Chelonian Conservation and Biology, 2002, 4(2):366–379

A Landscape Sampling Protocol for Estimating Distribution and Density Patterns of Desert Tortoises at Multiple Spatial Scales

Anthony J. Krzysik[1,2]

[1]*U.S. Army ERDC-CERL, P.O. Box 9005, Champaign, Illinois 61826 USA;*
[2]*Present Address: Ecological Research Institute, Prescott College, 220 Grove Avenue, Prescott, Arizona 86301 USA*
[E-mail: krzysika@cableone.net]

Abstract. – A sampling protocol was pilot-tested to estimate distribution and density patterns of desert tortoises (*Gopherus agassizii*) at multiple spatial scales. The value and uniqueness of the protocol is that it provides land managers with information on local small scale distribution and density patterns of tortoises, while concurrently monitoring long-term temporal density trends on landscape scales. The design is statistically rigorous and unbiased, and is valid at any population density or distribution in the landscape. The protocol is based on the integration of four design elements: defining the sampling universe(s), designing a landscape sampling frame, selecting a method for density estimation (distance sampling), and applying spatial modeling to develop a landscape distribution-density surface for the desert tortoise population of interest. Distance sampling is used to directly estimate tortoise density on a landscape scale. Small scale tortoise densities in this landscape are developed as a tortoise density surface by using unbiased estimates of burrow and scat densities at decreasing sampling scales to calibrate the overall tortoise density to local scales. The pilot study was conducted in the southcentral Mojave Desert at a lightly-used military training area and in a designated wilderness area in Joshua Tree National Park. Although tortoise density patterns were similar at the two sites, burrow/tortoise ratios, and other related parameters differed. Estimated tortoise densities were scale dependent and more variable at smaller spatial scales, indicating that tortoises were patchy in landscape distribution.

Key Words. –Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; density estimation; population monitoring; distance sampling; sampling protocol; distribution patterns; landscape scales; tortoise sign; management; Mojave Desert; USA

The purpose of this paper is to introduce a landscape scale sampling protocol for the desert tortoise (*Gopherus agassizii*) based on the integration of four individual design elements: defining the sampling universe(s), designing a landscape sampling frame, selecting a method for density estimation (distance sampling), and applying spatial modeling to develop a landscape distribution-density surface for the desert tortoise population of interest. The value and uniqueness of the protocol is that it provides land managers with information on local small scale distribution and density patterns of tortoises, while concurrently monitoring long-term temporal density trends on landscape scales. I also used the data generated by the sampling protocol to assess the spatial patchiness or landscape variability in the densities of two desert tortoise populations.

Population densities of animals are notoriously difficult to estimate (Seber, 1982). Indeed, statistically sufficient and unbiased sampling represents a major challenge for field biologists, despite good theoretical foundations (Cochran, 1977; Thompson, 1992) and practical guidance (Green, 1979; Hayek and Buzas, 1997). Mark-recapture techniques have typically been used and their theoretical foundations (Seber, 1982; Skalski and Robson, 1992) and applications are well established (White et al., 1982; Thompson et al., 1998; Young and Young, 1998; Krebs, 1999; Williams et al., 2002). Mark-recapture techniques provide accurate population numbers, but there is the persistent and significant difficulty of calculating "effective trapping area" or "area of influence," necessary to estimate population densities (number/area) (Seber, 1982). Even carefully designed mark-recapture studies have avoided estimating desert tortoise densities, because of the problems of calculating effective trapping area (Freilich et al., 2000). Mark-recapture studies are very labor and time intensive, and population estimates on local patches cannot be reliably extrapolated across large landscapes. Therefore, they are neither practical nor economical for estimating population densities of target organisms on landscape scales of hundreds to thousands of km^2.

The desert tortoise has undergone population declines from a large number of cumulative impacts including: Upper Respiratory Tract Disease and possibly other diseases; habitat loss and degradation from development, off-road vehicles, livestock grazing, and exotic plant invasions; highway and predator mortality (especially ravens on hatchlings); and direct human-induced mortality (casual shooting and collecting for pets and food) (U.S. Fish and Wildlife Service, 1994a). Populations of the desert tortoise west and north of the Colorado River were listed as federally threatened (USFWS, 1990), and whose recovery requires reliable and economic population distribution and density estimates to monitor temporal trends (USFWS, 1994a, b, c). The recovery plan recommended the Zippin maximum likelihood method (USFWS, 1994a). The Zippin method is a removal (marking) technique related to mark-recapture methods (Zippin, 1956, 1958). When using the Zippin method a large proportion of the population must be marked in order to obtain reliable density estimates. This method was ineffective in estimating desert tortoise densities, because adequate sample sizes could not be obtained when tortoise densities were low (USFWS, 1998).

Desert tortoise population densities are particularly difficult to estimate for a number of important reasons. All of the following pose challenging field sampling and statistical analysis problems. Desert tortoise populations are distributed throughout the Mojave and Sonoran deserts. Therefore, sampling as part of recovery efforts must occur on extensive landscape scales (USFWS, 1994a; Berry, 1997). Desert tortoises were probably never common in most of their range (Bury and Corn, 1995), and populations have declined, some dramatically, since the 1970s (USFWS, 1994a; Berry and Medica, 1995). Rare animals are not only more challenging to find, but result in smaller sample sizes for statistical analysis and modeling. Although tortoises prefer creosote-bursage scrub on gentle bajadas, the species can be found at lower densities in a wide variety of habitats at elevations from below sea level to over 2200 m (references in Hohman et al., 1980; Grover and DeFalco, 1995). "There is no 'typical' tortoise population because of the great variation among local populations" (Luckenbach, 1982). Tortoise distribution patterns exhibit high spatial variability at both local and landscape scales. In other words, tortoise individuals are aggregated and occur in patches or clumps. This is widely acknowledged and appreciated by tortoise field surveyors and researchers, and is specifically addressed in this paper and in Duda et al. (2002). Desert tortoises live in burrows, exhibiting a high degree of variability in surface activity both within and between days and years (Bulova, 1994; Zimmerman et al., 1994; Duda et al., 1999). Tortoises may spend over 95% of their lives in burrows, making sampling observations highly opportunistic. Nagy and Medica (1986) reported that in southern Nevada tortoises spent 98.3% of their time in burrows. This behavior is directly tied to their physiological responses to variability in temperature, precipitation, and food availability (Zimmerman et al., 1994; Henen et al., 1998).

Triangular strip-transects, 1.5 mi (2.4 km) long and 10 yd (9.1 m) wide, have been used extensively to sample tortoise burrows and scats (i.e., sign) in conjunction with 1 mi^2 calibration plots of "known tortoise density" (based on the stratified Lincoln Index) to estimate tortoise densities in unknown areas (e.g., Berry and Nicholson, 1984; Krzysik and Woodman, 1991). Krzysik (1997) incorporated Monte Carlo resampling and exact nonparametric statistics into the design to improve statistical inference by addressing the problems of small and unequal sample sizes and high sample variance, major inherent problems when using strip-transect sign counts. Nevertheless, strip-transects require the use of experienced surveyors, strong reliance on calibration by the mark-recapture method with its difficulties and economics, the use of surrogates to estimate tortoise density, and importantly, the tenuous assumption of a consistent relationship of sign/tortoise ratios between calibration and survey areas.

Distance Sampling

Line transect distance sampling (DS) is a statistically robust approach for estimating population densities. DS has a long history of theoretical development in addition to rigorous statistical foundations (Hayne, 1949; Gates, 1969; Eberhardt, 1978; Burnham et al., 1980; Buckland et al., 1993). DS has been used to estimate densities in a wide variety of wildlife, avian, fish, inanimate objects, and even cetacean populations. DS was compared with three other tortoise survey methods in 1994: triangular strip-transects, nested square strip-transects developed by U.S. Fish and Wildlife Service (USFWS, 1992), and the Zippen removal method (USFWS, 1998). Tortoises densities could not be determined, because sample size was inadequate. Sample sizes were sufficient for burrows and scats, and these were used to explore and statistically model patterns of burrow and scat densities. When modeling the data, only DS provided consistent and reliable transect and plot density estimates of tortoise sign. This was particularly evident for scats, objects that possessed low detectability.

The assumptions of distance sampling are reasonable and relevant to this sampling protocol, and I rank them as follows in relative order of importance. 1) Objects of interest must be correctly identified. Although desert tortoises are easy to identify, correct identification of tortoise burrows and scats may be difficult for inexperienced surveyors. 2) All objects on the centerline of the transect must be detected. 3) The perpendicular distances from transect centerline to surveyed objects must be accurately measured. Because this is the shortest possible distance, measurement errors result in longer distances, thus underestimating object densities. 4) The detection function must have a broad shoulder. In other words, virtually all objects near the centerline are detected, but as distance from the centerline increases detection falls off rapidly. This is required to efficiently fit the analytical detection function (the model) to the histogram of field data. This is a very reasonable assumption because, everything else being equal, surveyed objects close to the transect line are typically more readily visible to the surveyor than objects that are further away, where probability of detection is expected to decline. Detection function modeling is the central feature in distance sampling. The detection function varies with object visibility (e.g., size, color, and shape), skill and experience of observer, and habitat/environment specifics (e.g., vegetation density, substrate color and texture, topography and its complexity). 5) The transect line must be random to the distribution of survey objects. This is easily accomplished, and is particularly important when there is an underlying landscape pattern in the sampled area (e.g., soils, vegetation, topography), so that transect lines are not biased with relation to the pattern. 6) The transect survey line must be accurate in length. Shorter lines result in bias for lower densities, while longer transect lines result in bias for higher densities. 7) Objects on the same transect must not be counted more than once. However, what is not often appreciated is that survey objects may be counted more than once if they are associated with different transect lines (i.e., samples). 8) Survey objects must be detected at their original location. This is not a problem with tortoises or their sign, but mobile species are typically frightened by surveyors and have a strong tendency to move away. Contrastingly, some species are curious and may be attracted to surveyors. In these cases, DS estimated densities would be underestimated and overestimated respectively.

DS possesses a number of important advantages, including sampling design flexibility and no assumption that all survey objects are detected. As long as the above assumptions are met and survey objects are potentially detectable, objects can be missed and density estimates are accurate. The model innately incorporates variation in object detection based on: a) object visibility: size, color, cryptic pattern, shape; b) habitat and landscape complexity, environmental conditions; and c) surveyor skill, experience, fatigue, interest.

DS density estimates are independent of object density. This is not the case with strip-transects where density is not only always underestimated, but underestimated to a greater degree when object abundance increases (Krzysik, in prep.). DS has spatial scale flexibility and is also independent of the distribution of objects in the landscape. Surveyed objects can be random, uniform (even), or clumped in distribution. Or they could demonstrate each of the three distributions in different habitats, parts of the landscape, or at different spatial scales. With DS, sampling bias does not increase as w (truncated sampling distance) increases, and sampling variance does not increase as w decreases.

User calculation or calibration of detection functions are not necessary in DS, because field data directly determine the analytical form and metrics of the model. Analytical functions to estimate densities are based on robust estimators that possess desirable qualities (see Buckland et al., 1993:42). The size of the area sampled need not be known to calculate density. This is because the DS model estimates Effective Strip Width (ESW), which is the distance from the transect line that all sampled objects are detected, and therefore density is directly estimated. The larger the value of ESW the more visible objects are to surveyors.

The difficulties in the field application of DS are not unique, and are identical to the problems encountered when using strip-transects or any field sampling design. DS is particularly difficult to execute in complex habitats and terrain. This includes complex topography, cliffs, talus, the presence of boulders or scree fields, and of course, high shrub density. Another important problem that must be addressed regardless of method employed is the independent "adjustment" that must be made for objects that are invisible to the surveyor. This is referred to as the "g_0 problem" in distance sampling. Although not all objects need to be observed by the surveyors, as discussed above, they must all be potentially visible. If during a given survey time frame, half the females in a lizard population are underground brooding egg clutches, they are out of a surveyor's detection field, irrespective of how careful and detailed the survey is. In this scenario, assuming a 1:1 sex ratio, the population density would be underestimated by 25%. If the percent females that were brooding eggs were known from an independent study, the population density estimate could be adjusted accordingly using g_0.

Sampling Protocol Assumptions

Several important and reasonable assumptions are relevant to the protocol. The major assumption is that tortoises possess small and persistent home range sizes with respect to the smallest sampling scale (1 km^2), and within their home ranges tortoises construct burrows and deposit scats. This was verified from the concurrent radiotelemetry study where the mean home range (minimum convex polygon method) of adult desert tortoises (n = 29, males and females were statistically similar) at Sand Hill was 0.075 km^2, ranging from 0.0082 to 0.17 km^2 (Duda et al., 1999). Overlapping home ranges (which are very typical) have no effect on sampling, and burrow and scat abundances increase proportionally. Within scale-relevant spatial and time frames, tortoises maintain burrow/tortoise and scat/tortoise ratios that can be represented by a mean. Based on this logic, tortoises, burrows, and scats should be highly associated in the landscape (i.e., should exhibit highly significant correlations). Because of this association and the knowledge of their numerical relationships, tortoise densities could be estimated at increasingly smaller spatial scales from reliable estimates of burrow and scat densities.

METHODS

Study Sites. — I collected data from two study sites in the southcentral Mojave Desert: Sand Hill Training Area located in the southwest corner of the Marine Corps Air Ground Combat Center (MCAGCC), 28 km northwest of Twentynine Palms, California; and Pinto Basin located 64 km directly southeast of Sand Hill in the central portion of Joshua Tree National Park (JTNP). The vegetation, soils, and elevation were similar at both sites in creosote-bursage scrub (*Larrea tridentata - Ambrosia dumosa*). However, Pinto Basin had an increase (frequency and size) in white rhatany (*Krameria grayi*) and pencil cholla (*Opuntia ramosissima*), and the appearance of widely scattered ocotillo (*Fouquieria splendens*) and jojoba (*Simmondsia californica*), indicative of the transition into the Sonoran Desert. Sand Hill consisted of a broad plain with low relief hills. Sand Hill elevation ranged from 555 to 883 m, but most of its elevation contours were between 732–829 m. Soils were finely sorted, consisting of sandy-loams with some loose sands. Surveys were conducted in the spring and early summer of 1995, a productive year where the previous winter's precipitation was 225% greater than the long-term average for this specific region (Duda et al., 1999).

Sampling Universe. — The sampling universe (SU) is the spatial landscape unit or "stratum" that is being sampled. Although landscapes are characterized by complex mosaics (e.g., vegetation patches, soil classes, fluvial channels), here landscape units are defined as being fine-grained compared to other sampling universes (strata) that may also be of interest. Spatial extents are user defined (e.g., landforms or geomorphology, plant communities, land-use, political boundaries). Landscapes for assessing desert tortoise populations are on the order of 50 km^2 to thousands km^2. Typical examples of SUs for managing desert tortoise populations include: creosote-bursage scrub valleys, plains, and rolling bajadas of low relief; similar but more complex scrub on steeper bajadas and mountainous terrain; saltbush flats; sand dunes and aeolian sands; and disturbed landscapes (e.g., military training ranges).

Eleven ecosystems defined the 2413 km^2 landscape at MCAGCC (Krzysik and Trumbull, 1996). Sand Hill contained a large contiguous 80 km^2 portion of the Creosote-Bursage Scrub Plains ecosystem, and was selected as the sampling universe to pilot test the developed protocol. For comparative purposes, three square 9 km^2 plots were located in Pinto Basin. One was centered on the 2.6 km^2 Barrow Plot, a long-term desert tortoise monitoring plot with known tortoise densities (Barrow, 1979; Freilich et al., 2000). The other two plots were located 7 km northeast and 5 km northwest of the Barrow Plot. Preliminary surveys indicated high and low tortoise densities, respectively, in these areas.

Landscape Sampling Design. — The landscape sampling design consisted of a multi-nested systematic-random design. The combination of spatially-scaled systematic-random sampling has desirable properties. The systematic component insures representative cover of the spatial extent of the area of interest, while the random component insures unbiased sampling, independence of sampling errors, and unbiased variance estimation. For an introduction to sampling design, statistical analysis, and relevant references, see Krzysik (1998a).

Based on a preliminary landscape survey for tortoises and their sign, landscape densities of tortoises, burrows, and scats were approximately an order of magnitude apart. Therefore, the maintenance of recommended sample sizes for DS density estimates in the context of calculating sign/tortoise ratios required that survey sampling strata were also an order of magnitude apart. This was the rationale for the site–plot–transect scale of approximately 100–10–1 km^2.

I systematically placed five square 9 km^2 sampling plots 1 km apart in the Sand Hill SU, saturating the selected 80 km^2 portion of the training area. Four 4 km long square transects were located in a systematic-random fashion in each of the 9 km^2 plots (Fig. 1). The design randomly located the center point of each square transect within each of the four quarters of a given plot. The order of quarter selection was determined at random with two constraints. The center point had to be equal or greater than 0.5 km from the edge of the plot and any two of the four center points could not be closer than 0.5 km. The compass orientation of each transect was random, with values of 0–89°. Randomness was determined by a method I developed using two sets of dice. In the field, the southwest corner of each transect was located with a GPS unit. Although an accurate GPS receiver was used (< 5 m error), accuracy is irrelevant to the design, because GPS error represents another random component to the location of transects within plots. With this design, every point within the 9 km^2 plot, including its boundary, had an approximately equal chance of being sampled—an unbiased probability sampling design.

Survey lines were always initiated from the transect's southwest corner, the bearing was followed using a Suunto® sighting compass, and distance was determined by pacing. For consistency, the same surveyor (calibrated with a 100 m fiberglass tape) was used for pacing distance. Each of the four transect legs was established by ten 100 m paced segments, and the new bearings at the corners were determined by adding 90° to the current bearing. Closure of the square transect was always within 50 m, usually within 20 m, and was as close as 1 m.

When sampling small areas the distance between sampling plots (**S** in Fig. 1) could be reduced to zero, and in very large landscapes (e.g., major portions of the Mojave Desert) plots could be spaced at five or more km for economy and the monitoring of lower resolution distribution-density patterns. When the plots are not distributed throughout the entire sampling universe, the systematic plot grid should be randomly located on the landscape.

Desert Tortoise and Sign Counts. — Tortoise, burrow, scat, and carcass counts were surveyed simultaneously in the same place at the same time. This is an important requirement for calculating spatially scaled ratios of tortoises and

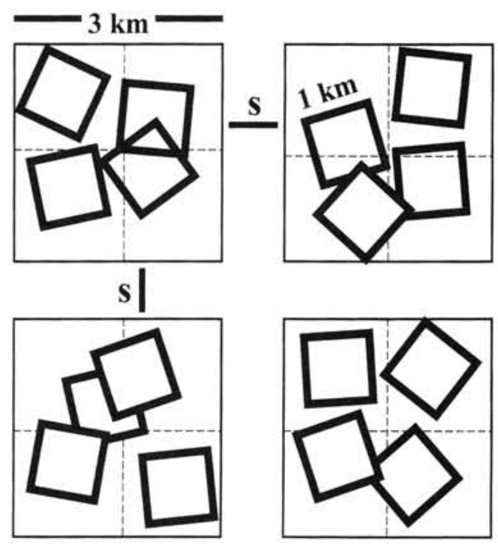

Fig. 1. Landscape scale sampling design of the desert tortoise distribution-density estimation protocol. The large squares are the 9 km^2 plots that were systematically placed at Sand Hill ($n = 5$). The distance between adjacent plots was **S** (1 km). The small squares are the 4 km long square transects that were randomly located within the plots. See methods section for a detailed explanation.

their sign. Burrows were assigned to ordinal classes 5 to 1 based on their condition (5 = currently active to 1 = deteriorated). The depth (length) of burrows was carefully measured to the nearest cm with a flexible steel tape. The relative age of scat was estimated on a scale of 5 to 1 (5 = fresh scat that appeared moist with no surface cracking to 1 = decomposing fibrous white scats). Carcasses included a wide range of items: complete tortoises, complete or partial carapaces or plastrons, skeletal bone fragments, isolated bony shell plates, and horny scutes. Because multiple scats or carcass fragments can actually represent a single incidence, care was taken to record these cases as single datum points. Within a locus of 10 m all located scats of the same age and size were recorded as a single incidence. Similarly, scats within or around burrows (a common occurrence) were not counted.

Association Between Tortoise, Burrow, and Scat Counts in the Landscape. — Transect counts of tortoises, burrows, and scats were statistically associated at three different scales: 0.25 km^2 (1 km transect legs), 1 km^2 (4 km transects), and 9 km^2 (16 km of transects within plots). Both study sites were combined in the analysis. All count data were transformed with the natural logarithm, $x' = \ln(x+1)$. For exploratory analysis, all analyses were also performed with untransformed raw data and the square root transformation of Freeman and Tukey (1950), $x' = x^{1/2} + (x+1)^{1/2}$. Data scatter plots were produced by SigmaPlot 4.0 (SPSS, 1997) and contour surface plots by AXUM 6 (MathSoft, 1999). All statistical analyses were performed with SPSS 9.0 (SPSS, 1999). Four analyses were used to test association strength among tortoise, burrow, and scat counts: 1) a linear regression model with tortoises as the dependent (response) variable and burrows and scats as independent (predictor) variables [tortoises = a(burrows) + b(scats) + constant]; 2) the preceding model with step-wise linear regression; 3) bivariate Pearson correlation coefficients were calculated for all three combinations of tortoise sign counts; and 4) the coefficient of variation (CV) was used to assess tortoise, burrow, and scat count variability at the three spatial scales. CV is the standard deviation expressed as the percent of the mean, CV = 100(SD/mean).

Density Estimation. — I used Line Transect Distance Sampling (DS) for estimating the densities of surveyed objects (Buckland et al., 1993). Surveyed objects included adult and immature (midline caparace length > 100 mm) tortoises, burrows, scats, and carcasses. The detectability of tortoises and burrows was similar (analytically assessed with DS). Therefore, counts of tortoises above ground and in their burrows were pooled. Tortoises were detected visually in their burrows with a stainless steel mirror and audibly when they responded to the disturbance by the measuring tape or to tapping. Tapping at burrow entrances (pounding open hand on the ground) elicited aggressive or curiosity behavior by tortoises causing them to move within or exit their burrows.

Three surveyors were necessary for this DS design. The center surveyor maintained the center line of the transect using the sighting compass to guide the surveyor who was pacing individual 100 m segments and placing florescent pink survey flags at 25 m intervals to delineate the transect centerline. The center surveyor walked the transect centerline and searched for tortoises and their sign at any distance on both sides of the transect, taking extra care to closely monitor the centerline and also the area close to both sides of the transect. The "pacer" surveyor and another surveyor respectively surveyed each side of the centerline by systematically walking back and forth between the centerline to approximately 30–40 m from the centerline, marking all survey objects with orange flagging. By this method, the centerline and the area very close to it were carefully surveyed by three surveyors. Distances away from the centerline, but still reasonably close to the centerline, were effectively surveyed by two surveyors; while increasing distances from the centerline were only covered by a single surveyor on each side of the transect line. The perpendicular distances from the centerline to the center of all located objects were carefully measured with a 50 m fiberglass tape, aluminum metric-stick, or 30 cm aluminum ruler to an accuracy of 1 cm.

I estimated the densities of all surveyed objects with program DISTANCE (Laake et al., 1993). Extensive exploratory analyses were conducted to estimate transect data truncation as a function of $g(x)$, the detection function of objects at x distance from the centerline. It is generally recommended to truncate 5–10% of the data or when $g(x) \sim 0.15$ (Buckland et al., 1993:50,106). Five, 10, 15, and 20% of transect data were truncated and various estimates of $g(x)$ were made. Guided by these results for optimizing the modeling of DS detection functions to the field data and maximizing sample sizes, DS estimates were based on truncation of 30 m for tortoises, carcasses, and burrows, and 20 m for scats. Additionally, these truncation distances showed lower coefficients of variation and 95% confidence intervals. Model development consisted of selecting at least five detection functions, each consisting of a prime function and a series adjustment term. The specific selection was based on modeling experience in using the software for similar data sets. The parameterization and fit of specific models to each histogram of DS field data used χ^2 goodness of fit and likelihood ratio tests to determine the values and number of parameters in specific models. The final selection among alternate models was based on the model with the smallest value of AIC (Akaike's Information Criteria) (Burnham and Anderson, 2002). The derived model (detection curve) represented the equation for object detectability as a function of distance (x) from the transect centerline. The solution of this equation at $x(0)$ provided the estimated density (D_i) of object i when detection is certain [$g(0)=1$].

Sample-Size Requirements for Surveys. — Desired accuracy, precision, and statistical power typically guide sample size requirements. Recommended sample sizes for DS density estimates are 60–80, but 40 may be adequate (Buckland et al., 1993:14). Extensive exploratory analyses found that sample sizes of 20–30 often produced stable density estimates. Nevertheless, unambiguous model development and statistical power dictate maximizing sample sizes. Sample sizes (after truncation) and associated scales for tortoises, burrows, and scats were respectively: $n_t = 60$ @ 107 km², n_b (mean) = 84 @ 9 km², and n_s (mean) = 101 @ 1 km².

Desert Tortoise Density Estimation at Multiple Scales. — I estimated tortoise, carcass, and burrow densities at the sampling universe scale, 80 km² at Sand Hill and 27 km² at Pinto Basin. I next estimated burrow densities for each plot (9 km² scale), and scat densities for each 4 km transect (1 km² scale) at each site. Scat densities on the 9 km² plots were estimated by averaging the densities on the four 4 km transects. Although virtually identical results were obtained when scat density was estimated directly with DS on the 9 km² plots, averaging more realistically represented spatial variability, because of unequal scat counts on individual transects.

Burrow/tortoise ratios were calculated separately for each site. Tortoise densities on each plot (9 km² scale) were estimated by dividing the DS estimated burrow density at each plot by the respective burrow/tortoise ratio for that site. Tortoise densities on each transect (1 km² scale) were estimated in two steps. First, the DS estimated scat density at each transect was divided by the respective scat/burrow ratio for the plot containing the transect. This value was the ratio-estimated burrow density on each transect. The final step consisted in dividing each transect burrow density by the respective burrow/tortoise ratio for the study site containing the transect. In other words, tortoise densities were sequentially estimated at increasingly smaller spatial scales from DS density estimates of tortoise sign and the knowledge of scat/burrow and burrow/tortoise ratios at comparable higher spatial scales where larger sample sizes were available for the respective DS density estimates.

Burrow densities and, in turn, burrow/tortoise ratios were estimated for all possible combinations of burrow condition classes during exploratory analyses. Essential results did not differ among all the combinations. The final analyses of burrow densities and burrow/tortoise ratio calculations used burrow classes 5 through 2, to maximize sample sizes and minimize any effects of classification judgment by different surveyors. A variety of scat condition classes were also modeled. Again, classes 5 through 2 were used for final scat density estimates and ratio calculations using the same rationale discussed for burrows and to minimize temporal differences among plots.

A desert tortoise radiotelemetry study was concurrent at the two study sites (Duda and Krzysik, 1998; Duda et al., 1999). Data from this study were used to estimate home ranges, provide patterns of burrow use by tortoises, and to directly test the accuracy of DS estimated burrow/tortoise ratios. Accuracy in this parameter was critical for assessing the potential of undetecting tortoises in their burrows during surveys, and therefore, violating an assumption of DS.

Landscape Distribution-Density Surface of the Desert Tortoise Population. — Desert tortoise density estimates at 1 km² scales were represented as point estimates in the center of each 4 km transect, whose UTM map coordinates were already established in the sampling frame. With this input, an interpolation and smoothing algorithm was used to define a population distribution and density surface on the landscape. Spline methods are a technique for fitting polynomial curves in the intervals between actual data points and deriving equation parameters to give continuity to a selected number of derivatives at each data point (Ripley, 1981; Cressie, 1993). U.S. Army, ERDC-CERL (Champaign, IL) has developed a robust three-dimensional interpolation and smoothing algorithm – Smoothing Thin-Plate Splines with Tension (TPS), for modeling watershed erosion dynamics and sediment yield. I have found the TPS algorithm useful for representing population responses on the landscape. TPS may have important advantages over kriging, a related technique that is commonly used in geological surveys and mineral exploration, but can also be used to represent biological data. I have generated desert tortoise density/distribution surfaces for Fort Irwin's 2600 km² landscape and demonstrated population trends between 1983–89 (Krzysik, 1997, 1998b). A more detailed discussion of TPS and important references can be found in Krzysik (1997). Tortoise density point estimates and their associated UTM coordinates were input into GRASS GIS (USACERL, 1993) and a landscape desert tortoise density surface was produced using the TPS algorithm.

Spatial Variability in Desert Tortoise Densities. — I assessed desert tortoise density variability at the sampling

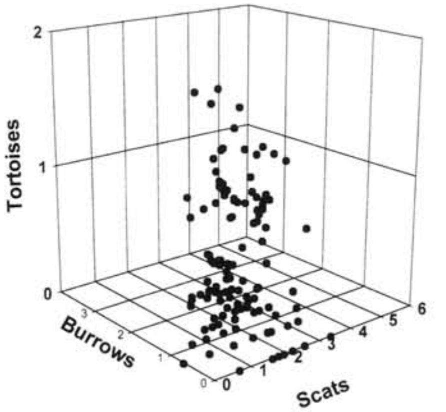

Fig. 2. Scatterplot of desert tortoise, burrow, and scat counts for 1 km transect legs, $n = 128$, data transformed as $x' = \ln(x+1)$.

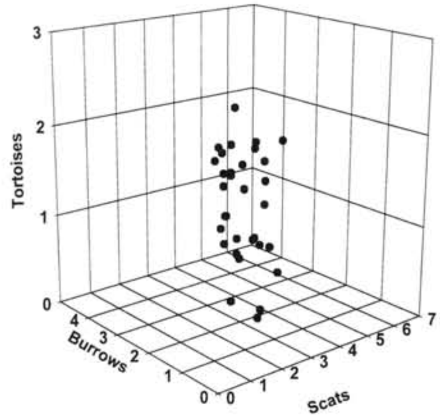

Fig. 3. Scatterplot of desert tortoise, burrow, and scat counts for 4 km transects, $n = 32$, data transformed as $x' = \ln(x+1)$.

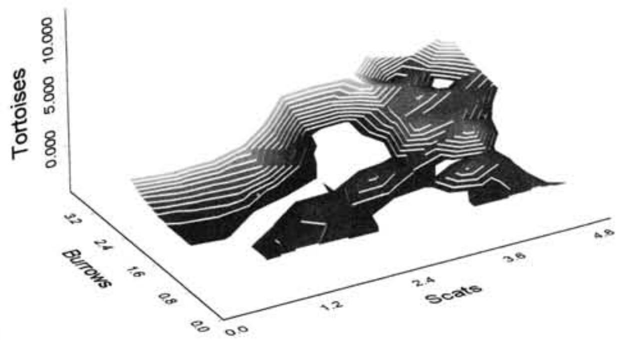

Fig. 4. 3-D filled-contour plot of desert tortoise, burrow, and scat counts for 1 km transect legs, n = 128, data transformed as $x' = \ln(x+1)$.

universe extent (80 km^2 and 27 km^2) using grains of 9 km^2 and 1 km^2, and at the 9 km^2 extent using 1 km^2 grain. Extent and grain are landscape terminology indicating respectively the largest and smallest spatial units for the analysis of interest (Turner and Gardner, 1991). Variability was calculated as the coefficient of variation (CV).

RESULTS

Association Between Tortoise, Burrow, and Scat Counts in the Landscape. — Desert tortoise, burrow, and scat counts were strongly associated in the landscape at multiple scales. Although both low and high burrow and scat counts were found on 1 km transect legs without finding a tortoise (observe burrow-scat 2-D plane), the converse was never true (Fig. 2). Finding a single tortoise, but especially finding more than one, always corresponded with increased burrow and scat counts. Despite the high innate variability of sign counts and the rarity in finding tortoises, there was a strong and persistent association among tortoises and burrow and scat counts in the landscape (see correlations in Appendix). Note that the loci of tortoise counts are strongly aggregated in the back corner of Fig. 2. This corresponds to high burrow and scat counts when tortoise counts increase. Tortoises were not found when burrow and scat counts were low.

When tortoise, burrow, and scat counts were associated at the scale of individual transects (4 km), a strikingly similar pattern emerged (Fig. 3). The only difference was that zero tortoise counts were almost eliminated because transect lengths increased by a factor of four, increasing the probability of finding at least one tortoise on individual transects. When the count data were modeled with a 3-D filled-contour plot the overall pattern in the data was enriched, particularly when tortoise counts increased (Fig. 4). Note the strong pattern in "tortoise peaking" toward the right (increasing scat counts) and rearward (increasing burrow counts).

The linear regression model predicting tortoise counts from burrow and scat counts was highly significant for both 1 km transect legs ($p < 0.001$, $n = 128$) and 4 km transects ($p = 0.001$, $n = 32$). Scat counts were a better predictor of tortoise presence than burrows for both 1 km and 4 km transects (step-wise linear regression, $p < 0.001$). Bivariate Pearson correlations on 1 km and 4 km transects were highest for burrows-scats, and lowest for tortoises-burrows, but all were highly significant ($p < 0.009$). The 4 km transect data, although of smaller sample size, represented a larger sample of survey values within individual transects, leading to larger correlation coefficients (Appendix). Plots represent even higher counts (16 km transect samples) and higher correlations, but the low degrees of freedom for the correlation analysis ($n = 8$) resulted in lower statistical power to assess statistical significance.

The variation of tortoises and their sign counts at three transect length scales (1, 4, and 16 km) were evaluated with the coefficient of variation (CV). At Sand Hill, variability was virtually identical at the scales of both transects (4 km) and plots (16 km) for tortoise, burrow, and scat counts (Fig. 5). However, at the scale of transect legs (1 km) where sample sizes were smaller, variability was higher, particularly for the scarcer tortoise counts where there were many zeros in the data cells. These data, along with the correlation data above, strongly suggest that tortoise/sign ratios at the scales of transects and plots can be used without concern for extensive variation among any of the parameters. Interestingly, at all scales, burrow counts showed the least landscape variability, suggesting more clumping with scat counts. With the exception of the 1 km transects, variability of tortoise and scat counts were very similar. The general

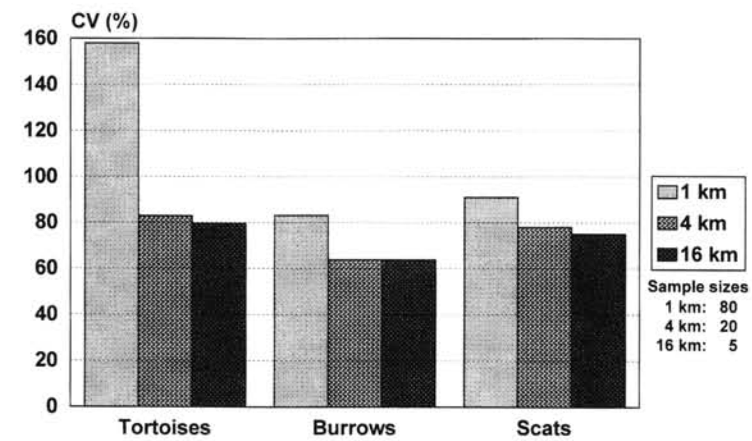

Fig. 5. Coefficient of variation (CV) at Sand Hill for desert tortoise, burrow, and scat counts at three sampling scales based on transect length: 1 km (transect legs), 4 km (transects), 16 km (plots). Sampling universe = 80 km^2.

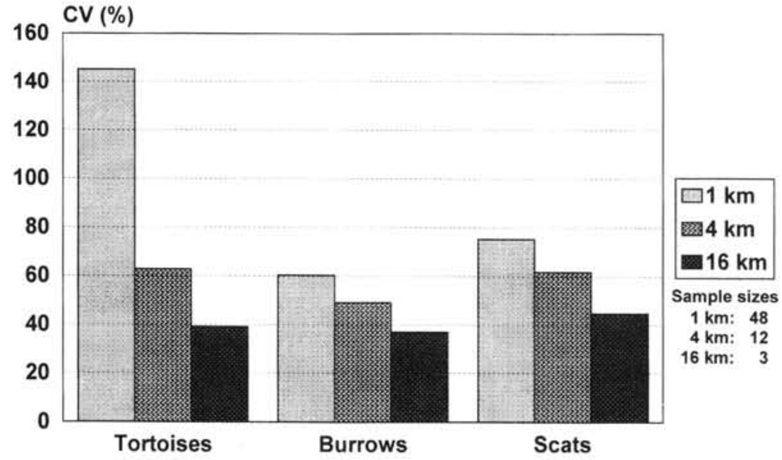

Fig. 6. Coefficient of variation (CV) at Pinto Basin for desert tortoise, burrow, and scat counts at three sampling scales based on transect length: 1 km (transect legs), 4 km (transects), 16 km (plots). Sampling universe = 27 km^2.

patterns in Pinto Basin were similar (Fig. 6). However, Pinto Basin exhibited less overall variability than Sand Hill for tortoises and their sign at all scales, and variability was consistently scale dependent (i.e., CV decreased as scale increased). These patterns were particularly surprising because two of the sample plots were *a priori* selected for their expected high tortoise densities, whereas the third plot was suspected of having low tortoise density.

Tortoise Burrow Depths. — The majority of tortoise burrows were shallow at both sites in both productive and drought years (Fig. 7). During the time period that DS surveys were conducted, approximately 50% of all burrows were less than 66 cm in depth, 75% were less than 1 m, 85–90% were less than 1.3 m, and 98% were less than 2 m in depth. Therefore, when tortoises were in their burrows they were usually readily visible.

Density Estimation. — Overall desert tortoise densities at the scale of the sampling universe were 8/km^2 at Sand Hill and 11/km^2 at Pinto Basin, with corresponding burrow densities of 64/km^2 and 224/km^2 (Table 1). Even though habitats were similar in the two study areas and they were separated by only 64 km, the burrow/tortoise ratio at Sand Hill was 8.06, but at Pinto Basin it was 20.7 (Table 2). These data indicate that burrow/tortoise ratios cannot be assumed to be consistent across large landscapes.

On the basis of DS estimated burrow densities on plots (Table 1) and the corresponding site burrow/tortoise ratios, tortoise densities were estimated on individual plots (Table 2). Tortoise densities at the scale of 9 km^2 ranged from 4.5/km^2 to 13/km^2 at Sand Hill, and from 3.7/km^2 to 15/km^2 at Pinto Basin. The DS estimated burrow/tortoise ratios for Sand Hill and Pinto Basin were 4.6 and 10.1 respectively, when only class 4 and 5 burrows were used. A concurrent radiotelemetry study at the same sites and time gave comparable values of 6.6 and 12.6 (Duda and Krzysik, 1998).

DS estimated burrow and scat densities at the scale of 9 km^2 were used to calculate scat/burrow ratios at this scale (Table 3). Burrow and scat estimated densities at plots were

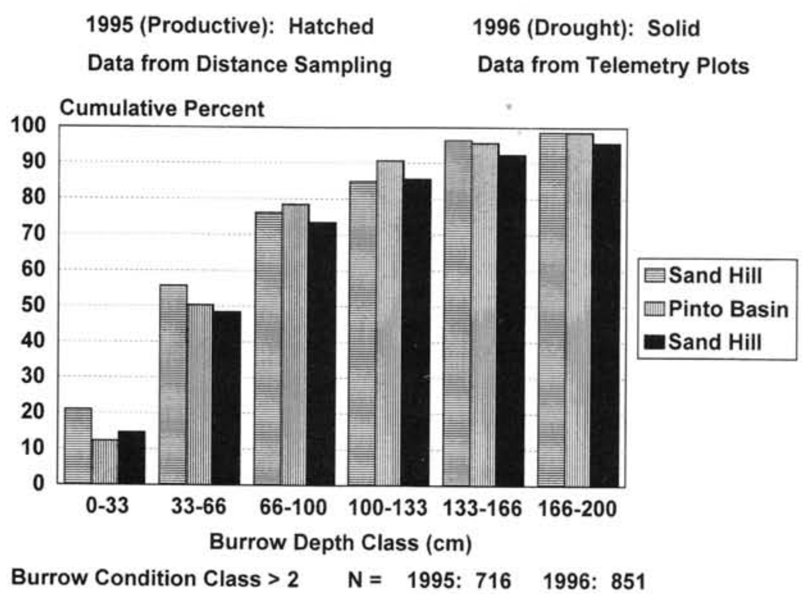

Fig. 7. Cumulative frequency of desert tortoise burrow depths at Sand Hill and Pinto Basin in the year of this study (1995, productive year) and in a drought year (1996, Sand Hill).

Table 1. Distance sampling density estimates of desert tortoises, carcasses/bone-scute fragments, and burrows at Sand Hill, Marine Corps Air Ground Combat Center (MCAGCC) and Pinto Basin, Joshua Tree National Park (JTNP). The tortoise encounter rate for both sites was 0.469 tortoise/km for 128 km (30 m truncation). n = sample size, CV = coefficient of variation, CI = confidence interval.

Site[1]	Plot[1]	Estimated Density (num/km²)	DS Model[2]	n	CV (%)	CI (95%)
Desert Tortoises						
Sand Hill	All	7.97	Haz/Cos	31	25.3	4.9-13.1
Pinto Basin	All	10.8	Haz/Cos	29	27.9	6.3-18.7
Carcasses						
Sand Hill	All	26.9	Uni/Cos	72	16.7	19.4-37.2
Pinto Basin	All	27.9	Uni/Poly	56	16.5	20.1-38.7
Burrows						
Sand Hill	All	64.2	HNor/Cos	192	11.5	51.4-80.3
	SE	106	Uni/Poly	78	12.1	83.0-135
	SW	69.2	Uni/Cos	37	16.5	49.5-96.9
	NW	58.4	Uni/Cos	31	25.8	34.3-99.4
	NE	52.9	HNor/Cos	23	31.9	27.7-101
	CE	35.9	Uni/Poly	23	29.5	19.8-65.2
Pinto Basin	All	224	Haz/Cos	476	10.4	183-276
	NE	307	Haz/Cos	214	14.1	229-412
	BA	283	Haz/Cos	189	12.7	218-369
	NW	76.0	Uni/Cos	73	18.6	51.4-113

[1]Sample units based on 1 km long transect legs, truncation width (each side of transect): 30 m. Sampling effort: number of plots x 4 transects/plot x 4 km/transect. Sand Hill: 5 x 4 x 4 = 80 km, Pinto Basin: 3 x 4 x 4 = 48 km. Condition 2 through 5 burrows used in analysis. Scale: sites = sampling universe: Sand Hill = 80 km², Pinto Basin = 27 km², plots = 9 km².
[2]Distance Sampling Model: Prime Function: Uni–Uniform, HNor–Half-Normal, Haz–Hazard Rate; Adjustment Term: Cos–Cosine, Poly–Simple Polynomial.

strongly associated (bivariate Pearson correlation = 0.87, p = 0.005). The corresponding burrow-scat counts correlation was 0.84, p = 0.009 (Appendix).

DS was used to estimate scat density in each 4-km transect in each sampling plot (Table 4, Column A). From these scat density estimates and the respective scat/burrow ratios in the eight plots (Table 3), burrow densities (Table 4, Column B), and in turn tortoises densities (Table 4, Column C) were estimated at the scale of 1 km². Burrow densities were calculated by dividing Column A by the corresponding plot scat/burrow ratio in Table 3. Tortoise densities were calculated by dividing Column B by the corresponding site burrow/tortoise ratio in Table 2. Tortoise densities at the scale of 1 km² ranged from 1.5/km² to 19/km² at Sand Hill, and from 1.9/km² to 23/km² at Pinto Basin.

Based on the estimated tortoise density at 1 km² (Table 4, column C) we created a landscape desert tortoise distribution-density surface at Sand Hill using the Thin-Plate Splines algorithm in the GIS environment. The southeastern portion of Sand Hill contained a high density of desert tortoises, while the central portion had a low density (Fig. 8). Tortoise densities were also low in two small localized portions of the landscape (southcentral and northeastern). The western portion of Sand Hill possessed a uniformly moderate density over its entire area.

Desert tortoise carcasses and carcass fragments (bones and scutes) were at a landscape density of 26.9/km² at Sand Hill, and 27.9/km² at Pinto Basin (Table 1). Whereas overall

Table 2. Estimated desert tortoise densities on 9 km² sample plots.

Sand Hill		
80 km² scale		
Tortoise Density (DS–Estimated)		7.97 tortoise/km²
Burrow Density (DS–Estimated)		64.2 burrow/km²
Burrow/Tortoise Ratio		8.06 burrow/tortoise
9 km² scale		
Plot	DS–Estimated burrow/km²	Ratio–Estimated tortoise/km²
SE	106	13.1
SW	69.2	8.58
NW	58.4	7.24
NE	52.9	6.56
CE	35.9	4.45
Pinto Basin		
27 km² scale		
Tortoise Density (DS–Estimated)		10.8 tortoise/km²
Burrow Density (DS–Estimated)		224 burrow/km²
Burrow/Tortoise Ratio		20.7 burrow/tortoise
9 km² scale		
Plot	DS–Estimated burrow/km²	Ratio–Estimated tortoise/km²
NE	307	14.8
BA	283	13.6
NW	76.0	3.66

tortoise densities were 35% higher at Pinto Basin, carcasses and their fragments were only 4% higher, suggesting that tortoise mortality is higher at Sand Hill than Pinto Basin. This could be attributed to land-use at the two study sites, the former a military training area (although lightly used when this study was conducted), and the latter a designated wilderness area in a national park.

Spatial Variability in Desert Tortoise Density. — Spatial variability in tortoise density at a grain size of 9 km² varied by a factor of 2.9 (4.5 to 13/km², CV = 40%) at the 80 km² contiguous landscape at Sand Hill, and by a factor of 4.0 (3.7 to 15/km², CV = 57%) at the 3 disjunct plots (27 km²) in Pinto Basin (Table 5). When grain size was reduced to 1 km², local tortoise density variability increased to a factor of 12.5 (1.5 to 18.9/km², CV = 55%) at Sand Hill, and by a factor of 12.2 (1.9 to 23/km², CV = 70%) at Pinto Basin (Table 5).

When spatial variability was assessed at the plot size (9 km²) with a 1 km² grain, within-plot tortoise density varied by factors of 1.6 to 7.5 (CV: 18 to 82%) at Sand Hill, and

Table 3. Scat/burrow ratios on 9 km² sample plots derived from DS estimated burrow and scat densities. Bivariate Pearson Correlation between burrows and scats is 0.87, p = 0.005. * Scat density based on transect means.

Site	Plot	Burrow/ km²	Scats/ km²*	n	Scat/Burrow Ratio
Sand Hill					
	SE	106	2403	662	22.7
	SW	69.2	1404	405	20.3
	NW	58.4	1250	234	21.4
	NE	52.9	730	107	13.8
	CE	35.9	639	145	17.8
	All	64.2	1285	1552	20.0
Pinto Basin					
	NE	307	2898	628	9.44
	BA	283	2594	756	9.17
	NW	76.0	1608	281	21.2
	All	224	2367	1665	10.6

Table 4. Estimated desert tortoise densities on 1 km² transects at Sand Hill and Pinto Basin.

Site	Plot	Transect	A DS Estimated Scat/km²	n	B Ratio Estimated Burrow/km²	C Ratio Estimated Tortoise/km²
Sand Hill						
	SE	SW	3469	248	153	18.9
		NW	1870	185	82.5	10.2
		NE	2217	113	97.8	12.1
		SE	2056	116	90.7	11.2
	SW	SW	1929	78	95.1	11.8
		NW	1041	116	51.3	6.36
		NE	1695	137	83.5	10.4
		SE	951	73	46.9	5.82
	NW	SW	1318	55	61.6	7.64
		NW	1278	61	59.7	7.40
		NE	938	44	43.8	5.43
		SE	1465	74	68.4	8.48
	NE	SW	500	27	36.2	4.49
		NW	1589	18	115	14.3
		NE	213	34	15.4	1.91
		SE	617	28	44.7	5.54
	CE	SW	1327	45	74.6	9.25
		NW	433	18	24.3	3.01
		NE	218	20	12.2	1.51
		SE	577	62	32.4	4.02
Pinto Basin						
	NE	SW	4484	254	475	22.9
		NW	3167	193	335	16.2
		NE	794	44	84.1	4.05
		SE	3147	137	333	16.1
	BA	SW	2911	205	318	15.3
		NW	1420	112	155	7.47
		NE	1911	121	208	10.0
		SE	4133	318	451	21.7
	NW	SW	823	42	38.9	1.88
		NW	2011	85	95.0	4.58
		NE	1164	76	55.0	2.65
		SE	2434	78	115	5.54

from 2.9 to 5.7 (CV: 46 to 53%) at Pinto Basin. At Sand Hill, within-plot tortoise density variability was highest at plots that possessed low overall tortoise abundance, whereas tortoise densities were more evenly distributed in the higher density plots (see CV and Highest/Lowest ratios, Table 5). There was no discernible pattern at Pinto Basin. Regardless of plot tortoise density, coefficients of variation and Highest/Lowest ratios were similar and had intermediate values compared to those at Sand Hill. Correlations (parametric and nonparametric) between tortoise density and CV on the study plots were not significant when either the sites were combined or analyzed separately ($p > 0.22$ to 0.90), but sample sizes (i.e., degrees of freedom) were low. Tortoise and burrow spatial variability or patchiness is directly addressed in Duda et al. (2002).

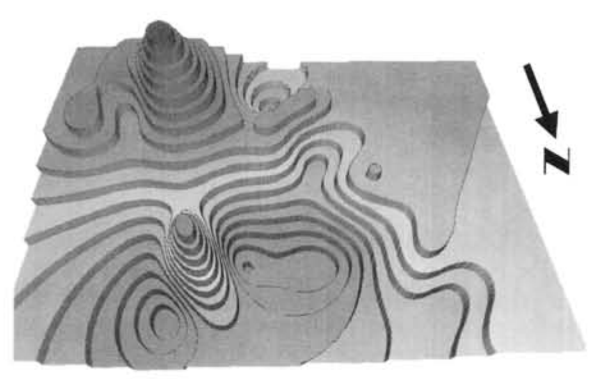

Fig. 8. Thin-plate spline representation of desert tortoise distribution-density surface at Sand Hill. Note that the orientation of the figure is to the south.

DISCUSSION

Land managers and researchers have been searching for a reliable method to estimate population densities of desert tortoises ever since the species was federally listed (USFWS, 1994a). Important problems with traditional methods included: coverage of landscape scales, sampling low density populations, estimating effective trapping area, detection of tortoises in burrows, representative sampling, assumption that tortoise density or distribution pattern does not bias sampling, bias in selection of plots or transects, assumption that sign/tortoise ratios are consistent over large spatial scales, and the validity of using burrows and scats (i.e., sign) as surrogates of tortoise abundance. This pilot-tested desert tortoise landscape sampling protocol, using the integration of four design elements with distance sampling as the density estimator, possesses important advantages and advances over other sampling methods. It balances accuracy and economy in the estimation of desert tortoise densities over extensive landscape scales, while simultaneously providing information on local small scale tortoise distribution-density patterns. The design is statistically rigorous and sampling unbiased. It does not require extensive field survey skills or experience, but adherence to simple and reasonable design parameters and survey assumptions. It is equally valid at any level of population density or spatial distribution pattern in the landscape. This is an important capability when desert tortoise population densities are very low, as is frequently the case. However, when tortoise densities are very low, larger portions of the landscape require sampling or transect density must be increased to insure adequate sample sizes for distance sampling estimates.

This sampling design does not use burrows and scats as surrogates for tortoise abundance. I used unbiased estimates of burrow and scat densities and their respective local variations at decreasingly smaller spatial scales to locally calibrate the tortoise density estimated for the entire landscape (sampling universe). It is important to emphasize that burrow/tortoise and scat/burrow ratios were only calculated at similar and appropriate spatial and temporal sampling frames. When just considering the accuracy of landscape density estimates, burrow and scat estimates are inherently more accurate than tortoise estimates, because of higher sample sizes and the irrelevance of the g_0 problem.

The protocol is immediately useful and relevant to land managers and planners who must make daily land-use decisions at multiple spatial scales. This capability is specifically addressed by the development of a population distribution-density surface throughout the landscape of interest. Although the development of this surface was based on estimated densities, absolute density values may not be as

Table 5. Assessing spatial variability in desert tortoise densities at the extent of sampling universes (80 km^2 and 27 km^2 scales) and plots (9 km^2 scale) with grains of plots (9 km^2) and transects (1 km^2).

Spatial	Extent (km^2)	Grain (km^2)	n	CV (%)	Lowest Density	Highest Density	Highest/ Lowest
Sand Hill	80	9	5	40	4.45	13.1	2.9
	80	1	20	55	1.51	18.9	12.5
SE	9	1	4	30	10.2	18.9	1.9
SW	9	1	4	34	5.82	11.8	2.0
NW	9	1	4	18	5.43	8.48	1.6
NE	9	1	4	82	1.91	14.3	7.5
CE	9	1	4	76	1.51	9.25	6.1
Pinto Basin	27	9	3	57	3.66	14.8	4.0
	27	1	12	70	1.88	22.9	12.2
NE	9	1	4	53	4.05	22.9	5.7
BA	9	1	4	46	7.47	21.7	2.9
NW	9	1	4	46	1.88	5.54	2.9

important to land managers as a reliable distribution surface of statistically valid relative densities. The tortoise density surface would remain identical in its shape contours even if overall tortoise density was underestimated (or overestimated) at the scale of the sampling universe. In the case of underestimation, the surface would simply be lower (have lower values for density everywhere in the landscape). What is relevant to the integrity of the density surface and for land managers, is that reliable and unbiased estimates can be made of tortoise sign parameters at different spatial scales. Additionally, because tortoise sign are on the surface, they are not subjected to errors of undetectability (i.e., the g_0 problem). The protocol is conceptually adaptable to a broad variety of population, community/ecosystem, and landscape sampling requirements.

The generated tortoise distribution-density surface (Fig. 8) was immediately beneficial to natural resources managers at MCAGCC, because they could make land-use military training and construction decisions while meeting their compliance with the Endangered Species Act. The high tortoise density in the southeastern portion of Sand Hill was unknown to the installation, while the central portion of Sand Hill, which possessed low tortoise densities, had previously been designated as a Desert Tortoise Conservation Zone.

Tortoises in Burrows and the g_0 Controversy. — The common perception of the "g_0 problem" refers to the fraction of tortoises undetectable in their burrows, and therefore, not observed on the transect centerline, a violation of a critical DS assumption. Of course, the actual probability of an undetected tortoise buried on the centerline is essentially "0" on any survey. The reality is that if tortoises cannot be detected in their burrows throughout the survey area, and thus unavailable for detection function modeling, this fraction of "lost" tortoises underestimates density proportionally. Because the detection function is evaluated at $x(0)$, g_0 mathematically represents the correction factor for "lost" tortoises that are in reality scattered in the underground realm of the area defined by the detection function.

The data demonstrate (at least in the southern Mojave Desert) that the majority of burrows used by desert tortoises during their spring-summer activity season, when surveys generally take place, are shallow enough to allow visible detection of their occupants. Tortoises burrows remained shallow even during a severe drought year (Fig. 7). For the small percentage of burrows that are deeper or strongly curved, tortoises can be acoustically detected by the use of a flexible steel measuring tape or tapping the soil at burrow entrances. These responses were observed in this study, and Medica et al. (1986) reported that both male (83%, $n = 144$) and female (82%, $n = 249$) tortoises responded to tapping by a wooden stick and emerged from their burrows. Their study was conducted over two successive years between March and July. They noted that tortoise response may increase as the season warms. Alice Karl (*pers. comm.*) tried tapping the soil in front of burrows that contained tortoises fitted with radiotelemetry transmitters, and 80% of her tortoises responded to the tapping and emerged from their burrows.

Therefore, on the basis of 2–3% deep burrows in the landscape and a 20% undetection of tortoises in deep burrows, only a very small percentage of tortoises avoid detection on surveys. Even these could be effectively sampled for occupancy with the use of a flexible probe mounted to a remote television camera. If burrow estimates were accurate because they were on the surface, but tortoise densities were underestimated (the g_0 problem), the calculated burrow/tortoise ratio would be inflated. However, DS estimates and radiotelemetry values were similar, but if there was a trend, it was in the opposite direction. DS estimates of active burrow/tortoise ratios were 4.6 and 10.1, respectively, at Sand Hill and Pinto Basin, while the corresponding radiotelemetry values were 6.6 and 12.6. These data support the accuracy of DS estimated tortoise densities without using g_0, at least in the southern Mojave Desert.

Tortoise surveys can be conducted in drought years, based on the data presented here. One would simply find a larger proportion of tortoises in burrows (Fig. 9). Tortoises were observed more frequently in their burrows during a drought year than during a productive year in both spring and summer, and were also more frequently found in burrows in summer compared to spring in both productive and drought years. Nevertheless, even in the spring of a productive year, tortoises spent over half of their diurnal time in their burrows. Additionally, they exhibited a great deal of both within and between daily variability in burrow use, actively and

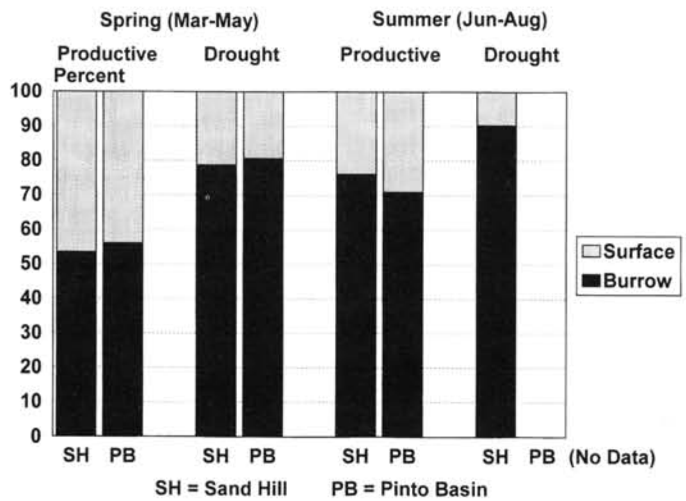

Fig. 9. Distribution of desert tortoises on the surface and in burrows at Sand Hill and Pinto Basin during the spring and summer of a productive year (1995) and a drought year (1996). The data were collected from tortoises that were fitted with radiotelemetry transmitters. Sand Hill $n = 29$, Pinto Basin $n = 9$; data from Duda and Krzysik, 1998.

rapidly responding to local environmental dynamics (Nagy and Medica, 1986; Zimmerman et al., 1994; Henen et al., 1998; Duda et al., 1999). Therefore, locating tortoises in burrows will always be inherent in any tortoise sampling strategy regardless of season, weather conditions, and annual productivity.

Tortoises in the northern part of their range (i.e., Nevada and Utah) possess deeper summer burrows and very deep winter burrows (Woodbury and Hardy, 1948; Rautenstrauch et al., 1998). Surveys cannot be carried out in the winter for three critical reasons: tortoises are in deep burrows where they are very difficult to visually detect, they are inactive and would not respond to tapping, multiple tortoises (possibly many) may occur is these burrows and they cannot be reliably counted even with a television camera probe.

Radiotelemetry studies are very resource-, time-, and energy-intensive (Duda and Krzysik, 1998; Duda et al., 1999). The dynamics of tortoises going in and out of their burrows, usually being detectable but sometimes not, may be rapid, and may be quite different over small spatial and temporal scales. In order to be meaningful, a telemetry project would have to be spatially and temporally simultaneous with the DS monitoring project occurring over large spatial scales. When tortoises occur in low densities (a common situation) the use of radiotelemetry over large spatial scales with adequate sample sizes may be impossible, and certainly would correspond with unreasonably low sample sizes for estimating the fraction of tortoises undetectable in burrows. Radiotelemetry does not appear practical nor economic, particularly when tortoises are visually or acoustically easily located in their burrows, and remote television probes can be employed in challenging circumstances or in the northern portion of their range.

Scale Variability in Desert Tortoise Densities. — Based on burrow and scat densities, desert tortoises were not uniformly distributed in the landscape, but varied in local density. This variability increased at smaller spatial scales. Tortoise densities were 8/km^2 at Sand Hill and 11/km^2 at Pinto Basin, but ranged from 3.7–15/km^2 at the scale of 9 km^2 (Table 2), and from 1.5–23/km^2 at the scale of 1 km^2 (Table 4). The dependence of tortoise density on spatial scale indicates a clumping or aggregation in their landscape distribution. The spatial distribution of burrows and tortoises are explored in more detail in Duda et al. (2002). The clumped distribution of tortoises is probably driven by social interactions and desired habitat patches related to food resources and soils for burrow construction. Specific mineral or element needs, or the availability or persistence of water puddles after precipitation may also be important. The consideration of seasonal availability of food resources may play a role—winter annuals in the spring and big galleta grass (*Pleuraphis rigida*) in the summer.

Future Research Issues. — This desert tortoise sampling protocol has addressed many complex and controversial density estimation issues. The protocol possesses important design features for monitoring both local and landscape scale populations of desert tortoises. Benefits can be gained by additional research in several areas. The current Thin-Plate-Spline algorithm requires sophisticated and expensive hardware, software, and a great deal of GIS expertise. It would be desirable to simplify and optimize the interpolation and smoothing of density estimates to obtain the landscape distribution-density surface so important to land managers. There are two important issues in field surveys. Additional information is required in the detectability of tortoises in their burrows as a function of geographical distribution, microhabitat parameters, season, and a number of environmental parameters. The 20% undetectable in deep burrows that I cite here may be underestimated. An important and persistent problem, in at least some areas, is the difficulty of finding desert tortoises in drought years (Freilich et al., 2000). Possibly a series of drought years drives tortoises deeper into deeper burrows, with some proportion of these burrows experiencing interior collapsing. This important issue requires additional investigations.

Tortoise densities are optimally estimated on large landscapes because distance sampling and statistical power require large sample sizes. Arguments against the use of tortoise sign (i.e., burrows and scats) as an aid to interpreting landscape scale estimated tortoise densities are counterproductive to the needs of land managers and the practicality and maximization of data acquisition and utilization. The use of burrow and scat densities in the context of this protocol does not replace the use of live tortoises for density estimation, but provides the data for reliable unbiased estimates of tortoise densities at smaller spatial scales and where tortoise densities are very low (i.e., where sample sizes are inadequate for distance sampling). In drought years, tortoises may be very difficult to find, whereas their sign is readily available. The decision to only sample live tortoises for density estimates would generate a single metric for a large landscape unit, while the land manager has no knowledge of the local distribution and densities of tortoises within the management unit. Nevertheless, the land manager is responsible for decisions and alternative choices for multiple-use requirements. In my protocol, the landscape population distribution-density surface would aid the land manager immensely. This represents a novel and desirable approach.

ACKNOWLEDGMENTS

Field surveys were conducted by Jeff Duda, Richard Stevens, and AJK. Additional field assistance was provided by Peg Gronemeyer and Jim Sechrest. Jeff Duda, Jerry Freilich, Kristin Berry, and an anonymous reviewer added immeasurably to the focus and clarity of the manuscript. Jocelyn Aycrigg conducted the GIS analysis. We all gratefully acknowledge Ester Hutchinson, Ken Kreklau, Rhys Evans, Sharon Jones, Roy Madden, and the range control officers at MCAGCC; and Jerry Freilich (JTNP) for field logistical support. This research was funded by the Directorate of Natural Resources and Environmental Affairs (MCAGCC). This desert tortoise research was conducted under a U.S. Fish and Wildlife Service Endangered Species Permit (PRT-702631).

LITERATURE CITED

BARROW, J. 1979. Aspects of ecology of the desert tortoise, *Gopherus agassizi*, in Joshua Tree National Monument, Pinto Basin, Riverside County, California. The Desert Tortoise Council Symposium Proceedings 1979:105-131.

BERRY, K.H. 1997. The desert tortoise recovery plan: an ambitious effort to conserve biodiversity in the Mojave and Colorado Deserts of the United States. In: Van Abbema, J. (Ed.). Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles – An International Conference. N.Y. Turtle and Tortoise Society, pp. 430-440.

BERRY, K.H. AND MEDICA, P. 1995. Desert tortoises in the Mojave and Colorado Deserts. In: LaRoe, E.T., Farris, G.S., Puckett, C.E., Doran, P.D., and Mac, M.J. (Eds.). Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. Washington, DC: U.S. Department of the Interior, National Biological Service, pp. 135-137.

BERRY, K.H. AND NICHOLSON, L.L. 1984. The distribution and density of desert tortoise populations in California in the 1970's. In: Berry, K.H. (Ed.). The Status of the Desert Tortoise (*Gopherus agassizii*) in the United States. Report to U.S. Fish and Wildlife Service from the Desert Tortoise Council on Order No. 11310-0083-81, pp. 26-60.

BUCKLAND, S.T., ANDERSON, D.R., BURNHAM, K.P., AND LAAKE, J.L. 1993. Distance Sampling: Estimating Abundance of Biological Populations. Chapman and Hall, New York, 446 pp.

BULOVA, S.J. 1994. Patterns of burrow use by desert tortoises: gender differences and seasonal trends. Herpetological Monographs 8:133-143.

BURNHAM, K.P. AND ANDERSON, D.R. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd ed. Springer, New York, 488 pp.

BURNHAM, K.P., ANDERSON, D.R., AND LAAKE, J.L.. 1980. Estimation of density from line transect sampling of biological populations. Wildlife Monographs 72:1-202.

BURY, R.B. AND CORN, P.S. 1995. Have desert tortoises undergone a long-term decline in abundance? Wildlife Society Bulletin 23:41-47.

COCHRAN, W.G. 1977. Sampling Techniques, 3rd ed. John Wiley and Sons, New York, 428 pp.

CRESSIE, N.A.C. 1993. Statistics for Spatial Data, revised ed. John Wiley and Sons, New York, 900 pp.

DUDA, J.J. AND KRZYSIK, A.J. 1998. Radiotelemetry study of a desert tortoise population: Sand Hill Training Area, Marine Corps Air Ground Combat Center, Twentynine Palms, California. USACERL Technical Report 98/39, U.S. Army ERDC-CERL, Champaign, IL, 75 pp.

DUDA, J.J., KRZYSIK, A.J., AND FREILICH, J.E. 1999. Effects of drought on desert tortoise movement and activity. Journal of Wildlife Management 63:1181-1192.

DUDA, J.J., KRZYSIK, A.J., AND MELOCHE, J.M. 2002. Spatial organization of desert tortoises and their burrows at a landscape scale. Chelonian Conservation and Biology 4(2):387-397.

EBERHARDT, L.L. 1978. Transect methods for population studies. Journal of Wildlife Management 42:1-31.

FREEMAN, M.F. AND TUKEY, J.W.. 1950. Transformations related to the angular and the square root. Annals of Mathematical Statistics 21:607-611.

FREILICH, J.E., BURNHAM, K.P., COLLINS, C.M., AND GARRY, C.A. 2000. Factors affecting population assessments of desert tortoises. Conservation Biology 14:1479-1489.

GATES, C.E. 1969. Simulation study of estimators for the line transect sampling method. Biometrics 25:317-328.

GREEN, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York, 257 pp.

GROVER, M.C. AND DEFALCO, L.A. 1995. Desert tortoise (*Gopherus agassizii*): status-of-knowledge outline with references. U.S. Department of Agriculture, Forest Service, Intermountain Research Station, General Technical Report INT-GTR-316, 134 pp.

HAYEK, L.A.C. AND BUZAS, M.A. 1997. Surveying Natural Populations. Columbia University Press, New York, 563 pp.

HAYNE, D.W. 1949. An examination of the strip census method for estimation animal populations. Journal of Wildlife Management 13:145-157.

HENEN, B.T., PETERSON, C.C., WALLIS, I.R., BERRY, K.H., AND NAGY, K.A. 1998. Effects of climatic variation on field metabolism and water relations of desert tortoises. Oecologia 117:365-373.

HOHMAN, J.P., OHMART, R.D., AND SCHWARTZMANN, J. 1980. An annotated bibliography of the desert tortoise (*Gopherus agassizi*). Desert Tortoise Council Special Publication No. 1, 121 pp.

KREBS, C.J. 1999. Ecological Methodology, 2nd ed. Benjamin Cummings, New York, 620 pp.

KRZYSIK, A.J. 1997. Desert tortoise populations in the Mojave Desert and a half-century of military training activities. In: Van Abbema, J. (Ed.). Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles – An International Conference. N.Y. Turtle and Tortoise Society, pp. 61-73.

KRZYSIK, A.J. 1998a. Ecological design and analysis: principles and issues in environmental monitoring. In: Lannoo, M.J. (Ed.). Status and Conservation of Midwestern Amphibians. University of Iowa Press, Iowa City, pp. 385-403.

KRZYSIK, A.J. 1998b. Geographic information systems, landscape ecology, and spatial modeling. In: Lannoo, M.J. (Ed.). Status and Conservation of Midwestern Amphibians. University of Iowa Press, Iowa City, pp. 404-428.

KRZYSIK, A.J. AND TRUMBULL, V.L. 1996. Biodiversity and wildlife management plan: an ecosystem approach - Marine Corps Air Ground Combat Center, Twentynine Palms, California. Final Report, 400 pp.

KRZYSIK, A.J. AND WOODMAN, A.P. 1991. Six years of Army training activities and the desert tortoise. Desert Tortoise Council Symposia Proceedings 1987-1991:337-368.

LAAKE, J.L., BUCKLAND, S.T., ANDERSON, D.R., AND BURNHAM, K.P. 1993. Distance User's Guide Version 2.0. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins, CO, 72 pp.

LUCKENBACH, R.A. 1982. Ecology and management of the desert tortoise (*Gopherus agassizii*) in California. In: Bury, R.B. (Ed.). North American Tortoises: Conservation and Ecology. Wildlife Research Report 12. U.S. Department of the Interior, Fish and Wildlife Service, pp. 1-37.

MATHSOFT. 1999. AXUM 6. Version 6.0. MathSoft, Inc., Seattle.

MEDICA, P.A., LYONS, C.L., AND TURNER, F.B. 1986. "Tapping": a technique for capturing tortoises. Herpetological Review 17(1):15-16.

NAGY, K.A. AND MEDICA, P.A. 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica 42:73-92.

RAUTENSTRAUCH, K.R., RAGER, A.L.H., AND RAKESTRAW, D.L. 1998. Winter behavior of desert tortoises in southcentral Nevada. Journal of Wildlife Management 62:98-104.

RIPLEY, B.D. 1981. Spatial Statistics. John Wiley and Sons, New York, 252 pp.

SEBER, G.A.F. 1982. The Estimation of Animal Abundance and Related Parameters, 2nd ed. Edward Arnold, London, 654 pp.

Skalski, J.R., and D.S. Robson. 1992. Techniques for Wildlife Investigations: Design and Analysis of Capture Data. Academic Press, New York. 237pp.

SPSS. 1997. SigmaPlot: Exact Graphs for Exact Science. Version 4.0. SPSS, Inc., Chicago.

SPSS. 1999. SPSS analysis software package. Version 9.0. SPSS, Inc., Chicago.

THOMPSON, S.K. 1992. Sampling. John Wiley and Sons, New York, 343 pp.

THOMPSON, W.L., WHITE, G.C., AND GOWAN, C. 1998. Monitoring Vertebrate Populations. Academic Press, New York, 365 pp.

TURNER, M.G. AND GARDNER, R.H. 1991. Quantitative methods in landscape ecology: an introduction. In: Turner, M.G. and Gardner, R.H. (Eds.). Quantitative Methods in Landscape Ecology. Springer-Verlag, New York, pp. 3-14.

USACERL. 1993. GRASS Version 4.1 Geographic Resources Analysis Support System. U.S. Army Corps of Engineers, Construction Engineering Research Laboratory, Champaign, IL.

USFWS. 1990. Endangered and threatened wildlife and plants: determination of threatened status for the Mojave population of the desert tortoise. Federal Register 55(63):12178-12191.

USFWS. 1992. Procedures for Endangered Species Act Compliance for the Mojave Population of the Desert Tortoise. U.S. Fish and Wildlife Service, Portland, OR.

USFWS. 1994a. Desert Tortoise (Mojave Population) Recovery Plan. U.S. Fish and Wildlife Service, Portland, OR, 73 pp.

USFWS. 1994b. Proposed Desert Wildlife Management Areas for Recovery of the Mojave Population of the Desert Tortoise. U.S. Fish and Wildlife Service, Portland, OR, 100 pp.

USFWS. 1994c. Endangered and threatened wildlife and plants: determination of critical habitat for the Mojave population of the desert tortoise; final rule. Federal Register 59(26):5820-5846 and corrections 59(37):9032-9036.

USFWS. 1998. A Comparison of Desert Tortoise Survey Techniques. Report for U.S. Air Force, Edwards Air Force Base, AFFTC/EM, California. U.S. Fish and Wildlife Service, Ventura, CA, 53 pp.

WHITE, G.C., ANDERSON, D.R., BURNHAM, K.P., AND OTIS, D.L. 1982. Capture-Recapture and Removal Methods for Sampling Closed Populations. Report LA-8787-NERP, Los Alamos National Laboratory, Los Alamos, NM, 235 pp.

WILLIAMS, B.K., NICHOLS, J.D., AND CONROY, M.J. 2002. Analysis and Management of Animal Populations. Academic Press, New York, 817 pp.

WOODBURY, A.M. AND HARDY, R. 1948. Studies of the desert tortoise, *Gopherus agassizii*. Ecological Monographs 18:145-200.

YOUNG, L.J. AND YOUNG, J.H. 1998. Statistical Ecology: A Population Perspective. Kluwer Academic Publishers, Boston, 565 pp.

ZIMMERMAN, L.C., O'CONNOR, M.P., BULOVA, S.J., SPOTILA, J.R., KEMP, S.J., AND SALICE, C.J. 1994. Thermal ecology of desert tortoises in the eastern Mojave desert: seasonal patterns of operative and body temperatures, and microhabitat utilization. Herpetological Monographs 8:45-59.

ZIPPIN, C. 1956. An evaluation of the removal method of estimating animal populations. Biometrics 12:163-189.

ZIPPIN, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82-90.

APPENDIX

Regression Model: Tortoises = a(burrows) + b(scats) + constant

		Direct	Best Predictor	Stepwise
Legs	$n = 128$	$p < 0.001$	Scats	$p < 0.001$
Transects	$n = 32$	$p = 0.001$	Scats	$p < 0.001$
Plots	$n = 8$	$p = 0.14$	Scats	$p = 0.040$

Bivariate Pearson Correlations

	Burrows	Scats
Legs, 1 km, $n = 128$		
Tortoises	0.29 ($p = 0.001$)	0.35 ($p < 0.001$)
Burrows		0.73 ($p < 0.001$)
Transects, 4 km, $n = 32$		
Tortoises	0.45 ($p = 0.009$)	0.60 ($p < 0.001$)
Burrows		0.80 ($p < 0.001$)
Plots, 16 km, $n = 8$		
Tortoises	0.57 ($p = 0.14$)	0.73 ($p = 0.040$)
Burrows		0.84 ($p = 0.009$)

Received: 26 September 2000
Reviewed: 10 August 2002
Revised and Accepted: 11 September 2002