

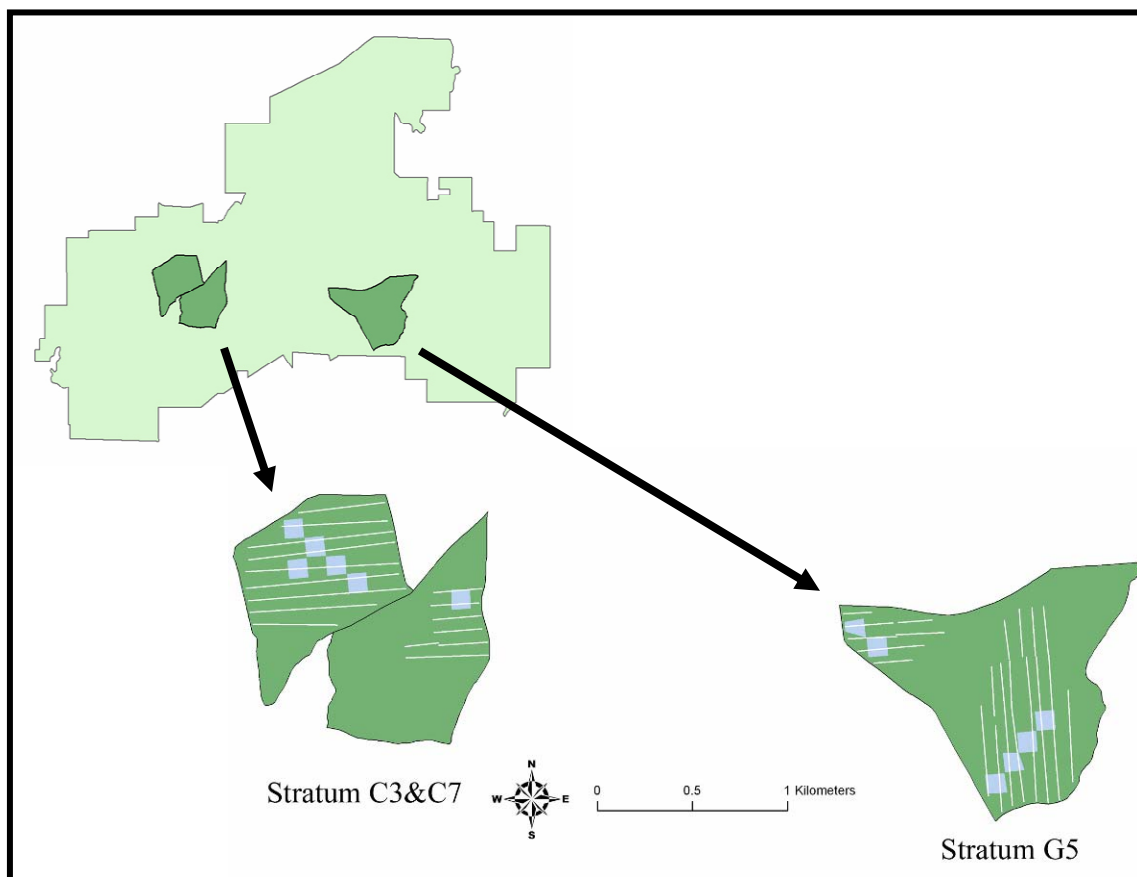


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Field Test of Gopher Tortoise (*Gopherus polyphemus*) Population Estimation Techniques

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Final Report

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Abstract: The Army is very concerned about the preservation of Threatened and Endangered Species (TES) that make their home on Army training lands. The gopher tortoise (*Gopherus polyphemus*) is one species that the Army is particularly concerned about. To ensure that the Army is able to execute its training mission requirements while mitigating adverse impacts to the gopher tortoise, installation natural resource managers have to track species abundance in a consistent and statistically comparable manner from year to year. This requires that the Army exercise great diligence in identifying methods to derive species abundance that can meet all levels of terrain and vegetative conditions present in a species habitat. The objective of this research was to conduct field tests to evaluate the line transect, total count, sample count, and double observer methods for estimating gopher tortoise burrow abundance and compare them in respect to efficacy, precision, and cost of application. This report contains a discussion of the field tests and recommendations for installation natural resource managers.

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Preface

This report is a deliverable product under military direct-allotted Department of the Army project A896, "Base Facilities Environmental Quality." This work was funded by Headquarters, U.S. Army Corps of Engineers. The Technical Monitor was Scott Belfit, DAIM-ED.

The work was completed under the direction of the Ecological Process Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). The CERL Project Manager was William D. Meyer. The Work Package Manager was Dr. Timothy J. Hayden. The Field Investigation work and resulting report was done by Mr. Saif Z. Noman, Dr. Raymond R. Carthy, and Dr. Madan K. Oli, of the U.S. Geological Survey, Florida Cooperative Fish and Wildlife Research Unit at the University of Florida under MIPR W81EWF32592004. Alan B. Anderson is Chief, CN-N, and Dr. John T. Bandy is Chief, CN. The associated Technical Director was Dr. William D. Severinghaus. The Director of CERL is Dr. Ilker Adiguzel.

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1 Introduction

Background

The Army is very concerned about the preservation of Threatened and Endangered Species (TES) that make their home on Army training lands. The gopher tortoise (*Gopherus polyphemus*) is one species that the Army is particularly concerned about. The gopher tortoise (GT) is a U.S. Fish and Wildlife Service (USFWS) listed TES species in the south-western portion of its range, which extends west from the Tombigbee and Mobile rivers in Alabama, Mississippi, and Louisiana (Carthy et al. 2005). East of these rivers, Federal protection does not extend to the tortoise in the remainder of its home range. These states include the area of Alabama east of the Tombigbee and Mobile Rivers, Georgia, South Carolina, and Florida. Several Army installations within these states have identified GT populations. To ensure that the Army is able to execute its training mission requirements while also preventing any further decline to the GT, installation natural resource managers have to track species abundance in a consistent and statistically comparable manner from year to year.

In 2005, the Engineer Research and Development Center, Construction Engineering Research Laboratory (ERDC-CERL), of the U.S. Army Corps of Engineers commissioned a study of Gopher Tortoise Population Estimation Techniques by the U.S. Geological Survey, Florida Cooperative Fish and Wildlife Research Unit at the University of Florida. The ensuing study evaluated existing techniques to estimate gopher tortoise populations and attempted to illustrate which techniques provided more reliable species abundance estimates under specific terrain and vegetation conditions.

One of the most relevant questions in conservation biology is: *How many are there?* Indeed, estimates of abundance are a prerequisite for listing or delisting of a species, and for monitoring recovery progress (Seber 1982; Cassey and Mcardle 1999; Williams et al. 2002;Carthy et al. 2005). Furthermore, estimates of abundance are needed for understanding density-dependent relationships, for parameterizing and evaluating population models, and for formulating or evaluating effective management programs (Williams et al. 2002).

Objectives

The objectives of this study were to:

1. Determine the accuracy of strip transects, line transects, sample count, and double observer for estimating gopher tortoise burrow density in three habitat types
2. Address detectability issues in sampling and total count methodologies
3. Compile cost estimates for each method in terms of work-hours of labor, and compile cost estimates for other aspects of gopher tortoise inventory, such as burrow scoping.

Approach

Phase one of this work involved Field Tests in which line transects, strip transects, sample counts, and double observer methods were evaluated in three occupied habitats of differing vegetative densities. One habitat was open with excellent visibility, the second was of moderate vegetation density with moderate visibility, and the third contained dense vegetation with limited visibility.

In each habitat, a series of 30- to 60-m wide transects were established. In each transect, burrow density was estimated by a single observer walking the centerline using the line transect and strip transect method. The transects were then completely searched by a team of observers to determine true burrow density. Accuracy of the sample method, which is essentially a total count within the plot, was measured by marking a sample of burrows in the transect in advance of the total count, and recording the number of marked burrows found during the total count. To control for sources of error/variance, replicates of the above techniques were conducted to test for error/variance in the approaches.

In addition to measuring accuracy, the time it takes to execute each method was recorded in terms of work-hours. The costs of burrow scoping, a separate element involved in many tortoise inventories, was measured by recording the time involved in scoping a minimum of 50 burrows. The labor data, together with the accuracy data, was collected to give two perspectives from which to judge the survey techniques, and to make recommendations on which technique is best for the conditions.

The data collected from the sampling techniques will be used to inform simulation models of the methodologies. The modeling approach, using the site-specific data obtained, may allow expansion of the inferences from the field trials and increase their robustness with iterative simulations and varying parameters (transect length, number, orientation, etc.).

Mode of technology transfer

This report has informed an ongoing study to produce guidelines for gopher tortoise inventory and monitoring.

This report will be made accessible through the World Wide Web (WWW) at URL: <http://www.cecer.army.mil>

2 Field Tests

The gopher tortoise is a species of conservation concern in the Southeastern United States. It is federally listed as a threatened species in the western portion of its range (western Alabama, Mississippi, and Louisiana) (Lohoefener and Lohmeier 1984; Federal Register 1987). Several state and Federal agencies in the gopher tortoises' range are charged with monitoring their presence, status, and population trends. Monitoring requires reliable estimates of abundance.

Gopher tortoises spend much of their time in the shelter of self-constructed underground burrows (Wilson et al. 1994), and direct observation of tortoises is difficult. The burrows are approximately 5 m in length. The burrow entrances generally match the dimensions of the occupant and are relatively easy to see due to their half-moon shape and the large mound of sand (commonly referred to as the apron) at the burrow entrance (Carthy et al. 2005). Because gopher tortoises are rarely seen outside their burrows, researchers typically estimate abundance of burrows, and use it as an index of tortoise abundance.

However, it is still not easy to estimate the abundance of animals (Seber 1982; Lohoefener 1990). Two key issues involved in abundance estimation are detectability and spatial sampling (Royle and Nichols 2002; Williams et al. 2002). Most surveys or sampling methods do not result in the detection of all animals present in a study area, so one must estimate detectability (the probability of observing an animal or object if it is present). Once detectability is estimated, one may estimate the population size as:

$$\hat{N} = \frac{C}{\hat{\beta}} \quad (1)$$

where:

$$\begin{aligned} \hat{N} &= \text{estimate of population size} \\ C &= \text{number of animals counted} \\ \hat{\beta} &= \text{estimate of detectability.} \end{aligned}$$

Similarly, a sampling method often cannot be applied to the entire study area due to time and resource limitations. Typically, $\hat{\alpha}$ fraction of the area is sampled and the population size is estimated as:

$$\hat{N} = \frac{C}{\hat{\alpha}} \quad (2)$$

where:

$\hat{\alpha}$ = estimate of the proportion of the area sampled.

Considering detectability ($\hat{\beta}$) and spatial sampling ($\hat{\alpha}$) simultaneously, the estimate of population size is:

$$\hat{N} = \frac{C}{\hat{\alpha}\hat{\beta}} \quad (3)$$

Both detectability and spatial sampling also apply to the estimation of gopher tortoise abundance. It is typically not feasible to survey entire study areas, and not all burrows within the sampled areas are going to be detected. An additional issue involved with the estimation of gopher tortoise abundance is the burrow occupancy. Burrow occupancy is the probability that a burrow is occupied by a gopher tortoise. Since each tortoise may use several burrows within an activity season (McRae et al. 1981), there are generally more burrows than gopher tortoises (Auffenberg and Franz 1982; Breininger et al. 1991; McCoy and Mushinsky 1992; Eubanks et al. 2003). Once the total number of burrows is estimated, managers need to be able to convert this number into an accurate estimate of gopher tortoise abundance using an estimate of occupancy rate.

Carthy et al. (2005) reviewed methods of estimating abundance of gopher tortoise burrows. A brief overview of these methods is presented here.

Line transects

Line transect is a distance sampling method and is statistically robust for estimating abundance (Buckland et al. 2001; Krzysik 2002; Williams et al. 2002). Within each study site, transects are laid out either randomly or systematically at predetermined distances. An observer then walks along the line transects detecting and recording sighting angles and sighting distances, or perpendicular distances of burrows to the line. This method assumes that the detection probability of burrows declines as one moves away from the transect line.

The challenge is to find a function that models probability of detection as a function of the distance of the object from the line. One critical assump-

tion of line transect sampling is that all objects located on the line transect are seen and recorded. There is a relatively high probability that this assumption will be met for gopher tortoise burrows lying close to the line due to the conspicuous nature of the mounds associated with the burrows (Lohoefer 1990; Doonan and Epperson 2001). Recommended sample sizes for distance sampling density estimates are 60 to 80 observations; however, it depends on the desired coefficient of variation (Buckland et al. 2001). The line transect method is useful under the following conditions: (1) survey regions are large, (2) objects close to the observer are easily detectable, (3) objects are sparsely distributed through the survey area, (4) locations of the individuals or objects are easily recorded (Buckland et al. 2001).

Total count

Total count is a comprehensive count of all burrows in an area; the assumptions are that detectability is 100 percent, and that the entire study area is sampled, i.e., $\hat{\alpha} = 1$ and $\hat{\beta} = 1$ (Krebs 1999; Williams et al. 2002). Total counts have the advantage of minimal survey design and setup time since the whole area will be inspected (Carthy et al. 2005). The obvious limitation is that they are impractical when the survey area is larger than a few acres.

Sample count

The sample count method is suitable for any habitat, does not require complex statistics, and if plots are randomly selected, this method results in unbiased estimates. This method uses randomly located plots of predetermined size and can be useful in areas of low population density or high vegetation density (Carthy et al. 2005). The plots are searched by teams of observers who attempt to locate all burrows. Total population size is then estimated by extrapolating proportions of randomly selected plots to the estimated total occupied area.

Double observer

The double observer method was developed by Nichols et al. (2000) to estimate detection probabilities for avian point counts using two observers. The approach is a modification of a method developed by Cook and Jacobson (1979) to estimate abundance using aerial surveys with two observers.

The double observer method uses counts from two observers (primary and secondary) to estimate the number of burrows each observer failed to detect; these data are then used to estimate detection probability and abundance. The observations of the two observers can be independent (detection of the burrow by the primary observer is independent of detection by the secondary observer) or dependent (the secondary observer is aware of the primary observer's detections).

Burrow occupancy rate

Typically, there are more burrows than gopher tortoises. The number of burrows does not always correspond to the number of tortoises, although the two tend to be positively correlated. Thus, a third issue relevant to tortoise abundance estimation is the burrow occupancy rate, which is defined as the probability that a burrow is occupied by a gopher tortoise. Most studies that have attempted to estimate burrow occupancy rate have used burrow cameras (Spillers and Speake 1988).

According to Breininger et al. (1991), who used bucket traps, sticks, burrow excavation, a burrow camera, and an experienced gopher tortoise hunter to estimate gopher tortoise burrow occupancy rates, burrow cameras were the best methods as far as accuracy and cost are concerned. However, several studies (Doonan 1986; Doonan and Epperson 2001) have used the occupancy rate reported by Auffenberg and Franz (1982), who found that 61.4 percent of all apparently active burrows were occupied, as the "correction factor" to convert burrow abundance into tortoise abundance. Old or abandoned burrows were assumed to be empty.

This method does not account for variation in occupancy rate with region, habitat, season, and year (Burke and Cox 1988; Breininger et al. 1991; McCoy and Mushinsky 1992; Moler and Berish 2001), and tends to overestimate gopher tortoise abundance (McCoy and Mushinsky 1992; Moler and Berish 2001). A single value is unlikely to be applicable to all tortoise populations under all circumstances. Several studies have been conducted to estimate occupancy rates, however these studies have not used recently developed statistically robust techniques, such as occupancy modeling (Mackenzie et al. 2002; Royle and Nichols 2002).

Several studies have been conducted to estimate the abundance of gopher tortoises using line transects and other methods (Doonan 1986; Mann 1993; Epperson 1997; Doonan and Epperson 2001), and these studies have been previously reviewed (Carthy et al. 2005). Rigorous field tests of these techniques are needed to evaluate the efficacy of the methods relative to costs.

3 Methods

Study area

This study was conducted in the Katharine Ordway Preserve – Swisher Memorial Sanctuary (<http://www.ordway.ufl.edu>) located in Putnam County, FL (29°41' N and 82° W). The entire area encompasses approximately 4000 hectares. The preserve offers over 1600 hectares of potential gopher tortoise habitat, with old fields, pine plantations, and sand hill habitats of several burn frequency categories (Carthy et al. 2005).

Stratification

The Ordway Preserve sites were stratified into two strata (G5, and C3 & C7) based on habitat maps, burn history, and visual observation. While edaphic conditions ultimately dictate the form of land cover and whether tortoises are able to construct burrows, existing habitat type and land management practices exert proximate effects on the visibility of tortoise burrows. This directly impacts observers' ability to detect burrows; therefore, vegetation density was made the principal criterion for the stratification of the study area.

Stratum G5, comprising management unit G5, covered an area of approximately 110.3 hectares and was last burned in 2003. Stratum C3 & C7, comprising management units C3 and C7, covered an area of approximately 116.5 hectares and was last burned in 2005.

The study was conducted in the two strata to investigate whether the probability of detection of burrows, abundance of burrows, and cost of the methods varied with burn history and habitat type (Buckland et al. 2001).

Field methods

Line transects

A pilot survey of the study area was conducted to estimate the transect length needed to obtain the desired coefficient of variation ($cv(\hat{D})$) of ≤ 15 percent. Two km of transect was sampled, 46 burrows were detected, and the total line length to be sampled (L) was estimated as:

$$L = \left(\frac{b}{\{cv_t(\hat{D})^2\}} \right) \left(\frac{L_0}{n_0} \right) \quad (4)$$

where:

- L = estimate of line length to be sampled
- b = dispersion parameter
- $cv_t(\hat{D})^2$ = desired coefficient of variation for the density estimate
- L_0 = transect length of the pilot study
- n_0 = number of burrows detected in the pilot study.

Burnham and Anderson (2002) recommended $b = 3$ for planning purposes. Using $b = 3$, it was estimated that to achieve a coefficient of variation of 5, 10, 15, and 20 percent, one would need to sample 69.9km, 17.4km, 7.7km, and 4.3km of line transects, respectively. Thus, approximately 8km of total transect length would be required in each stratum to achieve a maximum coefficient of variation of 15 percent.

Eight km of transects were placed in each stratum systematically at predetermined distances. Sufficient spacing (30 to 60 m) between transects was allowed to ensure that burrows would not be double-counted, while providing an adequate sample size for statistically valid results (Buckland et al. 2001). Transects were oriented so that they did not run parallel to roads or other linear topographical features. Sampling line transects parallel to topographic or other linear features can provide data that over- or underestimate burrow density because topography can affect the location of gopher tortoises (Buckland et al. 2001; Williams et al. 2002; Carthy et al. 2005). Flags were placed and GPS coordinates were recorded at the origin and end of all transects.

Two teams, each consisting of an observer and an assistant, walked along each transect line. The first observer identified all burrows detected and the assistant then measured the perpendicular distance from the transect line to the burrow (Nichols et al. 2000; Buckland et al. 2001). This measurement was taken from the transect line to either the burrow's mouth or the beginning of the burrow apron, whichever was closest to the transect line. The assistant recorded the GPS coordinates for the burrow and measured the burrow width. The burrows were classified according to width as: juvenile (<14 cm wide), sub-adult (14 to 23 cm wide) and adult (>23 cm)

(Alford 1980; Smith 1992). Additionally, using external characteristics, the observer classified burrows into one of four burrow status categories:

- *Burrow status 1:* Burrows classified as 1 were considered active burrows. They had burrow aprons and entrances with little or no debris. Additionally, there was evidence of tortoise occupation based on fresh signs such as flat sand at the entrance and inside the burrow (from a tortoise's plastron), footprints on the apron, and/or scat located on the apron or inside the burrow.
- *Burrow status 2:* Burrows ranked as 2 were also considered active burrows. Like burrows classified as 1, these burrows had entrances and aprons clear of debris. However, these burrows lacked fresh signs.
- *Burrow status 3:* Burrows ranked as 3 were inactive burrows. They had small amounts of debris and leaf litter present on the apron, at the mouth, and in the burrow tunnel. The mouth of the burrow and the tunnel were still intact.
- *Burrow status 4:* Burrows ranked as 4 were abandoned burrows. These burrows had large amounts of debris and leaf litter on the apron, at the mouth, and in the tunnel. Burrow mouths were degraded so that they did not have the classic half-moon tortoise shape. Instead they may have been partially collapsed, filled-in, or possibly in use by an armadillo.

The assistant did not participate in detecting burrows, but simply measured the distance for burrows detected by the lead observer. All detected burrows were temporarily marked with a numbered tag so as to avoid double counting of burrows. Tags were hidden from view of the second observer, but were placed in a consistent location at burrows so they were easily located by the second observer's assistant on close examination of the burrows detected by the second observer.

Once the first team completed sampling transects, the same transects were sampled by a second observer. Sampling was conducted independently by the two teams to test for the inter-observer variability in detection probability, estimates of abundance, and cost. Observers from each team were unaware of the results of the other team's observations for a particular transect. The second observer proceeded along the transect in the same direction as the first observer. The assistant for the second observer had access to the data and GPS coordinates from the first team, and recorded data for burrows detected only by the second observer. The presence of a

numbered tag indicated previous detection. Since data for tagged burrows had already been recorded, the assistant removed the tag and recorded the burrow as being detected by the second observer. If a burrow not detected by the first observer was found, the second observer's assistant measured and recorded all pertinent burrow data.

Time taken for the sampling by all observers was recorded in work-hours for determining the cost of the line transect method.

Total count and sample count

A total count of burrows was conducted after the line transect data had been collected. Six 1-hectare plots were selected in each stratum. These plots were overlaid on part of the area where transect sampling had already taken place. The four corners of the plots were flagged and their coordinates were determined using a GPS unit. The plots were further subdivided into 10, 20x50 m subplots and each subplot was comprehensively searched for burrows by two observers. The observers recorded all pertinent information for detected burrows. Initially, three observers were used; however, when the third observer consistently failed to identify any additional burrows, the number of observers was reduced to two.

The field methods for the sample count method and the total count method were identical. Estimates of abundance obtained from the sample count method can vary depending on the proportion of total area sampled and the choice of the sample plots. Thus, the effect of selecting different proportions of plots on estimated abundance was evaluated by using data collected in each plot for the total count method. Each of these plots was thoroughly surveyed by two observers as described previously.

Time taken for sampling by all observers involved was recorded in work-hours for determining the cost of the total count and sample count methods.

Double observer

The dependent double observer method was implemented following Nichols et al. (2000) and Williams et al. (2002). Each of the 20 x 50 m subplots was comprehensively searched for burrows by two dependent observers (primary observer and secondary observer, where the secondary observer

is the dependent observer and is aware of the primary observer's detections). The primary observer surveyed the plots, and flagged and called out burrows detected to the secondary observer. The secondary observer recorded the information and proceeded to survey the plots to detect additional burrows (Nichols et al. 2000). At the completion of sampling of each subplot, the data were comprised of burrows detected by the primary observer and burrows missed by the primary observer, but detected by the secondary observer. Observers alternated roles on consecutive subplots, as recommended by Nichols et al. (2000).

The assumptions for the double observer method are that the detection probability for all animals or objects is equal, there is no observer bias, and that detection probability is the same for the two observers (Nichols et al. 2000). Time taken for data collection for the double observer method was the same as the total count and sample count methods. The total count, sample count, and double observer methods were conducted on the same six 1-hectare plots.

Burrow occupancy rate

To estimate the probability that a burrow is occupied by a gopher tortoise (burrow occupancy rate) researchers conducted burrow occupancy surveys. Due to time and resource limitations, burrow occupancy surveys were conducted only in management unit C3 of stratum C3 & C7. A subsample of burrows from C3 that were marked during the total counts were examined with a burrow camera three times each on consecutive days with positive occupancy status indicated by at least one burrow camera observation of a tortoise. Researchers used the Econo GeoVision, Jr. camera system designed by Marks Products, Inc. for use in borehole and water well systems. Burrows were classified as "empty" if the operator was certain that she/he had reached the end of the burrow and no gopher tortoise was present. Burrows were considered "occupied" only if the operator could identify a gopher tortoise with absolute certainty. Burrow occupancy was considered "undetermined" if the operator could not maneuver the camera to the end of the burrow due to burrow architecture (e.g., dramatic turns or tunnel size) or debris (e.g., leaf litter and/or sand).

The advantage of using burrow cameras for burrow occupancy estimation versus other methods is that cameras can be used in any season, thereby

permitting winter surveying, whereas other methods are only helpful when tortoises are active (approximately 5 to 7 months of the year) (Carthy et al. 2005).

4 Data Analysis

Line transects

Researchers used Program DISTANCE (Thomas et al. 2003) to analyze the line transect data to estimate the abundance of gopher tortoise burrows. DISTANCE obtained the estimated density of gopher tortoise burrows as:

$$\hat{D} = \frac{n}{2wL\hat{P}_a} \quad (5)$$

where:

- $\hat{D}$ = estimated burrow density
- n = number of burrows detected
- w = width of the area sampled on each side of the transect
- L = transect length
- $\hat{P}_a$ = expected proportion of burrows detected.

The estimate for total number of burrows was then estimated as:

$$\hat{N} = \hat{D}A \quad (6)$$

where:

- $\hat{N}$ = estimate for total number of burrows
- A = size of the effective sampling area.

Additionally, DISTANCE estimated the variance of the density estimate as (Buckland et al. 2001):

$$\text{var}(\hat{D}) = \hat{D}^2 \cdot \{[cv(n)]^2 + [cv(\hat{f}(0))]^2\} \quad (7)$$

where:

- $\text{var}(\hat{D})$ = variance of the density estimate
- $cv(n)$ = coefficient of variation of the number of objects detected
- $cv(\hat{f}(0))$ = coefficient of variation of the estimated value of the probability density function of perpendicular distances, evaluated at zero distance.

The standard error of the density estimate ($se(\hat{D})$) was estimated by taking the square root of the variance of the density estimate ($var(\hat{D})$). The 95 percent confidence interval for the density estimate was estimated as:

$$\hat{D} \pm 1.96 \cdot se(\hat{D}) \quad (8)$$

Coefficient of variation of the density estimate ($cv(\hat{D})$) was estimated by dividing the standard error of the density estimate ($se(\hat{D})$) by the estimated burrow density ($\hat{D}$) and multiplying the result by 100.

DISTANCE (Thomas et al. 2003) provides a flexible framework for alternative model parameterization and comparison of various models (Buckland et al. 2001). Researchers ran several different parametric models, each consisting of a key function and a series adjustment term (Buckland et al. 2001), using DISTANCE (Thomas et al. 2003). For each model, DISTANCE calculated Akaike's Information Criterion (AIC) values (Buckland et al. 2001) as:

$$AIC = -2 \cdot \log_e(L_i) + 2q \quad (9)$$

where:

- L_i is the maximized likelihood for the candidate model i
- q is the number of estimated parameters.

Researchers used AIC values for model comparison and selected the model with the lowest AIC value (Burnham and Anderson 2002). Models that are within 2 AIC values of each other can be viewed as statistically equivalent. In such cases they selected the model with the least number of parameters. As recommended by Buckland et al. (2001), they excluded from analysis 5 percent of observations (furthest from the line) to remove obvious outliers.

Additionally, researchers used burrow width as a covariate in DISTANCE and examined its effect on detection probability for Observer 1 in both strata.

Total count and sample count

The estimated abundance of burrows using total count was calculated as the total number of burrows detected by both observers.

Estimates of abundance, coefficient of variation, and cost obtained from the sample count method can vary depending on the proportion of total area sampled and the choice of the sample plots. Thus, researchers evaluated the effects of selecting different proportions of plots on the abundance estimate by using data collected for the total count method. They randomly selected 50 to 100 percent of the plots. Each of these plots was thoroughly surveyed by two observers as described previously. The number of burrows was then extrapolated to the entire sampling area to obtain an estimate of the total number of burrows in the 6 hectares of plots. For analysis purposes, all possible combinations of 3, 4, and 5 out of the 6 plots were extrapolated to the entire sampling area to obtain estimates for the total number of burrows. Sampling 100 percent of the plots (6 out of 6) is equivalent to a total count.

Approximate variance for the estimates for the total number of burrows was calculated as:

$$\text{var}(\hat{N}) = \frac{1}{n} \sum_{i=1}^n (N_i - \bar{N})^2 \quad (10)$$

where:

$$\begin{aligned} \text{var}(\hat{N}) &= \text{variance of estimates for the total number of burrows} \\ n &= \text{number of estimates} \\ N_i &= \text{estimate } i \\ \bar{N} &= \text{mean of estimates.} \end{aligned}$$

The coefficient of variation $cv(\hat{N})$ was calculated as:

$$cv(\hat{N}) = \frac{\sqrt{\text{var}(\hat{N})}}{\bar{N}} \times 100 \quad (11)$$

where:

$$\begin{aligned} cv(\hat{N}) &= \text{coefficient of variation of estimates for total number of burrows} \\ \text{var}(\hat{N}) &= \text{variance of estimates.} \end{aligned}$$

Double observer—Nichols' method

Researchers used the DOBSERV Program (Hines 2000) to analyze the double observer data. The overall detectability (Nichols et al. 2000) was estimated as:

$$\hat{p} = 1 - \frac{x_{12}x_{21}}{x_{22}x_{11}} \quad (12)$$

where:

- $\hat{p}$ = estimate of overall detectability
- x_{i1} = number of burrows detected by observer i in a primary role
- x_{i2} = number of additional burrows detected by observer i in a secondary role.

This estimate of overall detectability ($\hat{p}$) was used to obtain the estimate for the population size for the sampling area ($\hat{N}$) by dividing the total number of burrows detected by all observers ($x_{..}$) by the estimate of detectability ($\hat{p}$) (Nichols et al. 2000).

Researchers divided the estimate of population size ($\hat{N}$) by the sampling area to estimate the burrow density ($\hat{D}$) (burrows per hectare). They multiplied the estimated burrow density ($\hat{D}$) with the area of the stratum to obtain the estimate of burrow abundance in the stratum ($\hat{N}(tot)$).

Double observer—ad hoc method

For comparative purposes, researchers also estimated the detectability ($\hat{p}_i$) of the two observers by dividing the number of burrows detected by observer i in the primary role (n_i) by the number of burrows detected by both observers ($n(Total)$). The data pooled across all plots, and the approximate variance of the detectability for each observer was estimated as:

$$\text{var}(\hat{p}_i) = \frac{(\hat{p}_i)(1 - \hat{p}_i)}{n_i} \quad (13)$$

where:

- $\text{var}(\hat{p}_i)$ = variance of the estimate of detectability for observer i
- $\hat{p}_i$ = estimate of detectability for observer i
- n_i = number of burrows detected by observer i in a primary role.

The approximate 95 percent confidence interval for the estimated detectability for each observer was estimated as:

$$\hat{p}_i \pm 1.96 \cdot \sqrt{\text{var}(\hat{p}_i)} \quad (14)$$

The abundance of burrows in each plot (N (Plot)) was estimated by dividing n_i by $\hat{p}_i$. Researchers then estimated the burrow density per hectare ($\hat{D}$) by dividing (N (Plot)) by the area sampled by observer i in hectares. The total number of burrows in each stratum ($\hat{N}(\text{Total})$) was estimated by multiplying $\hat{D}$ by the effective sampling area of the stratum in hectares. The lower and upper estimates of the 95 percent confidence interval for the estimated burrow density ($\hat{D}$) were estimated by dividing the number of burrows detected by each observer (n_i) by the upper and lower estimates of the 95 percent confidence interval of the detectability estimate ($\hat{p}_i$) respectively, and further dividing the resulting estimates by the area sampled by each observer.

Burrow occupancy rate

Researchers used a statistically robust occupancy modeling approach (Mackenzie et al. 2002) implemented in Program MARK (White and Burnham 1999) to estimate detection probability and burrow occupancy rate. Occupancy rates can be applied to the estimates of burrow abundance to estimate gopher tortoise abundance. Researchers grouped the burrows into two categories: active (burrow status 1 and 2, as defined previously) and inactive (burrow status 3 and 4, as defined previously). For analysis purposes they treated “undetermined” attempts as “empty.” They used Akaike’s information criterion (AIC) to select the most parsimonious model. Using the most parsimonious models identified in the preceding analyses, researchers tested for the effect of the width of the burrow entrance (in cm) on the detection probability and the occupancy rate by modeling the logit of each rate as a linear function of burrow width. If the 95 percent confidence interval for the slope parameter (β) did not include zero (0), the relationship was considered statistically significant (Williams et al. 2002).

Due to time and resource limitations, burrow occupancy surveys were conducted exclusively in management unit C3 of stratum C3 & C7. For analysis purposes researchers assumed that the burrow occupancy rates were the same in both strata (C3 & C7 and G5).

5 Results

Line transects

A total of 28 line transects was placed in stratum G5 with a total length of 8025m (Figure 1). The sampling area on each side of the transect was 27.5m wide (w). The first observer (Observer 1) detected 163 burrows and the second observer (Observer 2) detected 150 burrows). For Observer 1, the model with the lowest AIC was Uniform Cosine (Table 1, Figure 2A). Based on this model, the estimated burrow density ($\pm$ standard error) was 8.58 ± 0.94 burrows per hectare (Table 2) and the extrapolated number of burrows in the stratum was 946.40 with a 95 percent confidence interval of 757.62 to 1183.30. For Observer 2, the model with the lowest AIC was also Uniform Cosine (Table 1, Figure 2B). Based on this model, the estimated burrow density was 8.49 ± 0.98 burrows per hectare (Table 2) and the extrapolated number of burrows in the stratum was 936.25 with a 95 percent confidence interval of 742.18 to 1181.10.

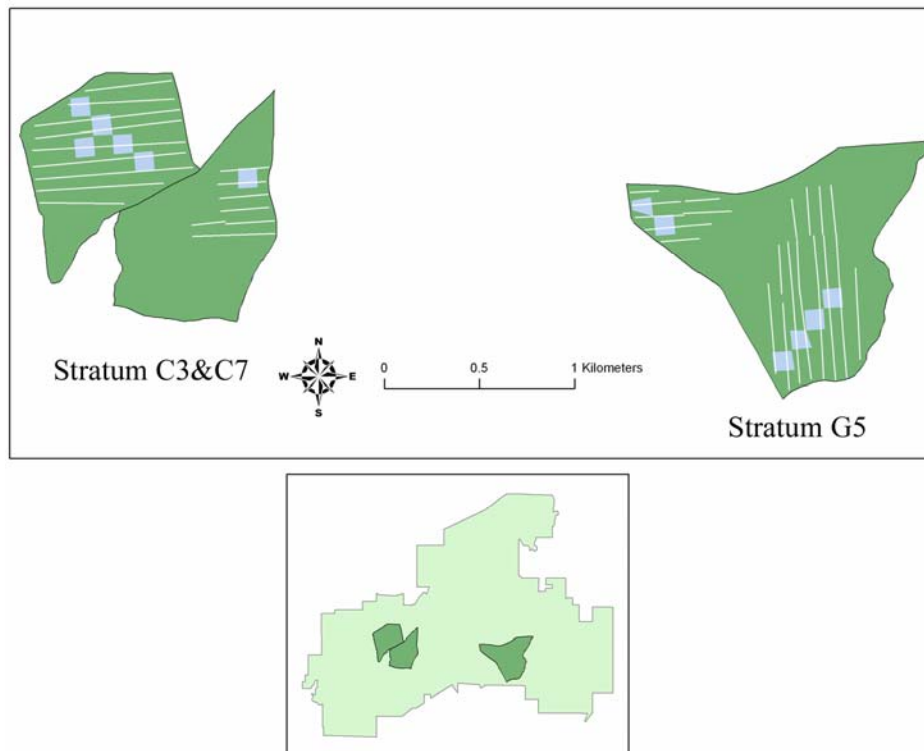


Figure 1. Map of Katharine Ordway Preserve – Swisher Memorial Sanctuary depicting stratum G5 and stratum C3 & C7 and locations of line transects and plots.

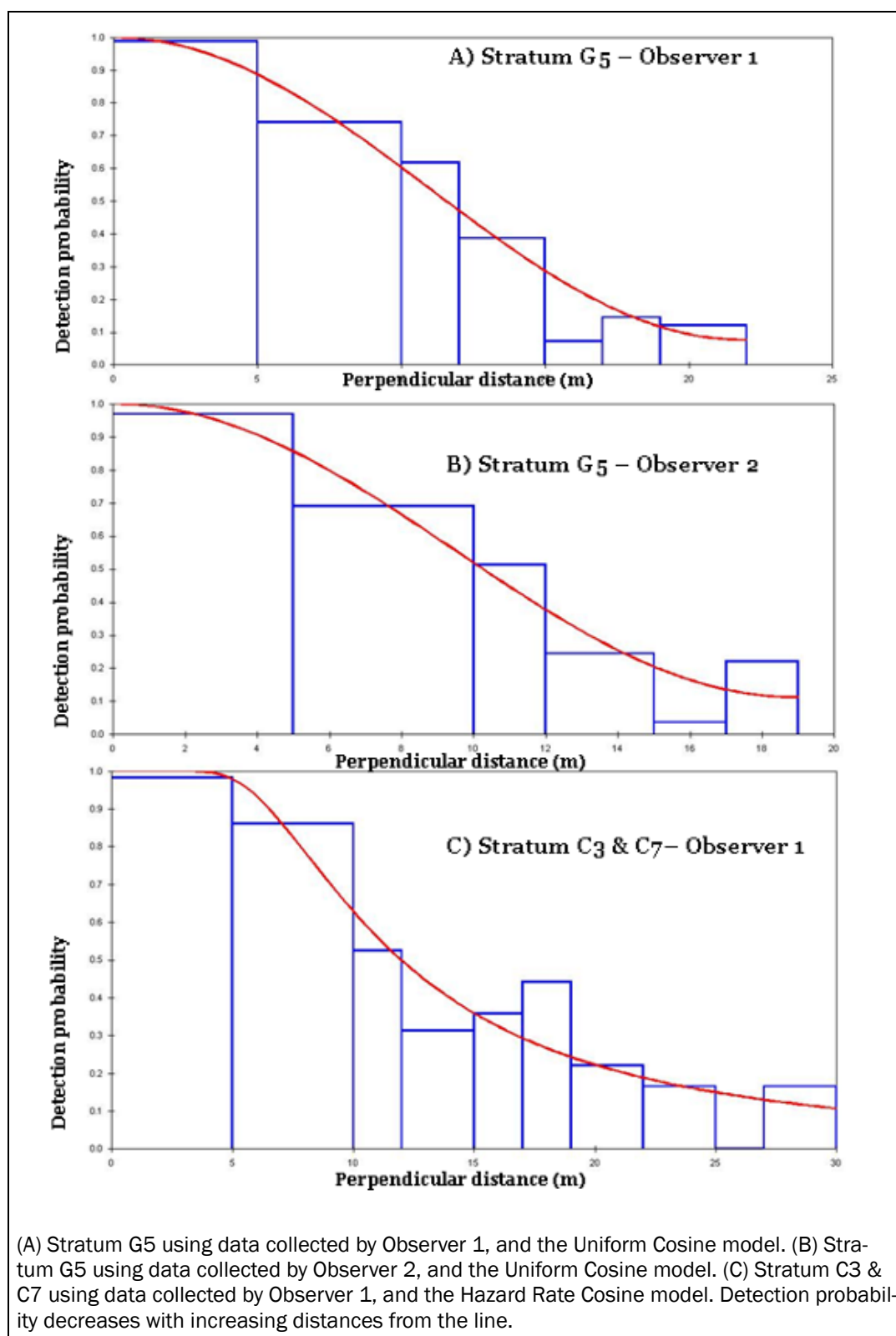


Figure 2. Detection probability plotted against perpendicular distance from burrows to the line transect.

Table 1. Comparison of models fitted to line transect data.

Stratum	Model	Observer 1		Observer 2	
		ΔAIC	Parameters	ΔAIC	Parameters
G5	Uniform Cosine	0	1	0.00	1
	Half Normal Cosine	0.13	1	0.93	1
	Half Normal Hermite	0.13	1	0.93	1
	Uniform Simple Polynomial	1.46	2	0.98	2
	Hazard Rate Cosine	2.22	2	2.31	2
	Hazard Rate Simple Polynomial	2.22	2	2.31	2
C3 & C7	Hazard Rate Cosine	0	2	—	—
	Hazard Rate Simple Polynomial	0	2	—	—
	Uniform Cosine	0.98	2	—	—
	Half Normal Cosine	1.32	2	—	—
	Half Normal Hermite	3.14	1	—	—
	Uniform Simple Polynomial	3.59	3	—	—

For each model the ΔAIC values and the number of parameters (total number of parameters fitted to the line transect data for the candidate models) are presented. ΔAIC is the difference in the AIC (Akaike's Information Criterion) values between each model and the model with the lowest AIC value. For each stratum and each observer, the model with the lowest ΔAIC value and the lowest number of parameters was selected (shown in bold).

Table 2. Estimated burrow density.

Stratum	Observer	$\hat{D}$	95% CI		$cv(\hat{D})$
			Lower	Upper	
G5	Observer 1	8.58	6.87	10.73	11.0%
	Observer 2	8.49	6.73	10.71	11.5%
C3 & C7	Observer 1	11.32	9.19	13.94	10.5%
	Observer 2	—	—	—	—

($\hat{D}$) (burrows per hectare), 95% confidence interval and the coefficient of variation ($cv(\hat{D})$) for each stratum and each observer. The results are based on the most parsimonious model for each stratum and each observer (Table 2).
Note: the line transect data for Observer 2 for stratum C3 & C7 were not analyzed because Observer 2 did not collect data independently of Observer 1.

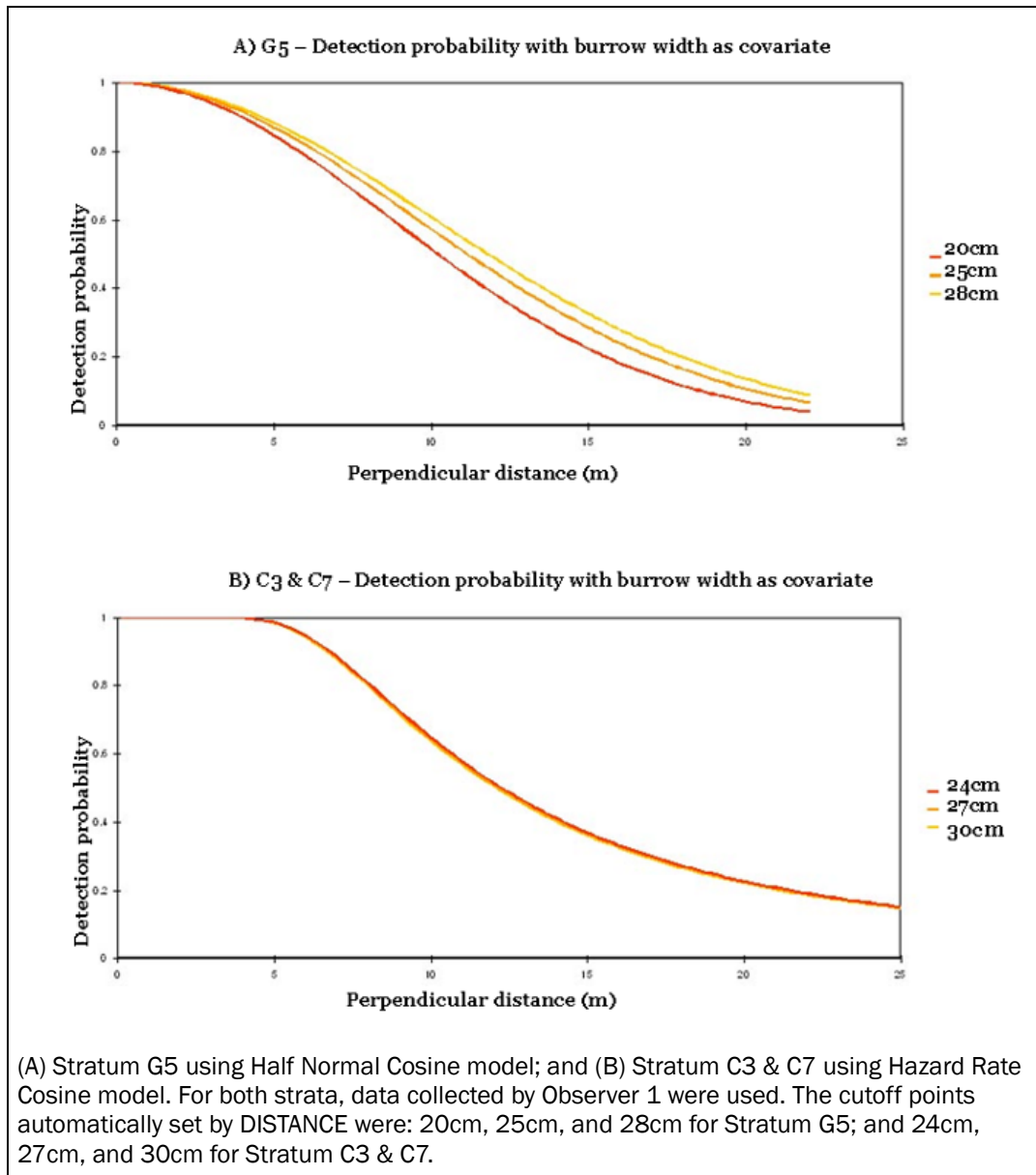


Figure 3. The effect of burrow width on detection probability plotted against perpendicular distance from burrows to the line transect

Researchers evaluated the effect of burrow width on detection probability using the Half Normal Cosine model. Although the probability of detecting smaller burrows was lower than that of detecting larger burrows, the difference appeared insubstantial (Figure 3A).

Sixteen line transects were placed in stratum C3 & C7 with a total length of 8003m. The sampling area on each side of the transect was 30m wide (w). The first observer (Observer 1) detected 262 burrows (Table 3).

Table 3. Data on transects, burrows, and time spent on line transect sampling.

Stratum	Transects		No. of Burrows Detected		Time Spent (work-hrs)	
	# of Transects	Transect Length (m)	Obs 1	Obs 2	Obs 1	Obs 2
G5	28	8025	163	150	18.35	—
C3 & C7	16	8003	262	—	22.17	—
Notes:						
1. The line transect data for Observer 2 for stratum C3 & C7 were not analyzed because Observer 2 did not collect data independently of Observer 1.						
2. Costs for Observer 2 in both strata were not analyzed because Observer 2 did not have to lay out the transects, and measure and record information for burrows that had already been detected by Observer 1.						

For Observer 1, the models with the lowest AIC were Hazard Rate Cosine and Hazard Rate Simple Polynomial (Table 1, Figure 2C). The results for these two models were exactly the same. Researchers selected the Hazard Rate Cosine model. Based on this model, the estimated burrow density was 11.32 ± 1.19 burrows per hectare (Table 3) and the extrapolated number of burrows in the stratum was 1318.31 with a 95 percent confidence interval of 1070.36 to 1623.60. They did not analyze the line transect data for Observer 2 for stratum C3 & C7 because Observer 2 did not collect data independently of Observer 1.

Researchers evaluated the effect of burrow width on detection probability using the Hazard Rate Cosine model. The burrow width did not seem to influence detection probability; the probabilities of detecting burrows in three different width categories were almost identical (Figure 3B).

Most of the burrows detected in both G5 (58.1%, $N = 167$) and C3 & C7 (76.5%, $N = 264$) were adult burrows ($>23\text{cm}$) (Figure 4).

Total count

In stratum G5, the total number of burrows in the 6 hectares sampled (six 1-hectare plots) was 68, with a density of 11.33 burrows per hectare; the extrapolated estimate for the stratum was 1249.44 burrows (Table 4).

In stratum C3 & C7, the total number of burrows in the 6 hectares sampled (six 1-hectare plots) was 78, with a density of 13.00 burrows per hectare; the extrapolated estimate for the stratum was 1514.09 burrows (Table 4).

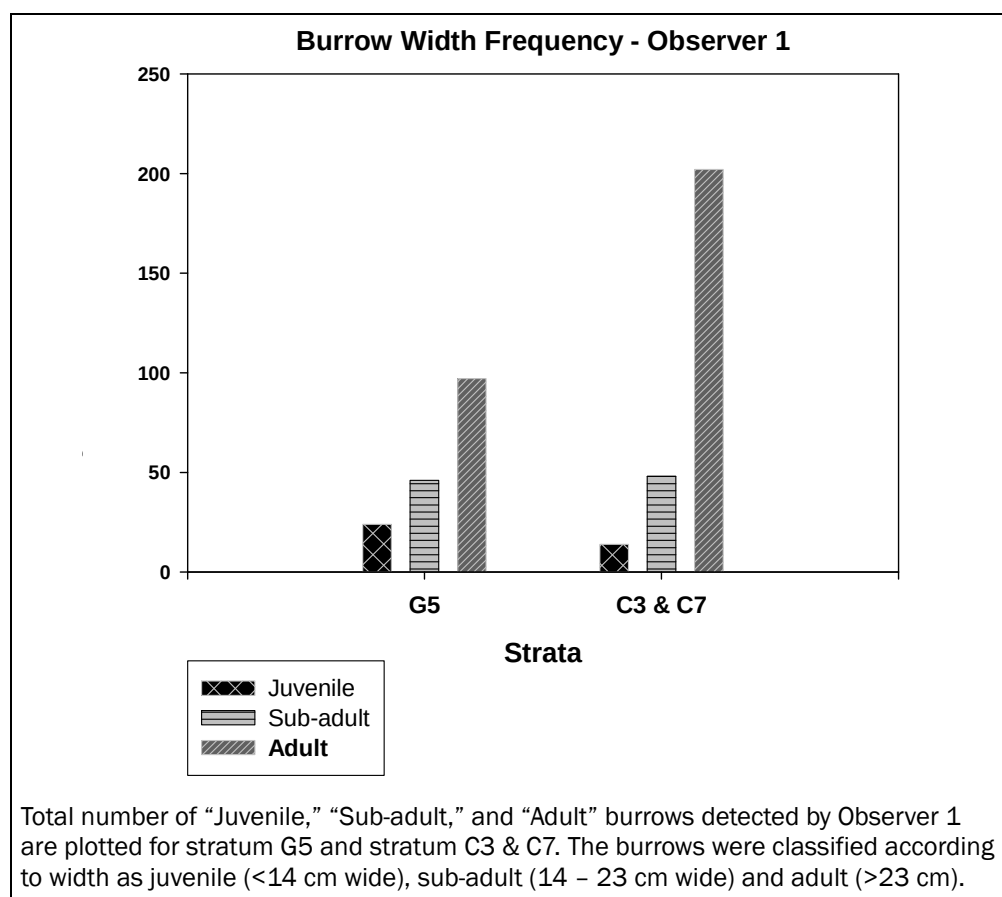


Figure 4. Frequency of burrow widths for burrows detected by Observer 1 in all strata.

Sample count

Table 4 and Figure 5A give the estimated abundance of burrows based on the sample count method for stratum G5 is presented. When 50 percent of the plots (3 out of 6) were sampled, the extrapolated number of burrows in the sampling area ranged from 48 to 88 burrows, burrow density ranged from 8 to 14.66 burrows per hectare, and the extrapolated estimate for the stratum ranged from 882.21 to 1616.66 burrows. When 66 percent of the plots (4 out of 6) were sampled, the extrapolated number of burrows in the sampling area ranged from 54 to 81, the burrow density ranged from 9 to 13.5 burrows per hectare, and the extrapolated estimate for the stratum ranged from 992.49 to 1488.74 burrows. When 88 percent of the plots (5 out of 6) were sampled, the extrapolated number of burrows in the sampling area ranged from 60 to 74.4, the burrow density ranged from 10 to 12.4 burrows per hectare, and the extrapolated estimate for the stratum ranged from 1102.77 to 1367.43 burrows.

Table 4. Estimates of gopher tortoise burrow density based on sample count method for each stratum.

Stratum	Proportion Sampled	Plots Sampled	$\hat{N}$	$SE(\hat{N})$	$\text{var}(\hat{N})$	$cv(\hat{N})$	$\hat{D}$ (per hectare)
G5	50%	3	48–88	0.54	117.9	16.0%	8.00–14.66
	66%	4	54–81	0.52	60.0	11.4%	9.00–13.50
	83%	5	60–74.4	0.86	26.9	7.6%	10.00–12.40
	100%	6	68	—	—	—	11.33
C3 & C7	50%	3	64–92	0.36	53.1	10.7%	10.70–15.30
	66%	4	69–87	0.35	27.0	7.6%	11.50–14.50
	83%	5	73.2–82.8	0.58	12.1	5.1%	12.20–13.80
	100%	6	78	—	—	—	13.00

The number of burrows detected in 50–83% of the plots was extrapolated to the entire sampling area to obtain estimates for the total number of burrows. “Sampling Proportion” is the proportion of plots sampled out of the 6 total plots in each stratum, $\hat{N}$ is the estimated number of burrows in the sampling area, $SE(\hat{N})$ is the standard error, $\text{var}(\hat{N})$ is the variance, $cv(\hat{N})$ is the coefficient of variation, and $\hat{D}$ is the estimated burrow density (burrows per hectare). The estimates of $\hat{N}$, $\hat{D}$, and $\text{var}(\hat{N})$ varied depending on the plots chosen. To capture this variance $\hat{N}$ and $\hat{D}$ were estimated based on all possible combinations of 3, 4 and 5 plots out of 6.

Note: Sampling 100% of the plots (6 out of 6) is equivalent to a total count.

Table 4 and Figure 5B present the estimated abundance of burrows based on the sample count method for stratum C3 & C7. When 50 percent of the plots (3 out of 6) were sampled, the extrapolated number of burrows in the sampling area ranged from 64 to 92 burrows, the burrow density ranged from 10.7 to 15.3 burrows per hectare, and the extrapolated estimates for the stratum ranged from 1246.24 to 1781.97 burrows. When 66 percent of the plots (4 out of 6) were sampled, the extrapolated number of burrows in the sampling area ranged from 69 to 87, the burrow density ranged from 11.5 to 14.5 burrows per hectare, and the extrapolated estimates for the stratum ranged from 1339.39 to 1688.79 burrows. When 88 percent of the plots (5 out of 6) were sampled, the extrapolated number of burrows in the sampling area ranged from 73.2 to 82.8, the burrow density ranged from 12.2 to 13.8 burrows per hectare, and the extrapolated estimates for the stratum ranged from 1420.92 to 1607.27 burrows.

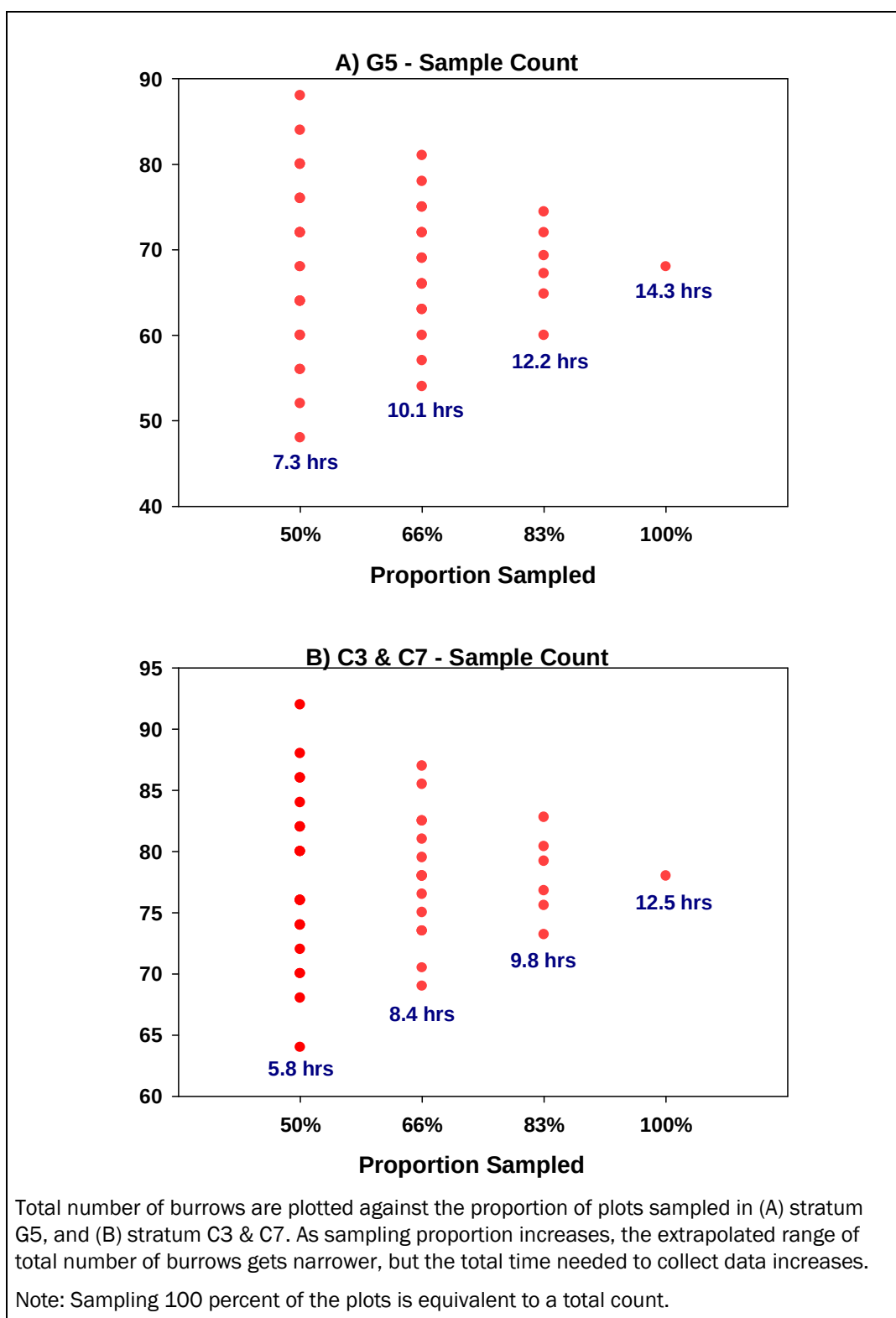


Figure 5. Effects of the proportion of plots sampled using sample count method on estimates of abundance and cost.

Double observer – Nichols’ method

In stratum G5, the estimated overall detectability ($\hat{p}$) was 1.000, total number of burrows detected ($x_{..}$) was 68, and the estimated number of burrows in the sampling area ($\hat{N}$) was 68 (Table 5). Based on these results, the estimated burrow density was 11.33 burrows per hectare, and the estimated number of burrows for the stratum (110.28 hectares) was 1249.44. Since the estimated overall detectability was 1.000, standard error or 95 percent confidence interval could not be estimated.

In stratum C3 & C7, the estimated overall detectability ($\hat{p}$) was 0.997 ± 0.003 , total number of burrows detected ($x_{..}$) was 78, and the estimated number of burrows in the sampling area ($\hat{N}$) was 78.23 ± 0.53 with a 95 percent confidence interval of 78.02 to 81.33 (Table 5). Based on these results the estimated burrow density was 13.04 burrows per hectare, with a 95 percent confidence interval of 13.00 to 13.56, and the estimated number of burrows for the stratum (116.47 hectares) was 1518.75.

Table 5. Estimates of overall detectability and abundance using the Nichols’ double observer method for both strata.

Stratum	$x_{..}$	Detection Probability		Abundance					
		$\hat{p}$	$SE(\hat{p})$	$\hat{N}$	$SE(\hat{N})$	$\hat{D}$	95% CI ($\hat{D}$)		$\hat{N}(tot)$
							Lower	Upper	
G5	68	1.000	—	68.00	—	11.33	—	—	1249.44
C3 & C7	78	0.997	0.003	78.23	0.53	13.04	13.00	13.56	1518.75

$x_{..}$ is the total number of burrows detected by both observers, $\hat{p}$ is the estimate of overall detectability, $SE(\hat{p})$ is the estimate of standard error for overall detectability, $\hat{N}$ is the estimated number of burrows in the sampling area, $SE(\hat{N})$ is the estimate of standard error, $\hat{D}$ is the estimated burrow density (burrows per hectare), 95% CI ($\hat{D}$) is the 95% confidence interval for the estimated burrow density, and $\hat{N}(tot)$ is the estimated population size in the stratum based on the area of the stratum.

Note: There is no standard error and 95% confidence interval for stratum G5 because the estimate of overall detectability ($\hat{p}$) was 1.

Double observer – ad hoc method

In stratum G5, the estimate of detectability for Observer 1 ($\hat{p}_1$) was 1.00, and the extrapolated estimated burrows for the stratum was 1029 (Table 6). Standard error and 95 percent confidence interval could not be estimated because $\hat{p}_1$ was 1.00. In stratum C3 & C7, the estimate of detectability for Observer 1 ($\hat{p}_1$) was 0.97 ± 0.03 , and the extrapolated estimated burrows for the stratum was 1320 (Table 6).

In stratum G5, the estimate of detectability for Observer 2 ($\hat{p}_2$) was 0.95 ± 0.04 , and the extrapolated estimated burrows for the stratum was 1470 (Table 6). In stratum C3 & C7, the estimate of detectability for Observer 2 ($\hat{p}_2$) was 0.93 ± 0.04 , and the extrapolated estimated burrows for the stratum was 1708 (Table 6). The double observer–ad hoc method indicated that detection probability differed between the two observers; consequently, estimated abundance of burrows also differed substantially between the observers.

Researchers extrapolated estimates for the total number of burrows for each stratum ($\hat{N}$) for each abundance estimation method and compared the estimates from the various methods (Table 7).

Burrow occupancy rate

The most parsimonious model indicated that the detection probability (probability of observing a gopher tortoise if it was in the burrow) was 0.92 ± 0.04 and did not differ between active (burrow status 1 and 2, as defined previously) and inactive (burrow status 3 and 4, as defined previously) burrows. However, the occupancy rates (the probability that a burrow is occupied by a tortoise) were significantly different between the two groups (active: 0.50 ± 0.09 ; inactive: 0.04 ± 0.04). The width of the burrow entrance did not significantly influence the occupancy rate or the detection probability. Burrow occupancy status could not be determined for 25 percent ($N = 168$) of the burrows examined.

Table 6. Estimated detection probability () for each stratum and observer using the double observer ad hoc method.

Stratum		Burrows Detected		$\hat{p}_i$	Detection Probability			Abundance				
		n_i	$n(tot)$		$SE(\hat{p}_i)$	95% CI ($\hat{p}_i$)		$N(Plot)$	$\hat{D}$	95% CI ($\hat{D}$)		$\hat{N}(tot)$
Obs 1	G5	28	28	1.00	—	—		28	9.33	—		1029
	C3 & C7	33	34	0.97	0.03	0.9129	1.0000	34	11.33	11.00	12.05	1320
Obs 2	G5	38	40	0.95	0.04	0.8807	1.0000	40	13.33	12.67	14.40	1470
	C3 & C7	41	44	0.93	0.04	0.8547	1.0000	44	14.67	13.67	15.99	1708

n_i is the number of burrows detected by Observer i in a primary role, $n(tot)$ is the number of burrows detected by both observers, $\hat{p}_i$ is the estimated detection probability for Observer i , $N(Plot)$ is the number of burrows in the plot, $\hat{D}$ is the estimated burrow density in burrows per hectare, $\hat{N}(tot)$ is the estimated total number of burrows in the entire stratum based on the area of the stratum, $SE(\hat{p}_i)$ is the estimate of standard error, 95% CI ($\hat{p}_i$) is the 95% confidence interval of the estimated detection probability ($\hat{p}_i$), and 95% CI ($\hat{D}$) is the 95% confidence interval of estimated burrow density ($\hat{D}$).

Notes:

1. The total area of stratum G5 is approximately 110.28 hectares and the total area of stratum C3 & C7 is approximately 116.47 hectares.
2. There were a total of 6 1-hectare plots in each stratum and the area sampled by each observer in each plot was 0.5 hectares.

Standard error and 95% confidence interval for stratum G5 for Observer 1 could not be estimated because $\hat{p}_1 = 1.00$.

Table 7. Overall summary of density estimates of gopher tortoise burrows for each method in both strata.

Method		Model	$\hat{D}$	95% CI		cv	$\hat{N}(tot)$
				Lower	Upper		
G5	Line Transect						
	Obs1	Uniform Cosine	8.58	6.87	10.73	11.0%	946.40
	Obs2	Uniform Cosine	8.49	6.73	10.71	11.5%	936.25
	Sample Count	50% *	8.00–14.66**	—	—	16.0%	882.21–1616.66**
		66% *	9.00–13.50**	—	—	11.4%	992.49–1488.74**
		83% *	10.00–12.40**	—	—	7.6%	1102.77 – 1367.43**
	Double Observer	Nichols' Method	11.33	—	—	—	1249.44
	Obs1	Ad Hoc Method	9.33			—	1029.00
	Obs2	Ad Hoc Method	13.33	12.67	14.40	—	1470.00
	Total Count	100% *	11.33	—	—	—	1249.44
C3 & C7	Line Transect						
	Obs1	Hazard Rate Cosine	11.32	9.19	13.94	10.5%	1318.31
	Obs2	—	—	—	—	-	
	Sample Count	50% *	10.67–15.33**	—	—	10.7%	1246.21 – 1781.97**
		66% *	11.50–14.50**	—	—	7.6%	1339.39 – 1688.79**
		83% *	12.20–13.80**	—	—	5.1%	1420.92 – 1607.27**
	Double Observer	Nichols' Method	13.04	—	—	—	1518.75
	Obs1	Ad Hoc Method	11.33	11.00	12.05	—	1320.00
	Obs2	Ad Hoc Method	14.67	13.67	15.99	—	1708.00
	Total Count	100%	13.00				1514.09
* Proportion of plots sampled.							
** Range of estimates.							

Abundance of gopher tortoises

Using these occupancy rates for active and inactive burrows, and based on the proportion of active and inactive burrows detected in both strata, researchers estimated the abundance of gopher tortoises (Table 8).

For stratum G5, with the line transect method, the estimated abundance of gopher tortoises for Observer 1 and Observer 2 was 227.02 and 229.82 tortoises, respectively. Based on the total count method, the extrapolated number of gopher tortoises in the stratum was 210.57 tortoises. Based on the sample count method, when 50 percent of the plots were sampled, the extrapolated number of gopher tortoises in the stratum ranged from 148.68 to 272.45 tortoises. When 66 percent of the plots were sampled, the extrapolated number of gopher tortoises in the stratum ranged from 167.26 to 250.90 tortoises. When 88 percent of the plots were sampled, the extrapolated number of gopher tortoises in the stratum ranged from 185.85 to 230.45 tortoises. Based on the double observer—Nichols' method, the extrapolated number of gopher tortoises was 210.57 tortoises. Based on the double observer – ad hoc method, the extrapolated number of gopher tortoises for Observer 1 and Observer 2 was 170.14 and 247.74 tortoises, respectively (Table 8).

For stratum C3 & C7, using the estimates of the number of burrows obtained from the line transect method, the estimated abundance of gopher tortoise for Observer 1 was 320.74 tortoises. Based on the total count method, the extrapolated number of gopher tortoises in the stratum was 310.58 tortoises. Based on the sample count method, when 50 percent of the plots were sampled, the extrapolated number of gopher tortoises in the stratum ranged from 255.63 to 365.56 tortoises. When 66 percent of the plots were sampled, the extrapolated number of gopher tortoises in the stratum ranged from 274.74 to 346.42 tortoises. When 88 percent of the plots were sampled, the extrapolated number of gopher tortoises in the stratum ranged from 291.47 to 329.70 tortoises. Based on the double observer—Nichols' method, the extrapolated number of gopher tortoises was 311.54 tortoises. Based on the double observer – ad hoc method, the extrapolated number of gopher tortoises for Observer 1 and Observer 2 was 270.77 and 350.36 tortoises, respectively (Table 8).

Table 8. The estimated number of gopher tortoises for both strata.

Method			Burrow Abundance		Gopher Tortoise Abundance			
			$\hat{N}_B$ (Active)	$\hat{N}_B$ (Inactive)	$\hat{N}_{GT}$ (Active)	$\hat{N}_{GT}$ (Inactive)	$\hat{N}_{GT}(tot)$	
G5	Line Transect	Obs1	411.23	535.16	205.62	21.41	227.02	
		Obs2	418.191681	518.058351	209.0958405	20.72	229.8181745	
	Sample Count	50%	246.5–451.71	635.71–1164.95	123.25–225.86	25.43–46.60	148.68–272.45	
		66%	277.31–415.97	715.18–1072.77	138.66–207.99	28.61–42.91	167.26–250.90	
		83%	308.15–382.08	794.64–985.36	154.06–191.04	31.79–39.41	185.85–230.45	
	Double Observer: Nichols' Method			349.11	900.33	174.55	36.01	210.57
	Double Observer: Ad Hoc Method	Obs1	287.51	659.62	143.76	26.38	170.14	
		Obs2	410.74	1059.26	205.37	42.37	247.74	
	Total Count		100%	349.11	900.33	174.55	36.01	210.57
C3 & C7	Line Transect	Obs1	582.62	735.69	291.31	29.43	320.74	
		Obs2	—	—	—	—	—	
	Sample Count	50%	447.36–639.68	798.85–1142.92	223.68–319.84	31.95–45.72	255.63–365.56	
		66%	480.81–606.23	858.58–1082.56	240.40–303.12	34.34–43.30	274.74–346.42	
		83%	510.07–576.97	910.84–1030.30	255.04–288.49	36.43–41.21	291.47–329.70	
	Double Observer: Nichols' Method			545.19	973.56	272.60	38.94	311.54
	Double Observer: Ad Hoc Method	Obs1	473.85	846.15	236.92	33.85	270.77	
		Obs2	613.13	1094.87	306.56	43.79	350.36	
	Total Count		100%	543.52	970.57	271.76	38.82	310.58

$\hat{N}_B$ (Active) and $\hat{N}_B$ (Inactive) are the estimates for the total number of active and inactive burrows in the stratum, $\hat{N}_{GT}$ (Active) and $\hat{N}_{GT}$ (Inactive) are the estimated numbers of gopher tortoises in active and inactive burrows, respectively, and $\hat{N}_{GT}(tot)$ is the estimated total number of gopher tortoises in all burrows in the stratum.

Notes:

- Burrows with Status “1” and “2” were considered “Active” burrows. Burrows with Status “3” and “4” were considered “Inactive” burrows.
- Burrow occupancy surveys were conducted only in stratum C3. For these analyses, it was assumed that the occupancy rates for burrows (0.50 for active burrows and 0.04 for inactive burrows) were the same in both strata (C3 & C7 and G5).
- The line transect data for Observer 2 for stratum C3 & C7 were not analyzed because Observer 2 did not collect data independently of Observer 1.
- Estimates for $\hat{N}_B$ (Active) and $\hat{N}_B$ (Inactive) were based on the proportion of active and inactive burrows in each stratum.
- For stratum G5, the proportion of active and inactive burrows detected using the line transect method for Observer 1 was 0.43 and 0.57, respectively. For Observer 2, it was 0.45 and 0.55 respectively. The proportion of active and inactive burrows detected using the total count methods was 0.28 and 0.72, respectively.
- For stratum C3 & C7, the proportion of active and inactive burrows detected using the line transect method for Observer 1 was 0.44 and 0.56, respectively. The proportion of active and inactive burrows detected using the total count methods was 0.36 and 0.64, respectively.
- For the sample count method, all possible combinations of 50–83% of the plots were selected and the number of burrows detected was extrapolated to the entire sampling area to obtain estimated total number of burrows.

Costs

Line transects

For stratum G5, the effective area sampled using the line transect method encompassed 35.3 hectares and data collection took 18.35 work-hours for Observer 1, with a mean sampling time of 0.52 work-hours per hectare (Table 9).

For stratum C3 & C7, the effective area sampled using the line transect method encompassed 48 hectares and data collection took 22.17 work-hours for Observer 1, with a mean sampling time of 0.46 work-hours per hectare (Table 9).

Costs for Observer 2 in both strata were not analyzed because Observer 2 did not have to lay out the transects, and measure and record information for burrows that had already been detected by Observer 1. Additionally, for stratum C3 & C7, Observer 2 did not collect data independently of Observer 1.

Total count and sample count

For stratum G5, the effective area sampled using the total count and sample count methods encompassed 6 hectares, and took a total time of 14.30 work-hours to sample, with a mean sampling time of 2.38 work-hours per hectare.

For stratum C3 & C7, effective area sampled using the total count and sample count methods encompassed 6 hectares and took a total time of 12.50 work-hours to sample, with a mean sampling time of 2.08 work-hours per hectare.

In both strata, the total effective area sampled using the line transect method was much greater than the effective area sampled using the other methods. Thus, the line transect method may have captured the spatial variability in the study area, which could explain some of the differences in the density estimates between the two methods (Table 9).

The cost for the sample count method was calculated by multiplying the proportion of the plots sampled with the cost for the total count method.

For stratum G5, the amount of time needed to sample 50 to 83 percent of the sample plots ranged from 7.15 to 11.87 work-hours. The rate of sampling ranged from 1.19 to 1.98 work-hours per hectare (Table 9).

For stratum C3 & C7, the amount of time needed to sample 50 to 83 percent of the sample plots ranged from 6.25 to 10.38 work-hours. The rate of sampling ranged from 1.04 to 1.73 work-hours per hectare (Table 9).

Costs of implementing the double observer methods (both Nichols' and ad hoc methods) were identical to the cost for the sample count and total count methods.

Burrow occupancy rate

The cost of sampling burrows with a burrow camera to determine occupancy status was 0.16 work-hrs per burrow camera observation. A total of 168 burrow camera observations were performed (three times for each of the 56 burrows scoped) requiring a total time of 26.88 work-hrs.

Table 9. Cost of implementing each method of abundance estimation for both strata.

	Method	Effective Sampling Area (ha)	Time Spent (work-hrs)	Cost (work-hrs/ha)
G5	Line Transect	35.3	18.35	0.52
	Sample Count (50%)	3	7.15	1.19
	Sample Count (66%)	4	9.44	1.57
	Sample Count (83%)	5	11.87	1.98
	Total Count (100%)	6	14.30	2.38
C3 & C7	Line Transect	48	22.17	0.46
	Sample Count (50%)	3	6.25	1.04
	Sample Count (66%)	4	8.25	1.38
	Sample Count (83%)	5	10.38	1.73
	Total Count (100%)	6	12.50	2.08

Overall costs are presented here for each method (along with proportion of plots sampled for the sample count and total count methods). "Area" indicates the area sampled (in hectares) using the method. "Time Spent" indicates the time spent sampling (in work-hours) by all observers using the method. "Cost" indicates the average number of work-hours spent sampling by all observers per hectare.

Notes:

1. Costs for Observer 2 in both strata were not analyzed because Observer 2 did not have to lay out the transects, and measure and record information for burrows that had already been detected by Observer 1. Additionally, for stratum C3 & C7, Observer 2 did not collect data independently of Observer 1.
2. The cost for the sample count method was calculated by multiplying the proportion of the plots sampled with the cost for the total count method.

6 Discussion

To monitor the population status and for appropriate recovery efforts for gopher tortoises, managers need good ways of estimating abundance. Over large areas, it is not feasible to conduct total counts of gopher tortoise burrows due to the great amount of time, manpower, and money required. Several methods have been used to estimate the abundance of gopher tortoises; however, these methods vary with respect to statistical rigor, accuracy, precision, and cost. Given the pivotal role of gopher tortoises in ecosystems where they are found, it is essential to use rigorous methods for estimating and monitoring tortoise abundance.

The objective of this research was to field-test the efficacy of line transect, total count, sample count, and double observer methods for estimating gopher tortoise burrow abundance. Researchers applied these methods to estimate burrow abundance in two strata in the Katharine Ordway Preserve – Swisher Memorial Sanctuary and compared the precision and cost of these methods. Additionally, they addressed the issue of gopher tortoise burrow occupancy, and used estimates of burrow abundance and occupancy rates to estimate abundance of gopher tortoises.

In the dense vegetation stratum (G5), the estimated burrow density using the line transect method for both observers (8.58 and 8.49 burrows per hectare, respectively) was nearly 3 burrows per hectare less than burrow density of 11.33 burrows per hectare obtained from the total count method. Estimates based on the total count method did not fall within the 95 percent confidence intervals of those obtained from line transect method (6.87 to 10.73 for Observer 1, and 6.73-10.71 for Observer 2). In the sparse vegetation stratum (C3 & C7), the estimated burrow density using the line transect method (11.32 burrows per hectare) was closer to the burrow density using the total count method (13.00 burrows per hectare). The total count number fell within the line transect 95 percent confidence interval of 9.19 to 13.94.

Mann (1993) compared estimates of tortoise burrow abundance obtained from line transect and total count methods, and found that the line transect method overestimated burrow abundance by as much as 49 percent in 13 sites and 32 percent on 7 sites. Results from similar studies suggest a tendency for line transects to overestimate abundance when compared to

total counts (Doonan 1986; Epperson 1997; Doonan and Epperson 2001). However, none of these studies convincingly demonstrated that probability of burrow detection was 1.0 (Carthy et al. 2005). It is therefore possible that the observed differences in estimates of burrow density between the line transect and total count methods could have been because of the violation of the assumption of total count method that all burrows were detected. Researchers used ≥ 2 observers to thoroughly search the sampling area, and ensured that detection probability was 1.0 or nearly so. The results contradict the claims of aforementioned studies that the line transect method tends to overestimate burrow. In fact, estimates of burrow abundance were lower than those obtained from the total count in stratum (G5); they did not differ significantly in stratum C3 & C7. These results indicate that the line transect method does not suffer from systematic bias as suggested by some authors.

One possible explanation for the difference in the estimated burrow abundance using the line transect and total count methods could be that the area sampled using the line transect method was much larger than the area sampled using the total count method. Therefore, the line transect method likely captured a greater amount of spatial variability in distribution and abundance burrows in the study area.

Using the sample count method, as the sampling proportion increased, the range of extrapolated estimates for burrow density became narrower, and the coefficient of variation decreased. However, there was a cost tradeoff in that the more time needed to collect the data (Figures 5A and 5B). Based on the double observer results, it would be possible to use only one observer and achieve detectability close to or equal to one, and thereby save on cost.

It has been recommended that sample sizes for distance sampling density estimates should be between 40 to 80 observations (Lohoefer 1990; Buckland et al. 2001). In this study, using the line transect method, each observer detected at least 150 burrows in both strata. Additionally, it has been suggested that coefficients of variation should not exceed 33 percent for tortoise density studies (Lohoefer 1990). All but one of the methods in our study gave us coefficients of variation less than 15 percent (Table 7), thus it is not expected that sampling variance adversely affected the reliability of the estimates of abundance.

Vegetation density and habitat type also affected the cost of the methods. The sparse vegetation stratum (C3 & C7) had a lower relative cost of sampling for all the abundance estimation methods. Lohoefer (1990) noted that the line transect method was probably the most cost effective method for estimating burrow abundance. In this study, sample counts and total counts were substantially more costly than line transects in both strata. Detection time may be substantially less in sparse vegetation (Lohoefer and Lohmeier 1984; Burke and Cox 1988; Diemer 1992) and prescribed burns prior to sampling may further reduce cost of sampling (Smith 1992; Mann 1993; Moler and Berish 2001).

Similar to previous studies (Epperson 1997; Marques et al. 2001), the estimates of burrow density varied with habitat type and burn frequency. Density estimates obtained from all abundance estimation methods were higher in stratum C3 & C7, which had comparatively sparse vegetation and was recently burned. The higher density of burrows and tortoises in stratum C3 & C7 likely indicates that this stratum offered a better habitat for the tortoises.

For burrow occupancy, the method employed gave estimates substantially lower than Auffenberg and Franz's commonly used 'correction factor' of 61.4 percent (Auffenberg and Franz 1982). Note, however, that burrow occupancy surveys were conducted only in management unit C3 of stratum C3 & C7, and empirical estimates of burrow occupancy rates for stratum G5 were not available. For analysis purposes researchers assumed that the burrow occupancy rate was the same in both strata (C3 & C7 and G5). This assumption needs to be further tested since occupancy rates may vary with habitat due to the ecological needs of gopher tortoises. For example, there may be less of a preferred forage type in the dense vegetation stratum (stratum G5) and tortoises could increase their movements, and increase the number of burrows they use in exploiting larger home ranges, leading to inaccurate estimates of abundance.

The total count and sample count methods were relatively straightforward to implement, and require no sophisticated software for data analyses. However, these methods are costly, particularly when a substantial proportion of the sites needed to be sampled. Moreover, these methods do not offer rigorous estimates of precision or meaningful ways of statistical inferences. The double observer method partially addressed some of these concerns by providing estimates of precision (when detectability is less than one), but is costly to implement.

The line transect method was the least costly of the methods and it was possible to sample a larger effective area with the same effort. The method is considered statistically rigorous and robust (Buckland et al. 2001; Krzysik 2002; Williams et al. 2002), and provides statistical measures of precision. However, a good understanding of the method and some understanding of underlying theory and working knowledge of program DISTANCE is needed for effective implementation of this method.

7 Recommendations

Burrow abundance

Among other factors, the selection of an abundance estimation method should consider the habitat type of the study area, and available time and resources (Ellis and Bernard 2005). With a stratified sampling design, and an adequate sample size, the line transect method is perhaps the most appropriate method because: (1) it is less costly than total counts and sample counts, (2) it is more likely to capture a wider range of spatial variation in the distribution and abundance of burrows, (3) it offers statistically robust estimates of measures of precision, and (4) it provides a flexible framework for evaluating effects of covariates on estimates of abundance.

Transects should be oriented so that they do not run parallel to roads or other linear topographical features. Sampling line transects parallel to topographic or other linear features can provide data that over or underestimate burrow density because topography can affect the location of gopher tortoises (Buckland et al. 2001; Williams et al. 2002; Carthy et al. 2005). In patchy habitat, and in areas with a high amount of topographical features, sample count may be a more appropriate method than line transects.

If one wishes to implement total (or sample) count method, it is recommended to use multiple observers, and provide evidence that detectability was 1.0. Nichols' double observer approach is a reasonable approach to estimating abundance if one wishes to implement a count-based method, but is unsure that detectability is less than 1.0.

Burrow occupancy

It is recommended that burrow cameras (or similar technologies) be employed, along with a patch occupancy modeling approach for data analysis, to obtain burrow occupancy rates and detection probability.

Occupancy rates likely vary spatially and temporally. Thus, it is strongly advised to *not* use a standard "correction factor" to convert gopher tortoise burrow abundance to abundance of tortoises. The application of the Aufenberg and Franz (1982) correction factor would have led to a gross overestimation of tortoise abundance in our study site. It is recommended that

burrow occupancy rates be estimated across the range of habitats in the study area to effectively capture the spatial variation.

Finally, it is suggested that gopher tortoise monitoring programs simultaneously consider burrow abundance and burrow occupancy rate. This is because changes in gopher tortoise abundance may be reflected in changes in one or both of these parameters (i.e., burrow abundance and burrow occupancy rate), and changes in one may not be interpreted as an indicator of changes in tortoise abundance.

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14. ABSTRACT The Army is very concerned about the preservation of Threatened and Endangered Species (TES) that make their home on Army training lands. The gopher tortoise (<i>Gopherus polyphemus</i>) is one species that the Army is particularly concerned about. To ensure that the Army is able to execute its training mission requirements while mitigating adverse impacts to the gopher tortoise, installation natural resource managers have to track species abundance in a consistent and statistically comparable manner from year to year. This requires that the Army exercise great diligence in identifying methods to derive species abundance that can meet all levels of terrain and vegetative conditions present in a species habitat. The objective of this research was to conduct field tests to evaluate the line transect, total count, sample count, and double observer methods for estimating gopher tortoise burrow abundance and compare them in respect to efficacy, precision, and cost of application. This report contains a discussion of the field tests and recommendations for installation natural resource managers.					
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