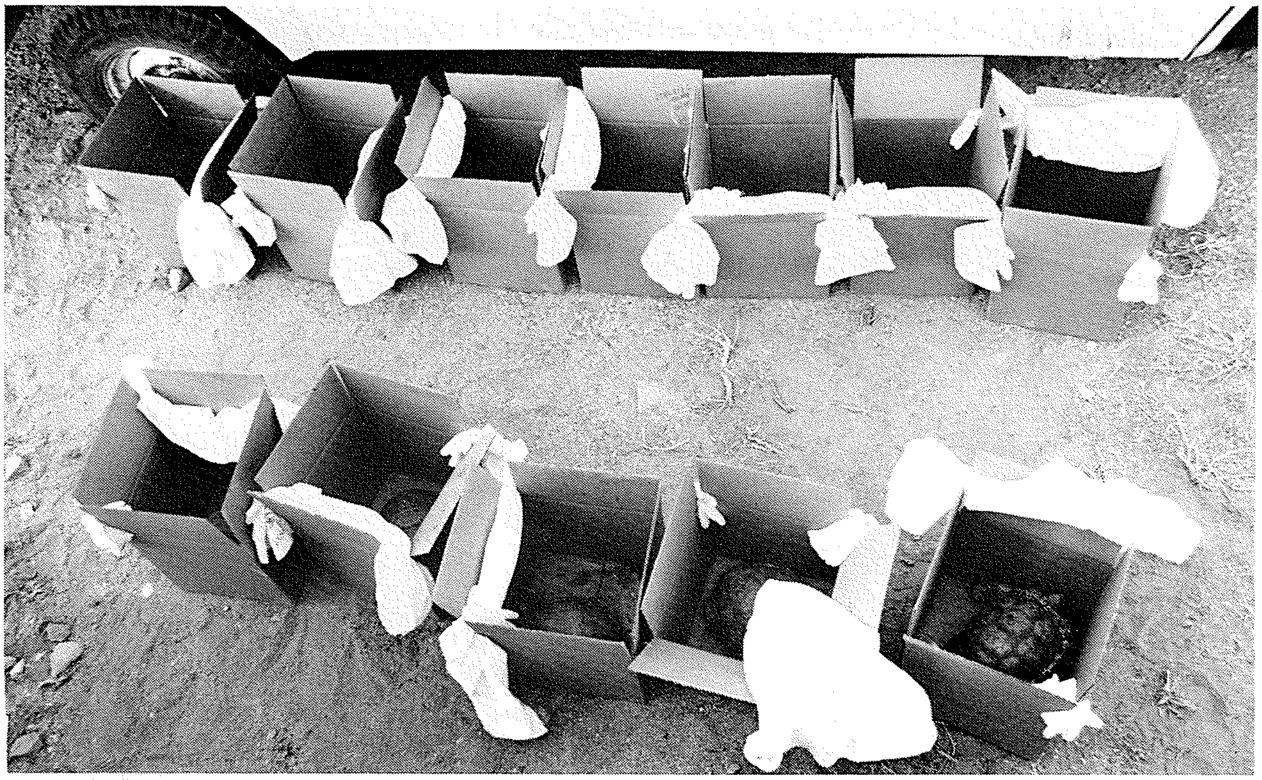


Figure 6. Desert tortoises kept in clean, individual cardboard boxes during sampling.

Figure 7. Veterinarian administering ketamine hydrochloride to immobilize a desert tortoise prior to sampling.

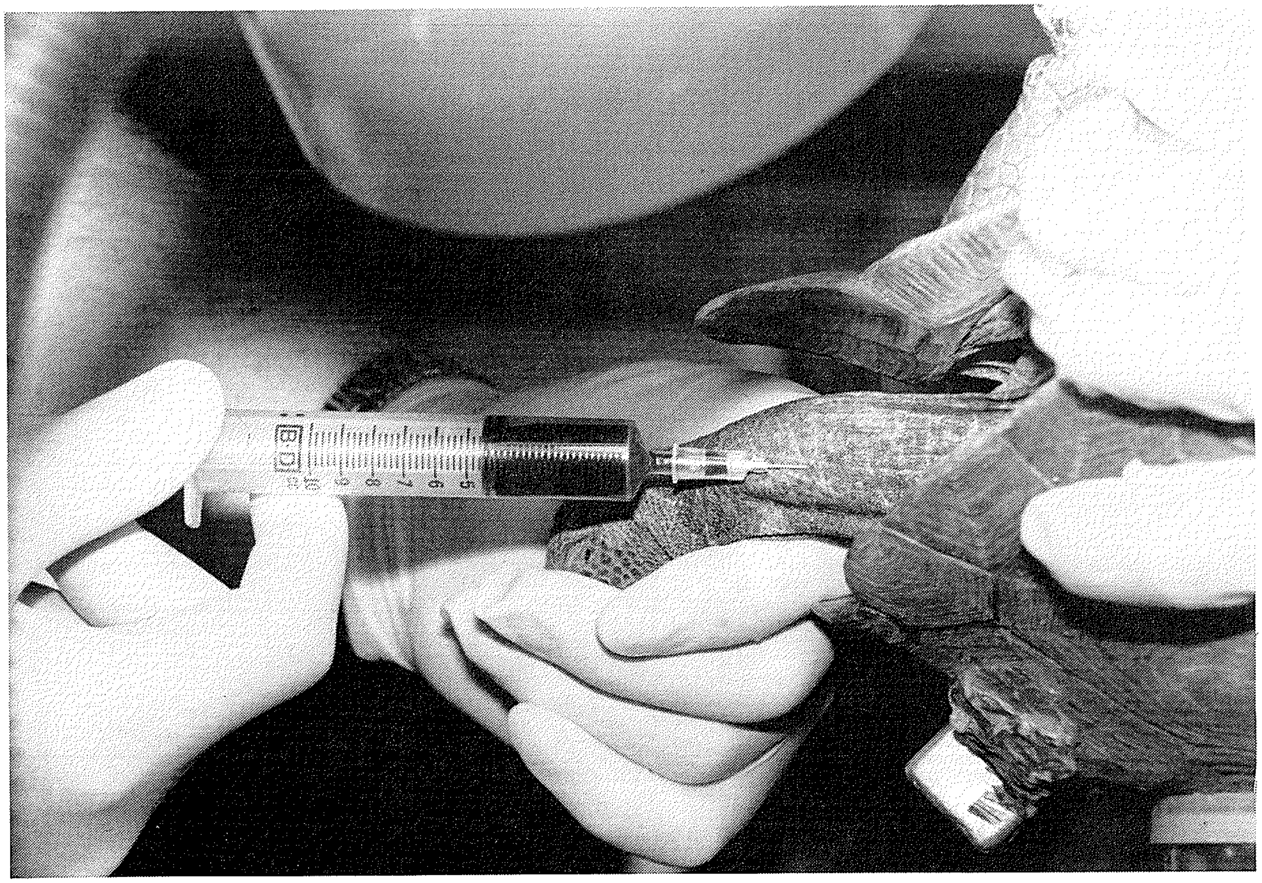


Figure 8. Collecting whole blood from the jugular vein of a desert tortoise.

off the plasma and then divided it into aliquots. In 1990, we placed the plasma in red top vacutainers (Becton Dickinson, Rutherford, N.J.) and placed them on dry ice. From 1991 to 1994, we placed the plasma in cryogenic vials (Whatman LabSales, Hillsboro, Oreg.) and immediately froze them in liquid nitrogen. We sent plasma samples on dry ice to the laboratory within 2 days of collection.

Plasma was divided into 4 aliquots. The first aliquot (1.0 ml) was analyzed for blood chemistry determinations with a 550 Express Analyzer (Ciba-Corning, Oberlin, Oh.). Plasma was analyzed for 24 blood variables: glucose, blood urea nitrogen (BUN), creatinine, uric acid, total protein, albumin, total globulins, bile acids, 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 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, 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 and zinc were measured by atomic absorption (Model Video 12, Instrumentation Lab, Waltham, Md.). The third aliquot (1.0 ml) was analyzed for corticosterone, estradiol, and testosterone by radio immunoassay (Lance et al. 1985). The fourth aliquot (0.5 ml) was analyzed by an enzyme-linked immunosorbent assay (ELISA) for *Mycoplasma agassizii*, as described by Schumacher et al. (1993).

We calculated normal reference ranges (Appendix 2) for tortoise blood parameters (including parameters from ill tortoises) as the mean ± 2 standard deviations (SD) following

Hoffman (1971) and Stewart et al. (1987). We separated data by sex to compile reference ranges. Outliers were values >2 SD from the mean. 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 as described by Christopher et al. (1992, 1993). Each tortoise with abnormal parameter values (>2 SD) for plasma was evaluated individually by examining blood and bacterial parameters, changes in weight, and rainfall patterns (Appendix 3).

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.*

Bacteria. From 1990 to May 1991, we took 1 choanal swab from each tortoise and stored them in Culturettes (Becton Dickinson, Cockeysville, Md.). We kept choanal swabs on ice and sent them to the laboratory within 24 hrs. Choanal swabs were used for gram stain and culture. Plates were incubated at both room temperature (23-25 C) and at 37 C. Bacterial cultures were grown using MacConkey agar (gram negative bacteria), Selenite agar (*Salmonella* spp., *Shigella* spp.), Hektoen agar (*Salmonella* spp., *Shigella* spp.), and Campylobacter agar (*Campylobacter* spp.). Bacteria were classified as gram positive or gram negative and, when possible, identified to species.

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 0.9% sodium chloride (Abbott Lab., Chicago, Ill.). We placed the aspirate from both nares in a cryogenic vial containing tryptic soy broth (MicroBio Products, Tempe, Ariz.), mixed the vial contents, and immediately froze the vial in liquid nitrogen. From 1990 to 1991, we used 0.5 ml of sodium chloride to flush each naris and 1.0 ml of tryptic soy broth for culturing the aspirate. We reduced the amount of sodium chloride (0.25 ml) and tryptic soy broth (0.5 ml) in 1992. Nasal aspirate was cultured for *Pasteurella* spp.

In 1994, we separated the nasal aspirate into 2 aliquots. The first aliquot was cultured for *Pasteurella* spp. The second aliquot was tested for *M. agassizii* using a polymerase chain reaction (PCR) test. The PCR test detected the 16S ribosomal ribonucleic acid (nRNA) gene of mycoplasmas (Brown and Brown 1994).

We took 1 cloacal swab from each tortoise and stored them in Culturettes (Fig. 9). In 1992, we replaced Culturettes with Transtube (Medical Wire Equipment Co., Dover, N.J.). Transtube was recommended after problems isolating bacteria with Culturettes (C. Reggiardo, Dep. Vet. Med., Univ. Ariz., Tucson, Ariz., pers. commun.).

Fecal. We collected fresh fecal samples from each tortoise and placed each sample in a separate glass vial. We sent the fecal samples on 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. 10). We injected a fluid volume equivalent to 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. 11).

Weather

Permanent U.S. National Oceanic Atmosphere Administration weather stations recorded ambient temperature (maximum, minimum) and rainfall, while temporary automatic stations recorded local ambient temperature, relative humidity, rainfall, soil temperature, soil moisture, and wind speed. Permanent weather data were collected daily at Hillside and Aguila, Arizona (Natl. Oceanic Atmos. Adm. 1989-94). The Hillside weather station was 17.7 km southeast of Little Shipp. The Aguila weather station was 20.9 km south of the Harcuvars. Permanent weather stations were in habitats similar to the study sites.

We installed 2 automatic weather stations (Model System 10, Rainwise, Inc., Bar Harbor, Me.), 1 at each site at random locations 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

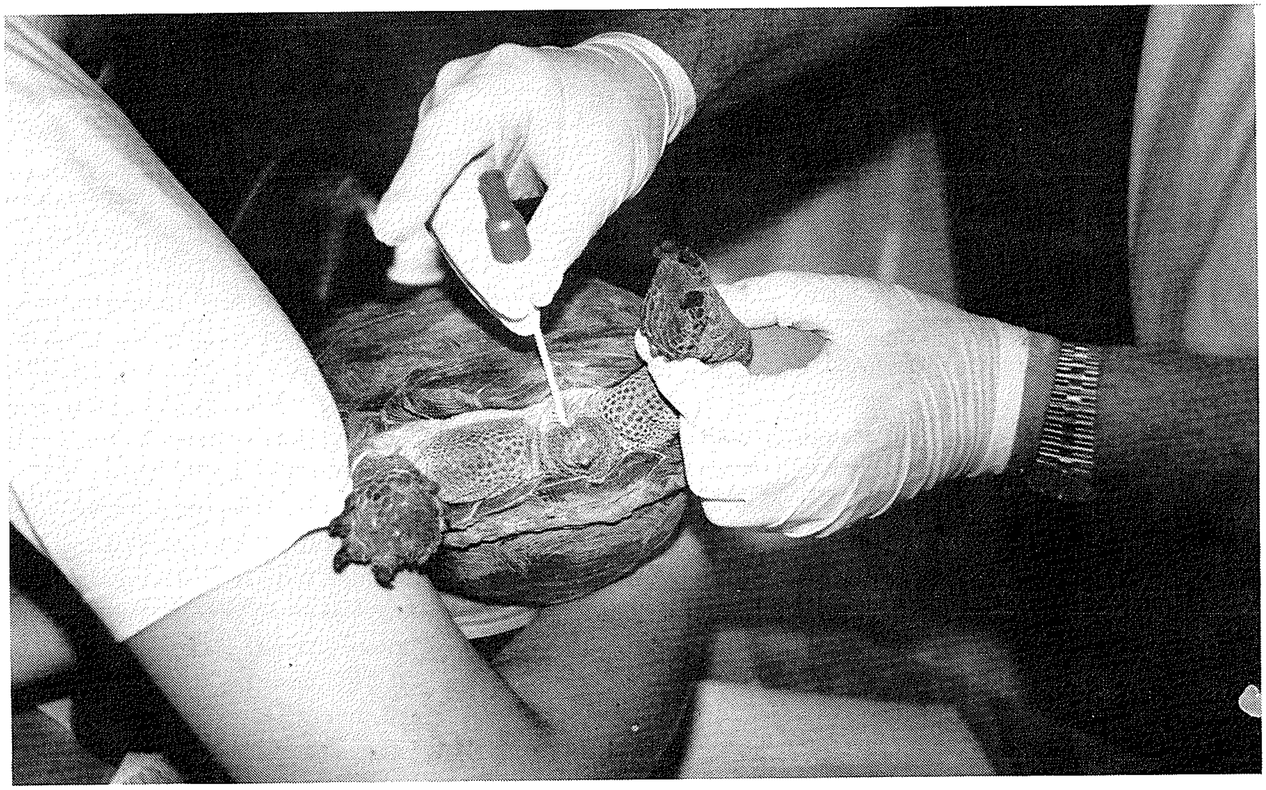


Figure 9. Collecting cloacal sample from a desert tortoise with a swab.

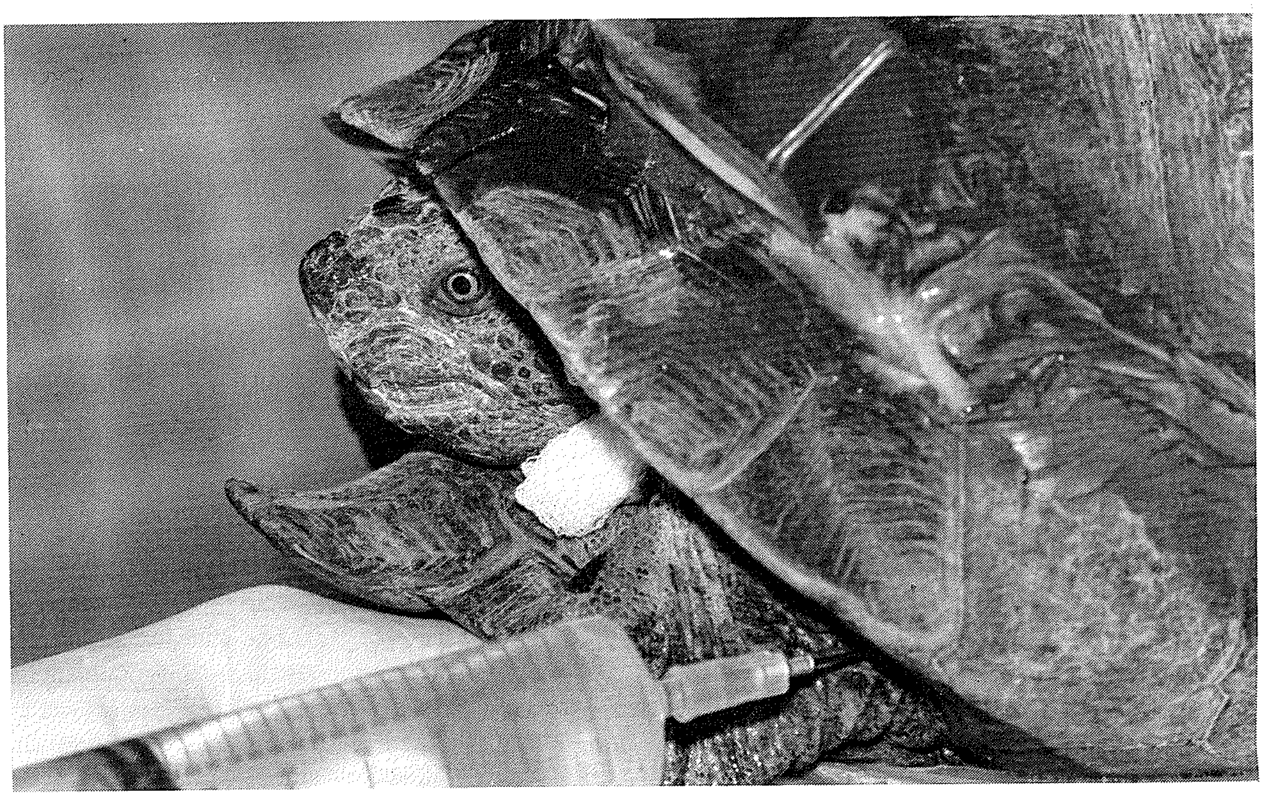


Figure 10. Rehydrating a desert tortoise by injecting equal parts Normosol and dextrose into its body cavity.

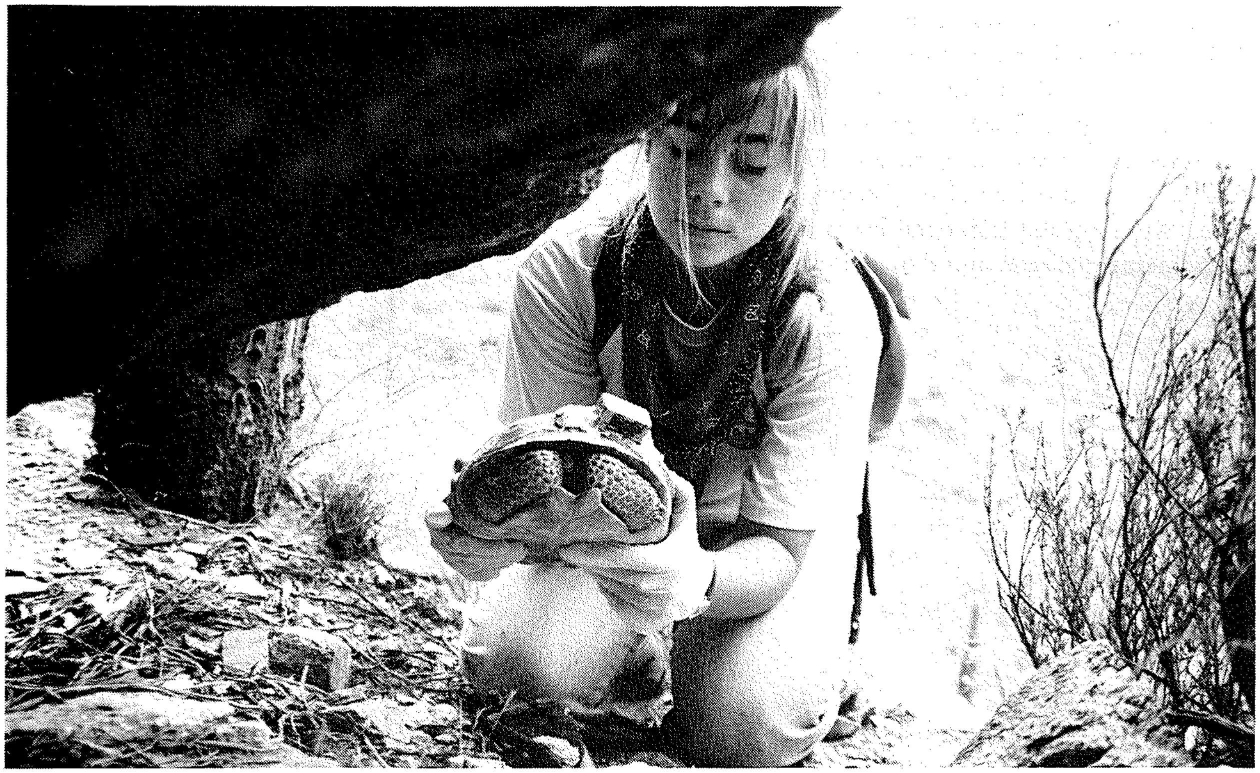


Figure 11. Releasing a desert tortoise at the point of capture in the morning after health assessment.

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 differences significant at $P \leq 0.01$. We tested data for normality with probability plots (SPSS, Inc. 1990) and used parametric statistics for all the data except bacteria and internal parasites.

Because the same tortoises were sampled repeatedly, the most appropriate statistical test was repeated measures multiple analysis of variance (RM-MANOVA). This type of analysis had 3 problems: (1) it is intolerant of missing values and none of the tortoises were sampled during each sampling period, (2) it simultaneously analyzes all treatment effects that are too complex to explain, and (3) it does not account for interactions among dependent variables. Instead we chose to test how each treatment (site, season, sex, year) affected each blood parameter individually using analysis of variance (ANOVA) with the program Statistica© (StatSoft, Inc. 1994).

We analyzed body mass and MCL, hematology, and plasma biochemistry using analysis of variance (ANOVA) for the effects of site, season, sex, and year. We used Tukey's honest significant difference (HSD) test to identify differences between means.

Body mass, MCL, PCV, and hemoglobin were analyzed for the effects of site, sex, season, and year. Differential WBCs (heterophils, lymphocytes, monocytes, eosinophils, basophils, azurophils) were analyzed for the effects of site, season, and year. We analyzed plasma (1991-94) for 11 biochemical parameters (BUN, creatinine, total protein, albumin, calcium, cholesterol, triglycerides, vitamins A and E, sodium, potassium) for the effects of site, season, sex, and year. We analyzed plasma (1993-94) for 11 biochemical parameters (glucose, uric acid, bile acids, AST, ALT, ALP, total bilirubin, direct bilirubin, indirect bilirubin, chloride, total carbon dioxide) for the effects of site, season, sex, and year.

We compared blood chemistry and differential WBCs between ill and healthy tortoises with a 1-way ANOVA. We analyzed nasal and cloacal bacteria and internal parasite presence or absence (0-1 response variable) using Kruskal-Wallis ANOVA for the effects of site, season, sex, and year. We compared eosinophil levels in tortoises with internal parasites and those without with a 1-way ANOVA. We analyzed automatic weather data (rainfall, ambient temperature, soil temperature, soil moisture, relative humidity, wind speed) using a 2-way ANOVA for the effects of site and year, and using a 1-way ANOVA for the effect of month.

RESULTS

We captured a total of 36 tortoises; 13 tortoises from Little Shipp and 23 tortoises from the Harcuvars. Of these total captures, we recorded 14 mortalities (4 at Little Shipp, 10 in the Harcuvars), 5 of which were due to predation (2 at Little Shipp, 3 in the Harcuvars) (Fig. 12). We suspect 2 Little Shipp tortoises were preyed upon by mountain lions (*Felis concolor*) based upon the width of canine puncture marks found on the tortoise carapaces. We suspect 2 of the 3 tortoises preyed on in the Harcuvars were taken by mountain lions based upon mountain lion scratch sign, while the third was preyed upon by coyotes (*Canis latrans*) based on the width of canine puncture marks. The other 2 Little Shipp tortoises were found dead on their backs wedged between rocks. These tortoises probably rolled off the steep Little Shipp slopes and became wedged between rocks on their backs and subsequently died.

We could not account for 7 of the 10 mortalities in the Harcuvars as there were no apparent signs of predation. Interestingly, all 7 were radio-tagged in October 1990; 3 died before sampling in April 1991, and 3 were found dead after being sampled in April 1991. Harcuvar tortoise 202 was also captured in October 1990 but survived until 1993. None of the 7 tortoises had signs of URTD. We found no difference in blood chemistry between tortoises which died in spring 1991 (n = 7) and all other tortoises (n = 29) ($P > 0.01$).

Body Mass and Median Carapace Length

Tortoise body mass and MCL (Table 1) differed between sites ($P < 0.001$) but not among seasons, sexes, and years ($P > 0.01$) (Table 2). We found Little Shipp tortoises were heavier (3.34 kg compared to 2.76 kg) and longer (264.1 mm compared to 249.9 mm) than Harcuvar tortoises ($P = 0.001$).

Hematology

PCV and hemoglobin values (Appendix 4) differed ($P < 0.01$) among seasons and years and to a lesser degree between sites. We found site, season, and year interactions in PCV and hemoglobin values (Table 2). Differential WBCs (Appendix 5) differed (P < 0.001) among seasons and to a lesser degree among years. We found season and year interactions in differential WBCs (Table 3).

We found Little Shipp tortoises had higher PCV in April 1992 and July 1994 compared to Harcuvar tortoises ($P = 0.01$), and Harcuvar tortoises had higher PCV in April 1993 and July 1993 compared to Little Shipp tortoises ($P = 0.01$) (Fig. 13). Hemoglobin values were higher in September compared to April and July for all 4 years combined (1991-94) ($P < 0.001$), and were higher in 1992 and 1993 compared to 1991 and 1994 ($P = 0.004$) (Fig. 14). We found no difference ($P > 0.01$) in PCV and hemoglobin for all other site, season, sex, and year combinations.

Lymphocyte counts were higher in September compared to April and July ($P < 0.001$) and higher in 1992 compared to 1991, 1993, and 1994 ($P = 0.005$) (Fig. 15). Azurophil counts were higher in 1994 compared 1991, 1992, and 1993 ($P < 0.001$) (Fig. 16). We found no difference ($P > 0.01$) for other differential WBC parameters (WBC estimate, heterophils, monocytes, eosinophils, basophils) with respect to all other site, season, and year combinations.

We observed 2 types of red blood cell abnormalities, polychromasia and anisocytosis. We observed polychromasia in 7 tortoises; 3 in 1994, 2 in 1993, and 2 in 1992. Harcuvar tortoise 218 was the only tortoise in which polychromasia was found twice (June 1992, November 1992). Six tortoises had mild anisocytosis; 5 in 1992, and 1 in 1991.

Clinical Chemistry

Plasma biochemistry (Appendix 5) and plasma electrolytes (Appendix 6) varied among seasons and years and to a lesser degree between sites and sex. We found site, season, sex, and year interactions in plasma biochemistry and electrolytes (1991-94) (Table 4), and plasma biochemistry (1993-94) (Table 5).

Plasma 1991-94. Seasonal and yearly changes occurred in total protein, albumin, cholesterol, and vitamin E (Fig 17). We also found seasonal changes in triglycerides and yearly changes in calcium and vitamin A (Fig. 18). Total protein and vitamin E were higher ($P < 0.01$) in July compared to April and September, and albumin and cholesterol were higher ($P < 0.01$) in September compared to April and July. Total

Figure 12. Desert tortoise carcass showing signs of predation.

Table 1. Body mass and median carapace length [$\bar{x} \pm SD$ (*n*)] for Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-94. Data from these 2 sites are combined.

Parameter	April	June	September
Body mass (kg)			
Male	2.97 ± 0.69 (29)	3.02 ± 0.80 (26)	3.03 ± 0.77 (55)
Female	3.17 ± 0.64 (28)	3.01 ± 0.65 (27)	3.07 ± 0.65 (44)
Median carapace length (mm)			
Male	254.4 ± 22.0 (29)	256.9 ± 25.2	259.2 ± 24.7 (55)
Female	256.5 ± 17.7 (28)	255.4 ± 18.7 (27)	258.4 ± 19.0 (44)

Table 2. Significant effects of site (Little Shipp Wash and Harcuvar Mtns., Ariz.), season (April, June, September), sex, and year (1991-94) on body mass, median carapace length, packed cell volume, and hemoglobin in desert tortoises (n = 36).

	F	df	*P*
Body Mass			
Site	27.5	132	<0.001
Median Carapace Length			
Site	14.9	132	<0.001
Packed Cell Volume			
Site	6.8	130	0.01
Season	4.4	130	0.01
Year	6.4	130	<0.001
Site x year	5.0	130	0.002
Site x season x year	4.9	130	<0.001
Hemoglobin			
Season	11.0	127	<0.001
Year	4.6	127	0.004
Season x year	3.9	127	0.001

Table 3. Significant effects of sites (Little Shipp Wash and Harcuvar Mtns., Ariz.), season (April, June, September), and year (1991-94) on white blood cell estimate, heterophils, lymphocytes, monocytes, azurophils, eosinophils, and basophils in desert tortoises (n = 36).

	F	df	*P*
Lymphocytes			
Season	19.5	151	<0.001
Year	4.3	151	0.005
Season x year	6.0	151	<0.001
Azurophils			
Season	34.2	151	<0.001

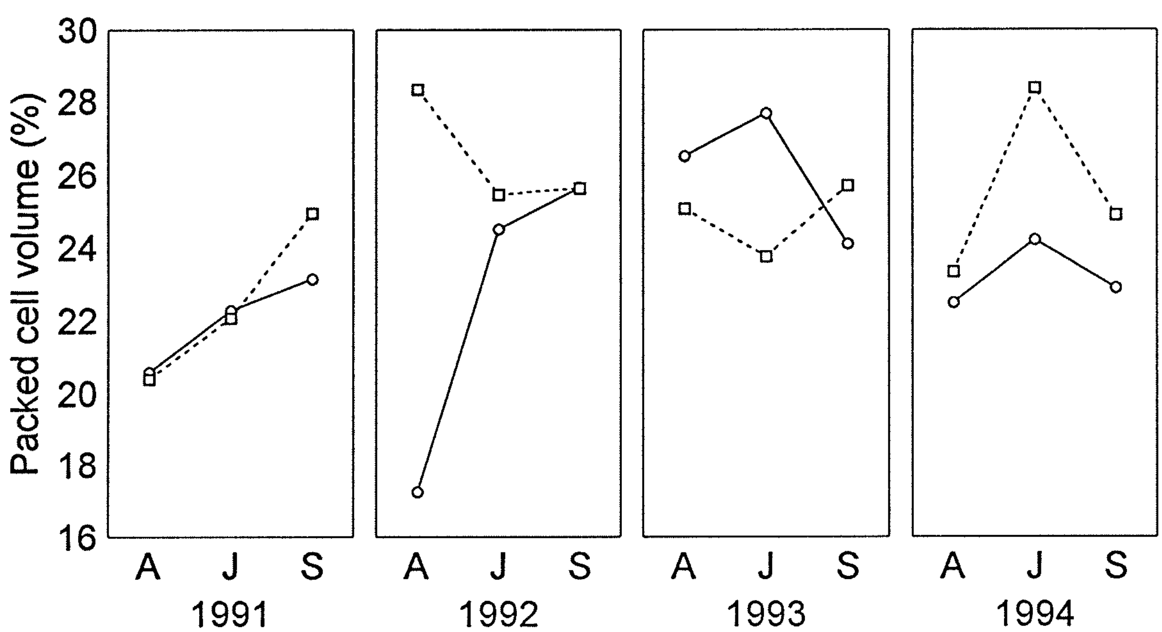


Figure 13. Seasonal change by year in packed cell volume in Sonoran desert tortoise (n = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1991-94. Dotted line = Little Shipp; solid line = Harcuvar Mtns.

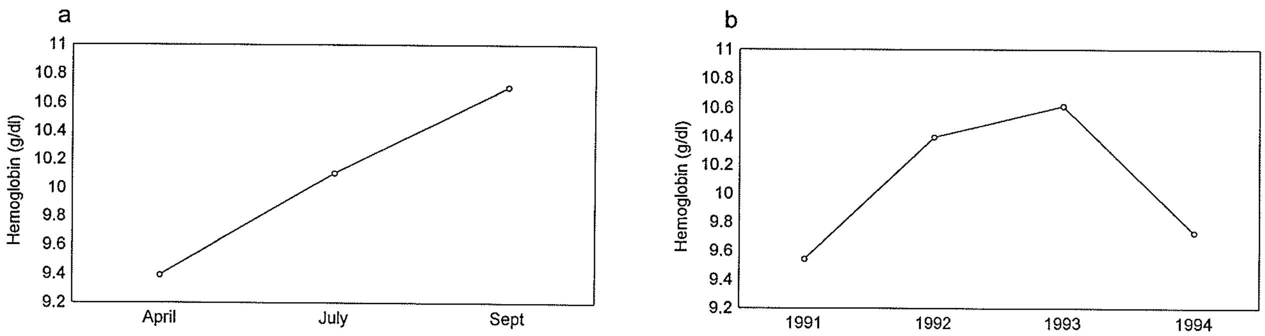


Figure 14. Seasonal (a) and yearly (b) changes in hemoglobin in Sonoran desert tortoises (*n* = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1991-94. Data from these 2 sites are combined.

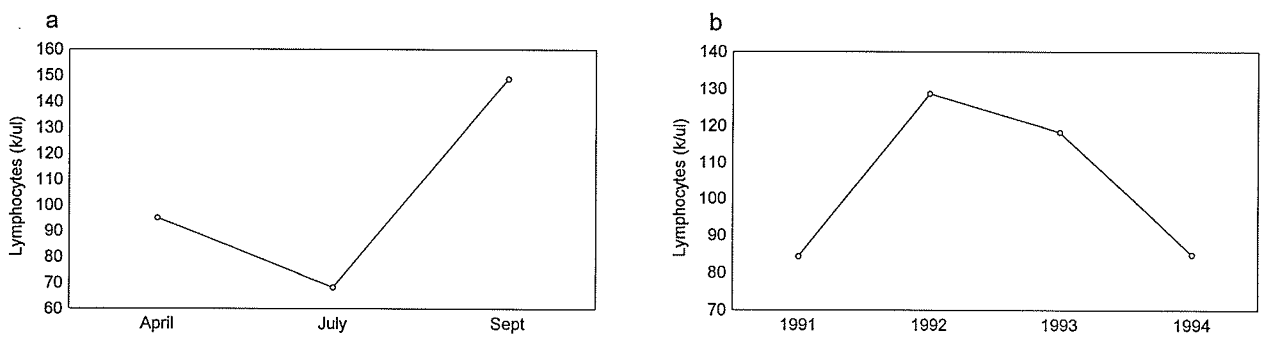


Figure 15. Seasonal (a) and yearly (b) changes in lymphocytes in Sonoran desert tortoises (*n* = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1991-94. Data from these 2 sites are combined.

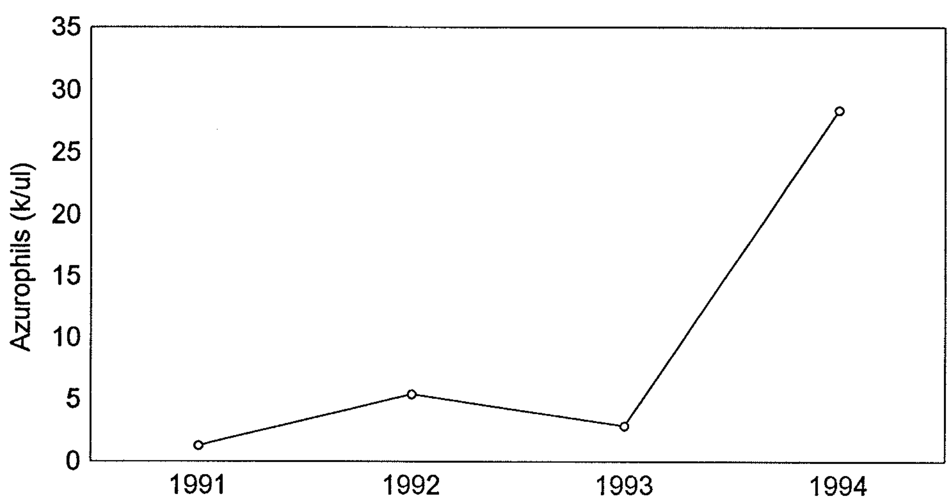


Figure 16. Seasonal (a) and yearly (b) changes in azurophils in Sonoran desert tortoises (*n* = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1991-94. Data from these 2 sites are combined.

Table 4. Significant effects of site (Little Shipp Wash and Harcuvar Mtns., Ariz.), season (April, June, September), sex, and year (1991-94) on total protein, albumin, calcium, cholesterol, triglycerides, vitamin A and E, sodium, and potassium in desert tortoises (n = 36).

	F	df	P
Total Protein			
Season	4.1	127	0.01
Sex	39.3	127	<0.001
Year	23.6	127	<0.001
Season x year	3.2	127	0.005
Albumin			
Season	5.7	129	0.004
Sex	22.8	129	<0.001
Year	146.0	129	<0.001
Site x season	11.0	129	<0.001
Site x year	7.2	129	<0.001
Season x year	10.6	129	<0.001
Sex x year	8.1	129	<0.001
Site x season x year	10.3	129	<0.001
Calcium			
Sex	97.0	129	<0.001
Year	6.4	129	<0.001
Site x year	10.0	129	<0.001
Cholesterol			
Season	8.2	130	<0.001
Sex	166.1	130	<0.001
Year	3.7	130	<0.01
Season x year	5.5	130	<0.001
Season x sex	10.0	130	<0.001
Triglycerides			
Season	32.6	130	<0.001
Sex	105.4	130	<0.001
Season x sex	22.1	130	<0.001

Table 4. (continued)

	F	df	P
Vitamin A			
Site	65.2	127	<0.001
Season	37.1	127	<0.001
Site x year	5.7	127	<0.001
Season x year	7.2	127	<0.001
Vitamin E			
Season	66.8	127	<0.001
Sex	139.8	127	<0.001
Year	14.1	127	<0.001
Site x season	5.6	127	0.004
Season x year	120.5	127	<0.001
Sodium			
Site	9.8	129	0.002
Season	33.1	129	<0.001
Year	33.0	129	<0.001
Season x year	19.1	129	<0.001
Potassium			
Season	12.3	129	<0.001
Year	6.7	129	<0.001
Season x year	4.5	129	<0.001
Site x season x year	3.4	129	0.003
Site x sex x year	4.0	129	0.009

Table 5. Significant effects of site (Little Shipp Wash and Harcuvar Mtns., Ariz.), season (April, June, September), sex, and year (1993-94) on fibrinogen, glucose, uric acid, aspartate aminotransferase, alanine aminotransferase, total bilirubin, indirect bilirubin, chloride, and total carbon dioxide in desert tortoises ($n = 36$).

	F	df	P
Fibrinogen			
Season	45.0	55	<0.001
Year	14.4	55	<0.001
Site x season	5.7	55	0.005
Season x year	85.7	55	<0.001
Glucose			
Season	12.0	58	<0.001
Uric acid			
Site	9.9	58	0.001
Season	53.5	58	<0.001
Year	97.0	58	<0.001
Site x season	5.2	58	0.008
Season x year	63.8	58	<0.001
Site x season x year	6.1	58	0.003
Asparatate aminotransferase			
Sex	8.3	58	0.005
Alanine aminotransferase			
Season	12.5	58	<0.001
Year	6.9	58	0.01
Season x year	8.7	58	<0.001
Total bilirubin			
Sex	22.1	58	<0.001
Year	11.2	58	0.001
Sex x year	13.3	58	<0.001
Indirect bilirubin			
Sex	20.4	58	<0.001
Year	12.6	58	<0.001
Sex x year	14.2	58	<0.001
Chloride			
Season	41.5	58	<0.001
Total carbon dioxide			
Site	14.5	57	<0.001
Season	6.1	57	0.004

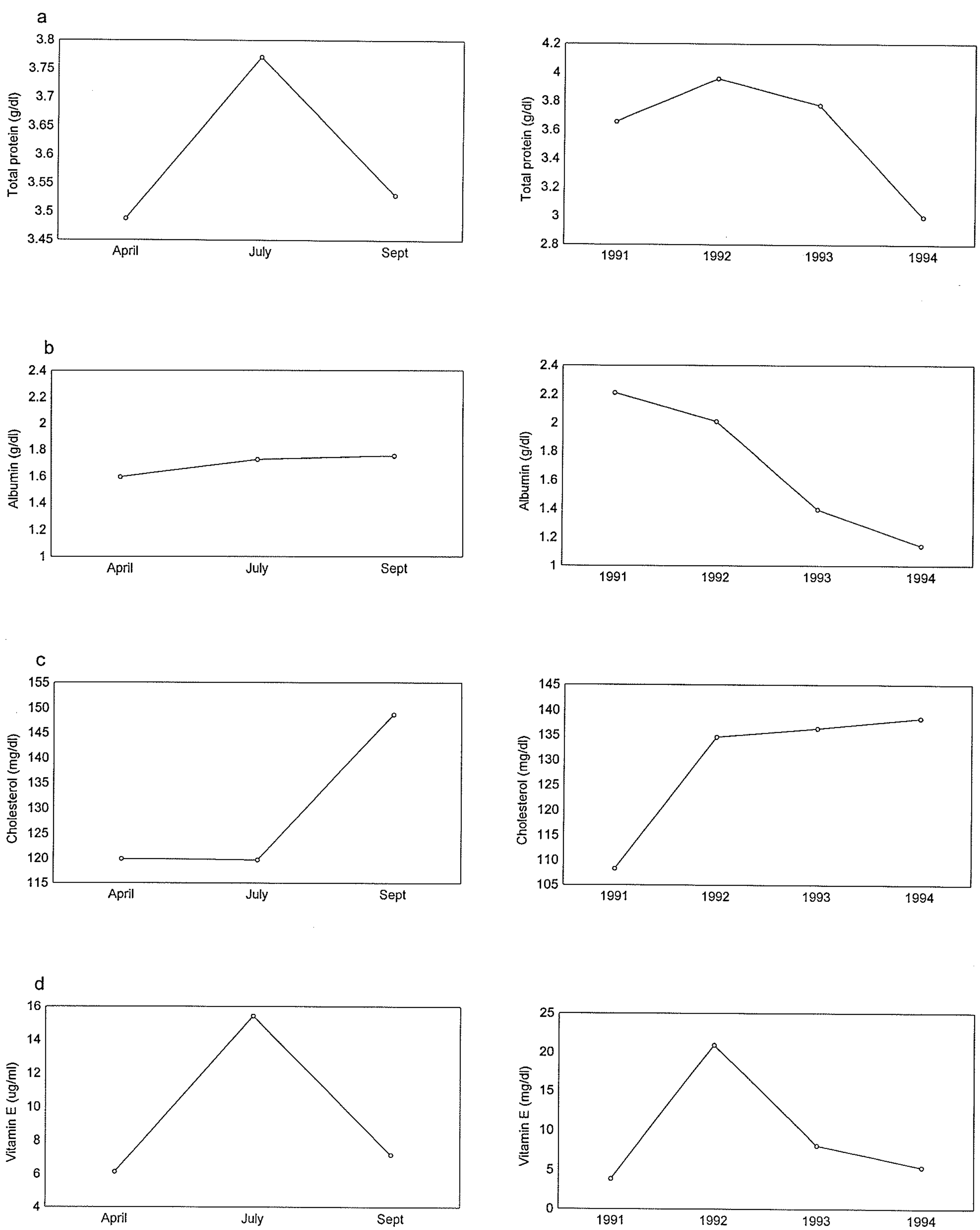


Figure 17. Seasonal and yearly changes in (a) total protein, (b) albumin, (c) cholesterol, and (d) vitamin E in Sonoran desert tortoises (n = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1991-94. Data from these 2 sites are combined.

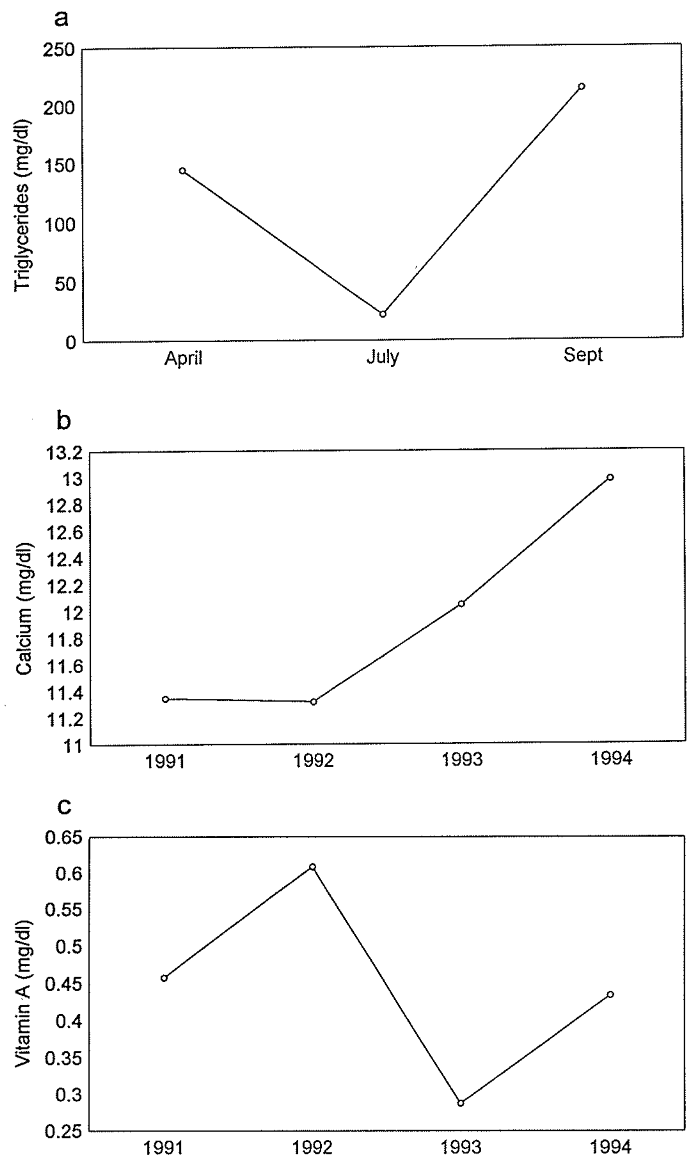


Figure 18. Seasonal changes in (a) triglycerides, and yearly changes in (b) calcium, and (c) vitamin A in Sonoran desert tortoises (n = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1991-94. Data from these 2 sites are combined.

protein and vitamin E were higher ($P = 0.01$) in 1992, albumin was higher ($P < 0.001$) in 1991, and cholesterol was higher ($P = 0.01$) in 1994 compared to all years. We found higher ($P < 0.001$) triglycerides in September. Calcium levels were higher ($P < 0.001$) in 1994, and vitamin A levels were higher ($P < 0.001$) in 1992 compared to all other years.

We did find site and sex differences in blood chemistry. Little Shipp tortoises had higher ($P < 0.001$) levels of vitamin A than Harcuvar tortoises and Harcuvar tortoises had higher ($P = 0.002$) levels of sodium than Little Shipp tortoises. Female tortoises had higher ($P < 0.001$) levels of total protein, albumin, cholesterol, triglycerides, calcium, and vitamin E compared to male tortoises. Cholesterol, triglycerides, and estradiol values peaked in April and September in all years.

We found plasma electrolytes, sodium, and potassium showed similar seasonal and yearly changes (Fig. 19). Sodium and potassium had higher ($P < 0.001$) levels in July compared to April and September, and higher ($P < 0.001$) levels in 1993 compared to 1991, 1992, and 1994. We found no difference ($P > 0.01$) for other blood chemistry values (BUN, creatinine) with respect to all other site, season, sex, and year combinations.

Plasma 1993-94. Seasonal and yearly changes occurred in fibrinogen, uric acid, and ALT (Fig. 20). Fibrinogen and ALT were higher ($P < 0.001$) in September compared to April and July, and uric acid was higher ($P < 0.001$) in April compared to July and September. Fibrinogen and ALT were higher ($P < 0.01$) in 1993 compared to 1994, and uric acid was higher ($P < 0.001$) in 1994 compared to 1993. We only found seasonal changes in glucose, chloride, and total carbon dioxide (Fig. 21). Glucose and chloride levels were higher ($P < 0.001$) in July compared to April and September, and total carbon dioxide levels were higher ($P = 0.004$) in September compared to April and July.

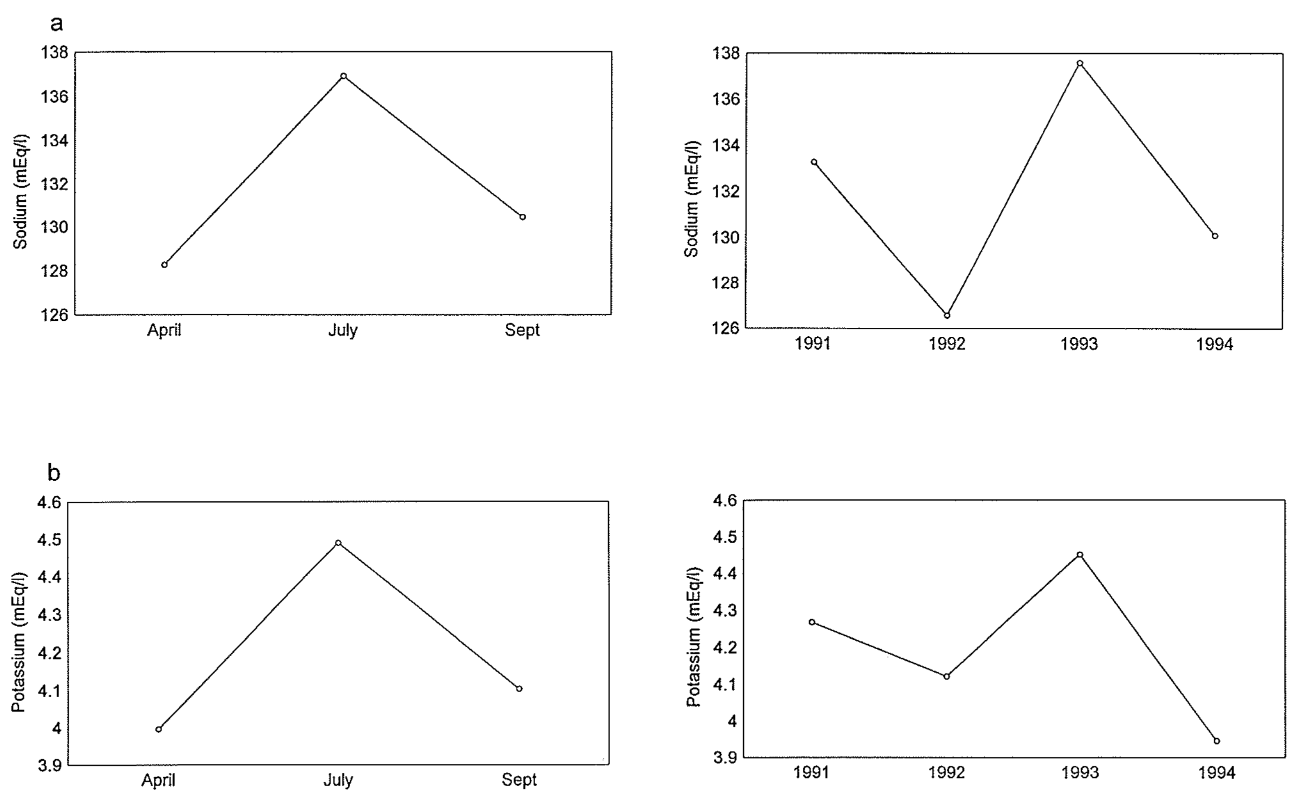


Figure 19. Seasonal and yearly changes in (a) sodium and (b) potassium in Sonoran desert tortoises ($n = 36$) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1991-94. Data from these 2 sites are combined.

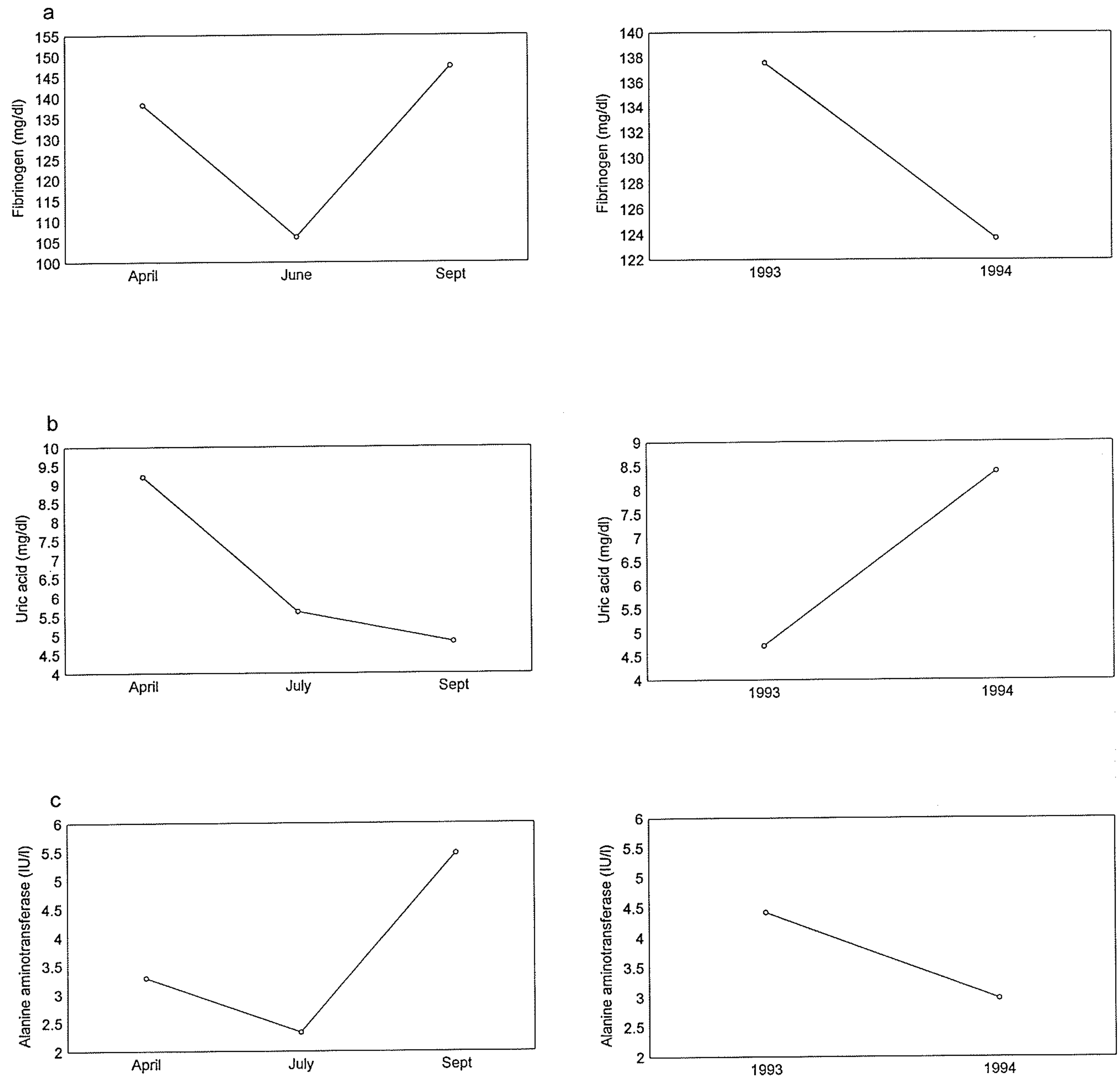


Figure 20. Seasonal and yearly changes in (a) fibrinogen, (b) uric acid, and (c) alanine aminotransferase in Sonoran desert tortoises (n = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1993-94. Data from these 2 sites are combined.

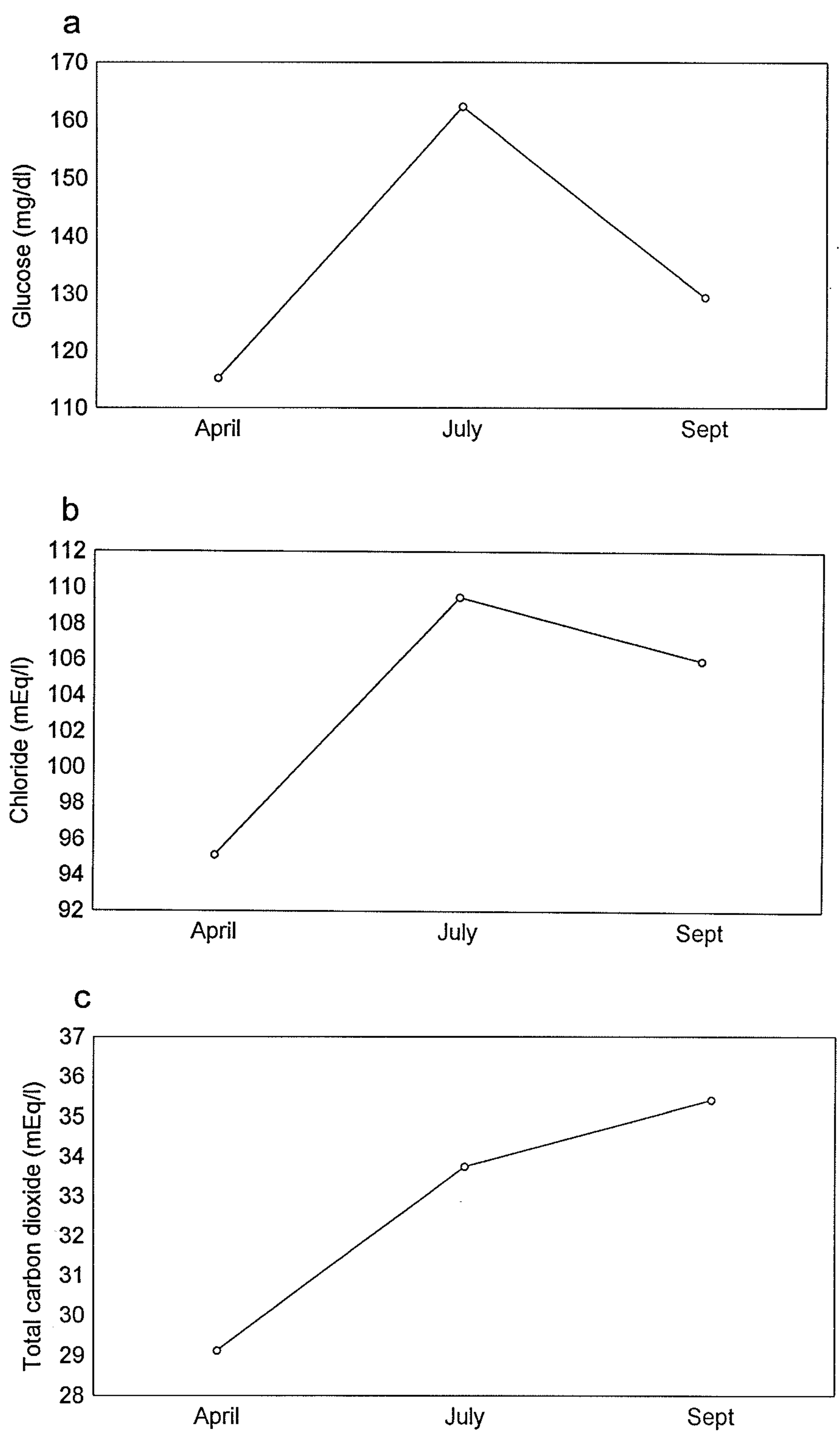


Figure 21. Seasonal changes in (a) glucose, (b) chloride, and (c) total carbon dioxide in Sonoran desert tortoises (n = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1993-94. Data from these 2 sites are combined.

Sex and yearly differences were found in total and indirect bilirubin (Fig. 22). Female tortoises had higher ($P < 0.001$) levels of both types of bilirubin and there were higher ($P < 0.01$) levels of total and indirect bilirubin in 1994 compared to 1993.

We did find site and sex differences in blood chemistry. Little Shipp tortoises had higher ($P = 0.002$) levels of uric acid than Harcuvar tortoises, and Harcuvar tortoises had higher ($P < 0.001$) levels of total carbon dioxide than Little Shipp tortoises. Male tortoises had higher ($P = 0.005$) levels of AST compared to female tortoises. We found no difference ($P > 0.01$) for other blood chemistry values (bile acids, ALP, direct bilirubin) and all other site, season, sex, and year combinations.

Health Profiles

We found all tortoises sampled in September 1990 ($n = 9$) had abnormally high levels of zinc. Zinc was not analyzed in November 1990. As indicated from ELISA and PCR results, we found 3 ill tortoises; 2 in the Harcuvars (203, male; 208, female); and 1 at Little Shipp (500, male). We found no difference in blood chemistry values between ill tortoises ($n = 3$) and healthy tortoises ($n = 33$) ($P < 0.01$).

Bacteriology

ELISA/PCR. ELISA results from 1992 to 1994 were the following: 4 tortoises with positive titers to *M. agassizii* and 8 tortoises with suspect titers ($n = 99$ samples). In 1995, all 99 samples were retested with ELISA and only 2 were suspect or positive (Fig. 23); Harcuvar tortoise 203 had a suspect titer for *M. agassizii* in July 1993 and Harcuvar tortoise 208 had a positive titer in September 1994. The PCR test in 1994 did confirm the presence of *M. agassizii* in Harcuvar tortoise 208, and also showed a positive test result for Little Shipp tortoise 500. Tortoise 500 tested ELISA negative for *M. agassizii* the same time it had a positive PCR.

Nasal. We found 4 species of bacteria in the choana (Appendix 7), with 1 possible pathogen (*Pseudomonas* spp.). We found 1 possible pathogen, *Pasteurella testudinis*, in the nasal cavity (1990-94;19%). We found higher ($X^2 = 17.0$, df = 4, $P = 0.002$) levels of *P. testudinis* in 1991 compared to all other years.

Cloacal. From 227 samples, we found 16 species of bacteria in the cloaca (Appendix 8), at least 2 of which were opportunistic pathogens (*Pseudomonas* spp., *Salmonella* spp.). The majority (75%) of cloacal bacteria were nonpathogenic *Staphylococcus* spp.

One cloacal bacteria showed site differences, 3 showed seasonal differences, and 2 showed yearly differences. Little Shipp tortoises had higher levels of *Shigella* spp. ($X^2 = 7.8$, df = 1, $P = 0.005$) compared to Harcuvar tortoises. Higher levels of coliforms ($X^2 = 29.0$, df = 2, $P = 0.00$) were found in July, while higher levels of diptheroids ($X^2 = 26.0$, df = 2, $P = 0.00$) and *Escherichia coli* ($X^2 = 9.3$, df = 2, $P = 0.01$) were found in September compared to all other seasons. We found higher ($X^2 = 31.0$, df = 4, $P = 0.00$) levels of *Enterobacter-Klebsiella* in 1992, and higher ($X^2 = 37.9$, df = 4, $P = 0.00$) levels of *Pseudomonas* spp. in 1993 compared to all other years. We found no difference ($P > 0.01$) for other cloacal bacteria (*Bacillus* spp., *Campylobacter* spp., *Citrobacter* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Pasteurella* spp., *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp.) with respect to all other site, season, sex, and year combinations.

Parasitology

Pinworms (*Trachygonetria* spp.) were the only parasites found. Infection rate did not differ ($P > 0.01$) between sexes and among seasons or years. Ninety-six percent of tortoise feces had pinworm eggs ($n = 121$ fecal samples). We found no difference ($P < 0.01$) in mean eosinophil numbers for tortoises with and without pinworms.

Weather

Permanent and automatic weather station data indicated above average rainfall at Little Shipp in 1992 and 1993, and below average rainfall at Little Shipp in 1994. These data also showed below average rainfall in the Harcuvars in 1991, 1993, and 1994 (Appendices 9-10). Mean annual rainfall at the Hillside (Little Shipp) permanent weather station was 40.0 cm over a 29-year period (1961-90). Mean annual rainfall at the Aguila (Harcuvars) permanent weather stations was 24.3 cm over the same period. Analysis of automatic station data (1992-94) showed higher ($F = 101.0$, df = 49, $P < 0.001$) temperature and soil temperature ($F = 100.9$, df = 44, $P < 0.001$) in

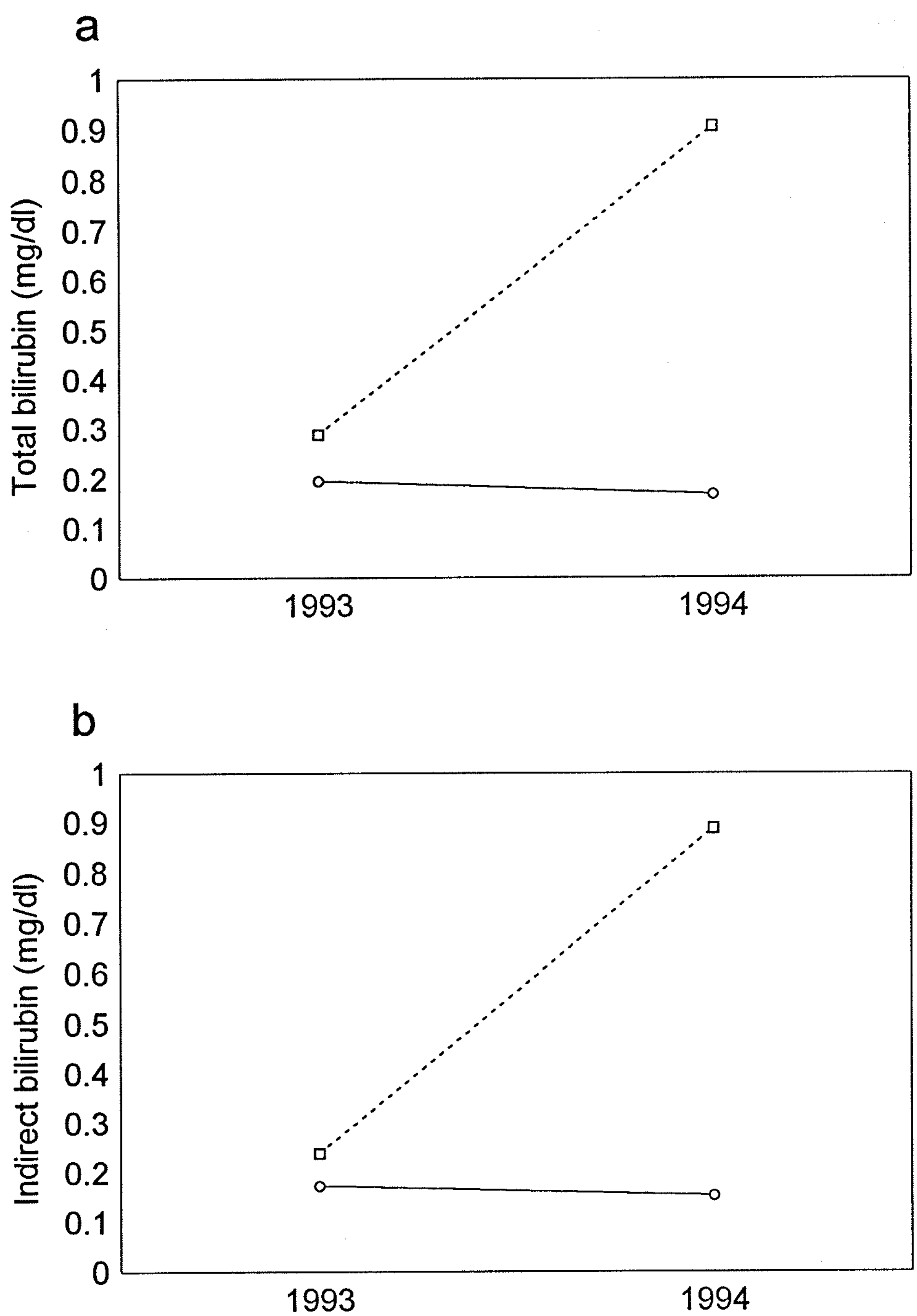


Figure 22. Yearly and gender differences in (a) total bilirubin and (b) indirect bilirubin in Sonoran desert tortoises (n = 36) at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) 1993-94. Solid line = males, dotted line = females. Data from these 2 sites are combined.

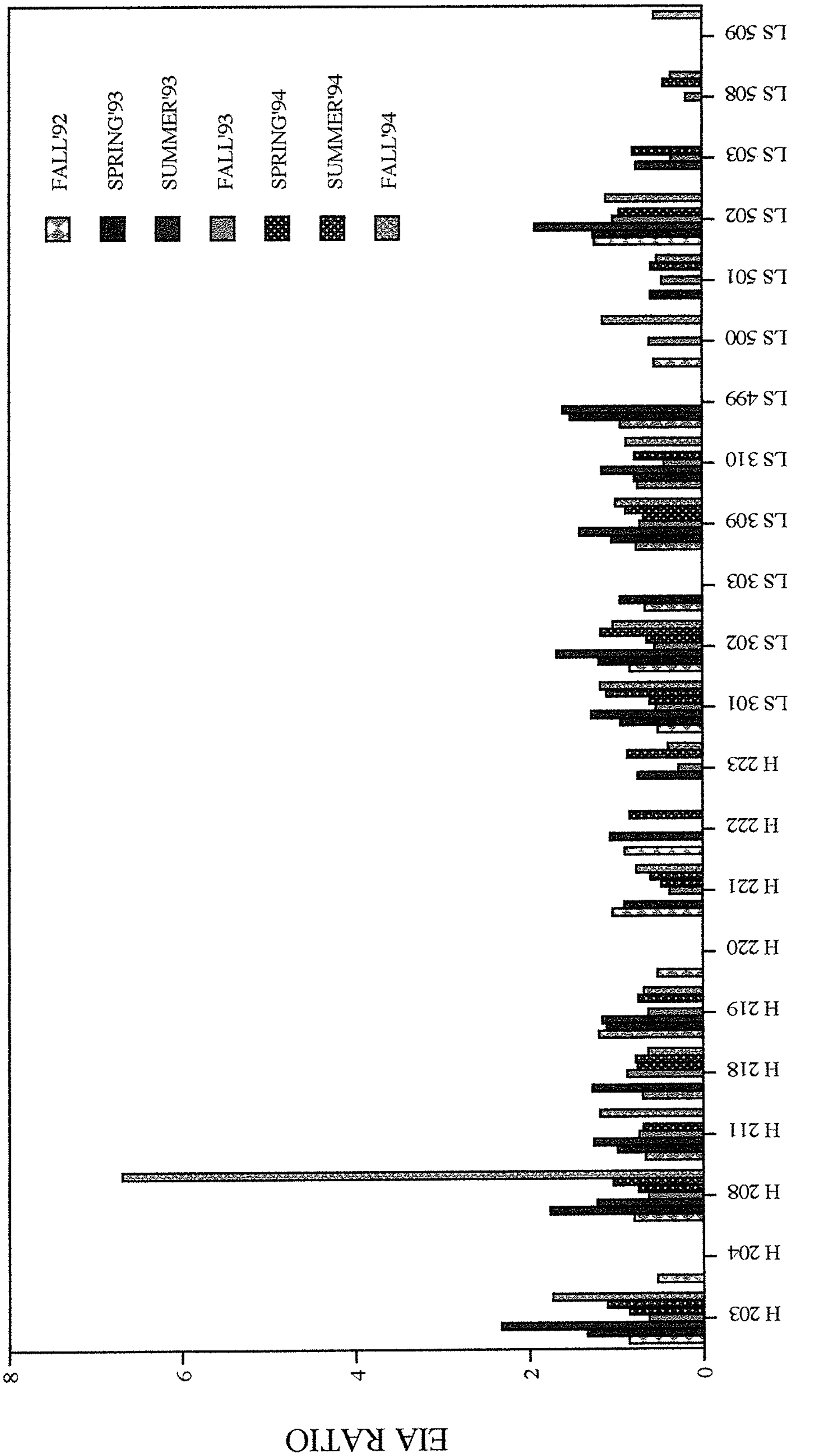


Figure 23. Plasma antibody titers for *M. agassizii* for 22 Sonoran desert tortoises from Little Shipp Wash (LS) and the Harcuvar Mtns. (H), Ariz., from September 1992 to September 1994. A suspect titer has an EIA ratio of sample >2, and a positive titer an EIA ratio of sample >3.

1994 compared to 1992 and 1993, and higher (F = 32.0, df = 49, $P < 0.001$) wind speeds in 1993 compared to 1992 and 1994. Automatic weather data also indicated higher (F = 2.5, df = 70, P = 0.009) rainfall in February and August compared to all other months (Fig. 24), and higher (F = 5.7, df = 70, $P < 0.001$) humidity in February, November, and December compared to all other months.

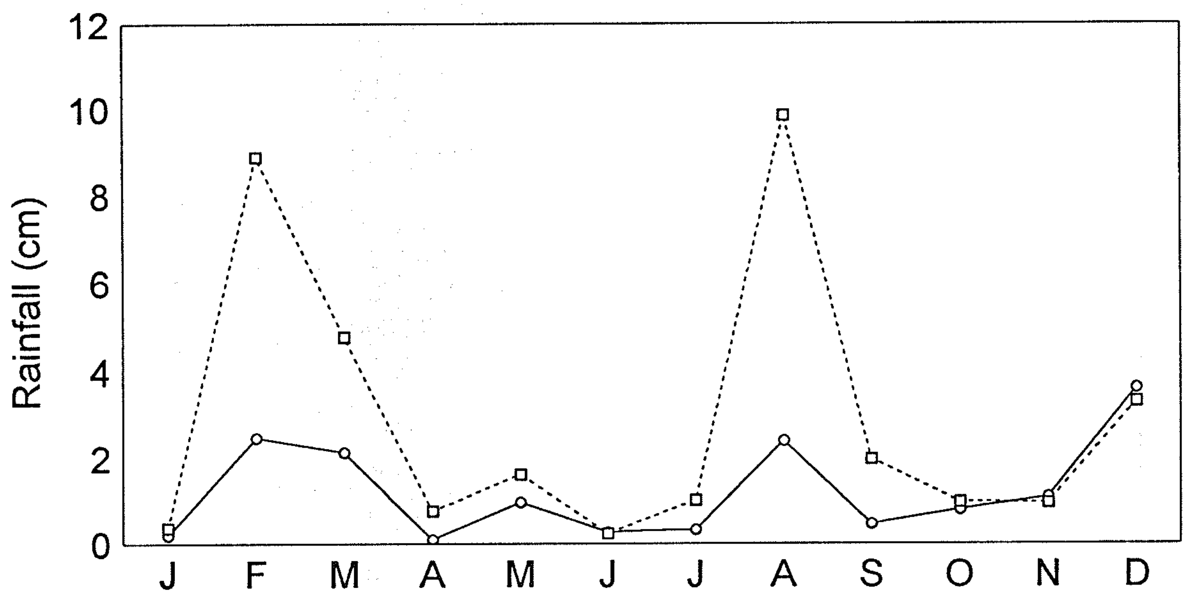
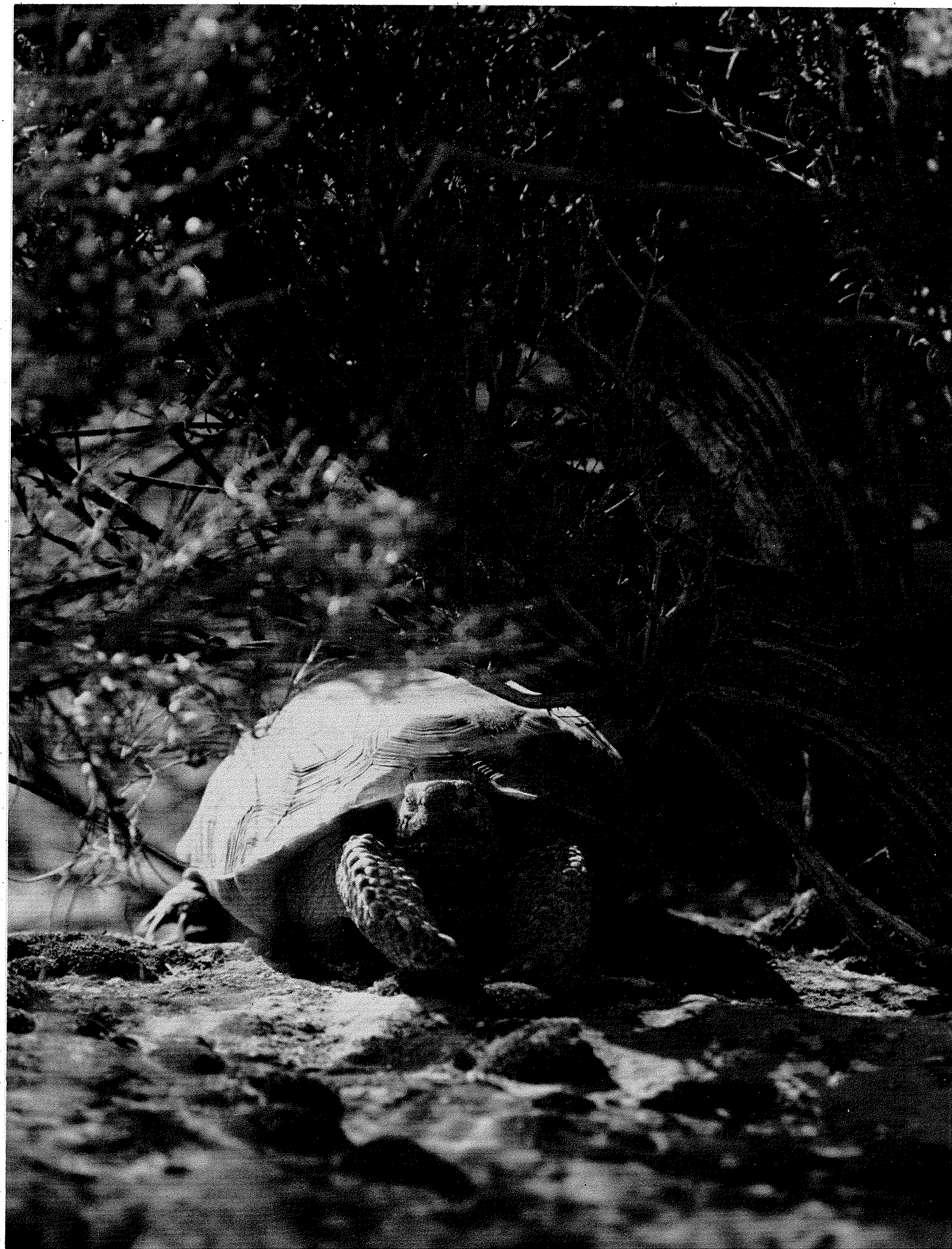


Figure 24. Monthly changes in rainfall at 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) in the Sonoran Desert, 1990-94 (data combined from permanent and automatic weather stations). Dotted line = Little Shipp, solid line = Harcuvar Mtns.



DISCUSSION

Mortality Factors

Four of 14 tortoise mortalities in this study were the result of predation by mountain lions. Although there is no published account of lions eating tortoises in limited areas of southwestern Arizona (Cashman et al. 1992), tortoises and lions have been found using the same mountain ridgetops in the Harcuvar Mountains (M. Peirce, Ariz. Game Fish Dep., Yuma, pers. commun.). M. Peirce found 1 felid scat in the Harcuvars with 2 tortoise feet in it. He could not determine if the scat was from a lion or a bobcat (*Lynx rufus*). In addition, researchers at the Little Shipp monitoring plot reported tortoises preyed upon by lions (Shields et al. 1990, Hart et al. 1992, Woodman et al. 1994).

We cannot explain the death of 7 Harcuvar tortoises in spring 1991. Poor winter rainfall was probably not a factor. Winter rains (October-March) in 1990-91 (11.45 cm) were below those for winters 1989-90 (18.46 cm), 1991-92 (21.1 cm), and 1992-93 (20.43 cm), but winter 1993-94 (2.65 cm) had less rainfall. We did not find any tortoise mortalities in spring 1994 despite the almost nonexistent previous winter rains. If capturing and/or sampling tortoises was a mortality factor we would expect to see similar mortality rates in all capture and sampling seasons.

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. The Sonoran Desert has rainfall in the winter and summer (Turner 1994). Tortoises forage in spring and late summer (Snider and Dickinson 1993), apparently in response to rainfall patterns. In our study, tortoises were probably foraging at the onset of the summer rains, as indicated by levels of total protein, vitamin E, sodium, and potassium in July.

During our study, there were above and below average years in terms of rainfall. Above average rainfall in 1992 and 1993 resulted in more available forage, while below average rainfall in 1994 resulted in less available forage. Cable (1975) found high precipitation led to high perennial grass production. In 1992 and 1993, levels of total protein and vitamins A and E suggested more foraging by tortoises. While in dry 1994, tortoises lost mass, and uric acid and PCV levels increased as tortoises became water deprived (see dehydration discussions by Guyton 1986). Increased levels of cholesterol during 1994 was probably a result of fat catabolism. From 1991 to 1993, excluding the drought year 1994, Little Shipp tortoises had increased body mass and uric acid compared to Harcuvar tortoises, which indicates foraging. Uric acid elevations have been reported in carnivorous reptiles after eating (Maixner et al. 1987).

Some Harcuvar female tortoises had higher levels of total and indirect bilirubin in 1993, a year of below-average rainfall. This may reflect liver disease in years of below average rainfall and in females whose physiology is stressed by vitellogenesis.

Sex Differences. Tortoise sex influenced seasonal changes in clinical chemistry. Most seasonal changes 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 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 higher levels of cholesterol with vitellogenesis 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). Vitamin E levels, first reported in this study, may be important in vitellogenesis.

The observed peaks in cholesterol and triglycerides in May and September suggest that females are in vitellogenesis from September through May. Rostal et al. (1994) reported vitellogenesis in the spring and fall in captive desert tortoises as indicated by increased calcium levels and follicular growth. In addition, Palmer and Guillette (1990) found increased levels of estradiol during vitellogenesis in free-ranging gopher tortoises.

Higher levels of AST in male tortoises possibly resulted from male aggression during mating. High levels of AST may be associated with muscle tissue damage because muscle cells contain a large amount of this enzyme (Coles 1986). O'Connor et al. (1994) suggested an increase in AST levels in male desert tortoises was associated with mating or fighting in the spring. Male tortoises have been observed fighting in the fall in the Sonoran Desert (Hart et al. 1992; Woodman et al. 1993, 1994, 1995).

Disease. Upper Respiratory Tract Disease. We did not observe any clinical signs of URTD at either site, but in 1992 1 tortoise on the nearby Little Shipp permanent monitoring plot had clear nasal discharge for 24 hrs (Woodman et al. 1993). Clinical signs of URTD are nasal discharge, ocular discharge, conjunctivitis, and palpebral edema (Jacobson et al. 1995); $\geq$ 1 of these signs indicates URTD. Monitoring plot observations of Harcuvar and Little Shipp tortoises with abnormal breathing and enlarged chin glands (Shields et al. 1990; Hart et al. 1992; Woodman et al. 1993, 1994, 1995), have not been attributed to URTD in clinical studies (M. Brown, Dep. Pathobiology, Univ. Fla., Gainesville, pers. commun.).

In our study, 3 tortoises had *M. agassizii*, the causative agent of URTD (Brown et al. 1994b). Harcuvar tortoise 208 (positive ELISA) did have *M. agassizii*, as this tortoise also had a positive PCR result. Harcuvar tortoise 203 (suspect ELISA, negative PCR) and Little Shipp tortoise 500 (negative ELISA, positive PCR) likely had *M. agassizii*. The conflicting ELISA/PCR results could be due to: (1) a poor nasal flush, (2) a new infection, (3) the tortoise had not yet developed an immune response, or (4) the number of *M. agassizii* antigens was insufficient to stimulate a response (M. Brown, Dep. Pathobiology, Univ. Fla., Gainesville, pers. commun.). Using revised cut-off values and refrozen plasma in retesting samples in 1995 was likely the cause of the different ELISA results from 1995 compared to those from 1992-94 (I. Schumacher, Dep. Pathobiology, Univ. Fla., Gainesville, pers. commun.).

Bacteria. Bacterial diseases may cause changes in hematology. For example, high numbers of lymphocytes and azurophils were associated in the presence of *Pseudomonas* spp. on cloacal culture. Healthy desert tortoises have many species of nasal and cloacal bacteria. *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). *Salmonella* spp. is an even more widespread commensal in chelonians, and, like *P. testudinis*, only produces disease opportunistically.

Parasites. The majority of tortoises in this study had pinworms. This is unremarkable, as pinworms are among the most common and numerous intestinal worms of lizards and turtles (Marcus 1981). The host can reinfect itself by breathing or ingesting pinworm 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.

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. Another type of intestinal parasite that our fecal sampling did not detect may be responsible for the high numbers of eosinophils.

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 1/3 the level of BUN, twice the level of ALP, and 3 times the level of total bilirubin than reported by Christopher et al. (1992, 1993). Our normal reference ranges for fibrinogen, creatinine, bile acids, triglycerides, copper, selenium, iron, zinc, and vitamins A and E are the first reported for desert tortoises.

We observed several physiological scenarios while evaluating the health of tortoises with abnormal blood values. Dehydration in tortoises was evident from elevated PCV and reduced body mass. High levels of electrolytes and plasma osmolality were found in water-stressed desert tortoises (O'Connor et al. 1994). We believe high levels of glucose indicated handling stress. In this study, tortoises exhibiting elevated uric acid with increased body mass are believed to have been foraging on protein-rich foods without the opportunity to drink sufficient water to flush their bladders. Elevated levels of total protein and vitamins A and E levels were found in foraging tortoises observed during bite count studies (Snider and Dickinson 1993). Some tortoises with liver disease later developed kidney problems and ultimately hyperphosphatemia. We suspect vitellogenesis is continuous from September until May and not biphasic during the calendar year. Rostal et al (1994) found captive tortoises in vitellogenesis in spring and fall indicated by increased calcium levels and follicular growth. Female tortoises also appear to lay eggs even if they have low body mass and signs of disease.

Sampling Techniques

Using different methods did not confound comparisons between tortoise health studies. Ketamine hydrochloride (Jacobson 1983, Bennett et al. 1992) and different autoanalyzers (monochromatic versus bichromatic) (Bolten et al. 1992) can result in differences in blood chemistry values. Our blood chemistry values were similar to those of Christopher et al. (1993) despite using ketamine hydrochloride and a monochromatic autoanalyzer; Christopher et al. (1993) did not use anesthesia to immobilize tortoises and used a bichromatic autoanalyzer.

Abnormally high levels of zinc in 9 tortoises sampled in September 1990 were likely due to plasma contaminated by the lubricant on red top vacutainer stoppers (D. Perry, Ariz. Vet., Diagnostic Lab., Univ. Ariz., Tucson, pers. commun.). Zinc contamination did not occur in sampling periods from 1991-94 as plasma was stored in cryogenic vials.

MANAGEMENT IMPLICATIONS

Assessing the physiological status of threatened populations can be important for development of appropriate management plans. Considering that Harcuvar Mountain tortoises had 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. In addition, the Harcuvar population should be monitored for URTD, as the presence of an ill tortoise may cause other Harcuvar tortoises to contract URTD. Because we found *M. agassizii* in Sonoran desert tortoises, we recommended sampling other tortoise populations in the Sonoran Desert for this organism.

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) follow a detailed protocol for sample collection, handling, and analysis; (2) use long-term monitoring of tortoise populations to assess spread of diseases such as URTD; and (3) use physical exams, laboratory tests, and microbiology to identify ill tortoises.

The following are recommended protocols for sample collection, handling, and analysis.

1. Conduct a detailed physical examination with emphasis on signs of malnutrition (see Glossary), dehydration, and URTD (Appendix 12). Take photographs of tortoise head and body to document signs of disease, as recommended by K. Berry (Natl. Biol. Serv., Riverside, Calif.).

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 WBC differentials.

4. Collect plasma in lithium heparin vacutainers for clinical chemistry analyses. Mix and centrifuge heparinized blood for 5 min. 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. Collect a duplicate aliquot for future ELISA testing and store at -80 C. Make sure the plasma is thawed only once prior to ELISA testing.

6. Collect nasal flushes and analyze for *M. agassizii* with a PCR to validate ELISA results.

7. Collect cloacal bacteria with swabs and analyze for *Pseudomonas* spp., *Salmonella* spp.

8. 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 to ensure proper statistical comparison and follow a univariate analysis approach.

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GLOSSARY

Alanine aminotransferase (ALT; SGPT) - An enzyme found in liver cells. 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 such as intestinal or renal loss.

Alkaline phosphatase (ALP) - An enzyme present in many body tissues and induced by growth, bone calcification, hyperadrenocorticism, and 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 cells size varies.

Anorexia - Lack of appetite.

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 in the liver. 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, kidney disease, and anorexia.

Chloride - Major extracellular anion. Changes in serum chloride can indicate dehydration, kidney disease, and severe vomiting/diarrhea.

Choana - The opening of the nasal cavity into the roof of the mouth.

Cholestasis - Condition where there is reduced bile flow through the bile duct; may occur with anorexia or obstruction.

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.

Conjunctivitis - Inflammation of the membrane that lines the inner eyelids and covers the exposed surface of the eyeball.

Copper - An important mineral necessary for hemoglobin formation, enzyme systems, bone development, and reproduction. Copper 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) - In this study, a 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 Immunosorbent Assay (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 ≤ 3, and a negative sample has an EIA ratio of sample ≤ 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 are associated with parasitism and hypersensitivity.

Geophagy - The act of ingesting soil.

Glucose - The end product of the metabolism of starch and glycogen. Glucose 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.

Hyperphosphatemia - Inorganic phosphorus is excreted by the kidneys.

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.

Malnutrition - Signs of malnutrition in desert tortoise include weight loss, sunken eyes and temporal muscles, pale oval mucosa, weakness (head flops from side to side), non-healing wounds, collapse of central carapace scutes, and shell pliancy (especially plastron). Collapse of central scute and shell pliancy are seen in adult tortoises with long-standing malnutrition.

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.

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. Selenium deficiency produces diseases similar to those observed in vitamin E deficiency. The concentration of selenium in plants depends upon 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 metabolism and catabolism in reptiles. Accumulations of uric acids may result from severe dehydration or renal disease, and can lead to visceral gout and death.

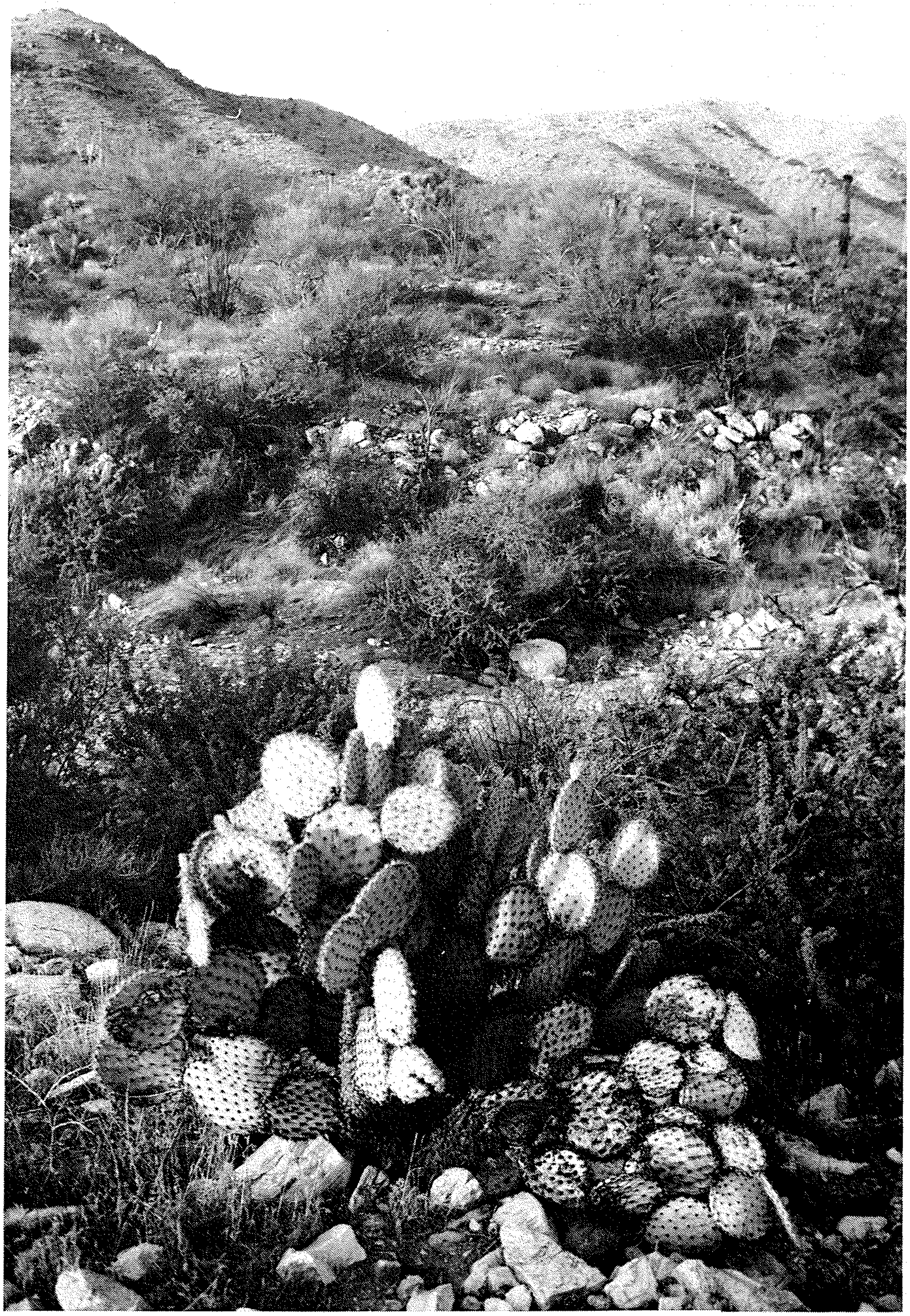
Vitamin A - A fat-soluble vitamin present as provitamin A carotenoids, mainly B-carotene, in all green parts of growing plants. It is stored in large concentrations in the liver and used during dietary inadequacy. Deficiencies result in vision problems, loss of integrity of the protective lining of mucosal 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. Summary of methods used in desert tortoise blood sampling by year from 2 sites (Little Shipp Wash and Harcuvar Mtns., Ariz.) in the Sonoran Desert, 1990-94.

Year		Methodology
1990	Collection	6.0 ml jugular venipuncture, 22-gauge needle
	Anesthetic	15 mg/kg body weight ketamine hydrochloride
	Whole Blood	0.6 ml whole blood in lithium heparin microtainer; packed cell volume field (PCV) and hemoglobin
	Blood Smears	None
	Plasma	2 aliquots on dry ice in red top vacutainers (1) blood urea nitrogen (BUN), total protein, albumin, aspartate aminotransferase (AST), calcium, cholesterol, triglycerides, sodium, and potassium, (2) vitamins A and E, copper, selenium, and zinc.
1991	Collection	Same
	Anesthetic	Same
	Whole Blood	Same
	Blood Smears	Two air dried blood smears examined for white blood cells (WBC) estimate, differential WBCs (heterophils, lymphocytes, monocytes, eosinophils, basophils, azurophils), platelet estimate, red blood cell morphology, hemoparasites, and evidence of anisocytosis and polychromasia.
	Plasma	Same; 3 aliquots in cryogenic vials 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.
1992	Collection	Same
	Anesthetic	Same
	Whole Blood	Same
	Blood Smears	Same
	Plasma	Same; 4 aliquots in cryogenic vials in liquid nitrogen (1) same as 1991 except 4 additions (glucose, uric acid, AST, alanine aminotransferase (ALT)), (2) same, (3) same, (4) ELISA for detection of *Mycoplasma agassizii*.
1993-94	Collection	Same
	Anesthetic	Same
	Whole Blood	additional test; fibrinogen
	Blood Smears	Same
	Plasma	Same; 4 aliquots in cryogenic vials in liquid nitrogen the same as those in 1992 except 9 additions to the first aliquot (total globulins, bile acids, alkaline phosphatase (ALP), total bilirubin, direct bilirubin, indirect bilirubin, chloride, carbon dioxide, anion gap).

Appendix 2. Overall means, standard deviations, and reference ranges for body mass, median carapace length, hematological, and plasma biochemical parameters in Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-94.

Parameter	$\bar{x} \pm$ SD (n)	Reference Range $\bar{x} \pm 2$ SD (n)
Body mass (kg)		
Male	3.01 ± 0.75 (110)	3.1 ± 0.68 (105)
Female	3.08 ± 0.65 (99)	3.1 ± 0.63 (98)
Median carapace length (mm)		
Male	257.4 ± 24.0 (110)	258.8 ± 22.1 (105)
Female	257.0 ± 18.5 (99)	256.6 ± 18.1 (98)
Packed cell volume field (%)		
Male	24.4 ± 4.6 (102)	25.0 ± 3.6 (98)
Female	24.4 ± 4.0 (90)	24.3 ± 3.2 (85)
Hemoglobin (g/dl)		
Male	10.2 ± 1.8 (99)	10.2 ± 1.5 (93)
Female	10.4 ± 1.5 (89)	10.3 ± 1.3 (85)
Fibrinogen (mg/dl)		
Male	134.2 ± 35.4 (36)	131.6 ± 32.2 (35)
Female	127.3 ± 39.9 (44)	130.2 ± 35.1 (43)
White blood cell estimate (k/μl)		
Male	9.1 ± 4.5 (84)	8.5 ± 3.7 (80)
Female	8.4 ± 4.6 (79)	7.8 ± 3.7 (75)
Heterophils (k/μl)		
Male	647.6 ± 398.3 (84)	582.3 ± 309.2 (79)
Female	554.5 ± 410.1 (79)	483.1 ± 307.3 (74)
Lymphocytes (k/μl)		
Male	110.8 ± 81.4 (84)	100.7 ± 62.6 (81)
Female	110.3 ± 97.0 (79)	93.9 ± 64.8 (75)
Monocytes (k/μl)		
Male	16.7 ± 25.2 (80)	13.0 ± 16.6 (77)
Female	13.5 ± 16.9 (78)	9.9 ± 11.7 (72)
Azurophils (k/μl)		
Male	10.4 ± 20.9 (84)	6.3 ± 12.3 (79)
Female	6.4 ± 12.5 (79)	4.2 ± 7.6 (75)
Eosinophils (k/μl)		
Male	39.5 ± 60.4 (80)	26.5 ± 33.0 (75)
Female	28.4 ± 43.1 (75)	16.8 ± 23.4 (68)
Basophils (k/μl)		
Male	88.5 ± 58.0 (84)	83.2 ± 46.6 (82)
Female	129.1 ± 96.0 (79)	116.1 ± 79.4 (75)
Glucose (mg/dl)		
Male	137.0 ± 38.3 (51)	132.6 ± 32.2 (49)
Female	132.5 ± 41.9 (62)	127.1 ± 34.9 (59)

Appendix 2. (continued).

Parameter	$\bar{x} \pm$ SD (*n*)	Reference Range $\bar{x} \pm$ 2 SD (*n*)
Blood urea nitrogen (mg/dl)		
Male	2.1 ± 3.1 (108)	1.6 ± 2.4 (102)
Female	1.7 ± 4.7 (94)	1.0 ± 2.0 (92)
Creatinine (mg/dl)		
Male	0.26 ± 0.15 (85)	0.24 ± 0.13 (81)
Female	0.25 ± 0.12 (86)	0.25 ± 0.10 (80)
Uric acid (mg/dl)		
Male	5.7 ± 3.2 (51)	4.8 ± 2.1 (46)
Female	6.2 ± 3.2 (62)	5.4 ± 1.8 (57)
Total protein (g/dl)		
Male	3.4 ± 0.53 (110)	3.4 ± 0.43 (104)
Female	4.0 ± 0.81 (97)	3.9 ± 0.69 (96)
Albumin (g/dl)		
Male	1.8 ± 1.1 (110)	1.7 ± 0.5 (109)
Female	1.9 ± 0.7 (96)	1.7 ± 0.5 (91)
Total globulins (g/dl)		
Male	1.6 ± 1.1 (110)	1.7 ± 0.5 (107)
Female	2.1 ± 0.7 (96)	2.1 ± 0.5 (90)
Bile acids (μmol/l)		
Male	5.3 ± 9.6 (28)	2.2 ± 3.1 (25)
Female	2.5 ± 3.8 (38)	2.1 ± 3.3 (36)
Aspartate aminotransferase (IU/l)		
Male	79.4 ± 44.0 (70)	73.2 ± 32.5 (67)
Female	67.2 ± 27.9 (72)	63.5 ± 21.0 (69)
Alanine aminotransferase (IU/l)		
Male	3.9 ± 4.1 (50)	2.9 ± 2.0 (46)
Female	2.9 ± 2.8 (61)	2.7 ± 2.4 (59)
Alkaline phosphatase (IU/l)		
Male	79.0 ± 39.3 (36)	72.5 ± 29.4 (34)
Female	127.8 ± 154.9 (46)	107.3 ± 68.8 (45)
Calcium (mg/dl)		
Male	10.3 ± 1.2 (110)	10.3 ± 1.0 (103)
Female	13.4 ± 2.9 (96)	13.2 ± 1.5 (92)
Phosphorus (mEq/l)		
Male	1.6 ± 0.7 (91)	1.6 ± 0.6 (86)
Female	4.8 ± 2.5 (87)	4.4 ± 1.9 (82)
Cholesterol (mg/dl)		
Male	80.7 ± 28.6 (110)	77.1 ± 20.8 (106)
Female	182.2 ± 66.6 (97)	175.8 ± 56.9 (94)

Appendix 2. (continued).

Parameter	x̄ ± SD (n)				Reference Range x̄ ± 2 SD (n)			
·iglycerides (mg/dl)								
Male	28.2	±	82.1	(110)	18.7	±	25.1	(108)
Female	265.2	±	219.0	(97)	237.4	±	187.7	(92)
al bilirubin (mg/dl)								
Male	0.2	±	0.2	(36)	0.1	±	0.1	(34)
Female	0.6	±	0.6	(46)	0.5	±	0.4	(44)
ct bilirubin (mg/dl)								
Male	0.02	±	0.01	(34)	0.02	±	0.01	(33)
Female	0.06	±	0.2	(41)	0.02	±	0.01	(39)
rect bilirubin (mg/dl)								
Male	0.2	±	0.2	(36)	0.1	±	0.1	(34)
Female	0.6	±	0.6	(46)	0.5	±	0.4	(44)
ɔper (ppm)								
Male	0.6	±	0.1	(55)	0.6	±	0.1	(53)
Female	0.5	±	0.2	(45)	0.5	±	0.2	(44)
enium (ppm)								
Male	0.04	±	0.02	(69)	0.03	±	0.01	(67)
Female	0.04	±	0.02	(51)	0.04	±	0.01	(49)
ıc (ppm)								
Male	1.9	±	1.5	(11)	1.9	±	1.5	(11)
Female	2.2	±	1.5	(11)	2.2	±	1.5	(11)
tamin A (μg/ml)								
Male	0.4	±	0.2	(95)	0.4	±	0.2	(91)
Female	0.5	±	0.2	989)	0.4	±	0.2	(86)
tamin E (μg/ml)								
Male	6.9	±	11.7	(95)	4.3	±	3.6	(90)
Female	11.2	±	12.6	(89)	7.9	±	5.1	(82)
·stosterone (ng/ml)								
Male	156.5	±	150.9	(29)	127.0	±	106.8	(27)
Female	2.4	±	3.5	(36)	1.5	±	1.6	(33)
tradiol (pg/ml)								
Male	98.1	--						
Female	139.0	±	141.6	(49)	113.6	±	102.7	(46)
orticosterone (ng/ml)								
Male	8.3	±	7.4	(44)	6.9	±	5.4	(41)
Female	5.9	±	5.1	(50)	5.1	±	3.4	(48)
odium (mEq/l)								
Male	130.2	±	8.7	(109)	129.2	±	6.8	(102)
Female	131.1	±	9.0	(95)	130.4	±	8.1	(92)

Appendix 2. (continued).

Parameter	$\bar{x} \pm$ SD (*n*)				Reference Range $\bar{x} \pm$ 2 SD (*n*)			
Potassium (mEq/l)								
Male	4.1	±	0.7	(103)	4.1	±	0.6	(99)
Female	4.3	±	0.6	(92)	4.2	±	0.5	(87)
Chloride (mEq/l)								
Male	104.4	±	7.8	(36)	104.4	±	7.8	(36)
Female	102.4	±	8.6	(46)	101.9	±	8.0	(45)
Total carbon dioxide (mEq/l)								
Male	33.2	±	8.5	(36)	34.6	±	6.0	(34)
Female	32.4	±	8.0	(45)	33.0	±	6.8	(44)
Anion gap (mEq/l)								
Male	-1.2	±	13.6	(36)	-2.6	±	10.8	(35)
Female	3.4	±	11.9	(44)	3.5	±	10.0	(42)
Osmolality (mOs/kg)								
Male	271.3	±	28.0	(85)	268.1	±	22.9	(82)
Female	277.7	±	28.3	(86)	275.8	±	24.5	(82)

Appendix 3. Summary and evaluation of abnormal blood parameter values for plasma from Sonoran desert tortoises from 2 sites (Little Shipp Wash and Harcuvar Mtns., Ariz.), 1990-94. 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 and NA means the test was not available. Incorporated into the interpretation of the laboratory data are results of rainfall, hematology (begun 4/91), ELISA (begun 9/92), and opportunistic cloacal bacterial pathogens (*Pseudomonas* spp. [Pseu], *Salmonella* spp. [Salm], begun 7/91). LS = Little Shipp Wash, H = Harcuvar Mountains.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS301 - F														
Packed cell volume field (%)				--	↑36.0									
Eosinophils (k/µl)	NA	NA		--							↑163.4			
Basophils (k/µl)	NA	NA	↑299.0	--										
Blood urea nitrogen (mg/dl)		↑34.0*		--										
Uric acid (mg/dl)	NA	NA	NA	--	NA	NA						↑13.5		
Phosphorus (mg/dl)	NA	NA		--						↑10.6*				
Total bilirubin (mg/dl)	NA	NA	NA	--	NA	NA	NA	NA	↑1.9					
Indirect bilirubin (mg/dl)	NA	NA	NA	--	NA	NA	NA	NA	↑1.8					
Vitamin A (µg/ml)				--			↑1.1*							
Vitamin E (µg/ml)				--			↑49.0*							
Osmolality (mOs/kg)	NA	NA		--										↑366.0
Body mass (kg)	3.8		4.1	--	3.65	3.9	4.0	4.0	4.1	3.8	3.9	4.3	4.1	4.05
Cloacal bacteria	NA	3.7		--	Pseu	Salm	Pseu	Pseu	Pseu	Pseu		Pseu		Pseu

Evaluation: High blood urea nitrogen levels in November 1990 perhaps a result of catabolic state. This tortoise was probably in hibernation at the time. Basophilia in April 1991 may indicate an inflammatory condition but the cause is unknown. In September 1991 this tortoise appears mildly dehydrated (packed cell volume up, body mass down) and had *Pseudomonas* spp. in the cloaca. In June 1992 this tortoise was probably foraging (elevated vitamins A and E, gain in body mass). In April 1993 total bilirubin and indirect bilirubin were elevated which may have been a due to a lack of foraging or cholestasis. In June 1993 a drop in body mass with elevated phosphorus may indicate reduced renal output. In September 1993 there was eosinophilia with undetermined cause. In April 1994 tortoise had elevated uric acid and a gain in body mass. These 2 events usually occur in May and Sept when tortoises are eating high protein forage and not urinating. This condition (urine stasis) probably due to insufficient water availability (summer rains had not arrived yet) to flush the bladder. In September 1994 this tortoise had increased osmolality and decreased body mass which indicates dehydration.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS302 - M														
Fibrinogen (mg/dl)	NA	NA	NA	--	NA	NA	NA	NA						↑225.0
White blood cell estimate (k/μl)	NA	NA		--			↑23.0*							
Heterophils (k/μl)	NA	NA		--			↑1,679.0			↑1,536.0				
Azurophils (k/μl)	NA	NA		--										↑50.4
Eosinophils (k/μl)	NA	NA		--			↑253.0*							
Uric acid (mg/dl)	NA	NA	NA	--	NA	NA						↑12.7		
Total protein (g/dl)				--										↓1.7
Aspartate aminotransferase (IU/l)			NA	--	NA						↑239.0*			
Viramin A (μg/ml)				--		↑1.0	↑1.0							
Vitamin E (μg/ml)				--			↑57.0*							
Testosterone (ng/ml)	NA		NA	--	NA						↑554.8*			
Osmolality (mOs/kg)	NA	NA		--					↑344.0					
Body mass (kg)	3.3	3.3	3.3	--	3.3	3.3	3.5	3.8	3.6	3.7	3.75	3.8	3.7	3.6
Nasal bacteria	NA	NA		--										
Cloacal bacteria	NA	Pseu		--		Salm			Pseu	Pseu	Pseu Salm		Pseu Salm	Pseu

Evaluation: In April and June 1992 tortoise was probably foraging as vitamin A (April), vitamins A and E and body mass (June) were elevated. There was an inflammatory response (leucocytosis, heterophilia, eosinophilia) at the same time. Inflammation may be a response to an earlier (May 1992) *Salmonella* spp. infection. In April 1993 tortoise showed evidence of mild dehydration (elevated osmolality, loss of body mass) and had *Pseudomonas* spp. in its cloaca. In June 1993 tortoise may have been responding to *Pseudomonas* spp. infection with elevated heterophils. In September 1993 aspartate aminotransferase and testosterone were both elevated which may indicate this male was searching for females and/or fighting other males. In April 1994 tortoise was probably in urine stasis (elevated uric acid, gain in body mass) after feeding on protein-rich forage. In September 1994 a *Pseudomonas* spp. infection may have resulted in elevated fibrinogen, azurophils, and decreased total protein.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS303 - M														
White blood cell estimate (k/μl)	NA	NA		--			--	↑20.0		--	--	--	--	--
Heterophils (k/μl)	NA	NA		--			--	↑1,600.0		--	--	--	--	--
Lymphocytes (k/μl)	NA	NA		--			--	↑340.0		--	--	--	--	--
Selenium (ppm)				--		↑0.09	--			--	--	--	--	--
Vitamin A (μg/ml)			↑1.0	--		↑0.9	--			--	--	--	--	--
Body mass (kg)	3.55	3.7	3.65	--	3.4	3.6	--	3.5	3.55	--	--	--	--	--
Cloacal bacteria	NA			--	Pseu	Salm	--	Pseu	Pseu	--	--	--	--	--

Evaluation: Elevated vitamin A in April 1991 and April 1992 indicate foraging. Elevated selenium in April 1992 also associated with foraging. In September 1992 leucocytosis, heterophilia, and lymphocytosis may indicate an inflammatory response to a *Pseudomonas* spp. infection which persisted until this tortoise's death in June 1993 from suspected mountain lion predation.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS308 - F														
White blood cell estimate (k/μl)	NA	NA	↑18.0		--	--	--	--	--	--	--	--	--	--
Heterophils (k/μl)	NA	NA	↑1,494.0		--	--	--	--	--	--	--	--	--	--
Eosinophils (k/μl)	NA	NA	↑162.0		--	--	--	--	--	--	--	--	--	--
Phosphorus (mg/dl)	NA	NA		↑8.2	--	--	--	--	--	--	--	--	--	--
Vitamin A (μg/ml)			↑1.0		--	--	--	--	--	--	--	--	--	--
Body mass (kg)	3.15	3.2	3.2	2.8	--	--	--	--	--	--	--	--	--	--

Evaluation: In April 1991 tortoise had leucocytosis, heterophilia, and eosinophilia together with elevated vitamin A. Suspect this tortoise was foraging with an active inflammatory condition. In June 1991 there was a drop in body mass and elevated phosphorus which indicates a possible diminished renal output. Tortoise was found dead in August 1991 with signs of mountain lion predation.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS309 - F														
Packed cell volume field (%)	--												↑37.0	
Azurophils (k/μl)	--	NA										↑66.0*		↑50.4
Eosinophils (k/μl)	--	NA		↑151.0										
Uric acid (mg/dl)	--	NA	NA	NA	NA	NA						↑13.3		
Total protein (g/dl)	--												↑5.3	
Alkaline phosphatase (IU/l)	--	NA	NA	NA	NA	NA	NA	NA						↑373.0
Calcium (mg/dl)	--			↑17.5										
Phosphorus (mg/dl)	--	NA		↑11.0*			↑8.6			↑9.7				
Cholesterol (mg/dl)	--							↑294.0						↑421.0*
Triglycerides (mg/dl)	--							↑663.7			↑560.0			↑578.0
Selenium (ppm)	--						↑0.1							
Vitamin A (μg/ml)	--						↑0.1							
Vitamin E (μg/ml)	--						↑42.5							
Body mass (kg)	--	3.6	3.45	3.4	3.05	3.5	3.1	3.6	3.6	3.6	3.7	3.5	3.7	3.5
Cloacal bacteria	--			Salm					Pseu	Pseu	Pseu			

Evaluation: In June 1991 tortoise had unexplained eosinophilia. During the same period this tortoise had elevated levels of calcium and phosphorus, decreased body mass, and *Salmonella* spp. Tortoise may be showing evidence of decreased renal output which is in turn increasing parathyroid activity. Phosphorus was again elevated in June 1992 and June 1993 indicating reduced renal output. During the same period tortoise was probably foraging as selenium and vitamin A were elevated, and probably laid eggs as indicated by a drop in body mass. Elevated cholesterol and triglycerides in September 1992, and elevated triglycerides in September 1993 were probably associated with vitellogenesis. In April 1994 elevated uric acid probably due to urine stasis. In June 1994 elevated packed cell volume, and total protein, could indicate mild dehydration from restricted water prior to egg-laying. In September 1994 tortoise had azurophilia from an unknown case, elevated alkaline phosphatase possibly from bone remodeling, and elevated cholesterol and triglyceride levels probably associated with vitellogenesis. The disappearance of *Pseudomonas* spp. and azurophilia in April 1994 may indicate improved immune response.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS310 - M														
White blood cell estimate (k/μl)	--	NA	↑20.0											
Heterophils (k/μl)	--	NA	↑1,820.0*											
Lymphocytes (k/μl)	--	NA						↑417.6*						
Azurophils (k/μl)	--	NA										↑44.0		
Eosinophils (k/μl)	--	NA		↑276.0*			↑182.0	↑208.0*			↑249.0*			↑44.0
Creatinine (mg/dl)	--	NA		↑0.6								↑13.3		
Uric acid (mg/dl)	--	NA	NA	NA	NA	NA	NA	NA						
Bile acids (μmol/l)	--	NA	NA	NA	NA	NA			↑33.0*		↑155.0			
Aspartate aminotransferase (IU/l)	--	NA	NA	NA	NA			↑13.0	↑150.0		↑15.0*			
Alanine aminotransferase (IU/l)	--		NA	NA	NA	NA								
Selenium (ppm)	--	NA					↑0.2*							
Vitamin E (μg/ml)	--						↑47.9*							
Carbon dioxide (mEq/l)	--	NA	NA	NA	NA	NA	NA	NA	↓3.1*					
Body mass (kg)	--	2.7	2.88	2.8	2.65	2.5	2.6	2.65	2.4	2.7	2.65	2.8	2.7	2.7
Cloacal bacteria	--	--		Salm				Pseu			Pseu	Salm		

Evaluation: In April 1991 tortoise had leucocytosis and heterophilia without apparent cause of infection. Tortoise had unexplained eosinophilia in June 1991, June 1992, and September 1992. Also in June 1992 there was elevated selenium, vitamin E, and increased body mass indicating the tortoise was probably foraging. In September 1992 lymphocytosis and elevated alanine aminotransferase may indicate an inflammatory response due to liver disease. *Pseudomonas* spp. was probably an opportunistic pathogen at the time. In April 1993 tortoise had elevated bile acids, aspartate aminotransferase, decreased total carbon dioxide, and decreased body mass indicating a progressive liver disease with acidosis or anorexia. In June 1993 tortoise appeared normal probably after foraging and drinking. In September 1993 tortoise had elevated aspartate aminotransferase, alanine aminotransferase, and *Pseudomonas* spp. indicating recurrent hepatic disease with opportunistic bacteria and an unexplained eosinophilia. In April 1994 azurophilia, elevated uric acid, and increased body mass indicate inflammation with urine stasis and increased protein intake. Azurophilia in April 1994 and September 1994 may be an inflammatory response to *Salmonella* spp. found in April 1994.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS499 - F														
Fibrinogen (mg/dl)	--	NA	NA	NA	NA	NA	NA	NA	--	--	--	--	--	--
Lymphocytes (k/μl)	--	NA						↑297.0			--	--	--	--
Total protein (g/dl)	--	↑5.3									--	--	--	--
Phosphorus (mg/dl)	--	NA					↑10.3				--	--	--	--
Cholesterol (mg/dl)	--							↑292.0	↑365.0*		--	--	--	--
Triglycerides (mg/dl)	--	↑582.0			↑612.0	↑714.0		↑795.0*	↑871.0*		--	--	--	--
Vitamin E (μg/ml)	--						↑47.9*				--	--	--	--
Body mass (kg)	--	3.6	3.98	3.6	3.6	3.7	3.7	3.8	3.8	3.6	--	--	--	--
Cloacal bacteria	--							Pseu	Pseu	Pseu	--	--	--	--

Evaluation: Elevated total protein and triglycerideín November 1990 probably associated with vitellogenesis. Elevated triglycerides in September 1991, April 1992, September 1992, and April 1993 probably indicate vitellogenesis as does elevated cholesterol in September 1992 and April 1993. In June 1992 elevated phosphorus indicates slow renal clearance, and elevated vitamin E indicates foraging. In September 1992 lymphocytosis may be a response to *Pseudomonas* spp. Tortoise probably laid eggs before June 1991 and June 1993 as there were a drops in body mass between the April and June sampling. Tortoise was found dead in August 1993 on its back at the base of hillside with no signs of predation.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS500 - M														
Blood urea nitrogen (mg/dl)	--	--	--	↑12.0		--	--		--	--		--	--	
Testosterone (ng/ml)	--	--	--	NA	NA	--	--		--	--	↑555.2*	--	--	
Body mass (kg)	--	--	--	4.0	4.5	--	--	4.2	--	--	4.3	--	--	4.6
Cloacal bacteria	--	--	--	Pseu		--	--	Pseu	--	--	Pseu	--	--	

Evaluation: Elevated blood urea nitrogen may be associated with catabolic changes and/or dehydration occurring before the late summer feeding.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS501 - F														
Monocytes (k/μl)	--	--	--	--						--	↑69.6	--		
Phosphorus (mg/dl)	--	--	--	--						--		--	↑10.2	
Cholesterol (mg/dl)	--	--	--	--					↑303.0	--		--		
Total bilirubin (mg/dl)	--	--	--	--	NA	NA	NA	NA		--		--		↑2.9*
Indirect bilirubin (mg/dl)	--	--	--	--	NA	NA	NA	NA		--		--		↑2.9*
Vitamin E (μg/ml)	--	--	--	--			↑43.0			--		--		
Carbon dioxide (mEq/l)	--	--	--	--	NA	NA	NA	NA	↓4.3*	--		--		
Body mass (kg)	--	--	--	--	2.5	2.5	2.5	2.8	2.55	--	2.6	--	2.8	2.6
Cloacal bacteria	--	--	--	--		Pseu Salm			Pseu	--	Pseu	--		

Evaluation: Tortoise was found with a large neck abscess (3 cm diameter) in September 1991 which was removed in September 1992. Elevated vitamin E in June 1992 probably associated with foraging. Catabolic tortoise (elevated cholesterol) with acidosis (elevated carbon dioxide) and low body mass in April 1993. Monocytosis in September 1993 possibly associated with *Pseudomonas* spp. Elevated phosphorus in June 1994 might be related to reduced renal excretion as there was increased body mass. In September 1994 elevated total bilirubin and indirect bilirubin and decreased body mass may suggest liver disease, hemolysis, or anorexia.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS502 - F														
White blood cell estimate (k/μl)	--	--	--	--	--	--		↑18.5					--	
Lymphocytes (k/μl)	--	--	--	--	--	--		↑314.0					--	
Aspartate aminotransferase (IU/l)	--	--	--	--	--	--				↑195.0*			--	
Phosphorus (mg/dl)	--	--	--	--	--	--				↑9.4			--	
Direct bilirubin (mg/dl)	--	--	--	--	--	--	NA	NA			↑1.0*		--	
Vitamin E (μg/ml)	--	--	--	--	--	--	↑44.9						--	
Chloride (mEq/l)	--	--	--	--	--	--	NA	NA		↑124.0			--	
Body mass (kg)	--	--	--	--	--	--	2.45	2.6	2.7	2.3	2.9	2.6	--	2.6
Cloacal bacteria	--	--	--	--	--	--		Salm					--	

Appendix 3. (continued).

Evaluation: Elevated vitamin E in June 1992 indicates the tortoise was foraging. Leucocytosis and lymphocytosis in September 1992 may be a response to *Salmonella* spp. In June 1993 elevated phosphorus, chloride, aspartate aminotransferase, and decreased body mass probably indicate decreased kidney function, dehydration, and a muscular catabolic process. In September 1993 there was an elevated direct bilirubin indicating reduced bile flow possibly from inadequate foraging. Increased body mass at this time may be the result of urine stasis or geophagy.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS503 - M														
Monocytes (k/μl)	--	--	--	--	--	--	--	--	--			↑64.0	--	--
Azurophils (k/μl)	--	--	--	--	--	--	--	--	--			↑56.0	--	--
Uric acid (mg/dl)	--	--	--	--	--	--	--	--	--			↑12.5	--	--
Aspartate aminotransferase (IU/l)	--	--	--	--	--	--	--	--	--	↑176.0	↑150.0		--	--
Alanine aminotransferase (IU/l)	--	--	--	--	--	--	--	--	--		↑19.0*		--	--
Body mass (kg)	--	--	--	--	--	--	--	--	--	2.6	2.6	2.8	--	--
Cloacal bacteria	--	--	--	--	--	--	--	--	--	Pseu	Salm		--	--

Evaluation: Elevated aspartate aminotransferase in June 1993, and elevated aspartate aminotransferase and alanine aminotransferase in September 1993 indicate possible liver disease. *Pseudomonas* spp. and *Salmonella* spp. were cultured during the same period. In April 1994 liver enzymes were back to normal but tortoise had monocytosis, azurophilia, elevated uric acid, and an increase in body mass indicating an inflammatory response and urine stasis and increased protein intake.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
LS509 - M														
Azurophils (k/μl)	--	--	--	--	--	--	--	--	--	--	--	--	--	↑79.8*
Body mass (kg)	--	--	--	--	--	--	--	--	--	--	--	--	--	3.6

Evaluation: Unexplained azurophila in September 1994.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H202 - M														
Monocytes (k/μl)	NA	NA				↑84.0*		--	--	--	--	--	--	--
Azurophils (k/μl)	NA	NA				↑56.0		--	--	--	--	--	--	--
Glucose (mg/dl)	NA	NA	NA	NA	NA	NA	↑246.0	--	--	--	--	--	--	--
Creatinine (mg/dl)	NA	NA		↑0.6				--	--	--	--	--	--	--
Vitamin E (μg/ml)							↑48.1*	--	--	--	--	--	--	--
Body mass (kg)	2.65	2.6	2.9	2.1	2.4	2.6	2.5	--	--	--	--	--	--	--

Evaluation: Tortoise had monocytosis and azurophila in April 1992 but the cause of the inflammation is unknown. In June 1992 tortoise had elevated glucose, which may have been a result of stress. Elevated vitamin E may indicate fat eatabolism or foraging. Tortoise was found dead in April 1993, cause unknown.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H203 - M														
Monocytes (k/μl)	NA	NA	↑144.0*											
Azurophils (k/μl)	NA	NA				↑68.0*						↑50.0		
Eosinophils (k/μl)	NA	NA						↑142.5						
Uric acid (mg/dl)												↑13.7		
Bile acids (μmol/l)	NA	NA	NA	NA	NA	NA	NA	NA			↑28.5*			
Alanine aminotransferase (IU/l)	NA	NA	NA	NA	NA	NA	↑17.0*							
Corticosterone (ng/ml)	NA	NA	NA	NA	NA			↑21.8					2.5	2.1
Body mass (kg)	2.3	2.3	2.5	2.4	2.1	2.3	2.2	2.6	2.5	2.3	2.3	2.6	Pseu	
Cloacal bacteria	NA	Pseu					Pseu		Pseu	Pseu		Pseu		

Evaluation: Monocytosis in April 1991 may have been associated with *Pseudomonas* spp. found in November 1990. Azurophilia in April 1992 from cause unknown. In June 1992 elevated alanine aminotransferase, reduced body mass, and *Pseudomonas* spp., may indicate liver disease or anorexia with an opportunistic infection. In September 1992 elevated corticosterone probably stress induced and eosinophilia from an unknown source. This tortoise had a suspect titer for *M. agassizii* with an ELISA in June 1993 but showed no signs of inflammatory response. In September 1993 polychromasia was evident, this and elevated bile acids may be a result of liver disease. In April 1994 elevated uric acid and body mass may indicate urine stasis and increased protein intake or changes in kidney function from previous liver disease. Azurophilia in the same period may have resulted from *Pseudomonas* spp.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H204 - M														
Packed cell volume field (%)				--		↓14.0			--	--	--	--	--	--
Monocytes (k/μl)	NA	NA		--		↑104.0*			--	--	--	--	--	--
Blood urea nitrogen (mg/dl)				--		↑12.0			--	--	--	--	--	--
Vitamin E (μg/ml)				--			↑53.6*		--	--	--	--	--	--
Corticosterone (ng/ml)	NA	NA	NA	--	NA		↑27.2*	↑28.0*	--	--	--	--	--	--
Body mass (kg)	2.3	2.6	2.8	--	2.5	2.4	2.45	2.7	--	--	--	--	--	--
Cloacal bacteria	NA			--				Pseu	--	--	--	--	--	--

Evaluation: In April 1992 decreased packed cell volume, monocytosis, decreased body mass, and elevated blood urea nitrogen indicating anemia, inflammation, possible dehydration, or catabolism. In June 1992 tortoise appears recovered as vitamin E levels are high and there is an increase in body mass (foraging), packed cell volume back to normal. Tortoise stressed in June 1992 and September 1992 probably due to handling. In September 1992 tortoise gained body mass and then was not found until 1995.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H205 - M														
Packed cell volume field (%)						↓13.0	--	--	--	--	--	--	--	--
White blood cell estimate (k/μl)	NA	NA	↑18.0				--	--	--	--	--	--	--	--
Lymphocytes (k/μl)	NA	NA	↑396.0*				--	--	--	--	--	--	--	--
Body mass (kg)	1.55	1.4	1.6	1.4	1.6	1.5	--	--	--	--	--	--	--	--
Cloacal bacteria	NA	Pseu				Salm	--	--	--	--	--	--	--	--

Evaluation: Leucocytosis and lymphocytosis in April 1991 indicate inflammatory reaction perhaps to *Pseudomonas* spp. In April 1992 tortoise had decreased packed cell volume and body mass and *Salmonella* spp. indicating anemia and an opportunistic infection.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H207 - M														
Blood urea nitrogen (mg/dl)	--	↑10.0	--	--	--	--	--	--	--	--	--	--	--	--
Body mass (kg)	--	2.9	--	--	--	--	--	--	--	--	--	--	--	--
Nasal bacteria	--	Pseu	--	--	--	--	--	--	--	--	--	--	--	--

Evaluation: Undetermined significance of elevated blood urea nitrogen in November 1990. Tortoise probably preyed on in April 1991. This tortoise's chewed transmitter was found in April 1991 (suspect time of death) but the carcass was not recovered until September 1994.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H208 - F														
White blood cell estimate (k/μl)	--	NA								↑22.0	↑23.4*			
Heterophils (k/μl)	--	NA								↑1,936.0*	↑1,848.6*			
Lymphocytes (k/μl)	--	NA						↑540.0*						
Monocytes (k/μl)	--	NA							↑58.8					
Eosinophils (k/μl)	--	NA									↑140.4			
Blood urea nitrogen (mg/dl)	--					↑10.0								
Uric acid (mg/dl)	--	NA	NA	NA	NA	NA						↑16.3*		
Calcium (mg/dl)	--	NA										↑27.0*	↑28.6*	
Phosphorus (mg/dl)	--	NA										↑8.6	↑14.6*	
Direct bilirubin (mg/dl)	--	NA	NA	NA		NA	NA	NA	↑0.6*					
Vitamin A (μg/ml)	--				NA	↑0.9								
Vitamin E (μg/ml)	--						↑35.6							
Body mass (kg)	--	2.1	2.35	2.15	1.95	2.3	2.4	2.8	2.79	2.8	2.7	3.2	2.9	2.6
Nasal bacteria	--		Pseu											
Cloacal bacteria	--						Pseu	Pseu		Pseu				

Evaluation: In April 1992 tortoise was foraging as indicated by elevated blood urea nitrogen, vitamin A, and body mass. Blood urea nitrogen may indicate high protein forage. Elevated vitamin E levels in June 1992 also indicated foraging. From September 1992 to September 1993 elevated white blood cell parameters indicate an inflammatory response. During this time *Pseudomonas* spp. was cultured from the cloaca. In April 1993 elevated direct bilirubin may indicate bile stasis from anorexia or a disease process. In April 1994 elevated uric acid, calcium, phosphorus, and increased body mass may indicate urine stasis during vitellogenesis. In June 1994 further elevations of calcium and phosphorus probably due to egg shell formation, possibly with water deprivation.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H209 - F														
Total protein (g/dl)	--	↑8.3*	--	--	--	--	--	--	--	--	--	--	--	--
Albumin (g/dl)	--	↑5.7*	--	--	--	--	--	--	--	--	--	--	--	--
Calcium (mg/dl)	--	↑18.1	--	--	--	--	--	--	--	--	--	--	--	--
Cholesterol (mg/dl)	--	↑313.0	--	--	--	--	--	--	--	--	--	--	--	--
Triglycerides (mg/dl)	--	↑800.0*	--	--	--	--	--	--	--	--	--	--	--	--
Body mass (kg)	--	3.1	--	--	--	--	--	--	--	--	--	--	--	--
Nasal bacteria	--	Pseu	--	--	--	--	--	--	--	--	--	--	--	--

Evaluation: Tortoise probably in vitellogenesis with signs of dehydration (elevated total protein and albumin). Female probably producing eggs at her own expense. Female was dug out of a sheltersite it had not moved from for approximately 9 months in June 1991 with signs of chronic dehydration. Tortoise died 6 days later at a veterinary hospital.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H210 - M														
White blood cell estimate (k/μl)	--	NA	↑18.0			--	--	--	--	--	--	--	--	--
Basophils (k/μl)	--	NA	↑360.0			--	--	--	--	--	--	--	--	--
Blood urea nitrogen (mg/dl)	--				↑10.0	--	--	--	--	--	--	--	--	--
Creatinine (mg/dl)	--	NA		↑0.6		--	--	--	--	--	--	--	--	--
Potassium (mEq/l)	--			↑6.4		--	--	--	--	--	--	--	--	--
Body mass (kg)	--	3.5	3.6	3.3	3.3	3.9	--	--	--	--	--	--	--	--
Nasal bacteria	--		Pseu				--	--	--	--	--	--	--	--

Evaluation: In April 1991 tortoise had leucocytosis and basophilia possibly from *Pseudomonas* spp. cultured from the choana. In June 1991 elevated potassium and creatinine probably a result of urine stasis and dehydration. In April 1992 increased body mass and polychromasia probably from the tortoise foraging. In September 1991 cause of elevated blood urea nitrogen undetermined. Tortoise was found dead in June 1992 from possible predation.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H211 - F														
Packed cell volume field (%)	--					↓14.0							--	
Lymphocytes (k/μl)	--	NA						↑377.0*			↑436.8*		--	
Basophils (k/μl)	--	NA						↑406.0*			↑386.4*		--	
Glucose (mg/dl)	--	NA	NA	NA	NA	NA	↑258.0			↑240.0			--	
Uric acid (mg/dl)	--	NA	NA	NA	NA	NA						↑17.9*	--	
Alanine aminotransferase (IU/l)	--	NA	NA	NA	NA	NA					↑13.0		--	
Phosphorus (mg/dl)	--	NA								↑8.3			--	
Selenium (ppm)	--					↑0.07							--	
Vitamin E (mg/ml)	--						↑57.6*						--	
Corticosterone (ng/ml)	--	NA	NA	NA	NA			↑27.8*					--	
Body mass (kg)	--	2.3	2.2	2.0	1.8	2.2	2.0	2.4	2.3	2.1	2.15	2..4	--	1.9
Cloacal bacteria	--										Pseu		--	

Evaluation: Decreased packed cell volume and increased selenium in April 1992 probably indicates tortoise was drinking and foraging, respectively. In June 1992 elevated glucose likely stress induced. Elevated vitamin E during the same time probably from foraging. In September 1992 an inflammatory resopnse is evident with lymphocytosis and basophilia, no apparent cause. During the same time elevated corticosterone probably from stress. In June 1993 elevated glucose probably from stress, and elevated phosphorus and decreased body mass probably from decreased renal output. In September 1993 tortoise exhibits inflammation (lymphocytosis, basophilia), and liver disease (elevated alanine aminotransferase). *Pseudomonas* spp. may have been reason for inflammatory response and liver inflammation. In April 1994 elevated uric acid and body mass indicate a diet of protein-rich forage and urine stasis. Polychromasia during the same time indicates red blood cell regeneration possibly following liver disease. In September 1994 tortoise appears to have recovered as parameters were within normal limits.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H215 - M														
Creatinine (mg/dl)	--	NA	--	↑0.7*		--	--	--	--	--	--	--	--	--
Body mass (kg)	--	3.3	--	3.6	3.1	--	--	--	--	--	--	--	--	--

Evaluation: Undetermined significance of elevated creatinine in June 1991.

Appendix 3. (continued).

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H218 - M														
White blood cell estimate (k/μl)	--	--	--		↑20.0					--				
Heterophils (k/μl)	--	--	--		↑1,760.0					--				
Uric acid (mg/dl)	--	--	--							--		↑14.7		
Total protein (g/dl)	--	--	--				↓1.9			--				
Aspartate aminotransferase (IU/l)	--	--	--	NA	NA					--	↑242.0*			
Vitamin E (μg/ml)	--	--	--				↑62.0*			--				
Osmolality (mOs/kg)	--	--	--							--				↑369.0
Body mass (kg)	--	--	--	3.6	3.0	3.6	3.95	4.2	4.21	--	3.7	3.8	4.0	3.5
Cloacal bacteria	--	--	--			Salm		Pseu		--	Pseu	Pseu		Pseu Salm

Evaluation: In September 1991 leucocytosis and heterophilia indicate an active inflammatory response but there is no apparent cause. Body mass was decreased at this time as well. In June 1992 there was decreased total protein, increased vitamin E, and increased body mass indicate increased water consumption and foraging. In September 1993 elevated aspartate aminotransferase and decreased body mass may indicate liver disease or sustained activity (searching for females, aggressive behavior with other males). *Pseudomonas* spp. was cultured from the cloaca at the same time but the significance is unknown. In April 1994 elevated uric acid and body mass indicate increased protein protein intake and urine stasis. In September 1994 increased osmolality and decreased body mass indicate dehydration. This was also a period of below average rainfall. Also in September 1994 this tortoise had *Pseudomonas* spp. and *Salmonella* spp. probably resulting from a suppressed immune response.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H219 - F														
Calcium (mg/dl)	--	--	--	--			--					--	↑18.6	
Cholesterol (mg/dl)	--	--	--	--			--			↑278.0		--		
Triglycerides (mg/dl)	--	--	--	--			--		↑704.0		↑563.0	--		
Estradiol (pg/ml)	--	--	--	--			--		↑555.4*			--		
Corticosterone (ng/ml)	--	--	--	--	NA		--	↑21.6				--		
Body mass (kg)	--	--	--	--	2.95	2.9	--	3.25	3.0	2.5	2.75	--	2.9	2.5
Cloacal bacteria	--	--	--	--			--	Pseu	Pseu			--		

Appendix 3. (continued).

Evaluation: In September 1992 elevated corticosterone was apparently stress induced. In April 1993 tortoise was in vitellogenesis represented by increased triglycerides and estradiol. Tortoise probably laid eggs prior to June 1993 as body mass decreased. Elevated cholesterol in June 1993 probably associated with catabolic effects of diminished foraging after egg-laying. In September 1993 elevated triglycerides probably signify vitellogenesis. Undetermined cause of increased calcium in June 1994.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H220 - M														
Corticosterone (ng/ml)	--	--	--	--	NA		--	↑27.7*	--	--	--	--	--	--
Body mass (kg)	--	--	--	--	2.4	3.0	--	3.1	--	--	--	--	--	--
Cloacal bacteria	--	--	--	--		Salm	--	Pseu	--	--	--	--	--	--

Evaluation: Elevated corticosterone probably from handling stress.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H221 - F														
Basophils (k/μl)	--	--	--	--		↑364.8*		↑330.0		--				
Blood urea nitrogen (mg/dl)	--	--	--	--	↑26.0*					--				
Uric acid (mg/dl)	--	--	--	--	NA	NA				--		↑14.2		
Albumin (g/dl)	--	--	--	--	↑3.7					--				
Cholesterol (mg/dl)	--	--	--	--					↑285.0	--				↑359.0*
Vitamin E (mg/dl)	--	--	--	--			↑64.7*			--				
Estradiol (pg/ml)	--	--	--	--					↑550.4*	--	↑478.0			
Potassium (mEq/l)	--	--	--	--					↑6.3	--				
Body mass (kg)	--	--	--	--	2.35	3.0	3.0	3.65	3.6	--	3.1	3.4	3.5	3.1
Cloacal bacteria	--	--	--	--	Salm				Pseu	--	Pseu			Pseu

Evaluation: In September 1991 elevated blood urea nitrogen, albumin, low body mass, and *Salmonella* spp. signifies dehydration and opportunistic bacterial colonization. Basophilia in April 1992 may indicate an improved immune response to *Salmonella* spp. Tortoise also gained body mass during this period possibly from rehydration. In June 1992 elevated vitamin E indicates foraging. Undetermined cause of basophilia in September 1992. Elevated cholesterol and estradiol in April 1993 indicates vitellogenesis. Elevated potassium during the same time possibly the result of eating potassium-rich forage. Elevated estradiol in September 1993 probably associated with reproduction (maturing follicles). In April 1994 elevated uric acid

Appendix 3. (continued).

and body mass indicates protein consumption and urine stasis. In September 1994 elevated cholesterol and decreased body mass may be a result of catabolism perhaps with vitellogenesis occurring at the same time.

Tort # - Sex	9/90	11/90	4/91	6/91	9/91	4/92	6/92	9/92	4/93	6/93	9/93	4/94	6/94	9/94
H223 - M														
Azurophils (k/μl)	--	--	--	--	--	--	--	--	--			--		↑114.0*
Glucose (mg/dl)	--	--	--	--	--	--	--	--	--	↑241.0		--		
Bile acids (μmol/l)	--	--	--	--	--	--	--	--	--		↑31.7*	--		
Testosterone (ng/ml)	--	--	--	--	--	--	--	--	--		↑376.0	--		
Osmolality (mOs/kg)	--	--	--	--	--	--	--	--	--			--		↑359.0
Body mass (kg)	--	--	--	--	--	--	--	--	--	2.1	2.3	--	2.4	2.1
Cloacal bacteria	--	--	--	--	--	--	--	--	--	Pseu	Pseu	--	Pseu	

Evaluation: Elevated glucose in June 1993 likely stress-induced. During the same period this tortoise had decreased body mass and *Pseudomonas* spp. In September 1993 elevated bile acids may indicate anorexia or liver dysfunction. Elevated testosterone in September 1993, cause unknown. Azurophila in September 1994 indicates inflammatory response while elevated osmolality and decreased body mass indicate dehydration. Azurophila may be associated with a more effective immune response as this was the only sampling period *Pseudomonas* spp. was not cultured.

Appendix 4. Hematologic values [x̄ ± SD (*n*)] for Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-94. Data from these 2 sites are combined.

Parameter	April	June	September
Packed cell volume field (%)			
Male	22.4 ± 4.5 (29)	24.3 ± 3.2 (26)	25.7 ± 4.8 (47)
Female	23.1 ± 4.5 (28)	24.9 ± 3.9 (27)	25.0 ± 3.4 (35)
Hemoglobin (g/dl)			
Male	9.3 ± 1.8 (28)	9.8 ± 1.6 (25)	10.9 ± 1.6 (46)
Female	9.8 ± 1.7 (28)	10.4 ± 1.2 (26)	10.8 ± 1.3 (35)
Fibrinogen (mg/dl)			
Male	137.0 ± 14.9 (10)	110.5 ± 43.6 (11)	149.7 ± 30.3 (15)
Female	128.7 ± 39.1 (15)	102.3 ± 45.1 (13)	146.2 ± 24.2 (16)
White blood cell estimate (k/μl)			
Male	8.3 ± 4.3 (27)	10.7 ± 4.6 (21)	8.8 ± 4.4 (36)
Female	7.1 ± 3.6 (27)	9.2 ± 4.8 (20)	9.0 ± 5.1 (32)
Heterophils (k/μl)			
Male	572.6 ± 387.9 (27)	841.7 ± 404.7 (21)	590.7 ± 374.8 (36)
Female	442.6 ± 343.0 (27)	712.6 ± 457.9 (20)	550.0 ± 411.0 (32)
Lymphocytes (k/μl)			
Male	115.8 ± 76.4 (27)	58.7 ± 42.6 (21)	137.6 ± 89.2 (36)
Female	80.9 ± 50.7 (27)	67.2 ± 50.4 (20)	162.1 ± 123.6 (32)
Monocytes (k/μl)			
Male	22.5 ± 36.4 (27)	19.8 ± 19.5 (21)	9.8 ± 13.5 (32)
Female	15.0 ± 16.0 (27)	11.6 ± 14.9 (20)	13.5 ± 19.1 (31)
Azurophils (k/μl)			
Male	14.5 ± 21.6 (27)	4.7 ± 7.5 (21)	10.5 ± 25.0 (36)
Female	9.8 ± 14.4 (27)	2.3 ± 7.3 (20)	6.1 ± 12.8 (32)
Eosinophils (k/μl)			
Male	21.1 ± 26.0 (27)	58.1 ± 84.6 (21)	42.7 ± 59.7 (32)
Female	22.9 ± 34.6 (27)	14.2 ± 33.7 (20)	43.9 ± 52.2 (28)
Basophils (k/μl)			
Male	82.3 ± 63.0 (27)	90.8 ± 61.8 (21)	91.7 ± 53.0 (36)
Female	138.5 ± 100.8 (27)	115.0 ± 75.2 (20)	129.9 ± 104.7 (32)

Appendix 5. Plasma biochemical values [x̄ ± SD (*n*)] for Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-94. Slash marks (--) mean the value was not sampled that period. Data from these 2 sites are combined.

Parameter	April	June	September
Glucose (mg/dl)			
Male	114.9 ± 26.9 (10)	154.1 ± 46.3 (17)	134.1 ± 31.3 (24)
Female	116.6 ± 31.4 (15)	148.7 ± 54.0 (22)	127.9 ± 30.2 (25)
Blood urea nitrogen (mg/dl)			
Male	3.2 ± 3.7 (29)	1.7 ± 2.8 (24)	1.6 ± 2.7 (55)
Female	1.7 ± 2.7 (28)	1.2 ± 2.1 (24)	1.9 ± 6.5 (42)
Creatinine (mg/dl)			
Male	0.3 ± 0.1 (23)	0.3 ± 0.2 (26)	0.2 ± 0.1 (36)
Female	0.2 ± 0.1 927)	0.3 ± 0.1 (27)	0.2 ± 0.1 (32)
Uric acid (mg/dl)			
Male	8.6 ± 5.2 (10)	4.7 ± 2.0 (17)	5.1 ± 2.2 (24)
Female	8.9 ± 5.1 (15)	5.7 ± 1.5 (22)	5.0 ± 1.6 (25)
Total protein (g/dl)			
Male	3.3 ± 0.5 (29)	3.5 ± 0.6 (26)	3.4 ± 0.5 (55)
Female	3.7 ± 0.7 (28)	4.1 ± 0.8 (27)	4.0 ± 0.9 (42)
Albumin (g/dl)			
Male	1.6 ± 0.3 (29)	1.6 ± 0.3 (26)	2.0 ± 1.5 (55)
Female	1.6 ± 0.5 (28)	1.8 ± 0.5 (27)	2.0 ± 1.0 (41)
Total globulins (g/dl)			
Male	1.7 ± 0.4 (29)	1.8 ± 0.5 (26)	1.3 ± 1.5 (55)
Female	2.0 ± 0.6 (28)	2.3 ± 0.7 (27)	2.0 ± 0.7 (41)
Bile acids (μmol/l)			
Male	6.9 ± 9.6 (10)	0.47 ± 0.89 (11)	10.5 ± 13.9 (7)
Female	5.6 ± 4.0 (15)	0 (14)	1.4 ± 3.1 (9)
Aspartate aminotransferase (IU/l)			
Male	96.7 ± 33.0 (10)	68.3 ± 32.3 (17)	79.8 ± 49.3 (43)
Female	75.4 ± 16.4 (15)	71.3 ± 36.3 (22)	61.1 ± 24.9 (35)
Alanine aminotransferase (IU/l)			
Male	3.0 ± 1.8 (10)	2.8 ± 4.1 (17)	5.2 ± 4.7 (23)
Female	3.5 ± 1.6 (15)	1.5 ± 1.9 (22)	3.9 ± 3.6 (24)
Alkaline phosphatase (IU/l)			
Male	65.2 ± 4.1 (10)	80.7 ± 27.9 (11)	86.9 ± 45.0 (15)
Female	141.3 ± 254.3 (15)	125.4 ± 71.7 (14)	117.9 ± 80.1 (17)
Calcium (mg/dl)			
Male	10.7 ± 1.2 (29)	10.4 ± 0.8 (26)	10.1 ± 1.4 (55)
Female	13.8 ± 2.9 (28)	13.2 ± 3.9 (26)	13.1 ± 2.2 (42)
Phosphorus (mEq/l)			
Male	1.3 ± 0.8 (29)	1.7 ± 0.5 (26)	1.8 ± 0.7 (36)
Female	3.4 ± 1.6 (28)	6.8 ± 3.1 (27)	4.4 ± 1.2 (32)
Cholesterol (mg/dl)			
Male	89.1 ± 25.9 (29)	81.6 ± 24.8 (26)	75.8 ± 30.9 (55)
Female	165.4 ± 70.9 (28)	156.5 ± 41.8 (27)	209.9 ± 209.9 (42)

Appendix 5. (continued).

Parameter	April	June	September
Triglycerides (mg/dl)			
Male	31.4 ± 42.6 (29)	8.9 ± 5.2 (26)	35.7 ± 111.4 (55)
Female	299.3 ± 206.1 (28)	37.3 ± 29.0 (27)	389.0 ± 182.0 (42)
Total bilirubin (mg/dl)			
Male	0.2 ± 0.3 (10)	0.1 ± 0.04 (11)	0.2 ± 0.3 (15)
Female	0.7 ± 0.4 (15)	0.4 ± 0.5 (14)	0.6 ± 0.7 (17)
Direct bilirubin (mg/dl)			
Male	0.02 ± 0.01 (10)	0.03 ± 0.01 (10)	0.02 ± 0.01 (14)
Female	0.06 ± 0.1 (15)	0.03 ± 0.01 (14)	0.10 ± 0.3 (12)
Indirect bilirubin (mg/dl)			
Male	0.2 ± 0.3 (10)	0.1 ± 0.04 (11)	0.2 ± 0.3 (15)
Female	0.6 ± 0.4 (15)	0.4 ± 0.5 (14)	0.6 ± 0.7 (17)
Copper (ppm)			
Male	0.6 ± 0.1 (10)	0.6 ± 0.1 (15)	0.6 ± 0.1 (30)
Female	0.4 ± 0.1 (9)	0.5 ± 0.2 (18)	0.5 ± 0.2 (18)
Selenium (ppm)			
Male	0.04 ± 0.02 (20)	0.05 ± 0.04 (13)	0.03 ± 0.01 (36)
Female	0.04 ± 0.02 (14)	0.05 ± 0.02 (9)	0.03 ± 0.01 (28)
Zinc (ppm)			
Male	--	0.05 ± 0.02 (4)	2.9 ± 0.7 (7)
Female	--	1.4 ± 1.5 (6)	3.1 ± 1.1 (5)
Vitamin A (μg/ml)			
Male	0.4 ± 0.3 (29)	0.4 ± 0.2 (25)	0.4 ± 0.1 (41)
Female	0.5 ± 0.2 (28)	0.5 ± 0.3 (27)	0.4 ± 0.2 (34)
Vitamin E (μg/ml)			
Male	4.6 ± 2.7 (29)	13.8 ± 20.9 (25)	4.3 ± 3.8 (41)
Female	7.3 ± 2.9 (28)	17.8 ± 20.7 (27)	9.1 ± 4.8 (34)
Testosterone (ng/ml)			
Male	60.5 ± 50.9 (5)	81.4 ± 47.2 (11)	257.1 ± 174.4 (13)
Female	8.2 ± 5.1 (6)	0.46 ± 0.41 (14)	1.9 ± 1.5 (16)
Estradiol (pg/ml)			
Male	--	--	98.1 (1)
Female	181.6 ± 177.9 (16)	3.3 ± 25.9 (16)	198.3 ± 114.6 (17)
Corticosterone (ng/ml)			
Male	4.0 ± 3.1 (14)	8.7 ± 6.9 (16)	12.1 ± 9.1 (14)
Female	3.9 ± 2.5 (14)	5.8 ± 4.0 (22)	7.9 ± 7.7 (14)

Appendix 6. Plasma electrolytes and osmolality [$\bar{x} \pm$ SD (n)] in Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-94. Data from these 2 sites are combined.

Parameter	April	June	September
Sodium (mEq/l)			
Male	126.8 ± 7.5 (29)	136.6 ± 10.2 (26)	129.0 ± 6.9 (54)
Female	129.7 ± 9.9 (28)	136.5 ± 9.7 (26)	128.8 ± 6.3 (41)
Potassium (mEq/l)			
Male	3.8 ± 0.47 (29)	4.6 ± 0.71 (26)	4.0 ± 0.60 (48)
Female	4.3 ± 0.67 (28)	4.4 ± 0.58 (26)	4.2 ± 0.59 (38)
Chloride (mEq/l)			
Male	96.0 ± 4.1 (10)	107.5 ± 5.9 (11)	107.7 ± 6.9 (15)
Female	93.8 ± 3.1 (15)	110.4 ± 6.0 (14)	103.4 ± 6.6 (17)
Total carbon dioxide (mEq/l)			
Male	26.3 ± 11.2 (10)	37.3 ± 5.4 (11)	34.8 ± 5.3 (15)
Female	30.7 ± 10.9 (14)	30.1 ± 6.5 (14)	35.7 ± 5.0 (17)
Anion gap (mEq/l)			
Male	13.7 ± 13.8 (10)	-3.2 ± 7.9 (11)	-9.5 ± 7.9 (15)
Female	13.7 ± 7.8 (14)	3.7 ± 5.7 (13)	-5.3 ± 11.7 (17)
Osmolality (mOs/kg)			
Male	275.3 ± 21.9 (23)	265.6 ± 21.5 (26)	272.8 ± 34.9 (36)
Female	281.5 ± 21.4 (27)	273.7 ± 25.2 (27)	277.8 ± 35.3 (32)

Appendix 7. Microbial isolates from the choana of Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-91. n = number of choana samples. Data from these 2 sites are combined.

Little Shipp Wash

Organism	Number positive	n
Corynebacterium spp.	1	6
Pasteurella spp.	3	6
Pseudomonas spp.	1	6
Streptococcus spp.	1	6

Harcuvar Mountains

Organism	Number positive	n
Corynebacterium spp.	1	19
Pasteurella spp.	9	20
Pseudomonas spp.	4	20
Staphylococcus spp.	2	20

Appendix 8. Microbial isolates from the cloacal cavity of Sonoran desert tortoises from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1990-94. n = number of cloacal samples. Data from these 2 sites are combined.

Little Shipp Wash

Organism	Number positive	n
Bacillus spp.	8	96
Campylobacter spp.	4	96
Citrobacter spp.	5	96
Coliforms	8	96
Diptheroids	13	96
Enterobacter-Klebsiella	35	96
Escherichia coli	21	96
Lactobacillus spp.	8	96
Pasteurella spp.	2	96
Pseudomonas spp.	30	96
Salmonella spp.	11	96
Shigella spp.	27	96
Staphylococcus spp.	74	96
Streptococcus spp.	5	96

Harcuvar Mountains

Organism	Number positive	n
Bacillus spp.	5	105
Campylobacter spp.	5	105
Citrobacter spp.	5	105
Coliforms	4	105
Corynebacterium spp.	13	105
Diptheroids	26	105
Enterobacter-Klebsiella	31	105
Escherichia coli	10	105
Lactobacillus spp.	10	105
Pasteurella spp.	5	105
Proteus spp.	3	105
Pseudomonas spp.	28	105
Salmonella spp.	7	105
Shigella spp.	15	105
Staphylococcus spp.	77	105
Streptococcus spp.	12	105

Appendix 9. Monthly rainfall (cm) and mean ambient temperature (maximum, minimum; C) data from permanent weather stations near 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.), 1989-94. Data from these 2 sites are combined.

Little Shipp Wash, Arizona
Location: Yavapai Co., Arizona
Elevation: 788-975 m

	1989			1990			1991		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	6.53	14.4	-4.6	4.62	14.3	-4.2	3.51	13.8	-4.1
February	1.40	17.3	-3.1	4.83	15.4	-3.4	3.63	20.7	-2.8
March	3.63	22.6	-0.6	2.95	20.7	-0.1	17.73	14.7	-0.7
April	0	28.3	2.1	3.17	24.1	3.4	0	22.5	-0.3
May	0.86	28.3	4.1	1.04	27.7	4	0	26.4	2.9
June	0	32.9	7.3	0.13	36.3	10	0.28	30.9	6.6
July	3.15	35.7	14.7	8.43	34.9	16.9	1.8	35.4	14.3
August	3.02	34.6	13.9	4.65	33.3	13.7	3.61	34.5	15.2
September	0	33.4	9.4	8.23	30.7	12.6	2.06	31.5	11.7
October	1.09	26.6	3.2	1.6	27	3.2	1.83	28.4	5
November	0	21.8	-2.5	2.54	20.4	-2.3	4.27	19.1	0.2
December	2.16	17.8	-6.7	0.63	13.1	-7.7	2.29	14.7	-1.6
Total Rainfall	21.84			42.82			41.01		

	1992			1993			1994		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	5.71	15.3	-3.8	20.68	11	-1.5	0.84	17.9	-6.3
February	6.93	16.8	0.6	17.5	12.5	-0.3	3.84	15.7	-3.6
March	9.12	16.4	1.0	7.54	19.2	0.4	3.23	21.3	0.2
April	0.48	24.9	2.1	0	23.5	1.4	3.23	24.4	2.8
May	4.60	26.3	6.1	1.04	28.2	6.2	1.45	27.9	5.9
June	0.38	30.0	5.8	0.13	32.2	7.3	0.30	36.9	11.2
July	1.96	31.6	11.3	0	32.8	11	0.84	36.9	15.2
August	14.94	30.6	13.8	11.15	33.7	13.6	2.16	37.3	17.6
September	0	30.1	7.5	0	32	7.8	3.10	33.1	12.4
October	0.51	25.0	2.4	2.6	25.7	5	3.86	24.8	2.9
November	0	16.0	-6.3	3.6	18.7	-2.3	1.27	16.2	-3.0
December	12.7	10.5	-6.2	0.4	16.5	-5.4	6.65	15.2	-1.8
Total Rainfall	57.33			64.64			30.76		

Appendix 9. (continued).

Harcuvar Mountains, Arizona
Location: La Paz Co., Arizona
Elevation: 792-1,006 m

	1989			1990			1991		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	6.25	16.1	-0.7	3.96	16.5	0.6	1.55	16.9	1.3
February	0	19.9	1.9	0.66	16.3	1.3	1.75	22.5	3.7
March	2.34	25.3	5.2	1.27	22.8	5.7	8.15	18.6	3.6
April	0	31.2	9.3	0.71	27.4	9.6	0	25.6	5.3
May	0.48	31.9	11.3	0.43	30.8	11.9	0	30.2	9.3
June	0	37.8	15.8	0.23	38.8	17.9	0	34.4	14.4
July	1.96	40.1	22.3	8.1	37.5	22.3	0	38.7	20.3
August	0.46	36.9	20.6	3.3	36.4	19.8	2.31	37.4	21.2
September	0	35	16.6	5.87	34.3	18.1	0	34.5	18.4
October	11.43	28.5	11.4	0	29.5	10.6	1.14	30.4	11.8
November	0	23.8	4.4	0	23	3.7	0.91	20.9	4.3
December	1.14	18.7	-0.6	0	15.7	-2.2	2.36	16.3	2.8
Total Rainfall	24.06			24.53			18.17		

	1992			1993			1994		
	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp	Rainfall	Max Temp	Min Temp
January	3.0	16.7	0.7	0	16.2	4.3	0.13	19.8	-0.6
February	4.88	19.4	5.1	12.14	15.4	3.6	1.96	17.3	-0.2
March	8.81	19.7	5.5	1.91	23.0	5.3	0.56	23.3	4.9
April	0.63	28.5	8.8	0	27.8	6.6	0	27.5	6.8
May	0.84	32.0	13.4	0	32.7	12.4	2.31	29.9	10.8
June	0	36.3	15.7	0	36.9	15.3	0.89	38.6	17.9
July	0.76	37.9	19.8	0	37.8	18.6	3.0	38.9	20.6
August	0	37.1	21.8	5	36.9	19.7	0.94	39.3	22.4
September	0	35.6	17.0	0	34.7	14.4	0	35.6	17.5
October	0	30.2	11.5	0	27.6	10.1	0	27.7	8.5
November	0	19.8	1.7	0	20.4	3	0	18.5	0.8
December	6.38	14.6	1.0	0	18.0	-0.6	3.81	17.3	1.7
Total Rainfall	25.3			14.05			13.59		

Appendix 10. Monthly rainfall (cm) and mean ambient temperature (C), soil temperature (C), soil moisture (centibars), relative humidity (%), and wind speed (km/hr) from automatic weather stations from 2 sites (Little Shipp Wash, Harcuvar Mtns., Ariz.) in the Sonoran Desert, 1992-94. A slash mark (--) means weather data was not collected that period. Weather stations erected in July 1992. Data from these 2 sites are combined.

Little Shipp Wash, Arizona
Location - Lat: 34° 31′ N Long: 113° 5′ W

	Rainfall			Temperature			Soil Temperature		
	1992	1993	1994	1992	1993	1994	1992	1993	1994
January	--	0.2	0.58	--	10.8	49.3	--	12.7	--
February	--	17.73	0.05	--	9.7	46.2	--	12.8	--
March	--	2.72	6.83	--	14.1	66.8	--	17.9	67.6
April	--	0	1.55	--	17.9	63.7	--	23.4	79.8
May	--	1.7	1.55	--	22.8	70.2	--	28.6	85.2
June	--	0.08	0.43	--	26.6	84.3	--	32.9	97.4
July	0.69	0	2.41	28.0	28.9	86.0	36.5	35.6	100.7
August	19	5.61	4.98	27.8	22.8	86.2	36.8	33.2	99.9
September	0	0	5.94	25.9	24.9	80.1	32.7	29.9	--
October	0.79	2.11	0.05	20.1	19.2	75.1	25.7	23.6	--
November	0	1.63	1.29	10.0	11.5	46.8	15.2	14.6	71.4
December	4.72	0	5.23	5.7	9.6	47.8	10.1	11.9	69.8
Total Annual Rainfall	8.5	13.57	30.89						

	Soil Moisture			Relative Humidity			Wind Speed		
	1992	1993	1994	1992	1993	1994	1992	1993	1994
January	--	98.9	229.3	--	54.5	39.9	--	2.7	1.4
February	--	79.2	228.5	--	70.5	46.8	--	2.6	1.7
March	--	122.0	230.1	--	60.8	38.7	--	2.6	3.0
April	--	167.3	186.3	--	40.7	37.7	--	3.5	2.4
May	--	216.8	202.1	--	36.7	35.4	--	3.9	2.3
June	--	231.5	229.3	--	24.8	21.3	--	3.7	2.3
July	--	233.8	232.1	44.5	27.6	29.9	2.6	3.7	2.0
August	229.9	231.2	225.1	52.3	44.9	42.6	2.2	3.4	2.0
September	--	233.1	223.7	43.4	21.2	51.8	1.2	2.6	1.6
October	233.7	232.3	224.5	38.6	46.2	36.8	1.4	2.7	1.9
November	229.1	229.2	186.8	36.3	47.2	54.2	1.9	2.3	1.7
December	224.4	228.2	100.5	66.4	37.2	63.3	1.5	1.8	0.5

Appendix 10. (continued).

Harcuvar Mountains, Arizona
Location - Lat: 34° 6′ N Long: 113° 17′ W

	Rainfall			Temperature			Soil Temperature		
	1992	1993	1994	1992	1993	1994	1992	1993	1994
January	--	--	0.20	--	--	51.5	--	--	50.5
February	--	4.9	0.05	--	10.8	48.3	--	--	49.8
March	--	1.66	2.62	--	15.8	58.9	--	7.1	61.3
April	--	0	0.20	--	19.4	65.2	--	9.9	71.8
May	--	0.81	1.17	--	24	71.3	--	28.4	78.8
June	--	0	0.56	--	27.6	85.6	--	33.6	90.8
July	--	0	0.69	--	29.3	86.6	--	36.3	93.7
August	3.48	--	1.32	26		87.4	30.1	--	93.4
September	0.94	0	--	27.2	26.1	--	30.9	27.3	--
October	0	1.63	--	21.6	20.1	--	24.8	22.7	--
November	0	2.46	0.83	13.9	12.2	49.1	16.4	13.2	53.6
December	6.05	0	4.50	8.6	11.6	50.1	10.0	10.6	50.4
Total Annual	10.4	11.4	12.4						
Rainfall	7	6	4						

	Soil Moisture			Relative Humidity			Wind Speed		
	1992	1993	1994	1992	1993	1994	1992	1993	1994
January	--	--	225.6	--	--	36.4	--	--	1.8
February	--	28.6	226.7	--	90.9	37	--	4.8	1.5
March	--	141.7	127.1	--	35	34.7	--	2.7	1.9
April	--	190.5	163.6	--	33.7	33.8	--	3.7	2.4
May	--	222.8	203.2	--	32	32.6	--	3.4	2.1
June	--	232.8	228.0	--	20.5	30	--	3.5	2.4
July	--	233.2	233.7	--	30	29.6	--	4.3	2.2
August	118.7	--	231.7	32.3	--	28.3	1.6	3.1	2.0
September	193.5	233.4	--	31.2	31.1	--	1.5	--	--
October	231.6	233	--	31.6	33.3	--	1.6	2.6	--
November	232.5	230	229.2	50.9	36	36.9	2.1	2.7	2.4
December	80.4	228.6	198.1	37.9	36.2	36.7	1.6	3.1	1.4

Appendix 11. Sonoran tortoise data form for collection of general and health information with an emphasis on signs of malnutrition, dehydration, and upper respiratory tract disease.

SONORAN DESERT
DESERT TORTOISE DATA SHEET

MONTH:________ DAY:________ YEAR:________ FOUND TIME (MST):____________
TORTOISE NUMBER:__________ FREQUENCY:___________ FINISH TIME (MST):____________
STATUS:____________ GENDER:__________

SITE NO:____________ STATE:____________ TOWNSHIP:____________ RANGE:____________
SECTION:____________ ELEVATION:____________

MCL:________ M-3:________ M-4:________ M7-8:________ GRWIDTH:________ (M)
HEIGHT:________ PLN:________ PLT:________

WEIGHT:________(kg) SHWR:____________ ANNUL:____________ TAIL:____________(mm)

INVESTIGATOR:__________________________

LOCATION:__________________________ SITE NAME:__________________________

PROJECT NAME:__________________________ LANDOWNER:__________________________

MARKING PATTERN:__________________________ INJURIES:__________________________

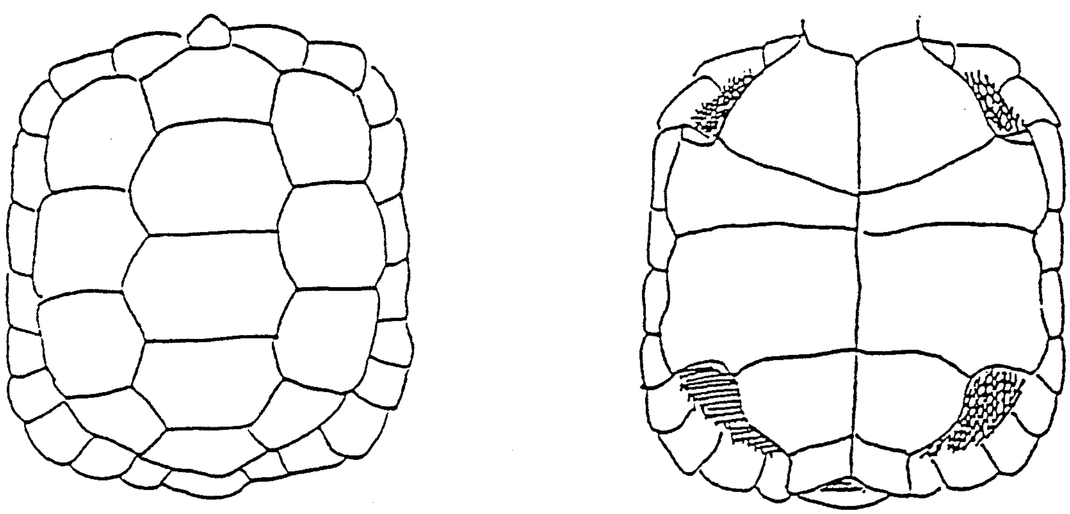
ANOMALIES:__________________________ MARGINAL SCUTE COUNT:______________

BEHAVIOR:__________________________ FOODS:__________________________

REMARKS:____
____
____

SHOW ALL NOTCHES, ANOMALIES, INJURIES

DRAW: shape of gulars, location of notches; chips, chews, shell damage, lesions; shell disease; shell abnormalities; scute concavities. Make new drawing at least once/year (spring).



(Adapted from Bureau of Land Management, AZ strip, Riverside, CA, forms) Revised 5/14/96

Appendix 11. (continued).

	YES	NO	UNK
BEAK & NARES Beak/nares wet			
Beak/nose damp			
Nasal exudate present			
Exudate color: clear			
cloudy			
white			
yellow			
green			
Bubble(s) from nares			
One nare occluded			
Both nares occluded			
Dirt on nose/beak			
Dirt in nares			
Depigmentation below nares			
FORELEGS (adjacent to face)			
Dried dirt on forelegs			
Moisture on forelegs			
Dried exud. on scales[1]			
BREATHING Smooth			
Wheezing			
Rasping, clicking			
Gurgling			
Tortoise extend head and neck to breath			
Tortoise forelimbs move in and out with each breath			
EYES, CHIN GLANDS (circle eyes or lids)			
Eyes/lids whitened or discolored			
Eyelids swollen			
Eyes/lids wet			
Discharge from eyes			
Eyes sunken			
Eyes clear, bright			
Eyes dull, cloudy			
Chin glands draining			

	YES	NO	UNK
INTEGUMENT Integument dull			
Integument glossy			
Normal elasticity[2]			
Abnormal skin peeling			
ORAL CAVITY[3] Observed			
Discharge present			
Membranes pink			
Membranes pale, white			
Smells/mouth rot			
EVIDENCE OF SHELL/BONE DISEASE			
Lesions present			
Scute laminae peeling			
Scutes missing/peeling			
Pitting			
Scutes depressed/concave			
EVIDENCE OF TRAUMA Head			
Gular			
Forelimbs			
Hindlimbs			
Shell			
Bone/scute replacement			
Describe: ______________________			
POSTURE/BEHAVIOR			
Alert, responsive			
Lethargic			
Can withdraw tightly into shell			
Limbs, head, hanging limp or loose			
TEMPORAL MUSCLES Full			
Sunken			

OTHER NOTES: ____

1. Shiny integument, glossy with dried exudate. 2. Difficult, but try. For normal elasticity, gently pull skin on limb, note how quickly skin returns to position. 3. Important. DO NOT try to open mouth. Make observations opportunistically, if tortoise opens mouth.

(Adapted from Bureau of Land Management, AZ strip, Riverside, CA, forms) Revised 5/14/96

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