Chelonian Conservation and Biology, 2002, 4(2):436–448

Deaths of Desert Tortoises Following Periods of Drought and Research Manipulation

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ABSTRACT. – **Droughts, or years in which precipitation falls below the long-term mean, are a frequent occurrence in the American Southwest. During or following droughts between 1990 and 1995, 11 (6 moribund, 5 dead) desert tortoises (*Gopherus agassizii*) were salvaged due to dehydration and starvation from three field sites in the Mojave Desert. The tortoises included 5 juveniles, 1 immature, and 5 adult males. Ten weighed 19.5 to 39.7% less than control animals, drought survivors of equivalent carapace lengths from the same or nearby study sites. In the weeks and months preceding salvage or death, the salvaged tortoises behaved abnormally for the season and weather conditions, e.g., not entering burrows for hibernation in fall, remaining above ground overnight exposed to freezing temperatures. In addition, rain sufficient to produce free-standing water fell in the vicinity of 9 tortoises, but only 4 showed evidence of drinking. Hematologic abnormalities included low packed cell volumes and heterophil counts. Abnormalities in the plasma biochemical analytes included hypocalcemia, hyperbilirubinemia, marked azotemia, and elevated sodium and chloride levels. Necropsy findings included atrophy or disappearance of the thymus, lack of subcutaneous fat adjacent to the proximal ends of the humeri, lack of coelomic fat, empty stomachs and upper intestines, and urolithiasis. Gross and histologic examination of tissues indicated osteopenia, skeletal muscle and liver atrophy, and mild to severe hemosiderosis of the liver. Other factors possibly exacerbating the debilitating effects of dehydration and starvation in 10 of 11 tortoises included young or old age, handling, research manipulation, and crowding in experimental desert enclosures. During droughts, scientists conducting research and procedures on wild desert tortoises can use abnormalities in behavior and laboratory data as early warning signs of stress and take appropriate actions to reduce impacts by modifying protocols, or delaying or terminating the procedures.**

KEY WORDS. – **Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; mortality; morbidity; research manipulation; management; drought; starvation; dehydration; crowding; handling**

The desert tortoise (*Gopherus agassizii*) is a threatened species protected under the Federal Endangered Species Act of 1973 (as amended) and California's Endangered Species Act. Tortoise populations are declining for numerous reasons, including degradation of habitat from anthropogenic influences, drought, and disease (U.S. Fish and Wildlife Service, 1994). To stabilize and recover declining tortoise populations, wildlife biologists and research veterinarians need a better understanding of the possible natural and anthropogenic causes of mortality.

Drought and subsequent dehydration and starvation are contributors to poor condition and death in wild, free-ranging desert tortoises (Turner et al., 1984; Peterson, 1994, 1996a, 1996b). Drought has also been implicated in low growth rates of young desert tortoises (Medica et al., 1975), reduced egg production and reproductive effort in female tortoises (Turner et al., 1984, 1986; Henen, 1997), reduced activity levels and movement (Henen, 1997; Duda et al., 1999), and low field metabolic rates (Peterson 1996a, 1996b; Henen, 1997; Henen et al., 1998). Drought, dehydration, and low food intake (implied by lack of forage) also alter hematologic and plasma biochemical analytes (Peterson, 1994, 1996a; Christopher et al., 1999).

In this paper we drew from case studies to evaluate the possible roles of drought, dehydration, starvation, handling, and research manipulation in the debilitation and mortality of 11 desert tortoises. We sought to determine if clinical signs of disease, hematological and plasma biochemical values, and specific behaviors could be correlated with weather patterns and used to predict illness and impending death.

METHODS

Source of Desert Tortoises and Field Sites. — We salvaged 11 tortoises (6 moribund, 5 dead) for necropsy from San Bernardino County, California, between May 1990 and March 1995 from three study areas: the Ft. Irwin study site (FISS), National Training Center, Fort Irwin, U.S. Army (35°06'N, 116°29'W, elev. 650 m) in the central

Mojave Desert (n = 5); Ivanpah Valley (35°39'N, 115°15'W, elev. 914 m) in the northeastern Mojave Desert (n = 2); and Goffs (34°52'N, 115°10'W, elev. 732 m) in the eastern Mojave Desert (n = 4) (Table 1). The sites have similar vegetation, Mojave Desert creosote bush scrub (Vasek and Barbour, 1988), specifically Big Galleta Shrub Steppe (U.S. Fish and Wildlife Service, 1994), and are on bajadas or hillsides with 2–3% slopes. Common perennial plants are creosote bush (*Larrea tridentata*), burrobush (*Ambrosia dumosa*), and galleta grass (*Pleuraphis rigida*).

At the Fort Irwin study site (FISS), tortoises were confined within an experimental 60 x 60 m predator-proof enclosure where densities of juveniles were artificially high (281–344 per hectare [ha]) compared with densities in surrounding natural habitats (0.43–0.75 per ha, see Spangenberg, 1996; Morafka et al., 1997). Adult captive density also was artificially higher (30 per ha) than in similar nearby habitats (< 1 per ha). Soils and vegetation within the enclosure were in a disturbed condition from tortoises kept at artificially high densities and trampling by research scientists. In contrast, tortoises at Goffs and Ivanpah were wild and free-ranging.

Weather Data. — We used data on freezing temperatures from the weather station at FISS and from the weather station maintained by the National Oceanic and Atmospheric Administration (NOAA) at Daggett Federal Aviation Administration Airport, California (34°52'N, 116°47'W, 585 m). Data on precipitation were taken from the Daggett station for FISS, and from the mean of Mountain Pass, California (35°28'N, 115°32'W, elev. 1442 m) and Searchlight, Nevada (35°28'N, 114°55'W, elev. 1079 m) for the Ivanpah site (NOAA, 1976–95). Similar data were averaged from weather stations at Mitchell Caverns, California (34°56'N, 115°32'W, elev. 1326 m) and Needles, California (34°46'N, 114°37'W, elev. 279 m) for Goffs. The NOAA data sets provided background information on weather patterns for the region. Field notes supplied additional details for local weather conditions.

We calculated 30-year means for winter rainfall (defined as October 1 through March 31) and total annual rainfall (defined as the water or hydrologic year from October 1 through September 30, see Manning, 1992) using the NOAA weather station records. For each weather station, we assembled monthly data for "normals". Normals were the climatological normals or long-term means based on the period 1941–70 (NOAA, 1980). Drought years were defined as hydrologic years with precipitation below the long-term annual mean. Drought seasons, such as a dry winter, were defined as an October–March period with precipitation below the long-term mean and little or no production of winter annual plants and herbaceous perennial plants. Under such conditions, fresh, green plants were rare or unavailable for the tortoises to eat. We compared the NOAA long-term means for winter and annual rainfall with precipitation data from 1989–95, when the tortoises were salvaged.

Histories, Field Evaluations, and Physical Examinations of Tortoises. — For each tortoise, we compiled histories of known captures and capture locations, carapace lengths at the midline (CL, mm), weights (g), evidence of growth, health profiles, clinical signs of disease, locations and signs of movement, activities, and behaviors. Histories of handling by field workers and research scientists were also summarized, including procedures such as transporting tortoises long distances in backpacks, holding tortoises overnight in plastic buckets, phlebotomy (Nagy et al., 1997; Christopher et al., 1999), cystocentesis sampling (Peterson, 1994, 1996a), urine collection at the cloaca, use of temperature probes for cloacal temperatures, and attachment of radiotransmitters.

We evaluated all tortoises in the field prior to salvage (Berry and Christopher, 2001). Moribund tortoises were shipped via air freight from the field to the University of Florida for necropsy; dead tortoises were shipped via 24-hour Federal Express on ice or frozen for necropsy. Just prior to necropsy, moribund tortoises received a complete physical examination. The integumentary system was evaluated by inspecting seams between adjacent scutes for new shell growth, searching for signs of cutaneous dyskeratosis (Jacobson et al., 1994) or other lesions on the shell or skin, and examining the eyes and oral cavity for lesions. The condition of the eyes (e.g., position of globes in the orbits, the presence of edema, mucus, or other drainage) and nares (patency, color and type of drainage) were noted, particularly for signs of upper respiratory tract disease (URTD) (Brown et al., 1994). The cloacal area was examined for signs of diarrhea, straining, or discoloration. Similar data, where applicable, were collected from dead tortoises.

Comparisons of Drought Effects on Source Populations. — To compare effects of drought and research manipulation on the populations from which the salvaged animals were taken, we used two methods. The first, at FISS, was the death rate of other juvenile tortoises (n = 110) living in the same enclosure during and immediately after the time the 4 juveniles were salvaged. The second method, used at Ivanpah Valley and Goffs, was to compare changes in densities of adult tortoises before, during, and after the tortoises were salvaged (from 1979 through 1994). The salvaged tortoises were adjacent to or part of long-term study populations at permanent plots (Berry and Medica, 1995; KHB, unpubl. data). We used mark-recapture data to calculate density estimates with 95% confidence intervals using the Stratified Lincoln Index (Overton, 1971).

Weight-Length Relationships. — We assembled historical data on changes in CL and weight for each salvaged tortoise retrospectively. For determining effects of drought and other factors on weight-length relationships of salvaged tortoises, we developed a data base of control tortoises to match each salvaged tortoise (Appendix 1). Control tortoises were defined as wild, free-ranging individuals living on the same study site for the tortoises salvaged from Ivanpah and Goffs. For FISS, the nearby study site was at Lucerne Valley, which was similar in elevation, perennial vegetation, and precipitation patterns (Berry, 1984; Berry

Table 1. Site, sex, age class, and weight history of 11 desert tortoises salvaged between 1990–95. Six tortoises were salvaged moribund, and 5 were dead*. FISS = Fort Irwin study site.

Spec. no.	Source	Sex	Age class (yrs or estimate)	Status at salvage Date mmddyr	CL (mm)	Weight (g)	Years of weight history	Highest weight date mmddyr	(g)	Lowest weight date mmddyr	(g)
1*	FISS	F	3.23, juvenile	121694	48	16	3	032994	26	082294	16
2*	FISS	?	2.33, juvenile	021695	53	27	3	070794	27	092294	21
3	Ivanpah	M	est. 0.9-1.5, juvenile	041894	56	31	–	–	–	–	—
4*	FISS	F	3.29, juvenile	121694	65	41	3	061293	67	092294	39
5*	FISS	?	4.42, juvenile	021595	70	50	4	032093	61	092294	42
6	Goffs	F	10-14, immature	051090	141	390	8	not applicable, rapidly growing tortoise			
7	Goffs	M	> 70, very old adult	052990	238	2600	14	041784	3750	052990	2600
8*	Ivanpah	M	> 70, old adult	072090	252	1835	4	052789	3040	061890	1945
9	Goffs	M	middle-aged adult	052790	265	2600	11	060283	4100	052790	2600
10	FISS	M	middle-aged adult	032095	265	2250	–	–	–	–	—
11	Goffs	M	> 70, very old adult	051090	277	3250	14	050483	4700	051090	3250

and Medica, 1995). Control tortoises were of the same sex, within 10–15 mm CL, and were of the same relative age (juvenile, immature, young adult, middle-aged adult, old adult (see Turner and Berry, 1984). The controls for 5 salvaged adults had survived many drought years, including the years in which our animals were salvaged. Adult controls included data for drought and non-drought years. Compared to adults, juvenile and immature controls were only a few years old and may or may not have experienced drought conditions. For each control group, we calculated linear regression equations (least squares fit), 95% confidence intervals, and coefficients of determination (R^2) for weight-length (CL) relationships. We compared the regression equations and 95% confidence intervals of the control tortoises with the weight-lengths of salvaged tortoises.

We developed a second set of control animals for the 4 salvaged juvenile tortoises from FISS by using tortoises that experienced the same conditions inside the FISS enclosure but survived the 1993–94 drought. We compared mean weight losses for the control juveniles ($n = 97$) during the drought, specifically between spring and fall of 1994. For the 4 salvaged tortoises, we also calculated prime condition index (Nagy et al., 1997) and mean growth per year from hatching until time of salvage using changes in shell length, i.e., midline plastron length (PL), from gular to anal notches.

Hematologic and Plasma Biochemical Evaluations. — Blood samples were drawn from the jugular vein of 5 moribund tortoises and from an intracardiac sample from the heart of a sixth tortoise at the time of death (Appendix 2). Samples were placed in lithium heparin microtainer tubes, and analyzed for hematologic and plasma biochemical profiles (Jacobson et al., 1992). A blood sample taken from one tortoise 14 mo prior to salvage was also used. Hematologic evaluations included: red blood cell (RBC) counts, white blood cell (WBC) counts, differential WBC counts, hemoglobin (Hb) concentration, and packed cell volumes (PCV) (Christopher et al., 1999). The RBC count was determined on a Coulter 2BI and the Hb on a Coulter hemoglobinometer (Coulter Diagnostics, Hialeah, FL). The WBC count was determined manually. Plasma was separated from whole blood and assayed for fibrinogen. Plasma biochemical determinations included: albumin, albumin/globulin (A/G) ratio, alkaline phosphatase (ALP), alanine aminotransferase (ALT), anion gap, aspartate aminotransferase (AST), bile acids, total bilirubin, blood urea nitrogen (BUN), calcium, total carbon dioxide (TCO_2), chloride, cholesterol, creatine kinase, creatinine, globulins, glucose, iron, total binding capacity of iron, percent saturation of iron, unsaturated binding capacity of iron, osmolality, phosphorus, potassium, sodium, total protein, and uric acid. Plasma biochemical evaluations were made using automated analyzers (Ciba-Corning 500 or Express 550, Ciba Corning Diagnostics, Oberlin, OH). Electrolytes were analyzed on a Ciba-Corning Fast 4. Hematologic and biochemical profile data from salvaged tortoises were compared with reference intervals developed from wild, free-ranging healthy male and female adult tortoises (Christopher et al., 1999). Analytes with values outside the reference intervals were considered abnormal.

Necropsies. — Gross necropsies were conducted on all the tortoises as described previously (Jacobson et al., 1991; Homer et al., 1998). Moribund tortoises (Table 1) were euthanatized with an intraperitoneal injection of a concentrated barbiturate solution and necropsied; one tortoise died prior to necropsy. Samples of tissue from all major organ systems were placed in neutral buffered 10% formalin for 24–48 hrs, embedded in paraffin, sectioned at 5–6 µm, and stained with hematoxylin and eosin and a variety of special stains as necessary.

Urine collected from the bladders of 5 tortoises (Appendix 2) were analyzed for color, transparency, specific gravity, sediment, pH, osmolality, glucose, protein, ketones, hemoprotein, bilirubin, nitrite, and urobilinogen (all analytes except osmolality by Ames N–Multistix Bayer Corp. Diagnostics Division, Elkhart, IN; osmolality by Wescor Osmometer Model 5500, Logan, UT). Cystic calculi from 2 tortoises were analyzed for chemical composition by crystallography (Louis C. Herring and Co., Orlando, FL).

Microbial Investigations. —Isolation and identification of aerobic bacteria consisted of swab specimens of: (1) nasal cavities for 7 tortoises; (2) colon for 6 tortoises; and (3) lung for one tortoise (Appendix 2). Bacterial isolation was performed on sheep blood agar and MacConkey's agar and

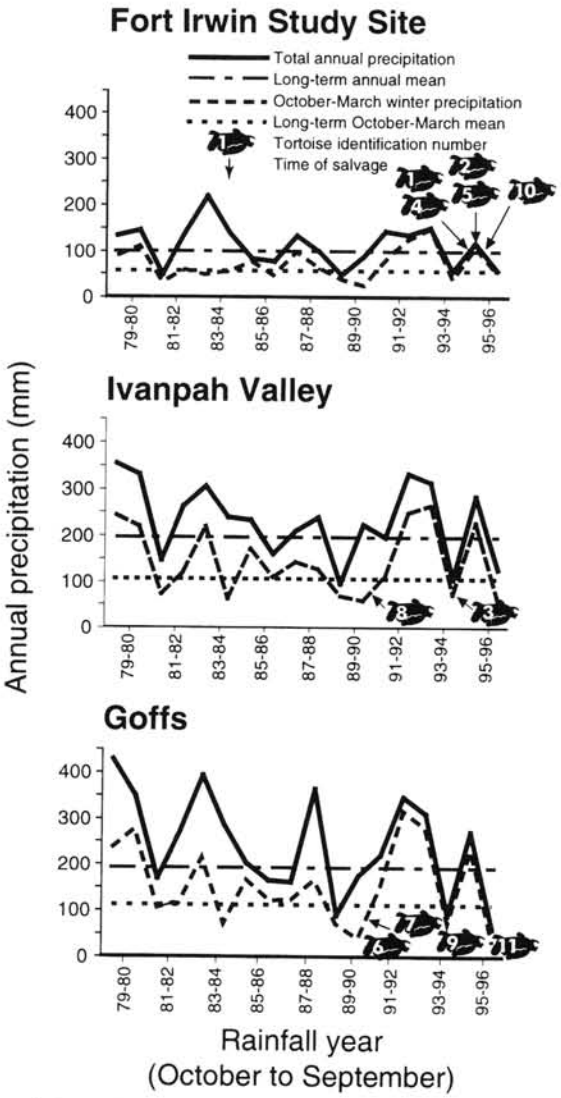

Figure 1. Precipitation data for the FISS, Goffs, and Ivanpah study sites. The data are from the National Oceanic and Atmospheric Administration, 1976–95. Tortoise symbols with specimen numbers indicate when specimens listed in Table 1 were salvaged.

identification of isolates was made utilizing a variety of biochemical tests including the API 20E system (BioMerieux Vitek, Hazelwood, MO) (Homer et al., 1998). In addition to microscopic examination of tissues for evidence of mycoplasmosis, swabs of nasal and choanal cavities were collected from 2 moribund tortoises for isolation of *Mycoplasma* sp. (Brown et al., 1994) (Appendix 2). Choanal swabs were obtained prior to sectioning the head. A polymerase chain reaction (PCR) amplification analysis was conducted using swabs of nasal cavity and sections of lung of 2 tortoises for detection and identification of *Mycoplasma* sp. (Brown et al., 1995), and an enzyme-linked immunosorbent test (ELISA) was used to measure the presence of *Mycoplasma agassizii*-specific antibodies in tortoise plasma (Schumacher et al., 1993).

Fecal flotation specimens from 8 tortoises (Appendix 2) were examined for the presence of helminth ova. Nematode parasites were isolated from the contents of the entire gastrointestinal tract by the Baermann method (Greiner et al., 1980). Direct smears of small intestinal contents were mixed with phosphate buffered saline and examined by microscopy for motile protozoa.

RESULTS

Drought and Salvage. — In the 10 to 15 years preceding salvage, below normal winter and annual precipitation occurred approximately once every 3 years (Fig. 1). With the exception of tortoise 3, all tortoises either died or were salvaged moribund in the months following a hydrologic year when rainfall was below the long-term means both for winter and for annual precipitation. At FISS, 5 tortoises died or were salvaged during the fall and winter of 1994–95, when fall and winter rains were ending a brief drought. In Ivanpah Valley, tortoise 8 was salvaged in July 1990, after 2 preceding years of drought, 1988–89 and 1989–90. Tortoise 3 was salvaged in a dry spring following a winter with precipitation levels that fell below the long-term mean. At Goffs, the 4 salvaged tortoises experienced 2 consecutive years of low rainfall (1988–89, 1989–90) and were salvaged after spring rains broke the drought.

Histories, Field Evaluations, and Physical Examinations Prior to Salvage. — With the exception of tortoises 3 and 10, histories of weight-length measurements and behaviors were available for the preceding 3 to 14 years. Tortoises were measured and weighed at intervals ranging from 12 times in 14 years to 10 times in one year. They experienced different types and frequencies of handling and research procedures. Five tortoises (3, 6–7, 9, 11) were handled minimally (weighed, measured, photographed). Tortoise 8 was handled more than others in this study in the 14 months prior to death. It was fitted with a 100 g radiotransmitter; repeatedly pulled, tapped, or dug from its burrows; carried for kilometers and hours in a backpack frame; and occasionally kept overnight in camp (C. Peterson, *pers. comm.*). In addition, brachial blood was drawn 10 times, cystocentesis was performed 7 times, and urine collected once when the tortoise voided. For tortoises 1, 2, 4–5, and 10, research procedures included blood sampling by cardiocentesis for 1 and 5 and by jugular phlebotomy for 10; urine sampling by capillary tube at the cloaca (4–5); and cloacal probes for body temperatures (1–2, 4). FISS tortoise 5 also carried a 5 g radiotransmitter, which was from 8% to 12% of its body weight, and was translocated to a site outside the enclosure. FISS tortoise 10 was moved from the wild to confinement in the enclosure.

During the weeks prior to salvage, all the tortoises displayed one or more abnormal behaviors for the time of day, season, or year (Berry and Christopher, 2001). All FISS tortoises (Table 1) exhibited atypical behaviors for use of burrows for the fall and winter seasons. Frequently more than one juvenile attempted to use a burrow (constructed for solitary use), resulting in exposure of one or more juveniles to extremes of temperature and moisture. All juvenile FISS tortoises (Table 1) delayed entering burrows for hibernation in fall or winter. Once in burrows, they failed to stay there,

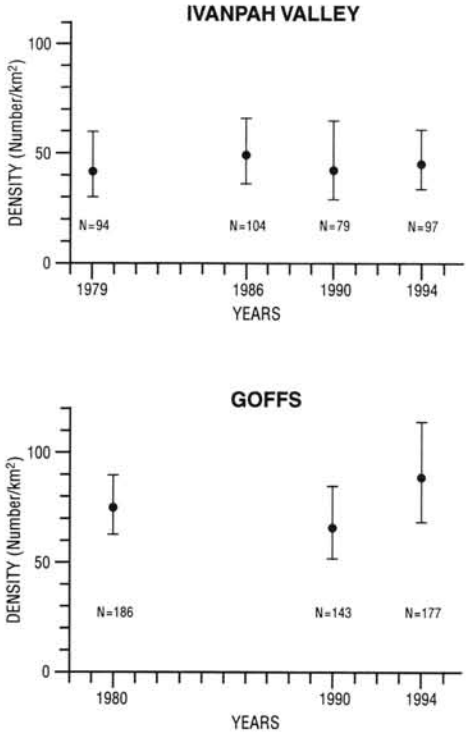

Figure 2. Densities of adult desert tortoises recorded at the Ivanpah Valley and Goffs study plots, California, between 1979 and 1994, using mark-recapture techniques. The filled circles are the midpoint of density estimates and the brackets enclose the 95% confidence intervals; N = sample size.

emerging to rest above ground overnight, sometimes in the same place without moving for several days at a time. These tortoises were probably exposed to below freezing temperatures from 1 to 14 days and for near-freezing temperatures for several additional days (Hillard, 1996). Tortoise 10 showed a similar pattern and remained above ground for several weeks, exposed to freezing temperatures and rain.

Nine of the 11 tortoises (1–2, 4–7, 9, 11) received sufficient rainfall to drink (standing water present, catchment construction possible) in the weeks and months prior to salvage and death. For the FISS tortoises, rain fell in the fall and winter of 1994–95, prior to the times that 4 of the tortoises died. For the Goffs tortoises, rain fell in spring, a few weeks prior to salvage. Five tortoises (1, 5–6, 10–11) showed no evidence of drinking the free water from the rain (mud or dried dirt on beak, forelimbs; no weight gain), but one (6) dug a drinking depression (Medica et al., 1980) during a light rain. Four (2, 4, 7, 9) drank and gained weight. One tortoise (10) did not eat or drink when offered supplemental food and water a few days prior to salvage. Two months prior to the winter rains and death, one tortoise (5) was offered water for rehydration and drank.

Of the 11 tortoises in the sample, 4 (3–4, 6–7) were inordinately inactive, sluggish, and lethargic, and 7 (1–2, 5, 8–11) were severely debilitated (abnormal postures, unable to walk or stand or retract heads or limbs into the shell). We interpreted inordinately inactive, sluggish, and lethargic behavior as signs of ill health, because the season and weather conditions were appropriate for the tortoises to be active and responsive. Lethargic and severely debilitated individuals were reluctant to move, inattentive and unresponsive. Signs of debilitation in most tortoises were intermittent or progressive. For example, 4 of these tortoises (6–7, 9, 11) were alert and able to retract into their shells when first encountered in the field in spring, but 2 (9, 11) were debilitated when seen again 21–37 days later, were salvaged, and subsequently died within 7–12 days.

All tortoises were emaciated and/or cachectic while still alive in the field. The spines and pleural ribs of juveniles 1, 2, 4, and 5 were prominently outlined through the semi-translucent scutes. Two juveniles (1, 4) shrunk in CL (1.1 and 1.3 mm, respectively) during the drought year. Eyes of 6 individuals (2, 4–5, 9–11) were sunken in the orbits and often closed; eyes for 3 (9–11) were dull and cloudy.

Other potential signs of poor health were: a clear ocular discharge (11); nares occluded with dirt and/or dried mucus (10); nasal discharge (6–7); dirt on the beak or nares not associated with rain or drinking free water (7, 9); wheezing or audible respiratory clicks (7, 9, 11); flaky, discolored, and dull shells (3, 9); and laminae peeling from scutes (3). Twenty ectoparasites (ticks, *Ornithodorus* sp.) were present on one tortoise (9).

Drought Effects on Source Populations. — At the FISS enclosure, the 4 juveniles were part of a group of 11 tortoises that died between March 1994 and March 1995 out of a population of 110 juveniles (EKS, unpubl. data). The remaining 90% survived the drought. At Ivanpah Valley and Goffs, populations of adults remained relatively stable in densities between 1979 and 1994 with no statistically significant changes (95% confidence interval) occurring between years (Fig. 2). At Ivanpah, only 2.3% of adults observed live ($n = 83$) in spring of 1990 were found dead in 1994; results were similar at Goffs.

Weight Changes and Growth in the Field. — Previous measurements of weight were available for 8 tortoises (Table 1). Weights taken at salvage or immediately pre-salvage were 0% to 38.8% lower than the highest live weight previously recorded (Tables 1, 2; Fig. 3). Weights for 10 (1–6, 8–11) of the tortoises were 19.6% to 39.7% less than expected for their CL (see theoretical weights for control tortoises, Table 2); for the remaining tortoise, the weight was no different than expected. With one exception (7), the weights of salvaged tortoises were outside the 95% confidence intervals for regression equations of the control groups (Fig. 3). The predictive powers of the regression equations for control tortoises, as measured by the coefficients of determination ($R^2 = 0.94, 0.79$), are high for the juvenile tortoises (Figs. 3a–b) and rapidly diminish with increasing CL ($R^2 = 0.46$ to 0.13). The wide variation in weight-length data points for the larger tortoises reflects the major weight changes that can occur within and between seasons and years for tortoises of the same CL (Figs. 3c–h).

Mean weight losses between spring and fall of 1994 for the 4 FISS juveniles ($\bar{x} = 30.5\%$, SD ± 5.3, range 23.8–36.5%, $n = 4$) fell within the range for similarly confined but not salvaged juveniles within the enclosures ($\bar{x} = 33.4\%$, SD

Table 2. Comparison of recorded weight loss in 11 salvaged desert tortoises from three sites in the Mojave Desert of California between 1990 and 1995 with theoretical or expected weight loss derived from control tortoises and the regression equations in Fig. 3.

Tortoise spec. no.	Salvage weight (g)	Recorded weight loss[1] (%)	Theoretical weight from regression equation[2] (g)	Salvage weight: % less than theoretical weight
1	16	38.4	23	30.4
2	27	0.0	36	25.0
3	31	–	44	29.6
4	41	38.8	68	39.7
5	50	18.0	81	38.3
6	390	–	561	30.5
7	2600	30.7	2610	0.0
8	1835	32.5	2850	35.6
9	2600	36.6	3231	19.5
10	2250	–	3282	31.4
11	3250	30.9	4110	20.9

[1]Recorded weight loss was based on the highest previously recorded weight minus the salvage weight, or the weight immediately prior to death (Table 1).
[2] See regression equations in Fig. 3.

± 7.2%, range 13.5–52.9%, n = 97). The annual growth histories for salvaged FISS juveniles showed a mean PL growth of 4.57 ± 1.97 mm, range 1.67–6.23 mm (n = 4) between late summer–fall 1990 and fall 1994. In September 1994, the prime body condition indices for the salvaged FISS juveniles were 0.32, 0.34, 0.31, and 0.32 g/cm^3, or 50% of prime.

Hematology and Plasma Biochemistry. — Hematologic and plasma biochemical data were available for 7 and 6 tortoises, respectively (Tables 3, 4). Hematologic values for one tortoise (10) were abnormal for 12 analytes. The PCVs were abnormally low (9.5%, 3.8%) in 2 tortoises (1, 11) and heterophil counts were abnormally low (12–76/µl) in 5 tortoises (6–7, 9–11) (Table 3). Each tortoise had 3 or more abnormal values for 23 plasma biochemical analytes (Table 4). Two or more tortoises were hypocalcemic (7, 9), hyperbilirubinemic (7, 9–10), or azotemic (6–8, 10). Two had abnormally elevated values for chloride (6, 9) and one had a high sodium level (9).

Gross Necropsies and Microscopic Lesions. — The 5 juvenile tortoises had several common pathologic changes. Four (1–2, 4–5) of the 5 juveniles were found dead in winter (Table 1) and were partially preserved by the cold ambient temperatures. Moderate to severe autolysis precluded many detailed evaluations. The stomach and small intestine of one tortoise (3) was empty, the bladders of 3 (2, 4–5) contained from 5 to 8 ml of dark brown, flocculent urine and gray sediment, and one (1) had an empty bladder except for ca. 2 ml of gray sediment. The shells of these tortoises were softer and more pliable than normal for the age and size. One tortoise (5) had scoliosis of the spine at vertebrae 3, 4, and 5. Adipose tissue was absent. The expected muscle mass was approximately 50% below normal in all juveniles. Moderate to severe segmental thinning of cortical and trabecular bone (osteopenia) of the shells was evident. Osteopenia was present to a lesser extent in the legs, spine, and head without any evidence of bony regeneration. In many areas of shell, only a thin strip of woven bone was present. In the most severe lesions, bone was replaced by fibrous tissue. Osteoblasts with prominent ovoid nuclei were often found within osseous lacunae, but they rarely were present at the edges of the bony trabeculae. The skin had more foreign debris than normal in the keratin layer, and occasional crusts contained multiple colonies of bacteria. Multifocal mild to moderate skeletal muscle atrophy and moderate to marked hepatocellular atrophy also were present. The ratio of liver mass to body weight was 1.1% for (3), 1.5% for (4), and 2.2% for (10). The livers of tortoises 1 and 3 had moderate deposition of hemosiderin and the serosal surface of the intestine of tortoise 3 had a focal area of chronic hemorrhage. Tortoise 1 also had acute pneumonia with intralesional gram negative bacteria, as well as acute, locally extensive, necrotizing nephritis with intralesional gram negative bacteria.

The immature (6) and adult (7–11) tortoises had several similar pathologic changes. Some organs of tortoise 8 were autolyzed, precluding evaluation. The thymus was atrophied and barely visible in 2 tortoises (6–7); in 3 (8–9, 11) it could not be found. Four tortoises (6–9, 11) had no subcutaneous fat adjacent to the proximal ends of the humeri. Stomachs and upper intestines were empty (7–11), with the exception of tortoise 8, which had fluid digesta in the small intestine. The large intestine of tortoise 10 contained ingesta; the colon of 4 tortoises had digesta, fecal balls, or hard, dry fibrous material. Livers of 5 tortoises (6–7, 9–11) showed mild to severe diffuse hemosiderosis, with hypertrophy of melanomacrophages. Microscopic examination of both lungs of tortoise 9 showed multifocal accumulations of bacterial colonies on the surface of the airways, interstitial edema, and multifocal proliferation of epithelial lining cells. The spleen in tortoise 11 was small with proliferation of periarteriolar lymphocytes; a focal pyogranuloma was noted in the caudal aspect of the right lung. Bladders contained dark brown and flocculent urine and/or gray sediment (Table 5), and 3 tortoises (7, 10–11) had uroliths. Tortoise 7 had 3 calculi, each 2 cm in diameter; tortoise 10 had a 3.3 cm diameter, 24.8 g calculus; and tortoise 11 had a single 6 cm x 5.5 cm calculus weighing 62.7 g. The crystallographic composition of the calculi from tortoises 10 and 11 was 12 and 70% ammonium acid urate, 85 and 15% uric acid salts, 3 and 4% protein, and 0 and 2% hydroxyl apatite, respectively. One tortoise (10) had lesions of articular and renal gout (details in Homer et al., 1998). Heads of 6 tortoises (6–11) were examined; none had inflammatory changes suggestive of URTD.

Microbial Evaluations and Mycoplasma Serology. — Potential pathogens were isolated from the nasopharynges of 7 tortoises: *Bordetella bronchiseptica* (5), *Pasteurella testudinis* (1, 4–5, 10–11), and *Pseudomonas cepacia* (5). An α-hemolytic *Streptococcus* sp. was iso-

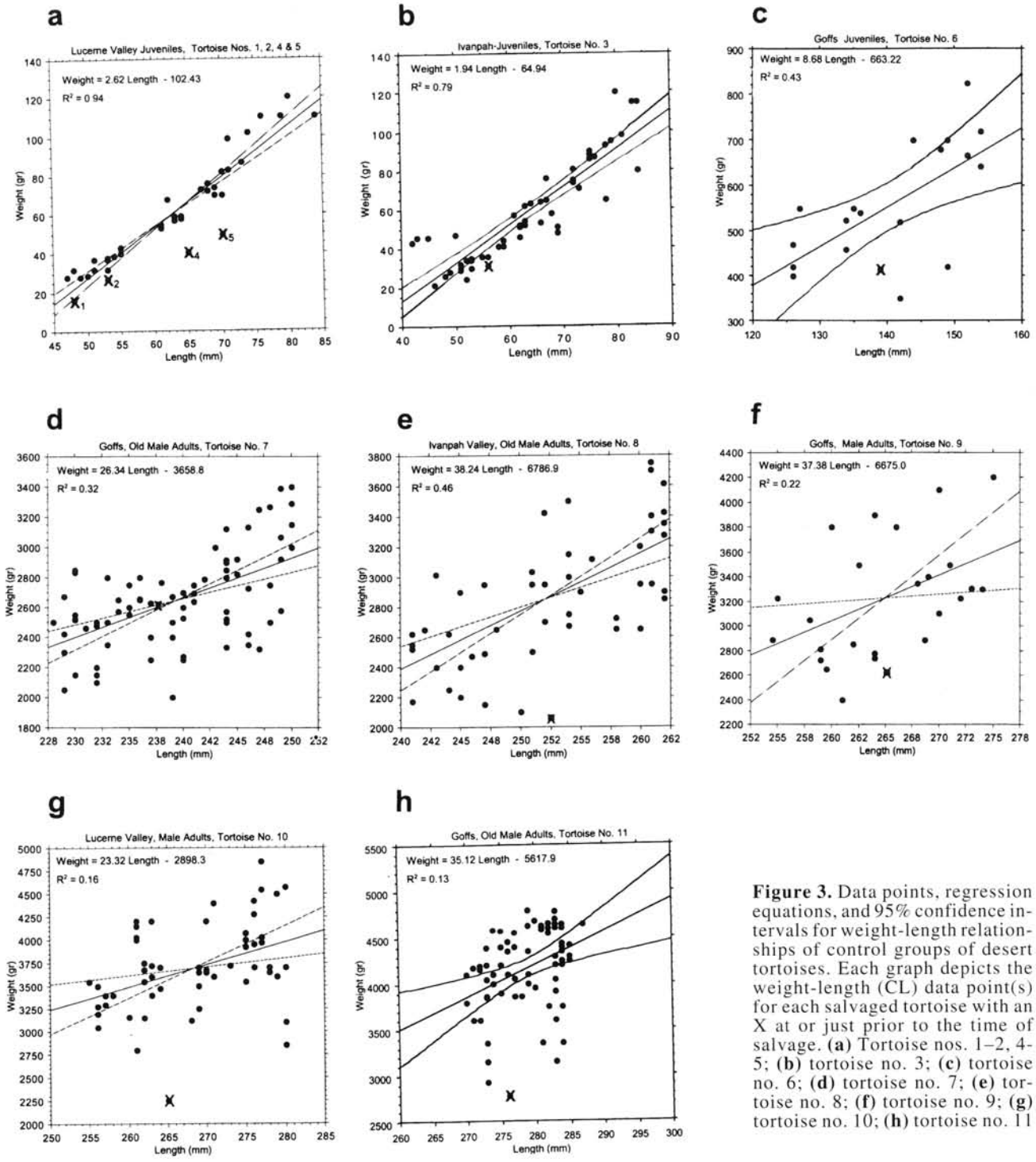

Figure 3. Data points, regression equations, and 95% confidence intervals for weight-length relationships of control groups of desert tortoises. Each graph depicts the weight-length (CL) data point(s) for each salvaged tortoise with an X at or just prior to the time of salvage. **(a)** Tortoise nos. 1–2, 4-5; **(b)** tortoise no. 3; **(c)** tortoise no. 6; **(d)** tortoise no. 7; **(e)** tortoise no. 8; **(f)** tortoise no. 9; **(g)** tortoise no. 10; **(h)** tortoise no. 11

lated from the lung of one tortoise (5), and *Pasteurella testudinis* was isolated from the colon of one tortoise (10). *Mycoplasma* was not isolated from cultures of the nasal passageways of 5 tortoises (3, 6–7, 9, 11). Of the 2 tortoises (5, 10) tested with the ELISA for *M. agassizii*, one (10) had values in the suspect range, but showed no evidence of inflammation in the nasal cavity. PCR tests were negative.

Parasitologic Evaluation. — Parasitic analyses were completed for 4 of the 11 tortoises (1, 3–4, 10). Two juveniles (1, 4) had mild pinworm endoparasitism, and a third (3) had moderate pinworm endoparasitism and a few balantidium-like protozoa in the colon. No parasites were observed in one (10).

DISCUSSION

The group of 11 tortoises constitutes a mix of size classes from three different sites and different drought years, and as such, is not a single, homogeneous sample but a collection of case studies. The sample composition should be kept in mind when interpreting the findings. Neverthe-

Table 3. Hematology for 7 of 11 desert tortoises salvaged between 1990 and 1995 from three sites in the Mojave Desert, California. The data for tortoise 1 are courtesy of R. Yates, who sampled the individual in October 1993, 14 months prior to salvage. *Outside the central 95th percentile for this analyte as defined by Christopher et al. (1999).

	Tortoise identification numbers						
	1	3	6	7	9	10	11
PCV (%)	9.5*	19.0	24.5	24.8	22.0	22.0	3.8*
RBC (x 10^6/µl)	—	—	0.6	0.5	0.5	0.5	0.1*
Hb (g/dl)	—	—	5.6	6.9	6.1	6.9	—
MCV (fl)	—	—	—	—	—	431.4	—
MCH (pg)	—	—	—	—	—	135.3	—
MCHC (g/dl)	—	—	—	—	—	31.3	—
WBC (/µl)	6000	—	1800	3800	3000	2900	2100
Heterophils (/µl, %WBC)	"high"	—	76*	55*	12*	53*	59*
Lymphocytes (/µl)	?	—	5	14	29	24	6
Monocytes (/µl)	—	—	—	1	17	6	3
Eosinophils (/µl)	—	—	—	—	—	1	—
Basophils (/µl)	—	—	2	13	24	1	19
Azurophils (/µl)	—	—	17	17	18	4	13
Icterus index (units)[1]	—	—	—	—	—	5-10	—
Plasma proteins (g/dl)[1]	—	<2.5	—	—	—	5.5	—
Fibrinogen (mg/dl)[1]	—	—	100	100	100	100	100

[1]No data for the central 95th percentile or range of minimum and maximum values, as defined by Christopher et al. (1999)

Table 4. Plasma biochemical analyses for 4 desert tortoises salvaged between 1990 and 1995 at three sites in the Mojave Desert of California with notes for a fifth tortoise (8). Data for a sixth tortoise (3) were limited to calcium and phosphorus which were within normal limits. *Outside the central 95th percentile for this analyte as defined by Christopher et al. (1999).

Analytes	Tortoise identification numbers				
	6	7	8[1]	9	10
Albumin (g/dl)	–	–	–	–	1.0
Albumin/Globulin Ratio	–	–	–	–	0.3*
Alkaline Phosphatase (U/L)	35.2	62.4	–	88.7*	74*
ALT (SGPT)(U/L)	3.9	2.0	–	2.1	37*
Anion gap (mmol/L)	–	–	–	–	18.2
AST (U/L)	146.6[1]	104.9	–	86.9	2960*
Bile Acids (µmol/L)	–	–	–	–	670*
Bilirubin, Total(mg/dl)	0.2	0.4*	–	0.4*	0.7*
BUN (mg/dl)	143.4*	88.7*	358*	5.8	640*
Calcium (mg/dl)	9.1	7.9*	–	8.4*	8.5
Chloride (mmol/L)	141.0*	117.0	–	134.0*	115
Cholesterol (mg/dl)	208.3	84.1	–	104.5	468*
Creatine kinase (x 10^4 U/L)[2]	–	–	–	–	20.9
Creatinine (mg/dl)	0.2	0.2	–	0.2	0.8*
Globulins (g/dl)	–	–	–	–	2.9
Glucose (mg/dl)	76.8	114.5	–	77.2	66
Iron (µg/dl)	–	–	–	–	98
Iron, Total Binding Capacity (µg/dl)[1]	–	–	–	–	339
Iron, Saturation (%)[1]	–	–	–	–	29
Iron, Unsaturated Binding Capacity (µg/dl)[1]	–	–	–	–	241
Osmolality (mOsm/kg)	–	–	494*	–	558*
Phosphorus (mg/dl)	1.0	1.4	–	1.8	4.4*
Potassium (mmol/L)	4.1	4.0	–	3.4	6.5*
Protein, Total (g/dl)	3.1	3.1	–	3.4	4.0
Sodium (mmol/L)	154.7	139.2	–	164.5*	164
Carbon dioxide, Total (mmol/L)	–	–	–	–	38.2
Uric acid (mg/dl)	4.4	3.0	–	5.7	20.1*

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less, the data, when evaluated collectively, provide an overall view of the types of parameters typical of tortoises experiencing starvation, dehydration, and other stressors.

Adaptations or Exaptations. — Desert tortoises have several adaptations and exaptations (Morafka and Berry, 2002) for living in an arid environment and tolerating frequent seasons and years without food and water. They spend much of the year in burrows hibernating or escaping temperature and moisture extremes (Woodbury and Hardy, 1948; Nagy and Medica, 1986); drink copious amounts of free water from catchments during and after rains (Medica et al., 1980; Nagy and Medica, 1986; Peterson, 1996a, 1996b; Henen et al., 1998); and forage opportunistically on selected winter and summer ephemeral plants, cacti, and herbaceous perennial plants during brief periods following rainfall events (Woodbury and Hardy, 1948; Nagy and Medica, 1986; Turner et al., 1984; Jennings, 1993; Henen, 1994; Peterson, 1996a, 1996b). They also have several critical physiological adaptations and exaptations for conserving water and can respond rapidly when water becomes available (Peterson, 1996a; Henen et al., 1998). They can reduce water flux, metabolism, and activity levels during dry seasons and years (Henen, 1994, 1997; Peterson 1996a; Nagy et al., 1997; Henen et al., 1998); tolerate high concentrations of metabolic wastes in their bodies (Nagy and Medica, 1986; Peterson, 1996a; Christopher et al., 1999); can accumulate water, ions, and nitrogenous wastes in the urinary bladder (Dantzler and Schmidt-Nielsen, 1966; Nagy and Medica, 1986; Peterson, 1996a); may resorb water from the urinary bladder (Peterson, 1996a); and can temporarily exist in a state of anhomeostasis (Peterson, 1996a). These adaptations or exaptations are essential for surviving in the deserts of the Southwest, because drought and lack of water and plant foods are frequent events. Most tortoises that live to adulthood (12 to 20 years: Woodbury and Hardy, 1948) have survived at least several droughts. The tortoises salvaged in this study had most recently experienced drought conditions for several months to over a year (Fig. 1) and 5 (6–9, 11) had lived through 2 consecutive years of drought. Nevertheless, if droughts are prolonged, tortoises are eventually going to succumb from lack of food and water.

Abnormal Behaviors. — The behavioral abnormalities observed in the tortoises prior to salvage signaled poor health or stress. The abnormalities were failure to drink, failure to hibernate, inactivity, atypical use of burrows, and lack of responsiveness. The failure to respond to the rains, and inability or unwillingness to drink free water during rains was unusual in a species that constructs water catchments for drinking (Medica et al., 1980), remembers locations of natural water catchments (Berry, 1986), drinks copious amounts of water after droughts, and is dependent on free water for physiological well-being (Nagy and Medica, 1986; Henen, 1994; Peterson, 1996a, 1996b; Henen, 1997; Henen et al. 1998).

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MCV (fl)	—	—	—	—	—	431.4	—
MCH (pg)	—	—	—	—	—	135.3	—
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Eosinophils (/µl)	—	—	—	—	—	1	—
Basophils (/µl)	—	—	2	13	24	1	19
Azurophils (/µl)	—	—	17	17	18	4	13
Icterus index (units)[1]	—	—	—	—	—	5-10	—
Plasma proteins (g/dl)[1]	—	<2.5	—	—	—	5.5	—
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Tortoises spend ca. 95% of the year underground in burrows (Nagy and Medica, 1986) where they are protected from temperature extremes and lack of moisture. Hibernation and inactivity within burrows are essential parts of the ecology and physiology of tortoises (Woodbury and Hardy, 1948; Burge, 1977; Zimmerman et al., 1994; Nagy et al. 1997; Henen et al., 1998). They thermoregulate, using the burrow as a refuge. For example, in mid-summer and early fall, adults may emerge in late afternoon and early evening to cool off during the night, thus gaining a thermal advantage at the beginning of the following day (McGinnis and Voight, 1971; KHB, unpubl. data). Failure to hibernate during late fall and winter (as observed in 5 tortoises in this study) exposes the tortoise to freezing temperatures and rain, predation, and metabolic stress. The cohabitation of burrows observed in FISS juveniles and commonly observed in captive juveniles (Bulova, 1992, 1994) is rarely seen in wild, free-ranging juveniles (Berry and Turner, 1986), although adults cohabit large, deep dens in the central and northeastern part of the Mojave Desert (Woodbury and Hardy, 1948; KHB, unpubl. data). Some cohabiting juveniles in this study were crowded out of the burrows and exposed to weather extremes. Behavioral abnormalities also have been reported in other crowded or ill chelonians (Oettle et al., 1990).

Weight Losses. — Body weights of healthy desert tortoises fluctuate by season and year, depending on rainfall and forage availability (Henen, 1994; Peterson, 1996a; Henen, 1997; Nagy et al., 1997; Christopher et al., 1999). The key issue is the greatest tolerable weight loss. We reported theoretical weight losses of 0.0 to 39.7% for all salvaged tortoises using control data bases and actual losses of 0.0 to 38.8% using historic weights for 8 tortoises (1–2, 4–5, 7–9, 11). The latter losses were slightly less than the maximum loss of 40% reported by Peterson (1996a) for adult tortoises; 41% of the tortoises in his study died (Peterson, 1994). Relationships between weight and CL are widely used as criteria for assessing health and condition of tortoises but have numerous limitations (Jacobson et al., 1993; Blakey and Kirkwood, 1995). Interpretations of the weight losses in this study were confounded by successful rehydration for 1 tortoise (5) and drinking and subsequent weight gain by 4 others (2, 4, 7, 9) shortly prior to death or salvage. An additional factor was the presence of cystic calculi in 3 tortoises (7, 10–11). Since the calculi contribute weight of nonliving material, the actual water content of these tortoises was likely to be less than expected for the weight. Without accurate, nondestructive measures of body condition (i.e., Henen 1994, 1997) body mass may only serve as a crude indicator of compromised health, in part because of the extreme physiological tolerances of desert tortoises (Nagy and Medica, 1986; Henen, 1994; Peterson, 1996a, 1996b; Henen, 1997).

Dehydration and Starvation. — The clinical signs of disease and pathologic findings from necropsies of juveniles were consistent with dehydration and starvation. The juvenile desert tortoises were in poor condition as evidenced by anemia (1) and hepatocellular and muscular atrophy. The ratio of liver mass to body weight for tortoises 3 and 4 were low, relative to a range of 2.1 to 6.0% for other tortoises (Homer et al., 1998). The stomach and small intestine of one moribund individual (3) were empty. The history of food scarcity in conjunction with weight loss, absence of coelomic adipose tissue, and atrophy of skeletal muscle and liver are indications of significant long-term malnutrition (Keymer, 1978; Jackson and Cooper, 1981). While the dermal bone of juvenile tortoises is normally incompletely developed (Wronski et al., 1992) the cortical and trabecular bone in this group of tortoises was markedly thin when compared with healthy juveniles. Osteopenia signifies a pathologic reduction in bone mass. Causes of osteopenia include starvation, disuse, old age (senility), intestinal parasitism, and deficiencies of calcium, phosphorus, or copper (Palmer, 1993). The

Table 5. Urinalyses results for 5 desert tortoises salvaged between 1990 and 1995 at three sites in the Mojave Desert, California. neg = negative, pos = positive.

Analytes	Tortoise identification numbers				
	3	6	7	10	11
Color	very dark brown	dark brown	medium brown	dark brown	dark brown
Transparency	cloudy	very flocculent	clear	very flocculent	medium cloudy
Specific gravity (g/ml)	1.020	1.040	1.020	1.035	1.013
Potassium (mEq/L)	–	39.9	7.0	–	46.9
Sodium (mEq/L)	–	0.8	0.7	–	9.2
pH	7.5	–	–	5.0	–
Protein	trace	–	–	trace	–
Glucose	neg	–	neg	neg	neg
Ketones	neg	–	–	neg	–
Bilirubin	neg	–	–	neg	–
Hemoprotein	+4	–	–	3+	–
Nitrite	neg	–	–	neg	–
Urobilinogen	0.1	–	–	0.2	–
Bacteria	pos	none	none	neg	numerous
Casts	none	none	none	none	none
Crystals	urates, tyrosine	abundant	occasional	urates, carbonates	granular material
Cells	–	moderate epithelial	occasional epithelial	–	neg
Erythrocytes	4-5	–	–	0-2	–
Leukocytes	2	–	–	0-2	–
Osmolality	291	–	–	580	–

shell osteopenia observed in the 5 juveniles was consistent with malnutrition. The dark brown urine in tortoises 2, 4, and 5 indicated concentrated urine, consistent with dehydration, and the empty bladder in tortoise 1 indicated dehydration (Peterson, 1996a). Tortoises 1–2 and 4–5 were moderately to markedly autolyzed, precluding determination of the cause of death in 3 of them. Tortoise 1 had evidence of multicentric bacterial infection that likely resulted in its death; *Pasteurella testudinis* was isolated from the nasopharynx of this tortoise. If these juveniles had not been in a predator-proof enclosure, they probably would have been preyed upon by coyotes, kit foxes, or ravens several weeks or months earlier.

For the immature and adult tortoises, the histories, clinical signs, plasma biochemical values, and urinalysis results strongly suggested that inadequate water and nutrient intake caused or were major contributors to poor condition and death. Although adult desert tortoises tolerate a wide range of hematological and biochemical values (O'Connor et al., 1994; Christopher et al., 1999), including electrolyte levels (Nagy and Medica, 1986; Peterson, 1996a), each of the salvaged immature and adult tortoises exceeded the range or 95th percentiles for 4 or more analytes for healthy tortoises. The observed departures were consistent with starvation and dehydration. For example, elevated levels of sodium, chloride, BUN, and plasma and urinary osmolality occurred in hydrically stressed tortoises (Nagy and Medica, 1986; O'Connor et al., 1994; Peterson, 1996a, 1996b; Christopher, 1999; Christopher et al., 1999). The dark brown, concentrated urine in 5 tortoises (3, 6–7, 10–11) and the high urine specific gravity and osmolality in 2 tortoises (6, 10) were indicators of dehydration. Low calcium levels may indicate a dietary imbalance (Palmer, 1993). Weights were low for CL and tortoises exhibited mild to severe emaciation and cachexia, atrophy of skeletal muscle, and weakness. Weight loss, sunken eyes, muscular atrophy and similar biochemical abnormalities were observed in a desert tortoise trapped underground for 11 months (Christopher, 1999). Similar degenerative signs and lack of activity were also observed in giant *Geochelone* sp. with wasting disease (Samour et al., 1986).

The consistent microscopic abnormalities in the tortoises included mild to severe diffuse hemosiderosis of the liver and atrophy or disappearance of the thymus. Hemosiderosis of the liver and atrophy of the thymus occurred in *G. agassizii* with URTD (Jacobson et al., 1991) but not in healthy animals. Hepatic hemosiderosis was a common finding in ill tortoises associated with a variety of diseases including urolithiasis (Homer et al., 1998) and appears to be a non-specific indicator of chronic disease or debilitation. Involution of the thymus has also been reported in chelonians with malnutrition (Borysenko and Lewis, 1979). Hepatocellular hemosiderosis was found in river cooters (*Pseudemys concinna*) with shell disease (Garner et al., 1997), and *Geochelone* sp. with wasting disease (Samour et al., 1986). The pneumonia in tortoise 9 probably developed within 2 weeks of death and was a terminal event in an already debilitated tortoise. Urolithiasis in the 3 older tortoises may have been linked to improper nutrition, limited access to water, and dehydration (Bennett and Mader, 1996). There were no other pre-existing lesions to explain the cause of death of adult tortoises.

Point of No Return. — Dehydrated and starved tortoises can reach a point at which recovery is impossible, even if water and/or food become available. We believe that 9 of the tortoises had already passed the point of recovery before rains arrived and water was available. Although some drank and a few gained weight, they were incapable of a positive physiologic response. For these tortoises, the rain came too late, and water alone was insufficient to reverse the overall physiologic decline. For example, despite tortoise 9 drinking free water a few days before salvage, chloride and sodium levels were high, although its BUN was in the normal range; the tortoise already had developed pneumonia.

Potential Effects of Handling and Research Procedures. — Droughts occurring in 1989–90 and 1993–94 did not result in mass deaths in the associated tortoise populations. The tortoises that we salvaged and found dead represented only a small fraction of their respective populations. In wildlife populations, the very young and old are usually the more vulnerable age groups (Elkan, 1981; Cooper and Laurie, 1987), and 8 of the 11 tortoises were in these categories. Since 3 of the tortoises were not in this age class, predisposing factors other than age may have contributed in one way or another to vulnerability of these individuals, and probably to others as well. Handling and research procedures which are invasive are possible factors. Potential stress from handling is likely to vary considerably depending on the amounts of time, frequency, and activity involved. Procedures likely to be minimally stressful are weighing, measuring, and photographing for < 15 minutes compared with somewhat more stressful procedures such as notching of the shell, transporting long distances in a pack or vehicle for processing, and storing overnight in buckets. More invasive procedures (e.g., attachment of radiotransmitters, phlebotomy, cardiocentesis, cystocentesis, insertion of temperature probes for cloacal temperatures, and urine collection at the cloaca with capillary tubes) can be even more stressful. Tortoises from FISS and Ivanpah Valley (1–2, 4–5, 8) which were salvaged dead, were previously handled frequently and experienced invasive procedures (Nagy et al., 1997; Peterson, 1994, 1996a).

At the FISS experimental enclosure, we suggest that crowding and deteriorated habitat contributed to the low growth rates, low weights, and deaths of these juveniles. Densities of captive juveniles were 375 to 800 times higher than densities recorded for free-ranging tortoises in similar nearby habitats (Morafka et al., 1997). The juveniles had histories of abnormally low mean annual growth. Although precipitation was above the long-term means for both annual and winter rainfall in 3 of the 4 years (Fig. 1), the mean annual change in PL was about 50% of the mean annual growth rate of 9.1 ± 0.4 mm PL (4.3–14.4 mm, $n = 22$) reported for juvenile and immature tortoises in similar habitat and enclosures in southern Nevada (Medica et al.,

1975). The Nevada tortoises were in three 9-ha enclosures at a density of 0.81 tortoises per ha, about 0.2% of the density of the FISS juveniles. Other reptiles exhibit lower growth and survival rates and lower weights when crowded (Tubbs and Ferguson, 1976; Elsey et al., 1990).

In Ivanpah Valley in 1990, tortoise 8 was part of a research program in which 41% of the 22 adult tortoises died (Peterson, 1994). Tortoises in this research program were often transported distances of a few kilometers for sampling, and blood and urine were collected almost monthly using jugular phlebotomy and cystocentesis, respectively. At that time, the accepted method of urine collection was cystocentesis. Subsequently, cystocentesis was discontinued as a procedure for collecting urine because of circumstantial evidence that it caused or contributed to peritonitis and bladder abscesses in a tortoise at another research site (Christopher et al., in press). The high death rates in adult tortoises in the Ivanpah Valley research program in 1990 are in sharp contrast to the stability shown in adult tortoise densities at an adjacent site where tortoises were sampled intermittently for long-term demographic research but minimally disturbed. At the demographic study site, densities of adults did not change significantly before, during, or after the drought (Berry and Medica, 1995; Fig. 2); only 2.3% of live adult tortoises (n = 83) observed in 1990 were found dead 4 years later, in 1994. However, the comparison of death rates between these adjacent sites is not ideal.

In summary, while desert tortoises are adapted to survive droughts, they can only survive droughts for limited periods. Deaths from drought may occur several weeks or months after the drought has broken. During droughts, field personnel can use behavioral abnormalities such as lethargy, failure to drink, and failure to seek shelter at appropriate times as cues to health status. Key clinical signs of stress are weight loss, weakness, cachexia, sunken eyes, and atrophy of skeletal muscle. Typical abnormalities in hematological and plasma biochemical analytes are likely to be low packed cell volumes and heterophil counts, hypocalcemia, hyperbilirubinemia, marked azotemia, and elevated levels of sodium and chloride.

Recommendations

We support recommendations in the Desert Tortoise Recovery (Mojave Population) Plan (U.S. Fish and Wildlife Service, 1994) that discourage manipulative or intrusive monitoring and research procedures on desert tortoises in important (critical) habitats (Desert Wildlife Management Areas), because of the potential for increasing mortality rates locally. We further recommend that controlled experiments be conducted to test hypotheses on effects of handling and research procedures, especially those considered invasive or intrusive, on wild, free-ranging tortoises of different sizes under a variety of climatic conditions. In some cases, wild tortoises removed from lands slated for development are likely subjects for this type of experimental research. We also recommend that control groups with minimal handling and no invasive procedures be included as part of research protocols when invasive procedures are planned.

The point of no return is an important research subject that needs attention. It is an elusive point, most likely with a series of contributing factors acting synergistically. With the wide physiological tolerances of tortoises, at what point will a simple rain allow a tortoise to retreat from the edge of death and at what point will a drink of water be insufficient? While the data in this paper provide a basic foundation for developing hypotheses, they are but a first stage in a complex process. Considerable field and experimental work will be required to develop a multivariate index or predictive model, including survivorship data and correlates for each variable.

In the meantime, scientists conducting research on wild desert tortoises need to be acutely aware of and record data on environmental stressors, as well as the potential stresses induced by handling and more or less invasive procedures. Since the determination of cause or causes of death and predisposing factors in research animals can be difficult, we recommend careful and detailed records of all handling procedures, clinical signs of health and disease, blood samples, and tests for infectious diseases. Moribund tortoises should be salvaged and necropsied prior to death. Together scientists and the permitting agencies can develop protocols to minimize negative impacts to tortoises. For example, in drought years, the frequency and time spent in handling tortoises and the number and type of invasive procedures could be minimized, or the project could be delayed until less stressful conditions prevail.

ACKNOWLEDGMENTS

Necropsies conducted by ERJ and J. Gaskin for tortoises salvaged from Goffs and Ivanpah Valley were funded by the Bureau of Land Management (BLM, Contract No. CA-950-CT9-28). Necropsies conducted for tortoises salvaged from the National Training Center at Ft. Irwin were funded by the BLM under Contract No. B950-C1-0062) to BLH, and by the National Training Center at Ft. Irwin through a Memorandum of Understanding to the National Biological Service (Research Work Order No. 138) to BLH. All desert tortoises were in research programs under Scientific Research Permits held by KHB or D.J. Morafka. Desert tortoises were salvaged through the BLM's salvage authority and under Scientific Research Permits to KHB. The manuscript especially benefitted from reviews by M.M. Christopher, B. Henen, D.J. Morafka, A. Rhodin, and C. Peterson. Thanks are due to C. Peterson, B. Henen, and K. Nagy for field work, to J.M. Gaskin for lab support, to W.P. Alley for statistical support, and W. Quillman for financial support and review.

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APPENDICES

Appendix 1. Control data bases used for regression equations comparing length/weight relationships of 11 salvaged desert tortoises from the Mojave Desert, California. N = number of samples; N_1 = number of individual tortoises used for N. Some sample tortoises have more than one data point over the range of years of data collection. Salvaged tortoises from FISS (1–2, 4–5) were matched with tortoises from the Lucerne Valley database and Goffs and Ivanpah tortoises were matched with tortoises from the same sites.

Sample no. of salvaged tortoise	Criteria for control tortoises CL range (mm)	Weight range (g)	N, (N_1)	Range of years over which data were collected
1, 2, 4, 5	47-80	28-120	36 (34)	1980-94
3	42-84	21-120	52 (50)	1978-94
6	125-155	400-825	19 (9)	1983-94
7	228-250	2000-3400	75 (15)	1980-94
8	240-262	2100-3750	49 (12)	1978-94
9	255-275	2400-4582	179 (20)	1978-94
10	255-280	3125-4850	56 (15)	1980-94
11	270-285	2775-4800	76 (8)	1978-94

Appendix 2. Laboratory tests performed (+) or not (–) for the 11 desert tortoises salvaged between 1990 to 1995 from three sites in the California deserts. a = PCV and plasma protein only; b = calcium and phosphorus only; c = tested in April 1994, before release from the enclosure at the Fort Irwin study site.

	Tortoise identification numbers										
Determinations	1	2	3	4	5	6	7	8	9	10	11
Hematology	–	–	+	–	+[a]	+	+	–	+	+	+
Plasma biochemistry	–	–	–	–	+[b]	+	+	–	+	+	+
Urinalysis	–	–	–	–	+	+	+	–	–	+	+
cystic calculi	–	–	–	–	–	–	+	–	–	+	+
Serology	–	–	+[c]	–	–	–	–	–	–	+	–
Mycoplasmosis: swabs ELISA, PCR, culture	–	–	+	–	–	–	–	–	–	+	–
Microbial exam	+	+	+	+	+	+	+	–	+	+	+
aerobic bacteria, nasal cavity	+	–	–	+	+	+	+	–	–	+	+
aerobic bacteria, colon	+	+	+	+	+	–	–	–	–	+	–
aerobic bacteria, lung	–	–	–	–	–	–	–	–	–	+	–
Intestinal parasitic exam	+	–	+	+	–	+	+	–	+	+	+

Received: 3 October 2000
Reviewed: 13 August 2002
Revised and Accepted: 9 October 2002