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Desert Tortoise Annotated Bibliography, 1991–2015

Open-File Report 2016–1023

U.S. Department of the Interior
U.S. Geological Survey

Cover: Photograph showing an adult male desert tortoise (*Gopherus agassizii*) from one of the long-term health and disease research sites in central Mojave Desert, San Bernardino County, California. Photograph by Sara Hanner, U.S. Geological Survey, spring 2012.

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SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Director

U.S. Geological Survey, Reston, Virginia: 2016

Suggested citation:
Berry, K.H., Lyren, L.M., Mack, J.S., Brand, L.A., and Wood, D.A., 2016, Desert tortoise annotated bibliography, 1991–2015: U.S. Geological Survey Open-File Report 2016-1023, 312 p., http://dx.doi.org/10.3133/ofr20161023.

ISSN 2331-1258 (online)

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Conversion Factors

International System of Units to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
kilometer (km)	0.5400	mile, nautical (nmi)
meter (m)	1.094	yard (yd)
	Area	
square meter (m^2)	0.0002471	acre
hectare (ha)	2.471	acre
square hectometer (hm^2)	2.471	acre
square kilometer (km^2)	247.1	acre
square meter (m^2)	10.76	square foot (ft^2)
square hectometer (hm^2)	0.003861	section (640 acres or 1 square mile)
hectare (ha)	0.003861	square mile (mi^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
milliliter (mL)	0.03382	ounce, fluid (fl. oz)
liter (L)	33.82	ounce, fluid (fl. oz)
	Pressure	
kilopascal (kPa)	0.2961	inch of mercury at 60°F (in Hg)
	Mass	
nanogram (ng)	3.52739	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.

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Chapter 1.—Introduction

Agassiz's desert tortoise, *Gopherus agassizii*, was considered a single species for 150 years after its discovery by James Cooper (1861), with a geographic range extending from southeastern California, southern Nevada, and southwestern Utah southward into northern Sinaloa, Mexico (Murphy and others, 2011). What was once *G. agassizii* is now recognized as a complex composed of three sister species, *G. agassizii*, *G. morafkai*, and *G. evgoodei* (Murphy and others, 2011; Edwards and others, 2016) (fig. 1). The geographic range of Agassiz's Desert Tortoise (*G. agassizii*) is now limited to north and west of the Colorado River (Murphy and others, 2011), with the exception of a small population in northwestern Arizona (Edwards and others, 2015). This annotated bibliography is based on peer-reviewed journal articles published between January 1991 and December 2015 on Agassiz's Desert Tortoise, with the geographic range as defined by Murphy and others (2011). Studies pertaining to other species of *Gopherus* (e.g., *G. morafkai*), were included only when associated with *G. agassizii*. In addition to articles pertaining directly to desert tortoises, we compiled articles concerning threats to desert tortoises and the habitats they occupy. Similarly, we only included studies that encompass other habitat types when they were directly compared with habitats of *G. agassizii*.

Agassiz's Desert Tortoise (hereinafter called desert tortoise) is a state- and federally-listed threatened species (U.S. Fish and Wildlife Service, 1990; California Department of Fish and Game, 2015). The first population federally listed as threatened occurred on the Beaver Dam Slope, Utah (U.S. Fish and Wildlife Service, 1980). In 1990, the entire geographic range north and west of the Colorado River was federally listed as threatened (U.S. Fish and Wildlife Service, 1990), with the exception being a small population in northwestern Arizona. The purpose of this annotated bibliography is to support recovery efforts for the species, because populations have continued to decline in spite of designation of critical habitat and publication of a recovery plan (U.S. Fish and Wildlife Service, 1994). For example, between 2005 and 2014, populations in critical habitats declined about 50% (U.S. Fish and Wildlife Service, 2015).

We obtained articles in this compilation through keyword searches applied within search engines available through the U.S. Geological Survey Library. Search criteria included the keywords Mojave, desert tortoise, and *Gopherus agassizii* in combinations with the following keywords: alien, burro, cattle, climate change, disease, exotic, fire, highway, invasive, livestock, mining, road, sheep, soil erosion, renewable energy, utility line, or vehicle. In an effort to be complete, search results were compared against known publications and additional references provided by research scientists who were or are working on the species or its habitats. Our focus was on published papers in the peer-reviewed literature, and includes both research articles and reviews. While we recognized that additional information is contained in proceedings, technical reports, agency reports, theses and dissertations, and the gray literature, these sources were beyond the scope of this annotated bibliography.

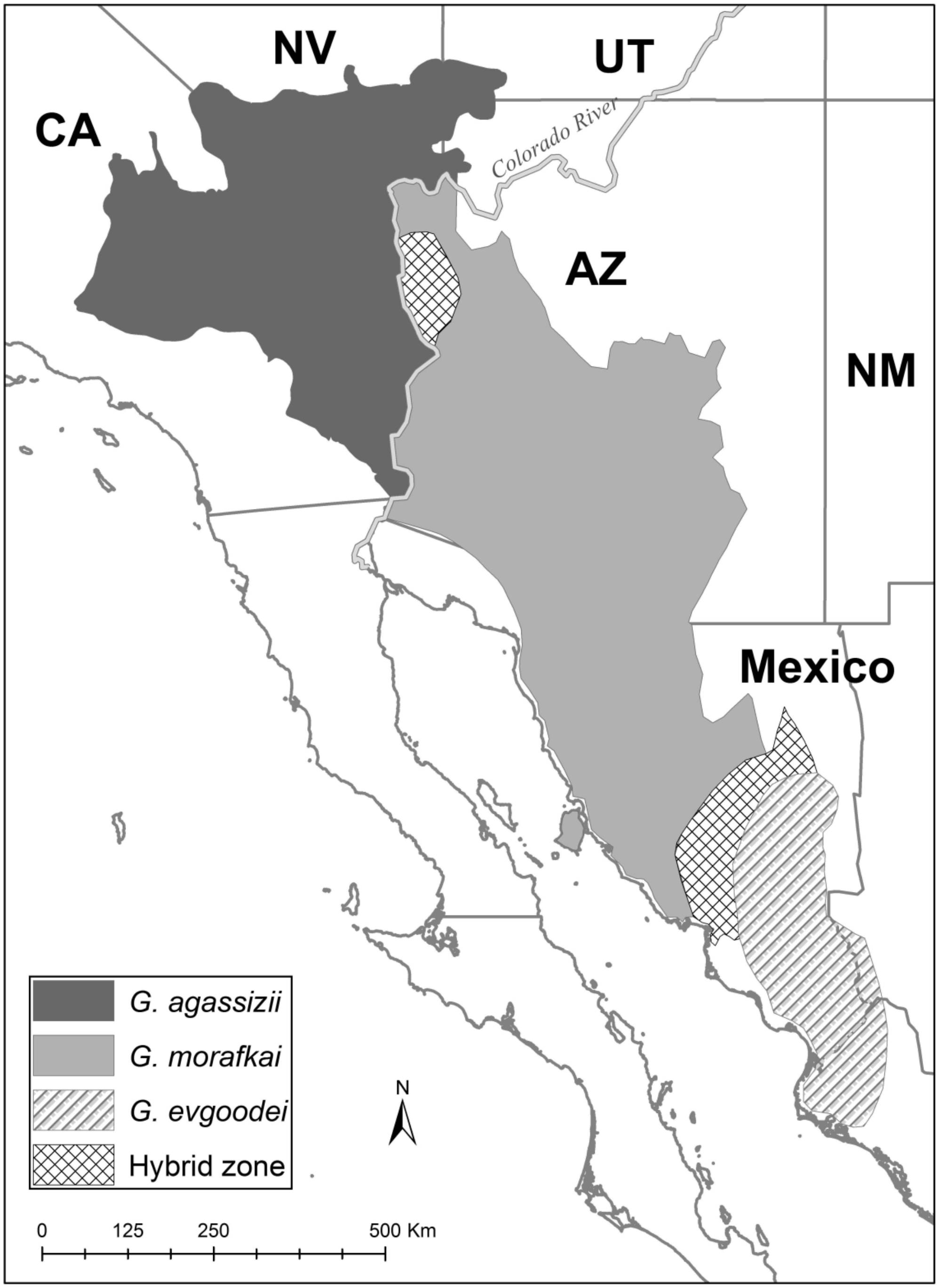

Figure 1. The geographic range of Agassiz's desert tortoise (*Gopherus agassizii*) once extended from southeastern California, southern Nevada, and southwestern Utah in the north through Arizona and into the states of Sonora and Sinaloa, Mexico, in the south (Murphy and others, 2011). This area is now occupied by three sister species: *G. agassizii*, *G. morafkai*, and *G. evgoodei* (Edwards and others, 2016). Agassiz's desert tortoise retains approximately one-third of its former geographic range in the north, where it occurs in the Mojave and western Sonoran or Colorado Deserts.

In an effort to provide an unbiased representation of findings or interpretation from a given study, we have often compiled the author's own words or phrases, spelling, or have summarized or paraphrased their original words for accuracy and brevity. We have not included such statements in quotation marks. For example, where authors used the spelling of Mohave for the place name Mohave Desert, we used the authors' spelling, even though the correct spelling is Mojave Desert.

This document is intended only for reference. While the annotations can provide an overview of relevant, available materials on a given topic for the period 1991–2015, we discourage direct citation from the summaries provided here. In particular, annotations from review articles are themselves dependent upon multiple sources. It is the reader's responsibility to obtain a complete account of the available information by referring to the primary literature.

The format provides annotations of the literature of Agassiz's Desert Tortoise by topic. We have not followed the format of the previous annotated bibliographies for this species (Hohman and others, 1980; Grover and DeFalco, 1995) because the type and amount of material available differs. This annotated bibliography is a stand-alone document for 1991 through December 2015, and begins with the end point of the Grover and DeFalco (1995) annotated bibliography. Our document will add to information published in the two previous annotated bibliographies. We use headings that accommodate the substantial literature developed in the 24 years following coverage in Grover and DeFalco (1995), such as additional work pertaining to taxonomy, genetics, health and disease, physiology, behavior, habitat deterioration, subsidized predators, restoration, and climate change. We also reviewed the substantial body of work on methodology and sampling design. Within a given subheading, the literature is treated chronologically from earliest to most recent. With each new author, we repeated the genus and species of the organism under discussion. Similarly, we did not use acronyms or initialisms except within the annotations of a particular article, because some users may seek a particular author or subject and may not read the entire document from the beginning.

In general, we avoided citing chapters in books; yet we made exceptions where the material is new, directly related to *G. agassizii* or its habitat, and is not completely available elsewhere. Several new and valuable books include information on health and diseases of desert tortoises (Mader, 2006; Jacobson, 2007), on the extant North American tortoises in the genus *Gopherus* (Rostal and others, 2014), and on the Mojave Desert, with relevant chapters on geology, vegetation, habitat deterioration, habitat recovery, and desert restoration (Webb, Belnap, and Thomas, 2009). We encourage our readers to make use of these books, in addition to the publications cited in the annotated bibliography.

Acknowledgments

We are very grateful for advice and reviews for one or more chapters to Scott Abella, William Boarman, Mary M. Christopher, Taylor Edwards, Brian Henen, Elliott R. Jacobson, Rebecca Jones, Lisa Hazard, Lawrence LaPré, Ed LaRue, Kenneth Nagy, Mark Massar, Robert Murphy, Michael Tuma, Andrew Walde, and James Weigand. Their advice, corrections, and proposed revisions enhanced the quality of the annotated bibliography and are much appreciated. Thanks are due to William Perry for creating figure 1. The U.S. Geological Survey provided funding for this endeavor and was additionally supported by a donation from the Desert Tortoise Council, Inc.

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Chapter 2.—Paleontology, Paleoecology, Taxonomy, and Genetics

I. Paleontology and the Fossil Record

A. Holman (1995) summarized Pleistocene fossil sites of special interest and containing species of *Gopherus*. He said that regional Pleistocene occurrences are in the Rocky Mountain/Great Basin and Pacific coastal regions.

1. Rocky Mountain, Great Basin region: Curtis Ranch Fauna, AZ, early Irvingtonian; Dry Cave Fauna, NM, 34,000 and 25,000 years before present (ybp); Shelter Cave, NM, late Wisconsinan; Gypsum Cave, NV, 11,690 ± 250 ybp; Conkling Cave, NM, late Wisconsinan; Robledo Cave, NM, Pleistocene; Rampart Cave, AZ, 19,000 and 9,500 ybp; Vulture Canyon, AZ, 13,000 and 12,000 ybp; and Welton Hills, AZ, 11,000 to about 9,000 ybp, very late Pleistocene to very early Holocene.

2. Pacific region: Costeau Pit, CA, 40,000 ybp (all taxa represent extant forms and all occurred in the vicinity of the deposit in historical times except for *Gopherus*, which occurs east of the site today); McKittrick Asphalt, CA, late Wisconsinan times; Mescal Cave, CA, late Pleistocene but possibly some Holocene species; Schuiling Cave, CA, late Pleistocene; and Whipple Mountain Site, CA, 10,500 and 10,000 ybp.

3. Changes in thinking about the fossil record: “One of the important outgrowths of the study of Pleistocene amphibians and reptiles in the Rocky Mountain/Great Basin region is the elimination of the scenario that the desert herpetofauna of the American Southwest retreated to Mexican refugia during glacial periods, as many desert species were found in their original ranges during the late Wisconsinan glacial age.” Desert species that remained in place were able to adapt to changes, from desert vegetation to pinyon-juniper forest. *Gopherus agassizii* also was found far east of its present range at the Dry Cave site near the Texas border in southeastern New Mexico.

4. Extinction patterns in the Pleistocene herpetological taxa: In North America, the Testudines conservatively had 6 unquestioned extinct taxa out of 33 identified taxa; all were tortoises in the genera *Geochelone* and *Gopherus*. In the Pacific region, the herpetofauna has remained remarkably stable for the past 14,000 years while vegetation has changed remarkably. From 40,000 years to the present, only 2 species—a rattlesnake and a possible tortoise (*Geochelone* sp.)—perhaps reached California, and became extinct. Predation by paleohumans may have been a cause.

B. Wang and others (1999) challenged the traditional view that Testudines should be regarded as the surviving clade of anapsid reptiles rather than be classified with the diapsid reptiles (snakes, lizards, and crocodiles). They analyzed neuroendocrine peptides (insulin, pancreatic polypeptide, neuropeptide Y, galanin, somatostatin, substance P, and neuropeptide γ) from *Gopherus agassizii*. Their results supported recent morphologically based cladistic studies that indicated Testudines shared a closer relationship with diapsid reptiles (Crocodilians, Archosauria clade) than the “classical” view that places them as the most basal living amniotes, anapsid reptiles.

C. Recent Fossil Reports and Evaluations

1. Jass and Bell (2010) reported that 2 fossil specimens from Cathedral Cave, NV, in the east-central Great Basin Desert were diagnosed as desert tortoises (*G. agassizii*) with maximum age 146,020–151,200 ± 4,400 years. This represents the first record on Pleistocene turtles from within the hydrographic Great Basin and suggests that turtles were extirpated from the Great Basin by environmental or other factors sometime between deposition of Cathedral Cave and the latest Pleistocene, when the reptilian assemblage across the Great Basin was devoid of turtles.
2. Jass and others (2014) regional, depositional settings, and richness of modern vs. Pleistocene turtles in North America. The authors based their study on the fossil record as compiled by Holman (1995) and others and statistically analyzed effects of 8 geographic regions, caves vs. open air settings, and time. They reported significant differences in regions by time but not for depositional settings. Pleistocene richness was significantly lower than for modern times, with the Pacific region lower in richness in both time periods than other regions. The authors state that low richness of species in some regions likely reflects actual low richness of past environments.

II. Paleoecology of Desert Tortoise Ancestors

Morafka and Berry (2002), drawing on a literature review of the fossil record of tortoises, the biogeography of testudinids as a group, and studies across the Mojave and northwestern Sonoran Deserts, addressed two questions: (1) Did *G. agassizii* evolve in direct response to selective pressures of a desert environment and is it a “relict” species? or (2) was it a more generalized omnivore in which pre-existing exaptations made survival possible as more arid climates developed in the Late Tertiary and Quaternary Periods? They proposed and evaluated five tests of the status of *Gopherus agassizii* as a desert species: (1) paleoecology, (2) anatomical and physiological, (3) climatic region association, (4) ecosystem association, and (5) forage exploitation. They reported:

A. Modern climate and vegetation typical for contemporary populations of desert tortoises have only developed during perhaps the most recent 1% of its 3–5 million year history as a distinct species, and especially during the last 7,000 years.

B. Ancestors of desert tortoises stabilized as a modern morph about 17–19 million years ago, 12 million years before the formation of deserts in North America. This suggested that current physiological and behavioral mechanisms for avoiding desert stressors were symplesiomorphies (primitive character states) or exaptations from various periods, including slow metabolic rates, high tolerance for osmotic flux, large brittle-shelled eggs, herbivory, expansive digestive tract, and burrow excavation, rather than new adaptations to desert conditions in the Mojave and northwestern Sonoran Deserts.

C. Testudinids as a group and *G. agassizii* in particular are not confined to deserts but range across a variety of climates, including warm temperate and tropical. They appear to be excluded from extremely arid zones with < 50 to 80 mm mean annual precipitation.

D. Tortoises are opportunistic generalists in terms of foraging, using five major groups of plants: (1) annual forbs (winter or summer ephemerals), (2) annual grasses (winter or summer), (3) cacti, (4) native perennial bunch grasses, and (5) herbaceous perennial shrubs. They do not forage on sclerophyllous vegetation, characteristic of much of the desert environments. Food plants have origins and ecogeographical associations from the Arcto-Tertiary and Madro-Tertiary geofloras as well as warm temperate and desert elements (a combination of C3, C4, and CAM plant species).

III. Phylogenetic Relationships

Lamb and Lydeard (1994) constructed a molecular phylogeny representing the four extant species of gopher tortoises (*Gopherus agassizii*, *G. berlandieri*, *G. flavomarginatus*, and *G. polyphemus*) and several outgroup taxa. They used a cladistics analysis of the mitochondrial cytochrome *b* sequence to obtain a matrilineal genealogy. In addition to *Gopherus*, they evaluated the maternal history in relationship to others within the family Testudinidae and two batagurids, *Manouria* and *Cuora*. They reported the following:

A. The mitochondrial DNA (mtDNA) phylogeny identified a monophyletic genus, *Gopherus*, that contained two well-supported clades: (1) the *agassizii* clade (*G. agassizii* and *G. berlandieri*), and (2) the *polyphemus* clade (*G. polyphemus* and *G. flavomarginatus*).

B. The proposed slow rate of mtDNA evolution in turtles (proposed by Avise and others, 1992) received additional support from cytochrome *b* sequence comparisons for other turtles, and indicated an average sequence divergence rate of about 0.4% per mya (million years ago) for turtle mtDNA. Using the divergence rate of about 0.4% per mya, the authors dated the divergence between the two major clades at 17–18 mya, a time frame consistent with environmental changes at the Lower-Middle Miocene interface. Additionally, they estimated that the genetically distinct Mojave and Sonoran populations of the desert tortoise diverged about 6 mya, a time frame consistent with the Pliocene marine transgression that extended along the Gulf of California north to southern Nevada.

C. Sequence comparisons of selected batgurid, emydid, and testudinid taxa (Testuinoidea) yielded a phylogeny demonstrating close phylogenetic affinities between the Testudinidae and the Bataguridae.

IV. Taxonomy

A. Taxonomic Classification of *Gopherus agassizii*

1. Berry and others (2002a), using genetic, morphological, physiological, behavioral, and ecological differences described in the literature, reported that *Gopherus agassizii* is best viewed as a composite of at least two and as many as four species.

2. Murphy and others (2007) conducted a genetic assessment of the six Recovery Units for desert tortoises (*Gopherus agassizii*). The Recovery Units were established by the U.S. Fish and Wildlife Service in the 1994 Recovery Plan for the species. The authors analyzed both the mtDNA from 125 tortoises and microsatellites from 628 tortoises and 31 study sites representing all Recovery Units. The mtDNA analysis revealed large divergence between populations west of the Colorado River and populations south and east of the Colorado River in the Sonoran Desert of Arizona.
3. Murphy and others (2011) used a suite of characters including morphological, physiological, ecological, and molecular data as a basis for splitting *G. agassizii* into two species: (a) Agassiz's desert tortoise (*G. agassizii*) to the west and north of the Colorado River, and (b) Morafka's desert tortoise (*G. morafkai*) to the east and south of the Colorado River. *Gopherus morafkai* was described as a new species.
    a. The type locality of the sole remaining specimen of *G. agassizii* was confirmed genetically and through historical records as from California. This specimen is now the lectotype of the species with the common name of Agassiz's desert tortoise.
    b. The specimen of *Gopherus lepidocephalus* from Baja, California, Mexico, was confirmed as being *G. agassizii* genetically; thus *G. lepidocephalus* was a junior synonym of *G. agassizii*.
    c. The holotype of the new species, *G. morafkai*, was a juvenile from the Tucson area, Pima County, AZ, collected July 9, 1912.

B. Traits Distinguishing Species

1. Germano (1993) measured 32 shell and scute characteristics of 4 species of *Gopherus* from North America. Because shell morphology varies greatly among populations of *G. agassizii*, the author separated *G. agassizii* into Mohave, Sonoran, and Sinaloan population groups. Based on carapace lengths, *G. berlandieri* was the smallest (155.3 ± 8.43 mm SD), about two times smaller than the largest, *G. flavomarginatus* (311.9 ± 8.38 mm SD). The Mojave population of *G. agassizii* was intermediate (220.2 ± 8.89 mm SD), but smaller than *G. polyphemus* (246.1 ± 11.7 mm SD), which was similar to the Sonoran (232.3 ± 10.4 mm SD) and Sinaloan (218.7 ± 10.2 mm SD) populations. For the Mojave population of *G. agassizii*, males are larger than females (226.8 ± 9.89 vs. 212.9 ± 9.87 mm SD). *Gopherus agassizii* from the Mojave Desert are significantly (a) wider than either the Sonoran or Sinaloan populations, (b) more domed than the Sinaloan population, and (c) longer in their gulars than the Sonoran population. The author said that differences in size and shape of North American tortoise shells were not related to genetic differences or regional climate.

2. Murphy and others (2011) reported that the recently described, new species of *Gopherus*, *G. morafkai*, can be separated from *G. agassizii* based on having a narrower shell, shorter gular scutes, shorter projections of the anal scutes, and in having a flatter, pear-shaped carapace.

    a. Ecologically, *G. morafkai* prefers slopes and rocky hillsides where it typically occupies excavated or eroded burrows underneath rocks or boulders, whereas *G. agassizii* primarily occurs in valleys and alluvial fans. In contrast with *G. agassizii*, *G. morafkai* exhibits both spring (mid-March–May) and late summer (late July–late September) activity periods following monsoonal rains.

    b. The locations of *G. agassizii* and *G. morafkai* are different, with the exception of a previously described population with two different mtDNA genotypes in the Black Mountains of northwestern Arizona (McLuckie and others, 1999): *G. morafkai* occurs south and east of the Colorado River and *G. agassizii* occurs north and west of the Colorado River.

C. Reiber and others (1999) conducted experimental studies with 21 juvenile tortoises maintained at 19, 28, or 37 °C for 4 months post-hatching. The objectives were to evaluate if thermal conditions influenced growth rate, shell morphology, and behavioral thermoregulation. After 4 months, tortoises held at 37 °C showed significant changes in body form when compared to the 28 and 19 °C groups. At 13 months of age, mean plastron length and maximum height of the 37 °C juveniles were reduced as compared to the 19 and 28 °C tortoises. This flat body form resulted in high surface area to volume ratio, significantly different from the two other recruitment groups. The study demonstrated phenotypic plasticity in growth, shell shape, and thermoregulatory behavior, and physiological set points in response to post-hatching maintenance temperature, which has long-lasting consequences. This study may explain the large amount of variation in shell morphology.

V. Genetics of Wild Tortoises

A. Development of Microsatellites for Evaluating Genera and Populations

1. Edwards and others (2003) studied variation in microsatellites in desert tortoise populations in the Sonoran Desert and compared findings with some Mojave populations. Of 6 microsatellite loci that exhibited variation among 170 individuals from 9 Sonoran populations in southern Arizona, all were polymorphic and 3 deviated significantly from Hardy-Weinberg expectations. These deviations were likely due to a population structure in tortoises characterized by isolation-by-distance. Of these 6 loci tested on 20–40 individual Mojave desert tortoises from 4 locations, one locus was monomorphic and 4 expressed alleles outside the range obtained for Sonoran samples. Only 4 of 6 loci for Mojave tortoises could be amplified using the same PCR conditions optimized for Sonoran tortoises, possibly due to sequence divergence between the western Mojave and Sonoran Desert regions.

2. Hagerty and others (2008) developed 14 novel tri- and tetranucleotide microsatellite loci for use in population genetic studies of the desert tortoise.
    a. Microsatellite loci were highly polymorphic across 3 populations (total of 87 individuals) from Nevada, Utah, and California populations in the Mojave Desert.
    b. Observed number of alleles ranged from 4 to 33.
    c. No linkage disequilibrium for any pair of loci or significant deviation from Hardy-Weinberg equilibrium was detected.
3. Edwards and others (2011) identified 9 dinucleotide microsatellite loci and assessed their utility in Mojave and Sonoran populations of desert tortoises (40 individuals each).
    a. Eight of the 9 loci were variable, and the observed number of alleles across all samples ranged from 8 to 16.
    b. The observed number of alleles for the Mojave populations ranged from 2 to 11.

B. Regional and Local Genetic Variation

1. Britten and others (1997) identified 5 genetic clusters (or Management Units) of desert tortoises in the northeastern Mojave Desert on the basis of allozyme and mtDNA variation that exhibited concordant geographic patterns with the ecologically based Desert Wildlife Management Areas within Nevada and Utah. The 5 Management Units (MU) were described as (a) the Southern Las Vegas Valley MU, (b) the Northern Las Vegas Valley MU, (c) the Piute Valley MU, (d) the Beaver Dam Slope MU, and (e) the Amargosa Desert/Pahrump Valley MU. They used blood samples from 236 tortoises representing 15 sites in Nevada and Utah.
    a. Geographic variation of genetic data was consistent with an isolation-by-distance model of gene flow and partial habitat isolation. The Piute Valley MU was noted to be partially isolated from the southern Las Vegas Valley by the McCullough Range to the west and north and from Lake Mead to the east. Further evidence for isolation was noted for the Amargosa Desert/Pahrump Valley MU due to the lack of tortoise habitat to the east by the Spring Mountains and the Spotted and Specter Ranges.
    b. A cluster of transitional populations lying between the northern and southern Las Vegas Valley MU was strongly suggestive of isolation-by-distance. Habitat isolation occurred at Piute Valley, which was partially isolated from the southern Las Vegas Valley by the McCullough Range to the west and north and Lake Mead to the east. The Amargosa Desert/Pahrump Valley was also almost completely isolated from tortoise habitat to the east by the Spring Mountains and the Spotted and Specter Ranges.

c. An important question pertained to how effective proposed Desert Wildlife Management Areas (DWMAs) were in preserving genetically delineated MUs among northeastern Mojave desert tortoises: four MUs (Beaver Dam Slope, northern and southern Las Vegas Valley, and Piute Valley) were represented by proposed Desert Wildlife Management Areas (DWMA). However, none of the three transitional samples between the north and south Las Vegas Valley were contained within a DWMA. Although these intermediate populations contained no unique genetic constituents, they provided important genetic and demographic links between the two segments of the Las Vegas Valley population in a rapidly growing urban area where absence of dispersal corridors or other mechanisms that promote gene flow eventually may isolate population segments. Populations in the Amargosa Desert/Pahrump Valley also were not contained with any proposed DWMA.

2. Latch and others (2011) identified landscape features that influence desert tortoise gene flow at the local scale differently than at a larger regional scale. They genotyped 859 tortoises sampled across the Fort Irwin National Training Center in the central Mojave Desert and identified two subpopulations.

    a. Although genetic differentiation between the subpopulations was low, landscape genetic analysis identified both slope and roads as variables significantly influencing gene flow within this local population.

    b. Although steep slopes affected the genetic structure of the population more than roads, the short time lag between emergence of roads as a barrier and detection of genetic effect indicates that roads may become increasingly important in shaping the evolutionary trajectory of tortoise populations. The ability to detect barriers to local gene flow is important to consider for translocations, captive breeding and release, and reserve and corridor design.

    c. The authors' results suggested that recent landscape changes can affect gene flow at a local scale, and that such effects can be detected using genetic data.

C. Genetics at a Landscape Scale

1. Murphy and others (2007) conducted a genetic assessment of the six Recovery Units for the 1994 Recovery Plan for the federally listed desert tortoise (*Gopherus agassizii*). Within the populations of tortoises occurring north and west of the Colorado River, they identified two major sublineages that included one broadly distributed haplogroup (A) and one haplogroup from the Northeastern Mojave (B), using mtDNA sequences from 125 tortoises. They:

    a. Reported that genetic structuring was strongly associated with geography, isolation-by-distance, and the limited dispersion of individual tortoises. Microsatellite analyses of 628 tortoises involving 31 study sites indicated a low frequency of private alleles, absence of panmixia (random mating), and a significant correlation between genetic and geographic distance among 31 sample sites. The results were consistent with an isolation-by-distance population structure across the Mojave and Colorado Deserts.

b. Reported that microsatellite variability was greater within than among sample groups, suggesting that the Mojave metapopulation was relatively homogeneous. The high degree of regional similarity in genetic structure among Mojave tortoises may have resulted in part from large-scale translocations of tortoises, captive releases, and habitat disturbance during the past century across the Mojave and Colorado Deserts. This included a substantial transfer of tortoises from the western Mojave Desert in California to Utah.

c. Concluded that regional genetic differentiation was complementary to the six Recovery Units of the 1994 Recovery Plan. Most allelic frequencies in the Recovery Units differed, and an assignment test correctly placed most individuals to their Recovery Units of origin. Of the six Recovery Units, the Northeastern and the Upper Virgin River units showed the greatest differentiation; these units may have been relatively more isolated than other areas and should be managed accordingly.

d. Stated that, in contrast to the six Recovery Units in the 1994 Recovery Plan, the Western Mojave Recovery Unit should be divided into three recovery units along regional boundaries into the Western Mojave, Central Mojave, and Southern Mojave Recovery Units. Theauthors based their proposal on new genetic data that differentiated tortoise populations, as well as differences in the amounts of summer rainfall, number of freezing days, mean temperatures, and floral composition.

e. Detected excess heterozygosity in two sample groups, in the Northeastern Mojave and Upper Virgin River Recovery Units; 24.5% of the data points showed deviations from Hardy-Weinberg expectations in the form of heterozygote deficiencies. Such deviations can result from a recent reduction in effective population size. Such deviations could also be due to other causes such as translocations or other instances of geographic mixing. More sampling of these two Recovery Units was needed to evaluate management strategies to ensure stable or increasing population sizes.

f. Noted the greatest differentiation in genetics of the in the northeastern Mojave and the Upper Virgin River units and said that they may have been more isolated than other areas and should be managed accordingly.

g. Emphasized the need for informed and carefully planned translocations of tortoises; otherwise translocations can compromise genetic integrity of a population by disrupting co-adapted gene complexes in local environments or reducing fitness through outbreeding depression.

   h. Identified restorative actions for populations that have become disjunct or mixed as a result of anthropogenic activities, e.g., remove translocated tortoises from critical habitat, genetically test and remove tortoises from areas adjacent to frequently used recreation sites where visitors often release tortoises illegally, or conduct augmentations or translocations in populations that have dropped below viable levels. However, using tortoises within a well-defined recovery unit or local geographic area for head-starting or augmentation was far better than translocating tortoises between Recovery Units.

2. Hagerty and Tracy (2010) used genetic assignment methods to delineate population structure among 748 sampled tortoises throughout the Mojave and Colorado Deserts and used this information to evaluate the Recovery Units of the 1994 Recovery Plan.

   a. Three basal groups represented the preferred number of genotypic clusters across the landscape, and corroborated the distribution of mtDNA haplotypes found previously (Lamb and others, 1989)

   b. These clusters followed a north–to-south gradient corresponding with regions surrounding the Northern Mojave, Las Vegas, and California areas, and in each cluster additional population structure was observed that corresponded to major geographical barriers.

   c. Geographic distance explained a large proportion of variation in genetic distance, which suggested that historical gene flow among populations was high and limited only on a regional scale.

   d. On the basis of the fine-scale genetic structure that was recovered, they recommended revising the Recovery Units for the Mojave desert tortoise to seven Recovery Units, including retention of the Upper Virgin River Recovery Unit.

3. Hagerty and others (2011) investigated three factors that potentially influence landscape connectivity among desert tortoise habitats across the Mojave and Colorado Deserts: (a) geographic distance, (b) dispersal barriers, and (c) landscape friction (defined as “a measure of the habitat’s resistance to flow of individuals through it”). The authors made a number of suggestions.

   a. The authors reported that the factors most associated with patterns of genetic structure were straight-line geographic distances and dispersal barriers.

   b. The authors suggested that dispersal barriers, such as mountain ranges (e.g., Spring, New York, Providence, and Sheep Ranges) and extremely low-elevation valleys (e.g., Death and Cadiz Valleys, and Baker Sink) likely served as potential barriers to gene flow.

   c. The authors suggested that elevation was an important determinate of barriers and served as an indirect measure of several variables that imposed thermal constraints on desert tortoises, such as differing thermal environments, soil types, and vegetation assemblages.

        d. This study reinforced the hypothesis that desert tortoises were well connected historically, despite restricted gene flow due to geographic distances and topographic barriers.

        e. The habitat models generated in the paper were best used for addressing large-scale patterns of gene flow, over many generations, and not for detecting the effects of recent habitat fragmentation or present-day barriers because of the long generation times within this species.

    4. Averill-Murray and Hagerty (2014) reported that tortoise populations within 200 km of each other are genetically correlated. Therefore, based on their results and previously published qualitative risk assessments that translocating tortoises from their original site to a recipient site < 200 km away has low probability of causing outbreeding depression.

D. Paternity

    1. Davy and others (2011) recovered a high incidence of multiple paternities in wild desert tortoises from Edward’s Air Force Base and the Marine Corps Air Ground Combat Center, California, on the basis of 17–20 microsatellite markers.

        a. Based on conservative criteria requiring evidence from at least two loci to determine multiple paternity, they recorded a minimum of 64% of females were polyandrous and a minimum of 57% of clutches from these females were sired by multiple males.

        b. This study was the first to report multiple paternities in wild desert tortoises, and formed one of the highest incidences of multiple paternities recorded to date in any species of tortoise.

        c. Polyandry and multiple paternities in desert tortoises may increase the lifetime reproductive success by increasing genetic variation within female’s clutches. This increased genetic variation, in turn, increases the phenotypic variability, which may aid a cohort of neonates adapt to fluctuating environmental conditions.

        d. Estimation of the true frequency of multiple paternities in a population is potentially complicated by the different number of clutches sampled, proportion of the clutch sampled, possible skew in male reproductive success, the number of loci sampled, and the power of these loci. These considerations highlight the potential pitfalls of quantitatively comparing paternity studies based on differing sampling strategies.

E. Populations with Both Mojavean and Sonoran mtDNA and Morphometric Phenotypes

1. McLuckie and others (1999) reported a unique population of desert tortoises with predominately Mojavean features in the Black Mountains of northwestern Arizona:
    a. Ten of 11 tortoises possessed Mojave mtDNA markers and only one tortoise possessed the Sonoran haplotype.
    b. A majority of the tortoises (24 of 37) exhibited Mojave morphometric phenotypes using discriminant analysis.
    c. Vegetation and topography provided supporting information.
    d. Mojavean tortoises appeared to have moved in a west–to-east direction from across the Colorado River in California to the Black Mountains of Arizona, possibly by active dispersal, river meandering, or human transport.
2. Edwards and others (2010) identified one microsatellite locus that exhibited a unique allele in congeners (*Gopherus flavomarginatus* and *G. berlandieri*), which the authors suggest may prove useful for hybridization or phylogenetic studies.
3. Edwards and others (2015), using new and more powerful genetic tools, followed up on the work of McLuckie and others (1999) in the Black Mountains of northwestern Arizona. They examined a secondary contact zone between *Gopherus agassizii* and *G. morafkai*. The taxa were isolated from a common ancestor 4–8 million years ago; however, an anomalous population of *G. agassizii* came into secondary contact with *G. morafkai* east of the Colorado River in the Black Mountains, AZ. The authors drew on 234 tortoises representing *G. agassizii* in California and *G. morafkai* in Arizona, as well as 53 individuals of undetermined genetic assignment in the contact zone and surrounding area.
    a. For the first time, hybrids were identified in the wild in this paper.
    b. The analysis revealed that the Arizona *G. agassizii* were more closely affiliated with tortoises in the Colorado Desert Recovery Unit of California than in the Eastern Mojave Recovery Unit.
    c. Most hybrids were identified as F2 and did not appear to be biased toward one species or gender. Most hybrids occurred within 3 mountain ranges (Buck, Hualapi, and Black Mountains).
    d. This analysis suggested a recent shared ancestry (about 2,400 years) with populations of *G. agassizii* directly across the Colorado River.
    e. The authors tested hypotheses about habitat use using habitat suitability models. They reported that *G. agassizii* and *G. morafkai* maintained independent taxonomic identities likely due to ecological niche partitioning.
    f. The maintenance of the hybrid zone was best described by a geographic selection gradient model.

VI. Genetic Affinities of Captive Desert Tortoises

A. Edwards and others (2010) used mitochondrial sequences and microsatellite genotype data to determine the source populations (i.e., Mojave Desert, Sonora Desert, or hybrid) of captive desert tortoises from three locations in Arizona (Kingman, Phoenix, and Tucson).

1. Of the 180 captive desert tortoise sampled, 70 exhibited mitochondrial haplotype variation that matched wild Mojave Desert haplotypes but were obtained from captive collections from Kingman (35 samples) and Phoenix (35 samples), AZ.
2. Captive desert tortoises from Kingman, AZ, exhibited the strongest microsatellite assignment to the wild Mojave Desert population.
3. The combined analysis of mitochondrial and microsatellite data assigned 45 captive samples of tortoises to pure Mojave Desert source populations, and 25 captive samples were assigned as potential Mojave-Sonoran Desert crosses.
4. The high assignment of captive tortoises from Kingman, AZ, to the Mojave Desert wild population may have reflected local collecting from the Black Mountains (near Kingman), where a small population of tortoises exhibit Mojave mtDNA haplotypes, or may have been an indication of local transport of Mojave Desert tortoises from California across the Colorado River and across State lines.

B. Edwards and Berry (2013) analyzed the mtDNA and microsatellites of 130 captive desert tortoises from two clinics (Ridgecrest and Joshua Tree, CA) and the Desert Tortoise Conservation Center, NV; they compared the DNA of captives with a large database of wild *Gopherus agassizii* and *G. morafkai*. They reported:

1. Based on analyses of population affinities, only 44% of 130 captive desert tortoises were assigned to local populations of wild tortoises based on genetic units derived from the reference database. Percentage affinity differed by facility, where captive individuals from Ridgecrest in Kern County, CA, had a higher affinity to their genetic unit of geographic origin (66.7%) compared with those from Joshua Tree, CA (34.9%) or the Desert Tortoise Conservation Center near Las Vegas, NV (41.0%).
2. A small proportion of tortoises appeared to have originated from distant regions within the Mojave Desert, e.g., two tortoises from clinics in the western and central Mojave appeared to have originated in the northeastern Mojave, and one individual from Joshua Tree was identified as *G. morafkai*. The genetic differentiation among the three captive tortoise populations emphasizes that tortoises in captivity differ among communities and cannot be considered a single genetic pool. These results suggest that captive desert tortoises kept within the native range of *G. agassizii* cannot be presumed to have genealogical affinity to wild tortoises in their geographic proximity.

Chapter 3.—Recent Descriptions of Distribution, Habitat Use, and Climate

I. Distribution of *Gopherus agassizii*

A. Murphy and others (2011), separated *Gopherus agassizii* into two species, *G. agassizii* and *G. morafkai*, and delineated the boundaries of *G. agassizii* as occurring north and west of the Colorado River in the Southwestern United States in the Mojave and western Sonoran Deserts in California, southern Nevada, northwestern Arizona, and southwestern Utah. *Gopherus agassizii* occupies predominantly valleys and alluvial fans in saltbush scrub, creosote bush scrub, desert scrub, and tree yucca woodlands.

B. Edwards and others (2015) described the location and genetic affinities of a *Gopherus agassizii* population in northwestern Arizona, including the contact zone with *G. morafkai* and hybrids between the two species.

II. Habitat (Vegetation Associations, Geology, Soils, Slope, and Aspect) Described by Location

A. Northwestern Mojave Desert, Nevada Test Site, NV. Rautenstrauch and O'Farrell (1998) conducted surveys for desert tortoises to assess relative abundance as a function of habitat type and reported that:

1. Tortoise sign was most abundant at elevations of 1,200–1,300 m. This elevation corresponded with upper alluvial fans and lower slopes of most mountain ranges in the southern part of the Nevada Test Site. Tortoise sign was found at elevations of 880–1,570 m, the latter of which is one of the highest elevations reported for desert tortoises. Tortoise sign was less abundant than expected at elevations of 900–1,000 m.
2. The amount of tortoise sign found in alluvial deposits was lower than in mountainous areas with exposed parent material of sedimentary or volcanic origin. Tortoises appear to have been more common on the upper alluvial fans and lower slopes of carbonate mountains than on mountains of volcanic origin. Tortoise sign was more than twice as abundant in areas of carbonate parent material than areas with volcanic ash-flow tuffs or tuffaceous parent material.
3. Relative abundance of tortoises was highest and more than expected in mountainous areas and in the *Larrea tridentata* association. The amount of sign was similar to that expected in the *Larrea tridentata-Coleogyne ramosissima* association and less than expected in both the *Coleogyne ramosissima* associations and adjacent to an ephemeral lake along Frenchman Flats with high clay and salt content in the soils.

B. Central Mojave Desert, National Training Center, Fort Irwin, CA. Andersen and others (2000) used a GIS-based model from field and spatial data collected on a 14,000-ha study area to analyze occurrence and abundance related to habitat variables. They reported that tortoises tended to occur in areas with abundant plant cover, exhibiting a preference for *Atriplex canescens*-dominated vegetation. Tortoises tended to occur on loamy soils and granitic conglomerate substrates and were less abundant in stony soils and medium-grained alluvial fan deposits, likely related to their ability to dig burrows. Desert tortoises tended to occur on southwestern exposures and were less abundant at sites with northern exposure, likely related to their need to maintain thermal balance.

C. Western Mojave Desert, CA. Jennings (2002) conducted a foraging study of adult desert tortoises equipped with radiotransmitters in creosote bush scrub habitat in landforms that included flat sandy areas, sandy washes, and low rocky hills. Tortoises used all these habitats for foraging (see also Jennings and Berry, 2015). Although tortoises ate at least 44 plant species, only 10 species comprised 81.4% of their diet. Tortoises preferred succulent to dry plants and selected plants during certain phenological states, suggesting that plant palatability varied with phenological state.

D. Central Mojave Desert, CA. Berry and others (2006a) studied tortoises on 21 plots associated with the National Training Center, Fort Irwin and Goldstone, CA. Tortoise sign and habitat occurred at elevations ranging from 512 to 1,223 m in numerous types of soils. Vegetation of 20 of the 21 plots was typical of creosote bush (*Larrea tridentata*) scrub communities, although more diverse vegetation was present on some plots than others. One plot was dominated by *Artemisia spinescens* and species of *Atriplex* typical of alkaline soils. Canopy cover of perennial vegetation ranged from 4.0 to 31.2%.

E. Northwestern Mojave Desert, CA. Berry and others (2013) described a tortoise study site at the southern end of the Argus Range in Salt Wells Valley, CA, at elevations of 597–732 m in topography ranging from valleys to low hills in white bursage and creosote bush (*Ambrosia dumosa-Larrea tridentata*) vegetation association.

F. Western Mojave Desert. Berry and others (2014a) modeled habitat variables (vegetation association, elevation), anthropogenic, and predator variables to determine relationships with tortoise abundance (tortoise sign) in tortoise populations under three different management strategies in the Fremont Valley and Rand Mountains, CA. Tortoises occurred in four vegetation associations, all with creosote bush/white bursage (*Larrea trdentata/Ambrosia dumosa*) present as two of the dominants: (1) creosote bush/white bursage with only two abundant species, (2) creosote bush/white-bursage/Anderson box-thorn (*Lycium andersonii*) with five abundant species, (3) creosote bush/white bursage/Mojave indigo bush (*Psorothamnus arborescens*) with nine dominant species, and (4) creosote bush/white bursage/Nevada ephedra (*Ephedra nevadensis*). Depending on the model and location, tortoise sign had the highest probability of occurrence in the association with Anderson box-thorn, double that of the associations with Nevada ephedra and indigo bush.

G. Mack and others (2015) studied radio-transmittered tortoises in the Soda Mountains, north-central Mojave Desert, CA, at elevations of 459–795 m in terrain of north-south trending, steep-sided ridges, 30–60 m in height. The ridges were separated by large and small ephemeral, generally dry stream channels. The surficial geology was old (550–1,000,000 years), and the predominant vegetation was creosote bush (*Larrea tridentata*) and white bursage (*Ambrosia dumosa*), with several other plant species. At this site, tortoises used 390 cover sites, including 314 caliche caves (80%), 39 burrows in soil (10%), 16 rock shelters (4%), 11 consolidated gravel caves (3%), and 10 pallets (3%).

H. Mapping Habitat

1. Wallace and others (2008) developed two models by coupling Moderate-Resolution Imaging Spectroradiometer (MODIS) Enhanced Vegetation Index (EVI) satellite data with vegetation data collected throughout the Mojave Desert. Their work yielded maps of perennial cover at 250 m spatial resolution. Validation and other checks of model stability and predictive power showed that the two models were indistinguishable from each other (R^2 of 0.82 and 0.81). Overall, the results showed that total perennial cover measured at the plot level can be correlated with MODIS–EVI data and with PCA data derived from MODIS–EVI images, which allows estimation of total perennial cover over a much larger area than is represented by the plots.

2. Wallace and Thomas (2008) developed models from Moderate-Resolution Imaging Spectroradiometer (MODIS) Enhanced Vegetation Index (EVI) satellite images and annual cover estimates from 50 sites within the Mojave National Preserve, CA. They found the strongest correlation between annual plant cover and MODIS–EVI derivatives using the satellite metric defined as the difference between the average spring greenness for 2005, a wet year, and the average spring greenness for 2002, a year of record drought. The spring difference metric showed correlations with annual plant cover of $R^2 = 0.61$ for 2005 and $R^2 = 0.47$ for 2003.

III. Climate and Precipitation Patterns

A. General Patterns

1. Berry and others (2006b), surveyed the literature and reported that temperatures in the Mojave Desert exhibit extreme ranges, with mean minimum January temperatures of -2.4 °C in Beatty, Nevada, and mean maximum July temperatures of 47 °C in Death Valley. Overall, the mean annual precipitation in the Mojave Desert is low, but varies throughout the region (42–350 mm). The distribution of precipitation varies from west to east and north to south, with > 85% of rain falling in winter in the northern, southwestern, and south-central regions of the Mojave Desert. In contrast, the central and eastern regions receive a substantial amount of precipitation in both winter and summer.

2. Hereford and others (2006) described long-term climate and precipitation patterns in the Mojave Desert, drawing on 52 weather stations across Mojave Desert and from examples of vegetation data from Death Valley and the Nevada Test Site, NV. Please note that these authors dealt with the entire Mojave Desert, and the findings are not focused on tortoises and habitat.

a. Precipitation varied substantially in the Mojave Desert throughout the 20th century. Historical precipitation was spatially consistent among the 52 weather stations across the Mojave Desert and varied on interannual and multidecadal timescales. Interannual variability produced irregular oscillations of about 5 years duration in the precipitation time series. This variability was partly related to the El Niño-Southern Oscillation, where El Niño conditions increase moisture and La Niña conditions typically decrease moisture. Multidecadal variability was expressed by three dry periods (1893–1904, about 1942–1975, and 1999–2003) and two wet periods (1905 to about 1941 and about 1976–1998), which probably resulted from alternating cool (dry) and warm (wet) phases of the Pacific Decadal Oscillation.

b. Historical precipitation data across the Mojave Desert revealed two distinctive patterns, approximately divided by the 117° W meridian; both patterns were biseasonal and winter dominant. The biseasonal pattern was more common east of 117° W and had 66 and 29% of the precipitation in cool months (October–April) and warm months (July–September), respectively. The winter dominant pattern was more common west of 117° W and had 82% of the precipitation in cool months versus 13% in warm months.

3. McAuliffe and Hamerlynck (2010) reported that 6–month Standard Precipitation Index (SPI) values calculated for the 1998–2003 period from 56 recording stations within the Sonoran and Mojave Deserts showed that the Mojave Desert stations had significantly higher SPI values than Sonoran Desert stations. Cool–season SPI was significantly lower than warm-season SPI.

B. Examples of Effects of Precipitation and Other Climate Variables on Vegetation (Tortoise Cover and Forage)

1. Perennial Vegetation. Hereford and others (2006) reported on studies conducted in the late 20th century in the northeastern Mojave Desert (Nevada Test Site, Death Valley). Their work demonstrated that fluctuations in precipitation affected populations of perennial vegetation, annuals, and small herbivores. Primary production of annuals was largest during periods of increased cool-season precipitation. Increased biomass from cool-season precipitation, in turn, improved resource availability for small herbivores and enhanced reproduction of small mammals and certain reptiles. Landscape rephotography during a relatively wet period (1978–1998), with periodic drought (e.g., 1989–1991), showed the variable responses of perennial plants. For example, some species such as creosote bush (*Larrea tridentata*) increased substantially in size and cover during the approximately 1976–1998 wet period. A brief but intense drought from 1989 to 1991 occurred; species within the Chenopodiaceae, particularly spiny hopsage and perennial grasses (e.g., desert needlegrass [*Achnatherum speciosum*]) decreased in size or suffered population mortalities up to 100%. Drought pruning, the shedding of above-ground biomass to reduce carbon allocation, occurred between 1999 and 2002, and increased substantially during drought.

2. Annual Plants

   a. Brooks (2000), in a study of the relationship between annuals and fire at three sites in the central, south-central, and southwestern Mojave Desert, CA, reported that in 1995–1996, winter rainfall averaged 94% of average (82 mm) and occurred in small increments throughout the winter. In 1996–1997, rainfall was 77% of average (67 mm), but occurred mostly in December, when rainfall was 307% of average for that month (46 mm). This high December rainfall stimulated mass germination of annual plants which resulted in high biomass and species richness during spring 1997.

   b. According to Longshore and others (2003), the biomass of winter annual plants at two sites on the Nevada side of Lake Mead National Recreation Area in the eastern Mojave Desert was positively and highly correlated with precipitation associated with the growing season precipitation (September–March).

   c. Medica and others (2012) conducted studies at the Nevada Test Site in the northeastern Mojave Desert. The authors reported that ephemeral production, measured as annual plant biomass (grams/m^2) between 1964 and 2003, increased dramatically as a function of the amount of winter rain. Ephemeral plant production was well-represented by a log-linear model ($R^2 = 0.84$) that showed a marked increase in production above 100 mm of rainfall.

IV. Effects of Climate Variables on Desert Tortoises

Climate, especially precipitation, has a profound impact on many aspects of tortoise life: e.g., health, physiology, reproduction, above-ground activity and other behaviors, forage availability, growth rates, and mortality. Publications on effects of precipitation or lack thereof and El Niño are cited throughout in the appropriate sections.

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Chapter 4.—Behavior, Shelters, and Home Ranges

I. General Postures and Action Patterns

Ruby and Niblick (1994) identified 80 behavioral actions in 8 behavioral categories (see below) and provided context for each group to help understand the meaning of the actions. The authors reported that the courtship sequence, which appeared to be only initiated by the male, had similar initial actions to the aggression sequence, with aggression occurring only between males. Dominance between males appeared to be related to the height of the head bob. Finally, the authors found no behaviors indicative of females displaying a "rejection" behavior towards courting males or that either sex scent-marked territories similar to mammals.

A. Maintenance behavior included resting, sitting, standing, scanning, looking around, being alert, closed eyes, or defecating.

B. Foraging behavior included sniffing substrate, exploring, mashing, biting food, chewing, or drinking.

C. Defensive or anti-predator behavior included all or head only defensive postures, freezing, gradual or quick lying down, getting back up, wedging, or dropping water.

D. Thermoregulatory behavior including basking or sitting at the burrow facing the sun.

E. Locomotor behavior included slow, normal, or fast walking; running, climbing, or yawning.

F. Social behavior included exploratory sniffing, exploring with the nose, throat pumping, high standing with head high or low, marking, rubbing the chin, sniffing face-to-face, jerking the head in, turning the head away, turning the body away, level or elevated head bobs, gaping, nipping, holding bite, approaching, trailing, side-display, soliciting, tail-wagging, rotating, circling, stroking, shell scratching, mounting, extending the head in and out, head swinging, hopping, copulation, walking away, aggressive climbing; front, side, or rear ramming; propping up, backing up, dropping the shell, flipping, falling over, biting foot, extending the neck, waving the foot while flipped over, or retreating.

G. Nesting behavior included digging, pushing dirt, laying eggs, or covering the nest.

H. Vocalizations included hissing, grunting, or moaning.

II. Defensive Behaviors and Camouflage

A. Defense by Juveniles. Marolda (2002), drawing on captive tortoises hatched in semi-natural pens in southern California, reported that posterior shell aperture reduction (PSAR) occurred in desert tortoises and was most prevalent in juveniles (12.5% reduction) and neonates (8.4%) compared with adults (2.4%). Laboratory-hatched desert tortoise neonates pecked with a raven model had PSAR of 14.6%. The author suggested that this inarticulate shell kinesis may protect the tail, soft tissues around the cloaca, and hind legs of young tortoises that are smaller and have softer shells, from predation by smaller avian and mammalian predators.

B. Nest Guarding and Nest Defense

1. Nest guarding. Agha and others (2013) approached two separate burrows and observed tortoise behavior consistent with nest guarding during the course of a larger nesting study at a wind energy facility near Palm Springs, CA. Female tortoises had moved to the entrance of their burrows and positioned themselves sideways, directly over their presumed nests. Researchers subsequently confirmed that the nests contained four and three eggs, respectively, behind the lip of the burrows. Nest guarding is rarely observed in Agassiz's desert tortoise.

2. Nest defense. Gienger and Tracy (2008) observed three different female tortoises unsuccessfully defend their burrows against entry by three (one male, two females) Gila Monsters (*Heloderma suspectum*) during separate events in May and June. On two occasions, tortoises chased Gila Monsters out of the burrow with one tortoise also biting at the lizard's tail and hind leg. Gila Monsters returned to or remained in two tortoise burrows overnight. Tortoise egg fragments were visible the next morning, suggesting that the Gila Monsters dug up and ate the eggs.

C. Camouflage. Nafus and others (2015a) studied camouflage and habitat selection in captive juvenile tortoises at the Desert Tortoise Conservation Center, NV, and in semi-wild juvenile tortoises raised in pens located at the Ivanpah Desert Tortoise Research Facility in the Mojave National Preserve, CA. In the experiment using six circular plastic pools at the Desert Tortoise Conservation Center, captive juveniles spent a greater amount of time near rocks. Juveniles fitted with radio transmitters were released from the Ivanpah Desert Tortoise Research Facility, were tracked and rocks of different sizes were placed near them in the wild. Juveniles in both experiments appeared to seek substrates with larger rocks. Additionally, juvenile tortoises were found to be less detectable on rock substrate by observers than they were on substrate lacking rocks. The authors hypothesized that rocks improved juvenile tortoise camouflage and, thus, that tortoises select for habitat containing rock substrate in part due to a survival advantage conferred by such use.

III. Social and Dominance Behavior

A. Conspecific Recognition and Chin Gland Secretions. Alberts and others (1994) carried out experiments at the Desert Tortoise Conservation Center, NV.

1. Monthly measurements of chin glands of 20 adult males were made over one year; gland volume varied seasonally, reaching a maximum in late summer.

2. Chin glands of socially dominant males tended to be larger than those of socially subordinate males. Among all males, there was a positive correlation between plasma testosterone levels and size of chin glands.

3. Tortoises of both sexes were able to discriminate between chin gland secretions of familiar and unfamiliar males, suggesting that chin gland secretions may function in conspecific recognition.
    a. Males approached unfamiliar male secretions sooner and spent more time investigating them than secretions of familiar tortoises, whereas females approached familiar male secretions sooner, and spent more time investigating and sniffing familiar male secretions than those of unfamiliar males.
    b. The authors suggest that the ability to recognize familiar conspecifics with whom dominance relationships have been established may help males avoid unnecessary and potentially costly aggressive encounters. Females may also benefit by recognizing conspecific males individually, which, in turn may facilitate mate choice.

B. The Tortoise Mating System. Niblick and others (1994) conducted behavioral experiments on captive desert tortoises at the Desert Tortoise Conservation Center in Las Vegas, NV, to determine the role of male-male dominance interactions and female choice.
    1. Dominance is affected by size. In trials in which two males and one female were allowed to interact freely, dominant males courted and mounted females significantly more frequently than subordinate males.
    2. Female choice experiments indicated that females preferred large males in their first encounter.
    3. Female cooperation appeared necessary for successful copulations, because the authors observed several mounting attempts in which no copulation took place.
    4. In trials where size of males was similar, dominance was based on residency and on previous interactions.

C. Ruby and others (1994a) conducted focal observations of tortoises raised in semi-natural pens at the Desert Tortoise Conservation Center in Las Vegas, NV. Over 2 years, they recorded 833 focal observations in which 114 (13.7%) resulted in encounters between two tortoises. Of those, 79 (69.3%) involved immature tortoises interacting with other immature tortoises or adults. A peak in interaction occurred in May of the second year following heavy winter rains and subsequent large amounts of available forage.

D. Davy and others (2011) conducted research on paternity of clutches in the western Mojave Desert, CA, and reported that clutches contained eggs with multiple fathers. See also Chapter 6, “Reproduction and Endocrinology.”

IV. Digging Behavior—Burrows and Dens

A. Cover Sites and Shelters

1. Bulova (1994) studied 28 transmittered tortoises adjacent to the Desert Tortoise Conservation Center, Las Vegas, NV, and reported that they used 141 soil burrows (67.5%), 24 dens (11.5%), and 44 pallets (21.5%) during a 5-month observation period. On average, soil burrow entrance height was 0.13 m, width was 0.30 m, length was 0.52 m, and declination was 20 °C. Dens were generally larger than soil burrows; on average den entrance height was 0.17 m, width was 0.76 m, length was 1.3 m, and declination was 9 °C. The majority of soil burrows and den entrances faced north.
2. Zimmerman and others (1994) examined daily fluctuations in mean operative temperatures on the surface and in burrows located within and adjacent to the Desert Tortoise Conservation Center, NV. Mean operative temperatures in burrows typically varied less than 1 °C per day, averaging 29 °C in late August and steadily declining to 7 °C in December.
3. Duda and others (2002) conducted surveys on the Marine Corps Air Ground Combat Center, CA, and located 592 (65.6/km^2) and 871 (96.8/km^2) burrows within two study plots. When data from the two plots were combined, 19.2% were active and an additional 20.0% of the burrows were classified as excellent. The majority of burrows (67.6%) were associated with perennial vegetation, mainly (52.1%) *Larrea tridentata*. The average depth of 851 burrows was 0.82 m. Burrows that were rated as active (mean = 1.1 m) were significantly deeper than those rated as excellent (mean = 0.88 m), which in turn were significantly deeper than burrows rated as good (mean = 0.56 m).
4. Berry and others (2006a) surveyed tortoises and tortoise sign on 21 plots at Goldstone and the National Training Center at Fort Irwin, CA, from 1997 to 2003. The plots were in several different vegetation communities and landforms, ranged in elevation from 512 to 1,223 m, and supported burrows, caves, rock shelters, and pallets. Tortoise use of the 484 cover sites differed significantly among plots because of habitat type. At 15 plots at Goldstone, tortoises primarily used burrows (70.1%); on Alvord Slope and Langford, tortoises primarily used burrows (97.3 and 100%, respectively). Caves were not available or rare at these sites. In contrast, at four other plots, caves and rock shelters were more readily available and used: (a) Eastgate 1, 80 and 10%; (b) Eastgate 2, 73 and 3%; (c) Tiefort Mountains, 43 and 17%; and (d) Soda Mountains, 57 and 4%, respectively.
5. Harless and others (2009) studied 34 desert tortoises in the southwestern corner of the National Training Center at Fort Irwin, CA. The number of burrows used was similar between years (n = 412 in 2004 and n = 418 in 2005) and independent of home range size. Males used a greater number of burrows per year compared to females, but relative burrow fidelity (the number of burrows used in both years divided by the mean number of burrows used in each year) was similar for both sexes.

6. Berry and others (2013) surveyed a 5.42 km^2 study area for tortoises and their habitat at the China Lake Naval Air Weapons Station in the northwestern Mojave Desert, CA, in 2010. They recorded 140 shelter sites of which 132 (94.3%) were burrows; there were 4 rock shelters, 2 caves in calcic soils, and 2 pallets.
7. Berry and others (2014a) surveyed 240 one-ha plots in the western Mojave Desert, CA, on adjoining lands managed with three different strategies: (a) the fenced and protected Desert Tortoise Research Natural Area, (b) private lands with no restrictions, and (c) critical habitat for the tortoise. They reported finding a total of 77 tortoise burrows and pallets.
8. Mack and others (2015) studied transmittered tortoises in the Soda Mountains, CA, between 2002 and 2004. They reported that the tortoises used 390 cover sites, including 314 caliche caves (80%), 39 soil burrows (10%), 16 rock shelters (4%), 11 consolidated gravel caves (3%), and 10 pallets (3%). The authors selected 30 of the sites in the proportion occurring on the site to measure temperature extremes and annual temperature fluctuations (24 caliche caves, three soil burrows, two consolidated gravel caves, and one pallet). Temperatures differed by cover-site type. Tunnel length had the greatest influence on cover-site temperatures.

B. Spring or Summer Burrows or Dens

   1. Description and Location

      a. Bulova (2002) studied microclimate of burrows near the Desert Tortoise Conservation Center, Las Vegas, NV. The author reported that during mid- to late summer between 1000 and 1200 h, burrow microclimate was generally more favorable than that on the surface with respect to water conservation by tortoises. This was illustrated by lower temperatures and higher humidities found in burrows, and by lower evaporative water loss predicted for tortoises in burrows. Path analysis of characteristics from 74 burrows indicated that longer burrows with smaller openings tended to be cooler and more humid.

      b. Rautenstrauch and others (2002) studied burrow use by 113 adult tortoises at Yucca Mountains, NV, from January 1992 through February 1995. Of 1,558 burrows used by the 113 tortoises, 21% were deep (> 1 m), 70% were shallow (< 1 m), and 9% were of an unknown depth. Deep burrows were mostly under boulders (51%), caliche (26%), or shrubs (13%), whereas shallow burrowswere mostly under shrubs (51%) or boulders (26%) or had no associated structure (19%).

   c. Lovich and Daniels (2000) measured 32 used and 32 randomly selected tortoise burrows at a wind energy generation facility in Riverside County, CA, and found that used burrows were located farther from *Yucca* spp. than random burrows. In addition, used burrows were closer to dirt roads than random burrows, perhaps because (i) the road berms mimicked banks of desert washes, (ii) elevated vegetative productivity occurs near unpaved road edges, or (iii) using unpaved roads allowed tortoises to move through the environment easier. The authors also noted that this site had limited public access (locked gates, barbed-wire fences, and no trespassing signs), which may have alleviated some detrimental aspects of roads. Roads and the concrete pads that supported the wind turbines and transformers were highly correlated; however, used burrows were also closer to the concrete pads than random burrows. In fact, tortoises often constructed their burrows under the pads. Based on this result, the authors suggested tortoises may have used the pads as “artificial caliches” to benefit from roof stability or thermal inertia relative to soil.

   d. Mack and others (2015), in a study of adult tortoise use in the north-central Mojave Desert, CA, measured temperatures inside and outside five shelter types throughout the year. Median average summer maximum temperature in soil burrows was higher than in caliche caves. Median average summer maximum temperature was significantly higher at the cover-site opening compared with inside the tunnel. Tunnel length had the greatest influence on providing cooler temperatures in summer and warmer temperatures in winter for the tortoises.

2. Patterns of Utilization

   a. Bulova (1994) radio-tracked 28 free-ranging tortoises for 5 months adjacent to the Desert Tortoise Conservation Center near Las Vegas, NV. Desert tortoises were observed using 3–18 shelter sites, and at least 35% were used by another tortoise. Tortoises exhibited a bimodal daily activity pattern; they used pallets, soil burrows, and dens during the hottest part of the day and also at night throughout active season. Patterns of burrow use and co-occupancy differed between the sexes and corresponded to the reproductive cycle. Most observations were of tortoises inhabiting shelters singly. During the nesting season in June, female tortoises used more shelters, switched more times, and moved to more new shelters than did male tortoises. During August and September, males used more shelters and switched more times; and male-female co-occupancy was commonly observed.

b. Bulova (2002), in a study of tortoises near the Desert Tortoise Conservation Center, Las Vegas, NV, reported that tortoises entered shelter between 0744 and 1512 h with T_{skin} of 28.3–43.1 °C. The mean burrow temperature was 30.5 °C. Skin temperatures of tortoises entering burrows were a mean of 5.3 °C greater than air temperature inside the burrow. Models predicted that a tortoise could maximize its total time for the day in microclimates that favor reduced water loss rates by spending the day in a burrow and the night on the surface. During mid-summer, humidity was significantly higher inside burrows than on the surface between 1000 and 1200 h. The range of variation in humidity, temperature, and evaporative water loss over 24 h was greater on the surface than inside burrows. Thus, surface conditions could be more favorable in certain cases, such as after rainfall events or during the night. These results suggest the importance of burrows not only to avoid lethal temperatures but also as a means decreasing water loss.

c. Freilich and others (2000) radio-tracked 10 adult tortoises from 1993 through 1996 on a plot at Joshua Tree National Park, CA, and found that burrow use varied annually. In years with higher rainfall and annual plant productivity, tortoises used more burrows and had larger home ranges. Males used more burrows than females. When tortoises were found in burrows, they were visible only 60% of the time.

d. Rautenstrauch and others (2002) radio-tracked 113 adult desert tortoises for 3 years on two plots in the Yucca Mountains, NV.

    i. Tortoises used an average of 11.7 burrows per year, with most (90%) tortoises using 7–17 burrows per year. Tortoises used an average of 4.8 new burrows per year and an average of 1.0 new deep burrow per year. The large number of total and new burrows suggested that burrows may not be limiting for this population.

    ii. The pattern of burrow use varied by season. Seasonally, burrow use was lowest in April (29% of observations, years combined), gradually increased from May (39%) through July (85%) and August (82%), decreased slightly in September (69%), and then increased again when tortoises began to hibernate. Tortoises were more often in shallow (<1 m) burrows from May to September and in deep (>1 m) burrows from November to February. The highest use was during summer, corresponding with high temperatures, and during winter, corresponding with low temperatures—results suggesting a thermoregulatory benefit of burrow usage.

    iii. Relative to female tortoises, males were observed in deep burrows more often, used a greater number of deep burrows, and were located deeper in burrows. Females were more likely to use shallow (< 1 m) burrows and a greater number of burrows during spring. This suggested that nutritional, reproductive, or other requirements influence cover use by sex.

e. Krzysik (2002) measured burrow depths at two sites, the Sand Hill Training Area and Pinto Basin, about 64 km apart in CA. The author reported burrow densities to be 64/km^2 and 224/km^2, respectively. The majority of tortoise burrows were shallow at both sites in both productive and drought years. Approximately 50% of the burrows were < 66 cm in depth, 75% were < 1 m, and 85–90% were < 1.3 m.

f. Nussear and Tracy (2007) tracked 150 tortoises near Las Vegas, NV, and reported that during the part of the day when tortoises were active, 60–75% of monitored tortoises were found in underground pallets or burrows, and tortoises were found in burrows at least two times more often than in pallets, under vegetation, or in the open.

g. Franks and others (2011) radio-tracked 60 tortoises across four study sites (western, central, and eastern Mojave Desert). They found that study site had a significant effect on the number of burrows tortoises used, but sex did not. More burrows were used at the two most eastern study sites (Ivanpah Valley, 5.4 ± 1.2 burrows SD, Fort Irwin 4.3 ± 1.3 burrows SD) than the two western study sites (Superior 2.7 ± 1.0 burrows, Fremont 3.6 ± 1.1 burrows SD).

C. Winter Burrows or Dens

1. Bulova (1994) radio-tracked 28 free-ranging tortoises for 5 months adjacent to the Desert Tortoise Conservation Center, Las Vegas, NV, and reported that tortoises were alone or in groups of two during hibernation. Tortoises used soil burrows or dens, and no tortoises spent the winter in a pallet.

2. Hazard and Morafka (2004) tested whether juvenile tortoises (8–9 years) and neonates (< 2 months) selected hibernation burrows with differing characteristics after release from head-start pens at the National Training Center, Fort Irwin, CA. After release, juveniles used hibernation burrows that had a mean direction of 162° (south-southeast); the burrows of neonates were not oriented in any particular direction. Selectivity of juveniles compared to neonates may have contributed to higher levels of movement by juveniles between release and hibernation.

3. Loughran and others (2011) confirmed that three female adult tortoises, which were hibernating in separate natural burrows that had partially collapsed due to slumping of soil that occluded the burrow entrances, were able to self-extricate by digging. For two of the females, extrication took about 1–3 months. The male, however, was completely encased in hard loamy soils and was unable to free himself, perhaps because the burrow was oriented towards the sun that baked the collapsed soil. This suggested that tortoises can normally self-extricate from naturally occurring entombment, but entrapment can occur.

D. Burrow Choice and Conspecific Chemical Cues

Bulova (1997) experimentally tested the influence of chemical cues on burrow choice by captive tortoises at the Desert Tortoise Conservation Center, NV, using a series of four two-choice tests of treated and untreated artificial burrows during nesting and mating seasons. The author reported that feces and chin-gland secretions deposited in the vicinity of burrows may influence burrow use patterns by free-ranging adults. For example, during the nesting season, significantly more females used the untreated burrow when the treatment was another female's feces.

E. Commensals and Co-Occupancy

1. Walde and Lindey (2009) observed a tarantula, suspected to be a male *Aphonopelma iodius* due to its small abdomen size and brown color, inside a burrow occupied by an overwintering adult female desert tortoise. This species of tarantula is a fall breeder, and the authors hypothesized the spider took refuge in the burrow while looking for a mate or food.
2. Gienger and Tracy (2008) reported that Gila Monsters co-occupied shelters, including tortoise burrows and others such as mammal burrows, rocky slopes/flows, or sandstone crevices, 16 times over 3 years. Only during three occurrences (18.8%) did the authors observe tortoise eggshell fragments in the burrows after the lizards departed.
3. Walde and others (2009) reported that in 2003, during surveys for desert tortoises (*Gopherus agassizii*) at the National Training Center at Fort Irwin, CA, four horned larks (*Eremophila alpestris*) were observed using desert tortoise burrows during summer. These observations add a third species of bird to the list of species documented to use burrows of desert tortoises, suggesting the importance of desert tortoise burrows as refugia for other species.
4. Lovich (2011) observed a red diamond rattlesnake (*Crotalus ruber*) and a desert tortoise co-occupying a desert tortoise burrow during the hibernation period at a wind energy facility near Palm Springs, CA. The range overlap for both species is comparatively small, occurring primarily west of the City of Palm Springs.
5. Germano and Perry (2012) reported cohabitation of a caliche cave by an American badger (*Taxidea taxus*) and an adult desert tortoise. The tortoise was unharmed even though badgers are known predators of desert tortoises.
6. Walde and others (2014) observed a speckled rattlesnake (*Crotalus mitchellii*) and a desert tortoise cohabitating in a desert tortoise burrow during July in the western Mojave Desert, CA. They suggested that the rattlesnake may have been foraging in the burrow or utilizing it for its thermally beneficial environment.
7. Walde and others (2015) reported observations of long-nosed leopard lizards (*Gambelia wislizenii*) using desert tortoise burrows in the western Mojave Desert, CA.

8. Walde and Currylow (2015) reported on desert tortoise burrow cohabitation by a desert banded gecko (*Coleonyx v. variegatus*) and a desert tortoise in the western Mojave Desert, CA.

9. Walde and others (2015) reported observations of long-nosed leopard lizards (*Gambelia wislizenii*) using desert tortoise burrows in the western Mojave Desert, CA.

E. Unusual Visitors to Burrows, Trampling of Burrows

1. Lovich and others (2014a) recorded a female black bear (*Ursus americanus*) and at least one cub at the entrance of an adult female tortoise's burrow. Radio-telemetry and camera trap data suggested that the female tortoise was inside and at least two male tortoises had visited. There was no evidence of the bears digging at the burrow. The researchers suggested the bears were "pushed" into the study area from a nearby wildfire.

2. Agha and others (2015a), using motion sensor trail cameras, documented collapse of a desert tortoise burrow by trampling of Nelson's big horn sheep (*Ovis canadensis nelson*) at a wind energy facility in Riverside County, CA. Three different sheep then proceeded to lie down and in the process compact soil, rocks and sticks on top of the newly collapsed entrance.

V. Behavior Relative to Culverts or Barriers

A. Ruby and others (1994b), using captive tortoise behavioral trials, reported that tortoises responded differently to solid and non-solid (e.g., mesh-like) barriers by interacting more extensively with barriers that were open by trying to insert their heads or feet through the barriers. Frequency of interaction declined during 2-h trials. When given the choice, tortoises showed no preference for following either solid or mesh barrier fences. Lighter weight fence (e.g., chicken wire), seemed to invite more pushing behavior by tortoises and a number of test animals became stuck in the fencing and in some cases could not extract themselves. These results suggest that barriers with openings large enough for a tortoise's head or foot would be ineffective in directing tortoises away from the barrier and could be a hazard. With respect to openings in the barriers constructed to represented tunnels, tortoises quickly walked past openings which were too small to enter. Tortoises also easily escaped from a barrier within 30 minutes when openings of an appropriate size were available. Tests indicated that screen mesh with small enough openings to exclude a tortoise's head (i.e., 1-cm hardware cloth) were most suitable barrier materials because the animal could see through the barrier and would follow along it to passageways (i.e., tunnels) allowing the tortoise to cross underneath a road; burying the hardware cloth 15 cm in the ground would clearly keep them off the road surface. Despite a tortoise's willingness to follow barriers and use tunnels, tortoises retreated from and never made contact with actual concrete barriers along Interstate Highway 15. It appeared the visual, vibrational, and noise stimuli from the road traffic deterred the animals. However, all five tortoises that were placed directly in front of a culvert opening were willing to enter the structure.

B. Boarman and others (1998) attached passive-integrated transponder (PIT) tags to 172 tortoises in the vicinity of two storm drain culverts along California State Highway 58. Using an automatic reading system (ARS), researchers documented five tortoises using the culverts to cross beneath the highway, or as refugia, a total of 75 times over 2 years. Tortoise crossings ranged from 1 to 29 times on 1 to 7 days per year, by individual tortoises. They found that placing the 18-mm cylindrical PIT tags vertically and the 18-mm disk tags horizontally on the tortoises allowed farther mean reading distances by the ARS readers (137.5 ± 4.01 mm SE for cylinders, 199.8 ± 9.15 mm SE for disks).

C. Lovich and others (2011a), in a study of *Gopherus agassizii* at a wind energy facility in the Colorado Desert, CA, described the entrapment of a radio-transmittered tortoise within a mud-filled culvert. The tortoise had used the culvert as shelter for almost a year and during winter brumation. Although rescued in late March, the tortoise died soon after, presumably of pneumonia.

VI. Daily and Seasonal Activity Patterns

A. Daily Activity Patterns

1. Ruby and others (1994a) found that tortoises kept in semi-natural pens without being supplemented with water and food (unsupplemented) moved farther than tortoises receiving supplementation. Morning activity periods were also shorter for unsupplemented tortoises, ending sooner (> 30 minutes) than supplemented tortoises. In addition, a greater proportion of unsupplemented tortoises were above ground and active than were supplemented tortoises. Overall, tortoises were more active for most of the day in April, exhibiting a unimodal activity period that switched to bimodal in late spring and remained so until October when they became active all day again. These results suggested that when stressed by lack of water and food resources, tortoises reduced the length of above-ground activity time but attempted to compensate for this decreased time by increasing amounts of movement and feeding while active. This also suggested that when faced with a trade-off between energetic cost of activity (including water loss) and the possibility of locating favorable habitat with food and water in prolonged drought, tortoises could be in a positive feedback loop wherein more animals will be active and travel to search for limited resources, expending more energy and causing a greater energy deficit.

2. Wilson and others (1999), in a study of juveniles in head-start pens at the National Training Center, Fort Irwin, CA, found that up to 88.7% (n = 63) of juvenile tortoises were active during any particular day from October 22 to February 18 (entire survey), whereas only as much as 12.7% (n = 9) were active during any particular winter survey day (November 27–January 23). Only 13 individuals (18%) were active during winter days. The authors reported a significant positive correlation between percentage of juveniles active and the minimum daily air temperatures for the entire survey period and the minimum, mean, and daily air temperatures for the winter survey period, but not annual rainfall for either survey period. Burrow lengths (n = 57) during winter days averaged 52.7 cm (range 5.0–130.0 cm) and significantly differed, with active juveniles occupying shorter burrows ($\bar{x}$ = 29.4 cm, SD = 23.6) and inactive juveniles occupying burrows two

times longer ($\bar{x}$ = 60.3 cm, SD = 35.5). Of the 13 active juveniles, most (about 9) were found at the mouth or on the mound of their burrow and were withdrawn inside their shells. The authors recorded 6 of 30 feeding events during the winter days and all occurred during the late afternoon surveys.

3. Freilich and others (2000) found that tortoise captures peaked between 0930 and 1130 h in Joshua Tree National Park, CA. Capture time became earlier in the day as the season progressed and as temperatures increased from March through May, with time difference being statistically significant between March and April, and March and May, and barely so between April and May.
4. Bulova (2002) studied 28 radio-telemetered tortoises near Las Vegas, NV, and reported that tortoises often retreated into burrows during the late morning, emerged in evening, and remained on the surface for the night. Patterns of shelter use by tortoises likely optimized conditions to regulate temperature and decrease water loss.
5. Nussear and Tracy (2007) found that tortoises near Las Vegas, NV, were generally more active during morning hours, but the proportion of tortoises active varied daily, weekly, seasonally, and annually.
6. Inman and others (2009) recorded activity of 34 tortoises surveyed in the Superior-Cronese and Ord-Rodman Desert Wildlife Management Areas, CA, during April and May 2004, a very wet year. The authors reported that 50–90% of the animals were active above ground in April compared to 20–40% in May, and that tortoises were generally not as active during the early morning (0500–0700 h) and late evening (1700–1900 h) compared with the middle parts of the day, especially in mid- to late May.

B. Seasonal Activity Patterns

1. Bulova (1997) conducted experiments on effects of chemical cues (chin gland secretions) on burrow choice by captive adult tortoises at the Desert Tortoise Conservation Center, Las Vegas, NV. The author used feces and chin gland secretions of males. During the mating season, more male tortoises used the burrow treated with chin-gland secretions than the control burrows but were less likely to use the burrow treated with another male’s feces. During the nesting season, more females used the control burrow than the burrow treated with another female’s feces. Feces and chin-gland secretions may influence burrow use.

2. Rautenstrauch and others (1998) monitored radio-telemetered desert tortoises at Yucca Mountain, NV, and reported that of 365 estimates of the date tortoises entered hibernacula, three tortoises entered hibernacula prior to September 17, whereas 98% of all tortoises entered hibernacula by November 15. Of 355 estimates of the date tortoises exited hibernacula, four tortoises exited prior to February 15, and 98% of tortoises were still in their hibernacula on February 15. Tortoises were found in burrows during 99.6% of 4,119 observations from November 15 to February 15. About one-half of the observations of tortoises out of burrows during that period were animals that were handled or otherwise disturbed. Adult males tended to enter and exit hibernacula later than adult females, and juveniles exited hibernacula earlier than other tortoises. The timing of entrance or exit from hibernacula also differed among some years, as tortoises may enter earlier in response to cooler temperatures, or exit earlier in response to warmer temperatures.
3. Hazard and Morafka (2002) released neonate and juvenile tortoises at the National Training Center, Fort Irwin, CA, and reported that juveniles were more active than neonates, occupying more locations per individual (4.3 versus 2.6) and taking longer to settle into a hibernation burrow. However, in the late winter (February and March), neonates moved more often than juveniles, suggesting that perhaps once juveniles establish burrows, they tend to not leave them, whereas neonates continue dispersing after hibernation.
4. Nussear and others (2007) studied hibernation among desert tortoises at four sites in the northeastern Mojave Desert. There was substantial individual variation in the timing and duration of hibernation among desert tortoises. Tortoises entered hibernation over as many as 44 days in the fall and emerged over as many as 49 days in the spring. Exogenous cues (e.g., temperature) did not appear to drive hibernation patterns at any site or within any year, but the onset of hibernation was early enough to prevent tortoises from being exposed to extreme and potentially lethal temperatures during winter.
5. Inman and others (2009) recorded activity of 35 tortoises surveyed in the Superior-Cronese and Ord-Rodman Desert Wildlife Management Areas, CA, and reported that above-ground activity varied throughout the activity season. The highest activity levels of an estimated 50–90% of animals occurred above ground in April and then decreased through May and June.
6. Agha and others (2015b) used motion sensor cameras at the entrances of tortoise burrows to investigate effects of temperature, sex, and day of year between June 1 and November 14, 2013, on activity of desert tortoises at the Mesa Wind Farm in the Colorado Desert, CA. They reported that males generally were more active than females. They also reported significant support for interactions between sex and day of the year and sex and temperature as predictors of the probability of activity.

VII. Spatial Relations

A. Home Range and Core-Use Area Sizes

1. O'Connor and others (1994a) monitored locations of desert tortoises adjacent to the Desert Tortoise Conservation Center, NV, and reported that Minimum Convex Polygon (MCP) estimates (corrected for the number of sightings) ranged from an average of 12.7 to 72.1 ha for 15 tortoises with greater than 17 recaptures. These home range area estimates were not significantly different from those estimated with other desert tortoise studies in the Mojave and Sonoran Deserts. Male tortoises had significantly larger and more variable home ranges compared with females in a statistical analysis of data from this study combined with those from Sonoran and Mojave populations. There were no data to confirm that gender differences in the home range area were due to reproductive movements. The authors indicated two limitations when interpreting the estimates. First, bootstrap analysis suggested substantial autocorrelation of tortoise sightings, which violated the assumption of independence likely causing underestimation of the true home range area. Second, comparison of "minimum" polygons created "by eye" with MCPs for the same tortoises suggested that MCPs included an average of 35% of "empty" space (i.e., areas with no evidence that tortoises used them).
2. Duda and others (1999) conducted radio telemetry surveys at the Marine Corps Air Ground Combat Center and Joshua Tree National Park, CA. Male desert tortoises had larger home ranges than females at both sites during both productive and drought years. Home range sizes of both male and female tortoises were significantly reduced during a drought year compared with a productive year. Home range size also varied by site. Home range sizes at Marine Corps Air Ground Combat Center averaged 7.7 ha for males versus 7.3 ha for females in the productive year, and 3.1 ha for males versus 0.9 ha for females in the drought year. At Joshua Tree National Park, home range size averaged 26.4 ha for males versus 8.5 ha for females in the productive year, and 6.7 ha for males versus 1.9 ha for females in the drought year.
3. Freilich and others (2000) observed four tortoises at the Pinto Basin, Joshua Tree National Park, CA. The tortoises made five forays (movement > 1 km) outside their normal home range boundaries and excluded these movements from the uncorrected Minimum Convex Polygon (MCP) home range calculations. Mean MCP home ranges for all years were 43.5 ha for males and 9.7 ha for females, which was significantly different between the sexes.
4. Hazard and Morafka (2002) released neonate and juvenile tortoises from head-start pens at the National Training Center, at Fort Irwin, CA. The tortoises moved 10–250 m from their release points during the first month, then entered hibernation. The distances moved did not differ between age classes, but neonates tended to move uphill to the northwest, whereas juveniles tended to move downhill to the northeast, in the direction of their natal pens.

5. Krzysik (2002), based on distance sampling and spatial modeling at the Marine Corps Air Ground Combat Center and at Joshua Tree National Park, CA, determined that estimated tortoise densities were scale-dependent and more variable at smaller spatial scales, indicating that tortoises were patchy in landscape distribution in the south-central Mojave Desert.
6. McLuckie and Fridell (2002) monitored 18 females at the Beaver Dam Slope, UT, and determined that mean home range (based on minimum convex polygon) was 25.6 ± 10.3 ha (mean ± SE) during the nesting season. Reproductive tortoises had smaller home ranges than non-reproductive tortoises, but not significantly so.
7. Harless and others (2009) radio-tracked 34 tortoises at Fort Irwin, CA, and reported that male tortoises exhibited different patterns of space and burrow use compared with females. Male home range and core area size averaged 65 and 73% larger than females, respectively. This suggested a lack of territoriality in this population.
8. Using data from 35 tortoises, Harless and others (2010) reported estimated home range sizes using all locations (mean number of locations per tortoise was 89 in 2004 and 105 in 2005) as 43–49 ha for males and 16–17 ha for females based on 100% corrected Minimum Convex Polygon (MCP), and 28–32 ha for males and 11–14 ha for females using 95% Fixed Kernel (FK) over 2 years. This was over two times larger compared to previous studies on desert tortoises in the western Mojave Desert. However, home range estimators were sensitive to sampling frequency and MCP estimates were consistently higher than FK estimates, suggesting that the validity of home range estimates is greatly affected by choice of estimator.
9. Franks and others (2011) radio-tracked 60 tortoises at four study sites across the western Mojave in 2001. The mean uncorrected Minimum Convex Polygon (MCP) home ranges for females from the most eastern study site westward was 7.6 ha ± 3.7 SD in Ivanpah Valley, 7.2 ± 5.7 ha at Fort Irwin, 2.1 ± 3.5 ha SD at Superior, and 1.6 ± 2.7 ha SD at Fremont. Males were tracked at three of the four sites and had uncorrected MCP home ranges of 16.2 ± 8.3 ha SD at Fort Irwin, 5.8 ± 7.0 ha SD at Superior, and 9.2 ± 7.3 ha at Fremont. Males had significantly larger home ranges than females at both Fort Irwin and Fremont. Lack of effect at the Superior site was probably due to the small sample size, as only four male and female tortoises each were tracked.
10. Lovich and others (2011b) reported that activity areas of eight female desert tortoises at a wind energy facility near Palm Springs, CA, did not differ between time intervals from a fire in 1995: proximate post-fire areas ranged from 2.85 to 42.63 ha and long-term post-fire areas ranged from 3.34 to 30.75 ha. The percentage of activity area that was burned also did not differ between time intervals.

B. Movements, Use of Space, and Sampling

1. O’Connor and others (1994a) tracked tortoises with radio transmitters at a study area south of the Desert Tortoise Conservation Center, Las Vegas, NV. The authors reported that tortoise movements varied with the interval between sightings, and sex (male > female), but was independent of maximum carapace length. The distance of movements between re-sightings for both sexes was distributed exponentially, with most movements covering relatively short distances (<200 m).
2. Boarman and others (1998) concluded that a passive integrated transponder (PIT) system, which included the tag and automated reading system (ARS), was effective to evaluate movements of desert tortoises through culverts (i.e., underpasses) along a highway. Numerous design constraints were overcome to successfully employ this method, including problems with security from environmental and human-caused hazards, and aspects of automated operation. Although the initial set-up costs were $1,250–$10,000, the authors felt this cost was reasonable given the high cost of observational behavior or radio-telemetry studies, especially for > 10 animals since each PIT tag cost $5–$8.
3. Duda and others (2002) studied tortoise movements at two sites at the Marine Corps Air Ground Combat Center, CA, and reported that the spatial patterns of desert tortoises and burrows (active and total) were aggregated, which was expected for at least two fundamental reasons—habitat quality and social interactions.
4. Krzysik (2002) integrated four sampling design elements, including distance sampling and spatial modeling, and found that estimated tortoise densities were scale dependent and more variable at smaller spatial scales, indicating that tortoises exhibited patchy distribution patterns across the landscape. At one of the study sites, the Sand Hill Training Area of the Marine Corps Air Ground Combat Center, tortoises where at a higher density, indicated by finding live tortoises and their burrows and scat (an indicator of space use) in the southeast corner while the central portion had the lowest density. This was an important finding for the Marine Corps as the central portion had been previously designated as a Desert Tortoise Conservation Zone, whereas the high tortoise density to the southeast of that area was previously unknown.
5. von Seckendorff Hoff and Marlow (2002) surveyed for tortoise sign (live, dead, cover sites, scat, etc.) by walking transects 500 m long parallel to the roads and spaced 10 m apart for up to 1 km. For distances greater than 1 km from the roads, transects were spaced 100 m apart. They found that total sign count was significantly correlated with distance from roads for all distances < 120 m, and therefore, selected transect intervals of 100 m for their research. However, they suggested that in areas where tortoise density or the influence from the road is suspected to be low that smaller distances between transects (i.e., more tightly packed transects), deeper transects (i.e., extending farther from the road), or longer transects be used to increase the probability of detecting an effect, if one exists. More tortoise sign was also found during the fall following a wet year compared to the summer of a drier year, suggesting wet year surveys might be more efficient in detecting trends in areas where tortoise densities are suspected to be low.

C. Effects of Drought or Increased Rainfall on Space Use

1. Ruby and others (1994a). See VI.A.1, this chapter.
2. Duda and others (1999) conducted radio telemetry surveys of tortoises at the Marine Corps Air Ground Combat Center and Joshua Tree National Park, CA. The authors reported that activity patterns (e.g., home range size, number of different burrows used, average distance traveled per day, and levels of surface versus burrow activity) were significantly reduced during a drought year compared to a productive year. These results suggested that differences in levels of winter precipitation between years and the resulting variation in winter annual biomass in spring appeared to play a significant role in desert tortoise movement and activity patterns.
3. Freilich and others (2000) reported that capture probabilities from the northern one-half of the plot in Joshua Tree National Park, CA, for 97 individual tortoises were greater in the 4 years with regular rainfall compared with the 2 years of drought conditions. Furthermore, tortoises were more likely to be found in burrows rather than above ground in years of drought when lower amounts of forage were available.
4. Krzysik (2002) surveyed for live and dead tortoises and their burrows and scat at the Marine Corp Air Ground Combat Center and Joshua Tree National Park, CA, during a productive (i.e., wet) year and compared his findings on burrows to a concurrent study that covered 2 years, including a drought year. The author found that during both productive and drought year, that the majority of tortoises burrows (75 %) were shallow (< 1 m). However, during the drought year, more tortoises were found in their burrows than above ground.
5. Franks and others (2011) radio-tracked 22 females at their Ivanpah Valley study site and 7 females at their Fort Irwin study site in 2000. The mean corrected MCP home ranges for females was 34.7 ± 33.7 ha at Ivanpah, which received four times more rainfall that year, compared to 10.8 ± 12.0 ha at Fort Irwin. When combining their data with those of Duda and others (1999) and Freilich and others (2000), they found a significant, but weak relationship between home range size and rainfall, with both males and females increasing their home range size with increased amounts of rain. The weak relationship suggests other study site specific factors, such as soil types, primary productivity, or predator abundance, are explaining the relationship more than rainfall.
6. Ennen and others (2012a), in a study of adult female tortoises at the Mesa Wind Farm near Palm Springs, CA, reported increased activity areas, more frequent movements, and increased number of burrows used in an El Niño Southern Oscillation (ENSO) year with substantially higher precipitation and spring wet biomass. This was presumably in response to increased resource availability. Tortoises also had higher activity during a drought year following the ENSO event, suggesting a possible lag effect.

D. Spatial Overlap in Home Ranges and Site Fidelity

1. O'Connor and others (1994a) measured locations of desert tortoises in a study area adjacent to the Desert Tortoise Conservation Center, NV, and reported relatively little overlap of home ranges of individual tortoises early versus late in the activity season (average overlap = 18%). Plots of locations of multiple individual tortoises revealed a dense interspersion of points from different tortoises consistent with non-exclusive home ranges. The overlap of home range polygons provided no support for any territoriality or exclusivity of home ranges between individuals.

2. Freilich and others (2000) found strong site fidelity for tortoises in Joshua Tree National Park, CA, with 53% of all captures made within 200 m and 75% within 300 m of the tortoises' previous location. When autocorrelation was removed and captures were ≥ 1 year apart, site fidelity was 22% within 100 m and 73% within 300 m of the tortoises' previous location.

3. Harless and others (2009) radio-tracked 34 tortoises at Fort Irwin, CA, and reported that female tortoise home ranges did not overlap. Female tortoises had a strong male bias in overlap of both space use and burrow sharing, whereas males overlapped and shared burrows with a similar number of tortoises of either sex. This suggested a lack of territoriality in this population.

E. Activity Areas in Disturbed vs. Undisturbed Habitats

Agha and others (2015c) evaluated sizes of tortoise activity centers as part of a 18-year study comparing survivorship of *Gopherus agassizii* at a wind energy facility and in a neighboring wilderness area, California. The sizes of activity centers varied but not significantly so between the wind energy area (6.25 ± 2.13 ha) and adjacent wilderness area (4.13 ± 1.23 ha).

VIII. Implications for Management or Recovery

A. Boarman and others (1998) documented five tortoises crossing through or using storm-drain culverts as refugia on 75 occasions along a California State Highway with barrier fences. This suggested that desert tortoise mortality could be reduced by using a combination of culverts to allow tortoise movement beneath highways and barrier fences to prevent tortoises from crossing over the road surfaces in the western Mojave Desert.

B. Nussear and Tracy (2007) mentioned how understanding the effects of behavior and its effect on tortoise detectability is important with methods used to estimate population sizes or densities and this should be given consideration when designing surveys.

C. Harless and others (2010) found that tortoise home range sizes were greatly affected by the choice of estimator and the chosen sampling frequency. Home range sizes were larger using the Minimum Convex Polygon (MCP) estimator compared with the Fixed Kernel (FK) estimator. Increased sampling frequency inflated MCP home range sizes but deceased home range sizes when using the FK estimator. Using the FK and MCP in combination may best represent the total land use patterns, as it will provide a comprehensive understanding since MCP will represent the total amount of area potentially used within and between years and FK will identify specific areas of intensive use. A broad understanding using both estimators is important for land management or conservation planning.

D. Mack and others (2015), drawing on studies of the thermal environment of tortoise cover sites in the Soda Mountains, CA, noted that:

1. Cover sites that buffer temperature extremes and fluctuations will become increasingly important for survival of tortoises with climate changes and warming.
2. Successful translocations may be limited by availability of suitable cover sites. The authors suggest that during periods of extreme temperatures, suitable cover sites should contain long tunnels and larger openings, and that the type of terrain and underlying substrate (e.g., old alluvial fans and conglomerate, limestone and dolomite formations). More information is needed about geographical distributions of geological formations likely to support optimal cover sites.

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Chapter 5.—Foraging Behavior, Digestion, and Nutrition

I. Digestive Tract and Digestion

A. Functional Anatomy of the Digestive Tract

Barboza (1995a) studied the digestive tracts of seven *Xerobates* [*Gopherus*] *agassizii* and identified the esophagus, stomach and fundic region, pyloric region, small intestine, proximal colon, and distal colon. The tortoise has a large digestive tract capacity up to 13% of body mass. The proximal colon was the longest section with the greatest apparent surface area, followed by the small intestine with the next highest surface area. The tongue, esophagus have thick cornified epithelia that resist sharp spines and fibers; mucus secretions facilitate swallowing coarse feeds. Gastric contents were acidic, whereas hindgut digest were near neutral pH. The colon was the primary site of fermentation. On diets of grass and high-fiber pellets, digestive capacity was large and estimated at 11–21% of body mass. The relatively simple hindgut anatomy may retain the widest flexibility in utilizing low-fiber fruit and herbage diets as well as coarse grasses. The capacious but simple digestive anatomy may provide the greatest flexibility in utilizing a variety of forages in its unreliable habitat. The versatile digestive strategy is consistent with the persistence of tortoises in many hot arid regions despite drastic changes in these habitats.

B. Digestion and Passage of Digesta in the Gut

1. Barboza (1995a) studied digestion and digesta passage of 22 tortoises in cages at the Desert Tortoise Conservation Center, Las Vegas, NV, in 1992; tortoises were fed separate diets of *Schismus barbatus*, *Sphaeralcea ambigua*, and two formulated pelletized diets (high and low fiber). Retention time was greater on grass (large particles) (15.95 days compared with herbage [9.57 days] and low-fiber pellets [5.29 days]). Tortoises were in positive nitrogen balance on all diets but near zero balance on the grass diet. On the grass diets, the tortoises experienced body mass losses, whereas they made gains on the herbage and pelleted diets. Large particles of grass were retained longer.
2. Meienberger and others (1993) conducted feeding trials on 14 tortoises using succulent green leaves, stems, and buds of a non-native forb, *Erodium cicutarium*, and dried leaves and stems of non-native grass, *Schismus barbatus*. Eight tortoises would not eat one or the other of the plant species and one tortoise would not eat either plant species. The authors determined transit times for most tortoises for the two plant species. Tortoises eating *S. barbatus* had lower transit times than did those eating *E. cicutarium*. Transit times were affected by body mass, whether females were carrying eggs, and if so, the number of eggs.

3. Tracy and others (2006a) conducted a laboratory experiment on hatchling and juvenile captive tortoises kept in plastic cages at Colorado State University to determine rates of passage of digesta in relation to body size and diet quality. The authors used labeled guinea pig and chick starter chows (high and low fiber). The size of tortoises influenced passage times, with food taking several days longer to pass through juveniles than hatchlings. Different passage times of liquid and particulate fractions of digesta may enhance rate of nutrient extraction. There was shorter mean retention time of the liquid than solid fraction, and particularly when higher in fiber. They thought that a diet consisting primarily of low-fiber foods could yield more energy per unit time than a high-fiber diet, and that it would be advantageous for young tortoises to have the opportunity to specialize on low-fiber diets. Invasion of weedy annual grasses that preclude access to low-fiber foods could constrain assimilation in young desert tortoises

C. Carbon Incorporation Rates and Diet-to-Tissue Discrimination in Growth of Desert Tortoises

Murray and Wolf (2012) conducted a diet switch experiment on captive-bred hatchlings using carbon isotopes in the laboratory. The average carbon retention times for red blood cells (RBCs) and plasma were 126.7 ± 40.3 and 32.9 ± 14.5 days, respectively. The authors analyzed growth using changes in straight line carapace, appearance of growth rings, and weight. Keratin samples were analyzed for $\delta^{13}C$ using a continuous flow isotope ratio mass spectrometer. Tissue carbon incorporation rates were affected by both growth and metabolism, with growth accounting for 50% of the carbon turnover in RBCs and 13% of carbon turnover in plasma.

II. Feeding Behavior

A. Limitations on Access to Food

Morafka and Berry (2002) pointed out that adult desert tortoises are confined to eating plants within approximately 15 cm of the substrate; neonates and small juveniles probably cannot reach much above 3 cm and tend to focus within 1–2 cm of the soil surface. The types of foods and capability of acquiring the foods differ according to age and size, with neonate and juveniles confined to the more delicate and tender shoots and flowers. Neonates and juveniles also are more constrained in movements and biting strengths than adults.

B. Types of Plant Foods Available to Desert Tortoises

Morafka and Berry (2002) reviewed the literature on ecogeographic origins of plants consumed by desert tortoises from across the Mojave and northwestern Sonoran Deserts. The desert tortoise forages on five major groups of plants: (1) annual forbs (winter or summer ephemerals), (2) annual grasses (winter or summer), (3) cacti, (4) native perennial bunch grasses, and (5) herbaceous perennial shrubs. The plants have their origins from the Arcto-Tertiary geoflora, Madro-Tertiary geoflora, Madrean-Tethyan elements, and California Floristic Province elements, and have C3, C4, and CAM photosynthetic pathways. The richness of the desert flora is the results of the accumulation of many taxa during the Tertiary and Quaternary, taxa pre-adapted to drought in the region.

C. Foraging Behavior and Evidence for Preferences in Wild and Semi-Wild Tortoises

1. Semi-wild Tortoises. Oftedal and others (2002) observed 15 juvenile tortoises (5–7 years of age, mean carapace length of 81.0 mm) foraging between April 24 and May 1 for 33.2 h inside naturally vegetated enclosures at the head-start pens, Fort Irwin, Mojave Desert, CA. Only bites that appeared to be productive were counted as feeding bites.

    a. Twenty-nine of the 38 (76%) annual plant species found outside the enclosed pens in the surrounding creosote/white bursage scrub were observed inside the pens. Tortoises encountered 18 of the 29 species and encountered *Schismus* spp. most often (239,400 plants, 98.06% of all plants), followed by species representing greater than 100 plants: *Cryptantha angustifolia* (1,741, 0.71%), *Camissonia claviformis* (1,054, 0.43%), *Erodium cicutarium* (688, 0.28%), *Chaenactis fremontii* (596, 0.24%), *Plantago ovata* (346, 0.14%), and *Malacothrix glabrata* (104, 0.04%). *Encountered* was defined as all plants that foraging tortoises walked past or approached within one body width on each side, whether eaten or not.

    b. The numbers of bites per foraging session differed among plant species, with tortoises foraging most often on *C. claviformis* (accounted for about 50% of all bites taken), *E. cicutarium*, *P. ovata*, and *M. glabrata*. Tortoises were selective in the parts of plants eaten, with leaves accounting for 72% of all bites. Of the four primary food species, the parts eaten were higher in water, protein, and potassium excretion potential (PEP), and lower in potassium than uneaten parts. Excluding *Schismus* spp. because tortoises rarely ate it despite its abundance (0.02% of plants encountered), plants eaten were higher in protein and potassium excretion potential (PEP) and lower in potassium than plants bypassed while foraging when considering all plants encountered. This suggested that in a year of abundant plant germination, juvenile tortoises were able to self-select a diet of high nutritional quality when there was sufficient access to species with high PEP parts such as *C. claviformis* and *M. glabrata*.

2. Wild Tortoises. Based on observations of 16 adult free-ranging tortoises foraging on the Desert Tortoise Research Natural Area, CA, Jennings (2002) reported that tortoises ate 44 plant species of seven shrub species and 71 annuals and herbaceous perennials detected.

    a. Ten species of native annuals and herbaceous perennials comprised 81.4% of their diet. The plant species included *Lotus humistratus* (29.7%), *Mirabilis bigelovii* (10.8%), *Chamaesyce albomarginata* (10.7%), *Astragalus layneae* (8.2%), *Prenanthella exigua* (5.6%), *Astragalus didymocarpus* (4.6%), *Camissonia boothii* (3.9%), *Erodium cicutarium* (3.3%), *Chorizanthe brevicornu* (2.6%), and *Phacelia tanacetifolia* (2.0%). These observations suggest that tortoise dietary requirements are quite specialized on these plants that are relatively rare in the western Mojave Desert, and that loss of these preferred plants due to habitat deterioration or invasive species would be quite detrimental.

  b. The author noted that flowering phenologies of preferred annuals and herbaceous perennial food plants were variable and that tortoises focused their foraging efforts on specific phenological stages of preferred food plants when available, such as first flowering or peak flowering for particular species. This resulted in dramatic seasonal variation in tortoise diet among the 10 preferred food plants and suggests that different phenological states may have varied in palatability and possibly nutritional value.

  c. The author noted that regardless of when tortoises initiated consumption of preferred food plants, they abruptly ceased to feed on any plants that become dry.

3. Wild Tortoises. Henen (2002a) conducted a study of nine female desert tortoises at Goffs, eastern San Bernardino Co., CA, between July 1987 and July 1989, and synthesized data on energy and water budgets, diets, and reproductive output. He reported that diets reflected adjustments to variable abundance and nutritive value of desert plants, especially winter annuals.

   a. Of 13,743 bites consumed (not attempted) by females during three years, 89, 7, and 4% were of annuals, perennials, and other materials, respectively. Annuals comprised the majority of the diet in summer and fall and included a relatively high consumption of dry *Schismus barbatus*, an alien annual grass, which the author suggested may have enhanced energy balance and lipid stores in tandem with drinking.

   b. The apparent preference for annual plants may be partly explained by high protein concentration of new annuals in spring, or high water content of new winter or summer annuals.

4. Captive and Wild Tortoises Combined in Reporting. Tracy and others (2006b), drawing on studies of 13 wild and 10 captive tortoises in the northeastern Mojave Desert, reported that 90% of tortoise diets were comprised of eight plants out of the approximately 100 available, and about one-half of those plants eaten were nonnative. Their results supported the specialist hypothesis, where select foods are eaten to maximize digestible energy.

   a. Their results may indicate a mechanism for observed preference for plants with longer phenologies.

   b. The authors found no evidence that tortoises foraged to specialize in particular nutrient intake, but there was a high correlation among nutrients in plants eaten by tortoises. Tortoises specialized in foods rich in protein and low in fiber, which generally would result in a diet with more digestible energy, calcium, magnesium, phosphorus, and potassium, important elements for producing bones, shells, and eggs.

   c. There was no evidence that desert tortoises foraged in ways that maximized their potassium excretion potential.

5. Wild Tortoises. Jennings and Berry (2015) observed 18 wild adult tortoises take 35,388 bites at the Desert Tortoise Research Natural Area in the western Mojave Desert, CA, during the spring season in 1992, an El Niño year with abundant wildflowers. One objective was to determine whether tortoises select particular plants for eating (selective foragers) or instead consume plants in relation to availability (non-selective foragers). They also evaluated abundance of plant species in three habitats where tortoises foraged (alluvial fan, low rock and gravelly hills, and ephemeral stream channels). The annual plant species had different phenologies and availabilities during early, mid-, and late spring. The authors reported that:

   a. Tortoises took 23,583 bites from annual plants, 10,660 bites from herbaceous perennial plants, 695 bites from a single dead leopard lizard (*Gambelia wislizenii*), 414 bites from unidentified plants, and 36 bites from tortoise scat.

   b. Plants eaten differed by phenological period (the spring season was divided into three phenological periods). Most of the recorded bites were on about 10% of the plant species available (8/78), with tortoises consuming plant species in different proportions and times during spring depending on their flowering phenologies.

   c. About 48% of bites from annual plants and 64% of bites from perennial plants were from plants not detected in plant surveys of the habitats. Overall, about two-thirds of bites were from annual species and one-third from perennials. Plants in a succulent state comprised 96% of bites and 45% of bites were from legumes. Two species of legumes, *Acmispon brachycarpus* and *Astragalus layneae* accounted for more than 73% of plants eaten and 71% of bites taken during the second phenological period. Some plant species (*Acmispon brachycarpus*, *Chamaesyce albomarginata*, *Astragalus layneae*, *Mirabilis laevis*) were rare in the habitats but still found and eaten by the tortoises.

   d. About 30% of the tortoise diet was of herbaceous perennials, which may help maintain tortoise health during drought years. Some of these species will regenerate stems, leaves, and flowers following rainfall outside of the normal winter and spring flowering seasons.

   e. Desert tortoises are selective foragers and selection of plant foods may be affected by nutrition and potassium levels. The tortoises in this study primarily consumed forbs and herbaceous perennials and not grasses.

   f. Activities that negatively affect key tortoise food plants (e.g., off-road vehicle use, livestock grazing, or climate change) may negatively affect tortoises.

D. Captive Tortoises and Potential Preferences—Laboratory Experiments

1. Okamoto (2002) conducted experimental feeding trials with captive tortoises from western Mojave (n = 16) and Sonoran Desert (n = 4) lineages. Juveniles ranged in size from 54 to 89 mm mid-carapace length. Juveniles showed a color preference for green food pellets (45.6% of recorded bites) over yellow (23.3%), red (17.8%), and orange (13.3%) pellets. Preference was given to eggshell (87.8%) over green (6.5%) and yellow (5.7%) pellets, as well as cuttlebone (91.5%) over green (7.0%) and yellow (1.5%) pellets. Preference was also given to live crickets (43.7%) and mealworms (41.0%) over green (15.3%) pellets and dead crickets (50.2%), and mealworms (38.9%) over green pellets (10.9%). Although causes for food preferences are unknown, they may be based on nutritional content, e.g., selection of white cuttlebone or eggshell as calcium sources needed for growing juveniles or insects for protein content.

2. Tracy and others (2006b). See I.B.3, this chapter.

3. Hazard and others (2009) reported that captive juveniles at the University of California, Los Angeles, fed dry grass (*Schismus barbatus*, *Achnatherum hymenoides*) on a voluntary basis, ate much less of it than they did of the two forbs (*Malacothrix glabrata, Erodium cicutarium*), suggesting that, when juveniles feed on dry grasses in summer, they may do so reluctantly.

E. Omnivory, Insectivory, Osteophagia, and Consumption of Bones

1. Morafka and Berry (2002), in a summary of the literature on foraging behavior, noted consumption of caterpillars, citing field observations of other ecologists from Ivanpah Valley, CA.

2. Okamoto (2002) reported use of mealworms and cuttlebone in experiments with captive tortoises.

3. Walde and others (2006) reported a tortoise to eat three pellets of scat of the black-tailed jackrabbit (*Lepis californicus*) in the western Mojave Desert, near Barstow, CA. Also, at widely separated locations in the study area, two separate observations of tortoise feces containing entire *L. californicus* scats were discovered, suggesting different individuals in each case.

4. Walde and others (2007a) reported separate behavioral observations in the western Mojave Desert, where desert tortoises (two females; one male) were observed seeking and consuming small bones of dead desert tortoises.

    a. Various other accounts of desert tortoise consumption of calcium rich deposits, reviewed in their paper, led the authors to suggest that desert tortoises seek out calcium-rich substances for mineral supplementation of their shell.

    b. The authors warned that removal of these shells from study areas for research purposes could be depleting an important, limited nutrient resource.

5. Brennan (2012) observed a tortoise (carapace length, 170 mm) in the eastern Mojave Desert eating portions of a disarticulated skeleton of a desert tortoise.

6. Jennings and Berry (2015), in a spring 1992 study at the Desert Tortoise Research Natural Area, CA, reported that a tortoise took 695 bites of a dead leopard lizard (*Gambelia wislizenii*).

F. Consumption of Non-Food Items

Walde and others (2007b) observed an adult male tortoise that had ingested a 108-cm portion of ribbon that was attached to a balloon fragment at a study area 40 km northeast of Barstow, CA, in desert tortoise critical habitat. When the researchers carefully extracted the ribbon, they found digested vegetative material on the ribbon indicating it had been far down the digestive tract. The researchers documented 178 new balloons had arrived in an 8-month period (22.3 balloons/month) from as far as >200 km away, and possibly from as far as >270 km away, from the study area.

III. Research on Nutritional Content of Forage and Value to Tortoises

A. Comparisons of Grasses and Forbs

1. Meienberger and others (1993), in experimental feeding trials on frozen green *Erodium cicutarium* and dried *Schismus barbatus* conducted in an outdoor laboratory at the University of California, Los Angeles, on 14 desert tortoises from Clark County, NV, determined that one tortoise refused to eat both diets, two refused the *E. cicutarium* and six refused *S. barbatus*. These animals were not included in the study.

   a. During the *E. cicutarium* feeding periods, all tortoises gained mass, whereas tortoises eating *S. barbatus* lost a small amount of mass each day.

   b. Flow through the digestive tract was tubular flow; tortoises that ate more had lower digesta transit times, which, in turn, affected digestibilities. Digestibilities of dry matter increased with increasing transit time.

   c. Total food consumption (total amount of food consumed between oral administration of marker and excretion of marker) was relatively stable over the measurement periods for any given tortoise fed either succulent *E. cicutarium* or dry *S. barbatus*, regardless of intake rate, but was related allometrically to body mass.

   d. Higher feeding rates and corresponding shorter transit times induced lower digestibility of dry matter, gross energy, and cell-wall components.

   e. The gastrointestinal tracts of tortoises eating green *E. cicutarium* contained nearly three times as much dry matter compared with tortoises eating a dry diet of *S. barbatus*, possibly related to the different properties of green and dry plant parts. The gut of a tortoise will hold more of the forb than the grass, and the transit time will be faster.

   f. Data on nutrient composition and digestibilities suggested that tortoises eating *S. barbatus* needed to invest large quantities of body water in digestion compared with *E. cicutarium*, which also contained about three times more nitrogen on a dry matter basis than *Schismus*. Tortoises eating *S. barbatus* did not digest enough of the dietary nitrogen to cover fecal nitrogen losses.

   g. Three gravid females observed had a reduced gut fill, presumably because eggs in the oviduct reduced the potential gut volume. Females with eggs were at a disadvantage for foraging if the only forage available was dry.

2. Barboza (1995b), in a study of nutrient balances and maintenance requirements for nitrogen and energy of eight adult tortoises fed *Schismus barbatus* and/or *Sphaeralcea ambigua*. Both plant species were collected in spring when green, frozen, and then thawed before providing to the tortoises in May. The author reported that:

   a. *Sphaeralcea ambigua* was higher in moisture content than the grass, contained more N but less fiber than grass, and tortoises ingested and digested more dry matter and energy from this herbaceous species than from the grass. Total mineral content as inorganic ash was greater in *Sphaeralcea*. Intakes and retention of N were greater from this herb than the grass, but amino acid proportions and true N digestibilities were similar between the diets.

   b. *Sphaeralcea ambigua* contained more Ca than the grass, especially in relation to P (Ca:P 14.5 vs. 1.9), but this was ameliorated by lower absorption of Ca from the herb. K intakes were greater from the herb than the grass and associated with digestive loss of Mg from the herb. Low Na content of both forages resulted in net losses of Na.

   c. Tortoises maintained similar body mass between diets, with small mass gains on *Sphaeralcea* and minor mass losses on *Schismus*.

   d. This paper contains considerable detail on amino acid concentrations in the two plant species and other topics. For the two species, the contributions of the sulfur amino acids to the N content of the forages were probably adequate for adult tortoises but could limit growth of young tortoises.

   e. The maintenance requirements of N in desert tortoises are lower than those of other herbivorous reptiles. Because of the slow loss of Na and Mg on these two forages, tortoises eventually would require complementary intakes of other foods to restore the loss of these minerals. Therefore the availability of a selection of forages within the home range may be critical to its nutrient balance in the long term.

3. Nagy and others (1998) conducted feeding trials on 15 captive desert tortoises to determine digestibilities of dry matter, energy, water, and nitrogen for four food plants in spring and the grass *Schismus barbatus* in summer. The grasses were the native grass (*Achnatherum hymenoides*) and the exotic grass (*Schismus barbatus*). The forbs were the native forb (*Malacothrix glabrata*) and for the exotic forb (*Erodium cicutarium*).

   a. The digestibility of nutrients in a native forb (*Malacothrix glabrata*) and an exotic forb (*Erodium cicutarium*) were similar, ranging from 63–70% for dry matter, 69–73% for energy, 72–79% for nitrogen, and 70–75% for water. The exotic forb provided 22% more nitrogen on a fresh matter basis than did the native forb. Which forb was more nutritious depended on whether tortoises ate to obtain a given volume of food (full stomach) or a given amount of digestible energy.

   b. The dry matter and energy digestibility of a native grass (*Achnatherum hymenoides*) and an exotic grass (*Schismus barbatus*) were both much lower than the forbs, but were similar to each other, ranging from 46 to 50%. Both grasses provided little or no nitrogen, and the tortoises lost more water than they gained while processing the grasses. Results suggest that dry grasses may be the least toxic foods available to tortoises in summer, and the type of food plant (forb or grass) and its phenological stage, rather than its geographic origin (native or exotic), best predicted its nutritional value.

4. Henen (2002b) reported that nitrogen or crude protein appeared to be the primary limiting resource for producing eggs, although lipid reserves were also important for female desert tortoises in remote sites in the eastern Mojave Desert. During drought years, water availability may also limit egg production.

5. Hazard and others (2009, 2010) conducted feeding trials on 20 juvenile, captive tortoises maintained in the laboratory, using four species: (a) one native grass (*Achnatherum hymenoides*), (b) one nonnative grass (*Schismus barbatus*), (d) one native forb (*Malacothrix glabrata*), and (c) one nonnative forb (*Erodium cicutarium*). The largest nutritional differences were between food types (fresh forb and dry grasses) rather than between native and exotic species.

   a. Forbs provided more energy, nutrients, and minerals (nitrogen, calcium, phosphorus, and magnesium) than did grasses. Forbs had less fiber and about five times more nitrogen than grasses. Juveniles gained weight rapidly (up to 0.5% of body mass added per day) when eating forbs. While eating grasses, tortoises lost body mass, shell volume, nitrogen, and phosphorus. Tortoises had a net loss of nitrogen and phosphorus on dry grass diets.

   b. Estimates of nitrogen requirements compared to annual nitrogen intake on these diets suggested that growth of juveniles may be limited in part by dietary nitrogen. Comparisons of nutrient availability to estimated requirements for growth and for egg production suggest that phosphorus is more limiting than calcium or magnesium. Excess dietary calcium may pose a significant osmotic challenge for excretion. The apparent avoidance of grasses may be important for juveniles to avoid nitrogen and phosphorus loss and to maintain growth.

B. Potassium Excretion Potential of Desert Plants

1. Based on a review of literature from studies conducted in the Mojave Desert, Oftedal and Allen (1996) reviewed the findings of their research on potassium and reported that:
    a. Tortoises were invariably able to select diets lower in potassium.
    b. The voluntary food intake of tortoises was negatively correlated to dietary potassium concentration when fed a single diet.
    c. The negative effect of potassium on food intake was more pronounced when nitrogen levels were low (e.g., 1.6% N or 10% crude protein).
    d. A diet high in nitrogen (e.g., 3.2% N or 20% protein), does not permit positive nitrogen balance in young tortoises if the diet also contains high potassium (e.g., about 3.8%).
    e. The results led the authors to hypothesize that tortoises in the wild may be naturally constrained to select plants by their nitrogen-to-potassium ratio (N:K), and that certain plants with high N:K ratios (including legumes) may be of particular nutritional importance.
2. Oftedal (2002) reviewed the literature of studies conducted in the Mojave and Sonoran Deserts, including his own field work, and noted several general features that may be important in tortoise food plants:
    a. Tortoise food plants vary in a wide array of nutrients, and tortoises face a difficult balancing act between one potentially toxic resource (potassium), and two scarce resources (water and protein). It is possible to calculate a potassium excretion potential (PEP) index integrating all three constituents to estimate how much potassium could potentially be excreted based on the amounts of water and nitrogen in the food, and to compare this with the amount of potassium contained in the food. A positive PEP index indicates there is more water and nitrogen in the food than is needed to excrete the potassium.
    b. PEP indices vary greatly both among plant species and within a plant species according to phenological stage. At the phenological stage at or near the transition from flowering to first fruit, major tortoise annual plant foods with high PEP indices (>15) are evening primroses (Onagraceae), filaree (Geraniaceae), legumes (Fabaceae), mustards (Brassicaceae), and spurges (Euphorbiaceae).
    c. Drought-adapted perennial shrubs generally have a low PEP index and are probably poor food for tortoises. There is some evidence that tortoises seek out high-PEP plants, at least in wet years, which provides a possible explanation for highly selective foraging behavior reported earlier by the senior author.

d. Tortoises rarely have the opportunity to drink because winter rains fall when tortoises are inactive. Thus, tortoises must obtain most water from winter annuals and spring-active herbaceous perennials. However, summer drinks allow tortoises to dump potassium-rich urates and urine from their bladders, reduce circulating potassium levels, and refill their bladders with dilute, hypo-osmotic urine. Such rehydrated tortoises are able to eat senescent grasses, deposit body fat, and are better prepared to withstand subsequent droughts.

e. Tortoise foods vary as a function of regions, climate, and year-to-year variability in rainfall. In the Mojave Desert, the biomass production of winter annual plants can range from zero in a drought year to more than 500 kg/ha in a wet year, thereby providing for very different foraging opportunities and choices for tortoises. In areas of both winter and summer rainfall, at least four different scenarios based on plant responses to rainfall patterns include (i) years of drought, (ii) low winter rainfall, (iii) high winter rainfall, and (iv) high summer rainfall, all of which affect foraging choices.

f. The replacement of C4 perennial grasses by invading annual C3 grasses such as Mediterranean and brome gasses may impact the nutritional status of the tortoises. This is due to the lower protein and PEP content of C3 desert grasses and their rapid phenological maturation with an associated decline in nutrient concentrations.

g. Based on a list of major plant species eaten by tortoises extracted from the literature, 43 major food species were observed. Twenty-six of these grew throughout the Mohave and Sonoran Deserts and, thus, could be of wider importance as tortoise food. However, only eight species were reported as major foods in both deserts, and only five were reported in both the western and eastern (or northeastern) Mohave Desert. The substantial differences in species eaten among areas cannot be solely attributed to regional patterns of plant distribution and may be due to different patterns of habitat use and floras between Mojave (in which tortoises typically inhabit broad basins and bajadas) and Sonoran Deserts (in which tortoises typically inhabit rocky and hilly terrain). Much of the variation reported is probably due to the limited duration and habitat coverage of most studies. The abundance and diversity of annual plants, and tortoise food choices, varies so greatly from year to year and even from one local site to another that it is difficult—if not misleading—to characterize an average tortoise diet.

IV. Contaminants in Soils and Plants—Elemental Contaminants, Amounts, Locations, and Sources

Chaffee and Berry (2006) studied concentrations of elements at six sites in the Mojave and Colorado Deserts, CA: (1) Western Mojave—Northern Section, (2) Western Mojave—Southern Section, (3) Goldstone, (4) Goffs, (5) Chemehuevi Valley, and (6) Chuckwalla Bench. They collected 256 samples of soils, 36 sediments from stream samples, and 366 samples of 35 different taxa of desert tortoise forage plants. Studies of concentrations of elements in soils were also presented from the same study areas. The studies were of elemental concentrations, not bio-available concentrations. The sources of some potentially toxic contaminants were dust (wind-borne) or systemic, from the substrate, or from anthropogenic sources, and varied by area.

A. Plants

1. The highest ratios of maximum concentration to geometric mean values were found for As, Au, Ce, Cr, Cs, Hf, La, Sb, Sc, Sm Th, U, W, and Yb in the western Mojave Desert study area (southern section) and As, Au, Ce, Mo, Na, and Sb in the western Mojave Desert study area (northern section). Because As and W are ore-related elements unessential for plant metabolism, the authors suspected that these two elements were present as contaminants on surfaces of plant parts. High As concentrations were found almost exclusively in plant samples collected in or near areas known to be contaminated by mining of As-rich ores (western Mojave and Goldstone). The highest As concentrations were found in 13 different plant species, of which five are legumes favored by tortoises.
2. In plants at the Goldstone study area, high values were found for most elements, with the highest values for Au and Mo, Rb, and W. Some of the plant material may include local surface contamination of eolian dust or substrate materials.
3. In plants at the Goffs study area, the highest values were for Mo, Re, Th, and U but high maximum concentrations were found in all species collected in this area. Some of the contaminants could have come from old Hwy 66 and the railroad right-of-way.
4. In plants at the Chemehuevi Valley Study area, elements with highest enrichments included Ag, B, Ba, Re, Se, and Zn. The highest concentrations occurred in samples collected near US Highway 95, suggesting that most elements were enriched from dust dispersed near the highway.
5. At the Chuckwalla Bench study area, high values occurred in plants for 13 elements (Au, Ca, Ce, Cs, Hf, La, Lu, Sb, Sc, Sm, U, W, and Zn). The primary source of contamination may have been eolian dust or substrate materials.

B. Soils

The authors note that only As, as well as the chemically associated Sb and possibly Eu, were anomalous regionwide.

1. In the western Mojave study area (southern section), only As was slightly enriched, which might be natural or a result of As dispersion from mining areas.

2. In the western Mojave study area (northern section), soil samples were generally highly enriched in at least six elements: As, Cr, Li, Ni, Sb and possibly Au. Concentration levels for As, Au, and Sb were found in mine dumps and tailings piles and were related to mineral deposits or ore processing. The other elements were the result of natural lithologic differences. Enrichments in certain elements at Goldstone were natural and due to lithologic differences in the area.
3. At the Chemehuevi Valley study area, soils and stream sediments were enriched in Ca, Cd, Cr, and Ni. The possible enrichments of the last three elements may represent local contamination.

C. Overall Enrichments and Anomalies

1. Western Mojave study area. In the Rand and Atolia mining districts, soil anomalies for As, Au, Cd, Hg, Sb, and (or) W and plant anomalies for As, Sb, and (or) W extended as far as about 15 km outward from the present mining areas. Soils containing anomalous Hg were found at least 6 km from old tailing piles. The anomalous concentrations of As and Hg may have been the source of elevated levels of these elements found in ill tortoises from the region.
2. In the Goldstone mining district, soil anomalies extended several kilometers from the mining areas, probably representing anthropogenic surface contamination of dust redistributed by wind, vehicles, and rainfall.
3. At the Chemehuevi Valley, one of two study areas transected by a paved road showed weakly elevated levels of Pb, which extended as far as about 22 m from the pavement edge and probably were related to vehicle exhaust.

V. Drought and Climate Change

A. Foraging During Drought

Oftedal (2002), drawing on a literature review of studies conducted in the Mojave and Sonoran Deserts, discussed how tortoises can show large among-year differences in foraging as a function of rainfall.

1. During times of drought, tortoises typically remain in burrows where they have a reduced metabolic rate.
2. During times of exceptional winter rainfall, such as El Niño years, tortoises can afford to bypass abundant species and focus on uncommon species that are particularly palatable.

B. Peterson (1996a) studied adult male desert tortoises at populations located in the western Mojave Desert (Desert Tortoise Research Natural Area) and eastern Mojave Desert (Ivanpah Valley). The two regions have different rainfall patterns. Calculations of field metabolic rates (FMR) and feeding rates during an extreme drought year suggested that tortoises experienced a net loss of energy on their spring diet of succulent annual plants. As such, tortoises required drier forage, such as dry grasses, to accrue an energy profit. Feeding rates were greater at the Desert Tortoise Natural Area than in Ivanpah Valley.

Overall, tortoises generally lost dry matter during the study period, but seasonal increases were registered at Ivanpah Valley in fall 1990 and at the DTNA in spring 1990, which corresponded to periods of highest annual plant density at the two sites. This study emphasizes the reliance of tortoises on drinking rainwater (which can be stored in the bladder to later hydrate dry forage). Furthermore, the author suggested that tortoises rely on free-standing water for drinking to achieve a net annual energy profit.

VI. Injuries to Tortoises from Plants

A. Homer and others (1998), in necropsies of 24 dead, moribund, or road-killed tortoises from California, reported that three tortoises had cactus spines embedded in the intestine, stomach or tongue and pharynx, associated with granuloma formation or acute inflammation and bacterial colonization.

B. Barboza (1995a), in a study and dissection of the digestive tracts of seven desert tortoises, reported that some individuals had sharp spines and fibres from the diet deeply embedded in the tongue, esophagus, and stomach linings and surrounded with chronic inflammations.

C. Medica and Eckert (2007) observed 4 of 18 recaptured tortoises from 1998 to 1999, following the El Niño rain event of 1997–1998, with injuries to their jaws from consuming fruits of the non-native grass, Red Brome (*Bromus madritensis*) at the Nevada Test Site. Injuries were observed to persist for 3–4 years in three tortoises and for 5 years in one tortoise, after which observers manually removed additional seed material. During 1998, the researchers also reported observing Red Brome fruit/seed embedded its full length in a nostril of one tortoise and impaled in the corner of an eye of another tortoise in Piute Valley, NV.

D. Smith and others (2015) described mechanical injury to an immature *Gopherus agassizii* at Joshua Tree National Park, CA, from a California barrel cactus (*Ferocactus cylindraceus*).

VII. Olfaction, Feeding, and Disease

Germano and others (2014) studied olfactory responses of three groups of penned tortoises at the Desert Tortoise Conservation Center, Las Vegas, NV, with a combination of clinical signs and results of enzyme-linked immunosorbent assay (ELISA) tests: (1) tortoises with no nasal discharge and antibody negative for *Mycoplasma agassizii*, (2) tortoises with no nasal discharge and antibody positive to *M. agassizii*, and (3) tortoises with a nasal discharge and antibody positive to *M. agassizii*. Because tortoises are known to use olfaction to identify food items, predators, and conspecifics, it is important to know if mycoplasmosis affects olfactory behaviors. Experiments were undertaken using a visually hidden olfactory food stimulus and an empty control. Tortoises with nasal discharge had a reduced sense of smell and, therefore, a reduced ability to locate food. The authors reported that moderate chronic nasal discharge in the absence of other clinical signs did not affect appetite.

Chapter 6.—Reproduction and Endocrinology

The reproductive cycles of male and female desert tortoises are not synchronized (Rostal and others, 1994a).

I. Characteristics of Tortoises at Sexual Maturity

A. Curtin and others (2009) proposed contrasting life-history strategies (age and growth rates) as strong indicators of differing ecological adaptations between tortoises from the Sonoran and Mojave Deserts.

  1. Based on skeletochronology of shell remains, female western Mojave Desert tortoises reached sexual maturity at 17–19 years, which was significantly earlier than female tortoises from the Sonoran Desert (approximately 25 years).

  2. Combined with adaptations for multiple clutches, more rapid sexual maturity may be an evolutionary adaptation for low juvenile survivorship and a significantly shorter life span resulting from lower and more variable annual rainfall than in the Sonoran Desert. The female Mojave Desert tortoises may have higher physiological stress compared with Sonoran desert tortoises.

B. Size of Females at First Reproduction

  1. Mueller and others (1998), in a study of radio-transmittered female desert tortoises monitored at Yucca Mountain, NV, reported that annual fecundity of tortoises ≥ 209 mm carapace length (CL) ranged from 0 to 16 eggs. The smallest female to reproduce was 209 mm CL.

  2. McLuckie and Fridell (2002), in a study of 12 reproductive females on the Beaver Dam Slope, UT, reported that the smallest reproductively active and monitored female was 192 mm in mid-carapace length.

II. Nests Locations and Egg Deposition (Oviposition)

A. Location of Nests

  1. Rostal and others (1994a), in a 14-month study of the reproductive cycle of penned adult female tortoises at the Desert Tortoise Conservation Center in Las Vegas, NV, reported that females placed nests in natural burrows, artificial burrows, and under vegetation.

  2. Baxter and others (2008), in a study under both semi-natural and wild conditions at the Fort Irwin Study Site in the central Mojave Desert, reported that:

     a. Adult female tortoises deposited their eggs 0.6–0.8 m inside burrow tunnels, regardless of the length of the burrow, a behavior suggesting that tortoises may select a particular range of distances from the burrow mouth into the tunnel.

     b. Mean temperatures of nests placed 0.6–0.8 m down the burrow tunnel did not differ between east- and west-facing burrows.

  3. Ennen and others (2012b) monitored reproductive behaviors of 15 females at the Mesa Wind Farm near Palm Springs, CA, in the Colorado Desert. They reported that:

   a. All first and second clutches were placed in different burrows.
   b. Three nests were deposited on burrow aprons, three directly below the burrow mouth, and the majority (17) deposited inside burrows; mean distance from the burrow mouth to inside the burrow was 13.3 cm.
   c. Nests oviposited outside and farther away from the burrow mouth were deeper than nests placed inside the burrow mouth.
4. Sieg and others (2015) monitored 20 nests of *Gopherus agassizii* at two sites in Ivanpah Valley, CA, in spring and early summer of 2003 and reported that all nests were within burrows dug into coppice mounds either directly underneath or near *Larrea tridentata* or *Ambrosia dumosa*.
5. Potential Factors Influencing Nest Location
   a. Temperature constraints on egg survival. Rostal and others (2002) studied temperature-dependent sex determination in eggs at the Desert Tortoise Conservation Center in Las Vegas, NV. The authors reported incubation and hatching times for eggs incubated under controlled conditions at temperatures of 26.0, 28.1, 29.0, 30.6, 31.3, 32.8, 33.0, 34.0, and 35.3 °C. Hatching success was 50% at 26.0 °C and 29% at 35.3 °C, but between 90 and 100% at other temperatures.
   b. Temperature constraints applied to nest location. Baxter and others (2008), in a study at the head-start pens at the Fort Irwin Study Site in the central Mojave Desert, CA, reported that six undisturbed females placed their nests 0.6–0.8 m inside burrows. In an experiment using pseudo-nests at 0.2 and 0.4 m inside burrows, temperatures were significantly cooler for egg nests than they were for the 0.2 m pseudo-nests. The proportion of 15-minute temperature data points above the critical threshold of 35.3 °C was significantly greater for the 0.2-m pseudo nests than for the 0.4-m pseudo-nests and the egg nests. (Critical threshold: desert tortoise eggs incubated at that constant temperature resulted in 72% mortality).
   c. Ennen and others (2012b) evaluated nest site selection for 15 female tortoises at the Mesa Wind Farm near Palm Springs, CA; they reported no evidence of nest sites associated with any anthropogenic structures. Additionally, nesting ecology was similar to other populations, suggesting that operation of the wind energy facility did not adversely affect nesting ecology of this population. However, alteration of habitat due to construction and maintenance would reduce plant biomass and diversity, which, in turn could adversely impact tortoise reproduction.

B. Timing of Nesting

1. Rostal and others (1994a) studied the seasonal reproductive cycle of penned adult desert tortoises at the Desert Tortoise Conservation Center in Las Vegas, NV, for 14 months. Desert tortoises displayed a distinct seasonal reproductive cycle, with intense mating activity occurring in the spring (April and May) and a second period in the fall (August through November). Nesting was observed during the late spring and early summer (May 12–July 3) when average monthly air temperatures ranged from 25.4 to 31.5 °C.
2. Wallis and others (1999) studied the reproductive output of 76 female desert tortoises from the Desert Tortoise Research Natural Area, CA, and at Goffs in the eastern Mojave Desert, CA. Over 2 years, clutches were observed in radiograph images from late April to late June at the Desert Tortoise Research Natural Area. At Goffs, the first images of eggs appeared in radiographs from April 21 to 29, and the last clutch appeared between June 24 and 29. The latest observation of egg-laying occurred between July 7 and 27, 1992. Larger females produced eggs earlier in the year, giving them a better opportunity to produce a second clutch that year, suggesting that the timing of first clutch was important to reproductive output.
3. McLuckie and Fridell (2002), in a 1989 study of 12 reproductive females on the Beaver Dam Slope, UT, reported that females laid their first clutch by late April (n = 6), early May (n = 4), or late May (n = 2). The earliest second clutch was laid by May 9, and the last clutch was detected on July 4. The interval between first and second oviposition was estimated at 24.8 ± 2.4 days (n = 4 days, range 15–30 days).
4. Bjurlin and Bissonette (2004) reported that mean clutch size was significantly larger for predated nests (5.91 ± 0.48 eggs SE) compared to non-predated nests (4.35 ± 1.31 eggs).
5. Ennen and others (2012b) monitored 15 females at Mesa Wind Farm near Palm Springs, CA, and reported that the earliest clutch was oviposited on May 12, whereas the latest clutch was laid on July 8.
6. Lovich and others (2012), at a wind energy facility near Palm Springs, CA, reported that the earliest to latest dates that first clutches (n = 65) were visible ranged from April 11 to July 13, and the earliest to latest dates that second clutches (n = 53) were visible ranged from May 1 to July 20.

III. Clutch Size, Frequency, and Annual Reproductive Output

A. Clutch Size

1. Rostal and others (1994a), in a study of the reproductive cycle of 50 penned adult tortoises (30 were females) at the Desert Tortoise Conservation Center in Las Vegas, NV, reported that mean clutch size was 4.68 ± 0.3 eggs (mean ± SE) and ranged from 2 to 7 eggs. Clutch size of first clutches was 5.07 ± 0.35 eggs, slightly larger than second clutches of 3.75 ± 0.63 eggs.

2. Mueller and others (1998), studied radio-telemetered female desert tortoises at Yucca Mountain, NV, and reported that clutch size ranged from 1 to 10 eggs. Clutch size for 10 tortoises sampled in all 3 years was 4.5 ± 0.4 eggs (mean ± SE) in 1993, 4.8 ± 0.3 eggs in 1994, and 4.8 ± 0.5 eggs in 1995. Mean clutch size for 25 tortoises sampled in 1994 to 1995 was 5.2 ± 0.3 eggs and 4.4 ± 0.4 eggs, respectively. For tortoises that had two clutches, mean size of the first and second clutches did not differ statistically. The adjusted mean clutch size of tortoises having one or two clutches was 5.7 ± 0.2 eggs and 4.8 ± 0.2 eggs, respectively. (Adjusted = predicted mean for the covariate mean of 247 mm carapace length).
3. Wallis and others (1999) measured reproductive output of 76 female desert tortoises from the Desert Tortoise Research Natural Area and Goffs, CA, over a 2-year period. The authors reported that variation in annual egg production was due mainly to variation in clutch size and not necessarily clutch frequency. Larger females produced larger clutch sizes, and the size of the second clutch was positively correlated to the size of the first clutch. This suggested that maternal body size strongly influenced annual reproductive output.
4. McLuckie and Fridell (2002) reported on a 1989 study of 21 female tortoises on the Beaver Dam Slope, UT. Three tortoises were excluded from some data analyses because of incomplete data. Of the 21 tortoises, 7 did not produce eggs. Twelve tortoises produced first clutches of eggs ranging from 2 to 8 eggs, with a mean of 5.2 ± 0.47 eggs; 4 tortoises produced a second clutch of 3 to 10 eggs with a mean of 5.5 ± 1.55 eggs. The mean clutch size of first and second clutches did not differ significantly.
5. Baxter and others (2008), in a study of 22 captive, gravid females in enclosures at the Fort Irwin Study Site in the central Mojave Desert, CA, reported that the mean (and SE) number of eggs oviposited per female was 3.9 ± 0.8 eggs (range 2 to 6 eggs).
6. Ennen and others (2012b) reported that of 15 females monitored at Mesa Wind Farm near Palm Springs, CA, clutch size ranged from 1 to 8 eggs with a mean of 4.3 eggs. Clutch size for first and second clutches did not differ significantly.
7. Lovich and others (2015), summarizing eight reproductive seasons of data from a 16-year study at the Mesa site, Colorado Desert, CA, reported that clutch size ranged from one to nine eggs with a mean that ranged from 3.25 to 5.73.

B. Clutch Frequency

1. Rostal and others (1994a) studied the seasonal reproductive cycle of desert tortoises at the Desert Tortoise Conservation Center in Las Vegas, NV. Of 30 monitored females, 22 females laid 1 clutch and 6 females laid 2 clutches.
2. Mueller and others (1998), based on radio-telemetered female desert tortoises monitored on Yucca Mountain, NV, over a 3-year period, reported that a mean clutch frequency for all tortoises monitored during the study was 1.5 clutches.

3. Wallis and others (1999) measured reproductive output of 76 female desert tortoises from Fenner Valley and the Desert Tortoise Research Natural Area, CA, over 2 years, and reported that approximately 70% of females laid a second clutch whereas apparently no females produced a third clutch. The proportion of mature females failing to form a clutch at all ranged from 0% at Goffs to 25% at the Desert Tortoise Research Natural Area.
4. McLuckie and Fridell (2002), in a 1989 study of adult female tortoises on the Beaver Dam Slope, UT, (22%), reported that 4 of 12 tortoises produced a second clutch. The mean clutch frequency per female was 1.33 and was significantly correlated with carapace length.
5. Ennen and others (2012b) reported that of 15 females monitored at Mesa Wind Farm near Palm Springs, CA, 13 produced 2 clutches and 1 produced 3 clutches.
6. Lovich and others (2015), reporting on eight reproductive seasons of data from the Mesa study area, Colorado Desert, CA, said that clutch frequency varied somewhat among years and ranged from 1.64 to 2.36 clutches/female/year.

C. Factors Influencing Clutch Size, Frequency, Reproductive Output, and Annual Fecundity

1. Size of female affects reproductive output
    a. Mueller and others (1998) studied radio-telemetered female desert tortoises at Yucca Mountain, NV, and reported that annual fecundity of tortoises ≥ 209 mm carapace length (CL) ranged from 0 to 16 eggs. The smallest female to reproduce was 209 mm CL, whereas all tortoises ≥ 209 mm CL produced eggs in at least 1 year. Linear regression analyses revealed a positive correlation of mean annual fecundity and female size, such that the predicted annual fecundity for a 209-mm CL tortoise was 3 eggs with an increase of 1 egg for each 7-mm increase in CL. Predicted annual fecundity for a 247-mm CL tortoise was 8.2 eggs.
    b. Wallis and others (1999) measured reproductive output of female desert tortoises from the Desert Tortoise Research Natural Area, western Mojave Desert and at Goffs, eastern Mojave Desert, CA, during 2 wet years (1992–1993). They reported that:
        i. Variation in annual egg production was due mainly to variation in clutch size, not clutch frequency.
        ii. Food supply probably limits reproduction only during drought years.
        iii. Annual egg production per female was lower at the Desert Tortoise Research Natural Area than Goffs because some adult females at the Desert Tortoise Research Natural Area did not produce eggs in some years. However, when correcting for body size, females at the Desert Tortoise Research Natural Area produced fewer but larger eggs than did females at Goffs.

iv. Females at Goffs were smaller and the eggs produced at Goffs were smaller in all dimensions than eggs produced at the Natural Area. Small eggs and presumably smaller neonates may be related to the greater predictability of summer rain and consequent greater food supply for emergent hatchlings at Goffs. Females at the Natural Area need to produce larger eggs because their hatchlings are likely not to have summer annuals to eat and must live longer on reserves from the eggs.

v. Maternal body size strongly influenced reproductive output through annual egg production, and, in turn, primarily through effects on clutch frequency, the size of the first clutch, and clutch volume.

vi. Results suggested a great reproductive advantage for females to grow large; larger females produced larger clutches and produced eggs earlier in spring, providing a better opportunity for a second clutch.

vii. The earlier a female's first clutch appeared, the greater the annual egg production.

c. McLuckie and Fridell (2002), in a 1989 study of 12 adult females on the Beaver Dam Slope, UT, reported that mean clutch size, clutch frequency, and mean annual fecundity (7.0 ± 1.16 eggs) were significantly correlated with midline carapace length. The regression equation predicted an increase of one egg for each 5.55-mm increase in carapace length.

2. Habitat changes

Lovich and others (2011b) in a study of eight female tortoises at a wind energy facility near Palm Springs, CA, reported that annual egg production did not differ following a 1995 fire (compared to the 1997–2000 and 2009–2010 post-fire periods). The authors noted that there was no pre-fire reproductive data with which to compare.

3. Precipitation and food supply

a. McLuckie and Fridell (2002), in a 1989 study of 12 reproductive females on the Beaver Dam Slope, UT, reported a mean annual fecundity 7.0 ± 1.2 eggs SE (range = 3 to 18 eggs). They noted that annual fecundity and percentage of tortoises producing second clutches was lower than in some other studies. The study, which took place in 1989, was in a year of below the 30-year mean annual precipitation and was also preceded in 1988 by a year of below average rainfall.

b. Nussear and others (2012), in a study of translocated tortoises at five sites in NV and UT, modeled several factors related to reproduction. The authors reported that model selection for all sites combined yielded a model with winter precipitation, body size (carapace length), and site as the best performing model. Both precipitation and body size were positively correlated with the total number of eggs produced. For the first year after translocation, mean reproductive effort for translocated tortoises was significantly less than that of residents. However mean number of eggs was not different between resident and translocated tortoises for 2 and 3 years after translocation.

c. Sieg and others (2015), in a study of reproductive success of 19 female *Gopherus agassizii* in spring of 2003 at two sites along a rainfall and elevational cline in Ivanpah Valley, CA, reported higher precipitation, herbaceous plant biomass, species richness of shrubs and total cover of plants at the higher elevation study area. There were no significant differences between plots in the proportion of transmittered tortoises depositing a second clutch or in the total annual number of eggs deposited per tortoise. Carapace length was not a significant covariate with clutch size. However, there was a significant interactive effect of plot and clutch number on egg volume, with the largest egg volumes in first clutches at the higher elevational site. Carapace length was positively correlated with egg volume.

4. Translocation

Nussear and others (2012), in a study of translocated tortoises at five sites in NV and UT, modeled several factors related to reproduction. For the first year after translocation, mean reproductive effort for translocated tortoises was significantly less than that of residents. However mean number of eggs was not different between resident and translocated tortoises for 2 and 3 years after translocation.

5. Climate warming

Lovich and others (2012) reported that clutch phenology exhibited inter-annual variation at a wind energy facility near Palm Springs, CA. However, most clutch phenophases (first clutch disappearance and mean number of days visible, second clutch appearance and disappearance) were correlated with climate, in that phenophases were shorter in warmer years relative to cooler years. Clutch phenology was significantly delayed and longer in cooler years relative to warmer years.

6. Multiple factors—Energy budgets and reproductive effort.

Henen (1997) synthesized relationships between energy budgets and reproductive efforts (the portion of available energy that is allocated to reproduction) of females at Goffs, eastern Mojave Desert, CA. Using data from nine tortoises and three techniques (gas dilution, doubly labeled water, and X-ray radiography) during a 2-year study (1987–1989). The first year (July 1987–July 1988) had moderate rainfall and annual plant production, but the second year (July 1988–July 1989) had extremely low rainfall and low annual plant production.

a. Egg production in 1988 (mean ± 1 SD: 3.56 ± 0.94 eggs) did not differ from 1989 (3.00 ± 2.69 eggs); both were lower than during 1983–1987 (6.75 ± 3.05 eggs, n = 100). Energy per se did not limit egg production in 1988 and 1989, but the availability of nonlipid energy, probably protein, limited egg production in 1988 and was limiting in spring 1989. Water was the primary resource limiting egg production in 1989.

   b. Amount of summer annuals can affect egg production because females stored nonlipid energy when eating these annuals and (summer 1988) allocated nonlipid energy (probably protein) to eggs in the following spring. Tortoises stored lipids during summer 1987 when consuming dry annuals. These lipids were critical for surviving the winter, but females forfeited body water and nonlipid dry matter to digest dry annuals.
   c. Reproductive effort (calculated as the ratio of reproductive expenditure vs. available energy) was higher during the drought year than during the wetter year because tortoises reduced field metabolic rate by 70 to 90% in the latter year.

7. Henen (2002a, 2002b) synthesized relationships between female diets and nutrient budgets, food abundance, and reproductive outputs drawing on a study of female reproductive output at Goffs, eastern Mojave Desert, CA. Using data from nine tortoises and three techniques (gas dilution, doubly labeled water, X-ray radiography) during a 2-year study, he tracked food intake and water content of food to measure food and water budgets. He reported that:
   a. Females need dietary protein for reproduction. The correlation between reproductive output and nitrogen consumption prior to brumation was greater than the correlation of reproductive output to annual nitrogen consumption. Egg yolks contain about 90% of nitrogen in tortoise eggs, and yolks are almost fully developed before females brumate.
   b. Females exhibited energy deficits only during winter and exhibited large tolerances to changes in body composition. Acquisition and use of protein was probably a limiting resource for reproduction and egg production, and along with water, appeared to be a limiting resource for egg production during a drought year.
   c. Water was most limiting during severe droughts. Water was essential for digestion. During rain, females emerged from burrows to drink rainwater equaling, on average, one-third of their mass. Tortoises can become conservative during droughts. Under drought conditions, females that produced eggs forfeited body water whereas females that abstained from egg production tended to increase their nonlipid energy and total body water. Thus, whereas water and protein appeared to be limiting resources for egg production during a drought year, the ability to relax homeostatic regulation of water budgets helped desert female tortoises conserve and store water and nutritional resources, and then draw upon these reserves to produce eggs in harsh, dry years.

d. Studies on clutch frequency, egg production, and winter annual biomass are consistent with both the "storage" hypothesis and the "winter rainfall-plant biomass" hypothesis (capital vs. income breeding). The desert tortoise strategy appears mixed between or intermediate to the capital and income strategies. Mean clutch frequency and egg production were correlated to the logarithm of winter annual biomass. The biomass of winter annuals is only a gross indicator of nutrient availability to desert tortoises because they are selective feeders (based on bite counts). Consuming dry *Schismus barbatus* can cause negative water and nitrogen balance for tortoises, but consuming this dry alien grass in tandem with drinking in summer can greatly enhance energy balance and lipid stores.

e. Consumption of perennial plant species and other materials was higher in spring than in summer. Availability of summer annuals probably reduced consumption of perennials. Annuals in spring have high protein content.

f. Females used large seasonal and annual changes in metabolism, body water, protein, and energy reserves to survive and produce eggs.

g. Females rarely achieved energy balance, illustrating relaxed homeostasis towards energy. Six of the nine females forfeited body water and nonlipid dry matter to produce just a few eggs during drought. The patterns observed indicated the importance of body water and nonlipid dry matter, more than lipids, to reproduction. By reducing metabolic rates by 90%, females conserved enough body reserves to produce eggs during extreme drought conditions. This was an effective reproductive strategy (bet-hedging) in an extreme and unpredictable environment of the eastern Mojave Desert.

h. Desert tortoises are mostly "capital" breeders because females forgo egg production and reabsorb follicles if food availability is poor and body reserves are small; accumulate water and protein reserves prior to winter and use these reserves to help produce eggs and full-sized follicles before entering hibernation; can emerge from winter dormancy and ovulate prior to eating; and egg production is correlated to winter annual biomass. Food or nutrient income also influences egg production.

8. Breeding Strategies. Henen (2004) compared capital and income breeding in *Gopherus agassizii* and the central Asian tortoise (*Testudo horsfieldii*). Here, we focus on the former species:

a. Female desert tortoise used an approach intermediate to capital and income breeding, relying on accrued body reserves and nutrients (capital breeding) and spring food consumption (income breeding) to produce eggs.

b. Nutrient budgets indicated that a relaxed homeostasis enabled female desert tortoises to store substantial reserves that ameliorate food and water shortages to enable egg production during drought conditions. Some females do not produce eggs in drought conditions. Protein and water are limiting during drought years.

    c. Additionally, spring nutrient income also influenced egg production. With increased food availability (moderate to high levels), egg production appears to approach an upper limit or asymptote. First clutches are dependent on body reserves (capital) whereas the production of second and third clutches may depend more on nutrient income, i.e., spring forage.
    d. Females were in energy balance or surplus in all seasons except winter, when lipid catabolism rates were high. Females replenish non-lipid protein reserves whenever they had access to fresh forage (late winter and spring, and with summer annuals). When in drought, females reduce activity field metabolic rates.
    e. Females exhibit bet-hedging. The smallest females have the least nutrient reserves and forego egg production in a dry year.

9. Lovich and others (2015), in a study of reproductive output of female desert tortoises for eight field seasons over 16 years at the Mesa site in the Colorado Desert, CA, confirmed findings of previously described bet-hedging in *Gopherus agassizii*. Winter precipitation had no significant effect on clutch frequency or annual egg production. Maternal size affected annual egg production, however. During an El Niño year, mean annual egg production and mean annual clutch frequency were the highest reported for the species.

IV. Egg Development, Incubation, and Hatching

A. Egg Development

1. Rostal and others (1994a), in a study of penned adult female desert tortoises at the Desert Tortoise Conservation Center, Las Vegas, NV, observed vitellogenesis and ovarian follicular growth during late summer and fall (July–October) following completion of nesting. Ovarian follicles had matured prior to hibernation. Shelled eggs were first observed in the oviducts in mid-April using ultrasonography, and 90% had ovulated by April 30 and were shelling eggs in oviducts.
2. Wallis and others (1999) measured reproductive output of 76 female desert tortoises from near Goffs and the Desert Tortoise Research Natural Area, CA, over 2 years, and after correction for differences in female body size between sites, noted that eggs produced at Goffs were smaller in all dimensions (egg width, length, volume) than eggs produced at the Desert Tortoise Research Natural Area. The authors reported that egg width and length were correlated to female body size but not to clutch size, suggesting that egg size is a physical constraint rather than tied solely to nutrient constraints. Smaller eggs and presumably smaller neonates also may be related to the greater predictability of summer rain and consequent greater food supply for emergent hatchlings at Goffs than at the Desert Tortoise Research Natural Area.
3. McLuckie and Fridell (2002), in a 1989 study of reproductive female tortoises on the Beaver Dam Slope, UT, reported a mean egg width of 37.2 ± 0.26 mm (SE) and mean egg length of 44.3 ± 0.33 mm (SE) for 81 eggs.

B. Incubation Period

1. McLuckie and Fridell (2002), in a 1989 study of 12 reproductive females on Beaver Dam Slope, UT, reported that the mean time from egg deposition to hatching was 89.7 ± 3.25 days (mean ± SE). The earliest observation of hatching was on August 21, and the latest on September 12.

2. Ennen and others (2012b) reported that, of 15 females monitored at Mesa Wind Farm near Palm Springs, CA, mean incubation time was 84.6 days, ranged from 74 to 100 days, and was significantly longer in first than in second clutches, possibly due to higher temperatures in second clutches.

C. Hatching Success and Survival

1. Spotila and others (1994) conducted experiments at the Desert Tortoise Conservation Center near Las Vegas, NV, to determine if incubation conditions affect post-hatching growth and survival. Hatching success and survival was very good between 28.1 and 32.8 °C in dry sand (-5,000 kPa). None of the eggs incubated under cool, wet (4.0% soil moisture) conditions hatched. Incubation time was inversely related to incubation temperature, i.e., 89 days at 28.1 °C to 68 days at 32.8 °C. Incubation time increased at 35.3 °C and survival decreased. Hatchlings from eggs incubated at 32.8 and 35.3 °C were significantly smaller than hatchlings from eggs incubated at 28.1 and 30.6 °C; hatchlings from eggs incubated at 30.6 °C grew significantly more than hatchlings incubated at 28.1 and 32.8 °C, whereas hatchlings incubated at 35.3 °C lost mass.

2. McLuckie and Fridell (2002), in a 1989 study of 12 females on the Beaver Dam Slope, UT, reported that of 71 eggs monitored, 52 (73%) hatched. There were no signs of predation on the 13 nests observed during the study, but these nests were protected.

3. Bjurlin and Bissonette (2004) sampled 25 female desert tortoises at the Sand Hill Training Area, Twentynine Palms, CA, and reported that:

    a. A relatively low overall rate of tortoise nest depredation (26% of 42 nests), but with higher depredation rate in 1998 (47%) compared with 1999 (12%). Some tortoise nests were near kit fox (*Vulpes macrotis*) natal dens, and kit fox scat and tracks were found around most depredated nests. The researchers thought their weekly nest monitoring in 1998 versus two total visits in 1999 might have facilitated predator detections of the nests. An estimated 40% of eggs laid survived as healthy neonates to hibernation (through mid–October) over two seasons although survival probabilities varied by year (1998–1999).

    b. Thirteen of 42 (30.9%) tortoise nests over 2 years were completed (i.e., eggs hatched and neonates emerged); completion increased to 50.0% when excluding depredated and nests of unknown fate.

    c. Presence of tortoise urine on top of the nest did not affect nest survival (8 nests with urine, 33 without). When depredated eggs were excluded, egg hatching success was 82.5% (109/132 eggs) and neonate emergence was 74.2% (98/132 eggs).

   d. Ten percent of 109 hatchlings were later found trapped or dead inside the nest perhaps because rainfall was twice the average the year when entombment was highest.

   e. Of 91 normal neonates that emerged during 1998 and 1999, mean weight was 21.3 ± 2.91 g SD, length was 43.8 ± 2.15 mm SD, width was 37.5 ±2.03 mm SD, and height was 22.3 ± 1.60 mm SD.

   f. Healthy neonate survivorship was 88% in the first year and 91% in the second year. Overall, an estimated 40% of eggs laid survived as healthy neonates to hibernation (through mid-October) over two seasons, although survival probabilities varied by year mainly due to heavy predation in the first year

   g. When effect of relative egg volume was removed, females >240 mm midline carapace length (MCL) had increased egg hatching success and consistency compared to smaller females. There was a linear relationship between neonate emergence and maternal carapace length. Neonates that emerged first were generally healthy and emergence of those nests were closely synchronized (mean 3.2 days between first and last neonate) while late emerging neonates appeared abnormal, as indicated by deformities, deficiencies, or illness, and emergence was more asynchronistic (mean 11.5 days).

4. Baxter and others (2008), in a study of female tortoises at Fort Irwin, CA, reported that of 59 eggs laid by 22 captive, gravid females in enclosures, 47 (79%) hatched.

5. Ennen and others (2012b), in a study of 15 females at Mesa Wind Farm near Palm Springs, CA, reported that:

   a. Hatching success was not significantly different between first and second clutches for non-depredated nests or when considering predation. Excluding 3 depredated nests, 74 hatchlings emerged from 20 nests; 12 eggs were non-viable from clutches and 5 from second clutches. Two of 74 hatchlings showed some form of deformity.

   b. Mean weight was 23.0 ± 3.9 g SD and midline carapace length (MCL) was 4.45 ± 0.19 cm SD for the 74 hatchlings. Neither weight nor MCL differed between first and second clutches. In addition, maternal MCL or egg width had no significant relationship with hatchling MCL or weight.

6. Sieg and others (2015) monitored 20 nests of female *Gopherus agassizii* at two sites in Ivanpah Valley, CA, and reported that predation levels were significantly higher at the higher elevation site than at the lower elevation site. The most common nest predators were *Vulpes macrotis*, as determined either by scat deposition, tracks, a lack of burrow opening enlargement, or a combination of characteristics. At least one nest was probably preyed upon either by *Canis latrans* or *Taxidea taxus*.

D. Maternal effects on offspring size, growth, and survival. Nafus and others (2015b) studied *Gopherus agassizii* at rearing pens at the Ivanpah Desert Tortoise Research Facility, CA, between 2011 and 2013. Larger mothers produced larger off-spring. First year survival was correlated with carapace length at hatching. For every 1 mm increase in initial carapace length, the odds of a hatchling surviving its first year doubled. The findings suggest that maternal body size may indirectly influence offspring fitness via growth and survival for at least the first year of life.

V. Recruitment of *Gopherus agassizii*: A Synthesis of Reproduction and First-Year Survival

Campbell and others (2015) synthesized data on annual recruitment of *Gopherus agassizii* and *G. morafkai* (only the findings of *G. agassizii* are presented here). Specifically, the authors quantified annual recruitment as a product of proportion of females that reproduced, number of eggs produced per reproducing female, hatching success, and hatchling survival. The estimated proportion of females that reproduced (0.81) and number of eggs produced pre year (6.90) were higher than for *G. morafkai*. Hatching success averaged 0.61. Average recruitment for *G. agassizii* ranged from 0.51 to 1.18 females/female/year and was dependent the figures used for hatchling survival.

VI. Sex Determination

A. Spotila and others (1994) conducted two sets of incubation experiments at the Desert Tortoise Conservation Center near Las Vegas, NV, to determine if temperature and soil moisture affect sex determination. They reported that low temperatures (26.0–30.6 °C) produced males and higher temperatures (32.8–35.3 °C) produced females, with pivotal temperatures at approximately 31.8 °C.

B. Rostal and others (2002) refined the chronology of sex determination in desert tortoises and reported that incubation temperatures of approximately 30.5 °C or below produced all males and temperatures of approximately 32.5 °C or above produced all females. An estimated pivotal temperature (temperature producing a 1:1 sex ratio) was 31.3 °C. Therefore, *Gopherus agassizii* possesses a Pattern Ia type of temperature-dependent sex determination. The pivotal temperature was relatively high and may reflect the relatively warm habitat of *G. agassizii*. Sex determination appeared sensitive to temperature as early as embryonic stage 15 or earlier and as late as stage 21 depending on specific incubation temperatures. Sexual differentiation of the gonads became histologically noticeable between stages 18 and 21, and the gonads showed distinct sexual dimorphism by stage 23. Incubation temperatures ranging from 28 to 34 °C resulted in similar incubation times (68–89 days) and high hatching success (90–100%). It is important to note that the incubated eggs were kept at constant temperatures, not typical of the natural environment where temperatures fluctuate.

C. Baxter and others (2008), based on 16 nests monitored at the National Training Center at Fort Irwin, CA, in the central Mojave Desert, reported that:

  1. Hatchling sex ratios were independent of east-facing versus west-facing burrow orientation.

  2. Early nests (May 22–June 2) were significantly cooler and produced four all- male nests and two mixed nests. In comparison, late nests (June 17– July 16) were significantly warmer and produced six all-female nests, suggesting that it may be possible to use nest date as a predictor of hatchling sex ratios of desert tortoises in the central Mojave Desert.

VII. Long-Term Sperm Storage

Palmer and others (1998), in a study of 50 penned male and female adult desert tortoises at the Desert Tortoise Conservation Center in Las Vegas, NV, used allozyme data to infer paternity and identify cases where stored sperm was used to fertilize eggs. They drew upon 12 clutches and 61 hatchlings from the clutches. Off-spring from 5 of 12 clutches appeared to be the result of fertilization with sperm stored from matings prior to the study. Tentative evidence for multiple paternity was found in an additional clutch. No reduction in fertility was observed in clutches resulting from stored sperm prior to the introduction of females into enclosures. Mean hatching success for clutches fertilized by sperm stored > 2 years was 97.1% (± 3.19, n = 5). The data were consistent with observations of viable hatchlings produced by females isolated from males for 2 years in other enclosures. Sperm may be stored in the albumen-secreting gland of the oviduct (observed in several turtle species). Fall mating behavior suggests that sperm are inseminated into the female and then stored in sperm-storage tubules until emergence from hibernation in spring.

VIII. Endocrine System and Reproduction

A. Annual Reproductive Cycle—Females

  1. Based on a 14-month study of the seasonal reproductive cycle of 50 adult desert tortoises maintained in pens at the Desert Tortoise Conservation Center in Las Vegas, NV, and using samples from jugular blood and ultrasound examinations, Rostal and others (1994a) reported that:

    a. Vitellogenesis and ovarian follicular growth were observed during the late summer and fall (July–October) following the completion of nesting (May–July). Ovarian follicles matured to ovulatory size range prior to hibernation. Female plasma calcium levels (indicative of vitellogenin levels) were significantly elevated during summer and fall when follicular growth was observed.

    b. Testosterone levels were much lower in females than males and ranged from a mean high of 6.22 ± 0.62 ng/mL (mean ± SE) during the spring mating period (April) to a mean low of 0.37 ± 05 ng/mL during the late nesting period (July). Females displayed a significant rise in testosterone (July–October), which coincided with onset of the fall mating period.

2. In research conducted by Lance and Rostal (2002) at the Desert Tortoise Conservation Center near Las Vegas, NV, female tortoises (20) in pens and additional free-ranging tortoises were sampled in 1992–1993; 16 were sampled in 2000 (findings are focused primarily on 1992 data):
    a. In April, females had elevated plasma estradiol, testosterone, corticosterone, and lipids. During April and May, ovulation and egg-laying occur and progesterone levels increase but rapidly return to baseline once eggs are laid.
    b. By May and June all hormones and lipids declined to lowest levels, except thyroxine, which was lowest in October.
    c. In August and September, plasma estradiol, lipid, and calcium increased coincident with vitellogenesis and follicular growth. Estradiol and total lipids were closely linked—both lowest in June and increased during July and August. Plasma lipids were higher in females than males at any time of year. (Rostal and others, 1994a; plasma lipids include triacylglycerol, phospholipid, and cholesterol).
    d. Calcium was highest when estradiol was highest in August but low in April when estradiol was still elevated. Testosterone was highest in April, declined to almost undetectable in June and July, then rose slightly from August through October.

B. Annual Reproductive Cycle: Males. All studies described below were conducted at the Desert Tortoise Conservation Center, Las Vegas, NV, and most animals were in pens.

1. Alberts and others (1994) performed measurements of chin glands of 20 adult male desert tortoises in pens on a monthly basis. They reported that chin gland volume varied seasonally, reaching a maximum in late summer.
    a. Chin glands of socially dominant males tended to be larger than those of socially subordinate males.
    b. Among all males, there was a positive correlation between plasma testosterone levels and the size of the chin glands.
    c. Behavioral experiments demonstrated that tortoises of both sexes discriminated the chin gland secretions of familiar males from those of unfamiliar males. This suggests that chin gland secretions may function in conspecific recognition.
2. Rostal and others (1994a), in a study of 20 penned adult male tortoises reported that plasma testosterone (measured using jugular blood) rose significantly during the summer from May to August into the fall mating period then declined prior to hibernation. Male testosterone levels ranged from a mean high of 243.60 ± 24.61 ng/mL (mean ± SE) during the fall mating period (August) to a mean low of 18.37 ± 3.14 ng/mL during the nesting period (May). Males also displayed a seasonal change in body mass, which coincided with the seasonal recrudescence of the testes: body mass was significantly higher from June to September. Male plasma calcium levels were lower in fall, prior to hibernation, and increased from April to May following spring emergence, then remained relatively constant from May to September.

3. Lance and Rostal (2002) reported a range of testosterone levels in males with a mean of 18.4 ng/mL in May to mean levels just under 200 ng/mL in August. Individual values of > 300 ng/mL were common in August, whereas values in females were < 8 ng/mL. Male tortoises had low testosterone after emergence from hibernation in April; testosterone then increased during June and July to maximum levels in August and September when male-male aggression, mating activity, and spermatogenesis were greatest. Plasma thyroxine levels followed a similar pattern to testosterone, highest in April after emergence, declined in May and June, then rose again in July and August, and lowest in October. Corticosterone levels showed an almost identical seasonal pattern to testosterone and were significantly correlated; corticosterone was higher in males than females at all times. Plasma total lipids were lowest in June and July at the beginning of the spermatogenic cycle.
4. Lance and others (2002) reported that total plasma lipid in males showed a negative correlation with testosterone and spermatogenesis, suggesting an association between lipid metabolism and seasonal testicular development.

IX. Thyroid, Thyroxine

A. Seasonal Fluctuations. Based on 2 years of sampling of 20 males and 30 females at the Desert Tortoise Conservation Center in Las Vegas, NV, Kohel and others (2001) reported that triiodothyronine (T_3) was nondetectable in any season, but that thyroxine (T_4) exhibited distinct cycles in both sexes.

1. Thyroxine (T_4) declined from August to January, was lowest during hibernation, and rose toward the time of emergence.
2. Females exhibited one peak in T_4 during early spring (April), but males peaked in early spring and with a lower plateau again in late summer (July–August).
3. July was the only month in which the age groups differed in T_4, with adults significantly higher than in juveniles or subadults.
4. Testosterone also increased from low levels in May to highest levels in August, with levels significantly different in each of the three age groups; adults males (>208 mm carapace length) had the highest T_4 levels, followed in descending order by subadults, then juveniles.
5. T_4 patterns corresponded with distinct activity patterns that included increased feeding, mating, and locomotor activity in the early spring and increased mating and combat for males in the late summer. The second seasonal peak of T_4 only in males suggests that male reproductive activity in late summer is associated with thyroid activation

B. In an experiment to determine effects of food intake on thyroxine levels, Kohel and others (2001) used 20 juvenile and subadult tortoises at the Desert Tortoise Conservation Center, Las Vegas, NV. T_4 declined significantly in unfed tortoises (food withheld for 2 weeks) compared with continuously fed controls, but increased significantly within 36 h of re-feeding, indicating that thyroid activity is responsive to nutrient intake. Elevated T_4 in desert tortoises was thus associated with periods of increased feeding and reproductive activity, supporting a role for thyroid hormones in these energy-demanding activities.

X. Plasma Corticosterone

In a study undertaken in penned adult male and female tortoises at the Desert Tortoise Conservation Center near Las Vegas, NV, Lance and others (2001) reported that:

A. Males had significantly higher plasma corticosterone in all months than females, with a low point in May, rising to highs in July, August, September, October, perhaps due to increased mating activity and male-to-male combat (see Ruby and Niblick, 1994). In the female, there were two peaks, one in May and one in September. The second peak was not significantly different.

B. In the male, there was a significant relationship between corticosterone and testosterone; the cycles for the two hormones were virtually identical (and were also seen in acutely stressed tortoises).Testosterone in males showed a low in May and peaked or plateaued in August, September, and October. Testosterone showed a significant elevation during the mating period.

C. Estradiol levels in females were at the lowest point in June, peaked in August with a gradual decline in September and October. Likewise to the male, estradiol showed a clear but less marked seasonal pattern for the mating period. Estradiol did not show a pattern suggestive of the female reproductive cycle.

XI. Plasma Lipids of Adult Tortoises—Triacylglycerol, Phospholipid, Cholesterol, Cholesterol Esters, Fatty Acids, and Total Lipids in Adult Desert Tortoises.

Lance and others (2002) conducted a study of lipids between April and October in adult tortoises using 30 females and 20 males at the Desert Tortoise Conservation Center, Las Vegas, NV. They reported that:

A. Females had significantly higher levels of all lipid fractions, except free fatty acids, than males in all months of the year. The seasonal pattern in estradiol secretion mirrored and was significantly correlated with triacylglycerol, phospholipid, cholesterol, and total lipids. Estradiol and the vitellogenesis-associated lipids were all significantly higher in August, September, October, and April than in June. In August, at the peak of vitellogenesis, 41% of the female plasma lipid was triacylglycerol, 29% phospholipid, and 23% cholesterol ester. The seasonal variation in cholesterol ester levels did not correlate with any of the reproductive events and did not appear to be involved in yolk precursor formation.

B. Male plasma, in contrast to female plasma, had higher free fatty acids in all months. A consistent pattern in males was that triacylglycerol, phospholipid, cholesterol, cholesterol esters, and total lipids all showed a decline during July and August but none of the differences were significant. The seasonal variation in testosterone was negatively correlated with total lipid and with cholesterol but not with any of the other lipid fractions. The authors suggest a potential association between lipid metabolism and seasonal testicular development.

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Chapter 7.—Physiology

I. Thermoregulation

A. Zimmerman and others (1994) studied body temperatures (internal and external) of four adult desert tortoises in and adjacent to the Desert Tortoise Conservation Center near Las Vegas, NV. Body temperatures varied widely depending on time of day, microhabitat selection, and time of the activity season.

1. The overall relationship between internal (T_{bi}) and external (T_{be}) body temperatures was quantified as $T_{bi} = 3.08 + 0.91*T_{be}$. During the activity season, internal body temperatures differed from external body temperatures by < 1 °C. External temperature predicted internal temperature less reliably when tortoises experienced marked thermal flux (e.g., entering or leaving a burrow).
2. Tortoises typically retreated to burrows when mean operative temperatures reached about 40 °C, exhibiting a mean body temperature of 34.9 °C before entering. Before emerging, tortoises were located near the mouths of burrows where body temperature often increased 3–4 °C and epigeal operative temperatures cooled to about 50 °C. After emerging in the afternoon, the rate of heating was 0.03 °C/min, which was less than the rate of heating during the morning activity period (0.04 °C/min).

B. Ruby and others (1994a), in experiments conducted on tortoises in pens at the Desert Tortoise Conservation Center, Las Vegas, NV, reported behavioral responses to treatments with and without supplemental water from sprinklers. Tortoises used the sprinklers as thermoregulatory devices by evaporative cooling. Tortoises started the morning at the sprinklers and then moved away from them to feed as the day heated, but when their shells dried off, they returned to and remained under the sprinklers as they cooled.

C. Reiber and others (1999) investigated effects of post-hatching maintenance temperatures on 21 captive-bred hatchling tortoises maintained at different temperatures (7 hatchlings each at minimum and maximum active temperatures of 19, 28, or 37 °C) at the University of Nevada, Las Vegas. Maintenance temperatures affected growth rates, shell shape, and thermoregulatory behavior.

1. Tortoises maintained at 28 °C increased in mass at a significantly higher rate than animals held at 19 and 37 °C. Once treatment groups were returned to the common holding pen at 28 °C, growth rates did not differ significantly between groups.
2. Maintenance temperature affected shell shape. Tortoises kept at 37 °C had flatter shells.
3. Tortoises kept at different temperatures had different thermoregulatory behaviors. Tortoises maintained at 37 °C maintained their cloacal temperatures at higher temperatures compared to those held at 19 and 28 °C. The 37 °C group also showed reduced cycle frequency within the chamber between 23 and 45 °C, remaining stationary in a basking position for long periods of time at an isopleth corresponding to 29 °C.

D. Nussear and others (2000) sampled a single tortoise from Clark County, NV, for a discussion on visible colour and long-wave infrared energy exchange between animals and their environments. They reported that the carapace of an adult desert tortoise had higher absorptance of visible wavelengths (400–700 nm) than the near infrared (700–2,600 nm), with slightly higher absorptance in dark areas between scutes than the light area in scute centers of the carapace.

E. Nussear and others (2002) placed customized temperature data loggers at five locations on a single desert tortoise to determine an external location for the thermistor probe that best approximated cloacal temperature. Temperatures measured above the tail were the least different from cloacal temperatures. The difference between above-tail and cloacal temperatures was greatest when the animals were warming (mean = 4.4 °C) and decreased as they approached a body temperature of 35 °C. Thermistors located at other sites on the tortoise did not stay attached.

II. Water Balance and Osmoregulation

A. Jørgensen (1998) reviewed the literature on urinary and cloacal bladders in chelonians, dating from the 1700s to the mid-1990s. There is a section on the desert tortoise, which includes work by C.C. Peterson (described in this annotated bibliography) and will not be repeated here. Cloacal bladders are present in several families and species of amphibious habits but not in desert tortoises.

B. Peterson (1996b) monitored 48 tortoises in the Desert Tortoise Natural Area (western Mojave Desert) and Ivanpah Valley (eastern Mojave Desert), and reported that:

1. Tortoises lost as much as 40% of their initial body mass, and mean total body water volume decreased below 60% of body mass, during an extreme drought year. When rainfall events occurred, tortoises at both sites drank copiously and body mass and total body water returned to hydrated levels. These results suggest that large fluctuations in body mass were almost entirely due to changes in hydration.
2. Based on monitoring blood and urine samples, tortoises stored wastes in, and apparently reabsorbed water from, their large urinary bladders during an extreme drought year. Osmolality of initially dilute bladder urine increased until it was isosmotic to blood plasma, after which osmolality of both fluids increased, eventually to some of the highest levels known for terrestrial reptiles. Bladder urine osmolality increased (owing primarily to soluble potassium) most rapidly during periods when tortoises ate annual plants. This suggested that their normal diet was osmotically stressful.
3. Drinking rainwater was as important for tortoises in the western Mojave, where summer rain is rare, as in the eastern Mojave, where it is predictable. Tortoises osmoregulate opportunistically, a tactic made possible by their capacity to tolerate temporary lack of homeostasis and by extremely low rates of water loss.

C. Bulova (2002) noted that factors affecting evaporative water loss in other reptiles include phase of skin shedding, activity, and age. The author noted that these factors remain to be studied in the desert tortoise.

D. Henen (2002a), in a synthesis of the literature which included research on nine female desert tortoises at Goffs, eastern Mojave Desert, CA, noted that females appeared to relax homeostatic regulation of their water balance. They accumulated water by drinking rainwater or consuming fresh annual plants during wet periods, and became extremely conservative with water efflux in dry periods primarily by reducing activity on the surface. The ability to relax homeostatic regulation of water budget allowed them to endure extremely harsh and variable desert conditions and for some individuals to produce eggs in dry years. He suggested that despite the extreme tolerances enabled by relaxing homeostasis, desert tortoises are vulnerable to extreme conditions that exceed their tolerances such as drought.

III. Deprivation of Food and Water—Drought

A. Rostal and others (1994a) tested effects of water stress behavior on desert tortoises in semi-natural enclosures at the Desert Tortoise Conservation Center near Las Vegas, NV. Tortoises received food and water supplementation or none. When stressed by lack of water and food resources, tortoises reduce the length of above-ground activity time but attempted to compensate for this decreased time by increasing amounts of movement and feeding while active. Length of morning activity period was shorter in unsupplemented pens for all months. Their observations confirmed hypothesis that activity of desert tortoises is strongly shaped by hydric constraints and that tortoises exposed to hydric stress changed their behavior.

B. Peterson (1996a) reported that field metabolic rates (FMR) were highly variable between and within 2 populations of male desert tortoises in the eastern and western Mojave Desert during extreme drought conditions. FMRs were generally lower than 1.5 L $CO_2 \cdot kg^{-1} \cdot d^{-1}$.

   1. Tortoises exhibited seasonal patterns where FMRs were very low in winter and peaked during the mid-to-late summer. These patterns differed both between and within sites. At Ivanpah Valley (eastern desert), seasonal patterns differed between 1989 and 1990, but there was no difference for tortoises at the Desert Tortoise Natural Area (western desert).
   2. In general, the largest differences in FMRs between populations corresponded to rainfall events in the eastern Mojave and subsequent larger FMRs at Ivanpah Valley.

C. Christopher (1999) observed abnormalities in an adult, male desert tortoise that was trapped in its burrow without food and water for 11 months, compared with the general population near Yucca Mountain, NV.

   1. Physical, biochemical, and hematological abnormalities were noted. Physical abnormalities included slight weight loss (4.2%), sunken eyes, and muscle atrophy.
   2. Biochemical abnormalities determined from blood sampling included marked azotemia (more than three times than previously described) and hyperosmolality with a marked increase in blood urea nitrogen, largely attributed to accumulation and retention of nitrogenous wastes.

3. Moderate hypercholesterolemia, hypophosphatemia, and increased aspartate transaminase activity, and mild hyperchloremia, hypocalcemia, hyperbilirubinemia and slight anemia in the trapped tortoise compared with the general population were probably due in part to dehydration and decreased nutrient intake.
4. The entrapped tortoise was seronegative for *M. agassizii*; thus abnormalities were likely due to lack of food and water rather than upper respiratory tract disease.
5. Despite the mild to extreme alterations observed in these hematological and biochemical analytes, many analytes remained within normal ranges. This exemplifies the high degree of physiological adaptation tortoises can undergo when deprived of food and water for a prolonged period.

D. Christopher and others (1999), in a 5-year baseline study at three sites in the western, eastern, and northeastern Mojave Desert, CA, evaluated hematologic and biochemical responses of desert tortoises to physiologic and environmental factors, such as lack of rainfall.

1. Marked seasonal variation was observed in most parameters in conjunction with reproductive cycle, hibernation, or seasonal rainfall. Year-to-year differences primarily reflected winter rainfall amounts (e.g., drought). Site differences were minimal and largely reflected geographic differences in precipitation patterns.
2. The effects of hydration and food intake were reflected in body weight and biochemical changes associated with hydration (decreased blood-urea-nitrogen [BUN], increased uric acid), ingestion of nutrients (increased glucose, total protein, albumin, phosphorus, cholesterol, iron, and potassium concentrations), and increased metabolic activity (increased alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase activities). The BUN concentration was the most sensitive indicator of availability of water and green forage.
3. Seasons of relative drought (usually summer or fall) were characterized by increased BUN, osmolality, electrolytes, and anion gap, and decreased body weight and total CO_2, consistent with dehydration. Only when urine osmolality reaches 300 mOsm/kg does plasma osmolality (and BUN concentration) begin to rise.

E. Wilson and others (2001) conducted field and laboratory experiments at the University of California, Los Angeles, Colorado State University, and the National Training Center at Fort Irwin, CA, to investigate impacts of environmental conditions and activity on water loss rates from neonate and juvenile desert tortoises.

1. In laboratory experiments:
    a. Water loss rates were unrelated to environmental vapor density differences and air temperatures that made up the different vapor densities.
    b. Active tortoises, on average, lost water twice as fast as sleeping neonates, and 20 times as fast as hibernating neonates.

2. In field experiments:
    a. Juveniles confined to long (70 cm) burrows gained water significantly faster than those in short (20 cm) burrows.
    b. Total evaporation rate was independent of burrow conditions, but tortoises in the longer, more humid burrows had higher rates of water vapor and total water input than those in shorter burrows. Thus, tortoises in long burrows lost body mass more slowly due to a higher humidity.
3. In field and laboratory experiments, rates of body mass loss due to evaporation from neonates were relatively high in the laboratory (0.4–0.8% of body mass per day) and the field (0.7–1.1% per day) compared with adults in the laboratory (0.17% per day) or the field (0.1% per day). This suggested that young tortoises were obliged to rely on behavioral means (drinking pooled rain; withdrawing into their shell; seeking long, deep burrows) to avoid lethal dehydration in relatively hot, dry seasons.

F. Berry and others (2002b) studied behavioral, hematological, and plasma biochemical variables of 11 moribund desert tortoises salvaged due to dehydration and starvation during or following droughts. The tortoises were from three field sites in the Mojave Desert, CA, and included juveniles, one immature tortoise, and five adult males. The clinical signs of disease and pathologic findings from juveniles were consistent with dehydration and starvation. 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 tortoises can tolerate a wide range of hematological and biochemical values, including electrolyte levels, each of the salvaged immature and adult tortoises exceeded the range or 95th percentiles for four or more analytes for healthy tortoises—departures consistent with dehydration and starvation. Examples follow:

1. Weight loss is a key clinical sign of stress; 10 of the 11 tortoises weighed from 19.5 to 39.7% less than comparable drought survivors.
2. In the weeks and months preceding salvage or death, the tortoises behaved abnormally for the season and weather conditions, e.g., lethargic, not entering burrows for hibernation, remaining above ground overnight exposed to freezing temperatures, and failing to drink when rain left free-standing water in the vicinity. Other signs are weakness, cachexia, sunken eyes, and atrophy of skeletal muscle.
3. Hematologic abnormalities included low packed cell volumes and heterophil counts. Plasma biochemical analytes included hypocalcemia, hyperbilirubinemia, marked azotemia, and elevated sodium and chloride levels.
4. Gross necropsies and microscopic evaluations produced somewhat different results for juveniles than adults, e.g., shells of juvenile tortoises were softer and more pliable than normal for age and size and expected muscle mass was ~50% below normal, osteopenia of some bones was evident, and the skin had more foreign debris. For the immature and adult tortoises, the abnormalities varied by individual but were numerous.

IV. Metabolism

A. Peterson (1996a) reported that field metabolic rates (FMR) and feeding rates (estimated from rates of water influx and rates of change in dry mass) were highly variable across seasons and years within and between two populations of adult male desert tortoises in the eastern Mojave Desert (Ivanpah Valley) and western Mojave Desert (Desert Tortoise Research Natural Area) during extreme drought conditions.

   1. FMRs were generally lower than 1.5 L $CO_2 \cdot kg^{-1} \cdot d^{-1}$.

      Tortoises exhibited seasonal patterns where FMRs were very low in winter and peaked during the mid-to-late summer. These patterns differed both between and within sites. At Ivanpah Valley (eastern desert), seasonal patterns differed between 1989 and 1990, but there was no difference for tortoises at the Desert Tortoise Natural Area (DTNA, western desert). In general, the largest differences in FMRs between populations corresponded to rainfall events in the eastern Mojave and subsequent larger FMRs at Ivanpah Valley.

   2. Much of this variation in energetic variables was associated with rainfall. Seasonal, annual, and inter-population differences in FMR and foraging rates corresponded to differences in availability of free-standing water from rainstorms.

B. Nagy and others (1997) estimated body water volumes, which are necessary for calculating field metabolic rate (FMR) and water influx rates, and measured body mass for 4–22 neonate tortoises raised in semi-natural pens for 2 years in the western Mojave Desert. They found that FMR was significantly correlated to body mass and water influx rate of neonates. Metabolic rates were higher in spring, which was consistent with the availability of green annual plants, than in summer and winter. During summer, tortoises were eating dry plants and, as indicated by the Water Economy Index, the tortoises were in a water deficit. During winter hibernation, tortoises were deep in burrows and relatively inert, having low FMR and water loss, and, thus, lost little body mass.

C. Henen (1997), studied energy budgets of nine adult female tortoises from Fenner Valley, CA, and reported that females produced eggs in years with low levels of winter annual plants by relaxing their control of energy and water homeostasis.

   1. Females tolerated large deficits and surpluses in body dry-matter composition on seasonal, annual, and longer timescales. They were able to increase body energy content (lipid and non-lipid energy) for winter and use this reserve the following spring to produce eggs.

   2. Females used high-protein foods and rainwater, when available, to achieve energy surpluses that helped them survive periods of low resource availability (e.g., during drought). They managed to produce eggs, even in a drought year, by adjusting seasonal and annual field metabolic rates and food requirements to levels of food availability.

   3. Water and annual plants in spring were primary resources limiting egg production in a drought year, and females forgoing egg production in that year accumulated body nonlipid energy and lost less total body water than did females producing eggs. Reproductive effort was higher during the drought than during the wetter year because tortoises reduced field metabolic rate by 70–90% in the drought year.

D. Henen and others (1998) studied field metabolic rates (FMR) and water influx rates (WIR) obtained from three sites in the western, eastern, and northeastern Mojave Desert, CA.

1. Male desert tortoises had higher FMR than females in high-rainfall years, perhaps due to larger home ranges and efforts by males to mate.
2. Field metabolic rates, WIR, and a water economy index (WEI) differed extensively among seasons, among study sites, and among years. Average daily rates of energy and water use by desert tortoises were highly variable, with 28-fold differences in FMR and 237-fold differences in WIR. In addition to variation in seasonal conditions affected by temperature, much of the variation was due to responses in year-to-year variation in rainfall, with higher annual spring peaks in FMR and WIR in wet years than in drought years.
3. Site differences in seasonal patterns appeared to be due to geographic differences in rainfall patterns (more summer rain at eastern than western Mojave sites). During an El Niño (ENSO) event, the WEI was greater than the maximal value obtainable from consuming succulent vegetation, indicating copious drinking rainwater at that time.
4. These results suggest that the physiological and behavioral flexibility of desert tortoises, evident in individuals living at all three study sites, appears central to their ability to survive droughts and benefit from periods of resource abundance. The strong effects of weather patterns on tortoise physiology suggest that tortoises are heavily dependent on the timing and amounts of precipitation locally, and that local manifestations of global climate events could have long-term influence on the physiological ecology and demographic pattern of Mojave desert tortoise populations.

E. Nagy (2000) conducted a literature review and reported that rapidly growing neonate reptiles do not increase their standard or field metabolic rates above those expected for adult reptiles, after correction for body mass differences, which raises questions for future research:

1. Do neonate reptiles select lower body temperatures than adults?
2. Do neonate reptiles spend less time on the surface than do adults? If so, are neonates more effective foragers?
3. Can neonates digest their food as effectively as can adults if they spend less time each day thermoregulating, or if they select lower body temperature?
4. Can a “minimum” time on the surface (to achieve energy steady-state) be determined from time-energy budgets of neonates and adults, and how do actual time budgets match the “minimum”?

F. See Henen (2002a), II.D., this chapter.

G. Brown and others (2005) studied captive tortoises in Los Angeles, CA, and reported that young, rapidly growing desert tortoises did not exhibit elevated standard metabolic rates or mass-adjusted field metabolic rates in captivity, which suggested that the cost of growth did not increase their field energy expenditures above those expected for adults. The authors suggested that long-term studies of individual reptiles from hatching through adulthood would be helpful to determine whether relative growth costs vary absolutely during different life stages.

V. Other Physiological Stresses, Handling

A. Rostal and others (1994a) in a study of captive and penned hatchling, juvenile, and immature tortoises at the Desert Tortoise Conservation Center in Las Vegas, NV, noted that prolonged handling and manipulation of tortoises prior to or during blood sampling (i.e., > 10 minutes) may influence plasma testosterone levels. Male and female tortoises may display increased testosterone secretion in response to prolonged handling stress (Rostal and others [1994a] drew on unpublished data).

B. Tracy and others (2006b) developed a hypothesis that physiological stress may decrease immunity to disease based on endocrine mechanisms. They proposed that free molecules of the stress hormone corticosterone, competes with levels of testosterone naturally occurring during the breeding season, and could inhibit immunity because the binding globulins that are responsible for controlling corticosterone also bind with testosterone. They suggested that this endocrine mechanism may be especially important for populations at high densities, where agonistic encounters are more frequent and excess testosterone may be released. Higher titers of testosterone compete with binding globulins leading to excess corticosterone in the blood, thereby increasing the vulnerability of individuals to disease.

C. Drake and others (2012b) measured cortisol levels as a stress response to activities associated with translocation of desert tortoises assigned to three treatment groups (resident, translocated, and control) in the central Mojave Desert, CA. Adrenocorticotropic hormone (ACTH), which controls the release of cortisol, challenge test results suggested that blood collection using subcarapacial venipuncture on tortoises induces minimal stress in the first 15 minutes of initial handling of the animals. Cortisol was higher for males than females, and values for both varied monthly throughout the activity season and among years. Female cortisol levels were higher in late spring, the time period associated with ovulation, egg production, nesting, and increased activity and movement. Comparisons among treatment groups showed that translocation explained little in terms of cortisol levels.

D. Agha and others (2015d), drawing on data collected between 1997 and 2014, studied effects of research activities and winter precipitation on voiding of *Gopherus agassizii* at the Mesa study area in the Colorado Desert, CA. The authors reported that 42 tortoises voided on 8.2% occasions (1,008 total capture events). The models indicated that increases in handling time led to significantly higher probabilities of voiding for juveniles, females, and males. Increases in precipitation also resulted in significant higher probabilities of voiding for juveniles and females, but not for males. Capture frequency was negatively correlated with voiding occurrence. Models showed negligible effect for voiding behavior and sex on survivorship.

Chapter 8.—Health and Disease

I. Collecting Field Data to Determine Health Status

A. Assessing Health Status

1. Brown and others (1999) noted that the health of individual tortoises, especially those tortoises with infectious diseases, can affect the outcome of research projects and interpretation of results. Therefore any studies should include health status of the tortoises to avoid confounding the results. Especially of concern are reproductive and nutritional studies.

2. Berry and Christopher (2001) described several guidelines for conducting field studies on health and disease:

    a. Preparations for the field include developing a familiarity with the full repertoire of postures, behaviors, and display patterns of healthy tortoises, because one of the clinical signs of ill health is abnormal behavior.

    b. The authors developed detailed, standardized guidelines for collecting and analyzing qualitative and quantitative data on clinical and physical signs of health for wild desert tortoises for demographic, ecological, health and disease research methods**.** They present data sheets (Journal, Live Tortoises, Health Profile Forms), developed and tested from >5,000 tortoises among 36 sites in California deserts, and instructions on how to mark tortoises.

    c. Procedures to prevent spread of infectious diseases and parasites include taking special precautions to prevent transmission of pathogens such as *Mycoplasma*, and parasites (e.g., ticks) by using a fresh pair of disposable gloves and disinfecting equipment with sodium hypochlorite solution (0.175%) immediately after each use. Jacobson (1994) noted that the presence of ectoparasites on tortoises should be considered significant.

    d. As part of field protocols, tortoises that are released, are escaped captives or from other research projects should be identified.

    e. The appearance of the head, limbs, beak, nares, eyes, chin glands, and shell provide subtle signs indicative of health or disease; clinical signs can be rated as none, mild, moderate, or severe. Lesions on the shell from cutaneous dyskeratosis or other disease-related lesions can be rated by distribution on the shell, severity of lesions, and chronicity (relative age of the lesion, whether new and fresh or healed).

    f. Photographs or digital images are valuable sources of information for health and for evaluating changes in lesions over time.

B. Obtaining Blood and Management of Blood in the Field

1. Sites for drawing blood and their limitations and sources of error

a. Jacobson and others (1992) reviewed the advantages and disadvantages of drawing blood from different sites (heart, jugular vein, brachial vein, ventral coccygeal vein, orbital sinus, and trimmed toenails), noting such problems as contamination with pathogens, damage to tissues, and dilution of blood samples with extravascular fluids, lymph, and secretions. Compared to other sites, the jugular vein provides an undiluted source of blood, although can be difficult to access. O’Connor and others (1994a), in a study of hydric stress on captive and free-ranging tortoises, used the jugular vein to obtain blood. Christopher and others (1999), in developing reference ranges for hematological and plasma biochemical attributes of blood, used the jugular vein to obtain blood.

b. Gottdenker and Jacobson (1995) conducted an experiment to compare quality of blood samples from the postoccipital venous plexus and jugular veins for hematologic and plasma biochemical analytes. They reported significant differences between hematologic and plasma biochemical values for the two sites and attributed the differences to hemodilution of the occipital region samples with extravascular fluid or lymph or both. Error in blood values attributable to sample collection site may also originate at other commonly used blood sample collection sites, such as the ventral coccygeal, brachial, and scapular veins. Accurate interpretation of hematologic and plasma biochemical analytes requires blood samples to be representative of actual blood composition of the animal tested. Error in blood values attributable to sample collection site in desert tortoises may also originate at other commonly used blood sample collection sites (ventral coccygeal, brachial, and scapular veins). From these experiments, the jugular vein appears most likely to give representative samples of actual blood composition.

c. Cardiocentesis. Wimsatt and others (1998) reviewed the literature on cardiocentesis and describes in detail the technique that he and his colleagues used for experiments dealing with maintaining a port and conducting dosage studies on antibiotics.

d. Schumacher and others (1999) used cardiocentesis to sample hatchling tortoises.

2. Management of blood in the field and laboratory

    a. Jacobson and others (1992) noted the importance of using lithium heparin as the anticoagulant of choice for determining hematologic and plasma biochemical health profiles of turtles to avoid hemolysis. Hematologic and plasma biochemical values are affected by temperature and time; therefore whole blood in lithium heparin tubes should be kept on ice and samples evaluated within 24 h. Plasma should be separated as quickly as possible from whole blood and likewise be evaluated as quickly as possible, otherwise concentrations of sodium and potassium and other analytes may change. Samples can be placed in cryotubes and frozen in liquid nitrogen, although some enzyme activity may be altered by cooling or freezing. Studies need to be conducted on effects of freezing on these samples.

    b. Christopher and others (1999) noted that ensuring high quality samples and optimal sample handling was a major challenge in the desert environment. They used jugular venipuncture to avoid contamination or dilution of blood with lymph. However, clots were a problem and differences and variations in packed cell volumes obtained in the laboratory vs. the field suggested red blood cell shrinkage or lysis during transport or operator differences. Some plasma specimens were unacceptable because of insufficient sample volume and fibrin clots. Drastic changes in iron and creatinine values coincided with upgrading the laboratory autoanalyzer. This forced exclusion of some values.

C. Nasal Flushes or Nasal Lavage. Christopher and others (2003) described the method of conducting nasal flushes in the field by introducing 0.5 mL of trypticase soy broth into the anterior nares using a sterile tomcat catheter and placing the flushes in sterile cryovials.

D. Bladder Puncture, Cystocentesis: see also Hernandez-Divers, S.J., and Innis, C.J., Chapter 66 in Mader (2006). This procedure has numerous issues, can have negative impacts on tortoises through unsterile leakage into the coelom. Samples may not be representative of renal electrolytes and osmolarity. Christopher and others (2003) noted that one necropsied tortoise, repeatedly subjected to cystocentesis as part of a field research project, had severe peritonitis and multiple bladder wall abscesses, presumable related to cystocentesis.

E. Salvage of Ill, Dying, and Recently Dead Tortoises Followed by Necropsy

1. Homer and others (1998) noted that necropsy is a key approach for determining causes of disease and mortality in different populations of tortoises and that salvage plans should be incorporated into field research protocols.

2. Berry and Christopher (2001) noted that sacrifice of live tortoises provides more information at necropsy than salvaged dead tortoises in which tissues often have deteriorated, and frozen remains are of limited value for most pathologic studies. The authors provided 12 criteria for salvage. Subtle behaviors can provide evidence of illness and justification for salvage, i.e., active and above ground at inappropriate times of year, failure to emerge or late in emerging from hibernation, failure to return to burrows, failure to drink during a warm rain.

II. Baseline Data and Diagnostic Laboratory Tools for Determining Health Status

A. Use of Body Weight to Length Relationships

1. Jacobson and others (1993) reported that reliable predictions of health, based on body weight to carapace length relationships have not been fruitful, probably because so many factors affect weight (sex, reproductive status, degree of hydration, morphology of shell). The authors tested this with samples of 40 healthy and 37 tortoises ill with upper respiratory tract disease. The regression equations were similar; on average, tortoises with clinical signs of disease weighed about 7% less than healthy tortoises. The usefulness of weight vs. carapace length relationships for predicting health are limited because of gender differences in shell shape and weight, oviposition in females, seasonal changes (e.g., emergence from hibernation), and effects of drinking, voiding and defecating.
2. Christopher and others (1999) carried out a 5-year study on wild adult male and female tortoises (n = 98) in three regions of the Mojave Desert (western, eastern, northeastern) of California and established reference intervals for hematological values by sex, season (four seasons), and years of near-, above- and below average rainfall. Body weight and carapace length at the midline were significantly greater in males than females at all sites all year. Body weight increased in all tortoises between emergence from hibernation (winter) and spring. Mean body weight decreased significantly between fall and winter of 1993–1994. Body weight (body mass) and carapace length fitted a second-order polynomial regression curve. The slope of the regression curve did not differ significantly between sites, seasons or years but did differ by sex.
3. Berry and others (2002b) calculated linear regression equations, 95% confidence intervals and coefficients of determination (R^2) for weight-length relationships of wild tortoises that had survived periods of drought. These equations were used as controls for comparisons with moribund and dead tortoises salvaged for necropsy (starvation and drought). The predictive powers of these regression equations for control tortoises (measured by coefficients of determination) were high for juveniles and rapidly diminished with increasing carapace length.

B. Condition Indices

1. Methods

a. Wallis and others (1999) used two means of calculating condition indices (CI) for each tortoise, by dividing body mass (g) by volume (cm^3), the latter determined either by (i) the cube of carapace length at the midline or (ii) the product of carapace length, width, and height.

b. Nagy and others (2002) described a condition index (CI) for wild desert tortoises (simulating a physical density value). The CI was calculated as the ratio of body mass to estimated shell volume (carapace length x height x width). The prime CIs had a range of 0.6 to 0.7 g/cm^3 and averaged 0.641g/cm^3. In general, prime CIs did not vary between sexes or between study sites in the eastern and western Mojave Desert, nor did they vary with body size among adults, or with age. However, females exhibited a geographic difference with females at the Desert Tortoise Natural Area having lower prime CIs compared to females at Goffs and Ivanpah. Changes over time in CIs of three populations revealed seasonal and geographic variations that corresponded with the local availability of green forage plants and rainwater for drinking. The CIs for free-ranging tortoises in the eastern Mojave Desert, for example, peaked in May and progressively lessened through the summer, reaching the lowest values in August or October. Hatchling tortoises in captive conditions at the Fort Irwin National Training Center, CA, had very low CIs (CI = 0.401 g/cm^3), averaging less than 65% of prime CI following 5 months of poor food availability and drought. In contrast, two free-ranging hatchlings measured at Goffs in the eastern Mojave Desert had CIs of 0.645 and 0.733 g/cm^3 (101 and 114% of prime CI, respectively). The authors suggest that a prime CI value of 0.64g/cm^3 is a useful standard for evaluating body condition of wild tortoises in the field. Dehydrated tortoises are expected to have CIs below about 0.45 g/cm^3 (70% of prime CI).

    c. Ennen and others (2012a) calculated a general body condition index (average biomass/mid-carapace length) for female tortoises after a 2 year drought, during an El Niño Southern Oscillation event year, and during a drought year following the ENSO. They did not find a significant difference among condition index values, possibly because of small sample sizes and also differences observed in individual reproductive status, dietary condition, and availability of water at the time weight measurements were taken.

2. Reports on use of condition indices in populations

    a. Peterson (1996b) reported that none of the 48 monitored tortoises at the Desert Tortoise Natural Area and Ivanpah Valley, CA, grew (based on change in carapace length) over an 18-month drought period (1989–1990). Tortoises had lower condition indices during extreme drought; when rainfall occurred, tortoises at both sites drank copiously and condition indices increased. The author concluded that condition index values reflected changes in body mass and were independent of sex, and that populations differed in the temporal pattern of change.

    b. Wallis and others (1999) collected measurements from 76 female desert tortoises from Goffs and the Desert Tortoise Research Natural Area, CA, over a 2-year period. Goffs tortoises had a lower average condition index in 1993 but there was no difference between sites in 1992. In 1992, Goffs tortoises hibernated in better condition than those at the Desert Tortoise Research Natural Area while the opposite occurred in 1993. The authors suggest that condition index estimates were determined primarily by fluctuations in body mass which in turn was indicative of hydration status.

    c. Nagy and others (2002) reported that condition indices of free-ranging desert tortoises in the eastern Mojave Desert, CA, peaked in May and progressively lessened through summer, reaching their lowest CI values in August or October. This pattern was expected solely from a nutritional perspective, because spring was when green forbs were available; as summer progressed, temperature and drought conditions increased, and food plants dried and withered. In contrast, hatchling tortoises in captive conditions at the Fort Irwin National Training Center, CA, had very low condition indices (CI; 0.401 g/cm^3), averaging less than 65% of prime CI. In contrast, 2 free-ranging hatchlings measured at Goffs in the eastern Mojave Desert had CIs of 0.645 and 0.733 g/cm^3 (101 and 114% of prime CI).

d. McCoy and others (2011) compared body condition indices of adult desert tortoises, *Gopherus agassizii*, and gopher tortoises, *G. polyphemus*. Body condition indices (CI) for 163 adult (86 males and 77 females) desert tortoises measured from 1992 to 1993 were not different between years or between sexes. When pooled together, body condition increased from March to May, reaching a maximum of 0.63 in spring, and then steadily declined through the remainder of the active season, resulting in a significant difference between seasons. The average body CI for the desert tortoises was significantly higher than that of 184 gopher tortoises, sampled over three periods.

C. Baseline Laboratory Data and Reference Ranges for Healthy Desert Tortoises

1. Hematology—Morphology, cytochemistry, and parasites

a. Normal blood cells: morphology and cytochemistry. Alleman and others (1992) provided basic information on the characteristics of circulating blood cells of the desert tortoise, important for determining differential blood cell counts and thus the health status of desert tortoises. The data were drawn from samples obtained from 23 clinically healthy, free-ranging desert tortoises from Kern County, CA. The authors identified three types of cells—erythrocytes, thrombocytes, and leukocytes and recognized five categories of leukocytes: heterophils, eosinophils, monocytes (including azurophilic monocytes), lymphocytes, and basophils. All cell types and categories are described by appearance, size, occurrence, and the type of staining used. Cytochemical stains may be useful in identifying inflammatory cell types in cytologic samples and in differentiating thrombocytes from lymphocytes from blood and bone marrow samples.

b. Blood parasites. Protozoa (*Haemogregarina* sp.) were observed within erythrocytes of 2 of 23 tortoises (Alleman and others, 1992). Intracellular red blood cell parasites were not observed in any tortoises (n = 98) in another study (Christopher and others, 1999).

c. Staining and morphologic features of bone marrow hematopoietic cells. Garner and others (1996) studied 16 ill and healthy tortoises from Goffs (1, eastern Mojave), Chuckwalla Bench (1, southern Colorado Desert), Chemehuevi Valley (2, northern Colorado Desert), and Las Vegas Valley (11, northeastern Mojave Desert). The objectives were to determine optimal location for clinical bone marrow sample collection, to determine cytochemical staining characteristics of bone marrow hematopoietic cells, and to describe the morphologic features of bone marrow hematopoietic precursor cells. The pelvis, proximal humerus, femur, and thickened portions of the peripheral cranial and caudal regions of the carapace and plastron are suitable sites to collect bone marrow hematopoietic cells for histologic and cytologic evaluation. Data were provided on both healthy and ill tortoises. For ill tortoises, see below.

2. Reference ranges: baselines for hematology. Christopher and others (1999) carried out a 5-year study on wild adult male and female tortoises (n = 98) in three regions of the Mojave Desert (western, eastern, northeastern) of California to establish reference intervals for interpretation of laboratory data on hematological and plasma biochemical values by sex, season (four seasons), and years of near-, above- and below-average rainfall. They reported reference intervals and physiologic alterations in hematologic and plasma biochemical analytes. For hematological analytes, a few examples of differences are given here; see the original paper for details and a complete summary.
    a. Males had significantly higher red blood cell mass (packed cell volume, red blood cell counts, hemoglobin concentration) than females all year. Females had more pronounced seasonal alterations in red blood cell mass than males with higher values in spring and summer. For both sexes, packed cell volume and hemoglobin concentrations were lower in drought years. Hormonal, dietary, and hydration influences affected RBC mass, as evidenced by annual, seasonal changes and increased values in years with above-average rainfall.
    b. Lymphocyte and basophil concentrations were significantly lower than those of other leukocytes.
3. Seasonal changes in total white cell numbers. Lance and Rostal (2002), in a study of penned tortoises at the Desert Tortoise Conservation Center in Nevada, reported seasonal changes in total white blood cell numbers in male and female tortoises. In both sexes there were significant increases in total cell numbers in August, September, and October as compared to April through July. This was not observed by Christopher and others (1999). The peak in white blood cell numbers in captive desert tortoises occurred when both testosterone and adrenal steroid secretion were greatest in male and when estradiol secretion was greatest in females. The authors raised the question as to whether steroids were modulating the immune system.
4. Reference Ranges: baselines for plasma biochemistry (Christopher and others, 1999). In a 5-year research project in the western, eastern, and northeastern Mojave Desert, the authors developed reference intervals and assessed physiologic alterations for plasma biochemical values in male and female adult tortoises (see C.1., Hematology above). The tests included glucose, blood urea nitrogen (BUN), creatinine, uric acid, total protein, albumin, calcium, phosphorus, total bilirubin, direct bilirubin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, total cholesterol, iron, magnesium, sodium, potassium, chloride, total carbon dioxide (TCO_2), bile acids, globulins, albumin/globulin ratio (A/G), anion gap, osmolality, sodium/potassium (Na/K) ratio, and indirect bilirubin. See original paper for details. Only a few examples are offered here:

   a. Significant differences occurred in biochemical analytes by sex, season, and year (rainfall), e.g., cholesterol and triglyceride concentrations were significantly higher in females; cholesterol concentration was significantly lower in winter. High concentrations of cholesterol, triglycerides, calcium, and phosphorus in females were consistent with egg production and vitellogenesis. ALT and AST were significantly higher in males and calcium and phosphorus were significantly higher in females.

   b. Many analytes varied by season and by rainfall, e.g., BUN concentration was high in all tortoises at emergence from hibernation but markedly and significantly decreased in spring; variable increases in summer and fall were dependent on rainfall pattern. Uric acid concentration increased between winter and spring and decreased in fall. There was an inverse relationship between uric acid and BUN. Hibernation was associated with the greatest number and magnitude of hematologic and biochemical changes, which were categorized as decreased immune function, production and retention of nitrogenous wastes, decreased food intake, and decreased metabolic activity.

   c. Hibernation also was characterized by lower concentrations of cholesterol, glucose, total protein, albumin, P, iron, and, in females, calcium consistent with decreased nutrient intake.

   d. Seasonal effects on reference values resulting from hibernation and availability of food and water were pronounced and significant from year to year. Thus seasonal reference intervals must be used in interpreting most laboratory values. In addition, sex-specific reference intervals should be used for some analytes.

5. Measuring hydric stress in experimental situations. O’Connor and others (1994b) studied the physiological effects of water stress in desert tortoises as assessed by hematologic and plasma biochemical assays. The authors used free-ranging tortoises near Las Vegas, Nevada, as controls and a high density population of captives at the Desert Tortoise Conservation Center. Tortoises were subjected to three levels of water supplementation (supplemented, unsupplemented, and intermediate) to mimic different levels of hydric stress. They were sampled in June–September 1991, February 1992, and April–October 1992 and the population included tortoises with upper respiratory tract disease. Examples of findings:

   a. Hematocrits were higher in tortoises at the control sites and lowest in tortoises at the unsupplemented site.

   b. Males had higher hematocrits than did females or immature animals; in general, larger animals had higher hematocrits than smaller animals.

   c. White blood cell counts in captive tortoises without water supplementation were higher than in free ranging and water-supplemented captive tortoises.

   d. Unsupplemented tortoises had the highest concentrations of Na, K, Cl, and HCO_3 and control tortoises had the lowest values.

   e. Calcium values were highest in females and lowest in males, consistent with changes in vitellogenesis.

   f. Enzymes differed by sex and season and glucose and serum. proteins varied by size, sex, and season, depending on the analyte.

   g. Overall, blood profiles could not be used to predict which tortoises were exposed to which water supplementation treatment or hydric stress. However, blood screens did identify several possible stress responses: high Na, Cl, HCO_3, and urea nitrogen concentrations suggested water stress in unsupplemented tortoises and rising electrolyte concentrations suggested negative water balance and hydration stress.

   h. Blood urea nitrogen (BUN) increased throughout the season and tortoises in unsupplemented plots had higher levels of BUN than others.

6. Christopher and others (1994) evaluated concentrations of β-hydroxybutyrate in plasma and urine samples of 17 male and 7 female tortoises to assess significance in relation to sex, season, and health status and to understand the comparative availability and utilization of fuel sources by tortoises. Some tortoises were seropositive for upper respiratory tract disease. Plasma β-hydroxybutyrate concentration was significantly higher in October compared to other months with lowest values in January; there were no significant differences between sexes. Urine β-hydroxybutyrate values remained very low throughout the year in all tortoises. Plasma glucose concentrations were significantly greater in July, the month of peak rainfall. Females had significantly greater total cholesterol levels than males with high levels in July concomitant with egg-laying. The authors compared β-hydroxybotyrate with several other plasma biochemical analytes. This study suggested that ketogenesis is not an important source of fuel during hibernation; rather, protein catabolism significantly contributes to hibernational azotemia and thus was likely an important fuel source during hibernation. Ketone bodies, specifically β-hydroxybutyrate, may be an important short-term source of energy during periods of decreased resources and food intake.

7. Peterson (1996b) measured concentrations of five plasma parameters (total osmolality, potassium, chloride, sodium, and urea nitrogen [BUN]) in 48 tortoises at the Desert Tortoise Natural Area and Ivanpah Valley, CA. He reported significant variations over an 18-month period.

   a. Mean total plasma osmolarity increased from typical levels for terrestrial reptiles (280–310 mOsmol) to 379–407 mOsmol in June 1990, with some individuals exceeding 500 mOsmol, before returning to a mean level of approximately 300 mOsmol, following rainfall events.

   b. Plasma sodium and chloride concentrations showed patterns similar to total plasma osmolality, except both decreased slightly over winter.

   c. Plasma potassium concentration showed a similar temporal pattern and varied from 4 to over 7 mmol L^{-1}.

   d. Blood urea nitrogen increased from 8 to 20 mg dL^{-1} to over 120 mg dL^{-1} (Desert Tortoise Natural Area) and over 160 mg dL^{-1} (Ivanpah Valley).

8. Peterson (2002), in studies of two populations of desert tortoises from the eastern and western Mojave Desert, CA, reported many simultaneous and sometimes opposing effects when evaluating the relationship of packed-cell volume with physiological indictors of nutritional health. Packed cell volume may be a poor indicator of physiological state in desert tortoises.

D. Bacteriology

1. Tests, protocols, and procedures for detecting *Mycoplasma* spp.

   a. Enzyme-linked immunosorbent assays (ELISA) for *Mycoplasma agassizii*

      i. Schumacher and others (1993) developed an ELISA for detection of *M. agassizii*-specific antibodies with amonoclonal antibody having specificity for desert tortoise immunoglobulin light chain. Plasma samples from 24 infected and uninfected tortoises, determined by presence or absence of clinical signs and pathologic lesions typical of upper respiratory tract disease (URTD), were tested. Plasma was also obtained from an additional group of 12 tortoises used in a transmission study. Antibody levels against *M. agassizii* were high in the known URTD-positive tortoises 1 month after challenge. Antibody levels were very low in the URTD-free tortoises and undetectable in the tortoises before challenge with the pathogen. Western blots were used to independently test the antigen-antibody reaction. The ELISA was able to detect seroconversion in individual tortoises. Lesions in the nasal mucosae were significantly correlated with detection of *M. agassizii*-specific antibody. This ELISA had a high degree of specificity for detection of antibodies against *M. agassizii* and a high degree of sensitivity for distinguishing presence of antibodies in infected vs. non-infected tortoises.

      ii. Wendland and others (2007) described a substantially refined ELISA for *M. agassizii* with more stringent quality control measures based on analysis of 5,954 samples from *Gopherus agassizii* and *G. polyphemus*. The assay was converted from an enzyme immunoassay ratio system to a titer-based system, consistent with other diagnostic serologic tests. The relationship between titer and A_{405} was validated from known positive and negative control tortoises. This ELISA now has a substantially improved performance compared with the earlier ELISA: sensitivity of 0.98, specificity of 0.99, and Youden index, *J*, of 0.98. A graph is available showing the positive and negative predictive values (PPV, NPV) as a function of seroprevalence.

   b. Enzyme-linked immunosorbent assays (ELISA) for *Mycoplasma testudineum* (Jacobson and Berry, 2012). The ELISA developed by Wendland and others (2007) was modified to detect anti-*Mycoplasma testudineum* antibodies. A nasal lavage was obtained from each desert tortoise and used for culture and identification of the *Mycoplsma* by polymerase chain reaction using species-specific DNA nucleotide sequences. Restriction fragment length polymorphism analysis of the 16S rRNA gene was conducted on all positive PCR samples to confirm the identity of the *Mycoplasma*.

c. Natural antibodies and interpretation of serological results.

   i. Hunter and others (2008) used a single isolate of *M. agassizii* (PS6) as an antigen for immunoblotting. They reported that the *M. agassizii* ELISA may misidentify true-negative tortoises as seropositive due to the presence of natural antibodies (predominantly IgM) and that Western blot assays should be used as a confirmatory test for *M. agassizii* exposure.
   ii. Wendland and others (2010) designed a study to determine whether strain variability in *M. agassizii* affected results of Western blot analyses described by Hunter and others (2008). Wendland and others (2010) used four distinct clinical isolates of *M. agassizii* as antigens to compare results from ELISA and Western blot analyses in their experiment. They used an ELISA based on multiple strains of *M. agassizii*. They reported that differences in *M. agassizii* strain variation and distinct host antibody responses explained the differences between ELISA and Western blot assays. They emphasized that it is necessary to use multiple strains of mycoplasmas as antigens in Western blots to avoid false negatives. Unlike the Western blot analysis, the reactions of tortoise sera in the ELISA (see Wendland and others, 2007) were similar, even when the antigens were prepared from different strains of *M. agassizii.* They emphasized that the current ELISA is the only diagnostic test for mycoplasmal URTD that has undergone rigorous validation and has been correlated with clinical disease, culture, and PCR as well as the presence of histological lesions in both naturally and experimentally infected animals. Western blot analysis, particularly when only a single isolate is used an antigen, is likely to lead to misidentification of 15 to 25% of truly infected animals as negative.
   iii. DuPré and others (2011) developed a quantitative PCR method for detecting and quantifying *Mycoplasma agassizii* and *M. testudineum* DNA from nasal lavage samples. This method did not amplify normal flora DNA and was able to detect low to high concentrations of *M. agassizii* DNA, including in tortoises that tested negative on the ELISA test (see Hunter and others, 2008) and that did not show evidence of seroconversion.
   iv. Jacobson and others (2014), reviewing the work by Hunter and others (2008) and Wendland and others (2010), noted that natural antibodies are generally irrelevant in immunological tests, since sera are usually diluted sufficiently to avoid nonspecific background interference.

d. Bacterial cultures of nasal lavage fluid. Brown and others (1995, 2004) reported that *Mycoplasma agassizii* and *M. testudineum* grow very slowly in SP4 broth or agar. Antibiotics are usually added to avoid growth of undesirable bacteria and fungi.

e. Flow cytometry of *Mycoplasma agassizii*. Mohammadpour and others (2010) demonstrated that a nonfluorescent substrate, 5-carboxyfluorescein diacetate acetoxymethyl ester, could penetrate cell membranes of viable *M. agassizii,* be converted to a fluorescent molecule, and thus label the bacterium. Viable *M. agassizii* could then be easily detected by flow cytometry and counted from cultures with as few as 100 viable cells/mL.

f. PCR, DNA fingerprinting; qPCR

    i. Brown and others (1995) and Wendland and others (2007) reported on a conventional PCR assay to detect tortoise mycoplasmas in culture medium and in nasal lavage fluid. They used restriction enzymes to obtain unique patterns for mycoplasmal 16S rRNA.
    ii. DuPré and others (2011) developed qPCR assays using primers specific for *M. agassizii* and *M. testudineum* to quantify mycoplasmas in samples and to correlate microbial burdens with clinical signs. They used qPCR and Western blot to detect *M. agassizii* DNA in 20 captive tortoises; they interpreted their findings to mean that the tortoises were colonized but not infected. However, according to Jacobson and others (2014) their study did not include ELISA results or address the limitations of using Western blot analysis.
    iii. Braun and others (2014) developed qPCR assays specific for agents causing clinical signs of URTD: *Mycoplasma agassizii*, *M. testudineum*, and Testudinid herpesvirus 2 (TeHV2). Fifty-five necropsied tortoises from the Desert Tortoise Conservation Center (DTCC) in Las Vegas, NV, and three wild tortoises were tested. The authors compared findings with ELISA tests for *M. agassizii*. They had no positive results for *M. testudineum*. When they evaluated compared ELISA and qPCR results in nasal flush and nasal mucosa specimens, the nasal mucosa samples yielded the largest number of *M. agassizii* qPCR positive tortoises (74%). In contrast, 26 tortoises (60%) were positive by nasal flush qPCR and 23 (53%) by ELISA. Overall 65% (28/43) of ELISA results agreed with the presence or absence of DNA via qPCR in both nasal flush and nasal mucosa samples. There was moderate agreement between the *M. agassizii* ELISA and nasal flush qPCR.

g. Review and application of diagnostic tests for mycoplasmal infections. Brown and others (2002) reviewed what was known about mycoplasmal disease, including disease in wildlife species other than the desert and gopher tortoises as of the publication date. They reported that:

    i. It is not clear whether naturally infected tortoises develop a protective immune response from exposure to *Mycoplasma* organisms; limited data suggest this does not occur. Most hosts do not clear mycoplasmal infections; *Mycoplasma agassizii* can cause severe changes in the upper respiratory tract; and different strains of *Mycoplasma agassizii* exist and may differ in virulence; some strains may not cause overt clinical disease.

   ii. The available diagnostic tests for *Mycoplasma* sp. and their applicability for conservation and management of tortoises do not give a complete picture of the type of infection (i.e., acute, chronic, subclinical, carrier) or relative virulence. The tests can be used to determine infection/exposure status, monitor progression of infection, and determine prevalence within populations.
   iii. The classical tests are culture, polymerase chain reaction (PCR) coupled with identification of DNA, and serology (ELISA). Each has advantages and disadvantages and, taken together, can provide complementary information that collectively can be used to define tortoise mycoplasmal status. The use of diagnostic tests depends on the specific goals of the user. Ideally, ELISA, culture, and PCR should be performed for all studies; if this is not possible, the next recommendation is ELISA serology on all animals coupled with culture and PCR on animals exhibiting clinical signs of URTD. If only a single test can be performed, then ELISA is the test of choice because it has a high sensitivity (>90%) and both the Positive Predictive Value and Negative Predictive Value are similar (>85%), meaning that the chance of either false positive or false negative results is similar and relatively low. The sample size needed to detect infection in a population is dependent on prevalence.
   iv. The available diagnostic tests can provide important information to assist in the decision making process related to relocation, repatriation, identification of healthy populations and populations at risk, and management of captive breeding programs.

2. Nasal and cloacal bacteria. Dickinson and others (2001) reported on studies of nasal and cloacal bacteria collected from adult tortoises (total n = 92) at three sites (City Creek and Paradise Canyon, UT; Littlefield, AZ) in the northeastern Mojave Desert between 1989 and 1993. The objectives were to determine differences by site, sex, and season and to determine if it was possible to differentiate between ill and healthy tortoises based on bacterial types. Some tortoises were sampled only once (51%). Bacteria were recorded as present or absent. Of 45 tortoises, 12 had clinical signs of upper respiratory tract disease (URTD). Three species of bacteria were found in the nasal cavity, with one potential pathogen (*Pasteurella testudinis*), *Flavobacterium* spp, and *Staphylococcus* spp. Significantly higher levels of *P. testudinis* were found in ill Mojave tortoises and in September. All tortoises with clinical signs of upper respiratory tract disease from 1992–1994 had positive ELISA and/or PCR results. Two of 17 species of cloacal bacteria were considered opportunistic pathogens (*Pseudomonas* spp, *Salmonella* spp.). The majority (94%) of cloacal bacteria were nonpathogenic *Staphylococcus* spp. Three cloacal bacteria showed site differences (coliforms), 4 showed seasonal differences and 3 showed yearly differences. There were no differences in numbers of cloacal bacteria by sex for ill and healthy tortoises. Complete list of cloacal bacteria: *Campylobacter* spp, *Citrobacter amolonaticus*, *Citrobacter* spp., coliforms, *Corynebacterium* spp. diptheroids, *Enterobacter-Klebsiella*, *Escherichia coli*, *Lactobacillus* spp., *Pasteurella testudinis, Pasteurella* spp, *Pseudomonas* spp., *Salmonella* spp., *Shiegella* spp. [spelling?], *Staphylococcus* spp., *Streptococcus* spp., yeast.

E. Bone and Shell Analysis

1. Osteology. In the first histomorphometric characterization of dermal bone for this species, Wronski and others (1992) compared carapaces from wild adult tortoises from a grazed habitat on Beaver Dam Slope (AZ and UT border) to tortoises in minimally grazed habitat (City Creek, UT), and dermal bones from adult wild tortoises to captive juvenile desert tortoises. Most animals collected from these sites were males. Samples were taken between the 2nd and 3rd marginal scutes. Juveniles were captives from Arizona. The authors concluded that the sample site on scutes was poor for detecting skeletal abnormalities induced by dietary, environmental, or hormonal changes in adult chelonians. They reported the following:
    a. Adult (wild) tortoises had significantly thicker, denser carapaces with decreased osteoid surfaces and relatively thin osteoid seams compared with captive juveniles; bone cells were lacking in dermal bone from most adult tortoises, therefore percentages of osteoblast and osteoclast surfaces could not be measured. In contrast, osteoclasts and osteoblasts were more common but variable in juveniles.
    b. Dermal bone thickness and porosity were nearly identical in adult tortoises from the grazed and ungrazed habitats. However tortoises from the grazed Beaver Dam Slope habitat had a significantly greater osteoid surface (may be evidence of mild osteomalacia) compared to tortoises from the ungrazed habitat. Greater osteoid surface in tortoises from the grazed habitat may be evidence of mild osteomalacia since osteoid increases as a consequence of dietary calcium deficiency or malnutrition.
    c. The organization of dermal bone in the carapace of adult tortoises was identical to that previously described for aquatic turtles; nearly all osteoid surfaces in juvenile tortoises were devoid of adjacent osteoblasts, in marked contrast to mammals.
2. Garner and others (1996) characterized hematopoietic tissue within tortoise bones and shell. The authors reviewed staining and morphologic features of bone marrow hematopoietic cells in ill and healthy tortoises from different parts of the Mojave and Colorado Deserts to determine optimal location for clinical bone marrow sample collection, to determine cytochemical staining characteristics of bone marrow hematopoietic cells, and to describe the morphologic features of bone marrow hematopoietic precursor cells. They provided data on both healthy and ill tortoises. For ill tortoises, see below.

3. Keratin and scutes. Homer and others (2001) analyzed soluble scute proteins of 20 tortoises: 7 ill tortoises with a normal-appearing shell, 6 tortoises with cutaneous dyskeratosis, and 7 healthy tortoises. Analytical techniques included separating proteins using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and analyzing proteins using densitometry, 2D IPG-PAGE and immunoblot analysis. The 14-kd proteins were analyzed for amino acid composition. The major protein components of scutes have amino acid composition and antigenic features of β keratins. Healthy tortoises had multiple 14-, 32-, and 45 kd proteins and proteins of 62 and 68 kd. The 14-, 32-, and 45-kd soluble proteins are β keratins and comprised 90% of total soluble scute protein. The 32 and 45 kd proteins have not been described in other species of reptiles.

4. Elemental analysis of scutes. Seltzer and Berry (2005), using laser ablation inductively coupled plasma mass spectrometery (laser ablation ICP-MS) to assess the utility in measuring differences in elemental content, reported interelement sensitivity factors for S, V, Mn, Fe, Co, Cu, Zn, As, Rb, Sr, Ag, Cd, and Pb. They found consistency in patterns of Zn concentrations and concluded that the technique was measuring endogenous patterns. They further concluded that patterns of Zn concentration might be useful as a map of the scute interior and as a spatial reference for the distribution of other trace elements. The authors compared the laser ablation ICP-MS transects across sections of scutes of one control (healthy tortoise) and three ill tortoises for As; they found that the method was useful in measuring As concentrations in scutes and in relative timing of uptake of As.

F. Urinary Crystal Analysis

Jacobson and others (2009) described techniques to confirm chemical composition of crystals in tortoise kidneys (oxalate crystals), including infrared spectra and a scanning electron microscope equipped with a ThermoNoran energy dispersive X-ray spectroscopy accessory.

G. Ultrasound (Ultrasonography)

Penninck and others (1991) evaluated the anatomic acoustic windows available for gaining information about the echoanatomy of the tortoise. The soft tissue areas of the integument provided three acoustic windows: mediastinal, axillary, and inguinal openings. The mediastinal and axillary windows allowed visualization of heart, liver and gallbladder, and the inguinal window offered images of intestines, urinary/accessory bladders, kidneys, and gonads. These windows provided the opportunity to describe the normal echoanatomy of the desert tortoise. The pancreas and spleen could not be visualized. Ultrasound is simple, fast, noninvasive, and easily repeatable. Small individuals with reduced shell openings cannot be effectively examined. Ultrasound can be used to diagnose some diseases in the tortoise.

H. Vascular Access

Young and others (2012) compared the efficacy of jugular catheterization with four intraosseous catheter sites (humerus, femur, plastocarapacial junction [bridge], and gular region of the plastron) for providing vascular access in captive tortoises. Compared to jugular catheterization, the humerus and femur sites provided the next best vascular access, with 84.4 and 61.8% of activity reaching the systemic circulation by 7 minutes, respectively. The bridge and gular catheter sites were less effective with 41.9 and 40.8% systemic activity, respectively. Intraosseous catheters were no more technically difficult to place than jugular catheters and were less frequently dislodged, making them a viable option for vascular access in tortoises.

I. Necropsies

Necropsies consist of gross and microscopic examination of tissues and ancillary serologic, microbiologic, and toxicologic evaluations (Homer and others, 1998). Berry and Christopher (2001) note that necropsies should be conducted by a veterinary pathologist with formal training, board certification by the American College of Veterinary Pathologists, and experience with reptiles.

III. Results of Field and Laboratory Studies of Ill or Moribund Tortoises

A. Behavioral Abnormalities

1. Starvation and dehydration (Berry and others, 2002b). All 11 tortoises (dead or moribund) salvaged for necropsy displayed ≥1 abnormal behaviors for the time of day, season or year, e.g., delayed entering burrows for hibernation; failed to stay in burrows during hibernation; spent overnight above ground, sometimes for several days at a time when they would be exposed to freezing or near freezing temperatures; failed to drink during rain; were inordinately inactive, sluggish and lethargic, or severely debilitated.
2. An adult male tortoise entrapped for 11 months near Yucca Mountain, NV, showed marked lethargy after being excavated (Christopher, 1999).

B. Abnormalities in Gross Appearance and at Gross Necropsy

1. Starvation and dehydration (Berry and others, 2002b). All 11 tortoises in this necropsy study were emaciated and/or cachectic while still alive in the field. Each of the 11 tortoises had ≥1 other clinical signs, e.g., spines and pleural ribs of juveniles were prominently outlined through translucent scutes; shrunken carapace length; eyes sunken in orbits and often closed, dull and cloudy; clear ocular discharge; nares occluded with dirt and/or dried mucus; wheezing or audible respiratory clicks; flaky, discolored and dull shells; and presence of ectoparasites.
2. An adult male tortoise entrapped for 11 months near Yucca Mountain, NV, exhibited weight loss, sunken eyes, and muscle atrophy (Christopher, 1999).
3. Berry and others (2002b) reported that five juvenile tortoises salvaged for starvation and dehydration had several common attributes at gross necropsy (examples only): bladders contained dark brown flocculent urine and gray sediment, or only gray sediment, or were empty; shells softer and more pliable than normal for age and size; muscle mass about 50% below normal; moderate to severe

segmental thinning of cortical and trabecular bone (osteopenia) of shells and to a lesser extent in legs, spine, and head; skin had more foreign debris than normal in the keratin layer; and multifocal mild to moderate skeletal muscle atrophy and moderate to marked hepatocellular atrophy. Immature and adult tortoises had similar pathologic changes, e.g., atrophied thyroid; no subcutaneous fat adjacent to the proximal end of the humerus; stomach and upper intestine empty with ingesta or digesta in the large intestine or colon; mild to severe diffuse hemosiderosis of liver with hypertrophy of melanomacrophages; multifocal accumulation of bacterial colonies on the surface of airways, interstitial edema; small spleen; bladders with dark brown and flocculent urine and gray sediment; and uroliths.

C. Abnormalities in Weight-Length Relationships, Growth, and Condition Indices

1. In 11 tortoises salvaged and necropsied for starvation and dehydration, Berry and others (2002b) reported that body weights at salvage or immediately prior to salvage were 0 to 38.8% lower than the highest live weight previously recorded; with one exception, weights were outside of the 95% confidence intervals for regression equations of the control groups. The prime body condition indices for salvaged juveniles were 50% of prime.
2. An adult male tortoise entrapped for 11 months near Yucca Mountain, NV, experienced a loss in body weight of only 4.2%, remarkably little after a long period of food and water deprivation (Christopher, 1999).

D. Abnormalities in Blood Analytes

1. Starvation and dehydration (Berry and others, 2002b). Tortoises salvaged for necropsy had ≥1 abnormal values for hematology and/or plasma biochemistry analytes. For hematology, examples included abnormally low packed cell volume and heterophil counts. For plasma biochemicistry, each tortoise had 3 or more abnormal values out of 23 analytes, e.g., hypocalcemia, hyperbilirubinemia, azotemia, and high chloride and sodium levels.
2. Physical and biochemical abnormalities in an entrapped adult male desert tortoise (Christopher, 1999). The tortoise was trapped underground without food or water for nearly 11 months near Yucca Mountain, NV. Biochemical abnormalities included marked azotemia, increased blood urea nitrogen (BUN) and hyperosmolality (attributable to accumulation and retention of nitrogenous wastes); moderate hypercholesterolemia, hypophosphatemia, and increased aspartate transaminase activity; and mild hyperchloremia, hypocalcemia, hyperuricemia, hyperbilirubinemia, and anemia. The tortoise experienced protein catabolism, evidenced by weight loss, muscle wasting, and atrophy. Dehydration was mild.
3. In a study of 108 tortoises at the Desert Tortoise Research Natural Area (DTNA) , Goffs/Fenner Valley, and Ivanpah Valley between 1990 and 1995, 58 tortoises were known survivors at the end of the study; 32 tortoises disappeared and were not relocated (Christopher and others, 2003). Diagnosis of disease in live, free-ranging tortoises by evaluation of clinical signs and laboratory test results is complicated by profound physiological adaptations that have evolved in a fluctuating, hostile environment.

a. Mortality and causes of death. The percentage of tortoises found dead ranged from 12.1% at Ivanpah to 25.7% at Goffs; annualized death rates ranged from 2.4% at Ivanpah to a high of 5.1% at Goffs. Goffs had about two times the crude and annualized mortality rate (combined effect of severe shell disease, culture-positive URTD, and oral lesions) compared with the DTNA and Ivanpah. Upper respiratory tract disease in the DTNA population has evolved from an acute, epidemic disease with high mortality to a chronic disease with variable morbidity, low mortality, and high seroconversion rate for antibodies to *M. agassizii*. Lymphocytosis, heterophilia, and azurophilia in DTNA tortoises were consistent with immunologic responses to infection. Poor correlation between *M. agassizii* culture and serology results suggested that Goffs tortoises may be infected with a different strain or isolate of mycoplasma or one less likely to be detected by the ELISA test. Of the tortoises that died, one at the DTNA had urolithiasis with azotemia and clinical signs of upper respiratory tract disease and one had bladder abscesses; one of the dead DTNA tortoises also had moderate shell disease. One dead tortoise from Ivanpah Valley had urolithiasis. Four of nine dead tortoises from Goffs and three of four Ivanpah tortoises had moderate to severe shell disease.

b. Abnormalities in hematology. Tortoises were observed with lymphocytosis, heterophilia, monocytosis, basophilia, heteropenia, lymphopenia, and basopenia. Tortoises with hematologic abnormalities were significantly more likely to show clinical signs of upper respiratory tract disease compared with tortoises with hematologic abnormalities. No hemoparasites were observed in any tortoise. Some tortoises had slight to marked polychromasia, and one tortoise had marked microcytic, hypochromic, regenerative anemia, heterophila, and basophilia, and was seropositive for *M. agassizii* for 4 consecutive years.

c. Plasma biochemical abnormalities were observed in 56% of tortoises at least once during the study, and included abnormalities in AST, bile acids or bilirubin concentrations, hypomagnesemia, hypophosphatemia, azotemia, hyperchloremia, and elevated blood urea nitrogen (BUN) levels. Tortoises at Goffs were significantly more likely to have increased AST activity and electrolyte abnormalities compared with tortoises at other sites. Compared with hydrated tortoises, dehydrated tortoises had significantly higher BUN, uric acid, total bilirubin, osmolality, sodium and chloride concentrations. Dehydrated tortoises had a greater prevalence of oral lesions and positive nasal cultures for *M. agassizii* but were not more likely to be seropositive for exposure to *M. agassizii*.

d. Sensitivity, specificity, and predictive values were developed for several analytes:

   i. Marked or persistent azotemia had low sensitivity but high specificity for mortality.
   ii. Leukocytosis and heterophilia had low sensitivity but higher specificity for seropositivity.
   iii. High AST had low sensitivity but high specificity for oral lesions. When tortoises with high AST, ALT, or ALP activity were considered together, sensitivity for oral lesions increased to 60% with only a slight decrease in specificity (92%).
   iv. Clinical dehydration and weakness had relatively high sensitivity (80%) for identifying tortoises with oral lesions.
   v. Positive nasal cultures for *M. agassizii* had relatively high positive predictive values for tortoises with moderate to severe shell disease.

E. Hematopoietic Abnormalities

Morphologic features of bone marrow hematopoietic cells in ill and healthy tortoises were compared (Garner and others, 1996). Heterophils were the most abundant hematopoietic cells in the bone marrow of ill and healthy tortoises, and percentages of different hematopoietic cells in ill tortoises were similar to those in healthy tortoises. However, ill tortoises had a mean 24% increase in the number of hematopoietic cells and a mean 88% increase in the total number of erythrocyte precursors in the bone marrow, compared with healthy tortoises. Mean cellularity of bone marrow was 24% higher in ill than in healthy tortoises. Bone marrow became hyperplastic in the tortoise with dermatitis and shell necrosis; both these tortoises had 25% more acidophils and acidophil precursors compared with the mean value for *Mycoplasma*-infected tortoises. The tortoise with acute bacterial pneumonia had hypoplastic bone marrow as well as 25% fewer acidophils and acidophil precursors compared with *Mycoplasma*-infected tortoises. These findings suggest that tortoises are capable of increasing bone marrow production in the face of certain infectious diseases (mycoplasmosis).

F. Abnormalities in Scutes

1. Cutaneous dyskeratosis (see Jacobson and others, 1994; Homer and others, 1998)

2. Soluble scute proteins differed in healthy and ill tortoises (Homer and others, 2001). Twenty tortoises were used in the analysis: six with cutaneous dyskeratosis, seven healthy tortoises, and seven ill tortoises with normal-appearing shells.

   a. Significant differences were found in the relative percentage amount of 14- to 16-kd proteins among control tortoises and tortoises with systemic illness.

   b. At least one protein component (62–64 kd) was not detected in ill tortoises with normal-appearing shells.

   c. In two tortoises with mycoplasmosis, the 45-kd protein reacted weakly with antisera against avian feather.

3. Seltzer and Berry (2005), using laser ablation (ICP-MS, see above) reported patterns of elevated As (arsenic) concentrations in scute tissues of diseased tortoises compared with controls. They compared As concentrations from three diseased tortoises and one control tortoise using laser ablation ICP-MS and conventional ICP-MS (in ppm) and found similar elevated levels. The three ill tortoises had (a) mycoplasmosis; (b) inflammation of chin glands, nasal cavity, eyelids and salivary glands; and (c) cutaneous dyskeratosis and necrotizing epidermitis. The laser ablation transect profile differed by tortoise, e.g., one tortoise had elevated As throughout the transect, including the early portion associated with neonatal areola tissues as well as throughout subsequent years. In another tortoise, the elevations occurred later in the laser ablation transect. The pattern of As in the profiles appears to reflect the timing of uptake. The inhalation of dust and ingestion of contaminated vegetation and soil particles are probably pathways for As intake.

G. Abnormalities in Microbial Evaluations (see also Christopher and others, 2003)

1. Necropsies of starving and dehydrated tortoises revealed the following potential pathogens: *Bordetella bronchiseptica, Pasteurella testudinis*, and *Pseudomonas cepacia*, and α-hemolytic *Streptococcus* sp. (Berry and others, 2002b)

2. Dickinson and others (2001) surveyed nasal and cloacal bacteria of desert tortoises at three sites in the northeastern Mojave Desert of Arizona and Utah. The authors reported significantly higher levels of *Pasteurella testudinis* in ill tortoises; higher levels were found in September. All tortoises with clinical signs of upper respiratory tract disease from 1992 to 1994 had positive ELISA and/or PCR results.

3. Christopher and others (2003), in a study of adult tortoises at three sites in the Mojave Desert (western, eastern, and northeastern regions), reported that 155 of 798 nasal cultures from 67 tortoises (62% of all tortoises) had moderate to heavy growth of *P. testudinis,* which was significantly more prevalent in Ivanpah tortoises (23.7%) than in Goffs (19.4%) or DTNA (14.5%) tortoises. Tortoises at Ivanpah with moderate to heavy *P. testudinis* growth had significantly higher heterophil counts than tortoises at other sites. *P. testudinis* was significantly more likely to occur in spring and winter and less likely in a dry year. Tortoises with moderate to heavy *P. testudinis* growth had significantly higher globulin concentrations.

IV. Identification and Description of Other Illnesses and Non-infectious Diseases

A. Starvation and Dehydration

1. Berry and others (2002b) noted that drought is a frequent occurrence in the Southwestern United States and affects growth rates, reproductive effort, activity, metabolic rates, and hematologic and plasma biochemical values. They reported that 11 wild tortoises (6 moribund, 5 dead) were salvaged for necropsy from three sites in California, one of which was a head-starting pen.

2. Christopher and others (2003), in a 5-year study of adult tortoises at three sites in the Mojave Desert (western, eastern, northeastern), reported laboratory values indicative of temporary or long-term dehydration:
    a. Azotemia was the most common laboratory abnormality observed in the study. In several tortoises at Goffs (eastern Mojave region), azotemia was likely evidence of physiologic extremes rather than disease because it occurred in multiple tortoises during dry years or seasons. Nevertheless, azotemia also can be an indication of lack of water due to urolithiasis or renal disease. Four of nine tortoises with severe or persistent azotemia died (three of four had necropsy evidence of urolithiasis or bladder infection).
    b. In response to periodic drought, there were also sharp changes in electrolyte, osmolality, blood-urea nitrogen (BUN) and anion gap values. Other laboratory abnormalities include anemia, heteropenia, hyperbilirubinemia, hyperosmolality, hypernatremia, hyperchloremia, hypocalcemia, and hypophosphatemia.
    c. Two tortoises with urolithiasis had high BUN values in the season preceding death. Both tortoises had had multiple incidences of mild but worsening azotemia in earlier seasons.
    d. Hypophosphatemia was likely due to decreased food intake and was usually observed in winter following drought.

B. Pneumonia. Three of 24 wild tortoises submitted for necropsy between 1992 and 1995 were diagnosed with bacterial or fungal pneumonia (Homer and others, 1998).

C. Cutaneous Dyskeratosis

1. A shell disease, prevalent in a population of tortoises on the Chuckwalla Bench, CA, was associated with very high mortality. The disease was described as cutaneous dyskeratosis (Jacobson and others, 1994) and characterized by discoloration (white, gray, orange), and dry, roughened, and extremely flaky scutes. The lesions appeared to commence at seams between scutes and spread outward to the middle of each scute. The disease was present on the carapace, plastron, and forelimb scutes, with the plastron more severely affected. There was loss of normal integrity of the horny material covering the scutes. In a retrospective evaluation of 35-mm color slides, the percentage of tortoises with shell lesions increased between 1979 and 1990: 56% in 1979, 68% in 1982, 90% in 1988, and 87% in 1990. A variety of aerobic bacteria and one fungus were cultured from a biopsy specimen. These organisms were considered secondary invaders and had little to do with the disease. The lesions may have been a peripheral manifestation of a more central disease (based on hematologic and plasma biochemical values). These findings suggest either a nutritional deficiency or a toxicosis, however, the exact cause of the disease could not be determined.

2. Homer and others (1998) reported that 7 of 24 wild tortoises submitted for necropsy between 1992 and 1995 exhibited lesions consistent with cutaneous dyskeratosis. Cutaneous dyskeratosis appears to contribute to or cause thinning of scutes and dermal bone, thereby rendering the tortoise more vulnerable to other diseases such as fungal infections and multicentric visceral inflammation.

3. Christopher and others (2003), in a 1990–1995 study of disease abnormalities in desert tortoises at three sites in the Mojave Desert, noted that four of nine tortoises at the Goffs, CA, site and three of four tortoises at the Ivanpah Valley site that died had moderate to severe shell disease consistent with cutaneous dyskeratosis. These shell lesions were observed in 85.4% of tortoises and were significantly more severe with increasing tortoise age. Significantly more Goffs tortoises had moderate to severe plastron lesions. Hyperglobulinemia, positive *Mycoplasma agassizii* cultures, and oral lesions—all seen primarily at the Goffs site—were significantly associated with this shell disease. There was a significant increase in the number of tortoises with moderate to severe plastron disease over time and the number of tortoises with active carapace lesions increased.

4. Berry and others (2006a), in a study of tortoises at 15 sites at Goldstone and 6 at the National Training Center, Fort Irwin, CA, reported that 10.9% of 182 tortoises showed signs of shell diseases and differences between plots were statistically significant. Goldstone plots had higher numbers and the Soda Mountains plot had lower numbers of tortoises with moderate to severe signs of shell disease than the other plots. Significantly more shell disease occurred on plots being used currently or recently for military maneuvers than on the plot with no history of military use. The higher mounts of surface disturbance, vehicle use, trash, debris from firearms and ordnance, and possibly dust in the military maneuver areas may be sources of potential toxicants or other stressors that contribute to shell disease.

5. Berry and others (2008), in a survey of a about 4 km^2 plot and 37 one-ha landscape plots in Red Rock Canyon, CA, found and evaluated nine live tortoises. One old adult male tortoise had active lesions typical of cutaneous dyskeratosis on <10% of the plastron and an immature tortoise had vermiculations under the carapacial scutes, advanced wear on scute laminae and depressions in several scutes.

6. Berry and others (2013), in a survey of tortoises and their habitats in the northwestern Mojave Desert, CA, at the Naval Air Weapons Station, China Lake, evaluated 28 tortoises for health. Twenty-four of the 28 tortoises had signs of active cutaneous dyskeratosis on and between the scales of the limbs covering up to 40% of the surface. For all but one tortoise, severity of the lesions was rated as mild. Ten tortoises also had mild, active signs of cutaneous dyskeratosis on either plastron or carapace or both.

D. Other Diseases of the Shell and Integument

1. Jacobson (1994) reported that captive desert tortoises in a private Arizona collection had hyperkeratotic skin lesions at multiple soft tissue sites. An organism resembling *Dermatophilus* was observed but microbial isolation attempts were negative and the identity remains unknown.

2. Homer and others (1998) reported a case of fungal dermatitis in an entombed wild desert tortoise and noted that it likely would have resulted in death of the tortoise.

3. Homer and others (1998) reported that 2 of 24 wild tortoises submitted for necropsy between 1992 and 1995 had shell necrosis, with several scutes detached, revealing discolored dermal bone.

E. Renal, Bladder, and Urinary Tract Diseases

1. Renal oxalosis, a new disease of wild desert tortoises described by (Jacobson and others, 2009), was found in a necropsied tortoise with compromised renal function and subsequently in a retrospective histologic analysis of the histology of 66 necropsied wild tortoises from the Mojave and Colorado Deserts. The initial tortoise was lethargic and had high blood urea nitrogen (BUN), uric acid, sodium and chloride levels; renal tubules were obstructed or obliterated with calcium oxalate crystals and similar crystals were present within the thyroid gland. Minimal amounts of calcium oxalate crystals were observed in thyroid glands (81% of tortoises) and kidneys (5% of tortoises) evaluated retrospectively. The source of calcium oxalate may be from plants.

2. Renal and articular gout. Homer and others (1998) reported that 1 of 24 wild tortoises submitted for necropsy between 1992 and 1995 had renal and articular gout; this tortoise was experiencing starvation and dehydration (Berry and others, 2002b). In a different study, Jacobson and Berry (2012) reported that 1 of 11 wild tortoises submitted for necropsy from the central Mojave Desert in 2007–2008 had polyarticular and visceral gout.

3. Urolithiasis (cystic calculi)

    a. Jacobson (1994) reported cystic calculi in several wild desert tortoises (as a personal observation).

    b. Homer and others (1998) reported that 3 of wild 24 tortoises submitted for necropsies between 1992 and 1995 had uroliths.

    c. Berry and others (2002b) reported that 3 of 11 tortoises submitted for necropsy because of starvation and dehydration had uroliths (composed of ammonium acid urate, uric acid salts, protein, and hydroxyl apatite).

    d. Christopher and others (2003), in a 1990–1995 study of tortoises with disease abnormalities at three sites in the Mojave Desert, CA, reported that two of five tortoises that died at the Desert Tortoise Natural Area had urolithiasis with azotemia and clinical signs of upper respiratory tract disease.

F. Parasites

1. Petter and Douglass (1976; cited in Jacobson, 1994) identified 15 species and subspecies of oxyurids in a study of gopher, Bolson, and desert tortoises: *Tachygometria macrolaimus tetrapapillata, T. dentata nearctica, Thaparia macrocephala, Thaparia microcephala, Alaeuris mazzottii, A. paramazzottii, A. gopheri gopheri, A. gopheri pudica, A. gopheri macrolabiata, A. caballeroi, A. kinsellai kinsellai, A. kinsellai sonorae, A. longicollis, Oxyuris* sp., and *Gopheruris aspicula*.

2. Jacobson (1994) reported an unidentified trombiculid mite on a desert tortoise in southwestern Utah.

3. Jacobson (1994) reported that the most frequent ticks seen are the argasid ticks, *Ornithodoros parkeri* and *O. turicata*; only *O. parkeri* appears to parasitize free-living desert tortoises in California.

4. Homer and others (1998) reported tissue cysts of *Sarcocystis*-like protozoa in skeletal muscles of 6 of wild 24 tortoises submitted for necropsy between 1992 and 1995.
5. Berry and others (2002b) reported deaths of *Gopherus agassizii* from starvation and dehydration from several sites in California. Parasitic analyses were completed for 4 of 11 wild tortoises submitted for necropsy because of starvation and dehydration: 2 juveniles had mild pinworm endoparasitism and a third had moderate pinworm endoparasitism and a few balantidium-like protozoa in the colon.
6. Christopher and others (2003), in a 1990–1995 study of disease abnormalities of tortoises at three sites in the Mojave Desert, reported that ticks (*Ornithodorus* sp.) were significantly more likely to be observed on tortoises that had oral lesions in the previous year.

G. Toxicosis

1. Jacobson and others (1991). In a study of desert tortoises at the Desert Tortoise Research Natural Area, mercury concentrations in livers of tortoises with upper respiratory tract disease (0.326 ppm) were significantly higher than in livers of healthy tortoises (0.0287 ppm).
2. Jacobson and others (1994) suggested that a toxicosis might be responsible for the lesions (cutaneous dyskeratosis) observed in desert tortoises from the Chuckwalla Bench, CA, but the cause of the lesions could not be determined.
3. Seltzer and Berry (2005) in a study of exogenous and endogenous composition of desert tortoise scutes, tested four specimens from necropsied tortoises (Homer and others, 1998), one healthy control and three diseased tortoises. Using a laser ablation technique on the lateral surface of scutes to avoid exogenous contamination, they reported elevated levels of arsenic for the three diseased tortoises. They compared their results with data from ICP-MS analyses and reported similarities.
4. Chaffee and Berry (2006) conducted a baseline and background chemical survey of soil, stream-sediments, and plants in the Mojave and Colorado Deserts. There were distinct variations from locality to locality that were generally consistent with the parent materials. However, some sources of anomalies were clearly anthropogenic and probably represented surface contamination of dust redistributed by wind, vehicles, and rainfall.
    a. In two mining districts in the western Mojave, soil anomalies for As, Au, Cd, Hg, Sb, and W and plant anomalies for As, Sb, and W extended as far as about 15 km from the present mining area; soils containing Hg were found at least 6 km away from old piles of tailings. The anomalous concentrations of As and Hg may have been the source of elevated levels of these elements in ill tortoises from the region.

b. One of two study areas transected by a paved road (Chemehuevi Valley, Colorado Desert) showed weakly elevated levels of Pb, which extended as far as about 22 m from the pavement edge and were probably related to vehicle exhaust.

c. No historically used military areas contained anomalous concentrations of the elements As, Cu, Cr, Fe, Pb, or Zn that could be ascribed to military maneuvers, vehicles or ordnance.

H. Nutritional Requirements, Deficiencies, and Health

1. Jacobson and others (1991). In a study of wild desert tortoises with upper respiratory tract disease and control tortoises, concentrations of serum and liver vitamin A were not significantly different.

2. Jacobson (1994) reported that osteopenia, which included thinning of trabeculae of the peripherals and lateral areas of the hypoplastron that produced a pronounced spongy appearance, was evident in an analysis of skeletal remains (shell bone) of 24 carcasses examined in 1987. The remains were from Utah and northwestern Arizona and displayed obvious signs of shell bone thinning. Malnutrition was considered responsible or the osteologic lesions observed in tortoises from the Beaver Dam Slope. Nutrient availability had declined and was considered a likely cause of recent tortoise mortality at the area.

3. Nutritional deficiencies may have contributed to the cutaneous dyskeratosis observed in desert tortoises on the Chuckwalla Bench; however, the cause of the disease was not determined (Jacobson and others, 1994; Homer and others, 1998).

4. Nagy and others (1998) conducted experimental studies on the nutritional quality of native and introduced food plants of wild desert tortoises, specifically the native forb, *Malacothrix glabrata* and the native perennial grass, *Achnatherum hymenoides,* and the non-native annual grass, *Schismus barbatus*, and non-native forb, *Erodium cicutarium*. The wild tortoises ranged from juvenile to adults and did not have upper respiratory tract disease. Digestibilities of nutrients in the two forbs were similar for dry matter, energy, nitrogen, and water; which forb was the more nutritious depended on what determined feeding rate (full stomach vs. digestible energy). The dry matter and energy digestibilities of the two grasses were similar but much lower than for the forbs. Both grasses provided little or no nitrogen and the tortoises lost more water than they gained while processing grasses. The type of food plant (forb or grass) and phenological stage rather than native or non-native origin best predicted the nutritional value to tortoises.

5. Hazard and others (2009, 2010), in an experimental research project on the nutritional quality of foods of desert tortoises, compared a native perennial grass species (*Achnatherum hymenoides*) and a native annual forb (*Malacothrix glabrata*) with two non-native forage species, an annual grass (*Schismus barbatus*) and an annual forb (*Erodium cicutarium*).

a. In Hazard and others (2009), the experiments were conducted on growing juvenile desert tortoises and the focus was on digestibility of dry matter, energy, fiber and nitrogen in the four foods. The two grass diets were higher in fiber content and contained less digestible energy than the two forb diets. The grasses contained little protein, and the juveniles actually lost mass and body nitrogen while eating them. The exotic forb yielded more energy and nitrogen per unit dry mass than did the native forb, but this may be related to differences in phenological stages and associated fiber contents of these foods when they were collected. Juvenile tortoises gained weight rapidly when eating forbs and showed no evidence of having a lower digestive capability than did adults, despite their small size and immaturity. Estimates of nitrogen requirements compared to annual N intake on these diets suggested that growth of juveniles may be limited in part by dietary nitrogen. An interesting point: 2 of 10 tortoises offered *S. barbatus* became ill and died early in the study and 2 others refused to eat and were excluded from the experiment. No other animals refused to eat or had to be removed from the project. Also, juveniles lost phosphorus on dried grasses and gained other nutrients (calcium, magnesium) only at low rates.

b. In Hazard and others (2010), juveniles were again used in the experiments and fed one of the four species of plants. In addition, data were presented for adults eating the non-native forb and grass species. Overall, tortoises gained more minerals from forbs than from grasses. Tortoises lost small but significant amounts of P when eating grasses, which may have contributed to nutritional differences between native and exotic forbs or between native and exotic grasses. There were few nutritional differences between native and exotic forbs or between native and exotic grasses. Comparisons of nutrient availability to estimated requirements for growth by juveniles and for egg production by adult females suggest that P is more limiting than Ca or Mg and that Ca may pose a significant osmotic challenge for excretion.

I. Handling and Research Manipulation

1. Berry and others (2002b). Wild tortoises salvaged dead from two sites were previously handled frequently and experienced invasive procedures. At one site (head-starting, Fort Irwin), the authors suggest that crowding and deteriorated habitat contributed to low growth rates, low weights, and deaths of juveniles. In addition, an adult tortoise, part of a research program in which 41% of 22 adult tortoises died, was often transported distances of a few kilometers for sampling; blood and urine were collected almost monthly using jugular phlebotomy and cystocentesis, respectively. This tortoise had severe peritonitis and multiple bladder wall abscesses, presumably related to previous cystocentesis done as part of a research study (Christopher and others, 2003, referring to Peterson, 1994). Cystocentesis was subsequently 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.

2. Agha and others (2015d) studied effects of research activities and winter precipitation on voiding behavior in *Gopherus agassizii* at a wind generation facility in Riverside County, CA, for 10 study seasons over a period of 18 years. They modeled effects of research manipulation, winter precipitation, voiding on long-term apparent survivorship. Voiding occurred on 8.2% of occasions; top models indicated that increases in handling time led to higher probabilities of voiding for juveniles, females, and males. Increases in precipitation also resulted in higher probabilities of voiding for juveniles and females but not for males. Their models demonstrated a weak effect on winter precipitation on survivorship but a negligible effect for both voiding behavior and sex.

J. Other Indicators of Disease in Wild Tortoises. Chronically ill tortoises had a history of weight loss and were emaciated at the time of collection: weight loss was an excellent indicator of chronic disease and was often associated with muscle, pancreatic and/or hepatic atrophy; liver degeneration; hepatocellular anisokaryosis; hemosiderosis; degenerative skeletal muscle changes; shrunken pancreatic acinar cells; and multi-centric inflammation of multiple coelomic organs (Homer and others, 1998).

V. Infectious Diseases

A book, *Infectious Diseases and Pathology of Reptiles: Color Atlas and Text* (Jacobson, 2007), is a comprehensive work with 13 chapters, 39 examples of data or studies on *Gopherus agassizii*, and 33 examples on the closely related *G. polyphemus*. Subject matter includes biology, anatomy, histology, immunology, circulating inflammatory cells, necropsy techniques, host response to infectious agents and identification of pathogens in tissue sections, use of the electron microscope, molecular diagnostics, serodiagnostics, viruses and viral diseases, bacterial diseases, mycotic diseases, parasites and parasitic diseases, and isolation of pathogens. Some material supplements previously published work and some data are new and previously unpublished. It is useful to scientists and veterinarians exploring illness or recent death in reptiles, including tortoises, through use of extensive diagrams and illustrations.

A. Mycoplasmosis. Species of *Mycoplasma* in tortoises. Jacobson and others (2014), in a review of upper respiratory tract disease of tortoises, reported that three species of *Mycoplasma* are known in *Gopherus agassizii*: *M. agassizii, M. testudineum*, and a new, unidentified species reported by Wellehan and others *Mycoplasma testudinis* has been isolated from a Greek tortoise but not from *G. agassizii*. Mycoplasmas have been identified in wild and captive tortoises in Europe and in the United Kingdom. On the basis of 16S rRNA sequences, Brown and others (1995) determined the phylogenetic placement of mycoplasmas cultured from the nares of desert tortoises diagnosed with upper respiratory tract disease. *Mycoplasma agassizii* was placed in the *M. hyorhinis* group and *M. testudinis* was placed in the *M. pneumonia* group. A third, previously unrecognized tortoise mycoplasma was detected and positioned in the *M. fermentans* group. The authors also developed a more rapid and sensitive method to detect exposure to *M. agassizii*, using a 16S rRNA gene PCR-based diagnostic test that complements the time intensive enzyme-linked immunosorbent assays previously used to detect host exposure.

1. *Mycoplasma agassizii*
    - a. Identification of the pathogen causing upper respiratory tract disease in *Gopherus agassizii*; clinical signs of disease; results of necropsies. Jacobson and others (1991) undertook a research project to determine the cause of upper respiratory tract disease in desert tortoises, using 17 ill tortoises from in and around the Desert Tortoise Natural Area, CA, and one from St. George, UT; four healthy, control tortoises came from Ivanpah Valley. They conducted hematologic and serum biochemical evaluations, pathologic and microbial investigations, and liver and metal determinations and concluded that *Mycoplasma* sp. might be the causative pathogen. They reported that this is a disease primarily of the upper respiratory tract, primarily affecting the nasal cavity (see necropsy findings under Necropsy section). Brown and others (2001) described this new species of mycoplasma, *Mycoplasma agassizii*, with type strain PS6^T (=ATCC 7000616^T) that caused upper respiratory tract disease in experimentally infected tortoises collected from the Mojave Desert.
    - b. Clinical signs of disease and evidence of subclinical disease
        - i. *Mycoplasma agassizii*: clinical signs of disease. Jacobson and others (1991) reported rhinitis characterized by intermittent serous discharge flowing or bubbling from nares, sometimes wiped by tortoises using the cranial surface of their forelimbs. Nares may appear dry on some days. As disease progresses, the discharge becomes more tenacious; nares may become occluded. Severely affected tortoises may be listless and anorectic; have a dull integument and palpebral edema and globes may be recessed into the orbits, indicating dehydration.
        - ii. Brown and others (1994) reported that in some tortoises, antibodies to *M. agassizii* may develop before any clinical signs of disease are observed.
        - iii. Jacobson and others (1995) evaluated 12 clinically healthy tortoises and 12 tortoises with signs of upper respiratory tract disease by clinical signs, serology, and pathology (necropsies). They developed criteria to classify lesions in the upper respiratory tract. Eight of 12 tortoises submitted as clinically healthy had lesions in the upper respiratory tract consistent with upper respiratory tract disease; of the 12, 5 were ELISA positive and 6 were culture positive with a total of 8 that were either culture or ELISA positive. The presence of lesions indicated that subclinical disease exists and that determining health status of tortoises requires more sophisticated approaches than clinical appearance alone. Conclusions: chronically infected animals serve as reservoirs, allowing transmission to naïve tortoises; because subclinical disease is present, determining health status of a tortoise by physical clinical examination alone is impossible. *Pasteurella testudinis* may contribute to the severity of the disease.

iv. Schumacher and others (1997) evaluated the relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* (ELISA test) in 144 free-ranging desert tortoises from Nevada. Presence of clinical signs of upper respiratory tract disease was positively related to positive ELISA results regardless of sex or age of the tortoise: 84% of tortoises with clinical signs tested seropositive. Mucous nasal discharge, the most severe and obvious of clinical signs, was highly predictive for exposure to *M. agassizii* based on ELISA: 93% of tortoises with mucous discharge tested seropositive. The authors detected subclinical infection (seropositive using ELISA) in 34% of 99 animals without clinical signs. They also noted that 7 tortoises had clinical signs but were seronegative (9.7% of all seronegative tortoises). In some tortoises, the appearance of clinical signs may precede production of detectable levels of *M. agassizii* antibodies. Tortoises without clinical signs but seropositive for *M. agassizii* may be silent carriers and act as reservoirs for the pathogen.

v. Brown and others (1999) reported on relationships between serological tests (ELISA) for *Mycoplasma agassizii* and clinical signs of disease in a 4-year study conducted at the Desert Tortoise Natural Area between 1992 and 1995. Findings suggested that (i) animals may remain ELISA positive without showing overt disease, a clinical pattern consistent with the chronic nature of most mycoplasmal infections (e.g., the percentage of animals that were ELISA positive yet free of clinical signs remained fairly constant (about 30%) until 1995, when it dropped to 4%; and (ii) clinical expression of the disease may be cyclical. See Brown and others (1999) below for more details.

vi. Dehydration. Christopher and others (2003), in a study of disease abnormalities in desert tortoises at three sites in the Mojave Desert, reported that one tortoise seropositive for *Mycoplasma agassizii* was dehydrated and had hypoglycemia, a low basophil count, and lymphopenia as well as general signs of illness, including poor body condition, lethargy and weakness. This tortoise subsequently disappeared. Additional tortoises were clinically dehydrated as evidenced by decreased skin elasticity or sunken eyes and most had clinical signs of upper respiratory tract disease. Clinical signs of upper respiratory tract disease were seen more often in 1990–1991 and again in 1994–1995 compared with other years; both of these years were normal to wet years following a year of below-normal rainfall. Clinical signs were significantly more likely to be observed at the time of emergence from hibernation in late winter and least likely to be seen in summer.

vii. Presence of oral lesions (see also section on herpesvirus). Christopher and others (2003, see above, also) noted that eight tortoises at one site and two at another had oral lesions in 1992–1993 (ulcerated or covered with whitish-yellow plaques). The tortoises either had concurrent signs of upper respiratory tract disease or signs at other sampling period; tortoises with oral lesions were significantly more likely to have positive nasal cultures for *Mycoplasma agassizii*.

c. Transmission of infection, tests fulfilling Koch’s postulates.

    i. Experimental Transmission. *Mycoplasma agassizii* causes upper respiratory tract disease (Brown and others, 1994). The authors demonstrated that a new species of *Mycoplasma*, proposed to be named *M. agassizii*, causes upper respiratory tract disease in desert tortoises through field and laboratory tests of five groups of clinically healthy animals with known antibody status. Tortoises challenged with *M. agassizii* alone or in combination with *Pasteurella testudinis* were significantly more likely to develop clinical disease than animals receiving *P. testudinis* alone or broth controls. These tortoises demonstrated a strong immune response to *M. agassizii*, seroconversion in all groups with clinical disease, and *M. agassizii* was isolated from the upper respiratory tracts. Antibody levels increased at 1 month following nasal inoculation with exudate from a tortoise having upper respiratory tract disease and up to 3 months following nasal inoculation of tortoises challenged with *M. agassizii* or a combination of *M. agassizii* and *Pasteurella testudinis*. Lesions in the upper respiratory tract were observed in all tortoises receiving exudate, in tortoises receiving *M. agassizii* alone, and in the group receiving both *M. agassizii* and *P. testudinis*. Lesions were not observed in controls.

    ii. Brown and others (2002) reported that under experimental conditions, the onset of clinical signs is as early as 2 weeks post-inoculation. Seroconversion lagged behind clinical signs with reliable detection of antibodies by 8 weeks post-inoculation (see Jacobson and others, 2014).

    iii. Natural transmission of *M. agassizii*: most likely method is direct contact and aerosol transmission (Brown and others, 1994). Transmission is more likely to occur when infected tortoises exhibit clinical signs; however tortoises with subclinical infections may be able to transmit *Mycoplasma* spp. under certain conditions (Jacobson and others, 1995, 2014).

    iv. Vertical transmission cannot be ruled out (Brown and others, 2002; Jacobson and others, 2014)

d. Serological tests for diagnosing mycoplasmal infections: Development and refinement of the ELISA (enzyme-linked immunosorbent assay) for detecting antibodies to *Mycoplasma agassizii* (see II.A.5., Diagnostic Tests)

e. Prevalence of clinical signs of upper respiratory tract disease in tortoise populations (no serology or cultures undertaken in these samples)

    i. Peterson (1994) monitored 33 radiotelemetered tortoises at the Desert Tortoise Natural Area in the western Mojave Desert. He attributed mortality of 20 tortoises to predation; however some of the high mortality could have been attributable to upper respiratory tract disease, as 3 symptomatic animals were removed from the site and 1 of the 17 tortoises monitored had signs of disease (nasal exudate).

  - ii. Berry and others (2008), conducted surveys for desert tortoises on a 4 km^2 plot and 37 one-ha plots in Red Rock Canyon State Park, CA, between 2002 and 2004. They found nine live tortoises all of which showed ≥1 mild to moderate clinical signs associated with upper respiratory tract disease or other diseases, e.g., mild swelling of the eyelids, wet lids, discharge from eyes, deposits of dried mucus or discoloration of the lids or periocular area or both, dirt on the beak, or occluded nares.
  - iii. Keith and others (2008) conducted a survey of the Jawbone-Butterbredt Area of Critical Environmental Concern and the adjacent Red Rock Canyon State Park, CA, between 2002 and 2004, using 751 one-ha plots. They reported finding five tortoises and evaluated four for health. Four were old adults and three had clinical signs of upper respiratory tract disease.
  - iv. Berry and others (2013), in a study of tortoises in the northwestern Mojave Desert at the Naval Air Weapons Station, China Lake, CA, evaluated 28 tortoises. None had wet nares or a purulent discharge typical of mycoplasmosis caused by *Mycoplasma agassizii* or *M. testudineum*. Some clinical signs were suggestive of mycoplasmosis, however: 11 tortoises had mild to moderate occlusions of one or both naris; 12 tortoises had moderate to severe edema of the palpebrae and/or periocular area; 7 had dried or wet mucus crusts on the palpebrae; and globes of 6 tortoises were bulging. Ocular signs may have been the result of abundant forage and plant sap.
  - v. Berry and others (2014a) surveyed tortoises in the western Mojave, CA, and evaluated three strategies for managing tortoises in three adjoining areas: the Desert Tortoise Research Natural Area, adjacent private lands, and adjacent critical habitat. The authors evaluated 34 tortoises for health: 2 had moderate clinical signs that were consistent with mycoplasmosis; the nares of 19 other tortoises were partially or completely occluded, potentially from dried exudate or from plant sap or dirt and mud from drinking during rainstorms.

- f. Epidemiology of upper respiratory tract disease caused by *Mycoplasma agassizii*: Evidence from clinical signs and serology and in some cases, cultures and necropsies
  - i. Lederle and others (1997) sampled 105 tortoises >140 mm carapace length using jugular blood at Yucca Mountain, NV, four times between September 1993 and September 1995, each time in either June or September. Sampling occurred during periods of average or above average rainfall. They reported that the likelihood of testing positive for mycoplasmal antibodies using ELISA did not differ between sexes; 19% of pooled data for 283 samples were positive. The percentage of seropositive samples ranged from 15 to 23%. Clinical signs were observed once in seven individuals (2%) and were minimal (slight nasal discharge, but no ocular discharge, palpebral edema, or conjunctivitis).

Change in antibody category status occurred in nearly 50% of tortoises that were tested two or more times.

ii. Clinical signs, serology and cultures. Brown and others (1999) monitored 35 tortoises in the Desert Tortoise Natural Area, CA between 1992 and 1995 using ELISA test and clinical signs of disease. Ten tortoises were monitored every year over a 4-year period. Specific antibody levels to *M. agassizii* were not influenced by sex of the tortoise. Levels of antibody and the distribution of positive, negative, and suspect animals classified with ELISA were also not consistently affected by season within years or by season among years. The ocular signs most commonly observed were swollen eyelids, wet eyelids indicative of an ocular discharge or mucus in the eyes, and wet or occluded nares. In 1992, only 16% of tortoises had clinical signs consistent with upper respiratory tract disease (URTD) at one or more sampling times, but by 1995, 76% of tortoises had clinical signs consistent with URTD at one or more sampling times. Significantly more tortoises presented with clinical signs in 1992 and 1995 compared with 1993 and 1994. The profile of animals that both tested positive with ELISA and showed clinical signs of URTD increased from 5% in 1992 to 42% in 1995. Conversely, the profile of animals that both tested negative with ELISA and showed no URTD clinical signs decreased from 52% in 1992 to 19% in 1995. These results suggest that URTD was not only present in this population as evidenced by animals that tested positive with ELISA, but also that the infectious agent was still present as evidenced by seroconversion of animals that previously tested negative with ELISA during the study. This study produced evidence suggesting that (a) animals may remain ELISA positive without showing overt disease, a clinical pattern consistent with the chronic nature of most mycoplasmal infections (e.g., the percentage of animals that were ELISA positive yet free of clinical signs remained fairly constant (about 30%) until 1995, when it dropped to 4%; and (b) clinical expression of the disease may be cyclical.

iii. Wallis and others (1999), in a study of annual egg production in tortoises from the Desert Tortoise Research Natural Area, Mojave Desert, CA, found that antibody status had no significant effect on any reproductive parameter including egg number, egg size, or time of appearance of shelled eggs in the oviduct (n = 16 seropositive out of a sample of 29 tortoises).

iv. Clinical signs, serology, and cultures. Christopher and others (2003), in a 1990–1995 study of tortoises at three sites (Desert Tortoise Natural Area, Goffs, Ivanpah) in the Mojave Desert, reported that tortoises at the Desert Tortoise Natural Area were significantly more likely to be seropositive for *Mycoplasma agassizii* than tortoises at Goffs and that 78% of seropositive and 75% of seronegative tortoises had clinical signs of upper respiratory tract disease. The percentage of tortoises with positive cultures for *M. agassizii* varied by site: 0% at the Desert Tortoise Natural Area, 39.4% at Goffs, and 28.1% at Ivanpah. Fifteen of

22 tortoises (68%) with positive nasal cultures for *M. agassizii* were seronegative for *M. agassizii*. The number of tortoises with positive cultures more than doubled between the beginning of the study and 1993–1994.

v. Dickinson and others (2005) reported on upper respiratory tract disease caused by *Mycoplasma agassizii* at three sites (City Creek and Paradise Canyon, UT; Littlefield, AZ) in the northeastern Mojave Desert. They sampled 19 adult males and 9 adult females three times per year in 1992 and 1993 but not all tortoises each time: Nine tortoises had clinical signs of upper respiratory tract disease (nasal or ocular mucous discharge, occluded nares, conjunctivitis or palpebral edema) and eight were seropositive for *M. agassizii*. Of the 68 ELISA samples, 26% were seropositive. There was 89% correlation (eight of nine) between clinical signs and positive serologic results.

vi. Berry and others (2006a) conducted a study of tortoises at 15 sites on Goldstone and 6 sites at the National Training Center, Fort Irwin, CA between 1997 and 2002. They reported that 34.6% of tortoises (63/182) had clinical signs of upper respiratory tract disease; more tortoises at Goldstone had moderate to severe signs than other plots (52.9%; 9/17). Ninety-one of 182 tortoises (50%) were tested for *Mycoplasma agassizii* using the ELISA test, cultures, and PCR. Only 2 of the 91 tortoises were positive (Goldstone tortoises); one also had a positive culture. The differences among plots were significant for clinical signs of upper respiratory tract disease. Mycoplasmosis and tortoise densities were negatively correlated and mycoplasmosis and death rates were positively correlated. Mycoplasmosis was also negatively correlated with distances from Fort Irwin offices, the cantonment (housing area), and paved roads.

vii. Berry and others (2014a) evaluated 34 tortoises for health in a study of tortoises occupying three contiguous areas with different land-use histories and land management strategies in the western Mojave Desert (Desert Tortoise Research Natural Area, adjacent portion of Fremont-Kramer critical habitat, and private lands recently acquired for conservation). Of the 34 tortoises, 2 adult males had moderate clinical signs of upper respiratory tract disease (damp/wet beak from exudate or bubbles) and the nares of 19 other tortoises were partially or completely occluded, potentially from dried exudate, plant sap, or dirt.

g. Epidemiological models of mycoplasmosis in wild populations

i. Sandmeier and others (2013), in a study of intrinsic and extrinsic factors affecting seroprevalence of *Mycoplasma agassizii* in 24 populations of *Gopherus agassizii* across the geographic range, used blood samples collected by a combination of toe-clipping, brachial and subcarapacial venipuncture. Western blots were used to measure exposure to *M. agassizii* via production of induced antibodies and seroconversion as per Hunter and others (2008). When samples were negative by Western blot or seronegative, a polyclonal ELISA was used to quantify relative levels of Nabs capable of binding to *M. agassizii* (Hunter and others, 2008).

The model variables were: proportion of population with signs of upper respiratory tract disease (URTD), seroprevalence, genetic identity, mean levels of natural antibody in a population, mean winter rainfall and annual freezing days. The authors reported that: (a) Within each population, the proportion of tortoises with signs of URTD ranged from 0 to 22.2% and seroprevalence from 0 to 73.3%. (b) Seroprevalence was best predicted by mean annual days below freezing but natural antibody and genotype group may be involved. (c) A population's mean natural antibody level was predicted best by genotype group.

ii. Berry and others (2015a) explored variables likely to affect presence of *Mycoplasma agassizii* and *M. testudineum* in 1,004 tortoises in a 1,183 km^2 study area in the central Mojave Desert, CA, between 2005 and 2008. They collected primarily subcarapacial blood samples which were analyzed using the ELISAs developed, refined, and validated at the University of Florida. They used covariates of habitat (vegetation, elevation, slope, and aspect), tortoise size and sex, distance from another test-positive tortoise, and anthropogenic variables (distances to roads, agricultural areas, playas, urban areas, and centroids of human-populated census blocks). They reported that (i) The prevalence of test-positive tortoises was low: 1.49% for *M. agassizii* and 2.89% for *M. testudineum*. (2) The spatial distributions of tortoises that were test positive for both mycoplasmas overlapped very little. (3) For both *M. agassizii* and *M. testudineum*, there was higher prevalence of test-positive tortoises with shorter distances to centroids of human-populated census blocks. For *M. agassizii*, other covariates of lesser importance included distances to urban edge, tertiary road, and test-positive tortoises. (4) The relationship between close proximity to human-populated census blocks and test-positive tortoises may be related to release or escape of captive tortoises, because prevalence of *M. agassizii* in captive tortoises is high.

h. Persistence of maternal antibody to *Mycoplasma agassizii*. Schumacher and others (1999) studied persistence of *M. agassizii* maternal antibodies in 43 captive-reared *G. agassizii* hatchlings using yolk, hatchling plasma, egg shell membranes, and nasal lavage fluid; studies on persistence of maternal antibody ranged from hatching to 29 months. The authors used hatchlings from adult females that were seropositive and seronegative for *M. agassizii.* Egg yolks from 0-day old hatchlings contained specific antibodies, although significantly less than plasma of adults; similarly, plasma of 0-day old hatchlings contained antibodies but less than in yolks. The antibody classes present in plasma and yolk were IgG and IgM. Antibody titers in hatchlings from seropositive females were significantly higher than in hatchlings from seronegative females and remained higher throughout the first year of life. Immunoblot analysis indicated the females transfer IgG and IgM into their eggs and that these antibodies are still detectable after 1 year. The *M. agassizii* specific antibodies seemed to be of the IgG class. Vertical transmission of infection was unlikely because of lack of increasing antibody titers over time and lack of clinical signs. The authors concluded that hatchlings were not infected with mycoplasmas and that the specific antibody in all hatchlings during their first year of life was maternal antibody. Note: After hatchlings of a *M. agassizii*-infected gopher tortoise (*G. polyphemus*) were exposed to chronically ill adult tortoises, the hatchlings developed severe URTD, suggesting that passively acquired antibodies may not be protective. The relevance of this study is that *M. agassizii* infections in hatchlings and young juveniles may be misdiagnosed if the hatchlings have persistent maternal antibodies. Wendland and others (2007) eliminated this problem by appropriate dilution of sera.

i. Effects of upper respiratory tract disease on olfactory behavior

    i. Alberts and others (1994), in studies of chin gland secretions conducted at the Desert Tortoise Conservation Center , NV, reported that two of seven tortoises with previous signs of URTD did not discriminate between chin gland secretions of familiar and unfamiliar males and the other five took longer to do so than did healthy tortoises. The authors hypothesized that if chin gland secretions mediated aggression among males or facilitated mate choice by females, then high incidences of URTD could disrupt social relationships, possibly resulting in reproductive dysfunction.
    ii. Germano and others (2014) conducted an experiment at the Desert Tortoise Conservation Center, NV, on three groups of confined tortoises: (a) no nasal discharge and *M. agassizii* negative, (b) no nasal discharge and *M. agassizii*—positive, and (c) nasal discharge and *M. agassizii*-positive. Tortoises were fed commercially available Mazuri diet. They reported that a nasal discharge reduced sense of smell and hence the ability to locate food.

j. Evidence of illness and presence of *Mycoplasma agassizii* from necropsies of wild tortoises

    i. Jacobson and others (1991) used 17 ill tortoises from in and around the Desert Tortoise Natural Area, CA, and 1 from St. George, UT; 4 healthy, control tortoises came from Ivanpah Valley. They conducted hematologic and serum biochemical evaluations, pathologic and microbial investigations, liver and metal determinations and concluded that *Mycoplasma* sp. might be the pathogen. They noted that (a) Ill tortoises had significantly lower hemoglobin and phosphorus (P) concentrations and significantly higher Na, blood urea nitrogen , creatinine, total cholesterol, mercury and iron concentrations and serum glutamic oxalacetic transaminase (AST). The values of some analytes may be related to dehydration and lack of forage. (b) Ill tortoises had moderate to large amounts of exudate in the nasal cavity and nasal passageways as well as diffuse loss of mucosal glands and other major tissue changes in the upper respiratory tract. Visible by transmission electron microscopy were pleomorphic organisms, present on the cell surface and occasionally forming chains, and ultrastructurally consistent with genus *Mycoplasma*. (c) Other organ systems affected in ill tortoises included trachea, bronchi, thyroid, thymus, and spleen. (d) Relatively few bacteria were isolated from controls compared to ill tortoises. *Pasteurella testudinis* was isolated from ill tortoises and one control.

ii. Drawing on necropsies of 12 clinically ill and 12 clinically healthy tortoises from Las Vegas, Jacobson and others (1995) reported that all 12 tortoises had clinical signs of upper respiratory tract disease, 11 had mild to severe lesions in the nasal cavity and one tortoise had a normal mucosa; 11 were seropositive for *M. agassizii* and 6 were culture positive. Of 12 healthy tortoises, 9 had lesions in the nasal mucosal epithelium, 5 were seropositive for *M. agassizii,* and 6 were culture positive. *Pasteurella testudinis* was isolated in a greater percentage of the total isolates from tortoises with moderate to severe lesions.

iii. Homer and others (1998) reported that five of 24 tortoises submitted for necropsy between 1992 and 1995 had mycoplasmosis; most were in moderately poor condition as evidenced by dehydration and low body weight; all had nasal lesions (Homer and others, 1998).

iv. Braun and others (2014) evaluated 55 necropsied desert tortoises at the Desert Tortoise Conservation Center, NV, and 3 wild tortoises for *M. agassizii* using a newly developed qPCR technique. They found a prevalence of 75% using a combination of nasal flush and nasal mucosa. By ELISA, the figure was 56%. No tortoise tested positive for *M. testudineum.* The presence of *M. agassizii* was not significantly associated with the presence of TeHV2. They also evaluated 3 wild tortoises from Nevada using a new qPCR technique (1 was positive for *M. agassizii* using qPCR from nasal mucosa and 2 were negative for *M. testudineum* using qPCR (nasal flush and nasal mucosa) and positive for TeHV2 (tongue and nasal mucosa).

k. Mortalities associated with upper respiratory tract disease.

   i. Jacobson and others (1991) described an acute mortality event that occurred at the Desert Tortoise Natural Area, CA, in 1989–1990.

   ii. Brown and others (1999) reported on changes in desert tortoise population densities at the Desert Tortoise Research Natural Area, CA, and noted that a significant decline in relative population density occurred during a 17-year period. Densities of adult tortoises from mark-recapture studies were >50 adults/km^2 in 1979, 1982, and 1988; however, by 1992 and 1996, densities of adults had declined to <10/km^2. At the 1988 sampling, the population was declining and the first clinical signs of upper respiratory tract disease were noted. Subsequently, the adult population declined 90%. At this site, upper respiratory tract disease evolved from an acute, epizootic disease with high mortality to a chronic endemic disease with variable morbidity, low mortality and high seroconversion rate for antibodies against *M. agassizii*.

    iii. Christopher and others (2003), in a 1990–1995 study of disease abnormalities at three sites in the Mojave Desert, reported that two of five tortoises that died at the Desert Tortoise Natural Area had clinical signs of upper respiratory tract disease.

l. Jacobson and others (2014) in a critical review of the mycoplasmosis literature, noted that mycoplasmosis is characterized by initial high mortality, followed by low mortality and high morbidity. The authors also reported that several tortoises infected with mycoplasmosis survived in captivity for many years with clinical signs varying over time. Morbidity from mycoplasmal disease may be more subtle and difficult to assess. Abnormal hormone profiles have been observed and could lead to alterations in foraging and reproductive behavior and decreased reproductive potential. Chronic inflammation in nasal and olfactory tissues could disrupt olfactory function and affect foraging and reproductive behavior (Jacobson and others, 1991; Homer and others, 1998). Soluble scute proteins may be affected (Homer and others, 2001).

m. Jacobson and others (1995) reported that host response to *Mycoplasma* spp. may differ from tortoise to tortoise; some tortoises with positive cultures and positive serology had normal nasal cavities, while most did not. Thus, not all tortoises respond to *M. agassizii* with a severe inflammatory response, suggesting that multiple strains of *M. agassizii* with variable pathogenicity may existor that different responses are related to different tortoise genotypes (summary in Jacobson and others, 2014).

n. Factors contributing to mycoplasmal disease in tortoises (reviewed by Jacobson and others, 2014) include:

    i. Heavy metals (e.g., mercury, Jacobson and others, 1991).
    ii. The desert environment and periods of drought (Peterson, 1996a, 1996b; Henen and others, 1998). Christopher and others, 2003) noted that tortoises emerging from hibernation in years following periods of drought had clinical signs of upper respiratory tract disease and heteropenia; suggesting that tortoises entering hibernation in a drought year may be physiologically compromised.
    iii. Human impacts and habitat degradation may contribute to mycoplasmal disease (review and summary in Jacobson and others, 2014).
    iv. Proximity to urban areas or places where people live and work may contribute to mycoplasmal disease in tortoises. Berry and others (2006a) reported that wild tortoises in remote areas of the Mojave Desert, distant from humans and paved roads were significantly less likely to be seropositive for *M. agassizii* than those in close proximity to human developments
    v. The capture, manipulation and transport of tortoises during research efforts, relocation, restocking and repatriation may be sources of stress that result in overt disease (Berry and others, 2002b)

        vi. The escape or release of captive tortoises living in urban and remote areas may be a significant factor in the epidemiology of disease. Examples and references include: (a) Thousands of captive tortoises have been released into wild lands (e.g., see summaries in Jacobson and others, 2014; also Field and others, 2007; Murphy and others, 2007;; Nussear and others, 2012). (b) Captive tortoises can be a reservoir of infectious diseases, e.g., captive tortoises in the Greater Barstow area had a high prevalence (82.7% of 179 tortoises) of positive ELISA results for *Mycoplasma agassizii*, 82.7% of 179 tortoises (Johnson and others, 2006). The escape of captive tortoises to the wild and the finding of two Russian tortoises in the wild during the 2000–2001 period indicate that release and escape of tortoises likely has resulted in the concomitant release of pathogens into the wild. (c) Captives may be the source of *M. agassizi* to free-ranging populations at three sites in Utah (Dickinson and others, 2005); captive releases were reported in the Red Cliffs Desert Reserve and on the Beaver Dam Slope in the 1970s.

    o. Review papers on *Mycoplasma agassizii* (see Sandmeier and others, 2009). A more up-to-date and comprehensive review of *M. agassizii* in *Gopherus* is in Jacobson and others (2014).

2. *Mycoplasma testudineum*

    a. Naming *Mycoplasma testudineum*. Brown and others (2004) identified and named the *Mycoplasma testudineum* strain BH29^T, which causes chronic rhinitis and conjunctivitis of tortoises. It was newly found in wild desert tortoises with upper respiratory tract disease from the Mojave Desert; *M. testudineum* was a new species and was estimated to have similar distribution but lower prevalence than *M. agassizii*.

    b. Jacobson and Berry (2012) provided data on nine wild tortoises from the central Mojave Desert, CA. These tortoises were necropsied in 2007–2008 and provided correlative serologic and pathologic findings that support a causal relationship between *M. testudineum* and upper respiratory tract disease in wild desert tortoises.

        i. Some tortoises had subclinical mycoplasmosis: five of the nine had a nasal discharge and two had wet, most or damp naris/nares or beak on one or more field evaluations. At necropsy, only two had a nasal discharge. Clinical signs included mild to severe rhinitis and changes in the palpebrae. Using light microscopy, three tortoises had no significant lesions of the palpebrae, three had mild lesions, and three had moderate lesions.
        ii. Microscopic evaluations of the nasal cavity indicated mild to severe lesions in one or both of the nasal cavities.
        iii. All nine tortoises had positive serology for *M. testudineum* and two were also positive for *M. agassizii*. Two tortoises that were serologically positive for *M. testudineum* were also PCR and DNA positive (nasal flushes).

iv. The following aerobic bacteria were cultured from the nasal cavities of the tortoises with *M. testudineum* and the outgroup of two additional tortoises: *Aerococcus urinae, Aerococcus viridans, Aeromonas hydrophila, Bacillus liceniformis, Bacillus pumilus, Chromobacterium violaceum, Chryseobacterium indologenes, Cornynebacterium aquaticum, Corynebacterium renale, Corynebacterium striatum, Empedobacter brevis, Enterococcus casseliflavus, Enterococcus raffinosus, Erysepelothrix rhusiopathiae, Klebsiella pneumonia, Lactococcus lactis, Micrococcus luteus, Micrococcus sedentarius Pseudomonas aeruginosa, Shewanella putrefaciens, Staphylococcus haemolyticus, Staphylococcus equorum, Staphylococcus sciuri, Staphylococcus simulans, Streptococcus agalactiae, and Streptoccus constellatus*. They probably represent normal bacterial flora.

c. Epidemiology of *Mycoplasma testudineum*. Berry and others (2015a) explored variables likely to affect presence of *Mycoplasma agassizii* and *M. testudineum* in 1,004 tortoises in a 1,183 km^2 study area in the central Mojave Desert, CA, between 2005 and 2008. They used covariates of habitat (vegetation, elevation, slope, and aspect), tortoise size and sex, distance from another test-positive tortoise, and anthropogenic variables (distances to roads, agricultural areas, playas, urban areas, and centroids of human-populated census blocks).

i. The prevalence of test-positive tortoises was low: 1.49% for *M. agassizii* and 2.89% for *M. testudineum*.

ii. The spatial distributions of tortoises that were test positive for exposure to both pathogens overlapped very little, indicating that researchers studying epidemiology of one or both species cannot assume that just because one species is present does not indicate the other species will also be present; similarly, the distributions of the two species is likely to be different.

iii. For both pathogens, there was higher prevalence of test-positive tortoises with shorter distances to centroids of human-populated census blocks. For *M. testudineum*, another but less important co-variate was distance to areas without habitat for tortoises (e.g., agricultural areas, playas).

iv. The relationship between close proximity to human-populated census blocks and test-positive tortoises may be related to release or escape of captive tortoises, because prevalence of *M. agassizii* in captive tortoises is high.

d. Review paper on *Mycoplasma testudineum* (and *M. agassizii*) in *Gopherus* (Jacobson and others, 2014).

B. Other Potential Bacterial Pathogens

1. Jacobson (1994), in a review of causes of mortality, identified *Pasteurella testudinis* as a potential cause of respiratory disease. In a study of 17 ill desert tortoises from the Desert Tortoise Natural Area, CA, one ill tortoise from Utah, and four healthy tortoises, *P. testudinis* was cultured from the nasal cavity of all ill tortoises and one of four healthy tortoises.

2. Snipes and others (1995) undertook molecular characterization of *Pasteurella testudinis* isolated from desert tortoises with and without upper respiratory tract disease in an attempt to identify strains associated disease. They collected 89 isolates and genomically fingerprinted and grouped them based on ribotype similarity. They identified an *Eco*RI ribotype comprised of multiple isolates exclusively recovered from tortoises with upper respiratory tract disease. Ribotypes were further studied using *Eco*RV; one of these *Eco*RI/*Eco*RV ribotypes was obtained only from ill tortoises. They failed to identify a particular "pathogenic" strain, which is somewhat in agreement with previous pathogenesis studies of *P. testudinis* in captive desert tortoises.
3. Homer and others (1998) reported on potential bacterial pathogens isolated from the choanae and colon of 24 wild tortoises submitted for necropsy between 1992 and 1995. These potential pathogens included *Citrobacter* sp., *Klebsiella oxytoca*, *Pasteurella testudinis*, *Pseudomonas* sp., and *Xanthomonas maltophilia.*
4. Dickinson and others (2001) studied Mojave tortoises at City Creek, UT, and Littlefield, AZ. They reported three species of nasal bacteria: *Corynebacterium* spp., *Staphylococcus* spp., and a potential pathogen, *Pasteurella testudinis*. Compared to healthy tortoises, significantly higher levels of *P. testudinis* were found in ill Mojave desert tortoises. Two of 17 species of cloacal bacteria were considered opportunistic pathogens (*Pseudomonas* spp., *Salmonella* spp.). Some cloacal bacteria showed site, season, and year differences. *Pseudomonas* spp. and *Salmonella* spp. were more prevalent in ill tortoises. All tortoises with clinical signs of upper respiratory tract disease had positive ELISA and/or PCR results. These same tortoises had significantly higher numbers of *P. testudinis* in their nasal cavities. The higher prevalence of *P. testudinis, Pseudomonas* spp., and *Salmonella* spp. in ill tortoises demonstrates their usefulness in identifying individuals with underlying pathology.
5. Christopher and others (2003), in a 5-year study of adult tortoises at three Mojave Desert sites (Desert Tortoise Natural Area, Goffs, and Ivanpah), reported that 62% of 798 nasal cultures had moderate to heavy growth of *Pasteurella testudinis*, which was significantly more prevalent in Ivanpah tortoises than in Goffs or Desert Tortoise Natural Area tortoises.
6. DuPré and others (2012) used a quantitative PCR method that amplifies 16S rRNA and rpoB genes of *Pasteurella testudinis* to test 20 captive desert tortoises that were currently presenting or had recently developed signs of URTD. Of 19 nasal lavage samples collected from wild desert tortoises in the Mojave Desert, 84% tested positive with *P. testudinis* DNA. Although preliminary, the authors suggested that their results may indicate that most wild tortoises may be colonized with the bacteria. Two tortoises with the highest levels of *P. testudinis* DNA also had the highest levels of *M. agassizii* DNA, which the authors interpreted as a possible sign of co-infection; co-infections may play a role in pathogenesis.

C. Herpesvirus

1. Jacobson (1994), in a summary of causes of mortality in desert and other tortoise species, described a report of a 6-year old captive desert tortoise with evidence of herpesvirus infection; and in another report, a desert tortoise was considered to have poxvirus infection because intracytoplasmic inclusions were seen in epidermal cells. However, no ultrastructural studies were performed for confirmation.
2. Pettan-Brewer and others (1996), in a necropsy of a captive desert tortoise ill with a respiratory infection at the University of California, Davis, identified herpesvirus particles in intranuclear inclusions and cytoplasm. The authors reported lesions in the oral cavity, trachea, and lungs and diagnosed viral stomatitis, tracheitis, and bronchopneumonia complicated by bacterial infection. The viral inclusions were unique and provided some evidence for viral participation in the pathogenesis of the oral and respiratory lesions. This may have been the first report of respiratory tract disease associated with a viral infection in a desert tortoise.
3. Oral lesions and plaques, a potential clinical sign of herpesvirus. Christopher and others (2003) reported on disease abnormalities in tortoises studied between 1990 and 1995 at three sites in the Mojave Desert, CA. Eight tortoises at one site and two at another had oral lesions in 1992–93 (ulcerated or covered with whitish-yellow plaques). The tortoises either had concurrent signs of upper respiratory tract disease or signs at other sampling period; tortoises with oral lesions were significantly more likely to have positive nasal cultures for *Mycoplasma agassizii*. Oral lesions indicated a new disease finding of substantial concern
4. Johnson and others (2005) identified a new herpesvirus, THV-2, from a captive *Gopherus agassizii*, using transmission electron microscopy, amplification of herpesvirus nucleic acid sequences using PCR, and indirect enzyme linked immunosorbent assay. The captive tortoise had necrotizing stomatitis, pharyngitis, and tracheitis. Four other tortoises were housed with the ill tortoise: two desert tortoises and one Texas tortoise were positive for anti-tortoise herpesvirus antibodies, and one desert tortoise was negative. This study shows that the current ELISA does not distinguish well between the two herpesviruses (THV-1, THV-2)
5. Johnson and others (2006), in a study of captive tortoises from 45 households in nine towns in the Greater Barstow Area, CA, between 2000 and 2001, reported that 26.6% of 109 tortoises samples were seropositive for herpesvirus, THV-1 using an indirect ELISA in which two isolates of THV-1 from Hermann's tortoises served as the antigen in the ELISA. None of the tortoises had oral plaques. No associations were found between tortoises with positive ELISA results and severity of clinical signs or with a positive mycoplasma ELISA result. Twenty-five percent of tortoises in this study had been exposed to exotic species of turtles and tortoises. This study indicated that release of desert tortoises has the potential of transferring pathogens to wild, naïve tortoises.

6. Jacobson and others (2012) confirmed the presence of Testudinid herpesvirus-2, TeHV2, in two wild *Gopherus agassizii*, one from the central Mojave Desert, CA, and the other from the northeastern Mojave Desert, NV, the first conclusive molecular evidence of herpesvirus infection in wild tortoises. They also reported serological results from surveys of two captive populations (n = 55) and 20 wild tortoise populations (n = 256) in California; 22% of captive tortoises were ELISA positive against both TeHV3 isolates and overall prevalence of wild tortoises with positive serology was 31%, varying by region from 15 to 84%. Additional data from 27 necropsied tortoises using available sera revealed that 6 were serologically positive for both TeHV3 isolates. Serologic findings indicate cross-reactivity between TeHV2 and TeHV3.
7. Braun and others (2014) used qPCR to evaluate 55 tortoises from the Desert Tortoise Conservation Center, NV, and 3 wild tortoises (1 of which is described in Jacobson and others [2012]). TeHV2 was detected in 20 of 42 samples (48%). Two of the wild tortoises were positive for TeHV2. All tortoises positive for TeHV2 by qPCR had positive tongue samples (n = 20) with the exception of 1 tortoise that was positive only by nasal mucosa qPCR. There was substantial agreement between the nasal flush qPCR and nasal mucosa qPCR results, moderate agreement between tongue and nasal mucosa qPCR, and fair agreement between tongue and nasal fluid qPCR results. The presence of *M. agassizii* was not significantly associated with presence of TeHV2.

VI. Implications of Infectious Diseases

A. Captive Tortoises: Implications for Care, Propagation, Relocation, Repatriation, and Release to the Wild

1. Jacobson (1993) noted that upper respiratory tract disease is thought to have been introduced into desert tortoise populations in the Mojave Desert by release of ill captive tortoises; a similar situation occurred with *Gopherus polyphemus* in Florida. The author reviews emerging and historically important infectious diseases in reptiles; only those reported in captive chelonians will be mentioned here, e.g., mycoplasmosis, herpesvirus, amebiasis. He discussed how intermixing of reptile species in the pet trade and in private and zoological collections has likely contributed to the epizootics. He emphasized the importance of isolating reptiles and progeny that may be returned to the wild.
2. Jacobson (1994) noted that many captives with respiratory tract disease exist in private collections throughout southern California, and an extremely pathogenic organism may have been introduced to wild populations at multiple sites by released pet tortoises.
3. Johnson and others (2006) conducted a study of seroprevalence of *Mycoplasma agassizii* and tortoise herpesvirus in captive desert tortoises in 45 households in 2000 and 2001 from nine towns in the greater Barstow Area, Mojave Desert, CA.

        a. They reported that antibodies to *Mycoplasma* (positive ELISA results) were present in 82.7% of the 179 tortoises sampled and that antibodies to herpesvirus were detected in 26.6% of 109 tortoises sampled. They also reported a positive association between tortoises with positive ELISA tests for *Mycoplasma agassizii* and severity of clinical signs and with age, with adults more likely to be seropositive than younger tortoises.

        b. Seven of the owners kept seven other exotic species of turtles and tortoises and four of the seven owners had exotic species in direct contact with desert tortoises. Four (2.2%) of tortoises that were owned in 2000 had escaped by 2001, and two Russian tortoises were found wild during the same time. The authors estimated that 4,400 captive desert tortoises could escape in any given year.

        c. This study indicated that release of desert tortoises may and likely has resulted in the concomitant release of pathogens into the wild.

    4. Edwards and Berry (2013), in a study of the genetics of captive tortoises presented to two clinics (Ridgecrest, Kern Co., and Joshua Tree, San Bernardino Co., CA) and the Desert Tortoise Conservation Center, NV, reported that captive tortoises had not been in the custody of their caretakers for long: 3.6 years for Ridgecrest and 6.5 years for Joshua Tree. Many caretakers had few details about the sources of their tortoises, and many tortoises had been transferred from one to another adoption group, rescue group, person or family over time. Most caretakers reported keeping more than one desert tortoise, and many desert tortoises lived in households where other chelonian species were present.

    5. Berry and others (2015a) conducted epidemiological research on 1,004 adult tortoises for two pathogens known to cause upper respiratory tract disease (*Mycoplasma agassizii, M. testudineum*). They modelled several biological, physical, population, disease, and anthropogenic variables. They consistently found higher prevalence of test-positive tortoises within shorter distances to the centers of human-populated census blocks (census blocks established by the U.S. Census Bureau, e.g., houses). The relationship between test-positive tortoises to close proximity of people suggests that the two upper respiratory diseases in wild tortoises may be related to release or escape of captive tortoises, because prevalence of these diseases is high in captive tortoises.

B. Wild Tortoises: Implications for Translocations and Relocations

    1. Berry and others (2006a), in a study of 21 desert tortoise study plots in the central Mojave Desert, reported that infectious disease (mycoplasmosis) was negatively correlated with distances from Fort Irwin offices, the cantonment (housing area), and paved roads; specifically the ill tortoises were found close to offices and the housing area. If translocations of tortoises were to occur, release sites remote from human activity would be more likely to be free of infectious disease.

    2. Aiello and others (2014) discussed common features observed following translocations that can affect transmission of infectious diseases. The authors used preliminary data from the translocation of tortoises to three sites from Fort Irwin in 2008 to illustrate potential consequences.

a. The authors reviewed dynamics of disease invasion; stress, virulence and susceptibility; release strategies, host density thresholds and contact rate; dispersal and contact network structure

b. The study employed three translocation and three control plots and used geographic locations for tortoises prior to and following translocation at the plots between April and October 2008 to model dynamic contact networks and identify changes in contact rates due to translocation. If tortoises were within 100 m, the potential for contact was inferred as high.

c. The modeling and simulations revealed that a temporary change in spatial network characteristics occurred at translocation plots but not control plots. Ninety percent of translocated tortoises were potentially connected in one large subgroup compared to 33% at control plots at the time of releases. The modeling indicated that translocation sites often had a higher percentage of resident tortoises infected at the end of simulations than at control sites.

d. This is only a first step for obtaining a more structured risk assessment for disease invasion after translocation. Contacts may have been over-estimated, and tortoise-tortoise interactions may have occurred that were not observed.

e. The authors outlined a research agenda with three steps.

3. Berry and others (2015a) conducted epidemiological research on 1,004 adult tortoises for two pathogens known to cause upper respiratory tract disease (*Mycoplasma agassizii, M. testudineum*). They modelled several biological, physical, population, disease, and anthropogenic variables. They consistently found higher prevalence of test-positive tortoises within shorter distances to the centers of human-populated census blocks (census blocks established by the U.S. Census Bureau, e.g., houses). The relationship between test-positive tortoises to close proximity of people suggests that the two upper respiratory diseases in wild tortoises may be related to release or escape of captive tortoises, because prevalence of these diseases is high in captive tortoises. When wild tortoises are translocated, the proximity to human households and settlements should be a consideration.

VII. Immune Systems, Natural and Acquired Antibodies

A. Sandmeier and others (2012), in a study of natural and acquired antibodies of 20 captive desert tortoises, used injections of ovalbumin to determine natural antibody response with respect to season and gender and interactions between natural and acquired antibodies. They assayed ovalbumin-specific antibody titres using the polyclonal ELISA described by Hunter and others (2008). They reported:

1. All tortoises had relatively high levels of natural antibody to ovalbumin; natural antibody titers in winter were significantly lower than titers in spring. However, there was variability among individuals.

2. All tortoises exhibited a lag time of 4 weeks prior to increases in antibody titers and only produced consistent, maximum antibody titers at 27 weeks post-treatment; no drop in titers occurred between spring and fall.
3. Antibody titers > 1 year post-immunisation were significantly higher than pre-immunisation antibody titers.

B. Jacobson and others (2014) in reviewing the literature, noted that “natural antibodies are generally irrelevant in immunological tests, since sera are usually diluted sufficiently to avoid interference from so-called ‘nonspecific background.’

VIII. Effects of Stress, Measuring Stress

A. Experiments to assess the effect of stress on reproductive hormones.

Lance and Rostal (2002) conducted experiments on adult male tortoises at the Desert Tortoise Conservation Center, Las Vegas, NV, by (1) holding tortoises in boxes for several hours and then assaying for corticosterone and testosterone, and (2) testing effects of adrenocorticotropic hormone (ACTH) on circulating levels of corticosterone and testosterone. Tortoises held in boxes and taken from the field before blood was drawn had significantly higher corticosterone and testosterone concentrations than tortoises bled immediately in the field. They found a similar response to a single injection of ACTH; there was a rapid rise in corticosterone secretion and an equally rapid increase in plasma testosterone levels, but by 8 h post-injection, corticosterone had returned to baseline and testosterone had fallen to about 50% of the initial value.

B. Drake and others (2012a) evaluated a population of desert tortoises in 3 treatment groups (resident, translocated, and control) in the north-central Mojave Desert, CA, for stress responses using plasma total corticosterone. Corticosterone was higher for males than females and values for both varied monthly throughout the activity season and among years. Blood samples collected from adult tortoises for 1 year and prior to and 2 years after translocation showed that year and sex (but not translocation per se) were strong predictors of corticosterone levels.

IX. Using Gene Transcription-based Biomarkers to Understand Desert Tortoise and Ecosystem Health

Bowen and others (2015) described development of quantitative real-time polymerase chain reaction (qPCR) assays to measure differential transcript levels of multiple genes in the tortoise (genes related to immune function, response to pathogens, etc.), using 64 wild and 19 captive tortoises. The authors developed the first leukocyte gene transcription biomarker panel for the tortoise, enhancing the ability to identify specific environmental conditions potentially linked to declining animal health. Blood leukocyte transcript profiles have the potential to identify physiologically stressed animals in lieu of clinical signs.

X. Trauma: Injuries to Soft Tissues and Bones

A. Jacobson (1994) personally observed cactus spines embedded in subcutaneous tissues and tongues of desert tortoises and the resultant inflammatory response.

B. Homer and others (1998) reported that 4 of 24 wild tortoises submitted for necropsy between 1992 and 1995 were injured or dead from blunt force trauma (vehicles, 2 tortoises; predators, 1 tortoise) or burns (1 tortoise). Three tortoises had cactus spines embedded in the intestine, stomach or tongue and pharynx, associated with granuloma formation or acute inflammation and bacterial colonization.

C. Christopher and others (2003) in a 1990–1995 study of adult tortoises at three sites in the Mojave Desert, CA, reported that traumatic lesions were observed on 41.7% of tortoises. Traumatic lesions in general were more likely to be seen in tortoises with anemia.

D. Berry and others (2006a) surveyed 21 plots at the National Training Center, Fort Irwin, in the central Mojave Desert, CA. Fifteen of the plots were on the Goldstone portion of the base and figures are treated collectively. Trauma was noted for 3 of 17 Goldstone tortoises, 7 of 29 Alvord tortoises, 2 of 12 Langford tortoises, 11 of 72 Tiefort tortoises, 4 of 11 Eastgate 2 tortoises, and 6 of 39 Soda Mountains tortoises.

E. Medica and Eckert (2007) observed 4 of 18 recaptured tortoises from 1998 to 1999, following the El Niño rain event of 1997–1998, with injuries to their jaws from consuming Red Brome (*Bromus madritensis*) seeds at a study site on the Nevada Test Site, NV. Injuries persisted for 3–4 years in 3 tortoises and for 5 years in 1 tortoise, after which observers manually removed additional seed material. During 1998, the researchers also reported observing Red Brome seed embedded to its full length in a nostril of 1 tortoise and impaled in the corner of an eye of another tortoise in Piute Valley, Clark County, NV.

F. Walde and others (2007b) observed an adult male tortoise that had ingested a 108 cm portion of ribbon that was attached to a balloon fragment in the central Mojave Desert, CA. When the researchers carefully extracted the ribbon, they found digested vegetative material on the ribbon indicating it had been quite far down in the digestive tract. The researchers documented arrival of 178 new balloons in an 8 month period (22.3 balloons/month) from as far as > 200 km away, and possibly from as far as > 270 km away, from the study area.

G. Berry and others (2008), in a survey of a plot of about 4 km^2 and 37 one-ha landscape plots in Red Rock Canyon, CA, evaluated nine live tortoises. Five tortoises showed signs of old, healed trauma to the shell, such as old chews and chips on the marginal scutes, damage to the gular scutes, puncture wounds, and a depression fracture from a possible puncture wound. Lesions were evident on the posterior plastron and/or anal scutes for three adult tortoises (two males, one female).

H. Lovich and others (2011b) monitored eight female tortoises at a wind energy facility near Palm Springs, CA, for 15 years and reported that five adult tortoises showed scarring by fire on the scutes covering their carapaces. This was possibly caused by a large wildfire.

I. Drake and others (2012b) reported that after a wildfire in 2005, one male tortoise had what seemed to be melted and re-fused scales on its forelegs, and fire-related damage to the scutes and bones of the carapace. During the following 3 years, the exposed bone became depressed and covered with an opaque keratinaceous tissue. The authors reported on a second male tortoise that had a smooth mottled layer where the costal scutes were missing, similar in appearance to the tissue on the other fire-injured tortoise. However, at recapture 3 years later, the second tortoise had two more scutes that appeared to be in the initial stages of delamination. These injuries did not lead to death within the study period.

J. One of 11 wild tortoises submitted for necropsy from the central Mojave Desert in 2007–2008 had severe periocular hemorrhage and tissue damage suggestive of envenomation by a rattlesnake (Jacobson and Berry, 2012).

K. Berry and others (2013) sampled 28 live tortoises from the China Lake Naval Air Weapons Station in the northwestern Mojave Desert, CA, and noted that one adult tortoise had fresh injuries to the foreleg due to unspecified causes; one adult tortoise had healed severe injuries to the plastron and to the gular horn which had been chewed away, injuries typical of a domestic dog attack. One immature tortoise also had bites and tears to the gular horn, plastron, and carapace.

L. Berry and others (2014a), in a survey of tortoises in a study area of about 260 km^2 in the western Mojave Desert, CA, reported signs of predator attacks on all 25 adult tortoises that were handled. Of the 25, 12 had moderate to severe damage to the gular horn; the gular horn was severely reduced or chewed away completely in 9 tortoises. Some signs of trauma appeared typical of domestic dog attacks. One adult tortoise had a healed injury from crushing, potentially by a vehicle. Two juvenile tortoises had ant heads attached to soft parts of the integument.

M. Smith and others (2015) described mechanical injury to an immature *Gopherus agassizii* at Joshua Tree National Park, CA, from a California barrel cactus (*Ferocactus cylindraceus*).

XI. Medicine, Surgery, and Treatment of Infectious Diseases

A. Anesthesia. Rooney and others (1999) experimentally tested the effects of sevoflurane anesthesia on six captive *G. agassizii* and reported that it provides rapid, controllable, and reliable induction of anesthesia and recovery. While under the anesthetic agent, the tortoises experienced decreases in systolic, diastolic, and mean ventricular pressures but no change in heart rate or Na^+, K^+, and Ca^{++} blood concentrations. Sevoflurane appeared to provide safe and effective anesthesia with rapid induction and recovery.

B. Vascular Access. Wimsatt and others (1998) tested the efficacy of a cardiac access port for repeated collection of blood samples using 14 tortoises seropositive for *Mycoplasma agassizii*. They drilled a hole through the plastron at the junction of the pectoral and abdominal scutes, inserted a sterile rubber stopper and sealed with epoxy. Blood collection required a 27-gauge, 5/8 inch needle. Of the 14 tortoises, 13 appeared to tolerate the sampling well. Pericardiectomy was done in seven of the tortoises but does not appear to be necessary. The authors stated that sample collection under natural or semi-natural laboratory conditions would greatly reduce restraint-related stress (jugular).

The ports can be successfully removed and long-term complications are rare. Postmortem examination revealed evidence of mild fibrotic myocardial changes attributed to repeated cardiocentesis.

C. Treatment of Mycoplasmosis

   1. Wimsatt and others (1999) studied the pharmacokinetic effects of clarithromycin in tortoises seropositive for *Mycoplasma* by using single oral doses of 7.5 and 15 mg/kg and doses of 15 mg/kg given daily for 9 days. The suggested dose was 15 mg/kg p.o. every 24 h to achieve maximal blood levels of ≥ 1 µg for multiple dosing. However, the multiple dosings resulted in increases of plasma concentrations of the drug, reflecting a lower elimination time, and may need to be reduced. There were many qualifications to this study.

   2. Wimsatt and others (2008) reported on long-term oral and per rectum disposition of clarithromycin in treating *Mycoplasma* in *Gopherus agassizii*. They characterized plasma concentrations after a single dose, after 3.5 months of dosing, and after per rectum administration; all doses were 15 mg/kg. They drew on wild-captured tortoises from St. George, UT, that were seropositive for *M. agassizii*. The median maximal plasma concentration of 1.69 mg/mL occurred at a median of 6 h after administration, and half-life was 6.9 h. Plasma concentrations after long-term dosing varied; per rectum concentrations caused antibiotic retention below target values. Three months after the end of the long-term dosing study, the long-term treated tortoises started to exhibit signs of URTD again. Chronic dosing of tortoises leads to accumulation of the drug within the plasma space. Clarithromycin shows promise in treating this chronic progressive disease, providing that sufficiently high tissue levels can be sustained in animals that are not too debilitated.

   3. Kinney and others (2014) tested the pharmacokinetics of a single intramuscular injection of tulathromycin in 110 captive adult tortoises at the Desert Tortoise Conservation Center, NV. This was a first step in evaluating the clinical utility of the drug in desert tortoises for treatment of upper respiratory tract disease.

D. Husbandry, Diseases, and Surgery in Captive Desert Tortoises. Mader (2006), in his book, *Reptile Medicine and Surgery*, covered a broad range of topics with many species of reptiles in 90 chapters and 9 appendices. Husbandry and medical issues with desert tortoises are described in 28 different chapters, many of which are illustrated with images of procedures, clinical signs, or trauma and subsequent necropsy results. *Gopherus agassizii* is featured in chapters on general care, nutrition, and husbandry; clinical pathology, diagnostic imaging, ultrasonography, endoscopy, therapeutics, emergency and critical care; anorexia; aural abscesses; upper alimentary tract disease; cloacal prolapse; hyperglycemia; parasitology; upper respiratory tract disease; pneumonia and lower respiratory tract disease; reproductive biology and dystocias; perinatology; renal disease; urinary calculi; metabolic bone disease; neurologic disorders; fungal and bacterial diseases; and thermal burns. Please note that some topics do not have the latest summaries of published literature because of the many new journal articles published after 2006 (e.g., herpesviruses, upper respiratory tract disease).

E. Gastrointestinal Stasis. Tothill and others (2000) conducted a study at Colorado State University with 24 tortoises of varying ages to determine whether desert tortoises receiving any one of three gastrointestinal motility agents had shorter total transit times vs. controls. Marker passage rates were not significantly different between controls or treatment groups. The prokinetic agents were water (controls), cisapride, metaclopromide, and erythromycin.

XII. Effects of Diseases on Populations

A. Jacobson and others (1994) in reviewing background of desert tortoises with the newly described cutaneous dysteratosis (shell lesions and disease) in the Colorado Desert of southern California, noted that the population experienced a very high mortality rate of about 70% between 1982 and 1988.

B. According to Homer and others (1998), effects of disease on populations may be subtle: disease can inhibit or slow growth rates of individuals by reducing appetite, resulting in malnutrition, and thus reduced growth; for females, reduction in growth rates limits carapace length and size, thereby limiting numbers of eggs produced annually and reduced reproductive vigor; for juveniles, reduction in growth rates prolongs the period of vulnerability to predators and environmental stresses; and reduces survivorship of individuals and populations.

C. Brown and others (1999) reported trends in desert tortoise population densities at the Desert Tortoise Research Natural Area, CA, specifically, a significant decline in relative population density occurred during a 17-year period. From relatively high estimated relative densities > 50 adult tortoises/km^2 in 1979, 1982, and 1988, a dramatically lower population density was observed in 1992 and 1996 with estimated density of < 10 adult tortoises/km^2. This population decline was concurrent with, and subsequent to, the observation of clinical signs of upper respiratory tract disease in the population in 1988.

D. Berry and others (2002b), in a study of deaths from drought at the Fort Irwin enclosure for head starting juvenile tortoises and at sites for study of adult tortoises in Ivanpah Valley and Goffs, reported that 10% of juveniles (11/110) at the Fort Irwin enclosure died, whereas at Ivanpah, only 2.3% of adults observed live in spring of 1990 were found dead in 1994. Population of adults at Ivanpah Valley and Goffs sites remained relatively stable in densities between 1979 and 1994 with no statistically significant changes occurring between years, in spite of droughts. Tortoises have adaptations or exaptations essential for surviving in deserts, because drought is a frequent event. However, if droughts are prolonged, tortoises are eventually going to succumb from lack of food and water.

E. Christopher and others (2003), in a 5-year study of adult desert tortoises at three sites in the Mojave Desert (western, eastern, northeastern regions), reported that tortoises at Ivanpah (northeastern region) had disease types and severity intermediate between the other two sites, including intermediate severity of cutaneous dyskeratosis, oral lesions, and positive nasal mycoplasma cultures. Tortoises at the Goffs study site (eastern Mojave region) had the most severe cases of cutaneous dyskeratosis, more oral lesions, more positive *Mycoplasma* cultures, and dehydration. The Desert Tortoise Natural Area (western Mojave region) had more tortoises seropositive for *Mycoplasma agassizii.* The severe disease prevalence in Goffs tortoises likely contributed to the population decline that occurred during and subsequent to the Christopher and others study.

F. Berry and others (2006a), in a study of desert tortoise populations at 21 sites in the central Mojave Desert, reported that mortality of tortoises at five sites may have be due in part to disease because signs of disease were evident on the shells concurrent with signs of predation. The following percentages of remains with signs of disease and predation were noted for each of the five sites: Goldstone, 12.3; Alvord Slope, 6.4; Langford, 15.8; Tiefort Mountains, 16.3; and Soda Mountains, 3.5.

XII. Sterilization of Captive Male Tortoises as a Management Tool

Proença and others (2014) performed single surgeon coelioscopic orchiectomy on seven adult desert tortoises maintained at the Desert Tortoise Conservation Center in Las Vegas for population management. The authors used a bilateral prefemoral approach via sequential vascular clip ligation and radiosurgery. Six of seven animals survived; one tortoise (which eventually recovered) experienced significant hemorrhage.

XIII. Considerations and Actions for Management (by Year, from 1991 to Most Current)

A. Jacobson and others (1991) noted that the ease with which tortoises can be collected and transported probably has contributed to spread of disease caused by *Mycoplasma agassizii*; this problem should be managed through massive education. The authors noted that nutritional deficiencies, climatic factors influencing plant production, and grazing may affect nutrient availability and ultimately the immune system.

B. Jacobson (1993) recommended (1) development of sound preventive medical programs, including quarantine, treatment for parasites, physical exams, protocols for collecting blood and other biomedical samples, health assessments prior to release, and necropsies to determine causes of mortality; (2) long-term monitoring of relocation, repatriation, and translocation programs; and (3) protocols for breeding programs.

C. Schumacher and others (1993) recommended use of the ELISA for *Mycoplasma agassizii* for monitoring populations. For routine diagnostic use of this ELISA in the future, large collections of positive and negative plasma samples from necropsy-confirmed diseased and healthy animals need to be established for use as positive and negativecontrols (this has been achieved). Each plasma sample should be assayed for presence of *M. agassizii*-specific antibodies at 1:2 and 1:10 dilutions to ensure that animals with low levels of specific antibody are not missed.

D. Bulova (1994) suggested that tortoises may be most vulnerable to exposure to upper respiratory tract disease during peak occurrences of co-occupation during the mating season in August and September, based on a study conducted adjacent to the Desert Tortoise Conservation Center near Las Vegas, NV.

E. Brown and others (1994) reported that direct contact and aerosol transmission of *Mycoplasma* is the most likely method of spread, given the ability of *Mycoplasma agassizii*-containing exudate from donor tortoises to cause severe clinical disease.

However, the length of survival of *M. agassizii* in mucous droplets remains unknown. The authors described risks of relocating tortoises and release of captive tortoises into wild populations. *P. testudinis* does not appear to have a role in initiation of this disease but may have a synergistic effect. (There were some animals with stable antibody levels that did not show signs of clinical disease.)

F. Jacobson and others (1995) stated:

1. Regardless of clinical appearance, all seropositive tortoises from known "hot spots" such as Las Vegas Valley, should be considered potential carriers of *Mycoplasma* and should not be released in areas where the disease does not exist.
2. Clinically healthy seropositive tortoises originating from populations where URTD has not been seen may be relocated to other sites where clinically healthy seropositive tortoises have been identified.
3. Regarding ill or subclinically affected tortoises, euthanasia rather than relocation should be considered. These authors did not recommend euthanasia of clinically healthy tortoises (Jacobson and others, 1995, 2014).

G. Pettan-Brewer and others (1996), after identifying viral particles associated with a respiratory infection in a captive desert tortoise, said that further studies are needed to define the prevalence of herpesvirus infection in desert and other tortoises and its potential as a primary respiratory pathogen.

H. Schumacher and others (1997) recommended that interpretation of ELISA results used in population management to curb the spread of a contagious disease should err on the side of false positives rather than false negatives. The ELISA can be used to determine whether individual tortoises have been exposed to *Mycoplasma agassizii* as well as the prevalence of *Mycoplasma agassizii*-exposure within a population. However, a single ELISA test cannot be used to diagnose an active infection because a serologic test does not detect the presence of *M. agassizii* organisms in an animal; rather, organisms must be detected by culture or PCR. Nevertheless, the sensitivity and specificity of the ELISA merits its use as a reliable indicator of exposure to *Mycoplasma agassizii* in free-ranging tortoise populations. The authors noted that their findings reinforce the importance of serological testing for tortoises that will be relocated. It is importance not to miss tortoises that do not have clinical signs but that are infected with *M. agassizii* and able to spread the pathogen; even the most careful observer may miss clinical signs. There are cases where clinical signs are absent, either because infected chronically ill animals were visually assessed in between episodes of overt disease or because some tortoises were silent carriers that may, although infected, never show clinical signs. Although a single ELISA test cannot be used to diagnose active infection, rising titers between paired samples taken approximately 2 months apart would provide evidence of a recent infection.

I. Dickinson and others (2001) thought that nasal and cloacal bacteria screens could be used to identify debilitated or ill tortoises in populations experiencing underlying disease problems. The screening technique, while not stressful or invasive, does not, however, identify the cause of debility or illness.

J. Berry and others (2002b) emphasized the importance of experimental research (1) to test hypotheses on effects of handling and research, especially procedures considered invasive or intrusive and used on wild tortoises of different sizes under a variety of climatic conditions and (2) to establish the point of no return in terms of food and water. Finally the author emphasized the importance of salvaging and necropsying moribund tortoises prior to death

K. Brown and others (2002) wrote that it is important to understand the limitations of different types of tests (culture, PCR, and ELISA), especially with respect to relocation, repatriation, or captive management of tortoises. Each test provides different, complementary information that collectively can be used to define tortoise *Mycoplasma* infection status. While these tests provide information on the exposure of tortoise populations to *Mycoplasma* species, they do not provide simple positive versus negative results, or a complete picture of the overall health status of individual tortoises or populations. Ideally ELISA, culture, and PCR should be performed in all studies. If this is not possible, the next best would be to conduct ELISA serology on all animals coupled with culture and PCR on animals exhibiting clinical signs of upper respiratory tract disease. If only a single test can be performed, then ELISA is the test of choice because it has a high sensitivity (> 90%) and the chance of either a false positive or false negative result is similar and relatively low. However, a positive ELISA result indicates past exposure but not necessarily current infection. Additionally, false positives may be caused by cross-reaction of tortoise antibodies with other bacteria having similar antigens. The authors stated: “There are inadequate scientific data to provide definitive guidelines for the disposition of seropositive tortoises.” The sample size needed to detect infection in a population is dependent on the prevalence of the disease.

L. Christopher and others (2003) reported that:

  1. Re-evaluation of culture and serologic results on surviving Goffs tortoises would be useful to determine whether nasal growth of *M. agassizii* remained high and/or whether more Goffs tortoises are now seroconverting. The higher prevalent of positive cultures in male tortoises was consistent with increased contact between fighting males.

  2. In light of the high death rate in the Goffs population in the 1990s, further investigation into different strains of *Mycoplasma* organisms, histopathology of oral lesions, and research into the pathogenesis of shell disease, hepatic disease, and urologic disease are critical for better understanding of these problems.

M. Dickinson and others (2005) stated that more long-term monitoring of tortoises at the Mojave sites is needed to determine both the duration of URTD and the fate of infected tortoises.

N. Seltzer and Berry (2005) demonstrated the laser ablation ICP-MS technique as a successful method for examining trace elements in the incrementally grown laminate of scute tissues from shells of dead tortoises. It also can be used in analyzing shell-skeletal remains of tortoises from various regions of the desert as part of epidemiological surveys.

O. Chaffee and Berry (2006), in discussing future research, suggested that the distribution and abundance of elements in plants eaten by tortoises be evaluated for the respective roles of dust and systemic uptake; additional chemical data from tortoise necropsies and nutritional studies are needed to determine effects of potentially toxic elements in tortoise habitats on their health.

P. Wendland and others (2007) said that it is important that managers consider the seroprevalence of disease in the population and use methods that maximize assay specificity to reduce the probability of false-positive results when the potentially grave implications of culling or euthanasia are used to eliminate tortoises that test positive to *M. agassizii* with ELISA. When making management decisions on the basis of the assay, goals should be established for the tortoise population of interest, the necessary sample size needed to meet these goals should be considered, and the positive and negative predictive values should be evaluated before implementing any policy.

Q. Captive tortoises are likely responsible for release of pathogens (both *Mycoplasma agassizii* and herpesvirus (THV-1) to the wild (Johnson and others, 2006). Thus there is an urgent need for owner education to address the topics of appropriate animal husbandry, proper enclosures that will minimize interactions between wild and captive tortoises and prevent escape, and potential problems that might result from multi-species housing of tortoises.

R. Wendland and others (2007) wrote that disease surveillance is fundamental to disease prevention and control; there is an increased need for development of diagnostic assays. It is critical that diagnostic tests be appropriately validated and have quality control mechanisms established—this consideration is of even greater importance when potentially severe consequences exist for individual animals (see P) or for introduction of infectious agents into environmentally sensitive populations.

S. Sandmeier and others (2009) recommended an end to the euthanasia policy for tortoises classified as “suspect” or “positive” by the ELISA, given the uncertainty in the ability to diagnose URTD in tortoises and the possibility that high levels of natural and/or induced antibodies may occur in individuals most resistant to development of severe URTD. Further, to reduce the incidence of disease, the authors recommended managing mycoplasmosis (a context-dependent and opportunistic pathogen) by managing environmental parameters that affect disease prevalence, and not solely by managing the size and density of the host population.

T. Mohammadpour and others (2010) demonstrated a method for fluorescent labelling of viable *M. agassizii* organisms for flow cytometric detection. The authors stated that further studies are needed to determine whether this method can be used to detect mycoplasma infections in the desert tortoise.

U. Hazard and others (2010) noted that management practices promoting availability of forbs could increase growth rates and shell ossification, which would enhance resistance of juvenile tortoises to predation.

V. Jacobson and others (2012) said that further field investigations need to be conducted to determine: (1) the scope of diversity of endemic herpesvirus species, (2) the prevalence of virus and viral antibodies to TeHV2, (3) the frequency of TeHV2 shedding, (4) the distribution of the TeHV2, and (5) the transmission and pathogenesis of TeHV2. There also is an urgent need to develop more specific assays and protocols for testing and monitoring herpesvirus infection in tortoises expected to be translocated.

W. Jacobson and Berry (2012) recommended evaluation of both nasal cavities microscopically during necropsy for lesions, because they found that lesions could be confined to one cavity.

X. Sandmeier and others (2012) noted that quantification of natural antibodies is needed to expand our knowledge of the immune system of the tortoises and other vertebrates. Understanding the interactions between natural and acquired antibodies and immunity will lead to a better understanding of biology and ecology of host-pathogen interactions.

Y. Sandmeier and others (2013) recommended two additional directions of research: genetic sequencing to allow for identification of various genetic strains of *M. agassizii*; and development of simple tools to quantify immunocompetence in host populations.

Z. Jacobson and others (2014) stated that euthanasia of seropositive tortoises results in elimination of animals that might otherwise provide valuable reproductive and genetic contributions to wild populations and is not recommended. However relocation of seropositive tortoises could result in spread of mycoplasmosis to susceptible animals, with detrimental impacts on recipient populations. Likewise, healthy tortoises that have not been exposed to *Mycoplasma* spp. should not be relocated to populations with extensive clinical disease or those undergoing increased mortality events. The authors also stated that ELISA testing is an important tool for making management decisions, but should not be used as the sole means of evaluating health of an individual animal.

AA. Braun and others (2014) said that investigations of upper respiratory tract disease should not focus exclusively on *M. agassizii* infections. Their studies indicated a high prevalence of TeHV2 in tortoises from the Desert Tortoise Conservation Center and the wild. The relatively poor correlation between tests suggests that individual test results should not be used for decisions on animal disposition or translocation. Additional studies would be required to evaluate the positive and negative predictive values or likelihood ratios of various test combinations before testing recommendations could be made.

BB. Germano and others (2014) said that further studies on the indirect effects of reduced olfactory ability due to nasal discharge are warranted, specially noting effects on breeding success and survival.

Chapter 9.—Population Attributes

I. Size, Age, Growth Rates, and Longevity

A. Methods of Measuring Age and Longevity

1. Scute rings (laminae) for estimating age.

a. Tracy and Tracy (1995) assessed California desert tortoises raised in a laboratory and fed diets differing in concentrations of fiber and protein. They reported that from zero to seven scute rings were accumulated annually in 1–2 year-old tortoises, rather than one ring per year as observed in previous studies. Body size, rather than age, predicted the number of scute rings on desert tortoises. The authors suggested that variability in environmental conditions, such as variation in primary productivity due to precipitation or the length of the growing season, likely render the number of scute rings produced per year unpredictable. They authors indicated that this variability limited value in the scute-ring counting method for estimating age of desert tortoises.

b. Germano (1998) used data from six free-ranging tortoises from the Nevada Test Site and 20 captive tortoises raised in outdoor pens in Bakersfield, CA. The author reported a significant correlation between age and scute layers and that the correlation was not significantly different from one. Hatchling tortoises and tortoises 1 and 7 years of age had the same number of layers as age, but the number of layers was generally one layer less by the time tortoises were 15 to 20 years old. The number of layers was never more than the age of the tortoise or more than two layers less than age, through an estimated 28 years of age. The author suggested that that use of scute layers remained a useful technique in determining age of young, free-living and captive desert tortoises experiencing natural environmental fluctuation.

c. Berry (2002) evaluated the number of growth rings produced per year for 192 wild, free-living juveniles < 140 mm in carapace length at 11 sites and four regions of the Mojave and Colorado Deserts, CA. Using 35 mm slides, the author tested whether one ring was produced per year, ring counts accurately reflected age, and relationship of ring counts to the environment. Depending on the region, 43.4 to 72.9% of tortoises produced less than or more than one ring per year. Rings produced per year differed significantly by region. Tortoises in the western Mojave generally produced less than one ring per year, and tortoises in the northeastern and eastern Mojave, and northern and eastern Colorado Deserts with greater precipitation in both winter and summer were able to produce two rings per year. The mean number of growth rings varied by year and was positively correlated with increased precipitation and production of spring forage. The author concluded that environmental factors and geographic location likely preclude accurate age determination using counts of growth rings.

d. A critical evaluation of estimating age from growth rings. Wilson and others (2003) evaluated 145 papers comprising 49 case studies of turtles and concluded that there is no justification (currently) for generalizing use of growth rings to estimate age for many species of turtles. For *Gopherus agassizii*, the authors presented unpublished data from ring counts of juvenile tortoises from the Fort Irwin head-start pens, CA. They reported that counts of plastral growth rings tended to underestimate known ages of juvenile desert tortoises hatched in semi-natural enclosures without supplemental food or water.

2. Skeletochronology. Curtin and others (2008) evaluated shell remains collected from a long-term study of *Gopherus agassizii* in semi-wild pens in Rock Valley, NV, and from Piute Valley, NV. The tortoises from Rock Valley were of known age. Using two skeletochronological protocols, the authors obtained age estimates for humerus, scapula, femur, and illium. The highest accuracy was from the ilia.

3. Mark-recapture techniques. Medica and others (2012) used mark-recapture techniques to measure age and longevity of semi-wild tortoises in pens at the Nevada Test Site, NV.

4. Isotopes and carbon incorporation. Murray and Wolf (2012) conducted experimental studies with captive tortoises maintained at the University of New Mexico using isotopes to determine carbon incorporation rates and different diets. Stable isotopes of carbon ($\delta^{13}C$) exhibited a level of dilution in scute keratin (i.e., carbon creep), where the $\delta^{13}C$ of new keratin continued to contribute significant material to previously deposited rings. Tortoises produced two to four rings during 371 days after the diet switch; the rings were not biologically inert. The authors reported that new keratin continued to contribute significant material to previously grown keratin rings on the shell. Samples indicated that old rings closest to the region of new growth received about 73% of the carbon from the current diet. These data suggest that the interpretation of dietary history using growth rings must recognize that each ring may represent the weighted average of the diet over several seasons.

B. Sizes of Tortoises

1. Hatchlings

a. Spotila and others (1994) conducted incubation experiments on eggs and subsequent growth of hatchlings at the Desert Tortoise Conservation Center, NV. They reported that incubation temperature had an effect on egg size and large eggs produced large hatchings. Further, large hatchlings were still larger 120 days later and small hatchlings did not catch up in size to their larger siblings.

b. McLuckie and Fridell (2002), in a study of 12 reproductive females on the Beaver Dam Slope, UT, reported that of 51 monitored hatchlings, mean hatchling carapace length was 45.0 ± 0.33 mm SE and was significantly correlated with the carapace length of adults. Hatchling size was not correlated with egg width or length.

   c. Lovich and others (2011c) determined that the mean carapace length was 44.5 ± 2.6 mm SD for 74 hatchlings at a wind energy facility near Palm Springs, CA, in 2000.

2. Estimated size when tortoise shell hardens. Nagy and others (2011) found that shell hardness increased with both age (n = 39) and shell length (n = 41) among juvenile tortoises raised under natural conditions at the Fort Irwin and Edwards Air Force Base headstart programs, CA. The predicted relationships between age and size with shell hardening indicated that juveniles obtained a shell-hardness index (SHI) value of 98% at 11.5 years and 109 mm carapace length at the midline (MCL), and an SHI value of 99% at 15 years and 130 mm MCL. This suggests that juvenile shells became essentially incompressible (under a moderate force of 11.2 g/mm^2) by about 11 years and 110 mm MCL, which could make them less vulnerable to predation at that age. The tortoises were first measured at about 7 months of age, at which time they exhibited high variability in SHI but averaged about 85% of full hardness.

3. Adult tortoises.

   a. Estimates of age at maturity.

      i. Germano (1994) based age at maturity on estimates of scute annuli from shells of dead North American tortoises from multiple sites across their geographic ranges. The author estimated mean age at maturity for desert tortoises at 14.4 years for the western Mojave Desert and 15.4 years for the eastern Mojave Desert, which were intermediate to other species of North American tortoises.
      ii. Medica and others (2012), in a long-term study of tortoises in outdoor pens at the Nevada Test Site, reported that six female desert tortoises of known age reached sexual maturity between 16 and 21 years, with an average age of 18.8 years.

   b. Sizes of adult tortoises in studies (examples only)

      i. Christopher and others (1999) studied reference ranges for hematology and plasma biochemistry of adult tortoises at three sites in the Mojave Desert, CA (Desert Tortoise Research Natural Area, Ivanpah Valley, and Goffs). The authors measured carapace lengths at the midline and weights four times per year for multiple years. Sample sizes for each sex and study area ranged from 16 to 45.
      ii. Wallis and others (1999) measured 76 female desert tortoises from Fenner Valley (eastern Mojave Desert) and the Desert Tortoise Research Natural Area, CA, over a 2 year period for a reproductive study. Females at Fenner Valley were smaller (midline carapace length, MCL = 214 mm) than females at the Desert Tortoise Research Natural Area (234 mm MCL).
      iii. McLuckie and others (2002) encountered 850 live tortoises in 4 years; 702 of these tortoises were ≥ 180 mm (57 of these had sizes estimated because they could not be extracted from shelters). For all live tortoises measured, median carapace lengths ranged from 41 to 316 mm.

iv. Lovich and others (2011c) reported that male tortoises near Palm Springs, CA appeared to be mature when carapace length (CL) was >200 mm. The smallest mature female as shown by egg presence was 221 mm carapace length.

c. Sexual dimorphism (adults)

i. Germano (1994) reported that males are significantly larger than females in terms of both mean carapace length and upper quartile carapace length for desert tortoises from both the western and eastern Mojave Desert.

ii. Curtin and others (2009) evaluated shell remains from samples collected in the western Mojave Desert, CA, and reported that tortoises were sexually dimorphic in adult size. Females had significantly smaller carapace length compared with Mojave males. Energy savings from small-sized female tortoises may be invested into increased reproduction and survival during periods of low rainfall and limited forage in the western Mojave Desert.

C. Growth in Desert Tortoises

1. Estimates of growth rates using scute annuli (see also I.A., this chapter)

a. Germano (1992) used scute annuli from shells of live and dead desert tortoises originating from multiple sites across the eastern and western Mojave Desert, Sonoran Desert, and Sinaloan scrub, to estimate growth rates. Growth rates varied by region. Comparisons of carapace length to age showed the highest rates of growth (0–25 years) for tortoises from the western Mojave Desert and Sinaloan habitats, with lowest rates of growth in the eastern Mojave and Sonoran Deserts. The slope of the linear regressions for tortoises from the western Mojave Desert was significantly steeper than for tortoises from the eastern Mojave Desert. Based on these regression equations, desert tortoises grow at the highest rate (61–76.5%) from ages 1 to 5 and at the lowest rate (24.6–25.8%) from ages 15–20 years. NOTE: after this paper was published, three different species of desert tortoise were identified.

b. Germano (1994) estimated ages of North American tortoises from multiple sites across their geographic ranges using scute annuli from shells of dead animals.

i. *Gopherus agassizii* was intermediate in size and growth rates compared with *G. flavomarginatus* (largest/fastest) and *G. berlandieri* (smallest/slowest) but similar to *G. polyphemus.* All species grew relatively fast for 18 to 22 years, after which time growth rates decreased greatly.

ii. Growth rate differences between male and female tortoises occurred by about 20 years. Females showed distinctly slower growth rates than males in *G. agassizii* in the western Mojave starting at about 9 years and in the eastern Mojave starting as early as 3 years.

   iii. Several measures of growth did not correlate with precipitation among species of *Gopherus*, although mean yearly growth was negatively correlated with mean annual precipitation in populations of *G. agassizii.* These preliminary analyses suggest that the environment is not the main determinant of growth rates in desert tortoises or other North American tortoises.

2. Curtin and others (2009) evaluated shell remains collected from the western Mojave, CA (Highway 58 study, William Boarman) and from sites in the Sonoran Desert, AZ. Using skeletochronology, the authors reported that both male and female tortoises from the western Mojave Desert grew significantly faster than Sonoran desert tortoises, which may be evolutionary adaptations for a shorter life span and increased physiological stress, resulting from low annual rainfall and high variability in rainfall in the Mojave Desert.

3. Reiber and others (1999) studied effects of temperature on growth rates of hatchling tortoises maintained at different temperatures at the University of Nevada, Las Vegas.

   a. Tortoises maintained at 28 °C increased in mass at higher rates compared to those held at 19 and 37 °C. Once treatment groups were returned to a common holding pen (28 °C) growth rates were similar between groups.

   b. Tortoises maintained at 37 °C showed significant changes in body form compared to the 19 and 28 °C groups, exhibiting a “flat” body form (i.e., reduced length and height) that resulted in a high surface area-to-volume ratio.

4. Lovich and others (2011c), in a study of tortoises near Palm Springs, CA, developed a growth equation for male carapace length (CL) in millimeters as a function of tortoise estimated age. Using their equation, the authors suggested a slower male growth rate than reported elsewhere despite the larger body sizes. The growth equation for female carapace length was provided but parameterization did not result in biologically realistic estimates based on previously published data.

5. Medica and others (2012) conducted a long-term study of growth rates in semi-wild desert tortoises in 9-ha pens at the Nevada Test Site using mark-recapture techniques. Growth of a cohort of 17 males and females did not differ significantly until they reached 23–25 years. After that age, the differences between the sizes of males and females became significantly different as males continued to grow slowly while female growth leveled off. This may be due to females allocating more energy to reproduction than growth after reaching sexual maturity. Annual tortoise growth was correlated with winter rainfall, which in turn was highly correlated with growth of ephemeral vegetation. Growth rates appeared to be correlated with changes in year-to-year fluctuations in winter precipitation and tortoises grew minimally in years where winter rainfall was < 26 mm. Years of highest growth coincided with years of highest rainfall and concomitant high ephemeral plant production.

6. Indeterminate growth. Nafus (2015) measured growth rates a colony of 999 captive and formerly wild, adult (≥ 175 mm carapace length) *Gopherus agassizii* (this group of tortoises may have contained some *G. morafkai*) at the Desert Tortoise Conservation Center, NV. The author concluded that growth rate was related to size, larger individuals grew less than smaller ones, and the majority of individuals appeared to stop growth at some point during adulthood. Tortoises appeared to display a finite growth potential that is regulated by size, sex, and potentially age, but both sexes can grow well past sexual maturity.

D. Longevity

1. Germano (1992), using scute annuli from live and dead tortoise shells (n = 574) from the Mojave and Sonoran Deserts and Sinaloan scrub, reported the maximum age rarely exceeds 40 years.
    a. The greatest estimates of longevity from the different regions were 48 to 53 years from the eastern Mojave Desert, 32 years from the western Mojave Desert, and 32 years from the Sonoran Desert.
    b. Approximately 29% of tortoises from the Sonoran Desert, 11% of tortoises from the eastern Mojave Desert, and 5% of tortoises from the western Mojave Desert were estimated to be over 25 years.
2. Curtin and others (2009) used skeletochronology to compare estimated ages of tortoises from different regions. Specimens were from the western Mojave Desert (Highway 58 study, William Boarman) and the Sonoran Desert, AZ. The authors reported that the oldest western Mojave males reached 56 years compared with 27 years for females. In comparison, the oldest Sonoran males reached 47 to 54 years and the oldest Sonoran females reached 42 to 43 years, the latter significantly older than western Mojave females.
3. Medica and others (2012), in a 47-year study of semi-wild tortoises in pens at the Nevada Test Site, reported that the average age of long-term surviving tortoises was 43 years (range = 39–47 years). Approximate ages may be estimated using body length with some reservations, e.g., one female could not be reliably aged using body length as she became stunted and did not grow much beyond the age of 13 years. The lifespans described for tortoises in this study were shorter than those assumed (e.g., up to 83 years) and may alter assumptions for population modeling and viability analyses.

II. Sex Ratios in Wild Populations

A. Sex is Determined by Temperature of Incubated Eggs

Rostal and others (2002) conducted experiments on incubated eggs at the Desert Tortoise Conservation Center, Las Vegas, NV. Incubation temperatures of approximately 30.5 °C or below produced all males, and temperatures of approximately 32.5 °C or above produced all females. The estimated pivotal temperature to produce a 1:1 sex ratio was approximately 31.3 °C. The relatively high pivotal temperature relative to other turtles may reflect the warm habitats of desert tortoises. Incubation temperatures provide a basis for predicting hatchling sex ratios in natural populations of desert tortoises.

B. Determining Sex of Tortoises at an Early Age

1. Rostal and others (1994b) conducted a study at the Desert Tortoise Conservation Center in Las Vegas, NV, and evaluated two methods for sexing hatchlings (48.1–59.5 mm straight carapace length [SCL]), juvenile (57–90 mm SCL), and immature desert tortoises (136–190 mm SCL): laparoscopy and 2b plasma testosterone with blood samples from the jugular vein. Laparoscopy was 100% accurate and could be used on hatchlings as small as 28 g total body mass, whereas plasma testosterone was 98% accurate for juvenile and immature tortoises ranging from 69 to 190 mm straight line carapace length. Juvenile and immature male tortoises ranging from 57 to 190 mm SCL displayed significantly higher plasma testosterone levels than females in the same size range. Female levels were consistently < 200 pg/mL whereas male levels ranged >200 pg/mL to 9.48 ng/mL. Their results suggest that plasma testosterone was the most suitable methodology for field studies in that only a small blood sample was required for sexing purposes while laparoscopy may require holding animals for an extended period of time. Male and female tortoises may display increased testosterone secretion in response to prolonged handling stress. These non-lethal sexing techniques can aid understanding of sex ratios in desert tortoises.

2. Boone and Holt (2001) took 22 morphometric measurements on each of 105 tortoises (range in carapace length = 52 to 299 mm) at the Yucca Mountain study area in northern Nevada. The objective was to be able to use external morphology to identify sex in young tortoises. They used discriminant function analysis and were able to unambiguously assign gender to individuals as small as 140 mm carapace length, and potentially even smaller individuals.

C. Sex Ratios Reported in Wild Populations of Desert Tortoises

1. Berry and others (2006a) surveyed tortoises between 1997 and 2002 at 15 Goldstone sites and 6 sites at the National Training Center, Fort Irwin, CA, and reported sex ratios for subadult and adult tortoises (≥180 mm MCL). The Goldstone sites were treated as one because of sample size; sex ratios only differed significantly from the expected 1:1 ratio at 1 of 7 sites (Langford), which had a male to female ratio of 9:2.

2. Nussear and others (2008) reported a high ratio of male to female tortoises (2.56:1) found by both detector dogs and human teams at the Fort Irwin Army National Training Center, CA. While this may have reflected true differences in sex ratio, it may also have reflected physiological or behavioral differences, such as earlier hibernation in females.

3. Berry and others (2008) studied tortoises on a 4 km^2 plot at Red Rock Canyon State Park, CA, and found five tortoises >180 mm midline carapace length. Three were male, one was female, and one was unidentified as to sex.

4. Keith and others (2008) studied tortoises in the Jawbone-Butterbredt Area of Critical Environmental Concern, CA, on 751 one-ha plots. Tortoises were very rare; only five live individuals were located: three adult females, one adult male, and one unidentified tortoise in a burrow. All four tortoises were old adults and exhibited advanced stages of shell-wear or aging.

5. Lovich and others (2011b), in a long-term study of tortoises at a wind-energy developed site in the western Colorado Desert, reported that the sex ratio of marked tortoises (n = 51) did not differ statistically from 1:1.
6. Berry and others (2013) sampled live tortoises at the China Lake Naval Air Weapons Station in the northwestern Mojave Desert, CA. The authors reported that the sex ratio of adult tortoises was 3:10 (males to females) and differed significantly from the expected 1:1 sex ratio.
7. Berry and others (2014a) surveyed an area of about 260 km^2 using 240 randomly located 1-ha plots in the western Mojave Desert, CA. The study objectives involved evaluating the results of three different management strategies on desert tortoise abundance. The three equally sampled areas were: the Desert Tortoise Research Natural Area, critical habitat in the western Rand Mountains and Fremont Valley, and recently acquired lands for tortoise conservation. Seventeen live tortoises were found on plots and 27 tortoises off the plots. For on-plot tortoises the male to female subadult and adult sex ratio was 4:9 and not statistically different from 1:1; for the off-plot tortoises, the sex ratio was 12 to 10 and not significantly different from the expected 1:1 ratio.
8. Berry and others (2015a) evaluated 1,004 desert tortoises in an epidemiological study conducted on and adjacent to the National Training Center at Fort Irwin in the central Mojave Desert, CA. Of the 1,004 tortoises evaluated, 389 were females and 615 were males, with the exceptions of 2 large immature females and 1 large immature male, all tortoises were subadult to large adults ≥ 180 mm carapace length at the midline.

III. Age Structure Observed in Wild Populations

A. McLuckie and others (2002) encountered 850 desert tortoises over 4 years at the Red Cliffs Desert Reserve near St. George, UT. The age structure of tortoises that could be measured (n = 793) was 60 (7.1%) juveniles (carapace length, CL < 99 mm), 88 (10.4%) immature (CL 100–179 mm), 51 (6.0%) subadult (CL 180–207 mm), and 594 (69.9%) adult (CL ≥ 180 mm).

B. Keith and others (2008) studied tortoises in the Jawbone-Butterbredt Area of Critical Environmental Concern, CA, on 751 one-ha plots. Tortoises were very rare; only five live individuals were located: three adult females, one adult male, and one unidentified individual in a burrow. All four tortoises were old adults and exhibited advanced stages of shell-wear or aging.

C. Berry and others (2008) studied tortoises at Red Rock Canyon State Park, CA, in a 4 km^2 plot and recorded nine live tortoises: four were immature and five were adults (range = 205–296 mm in midline carapace length).

D. Lovich and others (2011c), in a population of tortoises at the Mesa Wind Farm near Palm Springs, CA, found that the population had predominantly more adults and hatchlings than two other California populations.

E. Berry and others (2013) studied tortoises on a 5.42 km^2 area at China Lake in the northwestern Mojave Desert, CA, and found 28 live tortoises, of which 3 were juveniles < 60 mm midline carapace length (MCL), 5 were juveniles 60–99 mm MCL, 5 were immatures 100–139 mm MCL, 2 were immatures 140–179 mm MCL, and 13 were adults ≥ 208 mm MCL. Overall, 46.5% were adults and 53.6% were immature and juvenile tortoises. The immature 2 class was poorly represented and no subadults were observed.

F. Berry and others (2014a) survey an area of about 260 km^2 using 240 randomly located 1-ha plots in the western Mojave Desert, CA. The study objectives involved evaluating the results of three different management strategies on desert tortoise abundance. The three areas, sampled equally, were: the Desert Tortoise Research Natural Area, critical habitat in the western Rand Mountains and Fremont Valley, and recently acquired lands for tortoise conservation. Seventeen live tortoises were found on plots and 27 tortoises off plots. On-plot counts were 12 tortoises on the Natural Area, 2 tortoises in critical habitat, and 3 tortoises on private lands. Adults composed the majority of samples for both on- and off-plot tortoises. For the 17 on-plot tortoises, the composition was two juveniles, two large immature tortoises, two small adults, and 11 adults ≥ 208 mm MCL.

IV. Densities and Trends Observed in Wild Tortoise Populations

A. California

1. Jacobson and others (1994), in a review of the literature, reported downward trends in the western Mojave Desert population of tortoises following declines, decreasing proportions of juveniles, high rates of deaths in the breeding population, and human-induced sources of mortality.
2. Brown and others (1999), monitored health and epidemiology of upper respiratory tract disease at the Desert Tortoise Research Natural Area, CA, and reported a significant decline in population density occurring over a 17-year period. Populations of adult tortoises were >50/km^2 in 1979, 1982, and 1988, compared with 1992 and 1996 (estimated density of <10/km^2). This apparent population decline was concurrent with and subsequent to the observation of clinical signs of upper respiratory tract disease in the population in 1988.
3. Freilich and others (2000) considered a population of tortoises on a plot in Joshua Tree National Park, CA, to be stable at 67 tortoises (42 adults / km^2) between 1991 and 1995. In 1996, they estimated a lower population size (43 tortoises) and attributed the lower figure to reduced observed movement and detection during drought conditions rather than a change in population size.
4. Krzysik (2002) using distance sampling and spatial modeling reported that overall desert tortoise densities were 8/km^2 at the Marine Corps Air Ground Combat Center and 11/km^2 at Joshua Tree National Park, CA. However, estimated tortoise densities were scale dependent and more variable at smaller spatial scales, indicating that tortoises were patchy in landscape distribution.

5. Berry and others (2006a) surveyed 21 plots at the National Training Center, Fort Irwin, CA. Fifteen of the plots were on the Goldstone portion of the base and figures were treated collectively. Densities of all sizes of tortoises are presented as number/km^2 with the 95% confidence interval in parentheses: in the Goldstone unit, 1.146 (0.33–2.20); for Alvord Slope, 17 (9–31); for Langford, 6 (2–16); for Tiefort Mountains, 28 (18–44); for Eastgate 1, 1.4 (0—3.3); for Eastgate 2, 13 (3–50); and for Soda Mountains, 28 (17–45). Densities for subadults and adults (number/km^2 with the 95% confidence interval in parentheses) were: 0.788 (0.13–1.6) for the Goldstone unit; 14 (7–26) for Alvord Slope, 5 (2–16) for Langford; 15 (9–25) for Tiefort Mountains; 1.4 (0–3.3) for Eastgate 1; 12 (3–43) for Eastgate 2); and 11 (7–19) for Soda Mountains. Death rates for subadult and adult tortoises ranged from a low of 1.9% in the Tiefort Mountains to a high of 95.2% for the 15 Goldstone plots. Densities of live tortoises were negatively correlated with death rates.

6. Keith and others (2008) evaluated 187.7 km^2 of habitat for desert tortoises using 751 one-ha plots in the Jawbone Butterbredt Area of Critical Environmental Concern and Red Rock Canyon State Park, CA. The authors reported densities throughout the entire study area to be 0.4 (95% confidence interval [CI] = 0.08–1.16) tortoises/km^2. Two areas of higher densities were present: 3.6 ± 2.32 SD (95% CI = 0.44–12.31) adult tortoises/km^2 in the Kiavah Apron region, and 2.7 ± 2.63 SD adults/km^2 in the Red Rock Canyon watershed.

7. Berry and others (2008) reported that the densities of tortoises in the western part of Red Rock Canyon State Park and watershed, CA, were probably ≤ 18 tortoises/km^2, the upper limit of one confidence interval (CI) estimate. For the landscape plots within the Park, the densities were estimated at 3.57 (95% CI = 0.09–18.35) tortoises/km^2 and, for the Red Rock Canyon watershed, 2.7 (95% CI = 0.07–14.16) tortoises/km^2. Data from shell-skeletal remains of subadult and adult tortoises on a 1-km^2 plot suggest that numbers of subadults and adults were almost three times higher in 2000 than 2004, and that deaths in this size-age class exceeded recruitment.

8. Inman and others (2009) recorded activity of 35 adult tortoises in the Superior-Cronese and Ord-Rodman Desert Wildlife Management Areas, CA, and estimated density of 5.3/km^2 for the Western Mojave Recovery Unit. This estimate was not significantly different from other published estimates for the area. However, the Coefficient of Variation was higher when accounting for daily variation in g_0 compared with an estimate of g_0 that was pooled across individuals.

9. Lovich and others (2011b) sampled 69 marked tortoises for growth and demographic parameters at a wind energy facility near Palm Springs, CA, and estimated population size at 96 tortoises (95% confidence interval = 80–130 tortoises) or about 15.4 tortoises/km^2 using program CAPTURE.

10. Berry and others (2013) surveyed a 5.42 km^2 study plot in the northwestern Mojave Desert, CA, and reported that densities of live tortoises developed from predicted live tortoise density models, ranged spatially from 1.2/km^2 to 15.2/km^2 over the plot. The crude density of adult tortoises was 2.4 tortoises/km^2 (13 adults/5.42 km^2). The predicted density depended on location within the study area,

with lower predicted densities associated with the end of a paved road where military testing occurred and habitat was denuded. The crude annual death rate for subadult and adult tortoises during the 2006–2010 period was 1.8%. The authors noted that this study represented a low-density isolated population fragment vulnerable to extirpation, with positive demographic attributes, including a sex ratio favoring females, a high proportion of juvenile and small immature tortoises and relatively healthy individuals. However, the authors also suggested that the population remained vulnerable to stochastic events such as drought and hyperpredation.

11. Berry and others (2014a) surveyed tortoises in an area of about 260 km^2 to evaluate three types of management strategies used in adjacent areas in the western Mojave Desert, CA: the fenced Desert Tortoise Research Natural Area, critical habitat, and unfenced private lands recently acquired for conservation. Density estimates for all tortoises and adult tortoises only were calculated for all three areas separately. For all sizes of tortoises, density estimates and confidence intervals (in parentheses) for tortoises/km^2 were: 14.8 (14.6–15.1) at the Desert Tortoise Research Natural Area; 2.4 (2.3–2.6) in critical habitat, and 3.7 (3.6–3.8) on private lands. For densities of adult tortoises/km^2 only, the figures were 10.2 (9.9–10.4) for the Natural Area, 2.4 (2.3–2.6) for critical habitat, and 3.7 (3.6–3.8) for private lands. Juvenile and immature tortoises were only found on the Natural Area. The crude annual death rates for adults for the 4 years preceding the survey were 2.8%/year for the Natural Area, 20.4% for critical habitat per year, and 6.3% per year for private lands.
12. Lovich and others (2014a) estimated abundance of adult tortoises from 1991 to 2012 in Joshua Tree National Park, CA, and reported that populations remained stable from 1993 to 1996, but declined greatly from 1996 to 2012. Results indicate a decreasing density over time, and, combined with climate models that suggest the region will be subjected to longer droughts, the site may become unsuitable for continued tortoise survival over the long-term.

B. Nevada

Longshore and others (2003) studied tortoises at the Cottonwood and Grapevine study sites at Lake Meade National Recreation Area, NV. The authors estimated populations of adult tortoises at 61 and 63 tortoises, respectively, from 1992 to 1995. The Grapevine site also contained more young tortoises. Survival rates were high from 1992 to 1995, but started to diverge in 1996, with a 7-year (1994–2001) annual survival rate of 0.985 at Grapevine and 0.829 at Cottonwood. The die-off in 1996 at Cottonwood was 30% of the radio-monitored adults. Drought conditions with lack of annual production continued from 1996 to 1999.

C. Utah

McLuckie and others (2002) surveyed populations of adult tortoises using line-transect distance sampling in Red Cliffs Desert Reserve, UT. The authors estimated population size of adult tortoises at 3267 per 10,176 ha sampled in management Zone 3, or a density of 0.32/ha (95% CI, 0.29–0.36). The estimated population size of adult tortoises was 3354 tortoises per 11,457 ha pooled in management Zones 2, 3, and 5, or a density of 0.29/ha (95%CI, 0.26–0.33).

V. Mortality (for Injuries and Trauma, see Chapter 8 on Health and Disease; for Nest Predation, Hatching Success and Survival, see Chapter 6, IV.C.)

A. Causes of Death. This list is presented in descending order of the number of published papers with known or suspected case or cases where a cause of death was assigned.

1. Unknown predators.
    a. Homer and others (1998) conducted necropsies of 24 ill, dying, or recently dead tortoises from the Mojave and Colorado Deserts and reported that, five tortoises experienced traumatic injuries. Of these cases, one tortoise was attacked by an unknown predator, and had chew marks associated with partial amputation of the cranium, maxilla, and the dorsal neck region, ulceration of the tongue, and perforation of the trachea.
    b. Franks and others (2011) radio-tracked 60 tortoises across four study sites during the second year of their research and reported that three female tortoises were found dead (5% mortality rate) at their Superior and Ivanpah Valley study areas, CA. One was predated and the cause of death of the other two was unknown.
    c. Ennen and others (2012b) found relatively low overall tortoise nest depredation (12% of 29 clutches from 15 females), all of which were second clutches at the Mesa Wind Farm near Palm Springs, CA. Species of predator(s) were unknown.
2. Mammalian predators.
    a. Coyotes (*Canis latrans*)
        i. Peterson (1994) monitored 55 radio-telemetered tortoises at the Desert Tortoise Research Natural Area (DTRNA) and Ivanpah Valley in the northeastern Mojave Desert and found significantly different proximate causes of mortality between sites. In contrast with mortality attributed to starvation or dehydration at Ivanpah Valley, deaths of 20 of 33 tortoises at the DTRNA appeared to be due to predation or scavenging by coyotes. Peterson attributed the high mortality at the DTRNA to indirect effects of drought through functional responses of predators to a diminished prey base, but he did not disregard the possibility that tortoises lost to coyotes might have had subclinical Upper Respiratory Tract Disease that made them more susceptible to predation.
        ii. Esque and others (2010a) concluded from their study of nine sites across the range of desert tortoises in the Mojave Desert that high adult tortoise mortality was correlated with size of nearby human population, the surface roughness of the landscape, and the size and sex of tortoises with other potential contributing factors including distance to the human population and road density. Smaller tortoises, females, and those in flat and open areas were most likely to be found dead. Because these tortoises were overtly healthy when last observed and fresh predator tracks, scat, and chew marks were seen on the carcasses, the authors assigned cause of death as predation by coyotes. Tortoise mortality rates

ranged from 0 to 43.5% in 2008, although two sites had no mortality and seven sites had higher mortality than previous years across the range of the tortoise. In 2006 and 2007, average annual rainfall was below normal, resulting in drought conditions prior to 2008. The precipitation patterns likely reduced population levels of the normal prey base for coyotes, making alternative prey such as tortoises more vulnerable to predation, especially in areas near high human population densities (note potential influence of urban).

- iii. Berry and others (2013), in a study of tortoises at a site in the northwestern Mojave Desert, reported remains of three tortoises (1 juvenile in a coyote scat) at predator sign concentration areas: two sites were scat marking areas and the third was at a coyote rock shelter.
- iv. Lovich and others (2014b) conducted surveys in Joshua Tree National Park, CA, and reported that some live and many dead tortoises found in 2012 showed signs of predation or scavenging by mammalian carnivores. Coyote scats and other evidence from site confirmed their role as tortoise predators and scavengers. Predation may be exacerbated by drought if carnivores switch from preferred mammalian prey to tortoises during dry years.
- v. Nagy and others (2015a) conducted a 3-year study on 53 juvenile and immature tortoises released from the head-start facility at the National Training Center, Fort Irwin, CA. Of the 53 released tortoises, coyotes killed the majority of the tortoises in the largest size class, 111–175 mm carapace length.

b. Kit fox (*Vulpes macrotis*): possible kit foxes. Bjurlin and Bissonette (2004) reported a relatively low overall rate of tortoise nest predation (26% of 42 nests) at the Sand Hill Training Area, Twentynine Palms, CA, but with higher predation rate in 1998 (47%) compared with 1999 (12%). Some tortoise nests were near kit fox natal dens, and kit fox scat and tracks were found around most depredated nests. The researchers thought their weekly nest monitoring in 1998 versus two total visits in 1999 might have facilitated predator detections of the nests.

c. Bobcat (*Felis rufus*) and/or Mountain lion (*Puma concolor*)

- i. Field and others (2007) conducted a translocation study in Nevada and reported six deaths. One female appeared to have died and been partially buried either by a bobcat or mountain lion.
- ii. Medica and Greger (2009) found eight adults and one juvenile desert tortoise dead during a long-term study on tortoise growth at the Nevada Test Site. Seven adults had the central portion of their carapace broken open and three of the carapaces had puncture marks with intercanine distances consistent with an adult mountain lion.
- iii. Nagy and others (2015a) conducted a 3-year study on 53 juvenile and immature tortoises released from the head-start facility at the National Training Center, Fort Irwin, CA. Of the 53 released tortoises, evidence indicated that bobcats killed some of the tortoises the size range of 81 to 175 mm carapace length.

d. Badgers (*Taxidea taxus*). Emblidge and others (2015), in a study of desert tortoises in the central Mojave Desert, CA, reported the badger as a potential predator in high mortality at one of four study populations (22 and 84%, respectively) in 2012 and 2013.

e. Unidentified mammalian predators.

    i. Berry and others (2006a), in a study of desert tortoise populations at 21 sites in the central Mojave Desert, CA, reported that from 0 to 53.1% of shell skeletal remains showed signs of either trauma (tooth punctures, cracking of scute or bone, gnaws, chews) or possibly scavenging from coyotes, kit foxes and other mammals.
    ii. Berry and others (2008), in a study of tortoises at Red Rock Canyon State Park, CA, on a 4.1 km^2 plot and 37 one-ha landscape plots, reported that of 58 shell-skeletal remains found, as many as 19 tortoises were killed by unidentified mammalian predators and one of these could be confirmed with certainty.
    iii. Keith and others (2008) studied tortoises within a 187.7 km^2 study area in the western Mojave Desert, CA, and reported finding nine shell-skeletal remains. One adult, dead about 4 years, had chew and puncture marks typical of a carnivore, indicating a kill or scavenging.
    iv. Nussear and others (2012), in a study of translocated tortoises across five sites in UT and NV, reported that 16 of 30 tortoise mortalities appeared to be due to canid predation.
    v. Berry and others (2013), in a study of a tortoise population in the northwestern Mojave Desert, CA, on a 5.42 km^2 plot found shell-skeletal remains of 16 juvenile and immature tortoises. Most remains showed signs of predation by unidentified mammals and Common Ravens. Models suggested that densities of tortoise sign increased with slope and signs of mammalian predators and decreased with ravens, but also varied based on interaction effects involving these predictors. The authors suggest that predation may have limited recruitment of young tortoises into the adult size classes.
    vi. Berry and others (2014a), in a study of tortoise populations in the western Mojave Desert, CA, in three different management areas, reported deaths from mammalian predators.

f. Dogs (*Canis lupus familiaris*): Berry and others (2014a), in a study of tortoise populations in the western Mojave Desert, CA, in three different management areas, reported evidence of death from a domestic dog.

g. Rodents or ground squirrels: Nagy and others (2015b), in a study of head-started hatchling and juvenile tortoises at Edwards Air Force Base, CA, reported that rodents preyed on eggs and killed hatchling tortoises before they could be released from head-start pens. Of 15 yearling tortoises released from outdoor enclosures in 2004–2005, 1 was killed by a rodent. Of 32 yearlings released from enclosures in 2007, 1 was known to be killed by a rodent.

3. Avian predation.
    a. Common Raven (*Corvus corax*) predation
        i. Camp and others (1993) sampled 226 pellets from 39 active Common Raven nests to assess diet composition in the eastern Mojave Desert. Desert tortoise remains were found in pellets from two nests (1.3% of pellets). Tortoise remains observed included leg bones, nails, scales, and skull parts.
        ii. Bjurlin and Bissonette (2004) noted that at the Sand Hill Training Area, Twentynine Palms, CA, Common Ravens in flocks of 15–60 frequently coexisted with the 26 radio-tagged neonate tortoises each September, but ravens killed no tagged neonates.
        iii. Berry and others (2006a), in a study of tortoise populations at 21 sites on the National Training Center, Fort Irwin, CA, reported that a few tortoise remains showed signs typical of kills by *Corvus corax.*
        iv. Lovich and others (2011c) found 11 dead tortoises during a 4-year study at the Mesa Wind Farm near Palm Springs, CA, and noted that some of the juvenile and immature deaths showed signs of predation by Common Ravens.
        v. Berry and others (2013) studied a tortoise population in the northwestern Mojave Desert, CA, and reported that shell-skeletal remains of 16 juvenile and immature tortoises were found. Of the 16 juvenile and immature tortoises, most showed signs of predation by Common Ravens and unknown mammals. Models suggested that densities of tortoise sign increased with slope and sign of mammalian predators and decreased with ravens, but also varied based on interaction effects involving these predictors. The authors suggest that predation may have limited recruitment of young tortoises into the adult size classes.
        vi. Berry and others (2014a) conducted a study of tortoise populations in three adjacent areas, each with different management strategies, in the western Mojave Desert. The authors reported that three of seven recent juvenile and immature remains found on plots showed signs of having been killed by Common Ravens or small animals. Causes of death for dead tortoises found off plots included predation by ravens.
        vii. Nagy and others (2015b) reported that all yearlings that were released (in autumn) from head-start enclosures at Edwards Air Force Base, CA, were dead within 6 months regardless if they had supplemental “rain” (i.e., added water via irrigation) or not. The primary cause of mortality was predation by Common Ravens. Of 15 yearlings released in September of 2004 and 2005, 7 were killed by ravens. Of 32 yearlings released in September 2007, 21 were killed by ravens (one possibly killed by hawks).

viii. Hazard and others (2015) tracked 16 juvenile tortoises for 3 months after release from head start pens at the National Training Center, Fort Irwin, CA, in 2001. Between day 21 and day 64, seven tortoises with radio transmitters were killed, apparently by a single Common Raven. The carcasses with transmitters still functional were at the base of powerline pylons 3 km from the site.

ix. Nagy and others (2015a) conducted a 3-year study on 53 juvenile and immature tortoises released from the head-start facility at the National Training Center, Fort Irwin, CA. Of the 53 released tortoises, ravens were suspected of killing >70% of the tortoises the smallest size class (45 to 80 mm carapace length). All 7 tortoises that were killed had initial masses of ≤125 g and a carapace length of ≤84 mm.

b. Burrowing Owl (*Athene cunicularia*). Walde and others (2008) found vertebral and marginal scutes and bones of a desert tortoise at least a year old inside a Burrowing Owl pellet northeast of Barstow, CA.

c. Avian predation (general). Keith and others (2008), in a study of a 187 km^2 area in the western Mojave Desert, CA, reported locating nine shell-skeletal remains (six adults, three juveniles). One of the juveniles showed signs of avian predation.

4. Nagy and others (2015b), in a study of head-started *Gopherus agassizii* at Edwards Air Force Base, CA, reported that fire ants killed hatchling and juvenile tortoises in the head start pens.

5. Dehydration and starvation usually the proximate cause with drought as the ultimate cause

a. Peterson (1994) found that although the proximate causes of mortality appeared to differ between radio-telemetered tortoises at the Desert Tortoise Research Natural Area (DTRNA) in the western and Ivanpah Valley (IV) in the northeastern Mojave Desert, he attributed the ultimate cause of death as the effects of drought. Mortality of 9 of 22 tortoises at IV was due to starvation or dehydration. The dead tortoises previously had symptoms of dehydration (high plasma osmolality) and/or starvation (high blood urea), as compared to tortoises that survived. Essentially no annual forage was available in 1989 and 1990 and annual rainfall was low in 1989. In contrast, mortality of 20 of 33 tortoises at the DTRNA appeared to be due to predation by coyotes, suspected to be indirectly caused by either (i) functional responses of predators to a diminished prey base (prey switching), or (ii) tortoises becoming more susceptible to predation due to Upper Respiratory Tract Disease.

b. Peterson (1996b) sampled 48 adult desert tortoises from the Desert Tortoise Natural Area and Ivanpah Valley, CA, and reported that tortoises conserved water by retaining salts and metabolic wastes inside their bodies and tolerated consequent increases in body fluids solutes that would be lethal to many vertebrates. To rehydrate and balance electrolyte budgets, they were completely dependent on periodic drinks of water from unpredictable rains.

Because of their abilities to conserve water and tolerate temporary lack of homeostasis, as well as their behavioral propensity for fossorial inactivity, desert tortoises can wait out drought periods and opportunistically exploit unpredictable resources. However, the abilities to cope with extreme conditions are occasionally pushed to the limit and several tortoises died at the study site apparently from drought-related dehydration and starvation.

c. Berry and others (2002b) reported that clinical signs and hematological and plasma biochemical values suggested dehydration and starvation and were likely causes of mortality for 10 of 11 monitored tortoises in central, northeastern, and eastern Mojave Deserts, CA. Most tortoises either died or were salvaged moribund in months following hydrologic years when rainfall was below the long-term means for winter and annual precipitation, suggesting that drought conditions likely caused death. Such deaths may occur several weeks or months after the drought has broken.

d. Longshore and others (2003) reported that substantially and significantly lower annual survival of tortoises at one of two sites corresponded to limited rainfall and failure of annual plant growth. The limited rainfall and lack of annual plants appeared to cause mortality of almost one-third of adult tortoises likely due to starvation or dehydration within the Lake Mead National Recreation Area, NV.

e. Field and others (2007) found that providing supplemental water for captive desert tortoises prior to translocation was correlated with high rates of carapace growth and distant movements by males after release, especially within the first 2 weeks, but did not impact mortality. Although the mortality rate was 21.4% in 1997, data suggested that drought conditions at the site rather than the translocation itself negatively affected the tortoises, as none of the tortoises died during the second season after significant rainfall.

f. Lovich and others (2014b) conducted surveys in Joshua Tree National Park, CA, and reported a decline in apparent survival of tortoises from 1997 to 2002 occurred concurrent with persistent drought. The best model relating apparent survivorship of adult tortoises over time was based on a 3-year moving average of estimated winter precipitation. The postures and positions of the majority of dead tortoises found in 2012 were consistent with death by dehydration and starvation. Climate modeling suggested that the region will be subjected to even longer duration droughts in the future and the site may be unsuitable for continued tortoise survival.

g. Nagy and others (2015b) found that tortoise survivorship during the first year of life in head-start pens at Edwards AFB, CA, averaged 90% despite a record low rainfall year, although growth rates for juveniles that received supplemental “rain” via irrigation were 2 to 16 times greater than juveniles that were not supplemented. Body condition measurements for the non-supplemented juveniles indicated those individuals were able to maintain body condition similar to supplemented juveniles during 2 average rainfall years, but not during drought conditions. Older non-supplemented juveniles died during the latter part of the 16-month drought, suggesting that the high drought survivorship during the first-year may be related to the yolk they carried after hatching.

6. Vehicle strikes and crushing on paved and unpaved roads by off-road vehicles (ORVs), passenger, and military vehicles

    a. Homer and others (1998) conducted necropsies of 24 ill, dying, or recently dead tortoises from the Mojave and Colorado Deserts, and reported that two tortoises were struck by moving vehicles at the edge of a highway such that the carapace and plastron were fractured through the scutes and bone.

    b. Bury and Luckenbach (2002) in a study of an off-road vehicle area near Barstow, CA, found one immature tortoise crushed on a motorcycle trail.

    c. von Seckendorff Hoff and Marlow (2002) found tortoise carcasses on the road shoulders of two-lane SR-163 (n = 2) and four-lane US-95 (n = 4), both paved, in southern Nevada, suggesting the tortoises died from being struck by vehicles.

    d. Berry and others (2006a), in a study of tortoises on 21 plots at the National Training Center, Fort Irwin, CA, reported tortoise remains with signs of vehicle crushing on all plots, but were most common on five plots with recent or ongoing military maneuvers. The percent of shell-skeletal remains with signs of death from military vehicles ranged from 2.1% and 2.5% at the Alvord Slope and Goldstone plots, respectively to 15.8% at Langford and 45.5% at Eastgate 2. The authors noted that shell-skeletal remains are likely to be undercounted and death rates under-estimated on plots with military maneuvers, because remains are often crushed or buried with vehicular traffic. Deaths from vehicles were significantly correlated with surface disturbance, counts of trash, military ordnance, and proximity to paved roads and military offices.

    e. Berry and others (2013) modeled a tortoise population and multiple variables affecting distribution and abundance of tortoises at a site in the northwestern Mojave Desert, CA, drawing on data from 28 live tortoises, remains of 32 tortoises, and other tortoise sign. The models suggested that densities of tortoises increased with distances from paved roads, denuded areas, and ordnance, increased with slope and signs of mammalian predators, and decreased where Common Ravens were observed. Similarly, densities of live tortoises varied by interaction effects among distances to denuded areas and paved roads, density of ordnance fragments, and slope. The authors concluded that multiple factors predicted the density and distribution of this population.

    f. Hughson and Darby (2013) estimated that a minimum of 5.3 tortoises were killed on roads per year in the Mojave National Preserve, CA, between 2002 and 2012. The authors documented the depression in tortoise abundance adjacent to roads in the Mojave National Preserve.

   g. Berry and others (2014a), in a study of desert tortoise populations in three different but adjacent management areas in the western Mojave Desert, CA, reported that remains of tortoises likely to have been killed by vehicles were found in the Desert Tortoise Research Natural Area and on private lands.

   h. Lovich and others (2011c) reported finding 11 dead tortoises from 1997 to 2010 at a study area in the western Colorado Desert, CA (wind renewable energy site). One of these, a female adult with radio-transmitter, was killed by a vehicle.

   i. Nafus and others (2013) conducted a study of road effects in the Mojave National Preserve, CA, and reported that relative abundance of tortoise sign was greatest along roads with low traffic volume compared to roads with intermediate and high traffic volumes. Frequency of live tortoise encounters decreased with increasing traffic volumes and tortoise size also correlated significantly with traffic volume, such that tortoises near the highest traffic volume road were smallest. The authors indicated that cumulative mortality may play a role.

7. Wildfire

   a. Homer and others (1998) conducted necropsies of 24 ill, dying, or recently dead tortoises from the Mojave and Colorado Deserts, and reported that five tortoises experienced traumatic injuries. One tortoise was burned in a brush fire and subsequently died

   b. Review of the literature: see Brooks and Esque (2002)

   c. Esque and others (2003) sampled two Mojave Desert sites following large wildfires, and reported finding one dead tortoise. This finding suggests the potential for direct mortality from fire, although some tortoises at these sites did survive fire, perhaps by remaining underground.

   d. Lovich and others (2011b), studied tortoises during the first 15 years following a large fire at a wind energy generation facility, CA. One female tortoise was killed by the fire and four tortoises bore non-fatal scars.

   e. Nussear and others (2012), during a translocation study at five sites in Utah and Nevada, reported that 3 of 30 tortoises died as the result of wildfire.

8. Entrapment of tortoises, nest failure or burrow collapse caused by livestock, excessive rainfall, or flooding

   a. Homer and others (1998), necropsied a female adult tortoise from the western Mojave Desert, CA. The tortoise had been entombed and tightly packed in dirt within the burrow, was lethargic, and had a cutaneous fungal infection as well as multicentric visceral inflammation.

   b. Christopher (1999) reported a tortoise pinned in its burrow by rock fragments and had to be physically removed.

   c. Bjurlin and Bissonette (2004), in a tortoise study at Twentynine Palms, CA, reported entombment of some nestlings in the nest.

- d. Field and others (2007) reported six deaths of translocated tortoises in Nevada. One female was found dead in a collapsed burrow, encasing the carcass in soil and cobbles. The circumstances suggest that this tortoise remained in the burrow during a rainstorm and did not dig itself out when the burrow collapsed.
- e. Lovich and others (2011a) documented a large male tortoise that used a 60-cm (diameter) culvert underneath a lightly traveled dirt road as a brumation site near Palm Springs, CA, and was subsequently entombed in a large amount of sediment from winter storms. Tightly-packed sediment prevented the tortoise from being able to dig out. The tortoise died 18 days after excavation, and necropsy revealed the animal likely had pneumonia from being immersed in mud.
- f. Nussear and others (2012), during a translocation study at five sites in Utah and NV, reported that one tortoise died as a result of burrow collapse by livestock and one by flooding.

9. Gunshot
    - a. Berry and others (2006a), in a study of tortoise populations at the National Training Center (NTC), Fort Irwin, CA, reported that tortoise remains with gunshot evidence were observed at the 15 Goldstone sites (1.2% of 135 shell-skeletal remains) and at Alvord Slope (8.5% of 47 shell-skeletal remains), locations accessible to unauthorized personnel because of proximity to the NTC boundary.
    - b. Berry and others (2008), studied a population of tortoises at Red Rock Canyon State Park, CA, on a 4.1 km^2 plot and reported that 5 of 58 shell-skeletal remains showed evidence of death by gunshot.
    - c. Berry and others (2014a), in a study of tortoises within three different but adjacent management areas in the western Mojave Desert, CA, reported remains of tortoises with evidence of gunshots within both the Desert Tortoise Natural Area and critical habitat.
10. Disease. See examples of papers where disease was known or suspected to be responsible or implicated in deaths of tortoises: Jacobson and others (1991, 2009, 2012); Peterson (1994); Homer and others (1998); Brown and others (1999); Christopher and others (2003); Berry and others (2002b, 2006a); Field and others (2007); Nussear and others (2012); Jacobson and Berry (2012).
11. Other
    - a. Guzzlers for Upland Game in the Wild. Andrew and others (2001) found no remains of desert tortoises in artificial water catchments surveyed in July and August 1998 in the Sonoran Desert of CA, suggesting that artificial water sources of the passive design studied did not present a serious and direct drowning hazard to tortoises. However, researchers did retrieve carcasses of 4 mourning doves, 2 kangaroo rats, 1 house sparrow, 1 bat, and 165 skeletal fragments representing at least 30 individuals consisting of 14 mammal, 12 bird, and 4 reptile species. The authors stated the remains were consistent with

breakage associated with predation by birds and mammals and hypothesized that most of the remains came from scats deposited by canids or from pellets cast by owls or diurnal raptors that were subsequently blown into the catchments.

b. Research-related deaths. Berry and others (2002b) reported on deaths of 11 monitored tortoises from the central, eastern, and northeastern Mojave Desert, CA. Crowding, deteriorated habitat, and drought, likely contributed to low growth rates, low weights, and deaths of juveniles at experimental enclosures at Fort Irwin. For adults in other projects, manipulative handling and invasive research procedures (cystocentesis) were likely factors that contributed to deaths.

c. Nest predation by Gila monsters (*Heloderma suspectum*): Gienger and Tracy (2008) observed Gila Monsters appearing to co-occupy shelters (not just tortoise burrows) with desert tortoises, almost exclusively with females, during the nesting season on 16 occasions in 3 years. Yet, at three different times researchers observed tortoises unsuccessfully defending their burrows against Gila Monsters entering, with subsequent observations the following mornings of tortoise eggshell fragments inside, suggesting the nests were likely predated by the invading Gila Monsters.

d. Exposure to temperature extremes.

   i. Nussear and others (2012), in a study of translocated tortoises at five sites in Utah and NV, reported that 3 of 30 tortoises died as a result of exposure to temperature extremes.
   ii. Nagy and others (2015a) conducted a 3-year study on 53 juvenile and immature tortoises released from the head-start facility at the National Training Center, Fort Irwin, CA. Of the 53 released tortoises, 32 died; 3 of the deaths were due to exposure (freezing or dehydration).
   iii. Nagy and others (2015b), in a study of *Gopherus agassizii* at a head-start facility on Edwards Air Force Base, CA, reported that of 15 yearling tortoises released in 2005, 4 died from exposure (freezing). Of 32 yearlings released in 2007, 1 died of exposure.

e. Falling in mineshafts: Nussear and others (2012), in a study of translocated tortoises at five sites in Utah and NV, reported that 2 of 30 tortoises died as a result of falling into mineshafts.

f. Rattlesnake bite. Jacobson and Berry (2012) reported that 1 of 11 necropsied tortoises from the central Mojave Desert, CA, showed tissue damage suggesting envenomation by a rattlesnake around the eyes.

10. Failed predation on a juvenile

Spenceley and others (2015) observed a failed attempt of predation on a head-started juvenile desert tortoise by a glossy snake, *Arizona elegans*, at Edwards Air Force Base, CA.

11. Potential responses to predation

    Marolda (2002), in a study of captive tortoises in southern California, reported that posterior shell aperture reduction (PSAR) likely reduces predation risk in young tortoises with small size and soft shell, and was most prevalent in juveniles (12.5% reduction) and neonates (8.4%) compared with adult (2.4%) tortoises. When desert tortoise neonates were pecked with a raven model their PSAR increased from 8.4 to 14.6% which suggests this inarticulate shell kinesis may protect the tail, soft tissues around the cloaca, and hind legs from predation by smaller predators.

VI. Mortality Rates; Survivorship

A. Mortality Rates

1. Peterson (1994) monitored 55 radio-telemetered adult tortoises at the Desert Tortoise Research Natural Area (DTNA) and Ivanpah Valley, CA, between 1988 and 1990. During the 3 years, 20 of 33 (39.4%) at the Natural Area and 9 of 22 (40.9%) at Ivanpah Valley died or were lost by the end of the 3-year study. These annualized adult mortality rates were significantly higher than earlier published mortality rates from 14 Bureau of Land Management study sites, including the DTRNA and IV, which ranged from 0 to 17%. All tortoises at Ivanpah died in 1990, the third year, from dehydration and starvation. At the Natural Area inclusion of known deaths only yielded mortality rate estimates of 25% for 1988, 21% for 1989, and 5% for 1990. Tortoises at the Natural Area died primarily from predation by coyotes and Upper Respiratory Tract Disease. The author attributed the high mortality to effects of drought, directly at Ivanpah through starvation and dehydration, and indirectly at the Natural Area in response to predators and possibly increased susceptibility to disease.
2. Freilich and others (2000) studied a population of tortoises at a single 2.59 km^2 plot in Joshua Tree National Park, CA, over 6 consecutive years. They estimated an annual mortality rate of 11.7%.
3. Krzysik (2002) reported that dead tortoises or their carcass fragments were at a landscape density of 26.9 tortoises/km^2 at the Marine Corps Air Ground Combat Center (MCAGCC) and 27.9 tortoises/km^2 at Joshua Tree National Park, CA. Whereas live tortoise densities were 35% higher at Joshua Tree National Park, carcasses were only 4% higher, suggesting that tortoise mortality was actually higher at MCAGCC than at Joshua Tree. This could be attributed to land use at the two study sites, the former a military training area and the latter a designated wilderness area in a national park.
4. Christopher and others (2003), as part of baseline research on health and disease, reported mortality rates for 108 adult male and female tortoises over the period 1990 through 1995 at three sites: Desert Tortoise Natural Area (western Mojave), Goffs (eastern Mojave), and Ivanpah (northeastern Mojave). At the end of the study, of the 108 tortoises, 53.7% were known survivors and 29.6% tortoises had disappeared and were not relocated. The annualized morality (for those known to be dead) was 2.5% at the Desert Tortoise Natural Area, 5.1% at Goffs, and 2.4% at Ivanpah. Goffs had about two times the crude and annualized mortality rate of the

other two sites. All nine Goffs dead tortoises were females, whereas females accounted for 40% of deaths at the Natural Area and 50% at the Ivanpah site. Several of the tortoises that died had identified health problems.

5. Berry and others (2006a) studied tortoise populations at 21 sites on the National Training Center at Fort Irwin, CA, and reported annual death rates of sub-adult and adult tortoises (> 180 mm MCL) for 7 of the 21 sites for the 4 years prior to the study. The 15 Goldstone sites were combined in a unit and had the highest death rate of 95.2%. Death rates at three sites with military use ranged from 1.9 to 23.8%. Two sites with recent military use had rates of 4.7 and 13.3%. One site, adjacent to the base, Soda Mountains, had a death rate of 9.7%.
6. Berry and others (2008) studied tortoise populations in the western part of Red Rock Canyon State Park and watershed, CA. The authors reported an estimated death rate of subadults and adults during the 4 years previous to the study of 67%.
7. Berry and others (2013) surveyed a 5.42 km^2 study plot in the northwestern Mojave Desert, CA, and reported a crude annual death rate of 1.8% for the subadult and adult size class during the 2006–2010 period.
8. Berry and others (2014a) surveyed tortoises in an area of about 260 km^2 to evaluate three types of management strategies used in adjacent areas in the western Mojave Desert, CA: the fenced Desert Tortoise Research Natural Area, critical habitat, and unfenced private lands recently acquired for conservation. The crude annual death rates for adults for the 4 years preceding the survey were 2.8% /year for the Natural Area, 20.4% /year for critical habitat, and 6.3% /year for private land.

B. Survivorship

1. Longshore and others (2003), in a study of adult tortoises at two sites in the Lake Mead National Recreation Area, NV, over a 9-year period, estimated survival rates derived from population surveys and radio-telemetry data. Survival rates were initially high from 1992 to 1995 at both sites (> 0.958; Grapevine, Cottonwood). Then, in 1996, survival rates diverged; the 7-year survival rates estimated from radio-telemetry monitoring were 0.900 for Grapevine and 0.269 for Cottonwood. The die-off and low survival at Cottonwood appeared to be related to drought, i.e., low precipitation and low productivity of annual plants.
2. Lovich and others (2011c) estimated the annual adult female survivorship at 91.6% (95% confidence interval = 90.5–93.5%) using a cohort of 11 telemetered tortoises near Palm Springs, CA. This high survivorship was considered surprising due to the highly altered industrial landscape, and was perhaps due to increased forage from coastal climate influence with high rainfall, limited human access provided by fences and gates used to protect the wind-energy facility, or the wind turbines that deterred presence of Common Ravens.
3. Nussear and others (2012) conducted a translocation study of tortoises at five sites in NV and UT. They reported that annual survivorship did not differ between resident and translocated tortoises and averaged 0.94 (range 0.72 to 1) over all seasons among sites.

4. Lovich and others (2014b) conducted surveys at a study site in Joshua Tree National Park, CA. Apparent survival for adult desert tortoises was estimated at 0.87 from 1991 to 2012, using a best fit model that assumed survival was a function of precipitation in the preceding 3 years. Estimates of survivorship agreed with estimates of adult abundances, which remained stable from 1993 to 1995, but declined greatly from 1996 to 2012, concurrent with persistent drought. According to the authors, the postures and positions of the majority of dead tortoises found in 2012 were also consistent with death by dehydration and starvation.
5. Agha and others (2015c), drawing on an 18-year study, modeled survivorship of *Gopherus agassizii* inside or near a wind energy facility and in a neighboring wilderness area, CA. The authors reported a significantly higher annual survival estimate for adult tortoises at the wind energy facility (0.96 ± 0.01) than in the neighboring wilderness area (0.92 ± 0.02).
6. Nagy and others (2015b), in a study of head-started tortoises released to the wild at the National Training Center, Fort Irwin, CA, reported a 32% survival of 53 released juveniles over a 3-year period. Body size was a better predictor of survival than age.
7. Nagy and others (2015b), in a study of head-started tortoises in a facility at Edwards Air Force Base, CA, reported heavy predation on hatchlings and yearlings. Annual survivorship in pens with natural rainfall only, over 4 years, averaged 89.7%, whereas survivorship in three unwatered pens during drought averaged 94.0%. The survivorship of six groups of eight yearlings released in September of 3 different years was very low; nearly all died within 6 months. Only four individuals were known to be alive a year after release.

VII. Methods of Sampling and Estimating Populations

A. Factors Affecting Detection of Tortoises

1. Observer experience. Freilich and LaRue (1998) grouped observer experience four alternate ways across five levels (novice, beginner, intermediate, advanced, and expert) in 1994 and three alternate ways across four levels in 1995. Novice or inexperienced observers had 0 h of previous tortoise survey experience while experienced observer's experience ranged from 1 to 7,200 h, across both years, depending on the groups used in the analyses. The authors found that: (a) the inexperienced and experienced groups did not differ in their ability to find styrofoam tortoises or tortoise sign; (b) longer search times did not equate to more tortoises or their sign being found; (c) observers tended to overestimate the number of burrows; and (d) observers also consistently under counted the number of tortoises and scat than were actually seeded in plots. These results suggested that observers differed widely in their searching ability, but that prior experience did not appear to improve their subsequent abilities in finding tortoises.
2. Season of searching. Freilich and others (2000) reported that capture probability of tortoises in Joshua Tree National Park, CA, was highest in spring (March–April), in wet years with ample above ground forage, and during mid-morning (930–1130 h) with moderate temperatures. These findings suggest that targeting the best times

is vital to a successful sampling design. In years with low rainfall and poor forage conditions, effort required to find tortoises increased, probability of capture decreased, a higher percentage of captures were made from tortoises in burrows, home ranges were smaller, and the reliability of estimates were lower due to low recapture rates.

3. Seasonal variation in patterns of use. Rautenstrauch and others (2002) radio-tracked 113 adult desert tortoises for 3 years on two plots in the Yucca Mountains, NV, and reported substantial variation in patterns of burrow use by season and year. Their findings suggest that counts of burrows must be considered as both site- and year-specific. Variation in patterns of burrow use also suggested that conversion factors required to estimate relative abundance from the number of burrows or other tortoise sign are best locally derived.
4. Krzysik (2002) tested sampling designs for live tortoises and their sign (burrows and scat) and dead tortoises at the Marine Corps Air Ground Combat Center and at Joshua Tree National Park, CA. The author stated that his sampling protocol proved to balance accuracy and economy, providing tortoise densities at both a landscape scale and the smaller local scale more useful to natural resource managers.

B. Tests of Different Methods and Their Efficacy for Locating and Sampling for Presence and/or Abundance of Tortoises

1. Use of tortoise sign
    a. Bulova (1994) noted that population density estimates based on counting burrows with evidence of use by tortoises can be improved by considering that individuals used an average of nine different burrows and 35% of these were used by other tortoises. Standard correction factors can be adjusted by accounting for burrows used by multiple tortoises.
    b. Krzysik (2002) tested different survey methods for live and dead tortoises, burrows and scat at the Marine Corps Air Ground Combat Center and Joshua Tree National Park, CA, and modeled associations between the different forms of tortoise sign on different scales (1, 4, and 16 km of transects within plots). He reported significant correlations between live tortoises and tortoise sign (burrows and scat) and a subsequent linear regression model predicting tortoise counts from burrow and scat counts was significant at two scales. In addition, scat counts were a better predictor of tortoise presence than burrows. At all scales, burrow counts showed the least landscape variability, suggesting more clumping than with scat counts. Finally, tortoises were never found when burrow and scat counts were low. The four-element design approach did not use burrows and scats as surrogates for tortoise abundance, but rather it was used to locally calibrate tortoise density estimated for the entire landscape. However, the author also stated that using burrow and scat estimates to estimate tortoise densities at a landscape scale provide more accuracy than using tortoises (presumably both live and dead) alone, because burrow and scats provide higher sample sizes and the g_0 problem (animals missed because they cannot be seen) becomes irrelevant. In addition, the author stated that his

sampling protocol proved to balance accuracy and economy, providing tortoise densities at both a landscape scale and the smaller local scale more useful to natural resource managers.

   c. Duda and others (2002) conducted surveys on two plots at the Marine Corps Ground Combat Center, CA. The authors reported a significant association between desert tortoises and both active and total burrows, with > 80% of the variability explained. This finding suggests that, within a given location and year, desert tortoise burrows could be reliably used as surrogates for desert tortoise density or to model population-level spatial structure. This finding could be useful in monitoring desert tortoise populations.

2. Mark-recapture data and analysis.
   a. Berry and others (2008) reported that study plots should have two complete, independent surveys in spring, thereby providing opportunities to calculate density using mark-recapture techniques or to estimate occupancy.
   b. Freilich and others (2005) showed through distance sampling simulations that density estimates were biased 80% of the time and had high coefficient of variation to density ratios largely because of insufficient encounter rates. Based on their results, considerable effort would be needed to ensure that sufficient detections are made. For example, they noted that surveys in areas with encounter rates of 0.57 detection probability would require 374 km to produce a 10% coefficient of variation to density ratio or 94 km to provide an estimate with 20% coefficient of variation.
3. Use of wildlife-detector dogs
   a. Cablk and Heaton (2006) concluded that trained detection dogs were a safe, effective (measure of the dog's ability to find tortoises), and reliable (measure of the dog in performing its trained alert after finding tortoises) at locating desert tortoises on the surface and in burrows under a range of environmental conditions. Dogs found > 90% of experimental animals in both surface and burrow trials. Reliability was 50% for surface and 69% for burrow trials; reliability was affected by the level and maintenance of the dog's training. Dogs also found tortoises as small as 30 mm carapace length whereas the smallest tortoises found by human survey teams were 110 mm. While dogs showed promise for conducting desert tortoise surveys, it is essential that a particular dog and its handler be suitable for wildlife detection work.
   b. Cablk and others (2008) reported that dogs found 184 tethered and "wild" (not tethered but living in the pens) tortoises and missed eight tortoises. The authors believed the dogs located 21 tortoises visually and 163 by olfaction. Of the 163, 99 were tethered or "known" tortoises and their sizes ranged from 89 to 300 mm. Detection distances ranged from 0.5 to 62.8 m with a median of 11.13 m for tortoises on the surface. No bias in detection distances was observed with tortoise sex, size, time of day, or the degree to which tortoises were handled prior to being found by the dogs (known or wild), although relative humidity (range 16–85%), temperature (12–27 °C), and wind speeds (0–9 m/s) were significantly different among trials. Dogs were able to

differentiate tortoise odor from other non-tortoise scent, and could differentiate live tortoises from tortoise scat and urine and never indicated on tortoise carcasses. Based on these results, dogs could be trained on residual scent from a relatively small number of captive tortoises and then generalized to locate unrelated, live tortoises outdoors in their natural habitat. However, the detection threshold of the dog is immeasurable until the molecular composition of tortoise scent can be described.

c. Nussear and others (2008) compared human and dog detection rates at the National Training Center, Fort Irwin, CA. The authors found no statistically or functional differences between human and dog team detection rates of tortoises, including for juvenile tortoises which were rarely detected in surveys. While the dog teams found a greater proportions of animals in vegetation than humans did (perhaps due to using olfactory cues to locate tortoises) and completed surveys more quickly, the cost of the human team was only 60% as much as the canine teams. The discrepancy was largely due to the cost of the dog handlers.

d. Heaton and others (2008a) reported no significant differences in predator sign, predation, sign of predator-inflicted trauma, or movements of desert tortoises after being found by humans versus being found by wildlife-detector dogs at the Marine Corps Air Ground Combat Center and National Training Center at Fort Irwin, CA.

C. Line Transect Distance Sampling

1. Important considerations. Duda (2002) noted that if the observation of live desert tortoises is essential for accurate population density estimates, then desert tortoise activity levels based on climatic factors will be required input for monitoring programs. Important climatic factors include years of high productivity versus drought. Study duration must also be used to evaluate studies detailing desert tortoise movement and activity.

2. Tests of the method for desert tortoises in Nevada. Anderson and others (2001) noted the following issues:

a. Based on field trials using model tortoises for which the true density was known, a $g_{50} = 0.3$ for adults and $g_{50} = 0.15$ for subadults indicated that detection was < 1 for this survey effort. These findings illustrated the importance of carefully considering field protocols to account for or minimize the effect of detection.

b. Failure of observers to detect tortoises on the line suggested violation of $g_0 = 1$ assumption and the need to improve field protocol and training, such as expending more effort near the center line, particularly in study areas with dense vegetation or uneven topography.

c. Field training is critical for line transect distance sampling; 1–2 days of training may be insufficient to ensure that all observers can detect 100% of smaller tortoises near the centerline. It is probably best to focus surveys on estimating sub-adult and adult tortoise density for individuals > 140 mm in carapace length.

3. McLuckie and others (2002) reported that the mean proportion of tortoises found above ground, g_0, was 0.79 ± 0.02 SE and varied from 0.65 ± 0.05 SE to 0.85 ± 0.03 SE across zones and years. Above ground activity was consistent for all years except 1999, when precipitation was 70.6 mm below the 100-year mean (210.8 mm). The estimated probability of detection, P_A, across the Red Cliffs Desert Reserve, which included all three zones, was 0.63 ± 0.02 SE, with average effective strip width (transect width) of 11.4 m.

4. Freilich and others (2005) reported that distance sampling simulations were biased 80% of the time and had a high coefficient of variation (CV) to density ratios largely due to insufficient encounter rates, even in moderate density areas such as Joshua Tree National Park and Ward Valley, CA. The authors suggested that considerable effort is needed to ensure sufficient detections are made to obtain precise results and reliable estimates. Surveys in areas with encounter rates of 0.57 require 374 km of transects to provide an estimate with 10% CV or 94 km of transects to provide an estimate with 20% CV, even in moderately high density areas. The authors, using simulated distance sampling data, conducted a power analysis. The results of the power analysis suggested a limited ability to detect population declines as high as 50% and that adequate trend detection would require estimates with 10% CV or less which is unlikely given encounter rates in moderately-high density areas. The authors suggest that present methods may not be capable of accurately estimating desert tortoise populations.

5. Nussear and Tracy (2007), using a power analysis, reported that coefficients of variation (CV) would have to be ≤12% around a population growth rate for tortoises of 1% annually over a 25-year time period. This is considered the maximum reasonable population growth rate under ideal conditions. Also, sample size had a large influence on the precision of estimates for g_0 (animals missed because they cannot be seen). About 95 animals would be required to achieve a <12% CV in the estimate of g_0, suggesting that focal populations may never be of sufficient size to estimate g_0 precisely. Estimated coefficients of variation from 2001 to 2005 ranged from 9.5 to 56.2%, meaning tortoises populations would have had to increase 4% annually to detect a trend over 25 years. Conversely, tortoises could decline up to 4% per year and that trend would still not be distinguishable from populations with no trend at all. These findings indicate that more precision in density estimates are needed to make sound management decisions. Environmental variables that improved estimation of g_0 were maximum daily and surface temperature in shaded habitat and daily average temperature in a sunny microhabitat..

6. Inman and others (2009) recorded activity of 34 tortoises surveyed in the Superior-Cronese and Ord-Rodman Desert Wildlife Management Areas, CA, and determined expected ranges of the coefficient of variation (CV) of g_0 (animals missed because they cannot be seen), P_a (decreasing detectability of tortoises with increasing distance from the transect), and *n* (number of tortoises detected) from

Line Transect Distance Sampling. Using those expected ranges, they calculated a possible range for the CV of the density estimates as 0.32 to 0.99. They then concluded that this degree of variation would not enable detection of what is thought to be the highest reasonable estimated of population growth in the desert tortoise of 1% per year.

VIII. Population Models and Vital Rates

A. Doak and others (1994), using data collected on eight study plots on Bureau of Land Management (BLM) land, developed stage-based demographic models with rates of survival and growth averaged over all the study plots and with four different estimates of reproduction.

1. All models predicted population decline, including the model using the most optimistic reproductive estimate rate. These predictions of a general decline in tortoises were in agreement with widespread population declines in the western Mojave suggested by BLM surveys.

2. When the authors projected population growth solely based on mean reproductive rates, estimated at levels consistent with field data, they obtained large population sizes of tortoises for 100 years into the future. However, when they included the estimated variation associated with the same reproductive rate in their analyses, they recovered a predicted risk of population extinctions within decades. While it is not possible to predict with any confidence what will happen to the tortoises over the next few years, even whether populations will increase or decrease, the most obvious conclusion was that populations of the desert tortoise in the western Mojave Desert are in grave danger, with a striking risk of population extinction within decades.

3. The authors quantified the relative importance of different vital rates on the rate of population growth and found that analyses were most sensitive to survival of adult females (carapace length >208 mm), whereas all other improvements to vital rates did not, alone, reverse predicted population declines.

4. They also investigated the consequences of two management scenarios—reducing human disturbance and removing ravens. They found that eliminating human-associated mortality in large tortoises might be more effective in reversing population declines than reducing raven predation on juveniles by one-third. Management actions to decrease the direct human harassment and killing of adult tortoises, along with cessation of livestock grazing on desert lands, might alone reverse population declines.

B. Wisdom and others (2000) developed a life-stage simulation analysis, to measure potential effects of uncertainty and variation in vital rates on population growth. The authors found that a variety of life stages could have a strong effect on population growth. They recommended the need for caution in the assessment of life stage importance for tortoise conservation, but noted that relatively small amounts of variation in the late stages of adult survival were associated with large changes in elasticity values.

Given the sparse amount of variation that was accounted for by any single vital rate, a comprehensive approach to improve all vital-rate values for the desert tortoise remains the single best solution to population recovery.

C. Bjurlin and Bissonette (2004) recommended prioritizing high female adult survivorship due to the relative importance of large females to increased neonates required for high population growth rates.

D. Berry and others (2008) proposed conservation actions to reduce mortality of tortoises. Densities of adult tortoises in the Red Rock Canyon State Park and watershed were slightly below a density of ≥ 3.86 adult tortoises/km^2, the figure recommended by the U.S. Fish and Wildlife Service in the *Desert Tortoise (Mojave Population) Recovery Plan of 1994* for a viable population.

E. Reed and others (2009) used a vital rate sensitivity analysis for a females-only mode. It was necessary to reduce mortality of older females (>52 years) more than reducing mortality of younger age classes to achieve population growth equivalent to a management option of increased nutrition. Further, regardless of initial population structure, population size, or time frame, management actions targeting reduction of juvenile mortality required a greater relative reduction in mortality than those targeting adults. And because small tortoises are difficult to detect, some management alternatives could result in increased population size but decreased numbers of individuals detected over short to intermediate (5–25 years) time frames because small tortoises are difficult to survey. This also suggests that effects of actions to reduce juvenile mortality, such as raven management, would not be apparent for >5 years, making evaluation of management effectiveness difficult or impossible before resources have been expended. Releasing adult females, regardless of which specific adult age class, had a greater effect on meeting target population increases than did releasing juvenile females. In addition, a single release rather than an annual release, which staggers the total number of animals released across years, required fewer total individuals to achieve the same population growth. Using specific management actions, however, depends on various site and situation specific factors, thus, the authors did not attempt to determine which management alternative was most cost effective.

IX. Opinions about Population Trends

Bury and Corn (1995) examined unpublished information and published (including “gray”) literature and questioned the widely-accepted, but unsubstantiated hypothesis of a once widespread, high-density population of desert tortoises found across the Mojave Desert that had experienced a long-term decline resulting in a few scattered, isolated fragments with reduced densities. The authors concluded that some of the conclusions previously drawn about tortoise abundance and distribution had been based upon circumstantial or anecdotal information or information that exceeded an appropriate scope of inference. Further, based on their evaluation of published evidence, the authors concluded that long-term data on tortoise populations were nonexistent, but noted that none of the published accounts support extraordinarily high historic abundance of tortoises. Rather, published accounts are generally neutral or support the alternative hypothesis of little change to abundance and distribution of tortoises.

Chapter 10.—Anthropogenic Impacts to Desert Tortoise Habitat with Management Recommendations: Part 1

I. General Effects of Disturbance on Desert Ecosystems

A. Soils. Literature review. Lovich and Bainbridge (1999) summarized the work of others until about 1998, drawing on the peer-reviewed literature in journals and other sources. The authors reported that undisturbed desert areas are characterized by the presence of natural soil stabilizers, including cryptobiotic crusts comprised of lichen, fungal, bacterial, and algal components; desert pavement; mechanical crusts; and chemical crusts. These desert crusts provide germination sites for vascular plants, conserve water, facilitate accumulation of organic material and soil nutrients, enhance soil moisture, and stabilize the soil, which, in turn, prevents erosion. These crusts occur at the surface and are easily disturbed by grazing and other agents of trampling, hiking, and off-highway vehicle use. In soils subjected to large shear stresses, a single pass by a vehicle is capable of destroying well-developed crust. Cryptobiotic soil recovery requires long time intervals without intervention, with specific functions of crusts projected to require 50–250 years to recover. The authors also reported that soil compaction reduces root growth of desert plants and reduces seedling survivorship. Soils that are most susceptible to compaction are loamy sand and course gravelly soils with variable particle sizes, particularly when wet. The effects of erosion can bury plants at some distance from the impacted area. Desert soils may take 10,000 years to develop and thus may not recover within human time frames, and recovery of natural vegetation would probably take centuries. Desert areas disturbed by human activities may take centuries to recover without active intervention, suggesting the critical importance of minimizing intensity, frequency, and area of disturbance. It is difficult and expensive to mitigate damage from disturbance, e.g., a major restoration program to improve recovery for the off-highway vehicle-damaged areas in the California desert region could exceed 1 billion dollars. Fences, signs, and enforcement to prevent further damage may be a better investment than intensive restoration.

B. Soils and Soil Crusts. Many papers are available on topics associated with deserts of the Southwest. Dr. Jayne Belnap is an expert on the topic and has a few publications annotated here, e.g., Belnap and Lange (2001). Biological crusts result from associaitons between soil particles and cyanobacteria, algae, microfungi, lichens, and mosses. These live within or immediately on top of the uppermost millimeters of soil. This book covers a wide variety of subjects including morphology and internal structure of biological soil crusts.

II. Urbanization

A. Human Population Growth.

1. Berry and others (2006b) summarized human population growth patterns and effects on resources. Human populations within and in the vicinity of the Mojave Desert have profound effects on land and resources. More than 30 million people live within a few hours' drive of the desert and many of them frequently visit for recreation. The Mojave Desert has several rapidly growing cities, some of which contain residents that commute to jobs within the Los Angeles Basin or Central Valley. In the California portion of the Mojave Desert, incorporated cities experienced population growth of >350% between 1970 and 1990; growth in the Las Vegas Valley, NV, and the St. George area, UT, followed a similar pattern. Population growth contributes to conflicts with natural resources, such as with rare, threatened, and endangered plant and animal species.

2. Hughson (2009), in a book chapter on human populations in the Mojave Desert, summarizes such topics as human population growth, water use and depletion of the water table by region, and a brief discussion of energy.

B. Nitrogen Deposition.

1. Allen and others (2009) summarized effects of air pollution, specifically oxidized and reduced forms of nitrogen, on soils and plants in the deserts. The authors presented new studies on effects of deposition of atmospheric nitrogen (e.g., nitric acid and ammonia concentrations in winter and summer) on vegetation and soils along a gradient at Joshua Tree National Park, CA. They also conducted fertilization studies and the effects on native vs. non-native plants (grasses). The studies indicated that long-term, low-level nitrogen inputs at the western end of the Naitonal Park may have already accumulated enough nitrogen in surface soils to affect nonnative grass productivity. Anthropogenically elevated nitrogen will cause a further imbalance if the invaders are nitrophilous and/or prolific seed producers.

2. Verburg and others (2013) conducted field experiments at the Nevada Test Site, NV, experimentally applying water to mimic increased precipitation, and adding nitrogen to mimic urbanization. The added summer precipitation did not have an overall significant effect on any of the fine root dynamics. However, increased winter precipitation appeared to increase root production, turnover, and depth, most likely due to stimulation of annual grasses. The authors suggested that increased winter precipitation and nitrogen deposition in response to climate change and urbanization were likely to affect fine root dynamics in these [northern] Mojave Desert ecosystems.

C. Studies of Spatial Change over Southwestern Ecosystems. Soulard and Sleeter (2012) analyzed remote sensing imagery to evaluate spatial change over the basin and range ecoregions of the United States, including Northern, Central, Mojave, and Sonoran ecoregions. They showed that 3.0% (21,161 km^2) experienced at least one land-use/land-cover (LULC) change between 1978 and 1980. Overall, LULC change increased between 1973 and 1986 (535 km^2), and 1986 and 1992 (1,285 km^2), but decreased slightly between 1992 and 2000 (944 km^2). In particular, the Mojave Basin and Range experienced a relatively low amount of change at 2.6%, which was less than the 5.8% experienced by the Northern Basin and Range and most other ecoregions of the Western United States. In 1973, the Mojave Desert Basin and Range contained 51% of developed lands in the basin and range province, which contained the Las Vegas, Palm Springs-Coachella Valley, and Lancaster-Palmdale metropolitan areas. By 2000, the Mojave Desert Basin and Range accounted for about 59% of the developed land in the Basin and Range province, with developed areas having increased by 1,673 km^2. New areas of developed land were most often associated with the conversion of grasslands/shrublands, disturbed transitional land, and agriculture for urban uses.

III. Grazing by Domestic Livestock and Feral Burros

A. Ecosystem Effects, General, from Livestock

1. Review of the literature. Fleischner (1994) reviewed the literature on livestock grazing in the Western United States and identified the general ecological costs of livestock grazing as: a) alteration of species community composition, b) disruption of ecosystem functioning, such as interference in nutrient cycling and ecological succession, and c) alteration of ecosystem structure such as altered vegetation stratification, soil erosion, and decreased water availability.

2. Knight and others (1998) reported that cattle were observed at all 20 stock tanks, 11 of 20 springs, and 10 of 20 control sites in the Mojave National Preserve, CA. Burros (*Equus asinus*) were found more often at springs (10 of 20 sites) than at stock tanks (n = 2 sites), and never at control sites.

B. Effects on Livestock Grazing on Soils

1. Review of the literature. Lovich and Bainbridge (1999) reviewed the literature and reported that areas trampled by grazing had greater soil compaction and lost cryptobiologic crusts leading, to increased soil erosion potential.

2. Brooks and others (2006), in a study of annual and perennial vegetation in the vicinity of livestock-created piospheres on the Pilot Knob Grazing Allotment in the west-central Mojave Desert, CA. (Piospheres surround watering sites and are denuded of vegetation next to the water source.) The authors reported that soil compaction appeared to increase with proximity to edges of areas denuded by livestock.

3. Beever and others (2006) found that surface soil stability decreased with increasing grazing intensity (measured as numbers of ungulate defecations) in the Mojave National Preserve, CA. Soil stability was greater under perennial vegetation than in bare interspaces. Stability of surface soil samples was more affected by time since removal of grazers than was stability of subsurface soil samples, and subsurface soil stability in bare spaces was not related to grazing intensity or time since removal of grazers.
4. Pietrasiak and others (2011) sampled biological soil crusts on 50 plots each that were either recently disturbed by hiking activities or historically grazed in Joshua Tree National Park, CA. They found that the grazed plots showed clear signs of recovery from past grazing disturbance, with higher visual cover of biological soil crusts. However, those crusts also had lower biomass values, supporting an earlier successional stage. The slightly lower cover values of lichens and mosses could be attributed to former grazing and animal trampling events. The dry conditions in the Mojave Desert could result in longer recovery rates for biological soil crusts.
5. See Belnap and others (2007) under VIII. Military Activities, this Chapter, for effects of grazing on soils and management recommendations

C. Effects on Livestock Grazing on Vegetation

1. Literature review. D’Antonio and Vitousek (1992) conducted a literature review of alien grass invasions and reported that alien grass invasions were most severe in the arid and semiarid West. The invader species included European annual grasses, e.g., *Bromus tectorum* and *B. rubens* The invasions appeared to be related to the historic introduction of sheep and cattle.
2. Literature review. Fleischner (1994) reviewed the literature covering the Western United States, documented studies that confirm the deleterious effects of livestock grazing on Mojave desertscrub in California by dramatically reducing above-ground biomass of annuals and cover of perennial shrubs. The prognosis for restoration of damaged rangeland acreage on arid and semiarid lands was poor.
3. Brooks and others (2006) studied native and alien annual plants and perennial plants in the vicinity of livestock watering sites (piospheres) in a wet year 1997–1998) and dry year (1999–2000) at the Pilot Knob Grazing Allotment in the west-central Mojave Desert, CA. They reported that:
    a. Total annual plant cover and species richness were higher in 1998 than in 2000.
    b. Cover and species richness of native annual plants decreased with proximity to livestock watering sites particularly within 200 m. Effects were greater in the interspace between shrubs than beneath shrub canopies.
    c. Cover of alien and native annual plants responded very differently to the gradient to the watering site: alien annual cover increased whereas native annual cover decreased with increasing proximity. In the interspace alien cover increased by 78% from 0 to 200 m, whereas in the beneath canopy it increased only 44%

   d. Total annual plant cover (native and alien, combined) increased with increasing proximity to watering sites when rainfall was high, particularly within 50 m from the edge of the denuded area (around the water site); total annual plant cover increased even more so in the interspace between shrubs than beneath shrub canopies.

   e. Total cover of alien annual species differed by species. Cover of *Erodium cicutarium* and *Schismus* increased with proximity to the watering sites and significant effects occurred within 200 m. In contrast, cover of the alien annual grass *Bromus rubens* decreased with proximity to watering sites.

   f. Native perennial plant cover, species richness, density, and structural diversity declined with proximity to livestock watering sites, or piospheres, particularly within 50m of the edge of the area denuded by livestock. This trend was due primarily to significant declines in moderate to small-sized shrubs and perennial grass. Cover of the largest shrub, *Larrea tridentata*, generally declined with proximity to water sites but the trend was not significant.

4. Abella (2008) synthesized seven diet and two exclosure studies conducted in the Mojave Desert on feral burros. He reported that burros are versatile foragers, as indicated by 175 total plant species found in their diets, but prefer graminoid and forb groups over shrubs when given the opportunity. *Plantago ovata* (native annual forb) constituted the greatest proportion of annual burro diets, followed by *Bromus rubens* (exotic annual grass) then by *Ambrosia dumosa* (shrub). Perennial grasses were three to nine times denser inside than outside exclosures, suggesting that grasses occurred in burro diets more than predicted based on availability. However, the effect of burros on habitat is unclear, as burros could decrease exotic plants by grazing them or increase exotics by eating competitive native plants, dispersing seed, or disturbing soil.

5. Reisner and others (2013) sampled 75 sites in the northern Great Basin Desert and found evidence that cattle grazing reduced prevalence of native bunchgrasses, increased gaps between vegetation, and trampled biotic soil crusts, actions that in turn weakened resistance to *Bromus tectorum* invasion within *Artemisia* ecosystems. The authors said that passive restoration of *Artemisia* ecosystems by reducing cumulative cattle grazing may be the most effective means of achieving (a) high bunchgrass cover and structure with spatially dispersed bunchgrasses and small gaps between them; (b) a diverse assemblage of bunchgrass species to maximize competitive interactions with *B. tectorum* in time and space; and (c) biological soil crusts to limit *B. tectorum* establishment.

D. Effects of Grazing on Desert Tortoises

1. Wronski and others (1992) analyzed dermal bone biopsies of carapaces from adult desert tortoises from Beaver Dam Slope and City Creek, UT. The authors reported that carapaces from habitat grazed by cattle were relatively normal with no signs of osteopenia (low bone density) but with mild osteomalacia (bone softening due to dietary calcium deficiency or malnutrition). However, the possible osteomalacia observed in dermal bone of desert tortoises from the grazed habitat was relatively mild compared with a prior, qualitative report of osteopenia in some carapaces of dead desert tortoises from the same grazed habitat.
2. Fleischner (1994) reviewed the grazing literature covering the Western United States, and reported studies documenting the deleterious effects of livestock grazing on desert tortoises. Studies included livestock trampling of young tortoises, damage to burrows and shrubs used for shelter, and removal of critical forage. The author concluded that the prognosis for restoration of damaged rangeland acreage on arid and semiarid lands was poor.
3. Lovich and Bainbridge (1999) reviewed the literature on grazing sheep and reported that sheep had direct effects on desert tortoises by trampling tortoise burrows. However, those burrows located under shrub cover were generally protected.
4. Keith and others (2008) sampled the Jawbone-Butterbredt Area of Critical Environmental Concern and Red Rock Canyon watershed using 751 one-ha plots. This 187.7 km^2 study area is in the western Mojave Desert, CA. The authors reported that the most prevalent sign of human impacts was livestock scat, which was found on 97% (n = 726 plots); significantly lower counts of livestock scat occurred in the Red Rock Canyon State Park and watershed than in other regions within the study area. Tortoise sign was found in areas with significantly lower impact from livestock, as measured by counts of livestock scat.
5. Berry and others (2008), in a 2002–2004 study of tortoises and anthropogenic impacts at Red Rock Canyon State Park, CA, reported that counts of cattle scat were significantly higher outside the Park and Red Rock Canyon watershed than inside these areas.
6. Nussear and others (2012) reported that 1 of 30 tortoises found dead during a translocation project in UT and NV was because livestock caused the burrow to collapse.
7. Berry and others (2014a) conducted a study on lands managed for the desert tortoise using three different strategies with an objective of identify natural and anthropogenic facts positively or negatively associated with tortoise abundance. The managed areas were the Desert Tortoise Research Natural Area, fenced from livestock grazing and recreational vehicle use since 1980; tortoise critical habitat adjacent to the Natural Area; and private lands recently acquired for conservation and adjacent to the Natural Area. Sheep grazing had occurred on all the lands until 1980, when it was excluded from the Natural Area. Sheep grazing was excluded from critical habitat in 1990. Sheep grazing occurred primarily on unfenced private lands. In models, sheep grazing (scat) was a significant predictor that tortoise sign

would be in low numbers or absent (21% decrease in odds of occurrence of tortoise sign per twofold increase in sheep scat). Sheep grazing had a negative effect on presence of tortoise sign. Tortoise abundance was significantly higher in the fenced Natural Area with a history of protection.

E. Recommendations for Management

1. Brooks (1995) reported that the floral and faunal community structure of the western Mojave Desert can profit from fenced protection from livestock grazing and off-road vehicle use, such as that observed at the Desert Tortoise Research Natural Area, with the primary benefit being greater biomass production by forbs than alien annual grasses.
2. Oftedal (2002) noted that habitat management decisions should take both the quantity and quality of tortoise nutritional resources into account. Particular attention should be paid to factors affecting the distribution and abundance of plants high in potassium excretion potential (PEP) index, especially in the western area of limited summer rains. It may be particularly important to protect tortoise food resources from livestock grazing in years of high winter rainfall, because high-PEP plants may only be abundant under such conditions.
3. Henen (2004), drawing on information from on nutrient budgets and reproductive data of nine female tortoises from Goffs, CA, reported that dry vegetation following a dry winter was a major component of the tortoise diet and may be necessary to increase survivorship and reproduction through droughts. Livestock compete with desert tortoises for this forage in spring. Managers could potentially reduce competition between cattle and tortoises by decreasing nutrient supplements (e.g., water and urea-salt licks) to livestock in dry years.
4. Brooks and others (2006) reported that artificial livestock watering sites in the Mojave Desert increased cover of aliens and decreased cover, species richness and structural diversity of native plants. Control of alien annual plants, and restoration of native annuals, should focus primarily in the area 200 m from watering sites at the Pilot Knob allotment. Special attention should be devoted to the interspace microhabitat where the negative piosphere effects were strongest. Restoration of native perennial plant cover, species richness and structural diversity should focus primarily on the area 50 m closest to the water sites and small shrub species hould be primarily used to restore plant structural diversity. These guidelines likely apply to watering sites in other livestock grazing allotments in the Mojave Desert.
5. Keith and others (2008) said that if managers wish to protect tortoises and tortoise habitat, they can use fencing to restrict or eliminate livestock from specific areas within the Jawbone-Butterbredt Area of Critical Environmental Concern, CA, such as the Kiavah Apron where relatively high densities of desert tortoises occur. Such actions may hasten the recovery of perennial shrubs and annual plants and result in more forage for tortoises, as well as protection from predators, and lessen trampling of cover sites and tortoises by livestock.
6. Abella (2008) noted that perennial grasses and other preferred forage species likely require protection from burro grazing in burro-inhabited areas if revegetation efforts in the Mojave Desert are to be successful.

7. Reisner and others (2013) reported that increased intensity of cattle grazing indirectly promoted increased magnitude of *Bromus tectorum* (cheatgrass) dominance in the Great Basin Desert. Therefore additional grazing would be unlikely to reduce hazardous fuel loads from *B. tectorum.* Use of additional grazing as a means to prevent catastrophic wildfires and restore perennial grasses and forbs in *Artemisia* ecosystems of the Great Basin was unlikely to be successful.

IV. Invasive or Alien Annual Plants

A. General Papers on Invasive Plants

1. Hunter (1991), in studies conducted at the Nevada Test Site, NV, reported that *Bromus tectorum* and *Bromus rubens* had greatly increased in frequency and density since quantitative studies began in 1957. By 1988 both species occurred in many places at densities exceeding 1,000 individuals/m^2.

2. Literature review. D'Antonio and Vitousek (1992) reviewed the literature on alien grass invasions. Effects of grass invasions included resource competition such as light absorption, water intake, and nutrient intake, effects on resource supply such as altered water holding capacity and altered rates of mineralization and immobilization, and other ecosystem effects. Grass invasions can have effects at multiple levels of ecological organization through impacts on resource use or supply, geomorphological processes, and microclimate effects. The most significant effects of alien grasses on ecosystems resulted from the interactions between grass invasion and fire. Grass invasion could set in motion a grass-fire cycle wherein alien grass colonized an area and provided the fine fuel necessary for the initiation of propagation of fire, which in turn increases the frequency, area, and perhaps intensity of fire, followed by more rapid recovery of alien grasses.

3. Literature review and personal observations. Kemp and Brooks (1998) reported that about 25 species of exotic pants are widespread in the deserts of California.
    a. They estimated that the number of recorded exotic species was from about 5 to 12% of the total flora in the California deserts. However only a small group of recorded exotic species were considered established and had spread into natural desert habitats.
    b. Most of the exotic species that had become established were likely to be pre-adapted to the dry habitats associated with their Mediterranean climate origins and were comprised primarily of annuals.
    c. The authors suggest that most exotic species likely entered the California deserts during the middle-to-late 18th century, and following the Gold Rush of 1849, became established as a result of the subsequent cattle and sheep grazing and road and railroad construction.

   d. In addition to disturbed habitats, exotic species have greatly increased in relatively undisturbed habitats. Exotic species, primarily *Bromus rubens, Schismus* spp., and *Erodium cicutarium*, now account for the majority of total annual plant biomass in many regions of the California's Mojave Desert.

4. Literature review. Lovich and Bainbridge (1999) reported that the following plants are considered invasive in the southern California desert ecosystem: saltcedar aka tamarisk (*Tamarix ramosissima*), Russian thistle (*Salsola iberica*), filaree (*Erodium cicutarium*) and the following grass species: split grass (*Schismus* spp.) and bromes (*Bromus* spp.). Exotic plants increase fuel load and fire frequency, and exhibit allelopathic properties that affect native annuals.

5. Brooks (1999a) conducted field work on alien annual grasses at 34 sites in the central, southern, and western Mojave Desert and reported that absolute frequency and cover of summer: spring ratios for eight annual plant taxa were highest for *Bromus* spp. (primarily *B. rubens*) and *Schismus* spp., and lowest for native forbs. Alien annual grasses contributed most to the continuity and amount of dead annual plants and the spread of summer fires. Fire spread rapidly and continuously across interspaces with *Bromus*, and slowly and discontinuously with *Schismus*, suggesting that *Bromus* spp. fuel fast-moving hot fires whereas *Schismus* spp. fuel slower moving cooler fires. Generally, the alien annual grasses *Bromus* and *Schismus* appear to be necessary for fire to spread across the Mojave Desert landscape.

6. Brooks (1999b) studied habitat invasibility and alien dominance, measured as alien richness and total biomass, respectively, at the Desert Tortoise Research Natural Area, CA. The author reported that species richness and total alien annual plant biomass were slightly higher where disturbance was high. This relatively slight difference between the two levels of disturbance may have been the result of only 15 years of differing sheep grazing and off-road vehicle use, leading to relatively small differences in plant community composition over that period. However, species richness and dominance of alien plants were significantly related with topographic position and microhabitat: alien richness was higher with higher soil nutrients in washlets than on hummocks and highest beneath creosote bushes during the high rainfall year and in interspaces during the low rainfall year. Precipitation played a role: absolute richness and biomass were lower during 1994 when rainfall was low, compared to 1995 when rainfall was high, when the pattern for proportional richness and biomass was high.

7. Brooks (2000) evaluated effects of two alien annual grasses (*Bromus* and *Schismus*) on native annual plants at three sites in the central, south-central and southwestern Mojave Desert, CA, during 2 years of contrasting productivity. The author thinned the two species of alien annual grass seedlings separately on the north side of *Larrea tridentata* for *Bromus* and in the open interspace between shrubs for *Schismus*.

   a. Thinning of *Bromus* and *Schismus* significantly increased density and biomass of native annuals at all three sites, only during a year of high annual plant productivity and species richness. Effects of thinning were greatest for *Amsinckia tessellata* and for a group of relatively uncommon native annuals.
   b. Thinning also significantly increased density and biomass of the alien forb, *Erodium cicutarium*.
   c. These results show that alien annual grasses can compete with native annual plants and an alien forb in the Mojave Desert and that effects can vary among years.

8. DeFalco and others (2001) quantified the influence of microbiotic soil crusts versus bare soils on production, species, diversity, nutrient content and water on annual plant species sampled near St. George, UT.
   a. The authors reported that microbiotic soils maintained about twice the soil organic matter and inorganic nitrogen, maintained higher density and shoot biomass of winter annuals, and had 53% greater water content during November and December when winter annuals become established, compared with bare soils lacking crust cover.
   b. Although species diversity of annuals did not statistically differ between the two soil types, the authors reported that native annuals comprised the greatest proportion of shoot biomass on bare soils and an exotic forb (*Erodium cicutarium*) and grass (*Bromus rubens*) produced more biomass on crusts likely as a response to the higher nitrogen availability on microbiotic soils, which allowed exotics to grow faster and potentially outcompete native annual species.
9. Review of the literature. Brooks and Esque (2002) evaluated the literature on alien annual plants, fire, and effects on tortoises. They reported that the most abundant and widespread invasive plant species in the Mojave and Colorado Deserts, the annuals *Bromus rubens, Schismus* spp., and *Erodium cicutarium*, invaded this region in the late 1880s and became widespread after the 1950s. Ecological effects of these invasive plants include their ability to compete with native species, inhibit germination of native species, and alter the fuel structure that in turn alters the fire regime by making sites more susceptible to fire. Management of alien plants and fire should be closely integrated, because alien plants can create fuel conditions that promote fire in otherwise fire resistant landscapes that predominate in the Mojave and Colorado Deserts.
   a. Minimizing the number of paved and dirt roads and maintaining nonroaded wilderness areas may reduce the dominance of aliens.
   b. Efforts to monitor the arrival of new species and eradicate them should be focused along roads and washes.

10. Brooks and Matchett (2003) sampled unburned and burned sites at Beaver Dam, UT, Spring Mountain, NV, and Joshua Tree National Park, CA. They reported that native species richness and cover decreased 6 to 14 years post-fire in *Coleogyne ramossisima* stands. In contrast, alien species richness and cover increased after burning, especially where the alien forb *Erodium cicutarium* was present.
11. Brooks (2003) conducted field experiments at three sites in the central, southern, and western Mojave Desert, CA. The responses of individual alien species (*Bromus rubens, Schismus* spp., and *Erodium cicutarium*) to nutrient additions (soil nitrogen) differed between microhabitats (beneath canopy and interspace) and between years (1996 and 1997). In response to nutrient additions (soil nitrogen) in experiments conducted in a year of high productivity, alien plant density and biomass increased whereas native density, biomass and species richness decreased. Species richness for native annual plants also was reduced by nutrient additions but only in 1 year (1997) of the study. The decrease of natives may have been due to increased competition for soil water and nutrients by aliens. These results indicated that increased levels of soil nitrogen from atmospheric nitrogen deposition from urban areas or other sources could increase the dominance of alien annual plants. Increased biomass of alien annual plants may in turn decrease the diversity of native annual plants or increase the frequency of fire. When new projects are evaluated for environmental threats, one important consideration is whether the project would increase the production of nitrogen pollutants and the potential to increase dominance of invasive alien plants and to facilitate invasion of new alien species.
12. Beever and others (2006) reported that in the Mojave National Preserve, CA, cover of all non-native plants averaged nine times higher in a high rainfall year after cattle had been removed for 1–2 years than in a low-rainfall year.
13. Brooks and Berry (2006) conducted a study of alien annual plants at 34 sites in critical habitat for the desert tortoise in the western, central and southern Mojave Desert, CA, in 1995 (wet year) and 1999 (dry year). They reported that:
    a. Alien annual plant species (*Bromus*, *Schismus*, and *Erodium*, combined) comprised a small fraction of the total annual flora in wet years (6%) but most of the annual plant community biomass (66%. In contrast, in dry years, alien annual plants composed 27% of the flora and 91% of the biomass. The results indicated that alien species comprised the majority of total annual plant biomass, especially in drier years.
    b. *Bromus rubens, Schismus* spp. (*S. arabicus* and *S. barbatus*) and *Erodium cicutarium* were the predominant alien species during both wet and dry years, comprising 99% of the alien annual biomass.
    c. Alien annual plant species richness was more reliably predicted by disturbance variables than productivity variables (annual rainfall, total soil nitrogen, native annual plant biomass, perennial plant cover) or plant community diversity (annual plant diversity, perennial plant diversity) variables across multiple sites.

   d. Alien annual plant species richness and biomass was positively correlated with density of dirt roads, particularly in wet years
   e. Alien annual plant species richness was positively correlated with total soil nitrogen, particularly in dry years.
   f. Total alien biomass was positively correlated with proximity to nearest urban area or dirt road during 1995 (wet year) and with native biomass and off-highway vehicle historical use during the dry year (1999). Total alien biomass was negatively correlated with annual plant diversity in 1995 (wet year).
   g. Total alien annual grass biomass was positively correlated with fire (number of fires and area burned between 1980 and 1994 within 5 km of each study site) in both wet and dry years. This was likely due to the increased presence and flammability of invasive grasses and indicates a positive link between fire frequency and size with alien annual grasses.
   h. Alien annual grass biomass was positively correlated with proximity to urban areas or paved roads and negatively correlated with annual plant diversity in a wet year.
   i. Alien annual grass biomass was also positively correlated with absolute biomass of native annual plants and off-highway vehicle disturbance during low rainfall.
   j. The prevention of new invasions should be priority for land-managers, because control is most feasible at the early stages of invasion. Monitoring to detect changes in the status of alien species should focus on regions of high road density or fire frequency, especially near urban or off-highway vehicle areas. Within monitoring sites, efforts to detect new species should focus on areas of high productivity such as washes, roadsides, and beneath-canopy microhabitats. Once new invaders are detected, decision support tools can help land managers evaluate them for their potential ecological impacts and prioritize them for control.

14. Brooks and others (2006), in a study of annual and perennial plants in the Pilot Knob Grazing Allotment in the west-central Mojave Desert, CA, reported that absolute and proportional cover of alien annual plants increased with increasing proximity to livestock watering sites (piospheres), particularly within 200 m, and more so in the interspace between shrubs than beneath shrub canopies.
15. Brooks (2009) described the spatial and temporal factors affecting nonnative plant distribution across six regions in the Mojave Desert using the themes of resource availability and propagule pressure to explain patterns. He described the six regions by their biophysical properties:
    a. Northern: low urbanization (Beatty, NV), extensive agriculture, one state highway that is a likely invasion pathway for nonnative plants associated with agriculture, low propagule pressure from adjacent regions;

   b. Eastern: very high urbanization (Las Vegas and Clark County, NV), significant agriculture, livestock grazing, three major highways, nonnative plants typical of urbanized and agriculture areas, relatively high propagule pressure from adjacent bioregions.
   c. Central: large urban center (Barstow, CA) where three major highways and a major railroad converge as well as functioning as the center of agricultural activity for region, high propagule pressure from transportation corridors.
   d. Western: urbanization present (Lancaster-Palmdale area, CA), one state highway that connects to the large agriculture area in the Central Valley which provides opportunities for agriculture-associated nonnatives, and propagule pressure from roads.
   e. South-central: extensively rural but transitioning to urbanization (Victorville and Yucca Valley area, CA) with agriculture and horse ranching, and a major highway providing a pathway for urban-associated nonnatives.
   f. Southeastern: least populated except around Colorado River featuring agriculture, horse ranching, recreation activities, and a major interstate highway providing a pathway for urban-associated nonnatives while a state highway allows for pressure from adjacent regions. Landscape variation within subregions is dependent on elevation, major vegetation types, microhabitat characteristics (e.g., topography, sizes of perennial plants, etc.), and amount and types of human disturbance.

16. Rao and others (2009) studied a nitrogen depositional gradient across 16 sites in Joshua Tree National Park, CA. The authors showed that soil nitrogen from atmospheric deposition was directly correlated with soil carbon and nitrogen, and that soil nitrogen from deposition was directly correlated with soil carbon and nitrogen. Exotic grasses (but not native forbs) were directly correlated with soil carbon, soil nitrogen and total mineralized nitrogen. The authors concluded that clear positive relationships exist between nitrogen deposition, soil nitrogen, soil carbon, and exotic grass cover in this region.

17. Abella and others (2009a) sampled 3,325 km of road, trail, and shoreline transportation corridors in southern Nevada, with 256 soil types and elevations ranging from 137 to 3,634 m as part of a Weed Sentry early detection program. They identified and mapped 43 exotic plant species in 3 years. Species such as *B. rubens* and *Schismus* spp. that were ubiquitous in the study area were excluded, because they were not part of the early detection. Most species 58% (25/43) occurred in 2% or fewer of 256 soil types. Surveyors chose to use herbicide or hand pulling; small incipient populations and populations in remote areas were most frequently treated upon discovery. The 15 most frequently encountered species (of the 43) were *Nicotiana glauca, Lepidium latifolium, Neium oleander, Tamarix ramosissima, Malcolmia africana, Brassica tournefortii, Hordeum murinum, H. vulgare, Sisymbrium irio, S. altissium, S. orientale, Bromus tectorum, B. berteroanus, B. diandrus,* and *Descurainia sophia. Malcomia africana* was an exception; it occurred on more than two times as many gypsum soil types as expected, as well as on 50 non-gypsum soil types. The authors treated 37,744

exotic plants of 26 species in incipient populations. Periodically resurveying to update exotic species distributions is one of the best known approaches this region has for curtailing widespread infestations by new invaders. In addition to surveying, however, a successful exotic plant information system will likely need to incorporate effectiveness monitoring, generate new or synthesize existing published research on invasibility and treatments, and be adaptive to new invaders or to changes in ecosystem invasibility.

18. Rao and Allen (2010) conducted multi-year field fertilization studies in Joshua Tree National Park, CA.
    a. The authors characterized the influence of precipitation timing on invasive grass and native forb cover. In low elevation, creosote bush habitat, native forbs had the greatest cover when November–December precipitation was > 28 mm, in combination with September–October precipitation of > 56 mm. Invasive grass cover was favored by a wet winter (January–February precipitation) and extended rainy season (March–April precipitation).
    b. In high elevation, piñon–juniper woodlands, native forbs were more influenced by late fall (November–December) and early winter (January–February) precipitation. Invasive grass cover was favored by September–October precipitation in high nitrogen sites and January–February precipitation in low nitrogen sites.
    c. Due to water limitations, biomass exceeded the fire threshold only under very wet conditions regardless of soil nitrogen status.
    d. The greatest production of *Bromus madritensis* and *Amsinckia tessellata* occurred under the highest soil nitrogen (inorganic N after fertilization = 2.99 g m^{-2}) and highest watering regime, indicating that these species are limited by both water and nitrogen.
    e. These results suggest that percent cover of invasive grasses and native forbs was primarily dependent on the timing and amount of precipitation and secondarily dependent on soil nitrogen and site-specific characteristics. The findings indicated that areas of high nitrogen deposition will be susceptible to grass invasion, particularly in wet years, potentially reducing native species cover and increasing the risk of fire.
19. Esque and others (2010b) conducted experimental fires of moderate temperatures in the Parashant National Monument, AZ. Following the fires, they reported that the invasive species *Schismus* spp., *Bromus madritensis*, *Bromus tectorum, Erodium cicutarium* and *Plantago* spp. made up > 95% of the seed bank at sampled sites.
20. Craig and others (2010) found no distinction between exotic and native annual plant cover relative to road type (paved or gravel) or distance from roads (ranging from 5 to 45 m) as part of a study of 3 microsites at 12 road sites sampled in Lake Mead National Recreation Area and adjacent Bureau of Land Management lands in the eastern Mojave Desert. Their results suggested that exotic plant species were not more prevalent near roadsides than in the adjacent desert. They did find more exotic annual cover and richness at microsites underneath *Ambrosia dumosa* and

Larrea tridentata shrubs than at interspace microsites. The authors stated that roadside surveys alone may not be adequate to detect exotic species, some of which may be dispersed independently of roads or may exploit natural disturbances farther from roads.

21. Rao and others (2011) established six study sites at Joshua Tree National Park, CA. They found that gradients in precipitation, nitrogen deposition, and wind were the most important factors to native richness and exotic species cover.
    a. The authors reported that the most dominant explanatory variable for native species richness was nitrogen deposition, while wind disturbance was most strongly associated with exotic annual cover.
    b. Similarly, they reported that higher native diversity was found on drier nitrogen-poor habitat gradients and that higher exotic species cover was found on the wetter, nitrogen-rich end of the habitat gradient.
    c. At high disturbance sites, the authors reported that nitrogen deposition and wind disturbance were positively associated with exotic grass and forb cover.
    d. The results suggested that large-scale patterns in disturbance and exotic species cover negatively affected native annual plant species diversity, but native species may also persist due to local microsite heterogeneity.
22. Steers and others (2011) conducted field experiments in Big Morongo Canyon Preserve, CA. The experiments were designed to test whether carbon additions could reduce invasive annual species. The experiments showed a large relative decrease in exotic annual grass cover, density, and biomass in response to carbon amendments in the first season (2006). However, in the third season (2008), exotic annual grasses were again impacted, but so were all other species groups, suggesting the relative abundance of native and invasive species at the time of application is critical to the success of carbon amendments.
23. Bykova and Sage (2012) conducted manipulation experiments on *Bromus rubens* and *B. tectorum*. The results of their experiments:
    a. did not support the hypothesis that winter cold tolerance accounts for the different range distributions of *Bromus tectorum* and *B. rubens*, because plants of each species growing outside during the winters of 2007–2008 and 2009–2010 responded similarly to winter conditions and artificial freezing treatments.
    b. demonstrated a slower acquisition of freezing tolerance in young *B. rubens* plants relative to mature *B. rubens* and both young and mature *B. tectorum*. These results support an alternative hypothesis that a slow rate of cold acclimation in *B. rubens* limits its ability to grow in the colder climates of the intermountain region where *B. tectorum* predominates, and at high elevations in the Mojave Desert region.
24. Suazo and others (2012), conducted an experiment at Lake Mead National Recreation Area, CA, and noted that invasion of species such as *Schismus* spp. could be facilitated by construction of solar energy facilities through removal of vegetation and soil disturbance.

25. Schneider and Allen (2012), conducted nitrogen fertilization experiments at field sites in Joshua Tree National Park, CA, and demonstrated that invasive species cover increased with nitrogen fertilization, but seed bank density of exotic invasive species in fertilized plots did not increase. However exotic invasive grass seeds dominated the seed bank at all sites, and sites with the lowest nitrogen deposition had the highest seed bank species richness.
    a. The authors also demonstrated that low invasion areas (by exotics) tended to have higher densities of native seed banks, while high invasion sites reflected the invasion in both the seed bank and aboveground vegetation.
    b. Their study provided insight into site-specific impacts of native versus invasive species composition on soil seed banks.
26. Schafer and others (2012) conducted studies at two sites (one at Fort Irwin National Training Center, CA and the other outside of *Gopherus agassizii* habitat in the Sonoran Desert, AZ). We limited our annotations to results from the Mojave Desert site. The authors reported that annual plant abundances were higher on the north side of *Larrea tridentata*, as predicted, and were more abundant in the microhabitats not influenced by shrubs (within canopy near *Larrea* stems and under the dripline). Native annuals on the north side of the shrubs increased from under the canopy to the canopy dripline and then decreased in open microhabitats. Abundance of non-native annuals increased from under the canopy into open areas, where abundance was predicted to be highest.

B. *Bromus rubens* (Red Brome)

1. Hunter (1991) reported on historic, as well as recent studies conducted at the Nevada Test Site, NV; he demonstrated that both frequencies and densities of *Bromus rubens* significantly increased over 30 years.
    a. The author estimated an increase from fewer than 10 individuals/m^2 in the 1960s and early 1970s to thousands/m^2 in the late 1980.
    b. Based on the author’s surveys in 1988, the mean population density was 362 ± 84 plants/m^2, and highest densities were found in areas where soils were disturbed by small mammal activity.
2. Literature review. D’Antonio and Vitousek (1992) synthesized the literature on alien grass invasions. The authors reported that grass invasions were most severe in the arid and semiarid West and included invasions by European annual grasses, including *Bromus rubens,* which appeared to be related with the historic introduction of sheep and cattle.
3. Literature review and personal observations. Kemp and Brooks (1998) reported that exotic species, primarily *Bromus rubens, Schismus* spp., and *Erodium cicutarium*, now account for the majority of total annual plant biomass in many regions of the Mojave Desert in California.

4. Brooks (1999b) sampled plants at the Desert Tortoise Research Natural Area, CA, and reported that *Bromus rubens* was more dominant in microhabitats with high nutrient levels (washlets and in *Larrea*-north shrubs) during both years of the study (1994–1995). Overall biomass of *B. rubens* was higher in the year (1995) of high rainfall.

5. Smith and others (2000), using free-air CO_2 enrichment (FACE) technology in experiments conducted at the Nevada Test Site, reported that:

    a. CO_2 enrichment substantially increased the production of new shoot biomass in *Larrea tridentata*, *Ambrosia dumosa*, and *Lycium andersonii* in a wet year (1998) but not a dry year (1999).

    b. *Bromus rubens* exhibited a 2.3-fold increase in above-ground biomass compared to control plots, due to a 50% increase in density coupled with a 53% increase in individual biomass at elevated CO_2 than did several species of native annuals. These results suggest that elevated CO_2 might enhance the long-term success and dominance of this exotic annual grass in the region.

6. Based on a glasshouse experiment, DeFalco and others (2003) demonstrated that *Bromus rubens* extracted soil moisture more rapidly and had greater biomass and nitrogen content than two native annuals, *Vulpia octoflora* and *Descurainia pinnata*.

    a. The authors suggested that this was due to its greater root-surface area and ability to penetrate deeper soils.

    b. The authors also demonstrated that *B. rubens* produced fewer but larger seeds that readily germinated compared with the two native species studied.

    c. The authors suggested that these advantages provide *B. rubens* the potential to persist in diverse habitats of the Mojave Desert, but with a susceptibility to deplete its seed bank during drought years.

    d. Understanding the allocation trade-offs and their associated environmental cues of *B. rubens* can help ensure the future of management and control.

7. Brooks (2003) conducted field experiments at three sites in the central, southern, and western Mojave Desert and reported that biomass of *Bromus rubens* was higher and its response to nutrient additions (nitrogen) was stronger where soil nutrient levels were naturally high in the beneath-canopy microhabitat of *Larrea tridentata* compared with the interspace microhabitat.

8. Salo (2005), drawing on herbarium records and published accounts in western North America, suggested possible modes for early introductions of *Bromus rubens*

    a. The earliest known collection dates comes from Plumas County, CA, in 1879.

    b. Possible modes for the early introduction included the California Gold Rush and Central Valley wheat production, southern California shipping activities, and northern California sheep pelage.

    c. Spread of *B. rubens* occurred rapidly between 1930 and 1942, with the greatest spread into new regions during the past 50 years. These events coincided with ‘warm’ Pacific Decadal Oscillation regimes, which are typically linked to increased winter precipitation in the Southwestern United States and northern Mexico.

  d. The author also suggested that global environmental change, such as increased atmospheric CO_2 levels and nitrogen deposition, may be contributing to the success of red brome, relative to native species.

  e. Droughts reduce densities of *B. rubens*, which does not maintain a seed bank, so that periods of adequate moisture after drought provide opportunities of native annual species to replenish their seed banks and for management of this exotic grass.

9. Brooks and Berry (2006) sampled 34 sites in desert tortoise critical habitat in the western, southern, and central Mojave Desert, CA, for alien annual plants. They reported that:

   a. *Bromus rubens* dominanted alien annual biomass during 1995, a wet year, but not during 1999, a dry year.

   b. *B. rubens* was positively correlated with elevation and the frequency and size of fires.

   c. Biomass of *B. rubens* varied by microhabitat; beneath-canopy contained most of the biomass of *B. rubens* compared with inter-shrub microhabitats.

   d. Biomass of *B. rubens* was positively correlated with elevation and perennial plant diversity in wet and dry years, though the relationship with perennial plant diversity was not significant when considered with elevation to, which plant diversity was correlated.

   e. Biomass of *B. rubens* was positively correlated with fire in a dry year.

   f. *B. rubens* and *Schismus* spp were negatively correlated with each other; *B. rubens* had higher biomass above 1000 m in 1995 and 1999.

10. Brooks and others (2006), in a study of livestock grazing effects on plants in 1997–1998 and in 1999–2000 at the Pilot Knob Grazing Allotment, CA, reported that cover of *Bromus rubens* increased beneath shrub canopies with increasing distance from livestock watering sites, particularly within 200 m.

11. Brooks and Matchett (2006) evaluated a fire occurrence database from numerous fire sites across the Mojave Desert, and reported that the ecological zone characterized by middle elevation shrublands was more susceptible than other areas of the Mojave Desert to increased fire size following years of high rainfall. This relationship was likely related to the flush of non-native annual grasses, *Bromus rubens*, that produced continuous fuel beds following years of high rainfall.

12. DeFalco and others (2007) found that growth of native perennials (*Larrea tridentata, Achnatherum hymenoides*, and *Pleuraphis rigida*) at the Nevada Test Site declined when *Bromus rubens* was established in winter. This decline was due to growth and high water use of *B. rubens* before perennial growth began (initiated approximately 2 to 3 months later).

   a. In comparison with winter establishment, *B. rubens* plants established in spring were smaller and thus did not effectively reduce growth of the native perennial plants. The authors suggested that this was likely because moisture delivery in spring months was less effective due to higher evaporative loss.

   b. The observed impact of *B. rubens* establishment on the evergreen shrub, *Larrea tridentata,* was lower than for the perennial grasses (*Achnatherum hymenoides* and *Pleuraphis rigida*) and suggested the likely cause of this reduced impact was because *Larrea* exhibited low growth rates throughout the year, even after *B. rubens* had completed its life cycle.

   c. The authors noted that mitigation of the effects of *B. rubens* will require an understanding of the environmental cues that drive its establishment and dominance.

13. Abella and others (2009b) compared unburned plots with those burned by wildfire from a lightning ignition outside of Las Vegas, NV, 2 years prior. The total mean cover of *Bromus rubens* was nine times lower on the burned versus unburned plots, a difference primarily driven by the significantly higher amount of dead *B. rubens* on the unburned plots (about 26% on unburned vs. about 2% on burned plots). *Bromus rubens* seed density was significantly higher under shrubs on the unburned plots while seed density was higher in the shrub interspaces on the burned plots, probably because greater fuel loads under shrubs allows a higher intensity burn—killing *B. rubens* seeds.

14. Abella and others (2011a) used greenhouse experiments to develop a competitive hierarchy of 27 native species with *Bromus rubens* and a field study to assess *in situ* responses of *B. rubens* to native perennial species in the Mojave Desert. Results indicate that interactions with *B. rubens* differ substantially among native species, that these interactions are not as closely linked to biomass production, and that potential exists for identifying native species that can reduce invasion of desert ecosystems.

    a. Native species most competitive with brome in competition experiments were the annuals Esteve’s pincushion (*Chaenactis stevioides*) and western fiddleneck (*Amsinckia tessellata*). Perennial species were the eastern Mojave buckwheat (*Eriogonum fasciculatum*), sweetbush (*Bebbia juncea*), *Thamnosma montana*, and brittlebush (*Encelia farinosa*), which reduced *B. rubens* biomass to 49 to 70% of its grown-alone amount.

    b. In the field, sweetbush and brittlebush supported among the least cover of brome. *B. rubens* attained its highest average cover (19%) below littleleaf ratany (*Krameria erecta*), significantly greater than all but 3 of the 16 species evaluated.

    c. Cover by brome was only weakly related to area of the perennial canopy.There was no clear difference in competitive abilities with brome between annual and perennial natives.

15. Abella and others (2011b) studied effects of burial depth (0, 2, 5, or 10 cm) and substrate (none, gravel, or litter) on emergence of *Bromus rubens* (and *Brassica tournefortii*) seeds from the Mojave Desert in a greenhouse experiment. *Bromus* displayed greatest emergence (70%) when sown on the soil surface. Emergence did not differ significantly at a 2-cm depth than at the surface but exhibited sharp declines at the 5- and 10-cm depths. Emergence of surface-sown seeds did not differ among substrates but seeds buried at a 2-cm depth exhibited sharp declines in emergence in gravel substrates. Only the litter treatment had appreciable seed emergence at the 5- or 10-cm depths. The findings suggest that seed fates in soil can be altered by manipulating soil surface conditions.

16. Brooks (2012) conducted a study in California on *Larrea tridentata* scrub in areas affected by multiple fires. The author reported that biomass of the invasive annual grass *Bromus rubens* increased following a first fire but did not increase further with additional fires. In contrast, density, cover and species richness of native perennial plants each decreased following fire and continued to decrease with subsequent fires, although not as dramatically. These results suggest that areas burnt once may need to be protected from additional burning and other significant disturbances to eventually recover the abundance and diversity of perennial species to pre-burn conditions. Control of *B. rubens* may be equally warranted after one, two, or three fires, but active revegetation of native perennial plants is most warranted following multiple fires.

17. Abella and others (2012a) conducted a study of *Bromus rubens* at 126 sites on the Lake Mead National Recreation Area and evaluated 97 biophysical variables, e.g. soil types, pH, elemental composition, elevation, precipitation, and temperatures. In an effort to develop distribution models for *B. rubens*, simpler models often portrayed its distribution as well as more complicated models, but varied in their ability to describe presence compared with absence.

    a. They found that *B. rubens* occupied 44% of 126 plots at elevations from 356 to 1222 m. For example, a simple classification tree using only elevation, soil group, parent material, and vegetation type improved estimates of *Bromus* presence for 55% of sites, absences for 87% of sites, and overall for 73% of sites compared with a naïve model containing the observed frequency of *Bromus* in the data.

    b. *B. rubens* distribution was not correlated with soil nitrogen. This finding was inconsistent with the supposition that nutrient-rich soils are more prone to invasion. Instead, there was a relationship to soil parent material and great-group taxonomic units. *B. rubens* was correlated with native perennial species richness, which is consistent with the postulate that exotic species abundance is correlated with species-rich habitats. However, the correlation was weak and similar in strength to many other environmental variables.

   c. When assessed on a relatively low-elevation landscape in the region of Lake Mead Recreation Area, areas most invaded by *Bromus rubens* included higher elevations (>777 to 1,222 m) but also the lowest elevation sites (149–490 m). Amount of precipitation in October was a factor in some models. Occurrence of *B. rubens* was more common in limestone-sandstone soils and with burrobush (*Ambrosia dumosa*) and mixed perennial communities. Areas least inhabited by *B. rubens* were low elevations (< 491 m), gypsum soils, and creosotebush (*Larrea tridentata*) and saltbush (*Atriplex* spp.) communities.

18. Barrows and Murphy-Mariscal (2012) conducted studies on plants and climate data from Joshua Tree National Park, CA. *Bromus rubens* occurred throughout all locations where Joshua trees (*Yucca brevifolia*) were recorded. Given this co-occurrence with Joshua trees as well as its broader occurrence at lower, warmer-drier elevations, it appears that *B. rubens* would shift in response to climate change much the way Joshua trees would. This suggests that an interaction between increased invasive grass-spread wildfires, and a climate change-related increase in severe wildfire conditions, will threaten the sustainability of Joshua trees even within Joshua Tree National Park refugia.

19. Bykova and Sage (2012) conducted controlled acclimation experiments using seeds collected in the Mojave Desert, Western United States, and Toronto, Canada. They demonstrated that 8-week-old plants of *Bromus rubens* and *B. tectorum* had similar responses to winter cold and artificial freezing treatments. However, *B. rubens* had a slower acclimation rate to subzero temperatures than *B. tectorum* and could not survive a rapid temperature drop from 1 to 14 °C even though 4-month-old *B. rubens* populations were as cold tolerant as *B. tectorum*. Results show that severe and sudden freeze events in late autumn can kill young plants of *B. rubens* but not *B. tectorum*. Such events could exclude *B. rubens* from the relatively cold, intermountain steppe biome of Western North America where *B. tectorum* predominates.

20. Jurand and Abella (2013) evaluated 12 previously burned sites at Red Rock Canyon National Conservation Area, NV. *Bromus rubens* constituted 92% of all seeds that emerged from seed banks 5 to 31 years post-fire (time since fire). *B. rubens* density in the soil seed bank was spatially variable and not related to TSF or burn status (burned and unburned). In unburned areas and in most TSF groups, *B. rubens* seed densities were lower in interspaces than under shrubs. *B. rubens* plant and seed bank densities can be greatly reduced immediately after fire, suggesting that a window of time may be available to limit *B. rubens* colonization and at the same time facilitate establishment of native species. However, this window is brief and once *B. rubens* is reestablished, soil seed densities in burned areas can be similar to those in unburned areas within 5 years.

21. Jurand and others (2013) conducted field experiments in Red Rock Canyon National Conservation Area, NV, and reported that while the *Bromus rubens* seed bank can be large, it is relatively short-lived and thus susceptible to population crashes in drought years. However, a small proportion of seeds will remain viable over time. The population crashes may represent windows of opportunity for managers to take advantage of reduced *Bromus* seed densities to apply control efforts such as herbicides specifically targeted to exotic annual grass.

22. Grossman and Rice (2014) conducted research to determine if *Bromus rubens* has evolved under experimental conditions elevated atmospheric CO_2. The study was conducted at the Mojave Desert, NV, free-air CO_2 enrichment facility. Within 7 years, field populations exposed to elevated CO_2 evolved lower rates of stomatal conductance, a physiological adaptation known to conserve water. Evolution of lower conductance was accompanied by reduced plasticity in upregulating conductance when CO_2 was more limiting, suggesting that genetic assimilation may be ongoing. The findings suggest that contemporary evolution may facilitate the spread of *B. rubens* in this desert ecosystem.

C. *Bromus tectorum* (Cheatgrass)

1. Hunter (1991), following historic studies conducted in 1957 at the Nevada Test Site, NV, demonstrated that both frequencies and densities of *Bromus tectorum* significantly increased over 30 years.

    a. Originally only present above 1,524 meters, the author documented an increased distribution throughout the lower elevations of Yucca Flat, 1,219–1,524 m, by the late 1980s. The author estimated an increase from fewer than 10 individuals/m^2 in the 1960s and early 1970s to thousands/m^2 in the late 1980s.

    b. The distribution was noted to be patchier and more strongly associated with disturbance. By the early 1980s, several near monoculture patches of *B. tectorum* became noticeably common below 1,524 m on some ground-zero nuclear bombing locations.

2. Literature review. D’Antonio and Vitousek (1992) conducted a literature review of alien grass invasions and concluded that these invasions were most severe in the arid and semiarid West and involved European annual grasses. *Bromus tectorum* was one of the alien species and its appearance was probably related to the historic introduction of sheep and cattle. The best documented example of ecosystem effects of alien grasses in North America was *B. tectorum* invasion into the intermountain West. On a regional level, *B. tectorum* increased both the size and number of fires. Overall, invasion by *B. tectorum* and attendant fires affected at least 40 million ha, making this perhaps most significant plant invasion in North America.

3. Brooks and Matchett (2003) sampled unburned and burned (6–14 years post-fire) sites at Beaver Dam, UT; Spring Mountain, NV; and Joshua Tree National Park, CA. Alien plant species increased while natives decreased with fire in stands of *Coleogyne ramossisima* across the Mojave Desert. The alien plant species that increased the most in cover differed among sites; at Joshua Tree National Park the increase was mainly caused by *Bromus tectorum*.
4. Abella and others (2009b) sampled > 3,000 km of transportation corridors in Clark County, NV, as part of a Weed Sentry program. The authors reported that *B. tectorum* occurred across a broad range of elevations from < 610 m to > 1,830 m.
5. Bykova and Sage (2012) conducted controlled acclimation experiments using seeds collected in the Mojave Desert, Western United States, and Toronto, Canada. They demonstrated that 8-week-old plants of *Bromus rubens* and *B. tectorum* had similar responses to winter cold and artificial freezing treatments, but *B. tectorum* had a faster acclimation rate to subzero temperatures than *B. rubens.* Results showed that severe and sudden freeze events in late autumn can kill young plants of *B. rubens* but not *B. tectorum*.
6. Reisner and others (2013) sampled 75 sites in the north Great Basin Desert and found no evidence to support the hypothesis that cattle grazing reduced cover of *Bromus tectorum*, even at high intensities. They found strong evidence that increased intensity of cattle grazing indirectly promoted increased magnitude of *B. tectorum* dominance by reducing bunchgrass abundance, increasing the size and connectivity of gaps between plants and reducing biotic soil cover that reduces susceptibility to *B. tectorum* invasion.

D. *Schismus* spp. (Mediterranean Split Grass)

1. Brooks (1995) sampled inside and outside the protective fence which excluded livestock grazing and off-road vehicle use at the Desert Tortoise Research Natural Area, CA. He reported that aboveground live annual plant biomass was generally greater inside than outside the fenced plots in the 3 years of the study. A notable exception was the alien grass *Schismus barbatus* that produced greater biomass in the unfenced area.
2. Kemp and Brooks (1998), based upon a review of the literature and personal observations, reported that exotic species, primarily *Bromus rubens, Schismus* spp., and *Erodium cicutarium*, now account for the majority of total annual plant biomass in many regions of the Mojave Desert of California.
3. Brooks (1999b) sampled *Schismus s*pp. at the Desert Tortoise Research Natural Area, CA. *Schismus* spp. was higher in washlets than hummocks but the differences were not significant over both years. *Schismus* spp. and had higher abundance in the year of high rainfall (1995). Biomass of *Schismus* spp. was also higher outside than inside the Desert Tortoise Research Natural Area in 1994.

4. Brooks and Esque (2002) reported that the more common, abundant and widespread invasive plant species, including the annual grass *Schismus* spp., invaded these deserts in the late 1880s and became widespread after the 1950s. These invasive species have an ability to compete with native species, inhibit germination, and alter the fuel structure—in turn altering the fire regime by making sites more susceptible to fire.
5. Brooks (2003) conducted field experiments at three sites in the central, southern, and western Mojave of California and reported that nitrogen addition increased biomass of *Schismus* spp., particularly in the lower productivity inter-shrub spaces.
6. James and others (2006) found that density and biomass of *Schismus arabicus* were significantly affected by neighbor shrub density and biomass and when water was supplied for density and biomass). During early spring (February–March), *Schismus* density and biomass were significantly lower with *Atriplex confertifolia* than with *A. parryi* neighbors. *A. confertifolia* is known to have high rates of root growth and nutrient capture during this time period. However, there was no significant difference during mid spring (April–May). *Schismus* density and biomass were also greater under the continuous (February–June) water supply pattern than when averaged across both spring seasons, probably because the shallow rooted *Schismus* had greater access to water than the more deeply rooted *Atriplex* spp. Thus, the amount of water may be as important as the timing in determining invasibility of *Schismus* in the Mojave Desert.
7. Beever and others (2006) studied in the Mojave National Preserve, CA, and reported that cover of *Schismus barbatus* was strongly related to distance from water, and this relationship changed with elevation and years since removal of grazers. For every 100-m increase in elevation and 10-fold increase in distance, *S. barbatus* cover was reduced by 14 and 29%.
8. Brooks and Berry (2006) sampled 34 sites in desert tortoise critical habitat in the western, southern, and central Mojave Desert, CA, in both wet (1995) and dry (1999) years. They reported that:
    a. Elevation was the most robust predictor of the biomass of *Schismus* spp. with high levels at low elevations. *Schismus* had higher biomass below 800 m (1995) to 1,000 m (1999) than did *Bromus rubens*.
    b. Relative biomass of *Schismus* spp. varied by microhabitat, and was higher in interspaces compared with beneath-canopy microhabitats.
9. Brooks and others (2006), in a study of annual and perennial plants in the vicinity of piospheres in the Pilot Knob Grazing Allotment, CA, reported that *Schismus* spp. increased with proximity to livestock watering sites, particularly within 50 m of the edge of the denuded area.
10. Rodríguez-Buriticá and Miriti (2009), drawing on results from observational and manipulative field studies conducted in Joshua Tree National Park, CA, indicated that density of *Schismus* spp. was independent of *Ambrosia* shrubs, but enhanced near shrub canopies. The authors demonstrated that *Ambrosia* adults can enhance individual *Schismus* growth. Changes in densities of *Schismus* decreased performance of *Ambrosia* seedlings.

a. The authors reported that the density of *Schismus* was independent of *Ambrosia* shrubs, but growth was enhanced near shrub canopies.

b. Using greenhouse experiments, competition between seedlings was observed, where *Schismus* seedlings reduce growth of *Ambrosia* seedlings. Because *Ambrosia* is an important nurse plant for a variety of annuals and perennials, unmanaged *Schismus* may cause reductions in diversity of native plants.

c. The results also indicated a net positive effect of *Ambrosia* on *Schismus* on growth under *Ambrosia* canopies.

d. Although a reduction of seedling establishment is not usually expected to slow population growth of long-lived perennials, recent unprecedented adult mortality in this community, and the well documented facilitative role of *Ambrosia*, suggest that *Schismus* invasion may be of high ecological significance for native perennial plant populations

11. Steers and Allen (2010) reported that a raking treatment performed poorly but treatments utilizing Fusilade II, a grass-specific herbicide, nearly eliminated invasive grasses (*Schismus* spp.), achieved native annual dominance, and increased native perennial abundance. The authors reported that extreme caution must be used when applying Fusilade II where native grasses or native *Erodium* species occur. *Vulpia octoflora*, a native annual grass that did occur occasionally in plots treated with Fusilade II, did not appear to be damaged. These results indicate that in the absence of invasive grasses and forbs, the native annual community can be resilient to fire disturbance and native perennials can recover in burned *Larrea tridentata* shrublands.

12. Suazo and others (2012), drawing on a field experiment at the Lake Mead National Recreation Area, NV, reported that *Schismus* spp. showed the greatest invasive potential at experimental sites following manipulations to water availability and soil disturbance. Water pulses at approximately 2-week intervals stimulated high densities of *Schismus* seedlings over two field seasons (2008 and 2009). Density also increased with disturbance in non-watered plots in 2009. At a landscape scale, invasion by *Schismus* spp. could be facilitated by increased human activity. For example, the construction of solar energy facilities could create habitat conditions suitable for invasions through removal of vegetation and soil disturbance. Given the observed responses by *Schismus* to water manipulations, invasibility could be further enhanced by changes in precipitation patterns.

E. *Erodium cicutarium* (Stork's bill)

1. Kemp and Brooks (1998), drawing from a review of the literature and personal observations, reported that exotic species, primarily *Bromus rubens*, *Schismus* spp., and *Erodium cicutarium*, now account for the majority of total annual plant biomass in many regions of the Mojave Desert of California.

2. Brooks (1999b), based on 2 years of sampling at the Desert Tortoise Research Natural Area, CA, reported that biomass of *Erodium cicutarium* was higher in washlets than hummocks but that the differences were not significant over both years, primarily because their spatial patterns differed so strongly between years. Proportional biomasses of *Erodium cicutarium* (and three other species of aliens) were higher in the year of low rainfall than in the year of high rainfall.
3. Brooks (2003) conducted field experiments at three sites in the central, southern, and western Mojave Desert, CA. He reported that nitrogen addition increased the biomass of *Erodium cicutarium*, particularly in the inter-shrub spaces where soil nutrient levels were naturally low.
4. Brooks and Matchett (2003) sampled unburned and burned (6–14 years post-fire) sites at Beaver Dam, UT; Spring Mountain, NV; and Joshua Tree National Park, CA. The authors reported that alien plant species increased 6–14 years post-fire in stands of *Coleogyne ramossisima.* The alien plant species that increased the most in cover differed among sites; at Beaver Dam and Spring Mountain enhanced cover of alien plants was mainly due to *Erodium cicutarium*.
5. Brooks and Berry (2006) sampled 34 sites in critical habitat in the western, central, and southern Mojave Desert in wet (1995) and dry (1999) years. They reported that:
   a. *Erodium cicutarium* biomass was positively correlated with dirt roads in wet and dry years; biomass increased as density of dirt roads increased
   b. Relative biomass of *Erodium cicutarium* varied by microhabitat, and was higher in interspaces compared with beneath-canopy microhabitat.
   c. *Erodium cicutarium* was not significantly correlated with *Bromus rubens*, *Schismus* spp. or elevation during 1995 or 1999.
6. Brooks and others (2006), in a study of annual and perennial plants in the vicinity of piospheres at the Pilot Knob Grazing Allotment, CA, reported that *Erodium cicutarium* increased with proximity to livestock watering sites, particularly within 200 m of the edge of the denuded area.
7. Steers and Allen (2010) conducted field experiments in the Big Morongo Canyon Preserve, CA, to control invasive annual grasses and forbs in the first 3 years following fire. A raking treatment to remove all annuals reduced abundance of invasive annual grass by about one-half, had little effect on native annuals, but overall performed poorly. Treatments utilizing Fusilade II, a grass-specific herbicide were effective at reducing the invasive forb *Erodium cicutarium*, achieved native annual dominance, and increased native perennial abundance. Fusilade II was most effective when plants were at an earlier growth stage (e.g., not flowering) and under non-drought conditions. Further, no evidence of negative effects on native species was observed, although extreme caution must be used when applying this product, especially where native grasses or native *Erodium* species occur. Once invasive annuals were removed, passive perennial plant recruitment, particularly in the interspace, also increased. These results indicate that in the absence of invasive grasses and forbs, the native annual community can be resilient to fire disturbance and native perennials can recover.

F. *Brassica tournefortii* (Sahara Mustard)

1. Trader and others (2006) studied seed production of *Brassica tournefortii* in two microhabitats (beneath canopy of *Larrea tridentata* or *Ambrosia dumosa*) and in the interspaces) and at two habitats (roadside, 20 m from the berm) at three roadside sites in the Colorado Desert, CA (Coachella Valley, Highway 95 south of Needles), and in Highway east of Yuma, AZ. The authors reported that larger plants produced more seeds per plant and greater seed biomass per plant. Their results indicated that production of individual plants could be higher in plots with lower densities. Further, their findings suggest that control efforts that do not remove all individuals may reduce densities but inadvertently increase net seed production within treated areas.

2. Bangle and others (2008) performed five experiments on seeds collected from the eastern Mojave Desert, and found characteristics that make *Brassica tournefortii* a potentially vigorous competitor of native annuals in the Mojave Desert.

    a. Seeds germinated at a wide range of temperatures (16–32 °C), under moderate salt concentrations (up to 3.20 dS m^{-1}), in 24 h of light or darkness, and after 10 weeks of submergence in water

    b. *B. tournefortii* seeds remained viable after extended submergence and could float across large bodies of water with seed pods intact.

    c. The authors concluded that this species is a highly successful invader and may pose a considerable threat to native annuals because of its early seedling emergence and ability to germinate in moderately saline soils at a wide range of temperatures, and after extended storage and submergence. Control efforts appear to be most efficient early in establishment while plants are still in the rosette stage and can be uprooted without the need for bagging and removal. Managers should initiate surveys soon after rainfall events and focus on early detection around shoreline habitats that may be vectors for spread, including areas experiencing little or no human contact.

3. Barrows and others (2009) reported that the density and percent cover of native annual species increased following experimental removal of *Brassica tournefortii* in the Coachella Valley, Colorado Desert, CA. In a year of high rainfall, composition shifted increasingly toward *B. tournefortii.* High between-year variance in precipitation may be a key to maintaining biodiversity as *B. tournefortii* is less abundant in drier years. Without control measures, the long-term impacts of this species to desert biodiversity may rest on changing climate. Wetter conditions or increased periodicity of high rainfall years will favor *Brassica* and result in reduced biodiversity, especially of native annual plants. Drier conditions will keep *Brassica* from becoming dominant but may have other negative consequences on the native flora and fauna. Given increased abundance of *Brassica* in wetter years, control efforts applied during wetter years may be an appropriate management direction. However, less than complete control may have little impact on future *Brassica* abundance.

4. Abella and others (2009a) sampled > 3,000 km of transportation corridors in Clark County, NV, as part of a Weed Sentry program. The authors reported that *B. tournefortii* occurred in the most soil types (47% of 256) surveyed in the study area and on 91% of 45 soil types below < 610 m elevation. It was the most frequently detected species below 915 m elevation.
5. Ennen and others (2011) reported that seeds of *Brassica tournefortii* were observed on the carapaces of two desert tortoises at a utility-scale renewable energy wind park in California, suggesting that tortoises could be a mechanism of dispersal.
6. Abella and others (2011a) studied effects of burial depth (0, 2, 5, or 10 cm) and substrate (none, gravel, or litter) on emergence of *Brassica tournefortii* and *(Bromus rubens)* seeds from the Mojave Desert in a greenhouse experiment. *Brassica* displayed greatest emergence (52%) when sown on the soil surface. Emergence was significantly lower at 2-cm depth than at the surface but exhibited sharp declines at the 5- and 10-cm depths. Emergence of surface-sown seeds did not differ among substrates but seeds buried at a 2-cm depth exhibited sharp declines in emergence in gravel substrates. Only the litter treatment had appreciable seed emergence at the 5- or 10-cm depths. The findings suggest that seed fates in soil can be altered by manipulating soil surface conditions.
7. Suazo and others (2012) conducted field experiments at the Lake Mead National Recreation Area, NV, on *Brassica tournefortti*. Treatments of water and disturbance did not significantly affect the seedling density of *B. tournefortii,* though silique production was greater in disturbed plots. *B. tournefortii* showed little invasive potential following manipulations to water availability and soil disturbance.
8. Marushia and others (2012) conducted controlled experiments on three species of Brassicaceae in mesic, desert, and greenhouse locations in the Mojave Desert. The authors suggested that *Brassica tournefortii* had a more rapid phenology that allowed it to produce seed in a short period of time and under a wider range of temperature and drought conditions than two other closely related mustard species, *B. nigra* and *Hirschfeldia incana*.
    a. All three species had high fecundity under well-watered conditions. However, *B. tournefortii* was less affected by site and water availability than the other closely related mustard species, but was smaller and less fecund regardless of experimental conditions.
    b. The major difference among the three species was that *B. nigra* and *Hirschfeldia incana* required 2 more weeks to produce seed than did *B. tournefortii*, which enabled *B. tournefortii* to produce seed before peak summer temperatures. Rapid phenology allowed *B. tournefortii* to reproduce consistently under variable, stressful conditions such as those found in southwestern deserts.

   c. Rapid phenology and drought response partition invasion patterns of nonnative mustards, including *B. tournefortii*, along a gradient of aridity in the southwestern United States. These traits may be predictive traits for other potential invaders of arid and highly variable ecosystems such as the Mojave Desert.

9. Abella and others (2013) experimentally tested (a) effects on *Brassica tournefortii* seed size and germinability of four physical treatments (none, hand pulling entire plant, breaking plant off at base flush from ground; removing siliques by hand) and influences of timing and treatment at three different seed developmental stages (undeveloped, developing, developed); and (b) effects on seed germinability of 3 different herbicides (glyphosate, metsulfuron-methyl, and 2,4-D) at three different seed developmental stages. For uprooted plants, plant material was laid on the ground. Studies were conducted in washes at Lake Mead Recreation Area, NV.

   a. Effects of physical treatments on silique and seed size depended on seed development stage.

   b. Separating siliques from plants was the most effective treatment for reducing germination, but results depended on development stage at which treatments were applied. Separating and pulling also prevented at least 70% of undeveloped seeds from germinating, but these treatments had little impact on developing and developed seeds.

   c. All three herbicides inhibited all or nearly all germination in all three seed development stages.

   d. The findings suggest that physical treatments should occur at or before the flowering stage, before seed production, to avoid maturation of the seed on plant material after treatment. The plants need to be removed from the treated site.

   e. Other topics—solarization and burn piles.

10. Berry and others (2014b) reported on a long-term study of *Brassica tournefortii* in the Chemehuevi Valley, western. Sonoran Desert, CA, and modeled factors contributing to invasion and establishment of this non-native from 1979 to 2009. The authors said that:

   a. *B. tournefortii* invaded the valley through a major highway and the Chemehuevi Wash, a major axial valley ephemeral stream channel, probably in the 1970s and by 2009 had colonized 22 km into the eastern part of the valley. Significant predictor variables were type and age of surficial geology, proximity to the highway and axial valley ephemeral stream channel, and the number of small ephemeral stream channels. Overall, this non-native annual plant rapidly colonized and quickly became established in naturally disturbed areas, such as stream channels and in young soils. Older soils (140,000–300,000 years old), such as those with desert pavement, were less vulnerable.

   b. *B. tournefortii* favored microhabitats under shrubs.

   c. As *B. tournefortii* became established, the proportional biomass of native winter annual plants declined.

G. Effects of Non-Native Annual Forbs and Grasses on Desert Tortoises: see Chapter 5, Foraging Behavior, Digestion, and Nutrition, and Chapter 8, Health and Disease (IV.H., Nutritional Requirements, Deficiencies, and Health; and X. Trauma)

V. Wildfire

A. History of Fires in the Mojave and Western Sonoran Deserts

1. Literature review. Lovich and Bainbridge (1999) reported that unlike parts of the Sonoran and Chihuhuan Deserts, fire was not an important factor in shaping the prehistoric structure and dynamics of plant communities in the California deserts. However, the proliferation of exotic annual plant species such as *Bromus, Schismus,* and *Salsola* has dramatically increased the fuel load and fire frequency in parts of the California deserts.
2. Brooks and Esque (2002), drawing on studies conducted in the Mojave and Colorado Deserts in California reported that the annual number of fires across the deserts increased significantly between 1980 and 1995. While the number of lightning-caused fires remained unchanged over the period (about 25%), the remaining fires were caused by an increase in human-caused ignitions, increased fuel loads created by alien annual grasses, and increased rainfall.
3. Brooks and Matchett (2003) sampled unburned and burned (6–14 years post-fire) sites at Beaver Dam, UT; Spring Mountain, NV; and Joshua Tree National Park, CA. They reported that fire reduced *Coleogyne ramossisima* cover and increased species evenness. Fire also decreased species richness after burning, though results varied among spatial scales. Total cover was unaffected by fire, but native species richness and cover decreased, whereas alien richness and cover increased after burning. Fire had no effect on frequency, and had variable effects on cover of alien annual grasses. Results indicated that in *Coleogyne ramossisima* stands, species richness can vary among sites at local spatial scales, and the effects of fire can vary among plant life-forms and between natives and aliens.
4. Brooks and Matchett (2006) reported that between 1980 and 2004, 8,699 fires occurred in the Mojave Desert and burned 292,017 ha. Most fires occurred in middle elevation shrublands dominated by creosote bush (*Larrea tridentata*), Joshua tree (*Yucca brevifolia*), and/or blackbrush (*Coleogyne ramosissima*). Human caused fires prevailed in the low and middle elevation and the desert montane zones, where human visitation rates are generally higher. Lightning-caused fires were the norm in the high elevation woodlands and more prevalent in the central and eastern half of the Mojave Desert.
    a. The best fit model for fire size from 1980 to 2004 in the Mojave Desert included the variables for ecological zone and for rainfall 1, 2, and 3 years prior to the fire season. The main effects of rainfall generally followed a pattern of larger fires following years of high rainfall. There was also an interaction between rainfall and ecological zone where rainfall had its greatest effect on fire size in the middle elevation zone.

b. Increased fire size was likely related to the flush of non-native annual grasses, *Bromus rubens* in particular, that produced continuous fuelbeds following years of high rainfall. This dynamic also occurred at lower elevations but the cover of native perennial fuels was lower there and muted the effects of the ephemeral fuels. Fire size did not vary with rainfall at elevations above the middle elevation shrublands, indicating that native woody fuels dictate fire regimes. The analysis of fire data from 1980 to 2004 suggested that an invasive plant/fire regime cycle is currently establishing in the middle and possibly the low elevation shrublands of the Mojave Desert, but not at higher elevations.

c. Given the higher susceptibility of the middle elevation shrublands to increased fire in the Mojave Desert, this zone could be a focus for pre-positioning of fire suppression equipment or crews, or for prioritizing rehabilitation or restoration of burned lands.

5. Vamstad and Rotenberry (2010) conducted vegetation surveys of a chronosequence of historic burns in Joshua Tree National Park, CA. Their studies showed an expected reduction of the percent cover of long–lived perennial plants after fire, followed by a return to unburned cover levels between 19 and 65 years since the original fire. Sub–shrubs established in the 9 and 13 years since burned and then declined. Annual plant cover showed a general increase through time in sites that burned, predominantly fromexotic annuals (*Bromus tectorum*, *Bromus rubens, Schismus barbatus*, and *Erodium cicutarum*)

6. Brooks and Chambers (2011) reviewed studies in North American deserts and reported that the Mojave Desert has lower annual precipitation than the Great Basin or Chihuahuan Deserts. The Mojave is also a less productive desert ecosystem that tends to be less resilient to fire and to plant invasions. Pre-settlement fires in low to mid-elevation shrublands of the Mojave Desert were infrequent and small. Many plant species occurring in the Mojave are fire intolerant. However, fire frequencies increased after settlement, in which a feedback loop of non-native annual grass invasions increased fuel continuity, and repeated fires decreased the recovery potential of native species with low fire tolerances.

7. Van Linn III and others (2013) created models for predicting fire risk in Gold Butte, NV. The authors reported that the best predictive model for fire risk at Gold Butte included two surrogates for ignition (distance to roads and lightning density), modelled fuel loading, estimates of maximum spring and summer fuel moisture, and perennial vegetation. The model had an area–under–the–curve performance measurement of 0.88 and 0.85 for the study site and for a similar validation site, respectively, indicating that the model accurately predicted fire occurrence. This type of approach, combining remote sensing data with field surveys, could be a management tool to identify areas with high fire risk.

B. Effects of Fire on Soils

1. Steers and others (2011) conducted experimental additions of carbons to 12 plots located in burned and unburned creosote brush scrub within Big Morongo Canyon Preserve, CA. The authors showed, following rain events, that soil microbial biomass increased greatly in the high carbon treatment (100:1 soil carbon to nitrogen ratio). Consequently, nitrate and ammonium were both significantly lower than the control.
2. Soulard and others (2013) used terrestrial LiDAR scans to assess effects of fire on surface topography, soil roughness, and vegetation on burned and unburned plots in the Grand Canyon Parashant National Monument, AZ. Eleven years following prescribed burns, soil mound volumes and soil-surface roughness were significantly lower on burned vs. unburned plots. Altered soil surfaces likely had been smoothed via wind and water erosion following fire treatments.
3. Abella and Engel (2013) evaluated influences of fire on soils across a 2 to 29-year, time-since-fire gradient in 17 *Coleogyne* and 15 *Larrea* communities in the eastern Mojave Desert, NV. These sites were compared with paired unburned areas. Organic C and total N were two key properties significantly related to fire, being 25 and 44% greater in burned than unburned areas. Burned and unburned soils overall were more different in *Coleogyne* than in *Larrea* communities, and time since fire was not strongly related to soil properties.

C. Effects of Fire on Vegetation

1. See Habitat Degradation, Invasive Species
2. Literature review. Lovich and Bainbridge (1999) reported that the proliferation of exotic annual plant species such as *Bromus, Schismus*, and *Salsola* has dramatically increased the fuel load and frequency of fires in parts of the Mojave Desert, CA. Native perennial shrubs have low rates of recovery to low-intensity fires where, for example, *Larrea tridentata* scrub in the upper Coachella Valley has been replaced by open stands of *Encelia farinosa*, native ephemerals, and exotic *Schismus* and *Bromus* grasses. In addition, post-fire recovery of cacti was low.
3. Brooks (1999a) reported that alien annual grasses, primarily *Bromus* spp. and *Schismus* spp., contributed most to the continuity and amount of dead annual plants and the spread of summer fires. The rate of fire spread varied between species. Fire spread rapidly (12 m/min) and continuously across interspaces with *Bromus* and slowly (1 m/min) and discontinuously with *Schismus*. These results suggest *Bromus* spp. fuel fast moving hot fires whereas *Schismus* spp. fuel slower moving cooler fires.
4. Brooks (2002) sampled three sites in the central, southern and western Mojave Desert, CA, and concluded that fire initially decreased the biomass of *Bromus* spp., increased the biomasses of *Schismus* spp. and *Erodium cicutarium*, and had variable effects on native annual plants, depending on microhabitat.

   a. Alien and native species richness was decreased during all 4 post-fire years in the undercanopy microhabitat. In the drip line microhabitat, alien and native richness decreased during the first 2 years, but native richness increased beginning in the third year. Additionally, *B. rubens* and *B. tectorum* eventually returned to or exceeded pre-fire dominance during subsequent years.
   b. Peak fire temperatures varied horizontally and vertically within fires at experimental sites and this variation may explain post-fire patterns of annual plant biomass, which were most likely caused by differential seed mortality. Peak fire temperatures were higher beneath *Larrea tridentata* shrubs than in the interspaces. Beneath *Larrea*, lethal fire temperatures for annual plant seeds occurred above- and belowground. This resulted in 4 post-fire years of reduced annual plant biomass and species richness likely due to seed mortality, especially of *B. rubens* and native forbs. At the canopy drip line, lethal fire temperatures occurred only aboveground, reducing annual plant biomass for 1 year and species richness for 2 years, and increasing biomass of *Schismus* sp., the alien forb *E. cicutarium*, and native annuals after 3 years. Negligible changes were caused by fire in interspaces, and there were no substantial differences between spring and summer fires.
   c. Fire may reduce dominance of *B. rubens* during the first few post-fire years, especially if interspace densities are low and fires pread is homogeneous, leaving few unburned shrub islands. These few years of low alien densities may be sufficient for native plants introduced as seeds to become established.

5. Brooks (2003) sampled plants and fire temperatures at three sites in the central, southern, and western Mojave Desert, CA. He reported that fire can temporarily reduce seed densities of *Bromus rubens*, but dominance of *Schismus* spp. may quickly increase above pre-fire levels. Additionally, *B. rubens* and *B. tectorum* eventually return to or exceed pre-fire dominance during subsequent years.
6. Brooks and Matchett (2003) compared burned and unburned sites 6 to 14 years after fires occurred at sites in Beaver Dam, UT; Spring Mountain, NV; and Joshua Tree National Park, CA. The authors reported that fire reduced *Coleogyne ramosissima* cover and increased species evenness. Fire also decreased species richness after burning, though results varied among spatial scales. Total cover was unaffected by fire, but native species richness and cover decreased, whereas alien richness and cover increased after burning.
7. Abella (2009) synthesized data on recovery of plants post-fire at sites in the Mojave and Sonoran Deserts. Post-fire sprouting by desert perennials was generally limited but varied among species, e.g., only 3–37% of *Larrea tridentata* resprouted compared with 64–86% of *Yucca schidigera* in the Mojave Desert. In the Mojave Desert some studies reported close relationships between times since fire (TSF) and return of cover. Studies with the longest TSF (≥37 years) found that cover had returned to within 10% of unburned areas within about 40 years. However, species composition post-fire had low convergence with unburned composition even 47 years after fire. In general, cover of perennials re-established faster than composition of perennials.

8. Abella and others (2009b) compared plots burned by a wildfire 2 years earlier with adjacent unburned plots at a site near Las Vegas, NV.
   a. The authors reported that richness of live annual plant species was 3.4 times greater at the 1-m^2 scale and 26% greater at the 100-m^2 scale on the burned versus unburned plots. Exotic annuals comprised 50% of the species richness at the 1-m^2 scale and 18% at the 100-m^2 scale in the burned plots. Density of *Bromus rubens* seeds was four times lower at 0–5 cm in the burn compared to the unburned site.
   b. At both scales, only 7% of the species richness on both burned and unburned plots was due to native plants, with perennial forbs making up 34–35% on the burned plots and 2–13% on the unburned plots, while perennial shrubs exhibited an opposite pattern with 5–30% on the burned plots and 76–79% on the unburned plots.
   c. Composition of perennial species shifted from dominance by late-successional native shrubs (e.g., *Coleogyne ramosissima*) on the unburned plot to native forbs: *Sphaeralcea ambigua*, *Eriognum inflatum*, and *Baileya multiradiata*. Importance values of *Coleogyne ramosissima* and *Yucca baccata* were positively correlated with unburned plots. Only 3% of *Yucca* spp. had crowns that survived on the burned plots, however, 64% of them resprouted.
9. Vamstead and Rotenberry (2010) conducted vegetation surveys of a chronosequence of historic burns in juniper and Joshua tree woodland in Joshua Tree National Park, CA. The surveys showed an expected reduction of the percent cover of long-lived perennial plants after fire, followed by a return to unburned cover levels between 19 and 65 years after the burns.
   a. Sub-shrubs established 9 and 13 years post-burns and then declined. Annual plant cover showed a general increase through time in sites that burned, and was composed predominantly of exotic annuals (*Bromus tectorum, B. rubens, Schismus barbatus*, and *Erodium cicutarum)*. Bunchgrasses showed no significant pattern.
   b. Species composition of vegetation changed with time since fire but reestablished vegetation assemblages did not converge to the assumed pre-burn condition, even after 65 years. These differences probably relate to the slow rates of establishment of certain vegetation components that make up the pre-burn condition of the plots. *Yucca brevifolia* was still rare even in the oldest burn sites. These results suggest that invasion by exotic plant species, nitrogen deposition, and global climate change together may initiate a fire cycle in this ecosystem that will arrest succession so that Joshua tree woodland cannot reestablish. Thus, it is possible that the long-term reestablishment of plants and animals in these habitats, with a shorter fire-return interval, may never return to a pre-burned condition.
   c. The results of the vegetation and rodent surveys across a chronosequence of historic burns showed that fire does not seem to significantly impact diversity. However, if the goal is to manage these habitats for specific "old growth" species like the Joshua tree, California juniper (*Juniperus californica*),

blackbrush (*Coleogyne ramossissima*), little pocket mouse (*Perognathus longimembris*) or canyon mouse (*Peromyscus crinitus*), the rates of reestablishment of these species will have to be considered in the context of a shorter fire cycle. Full fire suppression tactics and/or fire prevention measures would be necessary to achieve this type of management.

10. Esque and others (2010c) conducted an experimental fire in the Parashant National Monument, AZ, and reported that total annual plant production increased in response to increased inorganic nitrogen, following experimental fires and, when analyzed separately, the invasive annual grass *Schismus* spp. significantly increased in response to elevated inorganic N while native annual plant biomass decreased. These results suggest that increased N availability following wildfire can favor invasive annuals over natives. Whether the short-term success of invasive species following fire will direct long-term species composition changes remains to be seen.

11. DeFalco and others (2010) compared burned and unburned areas 5 years after the Juniper Fire Complex of May 1999 at Joshua Tree National Park, CA. Exacerbated by protracted drought, approximately 80% of burned *Yucca brevifolia* died compared with 26% in adjacent unburned site. Post-fire sprouting prolonged survival, but only at the wetter, high-elevation sites. Many burned plants < 1 m tall died immediately, and survival of all but the tallest, oldest plants declined to the same low level by 5 years post-fire. During succeeding dry years, *Thomomys bottae* (pocket gopher) damage reduced plant survivorship at low-elevation, unburned sites and diminished survival of burned plants in all but the driest site, which already had low survival. These results suggest that continued El Niño Southern Oscillation episodes and more frequent wildfires expected for the desert Southwest will likely shift *Y. brevifolia* population structure toward tall, old adults with fewer opportunities for recruitment, thus imperiling the persistence of this unique plant community.

12. Esque and others (2010b) conducted experimental fires of moderate temperatures in the Parashant National Monument, northwest AZ, and reported that fire caused significant seed losses of native and alien annual plants (55 to 80%). Losses were similar among species and microsites with one exception: *Bromus* spp. and *Plantago* spp. had proportionately greater seed mortality than *Schismus* spp. and *Erodium cicutarium*, the latter of which could either lodge into soil cracks or drill into the soil to avoid lethal temperatures. Greater seed mortality occurred beneath shrub canopy microsites compared with interspaces for most species (*Plantago*, spp., *Bromus* spp., and *E. cicutarium*), but had little effect on *Schismus* spp. *Plantago* spp. were the only native species among the common seed types and ranged between 2 and 4% of total seeds in all treatment categories. There was a 59% loss of *Plantago* due to fire, which was moderate compared with the other species in this experiment. No species was extirpated on experimentally burned plots. The relative abundances of common species did not change dramatically as a result of fire or microsite.

13. Literature review. Allen and others (2011) reviewed the fire literature from North American deserts, and reported that hot deserts such as the Mojave have experienced slow recovery of native shrubs and increased growth of invasive grasses following fire. Invasive species may either increase or decrease soil nitrogen and carbon depending on fire temperature and site and species characteristics.
    a. A relatively high proportion of studies reported increases in extractable nitrogen after fire. Mineralization and fixation of nitrogen are often high enough after fire that subsequent productivity balances nitrogen losses.
    b. Elimination of fertile islands (i.e., desert shrubs that draw nutrients and water from deep soils or intershrub spaces) due to fire, along with post-fire erosion, may be a major impact after fire in grass-invaded shrub lands. In the long term, the interaction of fire and invasive species may result in more frequent fires that eliminate fertile islands and reduce the productivity of deserts.
    c. Managers may use fire as a tool to control desert invasive plant species without the concern that N will be irrevocably lost, but this must be done carefully to avoid eliminating shrubs and further increasing invasive species. Using fire as a tool in invaded desert communities is counterproductive, because invasive plants will eventually regain dominance and repeated burning will eliminate woody perennials.
14. Engel and Abella (2011) analyzed recovery of 32 burn sites (15 in *Larrea* and 17 in *Coleogyne*) communities in southern Nevada with burn dates ranging from 1980 to 2007 and time since fires spanning 2–29 years. *Larrea* communities recovered more rapidly than *Coleogyne* communities, with species composition similar to pre-fire levels in < 20 years. In *Coleogyne* communities, fire increased perennial diversity, although this increase was at the cost of converting mature shrublands to early successional forb-shrub communities. These communities were persistent for longer than 29 years and showed little trend for convergence with unburned species composition. Post-fire data suggest that direct intervention, i.e., seeding or planting, may be necessary in *Coleogyne* communities to restore keystone shrub species. Additionally, if *Coleogyne* communities are considered vital habitat, managers may want to consider actions aimed at fire prevention.
15. Steers and Allen (2011) evaluated perennial vegetation at seven paired burned and unburned sites in the western Colorado Desert, CA. The sites spanned a 2–28 year old chronosequence. The studies revealed that fire significantly reduced shrub richness and diversity regardless of time since fire. Total shrub cover and density returned to or exceeded unburned levels at least 20 years after fire, although species composition was almost entirely brittlebush (*Encelia farinosa*), a shortlived shrub and typical colonizer of burned sites. Longer-lived shrubs indicative of unburned vegetation, such as creosote (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), and white ratany (*Krameria grayi*), failed to recover, suggesting *Encelia* shrublands may form an alternate stable state following fire in this region. Shrub plus cacti richness and diversity were lower in burned stands regardless of time since fire. Further research is needed to identify what structural features in *Larrea* shrublands are most important for high plant biodiversity, and

what restoration actions could be employed to recreate those structural features to speed recovery.

16. Brooks (2012) conducted a study to evaluate effects of high fire frequency on *Larrea tridentata* scrub and the invasive annual grass *Bromus rubens* in the central Mojave Desert, CA. Density, cover and species richness of native perennial plants each decreased following fire and also continued to decrease with subsequent fires, although not as dramatically. Biomass of *B. rubens* increased following fire but did not increase further with additional fires. Initial recovery in areas burned once was dominated by *Hymenoclea salsola* and *Achnatherum speciosa*, but lack of recovery after repeated burning suggested there may be limits to resilience of these species. The results suggest that control of *B. rubens* may be equally warranted after one, two, or three fires, but revegetation of native perennial plants is most warranted following multiple fires.

17. Reynolds and others (2012) conducted field experiments in the eastern Mojave on *Yucca brevifolia* and reported that specific germination and establishment requirements limit recruitment rates. Limited recruitment coupled with infrequent seed availability of *Y. brevifolia* affect (delay) return rates to pre-fire densities; thus demographic structure may require decades to centuries, especially in light of potential changes to regional desert climates in combination with the potential for fire recurrence.

18. Soulard and others (2013) used terrestrial LiDAR scans to quantify differences between burned and unburned surfaces. The authors reported that perennial vegetation and paired soil mounds (volumes) were reduced in burned plots compared to unburned plots, following 11 years of post–fire recovery in the Grand Canyon-Parashant National Monument, AZ. Vegetation that had re-sprouted in previously burned plots was roughly 80% of the size of vegetation in unburned plots, while the mounds in burned plots were smaller by 50%.

19. Suazo and others (2013) studied seed removal patterns in burned and unburned desert habitats south of Las Vegas, NV.

    a. Seed removal for *Ambrosia dumosa*, *Hymenoclea salsola*, and *Larrea tridentata* was mostly by rodents, but overall was relatively low (20–30%). Both rodents and ants removed seeds of *Baileya multiradiata, Coleogyne ramosissima, Encelia farinosa, Eriogonum fasciculatum, Penstemon bicolor*, and *Sphaeralcea ambigua* at variable levels through space and time, ranging from 0 to 100%.

    b. Ants were attracted to heavy seeds of *C. ramosissima*, but also removed high proportions of light-weight seed species, indicating that ants can exploit a diverse range of seed masses. Rodents had a preference for heavy, large-seeded species, especially for *C. ramosissima*.

    c. The percentage of total seed removal was highest during spring (16% of offered seed) and summer (21%).

    d. Rodents removed a high proportion of seeds in both burned and unburned habitat, suggesting that rodents can potentially consume large amounts of seeds and maintain stable populations in burned areas. On the other hand, low proportions of seed removed by ants in burned habitat suggested that fire may have negatively affected their foraging.

e. Selective removal of *C. ramosissima* seeds by granivores might influence restoration efforts by limiting ecosystem processes (e.g., creation of fertile soils) that affect long-term plant population dynamics. Protecting seeds from granivores can increase seedling establishment and on-site seed retention. Methods to protect seeds could include (i) selecting species for seeding based on seed characteristics that are not preferred by seed consumers (e.g. small–seeded species), (ii) using 'decoy' seed with high preference value might alleviate granivore pressure on target species, and seeding in the Mojave Desert in late October or early November to avoid high levels of seasonal foraging by granivores.

20. Shryock and others (2014), in a study of several burned sites across the Mojave and western Sonoran Deserts, in CA, NV, and UT, identified life-history traits of perennial species and used them to predict how the species respond to fires. Six life history traits (growth form, lifespan, seed size, seed dispersal, height, and leaf longevity) varied significantly between burned and unburned areas in short and long-term post-fire datasets. Forbs and grasses consistently increased in abundance after fire while cacti were reduced and woody species exhibited variable responses. Woody species were classified into three plant functional types based on combinations of life history traits. Group 1 plants, characterized by short lifespans, low height, and deciduous leaves, increased in abundance after fire. Group 2 plants were reduced by fire and had longer lifespans and evergreen leaves. Group 3 plants decreased after fire, were characterized by long lifespans, large non-wind dispersed seeds, and taller heights. Dominant, long-lived species possess a combination of traits limiting their ability to recover.

D. Direct Effects of Fire on Desert Tortoises

1. Homer and others (1998) described the lesions on an adult male tortoise that was burned in a wildfire. It was found while still alive, shortly after the fire, and was moribund; parts of the shell were melted and skin over the head, one foreleg and hind leg peeled away. Other internal lesions were evident (severe visceral inflammation).

2. Brooks and Esque (2002), drawing on studies conducted in the Mojave and Colorado Deserts, concluded that early season fires are potentially more threatening than summer fires to desert tortoises since tortoises are more likely to be above ground and recently hatched. Small tortoises, such as hatchlings, are more at risk from lethal heating than large ones, because they have a higher surface to volume ratio, allowing heat to penetrate vital organs relatively quickly.

3. Esque and others (2003) conducted post fire surveys for tortoises (and other reptiles at two northeastern Mojave Desert sites (Bulldog and Mile Creek fires). In the burned areas, carcasses were found in the open, under thick vegetation or in shallow shelter sites. Six live tortoises and one dead tortoise were located. The authors concluded that some tortoises can survive fire, perhaps by remaining underground. Even though many tortoises in a given population might be

underground and survive a particular fire during the height of the wildfire season in June and July, fires occurring in habitats heavily infested with introduced grasses are likely to kill a disproportionate number of gravid female tortoises compared to males or non-reproducing females.

4. Drake and others (2012b) reported observations of an adult male tortoise injured by wildfire in the northeastern Mojave Desert. Although the fire damaged the scutes and carapacial bone, smooth keratinaceous tissue developed over the exposed area during the 3 years post fire.
5. Drake and others (2015) compared movement patterns, home-range size, behavior, microhabitat use, reproduction, and survival for 53 adult desert tortoises located in, and adjacent to burned habitat to determine how tortoises respond to recovering burned habitat in Nevada.
    a. Approximately 45% of home ranges in the post-fire environment contained burned habitat, and tortoises used both burned and unburned habitats. Tortoises moved progressively deeper into burned habitat during the first 5 years following the fire and returned to unburned habitat for cover provided by perennial vegetation.
    b. By years 6 and 7, live cover of short-lived herbaceous perennial desert *Sphaeralcea ambigua* that typically re-colonizes burned areas, declined, resulting in contraction of tortoise movements from burned areas.
    c. Health and egg production were similar between burned and unburned areas, indicating that tortoises were able to acquire necessary resources by using both areas.

E. Indirect Effects on Desert Tortoises

1. Literature review. Brooks and Esque (2002), drawing on compiled literature from the Mojave and Colorado Deserts, concluded that fire can negatively affect desert tortoises by reducing cover of perennial plants needed for shelter and by reducing abundance and diversity of native annual plants important as desert tortoise forage.
2. Esque and others (2003) sampled two wildfire sites in the northeastern Mojave Desert following burns and reported that plant tissues burned to ground level, including root crowns in some places. The authors said that fires may impact tortoises through loss of vegetative cover that provides protection from temperature extremes and predators. Changes in vegetative composition post fire may affect diet.
3. See papers on effects of fire on soils and vegetation, also Drake and others (2015).

F. Relationships between Annual Plant Productivity, Nitrogen Deposition, and Fire Size in California Desert Scrub Communities

Rao and others (2015) used a 28-year fire record of 582 burns from low-elevation desert scrub communities in the Mojave and Sonoran Deserts of California to evaluate the relationship of precipitation, nitrogen deposition, and biomass with the distribution of fire sizes using quantile regressions. The vegetation types chosen contained native perennial fuels that are too sparse to carry fire without non-native annual plants filling the interspaces between them and historically have been subject to lower fire frequency. Models using annual biomass have similar predictive ability to those using precipitation and N deposition at the lower to intermediate portions of fire size. No distinct biomass threshold was found, although within the 99th percentile of the distribution, fire size increased with greater than 125 g/m^2 of winter fine fuel production. The results of the study indicate that the fire size distribution can be predicted using precipitation received during the winter rainy season and the prior year's winter precipitation, and that not much additional predictive power is gained by using annual plant biomass modelled from winter precipitation and nitrogen deposition. The authors recommended that it would be prudent for land managers to sample peak annual biomass to be prepared for the largest and most ecologically and economically destructive fires.

G. No Apparent Effects of Burned Habitat on Female Tortoise Activities. Lovich and others (2011b) monitored eight female tortoises at a wind energy facility near Palm Springs, CA, 2–5 and 14–15 years post fire. They reported that:

1. Tortoise activity area, distances between activity areas, and their average elevations did not differ 2–5 years vs. 14–15 years post-fire. Six of eight activity area centroids remained inside the burned area while one moved from unburned to burned area post-fire, suggesting that resource availability was not detrimentally impacted or was compensated following burning, perhaps from high rainfall due to coastal influence at the study site.
2. Annual egg production and body condition did not differ 2–5 years vs. 14–15 years post-fire, suggesting that resource availability for reproduction did not change over the study period following burn. However, no pre-fire reproductive data were available for comparison.

H. Considerations and Actions for Management

1. Brooks (1999a) said that sources of ignition from human activities should be minimized especially where alien annual grasses are abundant and topography is conducive to spread of fire.
2. Brooks and Esque (2002) noted that management of alien plants and fire should be closely integrated, because alien plants can create fuel conditions that promote fire in otherwise fire resistant landscapes that predominate in the Mojave and Colorado Deserts.
    a. Reducing the number of fires started by humans may be accomplished by education and enforcing regulations limiting human activities that cause fires such as camping, target practice with firearms, and vehicle use.

   b. Most large fire and areas of recurrent fire occur within mountainous areas designated as wilderness. The lack of motorized vehicle access in these areas can hinder fire suppression efforts, and managers should consider allowing motorized access by fire crews to control fires. Prescribed fire early in the season may be used to temporarily reduce the dominance of alien annuals prior to revegetation of native plants. However, if alien annual grasses are abundant, fires should not be used because of the significant chance that the native shrubland will be replaced by alien annual grassland.

3. Brooks (2002) reported that fire may reduce dominance of *Bromus rubens* during the first few post-fire years, especially if interspace densities are low and fire spread is homogeneous leaving few unburned shrub islands. These few years of low alien densities may be enough for native plants introduced as seeds to become established.

4. Brooks and Matchett (2006) reported that, given higher susceptibility of the middle elevation shrublands to increased fire in the Mojave Desert, focus on this zone for pre-positioning of fire suppression equipment or crews, or prioritizing rehabilitation or restoration of burned lands would be beneficial.

5. Vamstad and Rotenberry (2010) reported results of vegetation and rodent surveys across a chronosequence of historic burns in Joshua Tree National Park, CA. Their findings showed that fire does not seem to significantly impact diversity. However, if the goal is to manage for specific “old growth” species like the Joshua tree (*Yucca brevifolia*), California juniper (*Juniperus californica*), blackbrush (*Coleogyne ramossissima*), little pocket mouse (*Perognathus longimembris*) or canyon mouse (*Peromyscus crinitus*), the rates of reestablishment of these species will have to be considered in the context of a shorter fire cycle. Full fire suppression tactics and/or fire prevention measures would be necessary to achieve this type of management.

6. Lovich and others(2011c) said that it is prudent to vigorously prevent and suppress wildland firest in tortoise habitat due to the known potential for direct mortality.

7. Abatzoglou and Kolden (2011) said that projected increases in the frequency and onset of extreme fire danger across deserts of theWestern United States, with climate change, is likely to increase mortality rates of native species less adapted to wildfire. This projection increases the importance of proactive reduction of invasive fuel loads.

8. Brooks and Chambers (2011) offered strategies for preventing or managing invasive plant/fire regimes cycles in desert shrublands, including:
   a. conducting periodic resource assessments to evaluate the probability of establishment of an altered fire regime;
   b. developing and understanding ecological thresholds associated with invasion resistance and fire resilience that characterize transitions from desirable to undesirable fire regimes; and
   c. prioritizing management activities that increase resistance to invasion and resilience to fire, including reducing stressors. These stressors include repeated

fires, inappropriate livestock grazing, controlling surface disturbances and invasion corridors like roads and trails. It is important to increase efforts to eradicate invasive species, conduct preventative vegetation management, and seed native plant species after fires.

9. Engel and Abella (2011) reported that post-fire data from the eastern Mojave Desert suggested that direct intervention, i.e. seeding or planting, may be necessary in *Coleogyne* communities to restore keystone shrub species. Additionally, if *Coleogyne* communities are considered vital habitat, managers may want to consider actions aimed at fire prevention.
10. Allen and others (2011) wrote that managers may use fire as a tool to control desert invasives without the concern that N will be irrevocably lost, but this must be done carefully to avoid eliminating shrubs and further increasing invasive species. Using fire as a tool in invaded desert communities is counterproductive, as invasive plants will eventually regain dominance and repeated burning will eliminate woody perennials.
11. Brooks (2012) reported that the first lines of defence against altered fire regimes should include:
    a. vegetation management strategies that minimize dominance of *Bromus rubens* and other non-native invasive plants that facilitate fire spread;
    b. law enforcement activities that reduce the frequency of anthropogenic sources of ignitions; and
    c. fire suppression plans that result in quick responses to fires and limit the size of fires.
12. Van Linn III and others (2013) reported that the best predictive model for fire risk in Gold Butte, NV, in 2005, included two surrogates for ignition (distance to roads and lightning density), modelled fuel loading, estimates of maximum spring and summer fuel moisture, and perennial vegetation. The model had an area-under-the-curve performance measurement of 0.88 and 0.85 for the study site and for a similar validation site, respectively, indicating that the model accurately predicted fire occurrence. This type of approach, combining remote sensing data with field surveys, could be used as a management tool to identify areas with high fire risk.

VI. Roads, Railways, Powerlines, Pipelines

A. Literature review. Lovich and Bainbridge (1999) reported the following impacts from linear corridors to habitat and wildlife: introducing exotic plants from construction equipment to often otherwise undisturbed habitat, disturbing soil crusts, concentrating erosion and runoff, fragmenting habitat, animal mortality on roads, restricting animal movement and gene flow, providing increased access to remote areas for such activities as vandalism and illegal collection of plants and animals, and providing artificial perches and nest site for Common Ravens.

B. Effects on Desert Tortoises

1. von Seckendorff Hoff and Marlow (2002) sampled seven roads in southern Nevada, and reported that the magnitude of the road impact zone, defined as the distance from the road at which 90% of the total desert tortoise sign count was found, varied between 2,150 to 4,250 m for two-lane to four-lane highways, 1,090 to 1,389 m for graded and maintained electrical transmission line access roads, and with no detectable trend on one poorly maintained, paved road. The zone of impact increased significantly with increasing traffic level that ranged from 25 to 5,000 vehicles per day. These results indicate that the cumulative impact of a network of roads significantly reduced the effective area of conserved habitat defined by management prescriptions that do not include road barriers.
2. Boarman and Sazaki (2006) found that tortoise sign counts, corrected for bias caused by two types of sign found in close association (e.g., tortoise scat inside tortoise burrow), increased with distance from the highway.
   a. Tortoise sign increased from 0.6 mean sign at the road edge to 12.5–17.4 mean sign at 400–1,600 m from the roadway edge.
   b. Significant differences also occurred between 400 and 800 m from highway, but the researchers were cautious about that result because the study sites and distances were selected based on accessibility, property ownership, and continuity of habitat rather than randomly.
3. Hughson and Darby (2013), in a study of road effects on tortoises in the Mojave National Preserve, CA, found that traffic volume and speed were significantly higher for Morning Star Mine Road (370.6 ± 15.1 vehicles/day and 112.8 ± 0.9 km/h with a maximum of 193.1 km/h), which connects southern CA to Las Vegas, NV, than for Essex Road (87.6 ± 4.8 vehicles/day and 96.4 ± 0.18 km/h and a maximum speed of 180.2 km/h) that dead ends into local attractions, in the Mojave National Preserve.
   a. The County of San Bernardino provided traffic volumes for Kelbaker Road as 183 vehicles/day and the authors estimated traffic on Lanfair Road as 35 vehicles/day; no traffic speeds were provided for these roads.
   b. Tortoise mortality, however, was not different between the four roads and ranged from 0.66 to 0.81 dead tortoises per transect over 3 years.
   c. Road signage, which increased from no warning signs to warning signs clearly visible to warning signs associated with flashing yellow lights, had no noticeable effect on driver behavior and only 4% of drivers on Morning Star Mine Road and 14% on Essex Road reacted to model tortoises placed on the road edges.
   d. A road-edge effect appeared to have occurred at a distance of $\leq$ 400 m, but small sample size and only one transect to characterize undisturbed desert likely weakened the significance compared to similar studies (e.g., Nafus and others, 2013). Nevertheless, results suggested that even roads with lower traffic speeds and volumes could negatively affect desert tortoise recovery.

4. Nafus and others (2013) found that the mean relative abundance of tortoises was significantly lower along medium (30–60 vehicles/day) and high traffic volume (320–1,110 vehicles/day) roads, as well as at 400 and 800 m distances from high traffic volume roads in the Mojave National Preserve, CA.
    a. Likewise, low traffic roads (≤ 1 vehicle/day) had a greater abundance of burrows, a measure of where tortoises were willing to settle, and burrow abundance along high traffic roads increased significantly only at 400 and 800 m. Traffic volume also correlated with distance to first burrow with the first burrow encountered closer to roads with low traffic and farther from roads with high traffic. Also as traffic volume increased, the number of live tortoises decreased while tortoise mortality increased.
    b. Tortoises found near high traffic roads were 32–38% smaller (146 ± 21 mm) than those found near medium (230 ± 10 mm), low (235 ± 9 mm), or no (213 ± 7 mm) traffic roads. Tortoises near low traffic roads were significantly larger than tortoises not associated with roads.
    c. Similarly, the proportion of juvenile tortoises within 500 m of the high traffic road was greater than other road types (low, medium, high traffic); the loss of large reproductive tortoises could reduce population growth rates. The proportion of males to females was similar across all roads types. Distance from roads or road type had no effect on perennial shrub volume, distance to the nearest three shrubs, or the number of small mammal burrows. Therefore, the correlation between tortoise demography or relative abundance of their sign and traffic volume is most likely due to the direct effects of mortality or road avoidance rather than indirect effects from changes in habitat quality due to the road.
    d. Low traffic roads may offer some benefit to tortoises though, as run-off may increase drinking opportunities and annual plant production. In fact, tortoises along low traffic roads were far larger than even those found at > 500 m from roads. However, benefits from low traffic roads could only be gained if there was no subsequent increase in tortoise mortalities from vehicle strikes. Further, most of the area within the desert tortoise's range supports roads with traffic volumes much higher than those found within the Mojave National Preserve.
5. Berry and others (2013), in a study of factors contributing to tortoise distribution and low densities in the northwestern Mojave Desert, CA, reported low densities of tortoises near an infrequently used paved road. Models of tortoise sign densities and several anthropogenic and predator variables showed increases in tortoise sign densities with increased distance from the paved road; other factors were also important in determining distribution and densities at the terminus of the road, a denuded area.

C. Effects on Tortoise Habitat

1. Sharifi and others (1997) studied physiological parameters of gas exchange in dusted versus control shrubs near an unpaved road at Fort Irwin National Training Center, CA. Dusted plants of three species of common desert shrubs (*Larrea tridentata, Hymenoclea salsola*, and *Atriplex canescens*) had reduced maximum rates of net photosynthesis, maximum leaf conductance, transpiration, and instantaneous water-use efficiency compared with control plants. Intrinsic water-use efficiency was also reduced except in *Atriplex*. Dusted leaves also had higher temperatures and increased photosynthetically active radiation reflectance. Heavily dusted shrubs had smaller mean leaf areas and greater leaf-specific masses. Finally, dusted plants showed no growth or branch senescence while control plants showed a steady increase in both shoot lengths and number of lateral shoots produced. This suggested that the short-term effects of reduced photosynthesis and decreased water-use efficiency may cause lowered primary production and shrub vigor in desert plants exposed to dust during seasons when the photosynthesis is occurring.

2. Sharifi and others (1999) sampled *Larrea tridentata* plants under three conditions (dusted non-irrigated, dusted irrigated, and undusted non-irrigated control along an unpaved tank trail used for transporting military vehicles) in the central Mojave Desert, CA, and found dust was associated with a 40–90% reduction in shoot growth and decreased water-use efficiency. Overall, experiments showed that heavy dusting had consequences on physiological performances and shoot growth of *L. tridentata*. However, this species was not killed by dust and responded by shedding old leaves and producing new shoots that were morphologically and physiologically indistinguishable from those of undusted individuals following abundant summer or winter-early spring rehydration.

3. Bolling and Walker (2000) found that none of the measured soil parameters varied significantly with road age (between 5 and 88 years) for eight unpaved, abandoned roads in the Lake Mead National Recreation Area, NV.

   a. However, differences were found between soils on the basis of road type. Soils on track roads (created by surface vehicular traffic and road bed with raised center berm) were more compacted and had higher clay content, lower total N, and lower available P than soils from either bladed roads (those created by bulldozing and road has lateral berms) or control plots at both 0–7.5 cm and 7.5–15 cm depths. Track roads also had higher organic matter, lower pH, and lower available N than bladed roads. Overall, soils on bladed roads were more similar to soil on control plots than to track roads.

   b. Perennial shrub cover was significantly lower on road plots than on control plots, largely due to the lower cover of *Larrea tridentata* on road plots, although *Larrea* did increase somewhat on the oldest roads, indicating an expected successional pattern. Cover of *Krameria* was also significantly lower on road than on control plots, but cover of *Ambrosia, Encelia,* and *Hymenoclea* did not differ between road and control plots. Species richness was lower on roads than on controls. Bladed or bulldozed roads had significantly lower total plant cover than either track roads or controls.

   c. Following abandonment of unpaved roads, de-compaction of roads, flattening out lateral and control berms, and restoration of soil horizons would increase probability of a more natural community developing. Without restoration efforts, successional trajectories will be permanently altered after disturbance.

4. Bolling and Walker (2002) examined fertile island development around *Larrea tridentata* shrubs occurring on three types of abandoned roads, varying in years since abandonment. The authors compared those to shrubs found on control sites at three spatial scales (one shrub, 10 shrubs, entire road) near the Lake Mead National Recreation Area, NV. In general, shrubs on control sites had a more pronounced fertile island effect in soil N than shrubs on abandoned roads. Spatial heterogeneity, as measured by the total variance in percent total soil N, was only apparent at the shallow depth (0–7.5 cm) and the variance decreased as time since road abandonment increased. Application type (i.e., “bladed” or lateral berms versus having an intact “center berm”) was more important than time since abandonment in determining spatial patterns and concentration of available soil P and organic matter, both of which were higher on the road with a central berm compared to the other two roads. The raised center berm may have facilitated aeolian deposition of organic materials and affected soil water relations and nutrient content.

5. Chaffee and Berry (2006) sampled soils, stream beds, and rocks for elemental toxicity at 6 study areas in the Mojave and Colorado Deserts, CA. One study area transected by a paved road (Highway 95) in Chemehuevi Valley in the Colorado Desert showed weakly elevated levels of Pb in soils, which extended as far as 22 m from the pavement edge and were probably related to vehicle exhaust. Other metallic elements (Cr, Cu, Mo, V, Zn) were also slightly anomalous in one or several samples collected near the highway. Soils were slightly enriched with Ca, Cd, Cr, and Ni. Stream sediments were generally enriched in 18 elements (As, Au, Cd, Co, Cr, Cu, Fe, La, Mn, Ni, Pb, Sc, Th, Ti, V, Y, Yb, Zn). Plants were relatively enriched in As, Ba, Cd, Cu, Mo, Sr, and Zn relative to soil concentrations and Ba, Cd, Cu, Mo, and Sr relative to stream sediment values. At a second study site (Goffs), high concentrations of several elements were found in plants (Au and Mo in non-ashed plants; Mo, Re, Th, and U in ashed plants) and may have been the result of air-borne contaminants introduced and dispersed as a result of highway and railroad traffic.

6. Brooks and Berry (2006) conducted a study of variables contributing to dominance, richness, and biomass of alien annual grasses and forbs in desert tortoise critical habitat in the western, southern, and central Mojave Desert, CA, in wet and dry years. Alien species richness was positively correlated with the total length of dirt roads in a township section during a wet year.

7. Berry and others (2014b) modeled invasion and establishment of the non-native Sahara or African mustard (*Brassica tournefortii*) in the Chemehuevi Valley (tortoise critical habitat) in the Colorado Desert, CA. The paved, two-lane highway was the likely originating source of Sahara mustard and a significant predictor variable in establishment of this noxious species.

D. Recommendations for Utility Corridors

Lovich and Bainbridge (1999), presented management strategies for minimizing the effects of linear corridor construction, including: placement of power poles closer to existing access roads, modifying construction techniques for buried pipelines, less frequent road grading, and limiting the width of motorcycle race corridors along powerlines. The authors cited a Lathrop and Archbold article, published in 1980, and repeated the Lathrop and Archbold recommendations for routing corridors to minimize environmental impacts included: (1) routing them through gently sloping areas to minimize erosion, (2) routing them through areas occupied by colonizing species such as *Hymenoclea salsola*, (3) avoiding areas dominated by high nitrogen fixation communities such as *Acacia greggii*, and (4) avoidance of undue soil compaction with implementation of soil loosening efforts to aid natural revegetation.

E. Recommendations to Reduce Mortality of Tortoises Along Roads, Including Fences and Culverts

1. Ruby and others (1994b) stated the importance of developing structures that permit tortoises to move safely around and/or under human structures and to maintain some natural movement and gene flow within and among tortoise populations. Solid barriers were the most effective in containing tortoises, and the most effective highway barrier was probably a 1 cm hardware cloth fence. Tortoises could see through it and would continue to walk along it seeking a passageway. Fences should be buried a short distance underground (15 cm) for stability and to discourage exploratory digging by tortoises or other desert animals. Due to behaviors like pushing or putting head and feet through the fence, open fences with spaces wider than a tortoise's head should be avoided. The authors indicated that there is a wide choice of barriers that can be used to restrict or direct the movements of desert tortoises, and that adequately designed fences will likely be effective in guiding tortoises to passageways away from hazards or across highways. Given that major highways are important sources of mortality in tortoises, well-traveled roads in good tortoise habitat should be fenced first. Less-used roads and roads in marginal habitat should be fenced next. Any construction site or any human facility that can attract tortoises, or where they can be lethal to tortoises, should also be fenced.
2. Boarman and others (1998) reported that four tortoises entered or passed through storm-drain culverts on 60 occasions along a California State Highway with barrier fences. This suggests that desert tortoise mortality could be reduced using fences and culverts, allowing movement beneath highways and could help to maintain gene flow.
3. von Seckendorff Hoff and Marlow (2002) recommended installation of fences and other barriers along roadways to reduce direct mortalities to tortoises, given the clear negative impact of roads on tortoise populations. The authors also noted that unfenced roads reduced effective habitat conserved for desert tortoises because of the zone of influence from roads.

4. Boarman and Sazaki (2006) suggested use of barrier fences and culverts near highways and along secondary roads in desert tortoise habitat to allow movement of tortoises and to reduce risk of death from vehicles (see also Berry and others, 2008).
5. Heaton and others (2008b) recommended that roads be fenced to increase area available for translocation (40% within the Western Mojave Recovery Unit).
6. Latch and others (2011) described the short time lag between the emergence of roads as a barrier and detection of genetic effect in tortoises and indicated that roads may become increasingly important in shaping the evolutionary trajectory of desert tortoise populations.
7. Lovich and others (2011a) noted that the negative effects of culverts could be largely mitigated by several best management practives, such as installation of larger diameter culverts, such as larger concrete box culverts or corrugated steel culverts with a diameter > 1 m to lessen the possibility of blockage with sediment and debris that may entrap tortoises with onset of rain. If smaller culverts are used, frequent inspections to prevent blockage or entrapment of tortoises could be implemented.

F. Natural Recovery of Tortoise Habitat after a Linear Disturbance

1. Review of the Literature. Webb, Belnap, and Thomas (2009) reviewed natural recovery of soil crusts, soils, vegetation (primarily perennial vegetation) from severe disturbances, including some linear disturbances and some ghost towns. The review covered several sites in the Mojave Desert; some sites are outside of or above the elevations where desert tortoises currently live. Estimates for recovery varied depending on the disturbance type and size and whether the subject was biological crusts, soil compaction and composition, or annual and perennial vegetation. Many factors affect recovery rates, such as size of the disturbed area, species composition, proximity of seed sources, and droughts.
2. Berry and others (2015b) studied a 97-m wide corridor for a buried pipeline with a central dirt road 36 years after construction in a *Larrea tridentata* and *Ambrosia dumosa* plant alliance in the western Mojave Desert, CA. They measured composition, density, biomass, and richness of annual plant species at the road edge, 20 m and 40 m into the disturbance and a nearby control. They described a bilateral process of recovery: from the central road to the outward edge of the disturbance corridor and from undisturbed habitat into the corridor. Species composition of native annuals differed significantly by distance, suggesting secondary succession. Native annuals were generally closer to achieving recovery on the 40-m plots than at the road edge. Recovery estimates were in centuries or over a millennium and dependent on location (intershrub space or under canopy of shrubs), and whether considering all annuals or natives only.
3. Berry and others (2016) studied a 97-m wide corridor for a buried pipeline with a central dirt road 36 years after construction in the western Mojave Desert, CA. (This was a paired study with F.2. above). The authors measured density, cover, species richness, and species diversity of perennial plants at the road edge, 20 m and 40 m into the disturbance and at nearby control. Total numbers of shrubs did

not vary significantly with distance from the road edge; however canopy cover of shrubs, species richness, and species diversity were higher in the control than at the road edge and other distances. Canopy cover of common shrubs (*Ericameria nauseosa, Ambrosia salsola, A. dumosa, Larrea tridentata, Grayia spinosa*) and perennial grasses (*Elymus elymoides, Poa secunda*) also varied significantly by location. Patterns of recovery were bidirectional: secondary succession from the control into the disturbance corridor and inhibition from the verge in the direction of the control. Time estimated for species composition to resemble the control is dependent on location within the disturbance corridor and could be centuries at the road verge. The shrub *Ericameria nauseosa* at the road edge may be inhibiting recovery.

VI. Off-Road Vehicles

A. Effects on Tortoise Habitat

1. Literature review**.** Lovich and Bainbridge (1999) reported that impacts of off-highway vehicles included destruction of soil stabilizers, soil compaction, reduced rates of water infiltration, increased wind and water erosion, noise, decreased abundance of lizard populations, and destruction of vegetation. Soil density, a result of soil compaction, increases as a function of the number of vehicle passes, while soil infiltration rate decreases. All areas of the desert, except barren sand dunes and clay flats of playas, are susceptible to the negative effects of off-road vehicles.
2. Belnap (2002) experimentally studied off-road disturbance at the Nevada Test Site, NV, and at Joshua Tree National Park, CA. The author reported that disturbance reduced nitrogenase activity, an indicator of nitrogen fixation, in biological soil crusts by 70% at the Nevada Test Site and by 100% at one of two sites in Joshua Tree National Park. Due to high rock cover at one site in Joshua Tree National Park, vehicle tracks could not be visually identified post disturbance and no decline in nitrogenase activity was observed. In both disturbed and undisturbed soils, nitrogenase activity declined as sand content increased, but increased as fine-textured soils increased. As indicated by all nine sites, higher nitrogenase activity before the disturbance resulted in less impact to nitrogenase activity post-disturbance.
3. See Belnap and others (2007) about losses to soils from off-highway, off-road vehicle activity.

A. Effects on Both Tortoises and Tortoise Habitat

1. Bury and Luckenbach (2002) sampled vegetation and tortoises at a site near Barstow, CA, in the western Mojave Desert. The authors reported that:
    a. An unused, natural plot had 1.7 times more live perennial plants (shrubs) and overall greater plant cover compared with a plot heavily used by off-road vehicles (ORV). The ORV plot had 2.4 times more dead perennials. Most (75%) of the dead perennials were burroweed (*Ambrosia dumosa*), with 64% occurring on the ORV plot. There was no difference in the amount of live perennials between the unused and ORV plot. Creosote bush (*Larrea*

divaricata) dominated both plot types, and although there was no difference between the number of live and dead creosote bush between plot types, turpentine-brush (*Acamptopappus* spp), box-thorn (*Lycium* spp.), Mormon tea (*Ephedra* spp.), and ratany (*Krameria* spp.) differed between the unused and ORV plots. Excluding creosote bush, the amount of plant cover for perennial shrubs was 24.7 m^2 for the unused compared to 1.8 m^2 for the ORV plot.

   b. Significantly more live tortoises occurred on the unused, natural plot compared with the plot heavily used by ORVs. Six of the tortoises on each plot type were either juveniles or immatures.

   c. Significantly more burrows occurred in the unused area that in the ORV-used plot. A greater proportion of actively used burrows were in the unused area than the ORV plot. The spatial distribution of burrows was significantly different between plot types; most of the plot had active burrows. In contrast, burrows in the OHV plot were restricted to one part of the plot. Most of the active burrows on the unused plot were also wider (≥ 15 cm) than those on the ORV plot. Likewise, 25 adult tortoises > 200 mm carapace length (CL) where found on the unused plot compared to the largest tortoise at 170 mm CL found on the ORV plot.

   d. Body mass of tortoises was significantly greater on the unused, natural plot compared with the ORV plot.

2. Keith and others (2008) conducted a study of desert tortoises in the western Mojave Desert, at a 187.7 km^2 study area in the Jawbone-Butterbredt Area of Critical Environmental Concern and Red Rock Canyon watershed, CA, in 2002 and 2004. They surveyed 751 one-ha plots. Fifty-two percent of plots had vehicle tracks, either 4-wheel drive or motorcycle or both; few tracks were on designated OHV routes; most tracks (92%) stemmed from cross-country travel and provided documentation of unauthorized use. The authors reported that tortoise sign was found in areas with significantly lower impacts from off-highway vehicles than elsewhere.

3. Berry and others (2008), in a study of tortoises and their habitat in the western part of Red Rock Canyon State Park and watershed, western Mojave Desert, CA, reported that plots outside the Park in the Red Rock Canyon watershed had more than five times as many off-highway vehicle tracks as plots inside the Park. The authors noted that the State Highway, secondary roads, and cross-country vehicle tracks contributed to degradation of habitat and to high mortality rates. Other factors also contributed.

4. Berry and others (2014a) compared tortoise distribution and abundance on three types of managed lands for tortoises in the western Mojave Desert, CA: the fenced Desert Tortoise Research Natural Area, where sheep grazing and recreational vehicle use are excluded; adjacent partially fenced critical habitat for the tortoise; and adjacent private lands acquired for conservation management. In the lands managed as designated critical habitat for the tortoise, recreational vehicle use (vehicle tracks) had a significant negative effect on presence of tortoise sign, with a negative 28% change per each two-fold increase in vehicle tracks.

C. Management Considerations Regarding Off-Highway and Off-Road Activities

1. Zimmerman and others (1994) noted that burrows are a habitat requirement of singular importance to the thermal ecology of desert tortoises. It is therefore essential to manage environmental variables (e.g., limiting off–highway vehicle use) influencing the integrity of burrows, including soil compaction, particle size, and vegetative cover.
2. Brooks (1995) reported that the floral and faunal community structure of the western Mojave Desert can profit from fenced protection from livestock grazing and off-road vehicle use, such as that existing at the Desert Tortoise Research Natural Area, with the primary benefit being greater biomass production by forbs than alien annual grasses.
3. Lovich and Bainbridge (1999) reported that desert soils may take 10,000 years to develop and recovery of natural vegetation would probably take centuries. Therefore, impacts related to off-highway vehicle use present a serious challenge to desert restoration projects in the southern California deserts for three reasons: (a) the potentially severe impact of off-highway vehicle use in desert ecosystems, (b) the widespread nature of off-highway vehicle impacts, and (c) the fact that off-highway vehicle areas are often located in or near environmentally sensitive habitats. Areas targeted for restoration should be closed to off-highway vehicle use prior to initiating procedures to ameliorate past damages.
4. Bury and Luckenbach (2002) noted detrimental impacts of off-road vehicles to tortoise abundance, burrow density, burrow size, vegetation cover, and tortoise body mass and recommended finding ways to minimize or remove the impacts of off-road vehicles on desert tortoises.
5. Belnap (2002) reported that the impacts of off-road vehicles, expressed in terms of nitrogenase activity, an indicator of nitrogen fixation, varies in different regions and in different soils as a function of high rock cover (no decline in nitrogenase activity), sand content (nitrogenase activity declined as sand content increased), and fine-textured soils (nitrogenase activity increased as fine-textured soils increased). At all sites, higher nitrogenase activity before disturbance resulted in less impact to nitrogenase activity post-disturbance. These results suggest that the impacts of off-road vehicles vary in different regions and different soils, information which can be used in the development of off-road vehicle management plans.
6. See Belnap and others (2007) about losses of soils from vehicle activity (in VIII. Military Activities)
7. Keith and others (2008) said that increased patrols and stricter enforcement of the designated route system would reduce much of the risk to desert tortoises from off-highway vehicle activity.
8. Berry and others (2008) discussed the benefits and detriments of fencing or seasonal closures of selected secondary roads and washes to prevent vehicle kills.

9. Berry and others (2014a) demonstrated that the fenced Desert Tortoise Research Natural Area, where off-highway vehicles were excluded, had significantly more tortoises and lower mortality rates than critical habitat or private, recently acquired conservation lands. Long-term protection has had a positive effect on the tortoise population.

VII. General Recreation—Trampling and Hiking

A. Pietrasiak and others (2011) sampled biological soil crusts on plots that either had been grazed or disturbed due to recreational hiking in Joshua Tree National Park, CA; the authors compared the two areas using a disturbance index. The authors reported that high foot traffic was associated with reduction in visible crust cover; however the area had more lichen and moss crusts, indicating that crusts in this area were more successionally mature. In contrast, the historically grazed area showed clear signs of recovery, with a higher visual cover of biological soil crusts. However, the crusts also had lower biomass values, supporting an earlier successional stage. Given the fact that recent human trampling was implicated in a reduction of visible crust cover in this study, Park managers could establish a more complete trail system with appropriate signage that provides access to climbing areas while restricting off-trail trampling to prevent further crust degradation, soil loss, and loss of microbial biodiversity.

B. Soil Losses. See Belnap and others (2007) under VIII. Military Activities.

VIII. Military Activities

A. Literature review. Lovich and Bainbridge (1999) reported that military-related operations, such as tent sites, roads, and tank tracks, can cause intensive damage including significant reductions in plant density and cover relative to control areas. Reductions of cover and density were greatest in tank tracks and least in tent areas. Recovery to pre-disturbance levels of cover and density varied according to disturbance type, where tent areas showed the greatest recovery, tank tracks were intermediate, and roadways showed the least, reflecting the intensity of disturbance. Percentage cover and density of long-lived species, such as *Larrea tridentata*, were reduced in disturbed or impacted areas, where dominant plants in disturbed areas included pioneer species such as *Ambrosia dumosa* and *Hymenoclea salsola*. Differences in vegetative structure between control and impacted plots were due to soil compaction, changes in soil texture, removal of top layer of soil, and alteration of drainage channel density. Areas receiving the greatest amount of soil compaction, such as roadways, require the longest recovery times.

B. Status of Knowledge about Impacts of Military Vehicle Activities. Anderson and others (2005) stated that although our understanding of the impacts of off-road and military vehicle activities has increased since 1983, the effective use of this information has been limited in land management decision making. In summarizing the publications of others, the authors reported that only about 13% of 19,000 environmental impact statements (EIS) published from 1970 to 1997 had sufficient information to assess impacts and that the quality of documents did not improve during this time period. Anderson and others found that the quality was particularly lacking in the environmental impact predictions portions of the EIS and suggested that besides being quantitative, measurable, and scientifically defensible, impact predictions must be easily integrated into the decision maker’s customary decision-support process.

C. Effects of Military Maneuvers on Soils

1. Belnap and Warren (2002) studied soil impacts and recovery in an area of the Colorado Desert (Chemehuevi Valley) approximately 55 years after disturbance by World War II military vehicles.
    a. Tracks were still visible particularly in areas of desert pavement. They found that soil penetrability was substantially lower and had fewer rocks in the top 10 cm of the soil profile in visible tracks compared with adjacent areas outside tracks.
    b. Changes in soil composition were noted when comparing areas inside versus outside the vehicle tracks. Soil outside the tracks was characterized by more gravel-sized particles which indicate an intact desert pavement, compared with no coarse gravel inside the tracks.
    c. Results from biomass estimates indicated that the cyanobacterial component of biological soil crusts had recovered 46 to 55% in tracks compared with outside tracks, but recovery of lichens was much lower.
    d. These results suggest that it may take centuries to millennia to restore desert pavement. Assuming recovery of biological soils crusts is linear and complete when the most sensitive lichen species has fully recovered, it may require almost 2 millennia for full recovery of biological soil crusts in areas that have undergone similar disturbance by military vehicles.
2. Evidence of elemental toxicity (or lack thereof) from military activities. Chaffee and Berry (2006) sampled soils, stream beds and rocks in study areas of the Mojave and Colorado Deserts, CA for elemental toxicity or elevated levels. They reported that no soil or plant samples from historically used military areas (Goldstone, Goffs, Chemehuevi Valley, Chuckwalla Bench) in the western Mojave or Colorado Deserts contained anomalous concentrations of the elements As, Cu, Cr, Fe, Pb, or Zn that could be ascribed to military maneuvers, vehicles, or ordnance.
3. Belnap and others (2007), using a wind tunnel experiment to examine factors influencing vulnerability of soils to wind erosion, conducted human trampling experiments using control and experimentally disturbed sites at the National Training Center, Fort Irwin, CA.
    a. Factors that significantly decreased wind erosion were increased rock cover, coarser soil particles, and subsurface soil stability. When all factors were evaluated together, soil texture was the best predictor of susceptibility to wind erosion.

b. Control (i.e. less recently disturbed) sites had greater cyanobacterial biomass, soil surface stability, threshold friction velocities (TFV; i.e., the wind speed required to move soil particles), and sediment yield than sites that had been more recently disturbed by military maneuvers.

c. After trampling, all sites showed a large drop in TFVs and a concomitant increase in sediment yield. Decline in TFVs and the rise in sediment yield were significantly correlated with cyanobacterial biomass, but other factors including soil texture, rock cover, and inherent soil stability were more important than cyanobacterial biomass in determining the overall site susceptibility to wind erosion. Results indicated that there was a threshold of biomass below which cyanobacterial crusts were not the dominant factor in soil vulnerability to wind erosion. Most undisturbed soil surfaces in the Mojave Desert region produced very little sediment but even moderate disturbance increased soil loss from these sites which was likely to have long-term effects due to low rates of soil formation.

D. Recovery of Soils and Vegetation after World War II Activities and in Ghost-towns.

1. Webb (2002) sampled 19 abandoned World War II sites and ghost towns across the Mojave and western Sonoran Deserts. The author evaluated recovery of severely disturbed desert soils and reported that poorly sorted soils, particularly those with a loamy sand texture were most vulnerable to soil compaction (soils common to alluvial fans). Recovery of compacted soil was expected to vary as a function of precipitation amounts, wetting and drying cycles, freeze-thaw cycles, penetration depth and bulk density. The recovery rate appeared to be logarithmic, with the highest rate of change occurring in the first few decades following abandonment. Some high elevation sites had completely recovered from soil compaction after 70 years. Estimates for 85% or full recovery ranged from 92 to 124 years, depending on method used.

2. Webb, Belnap, and Thomas (2009). See VI.F.1.

E. Effects of Military Activities on Vegetation

1. Gibson and others (2004) sampled four sites along a gradient of military vehicle disturbance that still maintained shrub cover (i.e., were not denuded with excavation of roots) at the Fort Irwin National Training Center, CA.

a. *Larrea tridentata* shrubs possessed a natural ability to recover following episodes of severe damage by military vehicles. This shrub recovered from physical damage via resprouts arising from meristems in stem bark below the severed or crushed canopy.

b. At higher levels of disturbance by heavy vehicles, nearly all individuals showed measurable breakage, but without additional damage, plants regained a full canopy within 5 years under arid field conditions. Resprouts exhibited more vigorous growth and doubled the biomass accumulation which was stimulated by high rainfall in an El Niño year. Resprouted individuals had different morphological traits (i.e., leaflets were longer/wider and internodes were longer) and had slightly higher predawn shoot water potentials than canopy old growth.

    c. *Larrea tridentata* was the only species to show reprouting from each individual that was damaged across the four sites surveyed, whereas *Ambrosia dumosa* and *Lycium andersonii* were typically killed when plant crowns were crushed by vehicles.

    d. The natural ability of *L. tridentata* to recover from cutting and crushing bodes well for re-establishment of creosote bush desert scrub communities following episodes of severe damage by vehicles.

2. Caldwell and others (2006) reported that disturbance from tracked vehicles during military training exercises at the National Training Center, Fort Irwin, CA, resulted in significant increases in both bulk density and penetrometer resistance and in the major degradation of the morphology of surface horizons, especially in terms of their pore-size distribution and structure.

3. DeFalco and others (2009) assessed soil seed densities across varying surface disturbances (compacted, trenched) and adjacent undisturbed control plots at Fort Irwin, CA, in the central Mojave Desert. The authors reported that seed densities were greatly influenced by changes to soil conditions, either surface disturbance or direct loss to seed-bearing plants.

    a. Viable seeds of perennial plants were rare in undisturbed areas (3–4 seeds/m^2) and declined to < 1 seed/m^2 within disturbed sites.

    b. In general, annual seed densities were an order of magnitude greater than those of perennials, yet annual seed densities on compacted sites had much lower densities (one-third the seed densities on undisturbed sites).

    c. Compacted sites were characterized by recurrent and concentrated military activity that increased soil bulk density, thereby shifting soils toward coarse textures and increased surface topography. This likely resulted in almost undetectable densities of perennial seeds.

    d. Given the extremely low densities of perennial seeds detected within compacted sites as a result of decades of sustained military activity, the authors highlighted the difficulty of shrub recruitment in heavily disturbed desert landscapes with active reseeding efforts.

F. Effects of Military Activities on Desert Tortoises and Tortoise Habitats

1. Modeling. Aycrigg and others (2004) developed a spatially explicit dynamic simulation model to assess how varying military training both spatially and temporally affected desert tortoises and their habitat, specifically at the Army's Fort Irwin facility, CA. The authors simulated six different training scenarios (baseline, new baseline, training varied temporally, training varied spatially, training varied both temporally and spatially, and potential tortoise reintroduction) 100 times each. The baseline model did not include any military training and basically simulated the recovery of the landscape from previous impacts. Results of this model showed tortoises distributed across the landscape at moderate densities in a clumped pattern, but with higher densities along the southern border. Over the 250-year time period, tortoise densities increased. The purpose of the new baseline model was to simulate tortoise densities across the landscape that had been

recovered from military training for 250 years by using the results from the baseline model as the initial input, before examining how military training affected tortoise densities. The new baseline model showed that tortoise densities were maintained and their distribution remained relatively constant. Although tortoise densities decreased with the addition of military training, training that varied spatially (3 levels of training intensity at low [238 tracked-vehicle-days/month], medium [833 TVD/month], and high [1,428 TVD/month] that occurred primarily toward the center of the study area) caused tortoise densities to decline less than training that varied temporally (moderate training November–February and low training March–October). Results from the model that combined both temporal and spatial effects indicated that temporal effects still caused a greater decline to tortoise densities, but the two treatments were not additive, and that impacts to tortoises may be minimized by altering the timing, location, and intensity of military training. The tortoise reintroduction model indicated areas where tortoises could be supported, but did not occur in the previous models, and suggested that dispersal plays a major role in maintaining tortoise populations.

2. Berry and others (2006a) conducted a study of tortoises and their habitats at 21 sites on Goldstone and the National Training Center, Fort Irwin, CA, between 1997 and 2002. They reported that all plots had some human-related surface disturbance, and the percentages ranged from 0.03 to 43.59%. Surface disturbance was higher at the plots recently used or currently used for military maneuvers than at plots not used or used in the past. The percent surface disturbance was significantly correlated with counts of trash and military ordnance. Deaths of tortoises from vehicles were significantly correlated with amount of surface disturbance, counts of trash, military ordnance, and proximity to offices and paved roads.
3. Berry and others (2013) surveyed a 5.42 km^2 study area for tortoises and their habitat at the China Lake Naval Air Weapons Station, CA. The authors modeled distribution and densities of live tortoises and tortoise sign with predictor variables and interactions. Densities of tortoise sign were best predicted by distances from areas denuded by munitions testing and the paved roads that led to testing areas and interactions between some variables. Common ravens and slopes were also important predictors. The two best models predicting live tortoise density included ordnance and slope. Very few tortoises or tortoise sign were in the vicinity of the road, end of the road and the associated denuded areas used for testing.

G. Management Considerations Regarding Tortoises and their Habitats

1. Doak and others (1994) commented on the following: the proposed expansion of the U.S. Army's Fort Irwin National Training Center, predicted to destroy up to 13% of the western Mojave tortoise population, is undeniably a major blow to the tortoise population. However, the effect of the expansion on long-term population viability depends on the management of the remaining habitat. If a substantial amount of desert land were to be effectively managed for the health of desert tortoises in the future, and if some of this land were to be in the expansion area, then the Fort Irwin expansion could be a major setback for the future size and viability of the western Mojave population. The Fort Irwin expansion must be gauged in light of management plans for tortoises throughout the Mojave Desert.

2. Duda and others (1999) noted that knowledge of desert tortoise home range, average distance traveled, and the number of burrows used, when placed in a seasonal and climatic context, is important input for planning military training or construction activities.
3. Belnap and Warren (2002) studied soil impacts and recovery in an area of the Mojave Desert approximately 55 years after disturbance by World War II military vehicles. See C.1. Effects of Military Maneuvers on Soils.
4. Gibson and others (2004) reported that there are sites at Fort Irwin National Training Center, CA, where the landscape is essentially barren because damage is so frequent that the resprouting strategy of *Larrea tridentata* cannot keep pace with root crown reinjury. Below this disturbance threshold, *Larrea tridentata* can regain a typical canopy within five growing seasons although more time may be required to gain full ecosystem function. Management of military bases in desert landscapes requires rotation of training site exercises and furlough for heavily damaged sites to give these scrublands time to recover.
5. Caldwell and others (2006) reported results of research on soils at degraded sites on the National Training Center, Fort Irwin, CA. The authors studied soil physical properties in areas subject to low and high disturbance at microsites in the interspaces between shrubs (*Larrea tridentata*) and on *Larrea* mounds; they compared the findings to undisturbed surfaces of both well-developed soils formed on Pleistocene age alluvial deposits and weakly developed soils formed on Holocene age alluvial deposits. In general young interspaces were less sensitive to disturbance than older well-developed interspace soils. The overall morphology of the soil was dramatically impacted. Disking and ripping are common methods to alleviate soil strength prior to restoration activities. However, results indicate that these practices may result in further destruction of the soil surface.
6. Belnap and others (2007) used a wind tunnel experiment to examine factors influencing vulnerability of soils to wind erosion. The authors reported that soil loss in the Mojave Desert is likely to have long-term effects because current rates and dust inputs are very low and in turn soil formation rates are low. Land managers need to consider loss of soil stability, and the resulting loss of soil when deciding whether to permit certain types of land use activities such as trampling by military vehicles or training exercises, off-road vehicles, livestock, or hiking. Susceptibility of soil surfaces to wind erosion depend upon site factors and the degree of trampling, and managers may need to establish an upper limit of allowable soil loss over a given time period and use a predictive equation to predict those losses. Some studies have suggested losses > 5 g soil/m^2 soil surface are to be avoided.

IX. Mining

A. Literature review. Lovich and Bainbridge (1999) reported that forms of degradation from mining include pits, ore dumps, toxic tailings and fugitive dust, as well as the historically high demand for fuel and timber, grazing, and road building associated with mines. Brine evaporation and dry lake mine operations also led to substantial wind erosion.

B. Ghost Towns (from mining). Webb (2002) sampled 19 abandoned World War II sites and ghost towns (from mining) across the Mojave Desert and recovery of severely disturbed desert soils and vegetation, as measured with penetration depth and based on the logarithmic model, ranged from 105 to 124 years for 85% recovery; the highest rate of recovery occurred within the first few decades after abandonment. Using bulk density to estimate recovery, neither the linear nor logarithmic models performed better, thus recovery for both models required 92 to 124 years. Although compaction at these sites was caused by a wide variety of forces, ranging from human trampling to military tank traffic, the data did not allow segregation of differences in recovery rates for different compaction forces. Recovery of soil compaction was significantly related to elevation, and in turn related with wetting-and-drying and freeze-thaw cycles that increase with elevation. Some higher elevation sites completely recovered from soil compaction after 70 years, substantially less than lower elevation sites.

C. See also Webb, Belnap, and Thomas (2009), VI.F.1.

D. Chaffee and Berry (2006) studied elemental toxicity at 6 study areas in the Mojave and Colorado Deserts by sampling bulk chemistries of soils (n = 256), stream sediments (n = 36), and tortoise foraging plants (n = 366) to determine whether high concentrations of various elements may be contributing to increasing mortality rates and signs of disease in desert tortoises associated with elemental toxicity. The chemistries of soil, stream sediments, and plants varied from site to site and were generally consistent with types of exposed rock bulk minerologies and chemical contents. Of elements in soils that might have been toxic to tortoises at the study sites, only arsenic (As) seemed to be anomalous region-wide though some soil and plant anomalies were clearly anthropogenic and likely related with mining.

1. In the area including the southern Rand Mountains and Desert Tortoise Natural Area (southern section), there were 17 elements that exceeded two times their respective geometric mean values (As, Ca, Ce, Cr, Eu, Hg, La, Mo, Nd, Ni, P, Pb, Sb, Th, Yb and possibly Au and U). As, Au, Hg and Ni were highest (5.9 to 112 times the mean values).

2. In the historic Rand and Atolia mining districts where extensive dumps and abandoned tailing piles are common, soil anomalies for As, Au, Cd, Hg, Sb, and/or W and plant anomalies for As, Sb, and/or W extended as far as 15 km outward from mining areas. The highest concentration of As in soils was 510 ppm. Soils containing anomalous mercury (Hg, ≥0.05 ppm) were found at least 6 km away from old piles of tailings in both the southern section of the study area (see 1. above) and in the Rand and Atolia mining districts. Many stream channels draining the periphery of these districts are still highly contaminated with transported mill tailings that contain mineral deposit-related elements, as well as Hg. Elements present in high concentrations in plants included As, Au, Ce, Mo, Na, and Sb. As

Au, and Sb were found near the most mineralized or contaminated areas; As and W were probably present as wind-borne contaminants on the surfaces of plant parts because they are not essential for plant metabolism.

3. In the Goldstone mining district, soil anomalies (e.g., arsenic) extended several km from the mining area, these likely from surface contamination redistributed by wind or occasional flash floods.

E. Gold and Silver Mining Has Left a Legacy of Exposed Mine Wastes, Some of Which Contain the Highest Naturally Occurring Arsenic Levels in the U.S. as well as Mercury (Kim and others, 2012, 2014).

1. Kim and others (2012) studied fluvial transport of mine wastes in the western Mojave Desert, CA, from the Randsburg Historic Mining District, Cactus Mine, Ruth and Calico mines. They found that only scorodite, arseniosiderite and As(v) sorbed to FeOOH was present in tailings. While airborne mobilization of mine tailings is diffuse and covers large areas, fluvial transport is more localized and directed down narrow and semi-linear washes into ephemeral lakes or playas. Based on field sampling and geospatial mapping of mine tailing and streambed sediments in the Randsburg Historic Mining District, intense and episodic precipitation events mobilized mine wastes several kilometers downstream and downslope, e.g., from the Randsburg Mine through Fiddler Gulch to Koehn Lake and from the Kelly Mine downstream to Cuddeback Lake. Samples also were gathered at the Ruth, Calico, and Cactus Mines and associated lands. Transport varied by site, but generally is via a series of discrete pulses, causing dispersion both down and lateral to washes with exponential decay behavior as distance from the source increased. Their work showed an inverse relationship between particle diameter and arsenic concentration, with enrichment in the finest sievable size fraction (≤20 µm) as much as three times as high as bulk concentrations and 40 times greater than the coarsest size fraction (>2,830 µm) (Kim and others, 2014). The authors prepared quantitative models of arsenic concentrations in wash sediments, represented as a series of overlapping exponential power-law decay curves.

2. Kim and others (2014) reported on windborne transport of mine tailings, which covers more area than fluvial transport and can be influenced by wind direction and speed, topography, and human activities such as vehicular traffic and off-highway vehicle use. The authors studied surface concentration and enrichment of arsenic as proxies for windborne transport of mine tailings throughout the Randsburg Historic Mining District and Western Mojave Mining District, CA, examining variables responsible for controlling arsenic release through in vitro simulated lung fluid extraction experiments. They used an integrative approach (field studies, laboratory extractions, risk assessment calculations) to provide information on distribution, bioaccessibility, and potential toxicity of arsenic for long-term management of contaminants. There are approximately 0.5 M tons of mill tailings and an equal amount of waste rock containing high levels of arsenic (1,490–13,300 ppm in tailings, 2,053 ppm in waste rock) in the Randsburg Historic Mining District. Similar findings were reported for the Cactus, Tropico Mill, and Standard Hill mines in the western Mojave Desert. The authors reported that both surface arsenic

concentration and surface arsenic enrichment at the Randsburg sites showed an east/southeast-trending deposition in the regions surrounding the tailings piles; in contrast, fluvial transport carries the particles downslope to Koehn and Cuddeback dry lakes. They reported that As surface concentrations and surface enrichments correlated more strongly with the prevailing wind directions than with topography and surface water drainage and exhibited exponential decay with distance strongly dependent on prevailing wind direction (primarily west to east).

F. Known and Potential Effects of Mines and Mining on Desert Tortoises

1. Seltzer and Berry (2005), using laser ablation ICP-MS, examined trace elements in tortoise scutes. Three tortoises with elevated arsenic levels were ill and two were from the Rand Mining District, western Mojave Desert, CA.
2. Nussear and others (2012) reported that 2 of 30 tortoises found dead died from falling in to mineshafts during a study conducted on survivorship of resident versus translocated tortoises in Utah and Nevada.

G. Considerations and Recommendations for Management

1. Chaffee and Berry (2006) reported that anomalous concentrations of Hg and As may have been the source of elevated levels of these elements found in ill tortoises in the western Mojave Desert (see Jacobson and others, 1991). Anomalous concentrations of these elements were observed as far as 15 km from current and past mining areas and 6 km from old piles of tailings. These sources of toxicants likely have been redistributed by wind, vehicles, and rainfall.
2. Kim and others (2012) reported that mine wastes and tailings with highly elevated arsenic concentrations remained exposed at a number of former mining sites, as a result of extensive gold and silver mining in the Mojave Desert, CA. A conceptual model of migration of mining waste suggested that any remediation of historic mine sites that contained both tailings at the mine site and in the wash that drains it need to address both problems concurrently. Their results suggest that:
    a. Effective remediation of contaminated mine wastes requires addressing the amounts of potential tailings released and the stored arsenic-enriched sediment in washes and the associated playa concurrently**.** Control of tailings at a mine site without addressing the stored arsenic-enriched sediment in the wash would lead to eventual erosion of these stored sediments and transport to the lower part of the wash and eventually the playa, due to the decreased sediment load at the mine.
    b. Removal of tailings from washes and alluvial fans would be more challenging than remediation at mine sites, but the consequences of dealing with these problems separately may lead to unintended release of tailings, ineffective remediation efforts, and longer-term contamination.

3. Kim and others (2014) in a follow-on study to Kim and others (2012) reported that depending on the site, bio-availability of arsenic ranged from 2.8 to >20%. All samples from the Randsburg mining district, CA, one-third of samples from the western Mojave mining district and nearly one-half the samples from Calico and Empire mines had the potential to exceed the *de minimis* cancer risk threshold. Residents and recreational off-highway vehicle users are also at potential risk for exposure.

X. Trash, Shooting Targets and Debris, Casings, and Ordnance

A. Trash: Distribution and Effects on Habitat

1. Berry and others (2006a), in surveys of 21 sites at Goldstone and the National Training Center, Fort Irwin, CA, reported that all plots had trash, ranging in counts from 2 to >607 pieces. The lowest counts of trash occurred at the Soda Mountains plot (outside the Training Center) and the highest counts on the five plots recently or currently used for military maneuvers. The percent surface disturbance was significantly correlated with counts of trash.

2. Walde and others (2007b) summarized records of litter and effects on reptiles. Then the authors described ingestion of a balloon by a tortoise in the central Mojave Desert, CA, and potential negative effects on tortoises. Researchers at the study site counted 178 new balloons arriving between March and November 2005. Most balloons were of unknown origin but the sources of two balloons were > 200 km away.

3. Berry and others (2008), in a study conducted at Red Rock State Park, CA, reported that 28 landscape plots (1 ha each) inside the Park had more than two times the amount of trash (general trash) than outside the Park and within the Red Rock Canyon watershed.

4. Keith and others (2008), in a 2002–2004 study of tortoises and their habitat in the Jawbone-Butterbredt Area of Critical Environmental Concern and Red Rock Canyon watershed (187.7 km^2 study area) in the western Mojave Desert, CA, reported that trash was present on 58% of 751 one-ha plots. The amount of trash differed significantly by region with the study area: it was higher in the Red Rock and Indian Wells regions than elsewhere.

5. Berry and others (2013) surveyed a 5.42-km^2 study area for tortoises, anthropogenic impacts and other variables on the Naval Air Weapons Station, China Lake, in the northwestern Mojave Desert, CA. They counted 10,292 instances of ordnance, trash, balloons, burned areas, utility lines, fences, and vehicle tracks. Trash accounted for 5.4% of these disturbances.

6. Berry and others (2014a) surveyed an area of 260 km^2 in three adjoining areas, with each of the three areas using a different management strategy: the Desert Tortoise Research Natural Area, protected for over 30 years with a fence from grazing and vehicle use; critical habitat for the desert tortoise, protected from sheep grazing for about 20 years and only recently from vehicles; and recently acquired private lands for conservation use (and with no history of protection). Counts of trash were highest on private lands and substantially lower (but similar) on the Natural Area and critical habitat.

B. Shooting Debris, Casings, and Shooting Targets

1. Berry and others (2006a), in surveys of 21 sites at Goldstone and the National Training Center, Fort Irwin, CA, reported that 15 plots had debris from firearms and shooting, and counts ranged from 1 to >615 pieces. The Soda Mountains plot had no debris from firearms and Goldstone had low counts of debris from firearms. The five plots recently or currently used for military maneuvers had the highest counts.

2. Berry and others (2008), in a study conducted at Red Rock State Park, CA, reported that 28 landscape plots inside the Park had significantly more shooting debris than outside the Park and within the Red Rock Canyon watershed.

3. Keith and others (2008) in a 2002–2004 study of tortoises and their habitat in the Jawbone-Butterbredt Area of Critical Environmental Concern and Red Rock watershed (187.7 km^2 study area) reported that shooting debris (bullet casings) was present on 37% of 751 one-ha plots. The amount of shooting debris differed significantly by region within the study area: it was higher in the Red Rock than in the Indian Wells region.

4. Berry and others (2014a) surveyed an area of 260 km^2 in three adjoining areas, with each of the three areas with a different management strategy: the Desert Tortoise Research Natural Area, protected for over 30 years with a fence from grazing and vehicle use; critical habitat for the desert tortoise, protected from sheep grazing for about 20 years and only recently from vehicles; and recently acquired private lands for conservation use (and with no history of protection). Counts of shooting debris were highest on private lands but not significantly different from counts on the Natural Area and critical habitat.

C. Ordnance

1. Berry and others (2006a) surveyed 21 plots on and adjacent to the National Training Center at Fort Irwin, central Mojave Desert, CA to determine status of desert tortoise populations and evaluate potential impacts from anthropogenic uses. They reported that ordnance occurred on 14 of 21 plots with counts ranging from 1 to > 615, and with more ordnance occurring on plots currently or recently used for military maneuvers.

2. Berry and others (2013) surveyed a 5.42-km^2 study area for tortoises, anthropogenic impacts and other variables on the Naval Air Weapons Station, China Lake, in the northwestern Mojave Desert, CA. They counted 10,292 instances of ordnance, trash, balloons, burned areas, utility lines, fences, and vehicle tracks. Ordnance accounted for 93.1% of these disturbances. They also counted ordnance throughout the study area and in denuded areas and found more ordnance in denuded areas.

D. Recommendations for Management

1. Berry and others (2008) noted that conservation and management actions with potential for reducing mortality of tortoises include removal of all old and recent trash in desert tortoise habitat to reduce ingestion by tortoises.

XI. Renewable Energy

A. Literature Reviews on Potential Effects to Tortoises and Habitat

1. Lovich and Ennen (2011) conducted a literature survey pertaining to effects of utility-scale solar energy in the Mojave and Sonoran Deserts. They concluded that evidence was insufficient to determine whether solar energy development was compatible with wildlife conservation. Because little research and scientific data exist at the local scale, extrapolating to larger scales with any degree of confidence was not feasible. However, the authors provided a list of possible direct and indirect effects of construction, operation, and decommissioning of utility-scale solar energy facilities on desert tortoises: direct mortality due to vehicles and equipment that may collapse burrows or entrap tortoises within burrows, paved and unpaved roads that may increase mortality, barriers to movement or genetic exchange; also, exposure to electromagnetic fields, and light pollution and off-site impacts from water and deposits of toxic waste. Potential effects on habitat include: habitat fragmentation; decreased foraging resources due to dust and dust suppressants that damage plants; destruction and modification of habitat via vegetation removal; increased soil erosion; noise from operations; increased albido or heat discharge; pollution spills and discharge in evaporative ponds; water consumption creating water shortages; and increased fire risk associated with invasive annual plants.

2. Lovich and Ennen (2013a) reviewed published studies of known and possible effects of wind energy facilities due to construction, operation, and eventual decommissioning. These effects included direct mortality, habitat fragmentation and barriers to gene flow, possible disturbance as a result of noise, vibration and shadow flicker effects, electromagnetic field generation, macro- and micro-climate change, predator attraction, and increased fire risk.

B. Effects (or No Effects) of Renewable Energy Projects on Desert Tortoises and Their Habitats

1. General. Cameron and others (2012) evaluated the potential effects of renewable energy projects on desert tortoises and their habitats using a mitigation hierarchical planning approach. The authors identified between 250,000 ha (using <1% slope angle) to 1.6 million ha (using <5% slope angle) of higher conservation value lands (termed Ecologically Core and Intact lands) that would be open to renewable energy development.

    a. The extent of suitable habitat for desert tortoises within the area affected ranged from 90,103 ha (<1% slope) to >1 million ha (<5% slope).

    b. The authors estimated that >220,000 ha of habitat were proposed for development as solar facilities under right-of-way applications on Bureau of Land Management (BLM) lands in the Mojave Desert.

    c. If all of these BLM proposals were approved and developed, the desert tortoise would lose 103,509 ha of suitable habitat.

    d. The authors reported availability of nearly 200,000 ha of land of lower conservation value that could meet California's renewable energy goal several times over.

    e. The supply of high quality habitat on private land may be insufficient to mitigate impacts from future solar projects, so enhancing public land management may need to be considered among the options to offset such impacts.

2. Mesa Farm Wind Energy Generation Facility, Riverside Co., CA.

    a. Lovich and Daniels (2000) sampled 32 used versus 32 random burrow locations and reported that burrows were non-randomly dispersed and were in close proximity to turbines and transformers associated with concrete pads and dirt roads. They suggested that concrete pads provided a type of "artificial caliche" and basking opportunities while unpaved dirt roads mimicked banks of desert washes.

    b. Lovich and others (2011c), in a study of tortoises at the Mesa Wind Farm near Palm Springs, CA, reported little difference in age of maturity, density, sex ratio, and survivorship of tortoises compared with populations in more natural environments. However, growth rates for males were lower but maximum body size was larger. The population had predominantly more adults and hatchlings than two other previously reported CA populations. Since the study area is located in a highly productive area for tortoise forage, this might have offset the negative effects of this industrial development.

c. Ennen and others (2012b) evaluated nest site selection for 15 female tortoises at the Mesa Wind Farm near Palm Springs, CA. The authors reported no evidence of nest sites being associated with any anthropogenic structures. Additionally, the nesting ecology was similar to other populations, suggesting that operation of the wind energy facility did not adversely affect the nesting ecology of this population. However, alteration due to construction and maintenance would reduce plant biomass and diversity which in turn could adversely impact tortoise reproduction.

d. Agha and others (2015c), as part of an 18-year study, compared sizes of activity centers and adult survival estimates of *Gopherus agassizii* living at a wind energy facility and at a neighboring wilderness area, CA. The authors found no significant differences in activity centers but the annual survival estimates for adults differed significantly. The wind energy facility had higher survival estimates (0.96 ± 0.01) than the wilderness area (0.92 ± 0.02).

C. Considerations and Actions for Management. Cameron and others (2012) provided several recommendations:

1. Based on the integration of solar energy potential and biodiversity spatial data across the Mojave Desert, there was substantial opportunity for planners to reduce development impacts on areas of higher conservation value. This would reduce trade-offs between converting to a green energy economy and conserving biodiversity. A systematic approach for proactively balancing solar energy production with protection of biodiversity better accounts for and can help reduce trade-offs. It can also provide greater assurances to agencies, developers and conservationists that their respective goals are being met.
2. Parcelization of private lands creates a challenge for siting large renewable energy projects. One strategy to enhance protection of areas of high conservation value in the Mojave Desert would be to develop policy incentives to develop degraded private lands, including brownfields and areas in former agricultural production that were retired due to salinity, contamination, or water limitation.
3. When evaluating renewable energy projects, it is important to account for cumulative impacts in siting and mitigation decisions, especially in light of the increased stress that climate change will exert on desert ecosystems. Impacts of projects should not only be evaluated comprehensively regarding ecological impacts, but also examined cumulatively in the context of all of the major stressors in the desert, including but not limited to other proposed energy projects.

XIII. Wind Erosion of Soils and Dust Emission

A. Sharifi and others (1997) tested effects of windblown dust on physiological performance of 3 species of desert shrubs (*Larrea tridentata, Hymenoclea salsola, Atriplex canescens*). Maximum rates of photosynthesis of dusted organs were reduced in dusted plants, as well as reduced maximum leaf conductance, transpiration, and water use efficiency; temperatures of dusted leaves and photosynthetic stems were 2–3 °C higher than those of control plants. Heavily dusted shrubs had smaller leaf areas. Primary production may be lowered in desert plants exposed to dust.

B. Grantz (1998a) studied wind barriers for suppression of sediment transport in abandoned agricultural lands the southwestern Mojave Desert, CA. Wind fences established along the leeward edge of an area of blowing sand, perpendicular to the prevailing wind, significantly decreased fugitive emissions. Establishment of shrubs may be effective also.

C. Sharifi and others (1999) tested phenological and physiological responses of heavily dusted creosote bushes (*Larrea tridentata*) to summer irrigation in the Mojave Desert, CA. The authors reported that irrigation, simulating heavy summer rainfall, can help creosote bushes recover rapidly from acute heavy dust deposition.

D. Okin and others (2001) synthesized remote sensing data, field data, and a mathematical model to create a wind-driven model to explain desertification of arid shrublands in southeastern California. The authors found that aeolian removal and transport of dust, sand, and litter are the primary mechanisms triggered by anthropogenic degradation, killing plants by burial and abrasion, interrupting natural processes of nutrient accumulation, destroying islands of fertility under shrubs, and allowing the loss of soil resources by abiotic transport. Wind erosion operates within feedback loops that decrease soil nutrients such as nitrogen and phosphorous. Arid shrublands with sandy, wind-erodible soils already converted to human uses should be managed to minimize effects of wind erosion, e.g., keep vegetation on fallow or abandoned agricultural fields to promote soil conservation. Any arid shrubland with wind-erodible soils that are not already converted to human uses are extremely fragile. Disturbance may preclude future successional processes, resulting in permanent landscape change, and could make recreation and habitation impossible. Where possible, development of land susceptible to wind erosion should be avoided.

E. van Donk and others (2003) measured erosion rates under typical conditions using field dust samplers across five sites at the Marine Corps Air Ground Combat Center, Twentynine Palms, CA. They reported that the site with greatest erosion had a sediment discharge of 311 kg m^{-1} over a period of 17 months. Other sites showed much less erosion because of significant rock cover or the presence of soil crusts. The authors recommended that future data collection should be conducted at much greater time resolution than a month to better understand wind erosion processes.

F. Belnap and others (2007) used a wind tunnel experiment to examine the factors that influenced vulnerability of soils to wind erosion when soils are trampled at Fort Irwin, CA.

1. Before experimental trampling at control sites, sites had greater cyanobacterial biomass, soil surface stability, threshold friction velocities (TFV, the wind required to move soil particles), and sediment yield than sites that had more recently been disturbed by military maneuvers.
2. Factors that significantly decreased wind erosion were increased rock cover, coarser soil particles, and subsurface soil stability.

3. However, when all factors were evaluated together, soil texture was the best predictor of wind erosion susceptibility.
4. Trampling experiments also revealed a significant decline in threshold friction velocities and soil stability when sites were compared before and after trampling disturbances (i.e., military maneuvers).
5. Most undisturbed soil surfaces in the Mojave Desert region produced very little sediment but even moderate disturbance increased soil loss from these sites. Soil loss was likely to have long-term effects due to low soil formation rates.

G. Urban and others (2009) observed that annual plant growth was critical in shutting down dust emissions at three study sites around Soda Lake, CA, over a 9-year period. Following high levels of rain in late winter/early spring 2001, annual grasses grew abundantly, which shut down dust emissions within 3 months. Although 2002 was dry with very little rain, the previous year's dead annual grasses helped stabilize the bare ground for several months. Very heavy rains over the next 3 years produced heavy annual growth, including the appearance of Sahara mustard (*Brassica tournefortii*) at one study site and Mediterranean grass (*Schismus barbatus*) at another, reaching an average total plant cover of 74%. By 2006, all annual vegetation was dead and total plant cover dropped to an average of 45%, however, it continued to provide soil stabilization. Low levels of rain, less plant growth, and decaying dead annual grasses through most of 2007 initiated another strong resurgence of particle saltation until heavy rains in the fall of 2007, which shut down dust emissions again.

H. Williams and others (2013) developed conceptual models of geomorphic controls on distribution of biological soil crusts (BSC) in the Mojave Desert, NV, distinguishing where cyanobacteria crusts grow vs. extensive tall moss-lichen pinnacled crusts. Sand and dust play roles in furthering type of crust propagation and where it is likely to occur. This study highlights the role of dust accumulation in BSC development, restoration potential, and air quality. Dust is important in establishment, health, and continued propagation of moss-lichen crusts. Complete restoration of late successional crusts and ecological function likely requires reestablishment of biologically-mediated dust capture and reformation of biogenic vesicular (Av) horizons and BSC micro-features which may occur over tens to thousands of years. Because BSCs protect fragile fine-grained surfaces, physical disturbance to crusts should be avoided to prevent wind erosion that causes dust emissions and air pollution, as well as loss of nutrient-rich and hydrologically-important surface soils.

XII. Drivers of Climate Change, Potential Effects of Climate Change, and Effects on Tortoises

A. Effects of Drought, Lack of Food and Water, and Potentially Future Climate Change on Tortoises

1. Peterson (1996a) monitored adult male desert tortoises from populations located in the eastern and western Mojave Desert, CA, with different rainfall patterns during an extreme drought year. He reported that seasonal, annual, and inter-population differences in field metabolic rates (FMR) and foraging rates corresponded to differences in availability of free-standing water from rainstorms. A possible explanation for the relationship between drinking rainwater and increased FMR was that drinking rainwater triggered increased activity over subsequent days and weeks. By drinking rainwater, tortoises could flush their systems of excess potassium and other solutes. This would, in effect, release them from drought-imposed estivation and allow them to resume or increase foraging activity. Periods of very low FMRs allowed tortoises to tolerate long periods of chronic energy shortage during drought.

2. Henen (1997), based on data from energy budgets of nine female tortoises from Fenner Valley, CA, reported that females produced eggs in years with low levels of winter annual plants by relaxing their control of energy and water homeostasis. They tolerated large deficits and surpluses in body dry-matter composition on seasonal, annual, and longer timescales. They were able to increase body energy content (lipid and non-lipid energy) for winter and use this reserve the following spring to produce eggs. Females used high-protein foods and rainwater, when available, to achieve energy surpluses that helped them survive periods of low resource availability (e.g., during drought). They managed to produce eggs, even in a drought year, by adjusting seasonal and annual field metabolic rates and food requirements to levels of food availability. Water was the primary resource limiting egg production in a drought year, and females forgoing egg production in that year accumulated body nonlipid energy and lost less total body water than did females producing eggs. Reproductive effort was higher during the drought than during the wetter year because tortoises reduced field metabolic rate by 70% to 90% in the drought year.

3. Duda and others (1999), drawing on data from radiotelemetry surveys conducted at Marine Corps Air Ground Combat Center and Joshua Tree National Park, CA, reported that home range size, the number of different burrows used, average distances traveled per day, and levels of surface versus burrow activity were significantly reduced in populations during a drought year compared with a productive year. There was also a shift favoring shorter-distance movements during the drought year. These results suggested that differences in levels of winter precipitation between years, and the resulting variation in winter annual biomass in spring, appeared to play a significant role in desert tortoise home range size, movement, and activity patterns.

4. Christopher and others (1999), in a 5-year study of hematological and plasma biochemical analytes in *Gopherus agassizii* at three sites in the Mojave Desert, identified changes in blood values that occurred during winters of below normal

rainfall and subsequent low forage production in spring (drought years). Blood urea nitrogen (BUN) was the most sensitive indicator of availability of water and green forage, with uniformly low values in hydrated tortoises in spring and high concentrations in dry seasons. Seasons of relative drought were characterized by increased BUN, osmolality, electrolytes, and anion gap, and decreased body weight and Total CO_2 concentration.

5. Freilich and others (2000) reported that capture probabilities for 97 individual tortoises at a Joshua Tree National Park, CA, plot were greater in the 4 years with regular rainfall compared with the 2 years of drought conditions. Further, tortoises were more likely to be found in burrows rather than above ground in years of drought when lower amounts of forage were available.
6. Morafka and Berry (2002), in summarizing a review of the literature from studies across the Mojave and northwest Sonoran Deserts, reported that the innate morphological and behavioral flexibility of desert tortoises may be viewed as substantial exaptations with which the species will be able to withstand changes in climates. However, climatic desiccation, either from short-term drought or long-term climate change, narrows options critical for tortoise survival. Climate change, coupled with ongoing anthropogenic impacts to desert tortoises and their habitats, reduces access and availability to cover of shrubs and specific forage items. Composition of native forage is changing from invasion of alien annual plants and could result in starvation or increased susceptibility to disease. Other anthropogenic impacts include habitat fragmentation, urbanization, agricultural development, lowering of the water table, livestock grazing, and off-road vehicle use.
7. Henen (2002a) synthesized relationships between female diets and nutrient budgets, food abundance, and reproductive outputs, drawing on a study of female reproductive output near Goffs, CA. He reported that despite tolerances from relaxing homeostasis, desert tortoises are vulnerable to extreme desert conditions such as extended droughts and reduction in availability of a nutritive diet. Under such conditions, as well as global environmental change, females would experience decreased survivorship and egg production—adding another anthropogenic impact to the list of threats.
8. Krzysik (2002) surveyed for live and dead tortoises and their burrows and scat at the Marine Corp Air Ground Combat Center and Joshua Tree National Park, CA, during a productive (i.e., wet) year and compared his findings on burrows to a concurrent study that covered 2 years, including a drought year. The author reported that during both productive and drought years, the majority of tortoise burrows (75%) were shallow (<1 m). However, during the drought year, more tortoises were found in their burrows than aboveground.
9. Berry and others (2002b), in a study of tortoises dying or found dead of drought in the central, northeastern, and eastern Mojave Desert, CA, reported that the tortoises exhibited abnormal behaviors likely indicative of starvation, dehydration, malnutrition or other stressors. Behavioral signs included delayed entrance of burrows for hibernation and failure to use burrows during low temperatures. The tortoises also showed clinical signs of disease or poor condition (inactivity,

debilitation, and lethargy), as well as weight loss, and abnormal hematological and plasma biochemical variables.

10. Christopher and others (2003), in a 5-year study of hematologic and plasma biochemical abnormalities in ill tortoises at three sites in the Mojave Desert, noted that drought is a natural part of the desert tortoise's environment, but when combined with disease or habitat loss, may contribute to additional disease problems and mortality. Clinical signs of upper respiratory tract disease, lower leukocyte counts, positive nasal cultures of *Mycoplasma agassizii*, and mild to moderate azotemia were more commonly seen in a year of below-normal annual and winter precipitation. Heteropenia has been associated with drought and starvation. Tortoises entering hibernation in a drought year may be physiologically compromised, because clinical signs of upper respiratory tract disease and heteropenia were noted at the time of emergence from hibernation following a period of drought. Most dehydrated tortoises and most deaths occurred in years following dry winters. Tortoises with severe or persistent azotemia more likely had decreased renal function due to severe dehydration, urolithiasis, or renal disease. Clinically dehydrated tortoises often have other laboratory abnormalities.

11. Longshore and others (2003) studied survival in adult tortoises at two eastern Mojave Desert sites in Nevada over a 9-year period. The authors associated decreased survival with drought conditions. They concluded that relatively short-term drought, combined with little or no annual biomass of plants, appeared to have caused severe reductions in tortoise survival.

12. McCoy and others (2011) noted that the average body condition index of 163 desert tortoises from the Mojave Desert, CA, increased from March to May and then steadily declined through the remainder of the active season. This result was likely due to a strong relationship to seasonal rainfall that influenced tissue hydration, bladder water, and food in the gut. This suggests that altered rainfall patterns with climate change may affect the well-being of desert tortoises that depends upon plants with high water content and high protein to potassium ratios in high-rainfall years.

13. Barrows (2011) projected that suitable habitat for the desert tortoise would be reduced by nearly 88% in the Sonoran Desert portions of Joshua Tree National Park, CA, and nearly 66% in the Mojave Desert regions. He developed these projections by modeling climate change sensitivity across a temperature-precipitation gradient in which simulated distributions shifted to higher elevations with a cooler-wetter climate.

14. Lovich and others (2012) noted that tortoise clutch phenology varied widely with year-to-year fluctuations in climate, but appeared to track temperature change in a predictable way. This suggests that tortoises could have the ability to respond to future climate change though plasticity in clutch phenology.

15. Medica and others (2012), conducted long-term studies on tortoises in fenced hectare plots at the Nevada Test Site, NV. The authors observed lower growth rates in tortoises when winter precipitation was low and noted that the prediction of hotter and drier conditions in the deserts of the Southwestern United States could affect long-term survival of desert tortoises as climate changes.

16. Jennings and Berry (2015) studied selection and availability of food in adult desert tortoises at the Desert Tortoise Natural Area, western Mojave Desert, CA, and concluded that desert tortoises are selective herbivores. The authors noted that climate change is a factor that could negatively affect the distribution and abundance of critical tortoise food plants, and in turn affect the well being of tortoise populations.

17. Mack and others (2015) studied variables affecting cover sites or shelters of adult *Gopherus agassizii* in the central Mojave Desert, CA. The authors explored variables including type of shelter, opening height and width, depth of soil cover at cover-site opening, length of tunnel, and aspect. The authors measured temperatures throughout the year external to and inside the cover site, focusing on times of year with maximal and minimal temperature extremes (summer, winter). They reported significant differences in summer temperatures between types of burrows and that tunnel length had the greatest influence on cover site temperatures. With climate change models predicting increased warming in the Southwestern United States, cover sites that buffer temperature extremes and fluctuations will become increasingly important for survival of tortoises. Cover sites with long tunnel lengths occurred in strongly cemented old alluvial fan deposits and Miocene sandstone and conglomerate. Information such as the geographical distributions of geological formations likely to support optimal cover sites will be useful for predicting long-term persistence of tortoise populations.

B. Effects of Climate Change, Specifically, Increases in CO_2, on Habitat

1. Huxman and others (1998) conducted a greenhouse experiment on *Bromus rubens* seeds collected from the Nevada Test Site, NV. Seeds harvested from parents grown in elevated CO_2 had lesser mass, a greater C:N ratio, greater surface area, and produced seedlings which had reduced relative growth rate and smaller final biomass compared to seeds taken from parents grown from ambient CO_2. Seed reserves were greater in ambient-seed-source seedlings, as indicated by longer retention of green foliage than seedlings grown in elevated CO_2. Since the invasive success of *B. rubens* is primarily due to its ability to rapidly germinate, increase leaf area, and maintain a relatively high growth rate compared to native annuals or perennial grasses, reductions in seed quality and seedling performance in elevated CO_2 may significantly impact future community composition in the Mojave Desert.

2. Huxman and others (1999) conducted a greenhouse experiment at the University of Nevada, Las Vegas, using seeds of *Bromus rubens* from the Nevada Test Site. *Bromus rubens* grown at elevated levels of CO_2 exhibited signs of growth allocation versus reproduction. For example, more seeds per unit leaf surface area were produced at higher CO_2, but the seeds were of lesser mass compared to those grown at ambient conditions. The number of seeds produced per unit leaf surface in elevated CO_2 significantly increased relative to the number produced at ambient

CO_2. There was also a parental effect of the CO_2 environment, where *Bromus* seeds produced from parents experiencing elevated CO_2 growth conditions resulted in seedlings that produced smaller leaves with delayed development and smaller roots as compared with those produced by seeds from parents grown at ambient conditions. These results suggest that there could be population level responses of increased atmospheric CO_2 for *Bromus* in natural conditions.

3. Hamerlynck and others (2000) conducted a field experiment at the Free-Air CO_2 Enrichment (FACE) facility at the Nevada Test Site to test photosynthetic responses of *Larrea tridentata* to increases in atmospheric CO_2. The authors reported that elevated CO_2 could increase net assimilation rate in *Larrea* during both moist and dry periods in the potential growing season. Elevated CO_2 did not reduce stomatal conductance in *Larrea*, even following significant rain. This lack of a response may affect desert soil characteristics since there was no increase in soil moisture content at either 0–15 cm or 15–30 cm depths in the elevated CO_2 treatments. Elevated CO_2 did not extend photosynthetic activity longer into the dry season or during more stressful times of the day. The CO_2 saturated rate of photosynthesis was lower in *Larrea* under elevated CO_2 after autumn rains. These findings suggest that predictions regarding desert plant responses to elevated CO_2 should account for seasonal patterns of photosynthetic regulatory responses, which may vary across species and plant functional types.
4. Smith and others (2000) applied free-air CO_2 enrichment (FACE) technology at the Nevada Test Site, NV, to dominant perennial shrubs. Elevated CO_2 substantially increased the production of new shoot biomass in *Larrea tridentata*, *Ambrosia dumosa*, and *Lycium andersonii* in a wet year (1998) but not in a dry year (1999). During 1998, both native annuals and *Bromus rubens* responded to elevated CO_2 with larger plants, but *B. rubens* became a higher proportion of total plant density. These results suggest that elevated CO_2 might enhance the long-term success and dominance of this exotic annual grass in the region, and has the potential to accelerate the fire cycle and alter ecosystem function in deserts in Western North American.
5. Steven and others (2012) conducted a manipulative experiment that applied 10 years of elevated atmospheric CO_2 at the Nevada Free Air CO_2 Enrichment Facility. The authors reported that quantitative PCR surveys suggested a reduction in biological soil crust cyanobacterial biomass and reduced representation of cyanobacteria in the total microbial community in the elevated CO_2 treatment relative to the ambient controls. Taxonomic composition of the cyanobacteria was similar under ambient and elevated CO_2 conditions, indicating the decline was manifest across multiple cyanobacterial lineages. Four subsystem categories, 3 related to oxidative stress, were differentially abundant in relation to the elevated CO_2 treatment. Taken together, these results suggest that elevated CO_2 affected a generalized decrease in cyanobacteria in the biological soil crusts and may have favored cyanobacteria with altered gene inventories for coping with oxidative stress.

6. Schaeffer and others (2003) conducted field experiments at the Nevada Test Site, NV. The authors suggested that microbial nitrogen cycling in the Mojave Desert ecosystem was limited by carbon and nitrogen availability, indicating ecosystem nitrogen dynamics could be substantially altered in the desert by invasions of exotic species and elevated atmospheric CO_2 concentration. They found carbon limitation to microbial nitrogen cycling in soils from underneath the canopies of *Larrea tridentata*, *Pleuraphis rigida*, and *Lycium* spp. Soils from plant interspaces also exhibited a nitrogen limitation.

7. Smith and others (2014) studied responses of annual grasses and forbs to increasing atmospheric CO_2 at the Nevada Desert free-air CO_2 environment facility for 10 years. The authors evaluated productivity, reproductive allocation, and community structure of annuals in response to long-term elevated CO_2 exposure. The dominant species of forb (*Lepidium lasiocarpum*) and grass (*Bromus rubens*) exhibited accelerated phenology, increased size, and higher reproduction at elevated CO_2 in a wet year. However, a multiyear dry cycle resulted in no increases in productivity or reproductive allocation for the remainder of the experiment. They concluded that (a) elevated CO_2 does not increase productivity of annuals in most years, and (b) relative stimulation of invasive grasses will likely depend on future precipitation, with a wetter climate favoring invasive grasses but currently predicted greater aridity favoring native dicots.

C. Effects of Climate Change, i.e., Severe or Prolonged Droughts, Changes in Temperature

1. Hamerlynck and McAuliffe (2008) conducted a study in the Mojave National Preserve, CA. They highlighted the importance of how soil variation and topographic position influence the way severe droughts affect desert ecological processes.

   a. They found that canopy die-back and mortality were more widespread in *Ambrosia dumosa* than in *Larrea tridentata*. Dead plants tended to be smaller than surviving plants, especially in channel and hillslope locations, and in younger, weakly developed soils, suggesting that smaller, juvenile plants were particularly vulnerable as a result of dependence on incident precipitation by runoff.

   b. Plants growing in older, well-developed soils showed lower branch and plant mortality, especially in *A. dumosa.*

   c. Differences in plant response may be related to soil-specific variations. They noted that the distributions of large rocks within intermediate and old alluvial sites versus young sites might affect soil hydrological heterogeneity and intensity of plant competition for water.

2. McAuliffe and Hamerlynck (2010) studied plant response to multi-years of drought in the Mojave and Sonoran Deserts. They showed that responses of plants to severe drought varied among species. The mortality of *Ambrosia* spp. and other small, drought-deciduous subshrubs was nearly 100% even at sites where the drought-enduring evergreen *Larrea tridentata* experienced < 10% mortality. The strongest predictor of plant condition and survival in both *Larrea tridentata* and *Ambrosia* spp. was the 5-year cumulative measure of drought severity (60-month Standard Precipitation Index [SPI]), suggesting the importance of the cumulative effect of successive drought years. A 60-month SPI of –1.5 was a threshold at which significant mortality occurred in *Ambrosia* spp. and SPI of –1.7 was associated with dramatic increases in mortality of *Ambrosia* spp. and significant canopy die-back and mortality of *L. tridentata*. Episodes of drought-induced, perennial plant mortality represent extensive, region-wide ecological disturbances and may be one of the most important processes affecting plant populations and community composition in deserts.

3. Rao and Allen (2010) postulated that primary production in deserts is limited by soil moisture and nitrogen availability and thus is likely to be influenced by both anthropogenic N deposition and precipitation regimes, altered as a consequence of climate change. They conducted a seeded garden experiment and a 5-year field fertilization experiment to investigate how winter annual production is altered by increasing nitrogen supply under a range of water availabilities at Joshua Tree National Park, CA. They reported that:
    a. The greatest production of invasive grasses and native forbs occurred under the highest soil N and water regimes, indication these species are limited by both water and N.
    b. Winter annual biomass was primarily limited by November-December precipitation. In creosote bush scrub, biomass exceeded the fire threshold only under very wet conditions regardless of soil N status.
    c. The results indicate that areas of high N deposition will be susceptible to grass invasion, particularly in wet years, potentially reducing native species cover and increasing the risk of fire.

4. Abatzoglou and Kolden (2011) noted that climate scenarios for the mid-21st century suggest conditions favorable to the spread of invasive annual grasses, including: (a) increased length of the freeze-free season that may favor cold-intolerant annual grasses, (b) changes in the frequency of wet winters that may alter the potential for establishment of invasive annual grasses, and (c) an earlier onset of fire season and a lengthening of the window during which conditions are conducive to fire ignition and growth furthering the fire-invasive annual grass feedback loop. The projected increases in frequency and onset of extreme fire danger across deserts of the Western United States with climate change will likely increase mortality rates of native species less adapted to wildfires. These projected findings indicate the importance of proactive reduction of invasive fuel loads.

5. Bykova and Sage (2012) conducted controlled acclimation experiments using seeds collected in the Mojave Desert, Western United States, and Toronto, Canada, to evaluate winter cold tolerances of invasive grasses. Climate warming could induce range expansion of *Bromus tectorum*. *B. tectorum* should track moderation of minimum winter temperatures, while migration of *Bromus rubens* should follow a loss of severe cold events during autumn. If this happens, *B. rubens* could spread into the intermountain steppe region now dominated by *B. tectorum*. If more competitive, *B. rubens* could displace *B. tectorum*, but if not *B. rubens* invasion would compound the problems already caused by *B. tectorum*. The tolerance thresholds observed for these two species could provide critical information for building physiological-based models predicting geographic distributions and range shifts following climate change.
6. Barrows and Murphy-Mariscal (2012) modeled projected Joshua tree (*Yucca brevifolia*) distribution under climate change scenarios in Joshua Tree National Park, CA. Joshua trees were projected to retain 65–70% of their current areal extent at a 1 °C increase in mean maximum July temperatures; 22–34% at an increase of 2 °C; and 2–10% at an increase of 3 °C. This reduction was due to up-slope elevation shifts in the mean occurrence of suitable habitat under warmer and drier scenarios. Nevertheless, refugia of suitable Joshua tree habitat still remained within Joshua Tree National Park even within the most severe climate scenario, which represents a more optimistic scenario than previously published models of the impacts of climate change on Joshua trees. Given the potential interaction between increased grass-spread wildfires (e.g., red brome, *Bromus rubens*) and a climate change-related increase in severe wildfire conditions, resource managers may need to focus weed control and restoration efforts within identified climate refugia to minimize stressors that could reduce Joshua trees.
7. Bai and others (2013) synthesized data from 75 sites originating from a long-term soil temperature measurement project (1982–2000) to evaluate effects of soil climate on spatial distribution of perennial plants. The results suggested that both soil temperature and aridity resulting from climate change could have significant impacts on perennial plant distribution in the Mojave Desert. Increased ambient temperature over the region is expected to rise and areas with warmer soil temperature are expected to expand, encouraging the distribution of species such as creosote bush (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), and yucca (*Yucca* spp.). In the meantime, the distribution of sagebrush (*Artemisia* spp.) that occupies the wetter and colder region (>1,500 m) and lower soil temperature (<18 °C) is expected to expand upward in elevation into the pinyon-juniper communities. These results suggest that both soil temperature and aridity resulting from climate change could have significant impacts on plant distribution in the Mojave Desert.

D. Considerations and Actions for Management

1. Peterson (1994) noted that high mortality in populations of desert tortoises at both the Desert Tortoise Natural Area in the west Mojave Desert and Ivanpah Valley in the eastern Mojave Desert, CA, were attributable to effects of drought. The effect of drought occurred indirectly in the western Mojave through functional responses of predators to a diminished prey base [and disease], and directly in the eastern Mojave through starvation and dehydration. Episodic, drought-related high mortality has probably occurred repeatedly in the evolutionary history of desert tortoise, but human exploitation of the desert may exacerbate natural stresses, and recovery of populations is likely to be slow.
2. Duda and others (1999) reported that future management and conservation plans for the desert tortoise should consider weather and productivity as important factors influencing annual home range size, number of burrows used, average distances traveled, and activity levels.
3. DeFalco and others (2010) reported that continued El Niño Southern Oscillation episodes and more frequent wildfires are expected for the desert Southwest and will likely shift *Yucca brevifolia* population structure toward tall, old adults with fewer opportunities for plant recruitment, thus imperiling the persistence of this unique plant community.
4. Abatzoglou and Kolden (2011) wrote that climate change information needs to be delivered on scales and in a format readily accessible to management.
5. Barrows and Murphy-Mariscal (2012), noted that resource managers may need to focus weed control and restoration efforts within identified climate refugia areas to minimize stressors that could reduce Joshua tree (*Yucca brevifolia*) sustainability in Joshua Tree National Park, CA, given the potential interaction between increased grass-spread wildfires [e.g., red brome (*Bromus rubens*)] and a climate change-related increase in severe wildfire conditions.
6. Averill-Murray and others (2012) provided recommendations to adapt land management in the face of climate change: improve regional coordination of management activities, broaden spatial and temporal perspectives in management, and emphasize simultaneously addressing multiple threats while being inclusive of diverse human communities.
7. Averill-Murray and others (2013) said that existing Tortoise Conservation Areas (TCAs) provide an initial framework upon which to focus recovery efforts. However, given uncertainties related to climate change such as projected changes in temperatures, desert wildlife may need to shift their distributions at a rate of 0.7km/year which would cross critical habitat units within 23–187 years. Buffering around existing conservation areas and the linkages between TCAs may allow for necessary shifts in species' distributions.

XIII. Atmospheric Pollution

A. Literature Review. Lovich and Bainbridge (1999) reported that atmospheric pollutants transported in smog from urban areas, such as Los Angeles, into the Mojave Desert include ozone (O_3), sulfur dioxide (SO_2), and various particulates. Impacts from smog include degradation of visibility and dryfall of nitrogen-rich compounds that can be a major source of supplemental nitrogen, which favors many exotic plant species over native annuals and perennials. Damage to plants has been shown to occur in desert plants exposed to ozone and sulfur dioxide in laboratory settings, and some types of cryptogamic soils are highly sensitive to SO_2. Studies of plants from the Mojave and Colorado Deserts reveal that perennial species vary in their response to SO_2 and NO_2, with *Larrea tridentata* sensitive, *Encelia farinosa* and *Ambrosia dumosa* intermediate, and *Atriplex canescens* resistant to these pollutants under experimental conditions. Sensitivity also varies among native annual plants.

B. Rao and Allen (2010) conducted nitrogen fertilization experiments inside and adjacent to Joshua Tree National Park, CA. They reported that native forb cover is reduced at high levels of soil nitrogen, suggesting that at high soil nitrogen native forbs are either directly harmed or outcompeted by nitrophilous species such as invasive grasses. A companion garden experiment indicated native forbs benefitted from intermediate levels of soil nitrogen between 1.5 and 3 g m^{-2}.

C. Newingham and others (2012) conducted field experiments at the Mojave Global Change Facility in the northern Mojave Desert on effects of enhanced summer precipitation, nitrogen deposition and increased soil disturbance on productivity, reproduction, and herbivory of *Larrea tridentata*. While the water-nitrogen interaction was significant at times, there were no consistent patterns. Induced soil disturbance, which was intended to decrease nitrogen inputs by biological soil crusts had negative effects on *L. tridentata* stem elongation but only in a wet year. Their results suggest that increased summer precipitation and nitrogen deposition may result in only small growth increases by *Larrea*; any biomass gains were likely to be lost by mammalian herbivory.

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Chapter 11.—Anthropogenic Impacts to Tortoises: Part 2. Subsidized Predators, Predator Ecology, and Implications for Tortoise Recovery and Management

I. Ecology and Behavior of Common Ravens (*Corvus corax*, aka raven), Use of Subsidies

A. Knight and others (1993), in a spring 1992 study encompassing the western and southern Mojave Desert and parts of the Colorado Desert, CA, observed a total of 168 ravens and found that the number of ravens/100 km in urban-suburban areas was greater than in either rangeland or control areas, though did not differ from irrigated agriculture. Ravens were either perched (54%) or flying. The researchers concluded that low densities of ravens associated with rangeland and control areas suggested that historically they were uncommon in the Mojave Desert and that land-use patterns influenced raven numbers. Greater abundance of ravens in urban-suburban areas and irrigated farmlands may have been due to the greater abundance, availability, and dependability of food, water, and potential nesting sites in those areas. The authors believed that increased raven numbers are strongly associated with either linear right-of-ways or with urbanization or agriculture. There was no evidence that ravens readily left urban-suburban or irrigated farmland habitats to forage in desert areas, thus the authors suggested that conclusions regarding the impact of raven predation on desert tortoises in particular land uses and adjacent areas are speculative.

B. Boarman (1993) explained that in the Mojave Desert, Common Ravens are subsidized predators because they benefit from resources associated with human activities that allow their populations to grow beyond their "natural" carrying capacity in the desert habitat.

1. These resources include food and water from landfills, reservoirs, sewage ponds, agricultural fields, feedlots, gutters, as well as perch, roost, and nest sites from power towers, telephone poles, light posts, billboards, fences, freeway or railroad overpasses, abandoned vehicles, and buildings. Subsidies allow ravens to survive in the desert during summer and winter when prey and water resources are typically inactive or scarce.

2. Ravens are known to prey on juvenile desert tortoises based on direct observations and circumstantial evidence, such as shell-skeletal remains with holes pecked in the carapace. According to Breeding Bird Surveys conducted by the U.S. Fish and Wildlife Service between 1968 and 1988, the number of Common Ravens increased by 1,528% in the Mojave Desert.

C. Knight and Kawashima (1993), conducted a study in the Mojave and Colorado Deserts (based on Fig. 1), CA, in spring 1989. Using a helicopter, they surveyed 97 transects ($n = 24$ powerlines, $n = 28$ paved highways, $n = 45$ controls) totaling 1,685 km. They found Common Ravens were equally abundant along highway and powerline transects, but were more abundant along these transects than along controls (i.e., no highways or powerlines within 3.2 km). Raven nests, as well as Red-tailed Hawks (*Buteo jamaicensis*) and their nests, were more abundant along powerlines than along either highways or controls. Ravens used power poles as nest sites, and Red-tailed Hawks used power poles for both nesting and perching (rather than telephone poles, trees, or cliffs), more than expected based on availability. Results suggested that ravens were more abundant along highways due to automobile generated carrion, whereas both Ravens and Red-tailed Hawks were more common along powerlines because of the presence of superior perch and nest sites. The authors noted that if linear right-of-ways result in increased predator populations or concentrate the activities of predators, managers should monitor possible population decreases in sensitive prey species like the desert tortoise.

D. Camp and others (1993) quantified items in 226 pellets from 39 active Common Raven nests. Desert tortoises were found in pellets from only two nests (1.3% of pellets), although reptiles were present in 73% of the pellets, whereas invertebrates occurred in 86% of the pellets and human refuse occurred in 22% of the pellets. These results suggest that ravens are omnivorous generalists, and that desert tortoises were an infrequent prey item in the raven pellets sampled. While digestibility can vary among prey types, the authors suggest that digestibility was unlikely to have affected detection for desert tortoises because tortoise remains found in pellets included leg bones, nails, scales, and skull parts.

E. Knight and others (1995) reported that Common Raven numbers did not increase with increasing road traffic volume and traffic speed, but did show a positive relationship with increasing number of linear rights-of-way, which ran in parallel. Specifically, the greatest raven numbers were found where four to five linear rights-of-ways ran parallel compared with less than three linear rights-of way. Results suggested that traffic volume or traffic speed did not necessarily account for increased levels of road-killed carrion that would presumably elevate raven numbers. However, the finding of increased raven numbers with increasing number of linear rights-of-way suggested that the presence of roads, transmission powerlines, and railway lines may have provided food or nesting and perching sites that might have increased raven numbers.

F. Knight and others (1998), in a study in the Mojave Desert, reported that Common Ravens (n = 20 individuals at 12 sites) and European Starlings (*Sturnus vulgaris*, n = 12 individuals at four sites), were only seen at stock tanks. Nine Brown-headed Cowbirds (*Molothrus ater*) were seen at five stock tanks while two cowbirds were detected at one spring. No birds were detected at the control sites. Sixteen of 20 (80%) of the ravens, 17% of the starlings, and only one cowbird were seen drinking from the stock tanks. Two juvenile tortoise shells were found at two different stock tanks.

G. Knight and others (1999), in a 12-month study (1992–1993) in the eastern Mojave Desert, CA, reported the following for avian associations and behaviors with human land-uses, linear rights-of-way, and perch-sites:

1. Common Raven was the most frequently observed species with an average of 6.9 sightings per 100 km per month followed by Turkey Vulture (*Cathartes aura*), Red-tailed Hawk, and Loggerhead Shrike (*Lanius ludovicianus*), which had 0.8-1.7 sightings per 100 km. American Kestrel (*Falco sparverius*), Prairie Falcon (*Falco mexicanus*), and Golden Eagle (*Aquila chrysaetos*; four total observations) were sighted less frequently with < 0.5 sightings per 100 km per month. Turkey Vultures were not present during winter months, Golden Eagles were present only in November and December, and the remaining five species were seen throughout the year (except Prairie Falcon was absent in April and American Kestrel in May).

2. Common Ravens, Turkey Vultures, and Red-tailed Hawks were most numerous on irrigated agricultural lands, while Loggerhead Shrikes were most common at urban areas when comparing land use for each species. Increased abundance in these areas was likely due to the availability and dependability of food sources. Ravens, however, were 25 times more abundant than Loggerhead Shrikes in urban areas, and were the most abundant species on the rangelands. Ravens increased with increasing number of linear rights-of-way parallel to the survey route. Perching was the most common behavior type for all species, except for the Common Raven that was most frequently observed flying or soaring. Transmission powerline towers and telephone poles were used as perch sites disproportionately to availability, and may provide birds with a wider range of vision.

H. Lovich and Bainbridge (1999) reviewed the literature and reported that towers associated with many electrical energy transmission corridors provide nest sites and hunting perches for Common Ravens, a native predator that has increased dramatically in recent years due to human subsidies. The towers may allow ravens to hunt more effectively for desert tortoises (see Boarman, 1993).

I. Kristan and Boarman (2003) conducted a study of ravens at the Edwards Air Force Base in the western Mojave Desert, CA. They reported that predation risk to Styrofoam models of juvenile desert tortoises was highest near anthropogenic subsidies such as landfills and artificial water bodies that attracted large numbers of nonbreeding Common Ravens and near successful raven nests with relatively small numbers of ravens nearby. These patterns were consistent with both “spillover predation” attributed to nonbreeding ravens near large anthropogenic resources and “hyperpredation” attributed to breeding ravens. Because locations of some successful nests changed year-to-year, consistent, low-predation refugia for desert tortoises were nearly non-existent.

J. Kristan and others (2004) found that the diet composition of breeding Common Ravens assessed by pellets reflected their generalist diet and varied with distance from roads and resource subsidies. Greatest incidence of trash in pellets occurred in those close to subsidies such as landfills and far from major roads. Greatest incidence of mammals and reptiles in pellets occurred in those close to roads but far from subsidies, suggesting the use of road-killed carrion. Greatest incidence of birds and amphibians in pellets occurred in those close to both roads and subsidies. Greatest incidence of plant material and arthropods in pellets occurred in those far from both roads and subsidies. Fledgling success also correlated with diet composition. Ravens with diets consistent with trash or road-kill subsidies fledged the greatest number of chicks, and ravens nesting far from human developments and with diets containing relatively little human-provided food reproduced poorly. These results suggest that human developments in the western Mojave Desert affect raven populations by providing food subsidies, particularly trash and road-kill.

K. Webb and others (2004) conducted a mark-recapture study of 240 raven nestlings in 1999–2000 at Edwards Air Force Base in the western Mojave Desert, CA, to determine whether survival of juveniles could be predicted by a set of environmental and morphological variables. The authors reported a positive effect of nest proximity to anthropogenic resources on survival for at least 9 months after fledging. Annual survival was 47% for the first-year, 81% for the second-year, and 83% for third-year birds. The results support the hypothesis that anthropogenic resources contribute to increasing raven numbers via increased juvenile survival to departure (from the nest) as well as increased post departure survival.

L. Boarman and others (2006) conducted weekly counts of ravens for 29 months at 10 sites on the Fort Irwin National Training Center in the central Mojave Desert, CA, during April 1995 to August 1997. The authors reported that Common Raven abundance varied diurnally, seasonally, and with human abundance. Raven abundance was greatest near resource subsidies (specifically the landfill and sewage ponds), where mean raven abundance equaled 78.9 (SE = 0.10), compared with light resource subsidies (mean = 4.0). Numbers of ravens at the landfill were particularly high, 18 times the number found at light resource sites and 225 times that of the open desert sites. One-year old ravens were more common than adults at the landfill. While substantially less than at the landfill, raven numbers were also relatively high at sewage ponds, compared with other anthropogenic and undisturbed sites. Numbers of ravens also correlated positively with overall abundance of humans in the cantonment (offices, military facilities, homes) and with coyote abundance. Significantly higher numbers of ravens were tallied during times of no human presence at survey points, regardless of month of the survey. Finally, raven numbers decreased from winter to spring, peaking in October and dipping in March. This study further documents heavy use of landfills and sewage ponds by ravens. The latter use had not been previously reported in the published literature.

M. Kristan and Boarman (2007), in a 1996–2000 study, found that Common Ravens at Edwards Air Force Base in the western Mojave Desert, CA, nested disproportionately near point sources of food and water (e.g., towns, landfills, and ponds within 1-km) but not near roads (sources of road-killed carrion) within 0.5–1 km. Both sources of subsidy enhanced the total number of years of raven fledgling success, although proximity to roads was the most consistent predictor, and the total number of fledglings declined from the most developed areas (1.72 fledglings ± 0.24 [mean ± SE], n = 33 nests) to the least developed areas (0.92 ± 0.07, n = 226 nests), with mixed development being intermediate (1.18 ± 0.21, n = 20 nests). The effect of access to human-provided resources (i.e., primarily food) on fledgling success was relatively consistent from year to year and accounted for 63% of the total effect of all environmental variables on the number of successful years of reproduction compared to only 27% attributed to just natural habitat-related variables, which suggests that humans provide consistent and high-quality breeding habitat for ravens.

N. Fleischer and others (2008) obtained genetic samples of the Common Raven in the western and eastern Mojave Desert, Coastal CA, and Great Basin.

1. The genetic analysis, using mtDNA control region sequences and microsatellite data, revealed recent population growth for the Common Raven in the western Mojave Desert, a bottleneck in the northern California populations, and population stasis in the Great Basin, Nevada, and southern California. The authors suggested that ravens in the eastern Mojave were more similar to populations from northern Nevada and that there was limited gene flow between eastern and western Mojave sampling areas as a result of current anthropogenic habitat gaps.

2. Although *in situ* population growth of western Mojave populations could not be ruled out, the inferred patterns of movement from the genetic data suggested that increases in raven populations in the western Mojave may have resulted from source population movements from southern California and the Central Valley.

O. Webb, Boarman, and Rotenberry (2009) sampled and marked juvenile Common Ravens at Edwards Air Force Base, CA, and reported that a combination of anthropogenic food and water sources and conspecific attraction were the strongest correlates that explained post-fledgling movement patterns.

1. The median dispersal distance from the nest was 7.9 km (ranged from 1 to 27 km) and did not differ by sex.
2. The most important resources for dispersing juvenile ravens were communal feeding operations such as dairies, hobby farms, and stables, which likely presented ravens with abundant and concentrated food and water sources (i.e., livestock feed, manure, refuse, water troughs, livestock carcasses). This represented 69% of all cases of first dispersal locations.
3. Juvenile ravens were rarely located in open desert.
4. Foraging cues by related adults and/or adult aggression toward unrelated conspecifics was suggested to be an influencing factor to explain movements of juveniles from their natal territories to the nearest communal point subsidy, rather than closer anthropogenic resources

II. Recommendations for Managing Common Ravens, A Subsidized Predator

A. Knight and Kawashima (1993) stated that roads benefit Common Ravens because they provide increased amounts of food from road-killed wildlife, whereas powerlines provide suitable perch and nest sites for ravens and Red-tailed Hawks. If linear right-of-ways result in increased predator populations, or concentrate the activities of predators, land managers should monitor possible population decreases in sensitive prey species like the desert tortoise.

B. Boarman (1993) recommended a hybrid approach to reduce the impact of ravens preying upon tortoises, including broad-scale removal of ravens at some localities where tortoise and raven densities were both high, and selective removal of all “known offenders” year round. The amount of resource subsidy provided to ravens must also be reduced to facilitate a smaller raven population in the desert. Control of ravens must also be concurrent with management actions to reduce other causes of tortoise mortality.

1. In 1989, the Bureau of Land Management (BLM) and other resource agencies, the Department of Defense, and Animal Damage Control implemented a pilot raven-control program to reduce raven predation using an avicide, a poison. The program was quickly shut down due to concerns of secondary poisoning of non-target wildlife. In response, the BLM moved to develop a Common Raven Management Plan based on the principles of integrated pest management. The BLM was confronted with several challenges in developing a raven control program including lack of data, ethical and philosophical differences among stakeholders, and undefined criteria in stated goals.

2. Justification of the need for a control program included (a) substantial increases in raven populations; (b) evidence that ravens prey upon juvenile desert tortoises, (c) evidence that raven predation significantly reduces desert tortoise recruitment into the breeding population, and (d) widespread predation. Recommended control actions included a hybrid approach, with broad-scale removal of ravens at localities where both tortoise and raven densities were high, and selective removal of all "known offenders", augmented by removing the availability of resource subsidies to ravens.

C. Boarman (2003) recommended approaches to reducing Common Raven predation on tortoises: (1) long-term: undertake habitat alterations in conjunction with research to develop raven management strategies (i.e., adaptive management), and (2) short-term: limited lethal control of ravens to reduce predation pressure on tortoises. Long-term actions, however, should be implemented at the same time as the short-term actions and until tortoises are recovered.

1. Alter habitat to reduce raven access to anthropogenic food and water resources. These resources include solid waste at sanitary landfills, open garbage (e.g., dumpsters at rest areas, restaurants, gas stations, grocery stores; bags for curbside pick-up; grain from trains, livestock carcasses at dairies; food left out for pets), agricultural environments (e.g., grains at feed lots, rodents and insects in fields, orchard and row crops), carcasses of road-killed animals along highways, and water resources at landfills, sewage and septic sources, in urban environments, and other sources of standing water. These sources allow ravens to expand their range up to 65 km into areas that they otherwise did not occupy and thus increase tortoise vulnerability to raven predation.

2. Discourage nesting by ravens in the spring when tortoises are most active and ravens are feeding young. Removing raven nests with eggs in them would probably have the greatest benefit because they are not likely to re-nest. Dipping bird eggs into corn oil prevents hatching and causes parents to continuing incubating rather than to re-nest. Remove or avoid construction of raven nesting structures such as power towers, telephone poles, billboards, abandoned buildings, and vehicles.

3. Implement lethal action focusing on removing individual birds known to prey upon tortoises while monitoring the effectiveness of the action. Especially consider lethal action where tortoises are faced with multiple threats, including raven predation, such as at the Desert Tortoise Natural Research Area, locations where captive release and translocations of tortoises have occurred, and in limited areas in the Desert Wildlife Management Areas.

4. Use an adaptive management framework, which includes determining if the programs goals and objectives have been met and must include use of research with treatments and controls, and replicates when possible. Establish two work groups to (a) implement the raven control program and oversee management direction, review information, coordinate with agencies and groups, solicit funding for specific management efforts, distribute information; and (b) oversee technical and policy progress regarding management actions, such as interpreting the scientific data and recommending changes.

D. Kristan and Boarman (2003) stated that anthropogenic resources for ravens could indirectly lead to the suppression, decline, or even extinction of desert tortoise populations. The authors observed both spillover and hyperpredation patterns in the western Mojave Desert. They said that decreasing the regional raven population size, or decreasing raven reproductive success in tortoise habitat may be necessary to reduce the predation risk from breeding ravens.

E. Kristan and others (2004) noted that fledging success of ravens was associated with diets with greater incidence of human-provided foods. The fledging success ultimately supports and maintains large raven populations in the western Mojave Desert, CA. A decrease in anthropogenic resource subsidies can be accomplished by covering trash in receptacles and landfills. Likewise, decreasing refuse dumping, and fencing roads to prevent road-kills reduce subsidies.

F. Webb and others (2004) noted that land managers should expect raven numbers and conflicts with humans to grow in parallel to the human population, unless raven access to anthropogenic resources is diminished. Eliminating or altering management practices that inadvertently provide resource subsidies to ravens should reduce their population density and associated negative impacts on biodiversity in the western Mojave Desert, CA.

G. Boarman and others (2006) noted that rapid growth of populations of Common Ravens resulting from human subsidies in the Mojave Desert has been a management concern because ravens prey on threatened desert tortoises. This study suggests that raven management should focus on reducing the availability of resource subsidies, especially at landfills and sewage ponds. However, these efforts may only be successful when coupled with similar efforts on a broader, region-wide basis.

H. Kristan and Boarman (2007) said that anthropogenic cover types promote raven population growth and allow ravens to occupy otherwise inhospitable parts of the desert. Predatory impacts of ravens in the Mojave Desert can be considered indirect effects of anthropogenic development. To the extent that human developments in the region (western Mojave Desert) are promoting the spread and persistence of a large number of Common Ravens in areas that are marginal breeding habitat, human activities can have effects on tortoises far from the actual developments that serve as attractions.

I. Fleischer and others (2008) reported that increased numbers of Common Ravens in the Mojave Desert represent a problem for threatened desert tortoises. Because the raven is a partially subsidized predator benefiting from anthropogenic subsidies, it is important for managers to know if limiting raven reproductive and feeding opportunities in the Mojave Desert will reduce raven population size and density, or whether immigration will nullify such management actions. Genetic data from this study show the possibility of large-scale, region-wide movements with reproduction (i.e., gene flow), supporting the need for regional-scale management efforts rather than just local ones. Current gaps in anthropogenic habitat are probably the only factor maintaining the identified genetic structure. Developments and other actions that subsidize essential resources for ravens provide more hospitable habitat thereby facilitating the incursion of ravens farther into the desert.

J. Webb, Boarman, and Rotenberry (2009) reported that land managers concerned with growing populations of Common Raven should reduce access to concentrated anthropogenic resources such as landfills and confined livestock feeding operations, which serve as important resources for juvenile ravens. Because juvenile ravens rarely venture into open desert, reducing their numbers by lethal removal or other means is unlikely to lessen raven predation of desert tortoises. If juvenile ravens prey on desert tortoises, then it is likely to be occurring mainly from chance encounters during transit between anthropogenic point subsidies.

K. Nagy and others (2015b) suggested that delayed releases be incorporated in the protocol for head-starting desert tortoises, because nearly all yearlings released were dead within 6 months primarily because of predation by Common Ravens.

III. Recommendations for Managing Known or Potential Subsidized Mammalian Predators

A. Mammalian predators, Coyotes. Esque and others (2010a) note that predator control is an option when local predation levels decimate the species of concern. Resource managers will need to consider efficacy and costs/benefits of potential management strategies. The authors acknowledged that implementing predator control can have short-term benefits to at-risk species such as the desert tortoise. However, predator removal could cause changes in community structure, cause compensatory predator migration or increased reproduction, or increase incidence of disease. The authors also said that limiting the amount of refuse and access to water in desert areas likely to be used by subsidized predators would be useful.

B. Mammalian predators, Bears. Lovich and others (2014a) stated that although interactions between tortoises and bears are rare, if a single black bear developed a taste for desert tortoises, it could have negative effects on a small population of tortoises similar to the mountain lion predation event reported by Medica and Greger (2009, annotated herein). Thus, researchers and resource managers should be aware of threats from novel introduced predators to federally listed species.

C. Mammalian predators, Badgers. Emblidge and others (2015) reported significant mortality in 2012 and 2013 (22 and 84%) on a sample population of adult desert tortoises at one of four study sites in the central Mojave Desert, CA. The degree of mortality at the site far surpasses the highest level reported to date for a wild population. The American badger (*Taxidea taxus*) was implicated in photographic evidence and may have been responsible, at least for some of the deaths. However, other sources of death should be considered. If badger predation is determined to be a problem requiring redress, wildlife managers will face a difficult situation of attempting to reduce predation on a threatened species by badgers, since they are a species of special concern in California.

Chapter 12.—Managing Desert Tortoises and Their Habitats

I. Management of *Gopherus agassizii* Individuals and Populations

A. Implications of Taxonomic Classification for Management or Recovery

1. Berry and others (2002a) noted that resolving taxonomic ambiguity is critical for prioritizing protection of genetically, ecologically, or biogeographically important subunits, to avoid inappropriate extrapolations among subunits, and to accurately designate tortoises within species, subspecies, Distinct Population Segments (DPSs), Evolutionary Significant Units (ESUs) or Management Units (MUs) for appropriate legal protection. Further, it is important to implement a standardized program of sampling that includes all major populations across the entire range of both *G. agassizii* and *G. berlandieri*, to complete the identification of populations and their assignments to ESUs and MUs based on mitochondrial DNA (mtDNA). Sampling populations in potential contact zones more extensively would be useful to determine absence or extension of gene flow among different ESUs, to resolve species identities, and to distinguish morphology, ecology, behavior and physiology among populations and to establish a complete and broadly based array of DPSs.
2. Murphy and others (2007) conducted a genetic assessment of the six Recovery Units for *Gopherus agassizii* and reported:
    a. The importance of conserving populations that show significant differences in genetics, such as the haplogroup from the northeastern Mojave, to help ensure unconstrained evolutionary processes.
    b. That translocation of tortoises should be minimized; translocations could compromise the genetic integrity of a population by disrupting co-adapted gene complexes in local environments or reducing fitness through outbreeding depression.
    c. That an appropriate recovery action would be restorative actions on populations that have become disjunct or mixed as a result of anthropogenic activities. For example, deliberately or inadvertently translocated tortoises could be removed from critical habitats where populations are declining and most tortoises could be located and genetically tested. Examples of such areas include the Upper Virgin River Recovery Unit and Beaver Dam Slope. Another restorative action would be to genetically test tortoises at frequently used recreation sites where visitors often release tortoises illegally, and place them in appropriate areas.
    d. That another restorative action would be carefully planned augmentations or translocations in populations that have dropped below viable levels; using tortoises within a well-defined recovery unit or local geographic area for head-starting or augmentation is far better than translocating tortoises between Recovery Units.
    e. That empirical studies need to be designed and tested to determine whether marker loci reflect specific adaptations with potential conservation value.

3. Murphy and others (2011) concluded that description of *G. agassizii* as a distinct species from *G morafkai* reduced the geographic range of *G. agassizii* to about 30% of its former range. Populations in Arizona and Mexico no longer could be considered as a genetic reservoir for Agassiz's desert tortoise. The authors contended that this reduction of the species' distribution could have dire consequences for risk of declines and extinction, and combined with population declines over several decades, could require a higher level of protection to ensure survival of the species.
4. Averill-Murray (2011) responded to Murphy and others (2011) about conservation implications of designating the two species, *G. agassiz*ii and *G. morafkai*, from the former *G. agassizii*. His first point was that many years prior to this designation, during the history of desert tortoise management and regulatory actions, the two species/populations had never been considered as a genetic reservoir for the other. His second point was that separation of the two species/populations and their associated ranges was incorporated into the threats analysis that went into the original federal listing and recovery plans, thus the new designation would not necessarily change prior findings with respect to conservation status.

B. Conservation of the Black Mountains Populations in Northwestern Arizona.

1. McLuckie and others (1999) described a population of *Gopherus agassizii* with unique morphological and genetic characteristics in the Black Mountains region of northwestern Arizona.
2. Edwards and others (2015), using both genetics and habitat suitability modeling, described the location of a population of *G. agassizii* currently outside the range of *G. agassizii* protected under the federal Endangered Species Act as threatened. This population has hybrids with *G. morafkai*. Increasing development in this region of Arizona may threaten the viability of this small population, particularly the alignment of a highway directly through primary habitat. The prudent approach to protecting the genetic and habitat diversity of the species is to protect this unique population.

C. The Importance of Protecting and Restoring the Food Supply for Tortoises. The background for the management recommendations on tortoise forage is described in several annotated articles in Chapter 5, Foraging Behavior, Digestion, and Nutrition.

1. Given the lower digestibility and nutritional content of grasses compared with forbs and the potential for growth of juvenile tortoises to be limited in part by dietary nitrogen and phosphorus, a desirable management goal is to reduce biomass of less nutritious, invasive grasses such as *Schismus* spp. that contain lower nitrogen and produce phosphorus loss, and to maximize diversity and abundance of native plant diversity that includes forb species. This would help tortoises obtain high nutritional foods without additional time spent searching that would increase their vulnerability to predators and temperature extremes (Hazard and others, 2009, 2010).
2. Management practices that promote availability of forbs over grasses could increase growth rates and shell ossification of juvenile desert tortoise, which in turn would enhance predator resistance (Hazard and others, 2010).

D. Translocation, Relocation, and Augmentation of Desert Tortoises

1. The decision process for translocation

    a. Using an ethical framework in the decision-making process. McCoy and Berry (2008) described the application of an ecological ethics framework that could make the reasoning and decision-making processes regarding relocation/translocation of desert and gopher tortoises and other animals more transparent. Use of an ecological ethics framework could help work through the variety of values, ethical positions and dilemmas faced by researchers and decision makers. Ethics can be individual-based, group-based, or survival-based or other. Examples include disease and parasite transmission, captive rearing and breeding, welfare of individuals, illness and euthanasia. The process involves multiple phases: (i) examine the dilemma; (ii) examine database and relevant facts; (iii) clarify the decision-making process; (iv) explore and assess values; (v) review key ethical principles, concepts or theories; and (vi) move toward a decision and plan of action.

    b. Heaton and others (2008b) developed a decision support system within a geographic information system (GIS) framework and identified seven suitable translocation locations for the translocation of tortoises from the National Training Center, Fort Irwin, CA. After natural resources managers conducted site visits, six translocation sites were selected as suitable. The model elucidated the importance of fencing additional roads to reduce tortoise mortality and increased available translocation areas by 40% within the Western Mojave Recovery Unit.

    c. Germano and others (2015) questioned whether mitigation-driven translocations are moving in the right direction (in general, using the gopher and desert tortoises as two examples, as well as other species).The authors noted that mitigation-driven translocations outnumber and receive more funding than science-based conservation translocations, with conservation benefits of the former unclear. Outcomes may be less successful in economically motivated mitigation translocations than releases designed to serve biological needs of species. Translocation as a regulatory tool may be ill-suited for biologically mitigating environmental damage caused by development. Evidence suggests that many mitigation-driven translocations fail, although the application of scientific principles and best practices would probably improve success rates.

2. General concerns. Sullivan and others (2015) stated that translocation of species for the purpose of mitigation (to avoid human-wildlife conflicts) can have population, community, and genetic consequences both at the site where the species was removed and introduced, such as disease transmission (e.g., *Gopherus agassizii*), destabilizing interactions among species in the area, and uncertain viability of the translocated species. Both high return rates or dispersal from release sites, with the potential to become a nuisance elsewhere, have been documented for translocated animals. The authors found that some long-lived reptile species have complex social interactions and have intimate knowledge of their resident environment, returning annually to known water resources and refugia, with

translocated animals often exhibiting significantly higher movement rates, larger home ranges, and greater mortality than resident animals. The authors found that carefully preplanning the translocation by considering the original habitat, finding or creating burrows at the new site, moving animals shorter distances, moving social groups together, moving them early in the active season or prior to aestivation, moving younger animals that have yet to establish a home range, vaccinating, using soft-release techniques, and releasing under protective cover and in the direction of intended travel– considerations similar to conservation translocations (to augment declining populations)–should improve the success of mitigation translocations.

3. Genetic considerations
   a. Murphy and others (2007) summarized thousands of known human-mediated translocations of desert tortoises, deliberate releases and escapes of captive tortoises, as well as commercial collections and transfer of tortoises to other states; these events occurred between 1935 and 2004 (maps are included). The authors noted that translocation of tortoises should be minimized; translocations could compromise the genetic integrity of a population by disrupting co-adapted gene complexes in local environments or reducing fitness through outbreeding depression. Further, an appropriate recovery action would be restorative actions for populations that have become disjunct or mixed as a result of anthropogenic activities. For example, deliberately or inadvertently translocated tortoises could be removed from critical habitats where populations are declining and most tortoises could be located and genetically tested. Examples of such areas include the Upper Virgin River Recovery Unit and Beaver Dam Slope. Another restorative action would be to genetically test tortoises at frequently used recreation sites where visitors often release tortoises illegally, and place them in appropriate areas.
   b. Davy and others (2011) noted that desert tortoises have the capability for polyandry. Long-term sperm storage allows females to carry the genetic material of several males. Thus translocation of a single, mature, multiply-mated female may be effectively equivalent to the more costly translocation of several males and could increase the speed at which translocation of these females can impact the genetic profile of the recipient population.
   c. Edwards and Berry (2013) said that it would be prudent to genotype and assign any captive individuals of unknown origin to the most appropriate genetic unit prior to any organized repatriation effort. There are many reasons, e.g., many captive desert tortoises have low genetic affinity with local wild populations. Such releases would likely result in genetic mixing of disparate populations with potentially detrimental consequences, such as outbreeding depressions or lower fitness. To maximize genetic and adaptive similarities of populations, the genetic identity of captive tortoises (as well as any potential progeny) should be resolved prior to release. Due to the high risk associated with repatriation of captive animals, captive populations should not be considered a reservoir for recovery of wild populations, and should only be employed where other conservation measures are unavailable.

d. Averill-Murray and Hagerty (2014) detected significant spatial autocorrelation between genetic and geographic distances even at the smallest distances (50 km) in a study of genetics and distribution across the range of *Gopherus agassizii*. The shape and intercept were similar for the 6, 10, and 15 km distance classes with intercepts ranging from 200 to 276 km. Least-cost path distance intercepts, which accounted for natural topographic barriers, ranged from 249 to 308 km. The authors concluded that the risk for outbreeding depression appeared to be low, particularly if desert tortoises are translocated no further than 200 km from their original site, as populations are considered genetically correlated within this distance.

4. Health and disease considerations

    a. Jacobson (1993) reported that relocation, repatriation, and translocation (RRT) programs involving reptiles have become an increasingly popular conservation strategy to mitigate habitat loss or declines of individuals or populations. However, such practices can spread infectious diseases such as herpesvirus and mycoplasmosis in desert tortoises that can, in turn, cause epizootics. The key for reducing the risk of occurrence of an epizootic is a sound preventative medical program, which begins with a 90-day quarantine of all new wild or captive animals. New animals entering the quarantine room should be given a physical examination and monitored throughout the quarantine period, in which anatomical and behavioral abnormalities are noted. Health assessments should be conducted prior to release from the quarantine room. All animals that die in quarantine should be necropsied. Caretakers should take precautions to avoid transferring pathogens.

    b. Wolff and Seal (1993) recognized that when evaluating the infectious disease risks of reintroduced or translocated captive animals into naïve wildlife populations, information on the following is inadequate: (i) incidence, distribution, and risks of disease in both the captive and wild populations, (ii) quarantine systems that will totally prevent disease transmission, and (iii) detection and monitoring systems that will identify disease without error.

    c. Brown and others (2002) wrote of the complexity of relocation and infectious diseases, with *Mycoplasma* as only one issue. Infectious diseases can spread with relocation. Efforts should minimize risk of *Mycoplasma* infections and other infectious agents in animals that are candidates for relocation. Among several recommendations, the authors stated that both donor and recipient populations should be tested and clinically ill animals or animals from populations experiencing unusually high mortality should not be relocated.

    d. Johnson and others (2006) wrote that release of captive desert tortoises should be controlled because a high percentage (86.6% of 179 tortoises) were exposed to *Mycoplasma agassizii*, herpesvirus, or both and could be a source of infection for wild tortoises.

   e. Jacobson and others (2012), in writing of Testudinid herpesvirus 2 and 3 and other herpesviruses in desert tortoises and other chelonians, described an urgent need to develop protocols for testing and monitoring desert tortoises expected to be translocated as a mitigation measure. The authors said that translocated tortoises need to be closely monitored, because this is a time when they are often stressed and consequently are likely to exhibit active infections or shed virus.

   f. Jacobson and others (2014), in a review of research on *Mycoplasma* in tortoises, concluded that translocation as a management tool should include the health status of translocated tortoises and those at the recipient site, as well as long-term monitoring of effects on translocated and recipient populations.

   g. Berry and others (2015a) conducted an epidemiological study of two species of *Mycoplasma, M. agassizii* and *M. testudineum* in desert tortoises, in a 1,183 km^2 study area in the central Mojave Desert, CA. The authors reported a consistently higher prevalence of test positive tortoises with closer distances to human households (centroids of human population census blocks) for both *Mycoplasma* specis. The relationship may be related to release or escape of captive tortoises, because the prevalence of *M. agassizii* in captive tortoises is high. The authors identified several points to consider: (i) because the two *Mycoplasma* species are distributed differently on the landscape, tortoises should be tested for both species; (ii) protocols for testing for infectious diseases should include both species of *Mycoplasma*; (iii) clinical signs may be absent or subtle, thus laboratory tests are essential; (iv) the association between test-positive tortoises and human households is of sufficient importance to develop management strategies to reduce disease transmission, such as signing and fencing boundaries of critical habitats in close proximity to human households and urban areas; (v) when wild tortoises are translocated, the proximity to human households and settlements should be a consideration; and (vi) management of captive populations of chelonian species is essential.

5. Other considerations

   a. Bulova (1994) suggested that success of relocations may be limited by availability of suitable shelters for introduced tortoises; also that provision of burrows may facilitate adjustment of relocated tortoises to a new area.

   b. Rostal and others (1994a) noted that the influence of biased sex ratios on chelonian populations is unknown and therefore it is important to understand the effect on future population dynamics and reproductive success. It is important to know sex ratios of young tortoise sand to use non-lethal techniques for sexing young tortoises.

   c. Andersen and others (2000) said that sites with loamy soils allowing tortoises to dig burrows, with sufficient areas with southern exposure likely to improve thermal balance, and with adequate plant cover should be selected when considering translocation sites.

   d. Heaton and others (2008b) developed a decision support system for determining location of feasible translocation sites. The model elucidated the importance of fencing additional roads to reduce tortoise mortality and increased available translocation areas by 40% within the Western Mojave Recovery Unit.

   e. Physiology. Drake and others (2012a), as part of a translocation program at the National Training Center, Fort Irwin, CA, measured cortisol levels as a stress response to desert tortoises assigned to three treatment groups (resident, translocated, and control). Results of challenge tests of adrenocorticotropic hormone, which controls the release of cortisol, suggested that blood collection using subcarapacial venipuncture on tortoises induces minimal stress in the first 15 minutes of initial handling of the animals. Cortisol was higher for males than females, and values for both varied monthly throughout the activity season and among years. Female cortisol levels were higher in late spring, the time period associated with ovulation, egg production, nesting, and increased activity and movement. Comparisons among treatment groups showed that translocation explained little in terms of cortisol.

6. Responses to translocation: reproduction, survivorship, and mortality ion

   a. Field and others (2007), in a Nevada translocation project, reported a mortality rate of 21.4% in 1997. The authors wrote that the data suggested that drought conditions at the site rather than the translocation itself negatively affected the tortoises. None of the tortoises died during their second season at the site, suggesting that translocation, including previously captive desert tortoises, could be a useful conservation tool. The authors suggested that translocating captive tortoises during drought years might be acceptable because drought likely affects mortality of both translocated and resident tortoises similarly (as evidenced by comparing results with similar nearby projects). However, they cautioned that tortoises of unknown histories probably should be translocated only during non-drought years. If a goal of the translocation project is to increase tortoise densities at the recipient site, then male tortoises should not be given supplemental water to increase the probability of remaining at the release site.

   b. Esque and others (2010a), in a Mojave Desert-wide study of predation on desert tortoises covering a couple of years, stated that the probability of adult tortoise mortality likely caused by predation did not differ between translocated, resident, and control tortoises.

   c. Nussear and others (2012) found that annual survivorship did not differ between resident and captive translocated tortoises and averaged 0.94 over all seasons among five sites in UT and NV.The authors also found no relationship between mortality of translocated tortoises and possible contributing factors of translocation group, sex, day or month of the year released, or the amount of time spent in captivity prior to translocation (ranging from 15 to 2,292 days).

In the first year after translocation, mean reproductive effort for previously captive translocated tortoises was an average of one egg less than resident tortoises but the number of eggs between translocated and resident tortoises did not differ in the second or third years post-translocation. The authors emphasized three issues to be considered when translocating tortoises:

   i. Consider the potential for long distance movements and evaluate the site for potentially risky features, such as roads with heavy traffic, unless the boundaries of unsuitable features are fenced.
   ii. Release tortoises in spring or fall and avoid summer months because animals may fail to find adequate shelter from high temperatures.
   iii. Consider prioritizing adult female tortoises as candidates for translocations given their importance for population demographics.

7. Responses to translocation: movements.

   a. Nussear and others (2012) reported that translocated tortoises moved greater distances in their first year compared to residents, but decreased their movement over time for up to 2–3 years after which they showed increasing site fidelity indicating establishment of home ranges. For tortoises translocated to atypical habitat (Great Basin scrub at the Shivwits and Pakoon sites), movement distances were 3–4 times those observed at sites with typical tortoise habitat (Mojave Desert scrub). Two seasons elapsed before their movements were similar to Nevada residents and their movements generally took the animals to more typical habitat types.

   b. Hinderle and others (2015) evaluated homing and movement patterns of 80 adult desert tortoises among two release groups that were translocated during the Fort Irwin Land Expansion Project, central Mojave Desert, CA. Tortoises were released in three treatment groups (translocated [displaced 2, 5, or 8km from their source location], handling control, or control). The authors reported that 44% of tortoises in the 2-km translocated group returned home; one tortoise in the 5-km group, and no tortoises in the 8-km translocated group homed. They found that translocated tortoises moved at least 1.5 times more than tortoises in control groups, with some individuals moving >10 km following translocation. The authors cautioned that behavioral responses of tortoises need to be considered during translocation efforts to minimize risk to individuals and maximize the success of translocation efforts.

   c. Farnsworth and others (2015) compared home range sizes and use of space in four study groups of tortoises (total n = 308): translocated, resident, control west and control east. The study area was in Ivanpah Valley, CA, and the time was in 2012 and 2013 active seasons. The tortoises were translocated a short distance. During the first active season post-translocation, home range size was greater and space-use intensity was lower for translocated tortoises than for resident and control groups. These patterns were not present in the second season. In both years, there was no difference in home range size or space-use intensity between control and resident groups. Across both years, the number of times a tortoise was found in a burrow was positively related to greater space-use intensity.

E. Head-starting Desert Tortoises, Augmenting Populations

1. Hazard and Morafka (2002) released juvenile and neonate tortoises fitted with radio-transmitters from a hatchery at the National Training Center, Fort Irwin, CA, in October and tracked movements for 34 days, when the tortoises presumably entered hibernation and no activity was observed. Total distance traveled and final linear distances from release sites did not differ between age groups. Neonates moved less frequently and settled into hibernation locations more quickly than juveniles. No known mortality occurred in the first 34 days.
2. Nagy and others (2011) recommended maintaining juvenile tortoises at head-starting facilities until tortoises reached a shell hardness index of 98%, which, in head-started individuals from Fort Irwin and Edwards Air Force Base, CA, occurred at about 11 years of age or 110 mm carapace length. Additionally, pairing knowledge of shell hardness development with data on local predator populations would help managers and biologists make informed decisions when releasing captive-raised tortoises, especially in areas of increased predation risk from Common Ravens. The authors additionally recommended selecting a release site greater than 546 m from the natal head-start enclosures.
3. Nagy and others (2015a) released and monitored 53 juvenile *Gopherus agassizii* aged 2–15 years, that were hatched and head-started inside predator-resistant field enclosures at the National Training Center, Fort Irwin, CA.
    a. Some individuals moved large distances following release but homing itself was undetectable. The lack of homing behaviors was likely due to release distance and selection of release sites out of the line-of-sight of natal enclosures.
    b. The use of small halfway-house enclosures (soft release) had no effect on subsequent movements or survivorship during the first year following release. Survivorship was not affected by distance of release from natal enclosures, which ranged from 546 m to 1.4 km.
    c. After 2 years, most small juveniles had been killed by predators, but survivorship increased with body size and age. Juveniles >100 mm carapace length and 9 years of age when released exhibited high survivorship.
4. Nagy and others (2015b) released six groups of eight head-started juvenile tortoises at Edwards Air Force Base, CA, and reported that:
    a. Juvenile tortoises treated to natural rainfall and supplemental “rain” via irrigation had growth rates 2–16 times greater than juveniles that were raised in natural rainfall only head-start pens.
    b. Some juveniles moved greater distances following release, but homing itself was undetectable. The lack of homing behavior was likely due to release distance and release sites being out of the line-of-sight of natal enclosures. The use of small “halfway house” enclosures for 4 months to accustom juvenile to release sites had no effect on subsequent movements or survivorship during the first year.

   c. Nearly all yearlings that were released (in autumn) were dead within 6 months regardless of whether they had received supplemental water or were released near or far from the head-start pens. In the future the authors suggested that "rain" supplementation (i.e., additional water provided by irrigation) and delayed release from head-start pens be incorporated into the protocol for head-starting, as non-supplemented older juveniles died in the latter part of a 16-month drought and nearly all yearlings were dead within 6 months after release, primarily due to Common Raven predation.

5. Hazard and others (2015) released 16 juvenile tortoises ranging in age from 8 to 9 years from natal enclosures at the Fort Irwin Study Site, CA, to determine effects of release distance on homing. The authors released the juvenile tortoises 500 m away and in various directions from their natal enclosures and tracked them for 3 months following release, monitoring their locations, habitat use, activity, and survival. None of the tortoises exhibited homing behavior, and all appeared to settle into their release area within 2 weeks. Seven tortoises were killed over the 6-week period, apparently by a single Common Raven, and predation risk was higher for tortoises < 125 g. The authors recommended releasing juvenile tortoises once they have attained a larger size, and at distances of at least 500 m from natal enclosures. The authors conclude that homing behaviors may be beneficial in situations where managers want tortoises to show fidelity to an area following release, and that further studies may be needed to determine the effects of raising juvenile tortoises in pens prior to their release.

F. Effects of Providing Supplemental Resources to Semi-wild Tortoises in Pens, Head-started Tortoises and to Tortoises Prior to Translocation

   1. Semi-wild Tortoises in Pens. Ruby and others (1994a), in a study of tortoises at the Desert Tortoise Conservation Center, Las Vegas, NV, reported that a lower proportion of tortoises who received supplemental water and food were active during the day. However, the tortoises remained active above ground for longer than unsupplemented tortoises.

   2. Pre-translocation. Field and others (2007), in a Nevada translocation project, reported that providing supplemental water for captive desert tortoises prior to translocation was correlated with high rates of carapace growth and distant movements by males after release, especially within the first 2 weeks, but did not affect mortality. Although the mortality rate was 21.4% in 1997, data suggested that drought conditions at the site rather than the translocation itself negatively affected the tortoises. None of the tortoises died during their second season at the site, suggesting that translocation, including previously captive desert tortoises, could be a useful conservation tool.

   3. Head-start Program. Nagy and others (2015b), in a head-start program at Edwards Air Force Base, CA, reported that juvenile tortoises that were treated to natural rainfall and supplemental "rain" via irrigation had growth rates 2–16 times greater than juveniles that were raised in natural rainfall only head-start pens.

G. Signs and Speed-limits Along Roads As a Means of Protecting Wild and Free-living Tortoises

Hughson and Darby (2013) tested the effectiveness of temporarily erected warning signs equipped with flashing lights on driver awareness of a potential tortoise on the road in the Mojave National Preserve, CA. There was no noticeable change in driver behavior after erection of warning signs at the entrance to tortoise Desert Wildlife Management Areas. The authors concluded that slowing traffic with lower posted speed limits, increased law enforcement, and improving driver awareness may not reduce impacts to tortoise populations.

H. Fencing and Culverts as a Means of Protecting Wild, Free-living Tortoises and Translocated Tortoises

1. Ruby and others (1994b) described the importance of developing structures that permit tortoises to move safely around and/or under human structures and to maintain some natural movement and gene flow within and among tortoise populations. Solid barriers were the most effective in containing tortoises, and the most effective highway barrier was probably a 1 cm hardware cloth fence. Tortoises could see through it and would continue to walk along it seeking a passageway. Fences should be buried a short distance underground (15 cm) for stability and to discourage exploratory digging by tortoises or other desert animals. Due to behaviors like pushing or putting head and feet through the fence, open fences with spaces wider than a tortoise's head should be avoided. The authors indicated that there is a wide choice of barriers that can be used to restrict or direct the movements of desert tortoises, and that adequately designed fences will likely be effective in guiding tortoises to passageways away from hazards or across highways. Given that major highways are important sources of mortality in tortoises, well-traveled roads in good tortoise habitat should be fenced first. Less-used roads and roads in marginal habitat should be fenced next. Any construction site or any human facility that can attract tortoises, or be lethal to tortoises, should also be fenced.

2. Brooks (1995) sampled mammals and plants inside versus outside the fenced Desert Tortoise Research Natural Area, CA, an area that had been fenced to keep out off-road vehicles and livestock grazing. The author reported that aboveground live annual plant biomass was generally greater inside than outside the fenced plots in the 3 years of the study. A notable exception was the alien grass *Schismus barbatus* that produced greater biomass in the unprotected area. Inside the fence, forb biomass was greater than alien annual grasses during 3 years of the study, but outside the fence, forb biomass was significantly higher than alien grasses only during 1 year. There was greater percent cover of perennial shrubs and more seed biomass inside than outside the fence, as well as a greater diversity and density of rodents in the fenced area, perhaps related with greater seed biomass. These results suggest that cessation of livestock and mechanical destruction of plants by off-road vehicles due to fencing has affected plant cover after only a decade of protection, with a primary benefit being greater biomass production by forbs than alien annual grasses.

3. Brooks and Esque (2002) noted that fencing reduced disturbances such as livestock grazing and off-highway vehicle use that promote dominance of alien plants.
4. Boarman and others (1998) reported that four tortoises entered or passed through storm-drain culverts on 60 occasions along a California State Highway with barrier fences. This suggests that desert tortoise mortality could be reduced using fences and culverts, allowing movement beneath highways and could help to maintain gene flow.
5. von Seckendorff Hoff and Marlow (2002) recommended installation of fences and other barriers along roadways to reduce direct mortalities to tortoises, given the clear negative impact of roads on tortoise populations. The authors also noted that unfenced roads reduced effective habitat conserved for desert tortoises because of the zone of influence from roads.
6. Boarman and Sazaki (2006) said that use of barrier fences and culverts near highways and along secondary roads in desert tortoise habitat would allow movement of tortoises and reduce risk of death from vehicles (see also Berry and others, 2008).
7. Heaton and others (2008b) recommended that roads be fenced to increase area available for translocation (40% within the Western Mojave Recovery Unit).
8. Latch and others (2011) described the short time lag between the emergence of roads as a barrier and detection of genetic effect in tortoises. The authors indicated that roads may become increasingly important in shaping the evolutionary trajectory of desert tortoise populations.
9. Lovich and others (2011a) said that the use of larger concrete box culverts or corrugated steel culverts diameter of 1 m or greater should be considered to lessen the possibility of blockage with sediment and debris that may entrap tortoises with onset of rain. If smaller culverts are used, they should be inspected frequently to prevent blockage or entrapment of tortoises.
10. Berry and others (2014a) studied three different management strategies for tortoises in the western Mojave Desert, CA. The three areas were the fenced and long-term protected Desert Tortoise Research Natural Area (DTRNA), CA, partially and recently fenced critical habitat for the tortoise, and private lands recently acquired for conservation but unfenced (also adjacent). The fenced DTRNA with the longest history of protection and legal exclusion of livestock and vehicles had significantly more live tortoises and lower death rates than the other two areas. Critical habitat had lower tortoise densities and high death rates; here, tortoise abundance was negatively associated with vehicle tracks and positively associated with mammalian predators and debris from firearms. The management area with the least protection that was unfenced with uncontrolled vehicle use, sheep grazing and high trash counts, also had low tortoise densities and high death rates.

I. Limiting Off-highway Vehicle Use

1. Zimmerman and others (1994) reported that burrows are a habitat requirement of singular importance to the thermal ecology of desert tortoises. Accordingly, it is essential to manage environmental variables influencing the integrity of burrows, including soil compaction, particle size, and vegetative cover. Limiting off-highway vehicles use in desert tortoise habitat is clearly warranted. Effective conservation efforts to preserve habitat of desert tortoises will focus upon managing variables associated with integrity of burrows.
2. Brooks (1995) reported that the floral and faunal community structure of the western Mojave Desert can profit from fenced protection from livestock grazing and off-road vehicle use, such as that observed at the Desert Tortoise Research Natural Area, CA, with the primary benefit being greater biomass production by forbs than alien annual grasses.
3. Lovich and Bainbridge (1999), a literature review. Given that desert soils may take ten thousand years to develop and recovery of natural vegetation would probably take centuries, impacts related to off-highway vehicle use present a serious challenge to desert restoration projects in the southern California deserts for three reasons:
    a. The potentially severe impact of off-highway vehicle use in desert ecosystems.
    b. The widespread nature of off-highway vehicle impacts.
    c. The fact that off-highway vehicle areas are often located in or near environmentally sensitive habitats. Areas targeted for restoration should be closed to off-highway vehicle use prior to initiating procedures to ameliorate past damages.
4. Bury and Luckenbach (2002) noted that ways to minimize or remove the impacts of off-road vehicles on desert tortoises should be found, given detrimental impacts to tortoise abundance, burrow density, burrow size, vegetation cover, and tortoise body mass.
5. Belnap (2002) reported that impacts of off-road vehicles, expressed in terms of nitrogenase activity, an indicator of nitrogen fixation, varies in different regions and in different soils as a function of high rock cover (no decline in nitrogenase activity), sand content (nitrogenase activity declined as sand content increased), and fine-textured soils (nitrogenase activity increased as fine-textured soils increased). These results suggest that the impact of off-road vehicles varies in different regions, and different soils and can be used in the development of off-road vehicle management plans.
6. Keith and others (2008) said that an increase in patrols and stricter enforcement of the designated route system would reduce much of risk to desert tortoises from off-highway vehicle activity. This statement was based on a 2002–2004 study of tortoises and their habitat in the Butterbredt Area of Critical Environmental Concern and Red Rock Canyon watershed, CA, and on results showing that 52% of 751 one-ha plots had vehicle tracks, most of which stemmed from unauthorized use.

7. Berry and others (2014a), in a study of three adjacent areas with different management strategies, demonstrated that the protective fence at the Desert Tortoise Research Natural Area, CA, benefited the abundance of tortoises, compared to adjacent lands without protection. The fence excluded off-road vehicles and livestock grazing.

J. Re-wilding Tortoises, Using Tortoise Taxon Substitutes for Restoring Dysfunctional Ecosystems

Hansen and others (2010) presented arguments for using taxon substitutes as a strategy for restoring biodiversity and dysfunctional ecosystems. The authors presented evidence that large and giant tortoises (family Testudinidae) are useful models because many are keystone species. They included *Gopherus agassizii* in their summary tables.

II. Habitat Management for Tortoises: Food, Cover, and Water

A. General Considerations Regarding Degradation of Soil and Vegetation

1. Spotila and others (1994) described important implications for habitat management and restoration of tortoise populations based on experimental research. The subjects were temperature-dependent sex determination and the effects of incubation temperature and water potential of soil on hatching success, hatchling size, and post-hatching growth. To ensure normal sex ratios of desert tortoises and the chances for long-term recovery of tortoise populations, the composition and structure of natural vegetation communities and native soils should be preserved or restored. The presence of perennial vegetation is important for tortoise nesting as it alters the quantity of solar radiation absorbed on the soil surface and the transpiration of water from soil.
2. Okin and others (2001) developed a mathematical model to create a wind-driven model to explain desertification of arid shrublands in southeastern California. The authors recommended that any arid shrubland with wind-erodible soils not already converted to human uses are extremely fragile. Disturbance may preclude future successional processes, resulting in permanent landscape change, and could make recreation and habitation impossible. Where possible, development of land susceptible to wind erosion should be avoided. High risk activities in arid shrublands include off-road vehicle use, military training, grazing, and agriculture. If the land is already under use from high risk activities, then use of natural and correctly positioned wind breaks, cover crops for fallow fields, and planting long-lived native plants before abandoning fields should be encouraged to minimize future wind-driven erosion.
3. Williams and others (2013) developed conceptual models of geomorphic controls on distribution of soil crusts in the Mojave Desert. They recommended that physical disturbance to crusts should be avoided to prevent wind erosion that causes dust emissions and air pollution, as well as loss of nutrient-rich and hydrologically-important surface soils.

B. Quantity and Quality of Tortoise Nutritional Resources

1. Oftedal (2002) noted that decisions on habitat management should take both the quantity and quality of tortoise nutritional resources into account. Particular attention should be paid to factors affecting the distribution and abundance of plants high in potassium excretion potential (PEP) index, water and protein, especially in western area of limited summer rains. It may be particularly important to protect tortoise food resources from livestock grazing in years of high winter rainfall, because high-PEP plants may only be abundant under such conditions. To maintain high quality and quantity of tortoise forage, the following are important actions: reduction in alien *Schismus* spp. and other invading plants that have poor nutritional quality; reduction in impacts of livestock grazing and other anthropogenic uses such as off-road vehicle use, air pollution near urban or industrial areas, and global warming as it affects patterns of rainfall in the desert. (See also Oftedal and Allen, 1996).

2. Henen (2004), based on research of nutrient budgets and reproductive data of nine female tortoises from Goffs, CA, reported that a major component of tortoise's diet, which may be necessary to increase survivorship and reproduction through droughts, was dry vegetation following a dry winter. Livestock compete with desert tortoises for this spring forage. Thus decreasing nutrient supplementation (e.g., water and urea-salt licks) to livestock could benefit tortoises in dry years.

C. Water Supplementation to Enhance Growth of Creosote Bushes

Newingham and others (2012) conducted a field experiment by supplementing water to vegetation at the Nevada National Security Site. Growth of *Larrea tridentata* increased with water additions to mimic increased summer monsoons in 2 dry years but decreased in a wet year. Nitrogen addition only increased branch production in the wet year, though combined treatments of water additions and nitrogen did not enhance growth or reproduction. Substantial branch-level herbivory by mammals was observed, and while nitrogen addition alone had no effect, the addition of water and nitrogen significantly increased branch removal. The authors reported that increased summer monsoons and nitrogen deposition may result in only small growth increases by *Larrea tridentata*, and that any biomass gains are likely to be lost due to extensive mammalian herbivory associated with water and nitrogen additions.

D. Reduction in and Management of Invasive Plants

1. Brooks (1995) reported that the floral and faunal community structure of the western Mojave Desert can profit from fenced protection from livestock grazing and off-road vehicle use, such as that observed at the Desert Tortoise Research Natural Area, CA, with the primary benefit being greater biomass production by forbs than alien annual grasses.

2. Kennedy and others (2001) tested the influence of a deleterious root-colonizing rhizobacterium (*Pseudomonas fluorescens* D7, *P.f.*D7) on seed germination and root growth of downy brome, *Bromus tectorum*, for biological control.

   a. *Pseudomonas Fluorescens* D7 significantly inhibits *B. tectorum* growth. Root lengths of *B. tectorum* were reduced by an average of 97% in agar plate bioassays. Root growth of seven other *Bromus* spp. was also inhibited an average of 87% compared with that of controls in the agar plate bioassay.
   b. Seed germination of *B. tectorum* was also inhibited by >61%.
   c. In soil bioassays, only *B. tectorum* root growth was reduced significantly among 42 plant species. However, *P.f.*D7 stimulated root growth in one dicot (*Brassica napus).*
   d. The authors concluded that *P.f.*D7 may be a promising biocontrol agent that will not harm non-target species.
3. Brooks (2003) concluded that when new projects are evaluated for environmental threats, one important consideration is whether the project would increase the production of nitrogen pollutants, thereby increasing the potential for invasive alien plants to increase and invasion of new alien species to be supported.
4. Salo (2004) reviewed life-history characteristics of *Bromus rubens*, and discussed characteristics that make it susceptible to management practices.
   a. Given that *B. rubens* does not maintain a soil seed bank and exhibits an early and uniform germination, the author suggested that winter droughts can dramatically reduce densities of *B. rubens* and provide opportunities for management of this exotic grass.
   b. The author suggested that management practices should focus efforts on removal of *B. rubens* where the grass would be able to survive and reproduce during droughts.
5. Tracy and others (2006a) reported that invasion of annual grass weeds that preclude access to low-fiber foods could constrain nutrient assimilation in young tortoises, because diets consisting primarily of low-fiber foods yield more energy per unit time than high-fiber diets in young tortoises.
6. Brooks and Berry (2006), in a study of factors affecting dominance of alien annual plants in the western, central and southern Mojave Desert, offered several actions and priorities for preventing invasions of alien annual grasses, reducing risk of fire, reducing potential for tortoise deaths, and reducing damage to habitat:
   a. Biomass of alien annual plants is high in the Mojave Desert and may seldom drop below 50% during years of above-average rainfall, thus, for restoration of habitat or alien plant control, the authors recommended setting a target of <50% alien biomass for alien plant.
   b. Minimize anthropogenic disturbances, particularly density of dirt roads because density of dirt roads affects species richness and biomass of the alien *Erodium cicutarium*.

   c. Monitor areas of high risk to new alien plant invasions, particularly disturbed areas of high productivity in washes and roadsides, in areas of high road density, near urban areas, or near off-highway vehicle areas.
   d. Once alien plants are detected, decision support tools can be used to evaluate potential ecological impacts and to prioritize control measures.

7. Bangle and others (2008) studied *Brassica tournefortii* and stated that control efforts appear to be most efficient early in establishment while plants are still in the rosette stage and can be uprooted without the need for bagging and removal. Managers should initiate surveys soon after rainfall events and focus on early detection around shoreline habitats that may be vectors for spread, including areas experiencing little or no human contact.
8. Abella and others (2009b) noted the importance of periodic surveys to update distributions of exotic species. A successful exotic plant information system is likely to require monitoring for effectiveness. Findings from existing published research on invasibility and controls can be incorporated into management plans and can be adaptive to new invaders or changes in ecosystem invasibility.
9. Barrows and others (2009) studied *Brassica tournefortii* and said that given the increased abundance of *Brassica* in wetter years, control efforts applied during wetter years may be an appropriate management direction. However, less than complete control may have little impact on future *Brassica* abundance.
10. Marushia and others (2010) conducted field experiments in the Colorado Desert, CA, to test effectiveness of the herbicide glyphosate on reducing cover of exotic annual species (*Brassica tournefortii*, *Erodium cicutarium*, and *Schismus* spp.).
    a. The authors found that a single year of treatment was inadequate to cause reductions in exotic annuals and highlighted the need to perform multiple year studies.
    b. Early glyphosate application did not affect native cover but did reduce exotic cover, whereas late treatment with the herbicide negatively impacted both exotics and natives.
    c. The authors noted that herbicide treatments did not completely remove exotics from the system and suggested that hand-weeding in combination with early herbicide treatment can be effective.
    d. Results indicate that the rapid phenology of exotic annuals may be exploited to control exotics while minimizing impacts on native plants in desert communities.
11. Steers and Allen (2010) conducted field experiments in San Bernardino County, CA, using herbicides to control non-native annual plants. The raking treatment performed poorly but treatments utilizing Fusilade II, a grass-specific herbicide, nearly eliminated the invasive grasses *Schismus* spp. and the invasive forb *Erodium cicutarium*, achieved native annual dominance, and increased native perennial abundance. These results indicate that in the absence of invasive grasses and forbs, the native annual community can be resilient to fire disturbance and native perennials can recover.

12. Craig and others (2010) stated that roadside surveys alone may be inadequate to detect exotic species, some of which may be dispersed independently of roads or may exploit natural disturbances farther from roads.

13. Steers and others (2011) conducted experimental additions of carbon to 12 plots located in burned and unburned creosote brush scrub within Big Morongo Canyon Preserve, CA, and showed, following rain events that soil microbial biomass increased greatly in the high carbon treatment (100:1 soil carbon to nitrogen ratio). Exotic and native species did not differ in traits associated with nitrogen use, and carbon amendments were capable of decreasing plant density, cover, and biomass of both native and invasive species. Early-germinating species, regardless of whether they were native or exotic, were the most impacted by decreased soil nitrogen resulting from carbon amendments. Because invasive annuals typically germinate earlier and exhibit a rapid phenology compared to most natives, these species are expected to be more competitive than native annuals, yet more susceptible to early-season carbon amendments. However, desert annual communities can exhibit high inter-annual variability in species composition and abundance so that the relative abundance of native and invasive species at the time of application is critical to the success of carbon amendments.

14. Abella and others (2011b) conducted a competition experiment in a greenhouse with native and non-native species. Native species most competitive with the non-native *Bromus rubens* included the annuals Esteve's pincushion (*Chaenactis stevioides*), western fiddleneck (*Amsinckia tessellata*), cleftleaf wildheliotrope (*Phacelia crenulata*), and desertbells (*Phacelia campanularia*), which reduced *Bromus* biomass by 50 to 78%, and the perennials eastern Mojave buckwheat (*Eriogonum fasciculatum*), sweetbush (*Bebbia juncea*), and brittlebush (*Encelia farinosa*), which reduced *Bromus* biomass to 49 to 70% of its grown-alone amount. There was no clear difference in competitive abilities with *B. rubens* between annual and perennial natives, and competiveness was not strongly correlated with the biomass of the native species. *Bromus* exhibited greater amounts of biomass when grown with *Baileya multiradiata, Pleuraphis rigida*, and *Stephanomeria pauciflora* than when grown alone. In the field, sweetbush and brittlebush supported the least cover of *B. rubens*, suggesting strong, early competitive abilities of these species. In contrast, *B. rubens* attained its highest average cover (19%) below little leaf ratany (*Krameria erecta*). This was significantly greater than all but 3 of the 16 species evaluated. Results suggest that interactions with *B. rubens* differ substantially among native species, and that there is potential for identifying native species that can reduce invasion of desert ecosystems.

15. Abella and others (2012a) experimentally compared five types of plant communities and 12 native plants species to determine which best resisted invasion by the exotic *Bromus rubens* or *Schismus* spp. and found that an early forb community best resisted invasion. The early forb community reduced exotic species biomass by 88% (nitrogen added) and 97% (no nitrogen added) relative to controls (no native plants). Of the 12 native species monocultures, *Sphaeralcea ambigua* (desert globemallow), an early successional forb, was the least invasible.

This species reduced exotic biomass by 91%, though the least-invaded vegetation types did not reduce soil nitrogen or phosphorus relative to other vegetation types, nor was native plant cover linked to invasibility. This suggested that other traits influenced interactions between native and exotic species. This study provided experimental field evidence that early-successional native vegetation types exist that may reduce exotic grass establishment in the Mojave Desert.

16. Brooks (2012) stated that control of *B. rubens* may be equally warranted after one, two, or three fires, but active revegetation of native perennial plants is most warranted following multiple fires.

17. Suazo and others (2012) noted that the construction of solar energy facilities could create habitat conditions suitable for invasions through removal of vegetation and soil disturbance. Given the observed responses by *Schismus* to water manipulations, invasibility could be further enhanced by changes in precipitation patterns.

18. Jurand and Abella (2013) evaluated 12 sites ranging from 5 to 31 years post- fire at Red Rock Canyon National Conservation Area, NV. Density of *Bromus rubens* in the soil seed bank was spatially variable and not strongly related with time since fire. The soil seed bank density of *B. rubens* also did not differ between burned and unburned areas under shrubs, but was greater in burned than unburned interspace areas at some fire sites. The authors suggest that *B. rubens* plant and seed bank densities can be greatly reduced immediately after fire, suggesting that a window of time may be available to limit *B. rubens* colonization.

19. Jurand and others (2013) conducted field experiments in Red Rock Canyon National Conservation Area, NV, on viability of *Bromus rubens* seeds related to burial depth. Viability of seeds was significantly reduced with greater burial depth and greater time since burial. A relatively small proportion of seeds remained viable for 6 months and declined further to low levels at 12, 18, and 24 months. These results suggested that while the *B. rubens* seed bank can be large, it is relatively short-lived. However, a small proportion of seeds will remain viable over time. The population crashes may represent windows of opportunity for managers to take advantage of reduced *Bromus* seed densities to apply control efforts such as herbicides specifically targeted to exotic annual grass.

20. Berry and others (2014b) described how the non-native invasive species, *Brassica tournefortii* invaded and became established in the eastern Colorado Desert, CA, and the topography (major and small ephemeral streams or “washes” in valleys) and ages of geological deposits and soils are important predictors of sites for invasion. Because this non-native species is most likely to invade and colonize areas through roads and active major and minor ephemeral stream channels, especially where these features are numerous on the landscape or intersect highways and roads, it is important to monitor for it in these places. The species favors disturbed places (roads, washes) and young soils and geological deposits, common in valleys and alluvial fans.

E. Protection of Habitat by Early Monitoring and Prevention of Exotic Species

1. Brooks (1999b) said that monitoring to detect the invasion of new annual alien plants should focus on regions of high rainfall, areas of high soil nutrients such as nitrogen deposition, and on washes and beneath canopy microhabitats.
2. Brooks and Esque (2002) evaluated the literature on alien annual plants, fire, and effects on tortoises. They concluded that management of alien plants and fire should be closely integrated, because alien plants can create fuel conditions that promote fire in otherwise fire resistant landscapes that predominate in the Mojave and Colorado Deserts.
    a. Minimizing the number of paved and dirt roads and maintaining nonroaded wilderness areas may reduce the dominance of aliens.
    b. Efforts to monitor the arrival of new species and eradicate them should be focused along roads and washes.
3. Jennings (2002) noted that tortoise diets in the western Mojave Desert, CA, were specialized on 10 relatively rare species of annuals and herbaceous perennials. This indicates that loss or diminished abundance of preferred plants from proliferation of exotic annual plant species such as *Schismus* spp. or *Bromus* spp. could have deleterious consequences for tortoise health.
4. Henen (2004), drawing on information from nutrient budgets and reproductive data of nine female tortoises from Goffs, CA, reported that dry vegetation following a dry winter was a major component of tortoise's diet and may be necessary to increase survivorship and reproduction through droughts. Livestock compete with desert tortoises for this spring forage. Thus managers could decrease nutrient supplementation (e.g., water and urea-salt licks) of livestock, which in turn, may contribute to competition between livestock and tortoises for forage in dry years.
5. Abella and others (2009a) described potential changes and additions to the Weed Sentry Program used in Clark County, NV: (a) include common exotics, such as *Bromus rubens* and *Schismus* spp., and add coarse measures of abundance; (b) add potential sites for incipient populations, e.g., washes and animal trails; (c) repeat the surveys to watch for new invaders; (d) establish a network of permanent monitoring plots; and (e) conduct follow-up monitoring of treated areas. This program could be expanded to other areas and regions of the deserts. In addition, new information is needed about many of the new exotics (seed bank formation, seed dispersal, propagule pressure, effectiveness of control measures, etc. A successful exotic plant information system will need to incorporate effectiveness monitoring, generate new or synthesize existing published research on invisibility and treatments, and be adaptive to new invaders or to changes in ecosystem invasibility.
6. See Berry and others (2014b), II.D.20, this chapter.

F. Protection of Habitat by Signing and Fencing or Other Measures

1. Brooks (1995) sampled inside versus outside the fenced the Desert Tortoise Research Natural Area, CA. Sheep grazing and off-road vehicle activity had been excluded by fencing within the Natural Area for years. Brooks reported that aboveground live annual plant biomass was generally greater inside than outside the fenced plots in the 3 years of the study. A notable exception was the alien grass *Schismus barbatus* that produced greater biomass in the unprotected area. Inside the fence, forb biomass was greater than alien annual grasses, but outside the fence, forb biomass was significantly higher than alien grasses only during 1 year. There was greater percent cover of perennial shrubs and more seed biomass inside than outside the fence, as well as a greater diversity and density of rodents in the fenced area, perhaps related with greater seed biomass. These results suggest that exclusion of livestock grazing and mechanical destruction of plants by off-road vehicles by the protective fence has positively affected vegetation, with a primary benefit being greater biomass production by forbs than alien annual grasses.

2 Abella (2008) noted that perennial grasses, and other preferred forage species, likely require protection from burro grazing in burro-inhabited areas if revegetation efforts in the Mojave Desert are to be successful.

3. Heaton and others (2008b), using a decision support system developed in a GIS framework to evaluate translocation sites in the West Mojave Recovery Unit, CA, included fencing (e.g., fence along roads to prevent tortoise deaths) in the modeling effort. The addition of fencing opened up an additional 877 square miles (2.59 km^2 sections) as suitable for translocation. Thus, inclusion of fencing in the model increased available translocation areas by 40% in the Western Mojave Recovery Unit.

4. Keith and others (2008) noted that if land mangers wish to protect tortoises and tortoise habitat, they can use fencing to restrict or eliminate livestock from specific areas within the Jawbone-Butterbredt Area of Critical Environmental Concern, CA, such as the Kiavah Apron where relatively high densities of desert tortoises occur. Such actions may hasten the recovery of perennial shrubs and annual plants and result in more forage for tortoises, as well as protection from predators, and fewer trampled cover sites and tortoises.

5. Hughson and Darby (2013) reported on the ineffectiveness of flashing lights and signs and increased law enforcement to protect tortoises from road kills in the Mojave National Preserve, CA.

6. Berry and others (2014a) evaluated three different management strategies for the desert tortoise on adjoining lands in the western Mojave Desert, CA, using 80 one-ha plots in each of three areas: the Desert Tortoise Research Natural Area, desert tortoise critical habitat, and private lands acquired for conservation but not fenced. Four different vegetation associations were identified in the three management areas where the vegetation associations occurred in different proportions. The vegetation associations had from 2 to 11 abundant species (levels of abundance referenced in the article). The fenced Natural Area, with the longest history of protection, had more plots in the diverse vegetation associations and the least

number of plots in the low diversity vegetation association with only two abundant species (creosote bush scrub). In contrast, more plots with low diversity vegetation were on private lands, followed by critical habitat. The long-term degradation and loss of perennial shrubs by grazing and off-highway vehicle use in critical habitat and on private lands probably accounted for significantly more plots in the least diverse creosote bush association.

G. Protection of Habitat by Preventing Wildfires

1. Brooks (1999a) said that sources of ignition from human activities should be minimized, especially where alien annual grasses are abundant and topography is conducive to fire spread.
2. Brooks and Esque (2002) stated that:
    a. Reducing the number of fires started by humans may be accomplished by education and enforcing regulations limiting human activities that cause fires such as camping, target practice with firearms, and vehicle use.
    b. Most large fires and areas of recurrent fires occur within mountainous areas designated as wilderness. The lack of motorized vehicle access in these areas can hinder fire suppression efforts, and managers should consider allowing access by fire crews to control fires.
    c. Prescribed fires early in the season may be used to temporarily reduce the dominance of alien annuals prior to revegetation of native plants. However, if alien annual grasses are abundant, fires should not be used because of the significant chance that the native shrubland will be replaced by alien annual grassland.
3. Brooks and Berry (2006) noted that reducing biomass of alien annual grasses such as *Bromus rubens* would assist in reducing frequency and size of fires.
4. Esque and others (2003) described direct mortality to tortoises from fires and hypothesized that indirect effects might result in changes in composition of diets and loss of cover from vegetation, resulting in an increase in predation and loss of protection from temperature extremes.
5. Vamstad and Rotenberry (2010), after a study of historic burns, stated that the results of the vegetation and rodent surveys showed that fire does not seem to significantly impact diversity. However, if the goal is to manage these habitats for specific “old growth” species like the Joshua tree (*Yucca brevifolia*), California juniper (*Juniperus californica*), blackbrush (*Coleogyne ramosissima*), little pocket mouse (*Perognathus longimembris*) or canyon mouse (*Peromyscus crinitus*), the rates of reestablishment of these species will have to be considered in the context of a shorter fire cycle. Full fire suppression tactics and/or fire prevention measures would be necessary to achieve this type of management.
6. Brooks and Chambers (2011) outlined strategies for preventing or managing cycles of invasive plant/fire regimes, including:
    a. Conducting periodic resource assessments to evaluate the probability of establishment of an altered fire regime.

   b. Developing and understanding ecological thresholds associated with invasion resistance and fire resilience that characterize transitions from desirable to undesirable fire regimes.
   c. Prioritizing management activities that increase resistance to invasion and resilience to fire, including reducing stressors. These stressors include repeated fires, inappropriate livestock grazing, controlling surface disturbances and invasion corridors like roads and trails. It is important to increase efforts to eradicate invasive species, conduct preventative vegetation management, and seed native plant species after fires.

7. Abatzoglou and Kolden (2011) said that projected increases in the frequency and onset of extreme fire danger across deserts of the Western United States, with climate change, are likely to increase mortality rates of native species less adapted to wildfire. This projection increases the importance of proactive reduction of invasive fuel loads.
8. Allen and others (2011) stated that managers may use fire as a tool to control desert invasives without the concern that nitrogen will be irrevocably lost, but this must be done carefully to avoid eliminating shrubs and further increasing invasive species. Using fire as a tool in invaded desert communities is counterproductive, because invasive plants will eventually regain dominance and repeated burning will eliminate woody perennials.
9. Engel and Abella (2011), writing about burned *Coleogyne* habitat, stated that direct intervention, i.e., seeding or planting, may be necessary in *Coleogyne* communities to restore keystone shrub species. Additionally, if *Coleogyne* communities are considered vital habitat, managers may want to consider actions aimed at fire prevention.
10. Lovich and others (2011b) said that it is prudent to vigorously prevent and suppress wildlife fires in tortoise habitat due to known potential for direct mortality of tortoises.
11. Brooks (2012) suggested that the first lines of defense against altered fire regimes in *Larrea tridentata* scrub should include:
    a. Vegetation management strategies that minimize dominance of *Bromus rubens* and other non-native invasive plants that facilitate fire spread.
    b. Law enforcement activities that reduce the frequency of anthropogenic sources of ignitions.
    c. Fire suppression plans that result in quick responses to fires and limit the size of fires.
12. Barrows and Murphy-Mariscal (2012), in a study of how climate change could affect Joshua trees (*Yucca brevifolia*) in Joshua Tree National Park, CA, reported on the potential interaction between increased grass-spread wildfires by *Bromus rubens* and a climate change-related increase in severe wildfire conditions. The authors noted that resource managers may need to focus weed control and restoration efforts within identified climate refugia areas to minimize stressors that could reduce Joshua tree sustainability in this National Park.

13. van Linn III and others (2013) created models for predicting fire risk in the northeastern Mojave Desert landscape, drawing on a study area in the Gold Butte area of eastern Nevada. Their study demonstrates that remote sensing techniques used in combination with field surveys can accurately predict wildfire risk in the Mojave Desert and may be applicable to other arid and semiarid lands.

14. Rao and others (2015) wrote that it would be prudent for land managers to sample peak annual biomass to be prepared for the largest and most ecologically and economically destructive fires.

H. Considerations for Shelter Requirements Associated with Climate Warming

Mack and others (2015) noted that translocations of desert tortoises have become more common and human-mediated migrations of tortoises to higher elevations or more northerly habitats may become an essential recovery tool. The authors reported that tortoises may be limited by suitable cover sites. They suggested that during periods of extreme temperatures, suitable cover sites should contain long tunnels and larger openings and that the ability for locations to sustain such cover sites may rely on terrain and surficial geology, e.g., areas supporting caves in old alluvial fans and conglomerate.

I. Protection of Habitat from Roads and Other Linear Disturbances

1. Lovich and Bainbridge (1999) presented management strategies for minimizing effects of linear corridor construction and routing corridors.

2. Brooks and Esque (2002) noted that reducing the number of paved and dirt roads where alien annual plants often dominate and maintaining non-roaded wilderness areas may reduce the dominance of alien plants.

3. Nafus and others (2013) provided evidence that tortoises, a long-lived species, are susceptible to road presence, even in the Mojave National Preserve, CA, where traffic volume is low. The impact of roads to a species already in decline is a growing concern. The authors noted that their results suggest that preventative measures (such as fencing) against road mortality along roads with as few as 300 vehicles per day may be beneficial at least in preventing initial population declines.

J. Recommendations to Restore Livestock Use Areas

1. Brooks and others (2006), after studying annual and perennial plants at artificial livestock watering sites (piospheres) in the Mojave Desert, CA, recommended:

    a. Targeting active control efforts for alien annual plants in denuded and semi-denuded areas and within 200 m from the edge of water sites associated with livestock use.

    b. Focusing restoration of native perennial plant cover, species richness, and structural diversity primarily on the area 50 m closest to the edge of the denuded areas (livestock watering sites); small shrubs should be primarily used to restore plant structural diversity.

    c. Focusing restoration of native annuals primarily on the area 200 m from watering sites (at the Pilot Knob allotment—distances may vary in other places).

2. Keith and others (2008), in a study of tortoises and anthropogenic uses within the Jawbone-Butterbredt Area of Critical Environmental Concern and Red Rock Canyon watershed, CA, reported indications that livestock use may have significant impacts on tortoises. The authors noted that if managers wish to protect tortoises and habitat, they can use fencing to restrict or eliminate livestock from specific areas, such as the Kiavah Apron, which has higher densities of desert tortoises than elsewhere in the region. Reduction or elimination of grazing in specific areas may hasten the recovery of perennial shrubs and annual plants, resulting in more forage for tortoises, as well as protection from predators, and less trampling of cover sites and tortoises by livestock.
3. Abella (2008) noted that perennial grasses, and other preferred forage species, likely require protection from burro grazing in burro-inhabited areas if revegetation efforts in the Mojave Desert are to be successful.
4. Reisner and others (2013), in synthesizing a model developed to determine conditions favouring *Bromus tectorum* dominance of sagebrush steppe ecosystems, reported that grazing exacerbates *B. tectorum* dominance by adversely impacting key mechanisms mediating resistance to invasion. If the goal is to conserve and restore resistance of the ecosystem, managers should consider maintaining or restoring: (a) high bunchgrass cover and structure characterized by spatially dispersed bunchgrasses and small gaps between them; (b) a diverse assemblage of bunchgrass species and (c) biological soil crusts to limit *B. tectorum* establishment. Passive restoration by reducing cumulative cattle grazing may be one of the most effective means of achieving these goals. The findings raise serious concerns regarding proposals to use cattle grazing to control *B. tectorum* in these systems where remnant bunchgrass communities persist.

K. Remediation of Toxic Mine Wastes

Kim and others (2012) reported that mine wastes and tailings with highly elevated arsenic concentrations remained exposed at a number of former mining sites, as a result of extensive gold and silver mining in the Mojave Desert, southern CA. Their results suggest that:

1. Effective remediation of contaminated mine wastes requires addressing the amounts of potential tailings released and the stored arsenic-enriched sediment in washes and the associated playa concurrently**.** Control of tailings at a mine site without addressing the stored arsenic-enriched sediment in the wash would lead to eventual erosion of these stored sediments and transport to the lower part of the wash and eventually the playa, due to the decreased sediment load at the mine.
2. Removal of tailings from washes and alluvial fans would be more challenging than remediation at mine sites, but the consequences of dealing with these problems separately may lead to unintended release of tailings, ineffective remediation efforts, and longer-term contamination.

L. Recommendations Regarding Trash

1. Walde and others (2007b) suggested that further investigation is warranted into the risks posed by garbage and litter to tortoises and other reptile populations.
2. Berry and others (2008) reported that conservation and management actions that have potential for reducing mortality to tortoises include removal of all old and recent trash in desert tortoise habitat to reduce ingestion by tortoises.

M. Recommendations Regarding Renewable Energy

1. Ennen and others (2012b), in a study of reproductive output for female tortoises at a wind energy site, suggested that the wind energy facility in operation at the site did not adversely affect nesting ecology of tortoises. However, habitat alteration due to construction and maintenance would reduce plant biomass and diversity which in turn could adversely impact tortoise reproduction.
2. Cameron and others (2012) provided several findings:
    a. Based on the integration of solar energy potential and biodiversity spatial data across the Mojave Desert, there was substantial opportunity for planners to reduce impacts of development on areas of higher conservation value. This would reduce trade-offs between converting to a green energy economy and conserving biodiversity. A systematic approach for proactively balancing solar energy production with protection of biodiversity better accounts for, and so can help reduce, trade-offs. It can also provide greater assurances to agencies, developers and conservationists that their respective goals are being met.
    b. Parcelization of private lands creates a challenge for siting large renewable energy projects. One strategy to enhance protection of areas of high conservation value in the Mojave Desert would be to develop policy incentives to develop degraded private lands, including brownfields and areas in former agricultural production that were retired due to salinity or other contamination problems or water limitations.
    c. When evaluating renewable energy projects, it is important to account for cumulative impacts in siting and mitigation decisions, especially in light of the increased stress that climate change will exert on desert ecosystems. Impacts of projects should not only be evaluated comprehensively regarding ecological impacts, but also examined cumulatively in the context of all of the major stressors in the desert, including but not limited to the other proposed energy projects.

III. Natural Recovery and/or Experimental Efforts to Restore Soils and Vegetation

A. General

1. Fleischner (1994) reviewed the literature on livestock grazing in the Western United States, and reported that the prognosis for restoration of damaged rangeland acreage on arid and semiarid lands is poor.

2. Review of the literature. Lovich and Bainbridge (1999) reported that revegetation and restoration work can help mitigate impacts following disturbance. However, restoration is challenging due to naturally slow plant growth and establishment; reduced infiltration of water and reduced soil moisture-holding capacity of the soil following disturbance; and low and unpredictable rainfall. Prior revegetation studies indicated varied success among plant species, with good establishment of *Ambrosia dumosa* and *Larrea tridentata*, but lack of success with other perennials. In one study, two methods of establishment, direct seeding versus transplanting, exhibited widely variable success rates from site to site and according to species, but were not enhanced by irrigation perhaps due to differences in substrate. Revegetation studies along a powerline transmission corridor and a highway showed low, long-term seedling establishment for a variety of species under several irrigation treatments, with low overall germination-establishment rates for transplanted seedlings. More successful revegetation of transplanted native shrubs and grasses was achieved in the northern Mojave Desert when plants were protected from predation and were irrigated.

3. See Webb, Belnap, and Thomas (2009) for a review of the literature; also annotated in Chapter 10, VI.F.1.

4. Abella (2010) noted that when old plant communities are disturbed, the literature suggests that recovery times for species composition is on the order of decades to centuries at a minimum. If the management objective is to maintain old perennial plant communities such as those containing Joshua tree (*Yucca brevifolia*), creosote bush (*Larrea tridentata*), and blackbrush (*Coleogyne ramosissima*), the best strategy is to avoid disturbing these communities in the first place. Strategies could include minimizing unauthorized off-road driving through the desert, limiting unnecessary land clearing, reducing damage by non-native animals such as burros, and actively suppressing and reducing wildfires.

B. Soils

1. Grantz and others (1998a) sampled abandoned desert agricultural land in Antelope Valley, CA, and concluded that discrete artificial wind barriers were effective in reducing wind erosion and fugitive emissions (>75% at 0.2 m) from a variety of surface conditions (hard-to-stabilized) that had resisted revegetation with conventional techniques.

   a. The discrete artificial wind barriers consisted of widely spaced roughness elements (e.g., plastic cones) to mimic shrubs.

   b. Wind fences constructed of ultraviolet-stabilized high density polyethylene and established perpendicular to the prevailing wind, significantly decreased fugitive emissions (greater than 90% at 0.2 m). These wind barrier techniques provided rapid and effective suppression of fugitive emissions of soil-derived particles under conditions that had resisted revegetation strategies.

2. Bolling and Walker (2000) believed that de-compaction of roads, flattening out lateral and control berms, restoration of soil horizons, and inoculation of soils with native microbes and mycorrhizae would increase probability of a more natural community developing following abandonment of unpaved roads. Without restoration efforts, successional trajectories will be permanently altered after disturbance.

3. Belnap and Warren (2002) noted that impacts to soil surfaces from military vehicles in the Mojave Desert are long-lasting, and recovery is expected to require centuries to millennia for biological soil crusts on desert pavement. Lack of resilience to disturbance is probably due to low rainfall and lack of freeze-thaw events. Because many critical ecosystem functions are affected by off-road vehicles (whether tanks or wheels), it is clearly important that these types of activities be restricted as much as possible, and that soil surface impacts be considered in land management decisions.

4. Pietrasiak and others (2011) studied biological soil crusts in Joshua Tree National Park, CA. The authors confirmed that biological soil crusts enhance surface stability even in the drier conditions of the Mojave Desert. In this desert, algae and cyanobacteria are the primary components, whereas lichens and moss are relatively minor components. Geomorphic features with a suite of soil properties clearly impacted crust development, in contrast with individual physical and chemical soil parameters associated with crust development in previous studies. Wash banks showed the best crust development (51–52% total crust cover), likely because fine soils and lack of erosion make ideal habitat for algae and cyanobacteria, and slopes showed the poorest crust development (<37% total crust cover), probably due to the higher runoff and susceptibility to erosion. Lichens and mosses were best developed in the pocket areas (1.1% and 1.5% cover, 25%–30% frequency), which can accumulate and retain moisture during and following precipitation events. Overall, the authors concluded that biological soil crusts of the Mojave Desert are very different in composition, form, and ecology than crusts of other desert regions of North America.

5. Williams and others (2013) developed a model that explained biological soil crust distribution in the Hidden Valley Area of Critical Environmental Concern, NV. The authors demonstrated how geologic and geomorphic processes controlled the ratio of fine sand to rocks and constrained the development of 3 surface cover types: cyanobacteria crusts, moss-lichen pinnacled crusts, and low to moderate density moss-lichen. Their proposed conceptual model can be used to help predict biological soil crust distribution within inter-montane basins and to identify biologically sensitive areas.

    a. Cyanobacteria crusts grew where abundant fine sand and negligible rocks formed saltating sand sheets.
    b. Moss-lichen pinnacled crusts grew on early to late Holocene surfaces composed of mixed rock and fine sand.
    c. Low to moderate density moss-lichen crusts grew on early Holocene and older geomorphic surfaces with high rock cover and little surficial fine sand.
6. Soulard and others (2013) used terrestrial LiDAR (acronym developed from a remote sensing technology that measures distance by illuminating a target with a laser and analyzing the reflected light), to quantify differences in soil topography in burned and unburned areas following 11 years of recovery in the Grand Canyon-Parashant National Monument, AZ. Soil mound volumes, plant heights, and soil-surface roughness were significantly lower on burned relative to unburned plots. These results suggest a linkage between vegetation and soil mounds, either through accretion or erosion mechanisms such as wind and/or water erosion. The biogeomorphic implications of fire-induced changes, including reduced plant cover and altered soil surfaces from fire, likely influence seed residence times, inhibit seed germination and plant establishment, increase erosion, and affect other ecohydrological processes.

C. Vegetation—Active Restoration Efforts

1. Grantz and others (1998b) conducted experimental studies on revegetating former agricultural areas across the western Mojave Desert, CA, using nursery-grown, native perennial plant species (*Atriplex canescens, A. polycarpa, Isomeris arborea, Prosopis glandulosa* var. *torreyana*, and *Chrysothamnus nauseosus*).
    a. There were significant differences in perennial plant performance. For high desert use, the authors recommended *A. canescens*, followed by *P. glandulosa* for revegetation, although *P. glandulosa* was highly susceptible to herbivory.
    b. Plastic cones were better than wire cages for plant vigor and survival, and survival was much greater with narrow augured holes relative to wide, hand-dug holes. However, overall survival remained low and was limited by ambient rainfall.
    c. These results suggest that transplanting without intensive irrigation does not guarantee survival, and the greater cost of using nursery-grown plants, relative to direct seeding, may not be warranted for large-scale restoration of arid and semiarid environments.
2. Sharifi and others (1999) sampled *Larrea tridentata* plants at the National Training Center, Fort Irwin, CA, and reported that irrigated dusted plants experienced rapid shoot growth and had significantly higher predawn shoot water potentials than dusted plants that were not irrigated. Growth was so rapid after irrigation that nearly all dusted leaves had abscised and the irrigated plants were nearly indistinguishable from control plants. All four measures of water-use efficiency were higher in controls than in dusted plants. Results suggested that *Larrea tridentata* shrubs could recover rapidly from acute heavy dust deposition if irrigation, simulating heavy summer rainfall, was provided. These results also

suggested that irrigation may be useful to minimize the ecological impact of dust from roads and other human activities. The authors stated that although summer irrigation improves the appearance of dusted *Larrea tridentata*, the shrubs return to the physiological status of the surrounding community once soil water is depleted. As long as the input of dust is not continual, irrigation programs may be unnecessary because dusted leaves are shed with renewed shoot production, which reliably follows recharge of soil water from seasonal rainfall.

3. Walker and Powell (1999) examined the effect of seeding an abandoned dirt road 2 years post-recovery in the eastern Mojave Desert. They compared road plots that had been seeded to those that remained unseeded and to control plots placed 0.5 m from the road edge in undisturbed desert. Mean total plant cover was higher on desert plots than road plots, and although there was no statistically significant difference between seeded and unseeded plots, the high variability of the desert plots (desert seeded 41.25 ± 7.18 SE, desert unseeded 35.50 ± 9.04 SE) obscured differences between seeded and unseeded plots (road seeded 16.19 ± 2.69 SE, road unseeded 2.23 ± 0.36 SE). Conversely, species richness and plant density were higher on road plots than on desert plots. Species richness was also higher on unseeded plots than on seeded plots. Plant density was higher on unseeded than seeded desert plots and higher on seeded than unseeded road plots, meaning the unseeded road plots were more similar to adjacent desert plots than seeded road plots. On both the seeded and unseeded desert plots, filaree (*Erodium cicutarium*), cheesebush (*Hymenoclea salsola*), and big galletta grass (*Pleuraphis rigida*) were the most abundant species. On the seeded road plots, Palmer's beardstongue (*Penstemon palmeri*) and desert marigold (*Baileya multiradiata*) were most abundant and on the unseeded road plots Mediterranean grass (*Schismus barbatus*), filaree, skeleton weed (*Eriogonum deflexum*), and red brome (*Bromus rubens*) were most abundant. Seeding seemed to have altered the natural colonization process. However, measures of revegetation success can differ and some possible goals could be (a) return disturbed area to pre-disturbance condition by matching the surrounding plant community in cover, species richness, density, composition, and visual appearance; (b) reestablish the vegetation to stabilize the soil without regard to species richness or composition; or (c) exclude dominant non-native species such as filaree, *Schismus*, or red brome.
4. Scoles-Scuilla and DeFalco (2009) examined densities of seeds following soil reclamation at Lake Mead National Recreation Area, NV, and reported that effective seed reserves declined by 79% of the original undisturbed germinable seed reserves. The reason was that the deep, seedless soil fractions had been mixed with the top higher density seed layers during road construction and soil reclamation efforts.
    a. They recorded perennial plant recovery at nearby sites where stored surface soil had been reapplied, ranging from 0.5 to 5 years after application, with <1% cover after 7 years compared to 5% cover in nearby undisturbed areas. The reduced abundance of germinable seed during reclamation was primarily due to dilution of seed reserves when deeper soil fractions without seed were mixed with the surface soil during collection.

   b. When surface soil cannot be collected and re-spread at very shallow depths (<5 cm), off-site seed collection from shrubs and distribution after disturbance may be the most feasible way to return native seeds to the landscape.
   c. They suggested that more precise techniques of surface soil collection should be explored for revegetating disturbed soils with a shallow seed bank, such as those found in the Mojave Desert.

5. DeFalco and others (2009) assessed soil seed densities across varying surface disturbances (compacted, trenched) and adjacent undisturbed control plots at the National Training Center, Fort Irwin, CA. The authors found that litter cover was the best indicator of site degradation and recovery potential in arid lands.
   a. The authors reported greater litter cover comprising the infructescences of the dominant spring annuals and low gravel content enhanced seed densities of both annuals and perennials at trenched sites.
   b. Litter cover and surface ruggedness were the best explanations for viable perennial seed densities on compacted sites, but litter cover and the presence of a common harvester ant explained annual seed densities.
   c. Restoration and rehabilitation of degraded sites may benefit from litter additions to facilitate the development of seed banks.
   d. The authors also noted that harvester ants and other granivores can enhance seed movements and densities, and therefore should be considered in relation to the goals of passive or active restoration.
6. Abella and Newton (2009) reviewed 18 studies that planted (15 studies) or seeded (eight studies) across a range of environments that included abandoned agriculture land, various types of road rights-of-ways, mine spoils, and dry lakes in the Mojave Desert. Post-treatment monitoring ranged from 1 year (33% of studies) to 5 years (22% of studies). For the planting studies, 33 species of shrubs, four grasses, two forb-shrubs, and two cacti were planted with 16 of 41 (39%) species planted in two or more studies and 50% survivorship in at least one treatment of one study. Although 12 treatment types were tested, only three types (irrigation, cages, and tree shelters) were also used in two or more studies. Shelters improved survival twice as much as cages or irrigation. For the seeding studies, 28 species were seeded with only six species in two or more studies. Measures of species performance used among studies were plant density, percent survivorship, and percent cover, although species performance was often qualitatively rather than quantitatively assessed. Only three studies tested treatments and only one treatment (irrigation) was tested in more than one study. In comparing planting and seeding, *Atriplex* spp. and *Ambrosia dumosa* performed well in both planting and seeding while *Larrea tridentata* performed better when planted. No other species were both planted and seeded. Overall, all plant groups except shrubs were poorly represented in these studies.

7. Brooks and Chambers (2011) reported that restoration of land is ecologically challenging and expensive, if the areas have already crossed ecological thresholds to states that are dominated by invasive species. Such lands are lower priority than preventive measures except in special situations due to the magnitude and expense of restoration or rehabilitation. Greater benefit will be obtained by maintaining or increasing invasive resistance and fire resilience of areas that have not yet crossed ecological thresholds.
8. Engel and Abella (2011) sampled 32 burn sites in the eastern Mojave Desert, for species richness, diversity and composition of perennial species. The 32 sites exhibited different post-burn recovery patterns between community types. Diversity in *Coleogyne ramosissima* communities was greater in burned than unburned areas. In contrast, diversity did not differ in *Larrea tridentata* communities. Trajectories of species composition in *Larrea tridentata* communities indicated convergence with unburned community composition after 19 years post-fire whereas *Coleogyne ramosissima* communities lacked convergence for > 29 years sampled since fire. These results suggest that the persistence of fire effects varied depending on the community vegetation type and upon community components.
9. DeFalco and others (2012) conducted treatments to revegetate degraded shrublands at the National Training Center, Fort Irwin, CA, and demonstrated that revegetation of disturbed desert habitat shrublands through supplementation of seed banks was more complex than previously thought.
    a. They showed that a large proportion of broadcast seeds were missing after just 16 weeks (63–82%) and overall seedling emergence was less than 3%.
    b. The harrowing vs. tackifier application experiments revealed significantly different results. Harrowing of compacted sites was associated with decreased seed movements and increased seedling establishment, especially for early-colonizing *Encelia farinosa*, but tackifier was largely ineffective.
    c. Surface roughness of trenched sites retained three times the number of seeds than compacted sites, but soil mixing during trench development likely resulted in poor seedling emergence.
    d. They also found that seeds left on the soil surface were vulnerable to seed predation. The prevalence of harvester ants increased seed removal on compacted sites, whereas rodent activity influenced removal on trenched sites.

10. Abella and others (2012a) reported that an early forb community best resisted invasion, compared with an experimentally assembled early successional grass and shrub and late-successional shrub community in the Lake Mead National Recreation Area, NV. The early forb community reduced exotic species biomass by 88% (nitrogen added) and 97% (no nitrogen added) relative to controls (no native plants). In native species monocultures, *Sphaeralcea ambigua* (desert globemallow), an early successional forb, was the least invasible. This species reduced exotic biomass by 91% though the least-invaded vegetation types did not reduce soil nitrogen or phosphorus relative to other vegetation types, nor was native plant cover linked to invasibility. This study provides experimental field evidence that early-successional native vegetation types exist that may reduce exotic grass establishment in the Mojave Desert.
11. Brooks (2012) reported that post-fire management of creosote bush scrub may include *Bromus rubens* control and perennial plant revegetation. Control of *B. rubens* may be equally warranted after one, two, or three fires, but revegetation of native perennial plants is most warranted following multiple fires. Decisions to actively control invasive species or to revegetate perennial species should not just consider the specific short-term effects on the targeted species, but should more importantly consider the ultimate multi-trophic and ecosystem level effects of these management actions.
12. Suazo and others (2013) studied seed removal patterns in burned and unburned desert habitats south of Las Vegas, NV, and said that selective removal of *Coleogyne ramosissima* seeds by granivores might influence restoration efforts by limiting ecosystem processes (e.g., creation of fertile soils) that affect long–term plant population dynamics. Protecting seeds from granivores can increase seedling establishment and on–site seed retention. Methods to protects seeds could include (a) selecting species for seeding based on seed characteristics that are not preferred by seed consumers (e.g. small–seeded species), (b) using ‘decoy’ seed with high preference value might alleviate granivore pressure on target species, and seeding in the Mojave Desert in late October or early November to avoid high levels of seasonal foraging by granivores.
13. Perennial Plant Recovery. Berry and others (2016) studied natural recovery of 21 perennial plant species 36 years after construction in a 97-m wide aqueduct corridor in a *Larrea tridentata-Ambrosia dumosa* plant alliance in the western Mojave Desert, CA. The only attempt at speeding recovery was disking this part of the corridor shortly after construction to reduce compaction. The authors measured plants at 0 m (verge of the central road), at 20 m and 40 m into the disturbance, and at a control, beyond the disturbance. The total number of shrubs per transect did not vary in the four locations but canopy cover of shrubs, species richness, and species diversity were higher in the control than at the disturbed locations.

The canopy cover of common shrubs varied significantly by location. Patterns of recovery were bidirectional: secondary succession from the control into the disturbance corridor and inhibition from the road edge into the disturbance. Time estimated for species composition to resemble the control is dependent on location within the disturbance corridor and could be centuries at the edge of the central road. Rabbitbrush (*Ericameria nauseosa*) appeared to be inhibiting recovery in the central area, along the road.

14. Recovery of Annual Plants. Berry and others (2015b) studied natural recovery of native and non-native annual plant species 36 years after construction in a 97-m wide aqueduct corridor in a *Larrea tridentata-Ambrosia dumosa* plant alliance in the western Mojave Desert, CA. The only attempt at speeding recovery was disking this part of the corridor shortly after construction to reduce compaction. The authors measured plants at 0 m (verge of the central road), at 20 m and 40 m into the disturbance, and at a control, beyond the disturbance and identified 41 species of natives and six exotic species. Native annual plants significantly increased in richness from road verge to undisturbed vegetation (control), but not in density, biomass or cover. In contrast, exotic annual plants increased in density, biomass, cover and richness with increasing distance from the central part of the disturbance corridor, the road edge. The species of colonizing shrubs and type of canopy cover affected density, biomass, and richness of annuals. Species composition of native annuals differed significantly by distance, suggesting secondary succession. Recovery estimates were in centuries and dependent on location, canopy type and whether considering all annuals or natives only.
15. Abella and others (2015a) tested experimental techniques (rooting hormone, slurry, and soaking treatments) for enhancing survival of salvaged perennial plants and their establishment on severely disturbed sites at Lake Mead National Recreation Area, NV. These treatments were ineffective; survival of salvaged plants after 1 year of nursery care was 48% (1,017 of 2,105 plants). Of these survivors, 50% survived 27 months after transplanting to field restoration sites. On restoration sites, irrigation increased transplant survival by 50% (DRiWATER, a slow-release gel) and 79% (hand watering) compared to no irrigation (35% survival). Providing salvaged topsoil as a growth medium, without irrigation, doubled survival, nearly equivalent to irrigating plants. Survival varied by an order of magnitude across 23 species.
16. Abella and others (2015b), in a study designed to improve forage quality and augment cover of perennial plants, tested seeding (pelletized or bare seeding), watering, and fencing for increasing a native annual forage species (*Plantago ovata*), a perennial forage species (*Sphaeralcea ambigua*), and two shrub species (*Hymenoclea salsola* and *Krascheninnikovia lanata*) that provide cover. Treatments were ineffective in establishing the perennial species but pelletized seeding quadrupled the density of desert plantain compared with not seeding or seeding with untreated seed. Fencing tripled density of plaint plants. Pelletized seeding plus fencing produced the highest average density of plantain plants among the treatment groups. Augmenting native annual forage plants favored by desert tortoises is feasible.

D. Vegetation—Natural Recovery after Disturbance

1. Recovery of Soils and Vegetation after World War II Activities and in Ghost Towns. Webb (2002) sampled 19 abandoned World War II sites and ghost towns across the Mojave Desert and evaluated recovery of severely disturbed desert soils and vegetation, as measured with penetration depth and based on the logarithmic model. Time for 85% recovery ranged from 105 to 124 years. The highest rate of recovery occurred within the first few decades after abandonment. Using bulk density to estimate recovery, neither the linear nor logarithmic models performed better, thus recovery for both models required 92 to 124 years. Although compaction at these sites was caused by a wide variety of forces, ranging from human trampling to military tank traffic, the data did not allow segregation of differences in recovery rates for different compaction forces. Recovery of soil compaction was significantly related to elevation, and in turn related with wetting-and-drying and freeze-thaw cycles that increase with elevation. Some higher elevation sites completely recovered from soil compaction after 70 years, substantially less time than for lower elevation sites.

2. Ghost towns, pipelines, off-road vehicle disturbances. See Webb, Belnap, and Thomas (2009) for annotations in Chapter 10, VI.F.1. and for the original book chapter for a review of the literature.

3. Abella (2010) conducted a comprehensive review of 29 individual studies of vegetation recovery across the Mojave and Sonoran Deserts. The author reported that the time estimated for full reestablishment of total perennial plant cover after disturbance, such as fire, road building, and land clearing, was 76 years. This time was substantially shorter than an estimated 215 years (among 31 individual studies) required for the recovery of species composition typical of undisturbed areas, assuming that recovery remained linear following the longest time since disturbance measurement. Overall, it appeared that recovery after fire versus disturbances involving land clearing (i.e., abandoned roads and towns, powerline corridors) did differ, with perennial cover generally rebounding faster after fire compared to other disturbances. Fire was the disturbance type in 31% of the Mojave studies and 64% of the Sonoran studies.

IV. Recovery Actions: Potential Applications and Management

A. Bury and Corn (1995) stated that before the tortoise was federally listed as threatened, the scenario of declining, fragmented populations of desert tortoises provided the rationale for management decisions that may not have been beneficial to tortoises. For example, areas of low tortoise density received little management and minimal mitigation of activities harmful to tortoises, a policy that could have promoted the fragmentation of the distribution of the tortoise. Tortoises now have a high level of protection, and the proposed large management areas defined as critical habitat, if implemented, should be adequate for continued survival of the species

B. Leuteritz and Ekbia (2008), using stakeholder surveys and literature reviews pertaining to tortoises in Madagascar, South Africa, and in the U.S., the Mojave Desert, discussed the role of biological diversity in enhancing resilience and suggested that social parameters were the dominant drivers of change in the overall social-ecological system.

    1. They noted that even though the U.S. is an advanced country with high gross domestic product (GDP) and use of modern ecosystem management practices for tortoise conservation, the threat upon tortoises was highest and resilience was lowest among the three Nations.
    2. They suggested that present management practices were not the best way to achieve resilience and recommended maintenance of a continuous dialogue among various stakeholders such as private land-owners, off-highway vehicle users, the military, and land-management agencies.

C. Averill-Murray and others (2012) offered recommendations for recovery actions including:

    1. Identification of threats most responsible for site-specific population declines and implement effective management actions that address those threats at locally relevant scales.
    2. Determine the effectiveness of management actions based on demonstrable increases in tortoise populations, but at least through surrogate indicators.
    3. Coordinate research and management to ensure that questions about management actions are answered, and that multiple-use land-management agencies maintain conservation of tortoises at regional scales where potential impacts occur at local scales.
    4. Implement a coordinated recovery program in which actions, management-related research, and monitoring are coordinated around making decisions based on objectives, potential actions and their consequences implemented within a decision-support system.
    5. Actions that include members directly able to implement these actions towards recovery goals across jurisdictional boundaries.

D. Averill-Murray and others (2013) suggested that their results from habitat suitability modeling provided an initial framework to develop a conservation linkage network for desert tortoises.

    1. Suitable habitat within Tortoise Conservation Areas (TCAs; 45,340 km^2, described in the 2011 Recovery Plan for *Gopherus agassizii*) and Department of Defense administered lands contain 68% of total current habitat and 55% of total historic habitat.
    2. Suitable habitat within linkages connecting the TCAs in the most restrictive (“base” model) contained 17,831 km^2 of additional habitat (27% of current, 21% of historic), while the less restrictive “binned” model, which assumed lower resistance to tortoise occupancy, resulted in broader linkages between TCAs. Suitable habitat within linkages connecting TCAs in a “binned” model that assumed lower resistance to tortoise occupancy resulted in broader linkages between TCAs.

3. Of 17,831 km^2 suitable habitat contained within modeled linkages connecting the TCAs, up to 18.3% of the area is impacted by existing high-intensity human activities that include military operations and off-highway vehicle recreation. Only portions of some linkages (17%) are protected by designations, specifically wilderness or U.S. Bureau of Land Management National Conservation Areas.

E. Darst and others (2013) assessed importance of threats and risks to tortoise populations.

1. The five most important threats to the desert tortoise range-wide were urbanization (direct contribution: 3.1%, indirect contribution: 19.7%), human access (2.4, 15.8%), military operations (2.8, 7.0%), disease (8.6, 0%), and illegal use of off-highway vehicles (2.6, 4.9%). Indirect effects were 2.5 to 6.6 times greater than direct effects.

2. The three most important stresses to desert tortoises and their habitats were habitat loss (18.4% total contribution of risk to populations), predation (13.9%), and crushing (13.3%), which accounted for 45.6% of the total risk to the population.

3. The five recovery actions ranked for the highest effectiveness were habitat restoration (9.4% decrease in population risk), topic-specific environmental education (5.9%), land acquisition (5.5%), installing/maintaining tortoise-exclusion fencing (5.3%), and reducing predator access to human subsidies (3.6%), which were robust to changes in modeling assumptions.

4. Of the top 10 recovery actions only “signing and fencing of protected areas” dropped out of the top 10 in over 30% of the model variations, and “removing grazing” was elevated from its 11th ranked position to the 10th position for 30% of the variations, suggesting land managers could be confident in investing their resources towards these recovery actions. Lastly, the authors repeated and discussed the assumptions of the model, which included: (a) a linear and complete model (i.e., all threats and their relationships among each identified, and how the stresses contributed to population effect and that to population change), (b) no time lag, (c) that experts are experts range-wide, and (d) that the species could be recovered by incremental reduction in stresses.

F. In a review of published literature on turtles from 1980 to 2009, Lovich and Ennen (2013b) discovered that desert tortoises (including the Sonoran desert tortoise) were among the best studied species of turtles in North America with exponential increases in citations.

1. They suggested that this increase in publication was, in part, due to the threatened status of *G. agassizii* and its large geographic range.

2. However, the authors questioned whether the increase in knowledge has been translated into effective action for species recovery, and provided suggestions to improve agency effectiveness in applying scientific knowledge including: interagency cooperation, strategic planning, and finding ways for scientists to participate in policy development and implementation.

Chapter 13.—Literature Cited

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Publishing support provided by the U.S. Geological Survey
Science Publishing Network, Tacoma Publishing Service Center

For more information concerning the research in this report, contact the
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Berry and others—**Desert Tortoise Annotated Bibliography, 1991–2015**—Open-File Report 2016–1023

ISSN 2331-1258 (online)
http://dx.doi.org/10.3133/ofr20161023