

ENHANCING LINE-TRANSECT DISTANCE SAMPLING TO INCREASE
DETECTABILITY OF GOPHER TORTOISES (*GOPHERUS POLYPHEMUS*) IN
POPULATION SURVEYS

by

HEATHER ELIZABETH GAYA

(Under the Direction of Clinton T. Moore)

ABSTRACT

Gopher tortoise (*Gopherus polyphemus*) populations are declining range-wide and accurate abundance and demographic estimates are needed for proper management of the species. I developed a series of Bayesian hierarchical models to estimate the density and size demography of gopher tortoise populations while accounting for size bias against smaller individuals. In conjunction with the estimation models, I designed a survey to incorporate randomly located double-sampling blocks and systematic vegetation measurements into the standard distance sampling methodology to better account for sources of variability known to affect detection of all tortoises. My results suggest that my hierarchical models and double-sampling survey method may increase the accuracy and precision of both density point estimates and the underlying burrow size distribution estimates of gopher tortoise populations.

INDEX WORDS: gopher tortoise, *Gopherus polyphemus*, distance sampling, Bayesian estimation, double sampling

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DEDICATION

This thesis is dedicated to my late great aunt, Virginia Randall. Thank you for showing me the power of a good sense of humor.

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CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

Introduction

The gopher tortoise (*Gopherus polyphemus*) is a burrowing reptile found in upland scrub and longleaf pine (*Pinus palustris*) habitats of the southeastern United States. The species is currently declining range-wide, likely due to habitat loss and habitat degradation (McCoy and Mushinsky 1992; McCoy et al. 2006; Smith et al. 2006). As a result, the gopher tortoise is federally listed as threatened under the Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana, and it is listed as a species of special concern by the remaining states in its range (U.S. Fish and Wildlife Service 1987; Yager et al. 2007). Line-transect distance sampling (LTDS) is widely employed by land managers to obtain important demographic information (abundance, density, size distribution) about tortoise populations. Considering LTDS to be a reliable method of monitoring populations, the U.S. Fish and Wildlife Service currently promotes LTDS as the standard monitoring method for populations across the range (U.S. Fish and Wildlife Service 2011).

Gopher tortoise surveys use gopher tortoise burrows as the object of interest, as the tortoises themselves are rarely observed outside of burrows (Smith et al. 2009). Burrow widths range from 5 cm (hatchlings) to > 35 cm (adults) and roughly correspond to the length of the resident tortoise's carapace (Martin and Layne 1987; Doonan and Stout 1994). However, studies indicate juvenile and adult burrows have different probabilities of detection during surveys, with juvenile burrows frequently missed regardless of distance to the transect (Ballou 2013). Juvenile

tortoises also tend to burrow under objects such as dense shrubs or downed trees that mask the outward signs of burrow presence, increasing detection difficulties (Alford 1980; Doonan and Stout 1994; Epperson and Heise 2003). Additionally, the presence of ground-level vegetation significantly decreases detection of tortoise burrows of all sizes, highlighting the importance of accounting for vegetation in gopher tortoise surveys (Berish and Leone 2014; Howze and Smith 2018). However, no field or analytical methods exist to control for vegetation obstruction as a source of variation in detection rate. Though covariates linked to the size of the tortoise (e.g., burrow width) are helpful in controlling for size-related detectability in LTDS models (Buckland et al. 2004; Marques et al. 2007; Thomas et al. 2010), it remains difficult to differentiate between a small proportion of juveniles in the population and low juvenile detection probability. As the gopher tortoise population continues to decline, improving the LTDS sampling design to account for both vegetation and size-biased detection will become increasingly important for making informed decisions about management and conservation practices throughout the tortoise's native range. The goal of this project is to reduce bias and increase precision of tortoise density estimates by incorporating regularly-measured detection covariates and employing a double-sampling scheme to improve detection of juvenile burrows.

Objectives and Justification

The purpose of this research is to modify the existing LTDS techniques to allow for reliable population density estimates that can be used for long-term gopher tortoise population monitoring across the southeast. I developed a series of Bayesian hierarchical models to estimate the density and size demography of the population while accounting for size bias against smaller individuals. I compared the density point estimates and precision produced from popular packages for LTDS data (e.g., DISTANCE 6.2, Rdistance) and the newly developed models for

datasets simulated under plausible forms of LTDS assumption violation as applied to gopher tortoise surveys. I further compared the raw distribution of burrow diameter found during LTDS – conventionally used as a portrayal of tortoise size distribution – and the estimated posterior distribution produced when detection probabilities were accounted for. In conjunction with the estimation models, I designed a survey to incorporate randomly located double-sampling blocks and systematic vegetation measurements into the standard distance sampling methodology to better account for sources of variability known to affect detection of all tortoises, especially juvenile tortoises. To evaluate the performance of this survey design, I tested my enhanced line-transect distance sampling method at 25 sites across Georgia and Florida as well as with simulated datasets.

This project fulfilled the following objectives:

1. Improve precision and accuracy of gopher tortoise abundance estimates (Chapters 2 and 4)
2. Incorporate regularly-measured vegetation covariates into line-transect distance sampling to account for vegetation obstruction (Chapter 3)
3. Reduce bias in estimates of burrow size structure, especially for small burrows (Chapters 2 and 4)

Literature Review

The gopher tortoise (*Gopherus polyphemus*) is a terrestrial reptile species that digs its own burrows in a variety of habitats within the coastal plain of the southeastern United States. It is often considered a keystone species due to the hundreds of invertebrate and vertebrate species documented using or occupying the tortoise's burrows (Eisenberg 1983; Lips 1991; Engle et al.

2006). Gopher tortoises generally create and use multiple burrows throughout the year, with males creating more burrows than females (Eubanks et al. 2003; Guyer et al. 2012). Commensal species may use the burrow microhabitat as refuge from fire, predators, or extreme temperature events (Lips 1991; Pike and Mitchell 2013).

Highest densities of gopher tortoises are primarily in southern Georgia and northern Florida, but tortoises also occur in parts of Alabama, Mississippi, Louisiana, and South Carolina (U.S. Fish and Wildlife Service 1987; R. Smith et al. 2005). Tortoises are most commonly found on deep, sandy substrate ideal for constructing burrows, but can occupy a variety of habitats including sandhill, scrub, pine flatwoods, coastal dunes, and mixed hardwood pine communities (McCoy and Mushinsky 1992; Smith et al. 2005; Yager et al. 2007). The gopher tortoise is thought to be declining range-wide because of habitat loss and urbanization (McCoy and Mushinsky 1992; Smith et al. 2005). The gopher tortoise is federally listed as threatened under the Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana, and it is listed as a species of special concern by the remaining states in its range (U.S. Fish and Wildlife Service 1987; Yager et al. 2007).

The U.S. Fish and Wildlife Service currently recommends LTDS as the standard method for estimating population size and monitoring population status of the gopher tortoise across the range (U.S. Fish and Wildlife Service 2011). The LTDS protocol is thought to provide more representative coverage of study areas than other plot-based methods, as the transects for LTDS are randomly distributed across large areas (Castellón et al. 2015). One of the major advantages of LTDS is that population inference is possible even if some tortoise burrows escape detection (Buckland et al. 1993). Imperfect detection of burrows is accounted for during data analysis by modeling the detection probability as a decreasing function of distance from the transect line

(Buckland et al. 1993; Buckland et al. 2001). In comparison to other tortoise survey techniques such as total count, sample count or double observer methods, LTDS is the most cost and time effective method, allowing researchers to sample a larger effective area with the same amount of effort compared to alternative methodologies (Nomani et al. 2008; Smith et al. 2009; Castellón et al. 2015).

Though the methodology for LTDS is largely standardized, the approach has two known issues when applied to gopher tortoise surveys. Firstly, juvenile burrows (≤ 13 cm) are frequently overlooked, leading to inaccurate estimates of population abundance and size demographics (Ballou 2013; Howze and Smith 2018). Juvenile tortoises have been shown to prefer forbs to grasses, suggesting that herbaceous ground cover of nutritious forbs may alter the spatial distribution of juvenile tortoises differently than adult tortoises (Mushinsky et al. 2003; Pike 2006; Halstead et al. 2007). Detection of juvenile burrows in the field remains difficult because of a tendency of juvenile tortoises to burrow under objects such as dense shrubs or downed trees that protect them from predation and mask the outward signs of burrow presence (Alford 1980; Diemer 1992; Epperson and Heise 2003). Burrows of juveniles are also more likely to collapse or erode during rain events than adult burrows (Berish et al. 2012). Additional detection difficulties result from small sample sizes in the wild due to low adult fecundity and high juvenile mortality (Wilson 1991; Pike and Siegel 2006; Smith et al. 2009). This problem raises concerns about using LTDS for long-term population monitoring and has led to a general lack of knowledge about juvenile gopher tortoises in the wild (Mushinsky et al. 2003; Smith et al. 2009).

The effectiveness of LTDS efforts is also compromised by variation in ground-level vegetation both within sites and between sampling locations. Dense vegetation or thick litter cover leads to difficulty in detecting burrows of all sizes, reducing precision of population

estimates (Berish et al. 2012; Berish and Leone 2014; Howze and Smith 2018). Improved detection rates are possible with increased time and effort (Berish et al. 2012; Howze and Smith 2018), but considerable plant cover or debris frequently render these efforts ineffective in terms of cost and labor. Howze and Smith (2018) have suggested burning areas prior to performing LTDS to improve detectability and remove variation in detection caused by heterogeneous vegetation, but weather conditions, timing or other management constraints may render this method unfeasible in many instances. To address variation in vegetation obstruction within surveys, standardized vegetation measurements need to be integrated into the LTDS sampling design to account for differing levels of detectability.

To further our understanding of gopher tortoise populations, I developed a flexible Bayesian hierarchical model to estimate the density and size demography of populations while accounting for size bias against smaller individuals. I used mixtures of gamma distributions to model tortoise burrow sizes from LTDS data and reduce bias in demography estimation (Chapter 2). To account for heterogeneous vegetation obstruction encountered during gopher tortoise surveys, I incorporated vegetation measurements into LTDS surveys and compared survey results when the same plots were surveyed before and after prescribed fire (Chapter 3). Finally, I designed a double-sampling method to improve detection of juvenile gopher tortoises and developed a Bayesian hierarchical model to analyze the results (Chapter 4).

Literature Cited

- Alford, R. A. 1980. Population structure of *Gopherus polyphemus* in northern Florida. *Journal of Herpetology* 14:177–182.
- Ballou, A. 2013. Aspects of gopher tortoise (*Gopherus polyphemus*) populations in Georgia: status, landscape predictors, and juvenile movements and burrow use. University of Georgia, Athens, GA.

- Berish, J. E. D., and E. H. Leone. 2014. Follow-up demographic survey of a Florida gopher tortoise population. *Southeastern Naturalist* 13:639–648.
- Berish, J. E., R. A. Kiltie, and T. M. Thomas. 2012. Long-term population dynamics of gopher tortoises (*Gopherus polyphemus*) in a pine plantation in northern Florida. *Chelonian Conservation and Biology* 11:50–58.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. Laake, D. Borchers, and L. Thomas. 2004. Advanced distance sampling. Volume 89. Oxford University Press, Oxford.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, and J. L. Laake. 1993. Distance sampling: estimating abundance of biological populations. Springer, New York.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. Introduction to distance sampling: estimating abundance of biological populations. Oxford University Press, Oxford.
- Castellón, T. D., B. B. Rothermel, and S. Z. Nomani. 2015. A comparison of line-transect distance sampling methods for estimating gopher tortoise population densities. *Wildlife Society Bulletin* 39:804–812.
- Diemer, J. E. 1992. Home range and movements of the tortoise *Gopherus polyphemus* in northern Florida. *Journal of Herpetology* 26:158–165.
- Doonan, T. J., and I. J. Stout. 1994. Effects of gopher tortoise (*Gopherus polyphemus*) body size on burrow structure. *The American Midland Naturalist* 131:273–280.
- Eisenberg, J. F. 1983. The gopher tortoise as a keystone species. The gopher tortoise: A keystone species. Proceedings of the 4th Annual Meeting of the Gopher Tortoise Council. Florida State Museum, Gainesville, Florida.
- Enge, K., J. E. Berish, R. Bolt, A. Dziergowski, and H. . Mushinsky. 2006. Biological status report: gopher tortoise. Florida Fish and Wildlife Conservation Commission. <http://myfwc.com/media/969926/Gopher_Tortoise_BSR.pdf>. Accessed 27 Feb 2018.
- Epperson, D. M., and C. D. Heise. 2003. Nesting and hatchling ecology of gopher tortoises (*Gopherus polyphemus*) in southern Mississippi. *Journal of Herpetology* 37:315–324.
- Eubanks, J. O., W. K. Michener, and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). *Herpetologica* 59:311–321.

- Guyer, C., V. M. Johnson, and S. M. Hermann. 2012. Effects of population density on patterns of movement and behavior of gopher tortoises (*Gopherus polyphemus*). *Herpetological Monographs* 26:122–134.
- Halstead, B. J., E. D. McCoy, T. A. Stilson, and H. R. Mushinsky. 2007. Alternative foraging tactics of juvenile gopher tortoises (*Gopherus polyphemus*) examined using correlated random walk models. *Herpetologica* 63:472–481.
- Howze, J. M., and L. L. Smith. 2018. Detection of gopher tortoise burrows before and after a prescribed fire: implications for surveys. *Journal of Fish and Wildlife Management* X:xx–xx.
- Lips, K. R. 1991. Vertebrates associated with tortoise (*Gopherus polyphemus*) burrows in four habitats in south-central Florida. *Journal of Herpetology* 25:477.
- Marques, T. A., L. Thomas, S. G. Fancy, S. T. Buckland, and C. M. Handel. 2007. Improving estimates of bird density using multiple- covariate distance sampling. *The Auk* 124:1229–1243.
- Martin, P. L., and J. N. Layne. 1987. Relationship of gopher tortoise body size to burrow size in a southcentral Florida population. *Florida Scientist* 50:264–267.
- McCoy, E. D., and H. R. Mushinsky. 1992. Studying a species in decline: gopher tortoises and the dilemma of “correction factors.” *Herpetologica* 48:402–407.
- McCoy, E. D., H. R. Mushinsky, and J. Lindzey. 2006. Declines of the gopher tortoise on protected lands. *Biological Conservation* 128:120–127.
- Mushinsky, H. R., T. A. Stilson, and E. D. McCoy. 2003. Diet and dietary preference of the juvenile gopher tortoise (*Gopherus polyphemus*). *Herpetologica* 59:475–483.
- Nomani, S., M. Oli, and R. Carthy. 2008. Comparison of methods for estimating abundance of gopher tortoises. *Applied Herpetology* 5:13–31.
- Pike, D. A. 2006. Movement patterns, habitat use, and growth of hatchling tortoises, *Gopherus polyphemus*. *Copeia* 2006:68–76.
- Pike, D. A., and J. C. Mitchell. 2013. Burrow-dwelling ecosystem engineers provide thermal refugia throughout the landscape. *Animal Conservation* 16:694–703.
- Pike, D. A., and R. A. Seigel. 2006. Variation in hatchling tortoise survivorship at three geographic localities. *Herpetologica* 62:125–131.

- Smith, L. L., T. D. Tuberville, and R. A. Seigel. 2006. Workshop on the ecology, status, and management of the gopher tortoise (*Gopherus polyphemus*), Joseph W. Jones Ecological Research Center, 16–17 January 2003: Final Results and Recommendations. *Chelonian Conservation and Biology* 5:326–330.
- Smith, L., J. Linehan, J. Stober, M. Elliott, and J. Jensen. 2009. An evaluation of distance sampling for large-scale gopher tortoise surveys in Georgia, USA. *Applied Herpetology* 6:355–368.
- Smith, R., A. Chambers, J. Berish, T. Tuberville, and K. Herpich. 2005. Gopher tortoise burrow surveys: External characteristics, burrow cameras, and truth. *Applied Herpetology* 2:161–170.
- Thomas, L., S. T. Buckland, E. A. Rexstad, J. L. Laake, S. Strindberg, S. L. Hedley, J. R. B. Bishop, T. A. Marques, and K. P. Burnham. 2010. Distance software: Design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology* 47:5–14.
- U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants: Determination of threatened status for the gopher tortoise (*Gopherus polyphemus*). *Federal Register* 52:25376.
- U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants: 12-month finding on a petition to list the gopher tortoise as threatened in the eastern portion of its range; Final rule. *Federal Register* 76:45130–45162.
- Wilson, D. S. 1991. Estimates of survival for juvenile gopher tortoises, *Gopherus polyphemus*. *Journal of Herpetology* 25:376–379.
- Yager, L. Y., M. G. Hinderliter, C. D. Heise, and D. M. Epperson. 2007. Gopher tortoise response to habitat management by prescribed burning. *Journal of Wildlife Management* 71:428–434.

CHAPTER 2

NUISANCE OR NECESSARY? A HIERARCHICAL MODEL FOR DISTANCE SAMPLING WHEN A DETECTION COVARIATE WITH AN UNKNOWN DISTRIBUTION IS ALSO A DEMOGRAPHIC PROPERTY OF INTEREST ¹

¹ Gaya, H.E., B. L. Nuse, and C. T. Moore. To be submitted to *Methods of Ecology and Evolution*

Abstract

1. Line-transect distance sampling (LTDS) is a popular method for estimating abundance and density of animal populations. Covariates occurring in statistical models, including models for LTDS data, typically serve as nuisance adjustments to improve inference about the response by accounting for sources of variability potentially affecting it. However, inference on the covariate itself, such as its distributional properties, is sometimes of interest if the attribute relates to population status.

2. To exemplify this sampling situation, we focus on the gopher tortoise (*Gopherus polyphemus*), a terrestrial reptile endemic to the southeastern United States that digs its own burrows. We present a Bayesian model for line-transect distance sampling in which a covariate associated with the animal, burrow width, helps to account for detection bias of all burrows present and improves precision of estimated density. By modeling the number of burrows missed, by size interval, as a latent quantity, we also produce a detection-adjusted size frequency distribution of occupied burrows, an indicator of population age structure. We compare the raw distribution of burrow diameter found during LTDS and the estimated posterior distribution produced when detection probabilities are accounted for. We further compare the density point estimates and precision produced by the frequentist approach and our own model for both a simulated and real dataset.

3. Approximated size distributions for the smallest covariate values (≤ 10 units) from our model were consistently closer to the true distribution than estimates produced from the raw LTDS data regardless of population density. Except for populations with a uniform covariate distribution at the lowest density (2 individuals ha^{-1}), the point estimates from our bias-adjusted model were nearer the true value than those under a conventional approach.

4. Successful conservation of animal populations may depend on accurate assessment of wildlife population abundance and demographics. Our covariate model provides a powerful tool for understanding the distribution of an individual-level attribute that dually affects the detection of organisms and is informative about population status.

Keywords: distance sampling, *Gopherus polyphemus*, hierarchical models, mixture models, population estimation

1. Introduction

Line-transect distance sampling (LTDS) is a popular method for estimating abundance and density of animal populations. It belongs to a class of sampling approaches that do not require physical capture or encounter of individuals, and not all individuals in the survey area need be detected to provide inference on density, saving the researcher the time and energy necessary to complete a full population census (Buckland, Anderson, Burnham, & Laake, 1993). In conventional distance sampling, objects can be missed in the survey for one of two reasons: The individual was not located in the area covered by the survey, or the individual was present but not detected (Buckland et al., 2001). Imperfect detection of objects is then accounted for during data analysis by modeling detection probability as a function of distance from the transect line (Buckland, Anderson, Burnham, & Laake, 1993; Buckland et al., 2001). Many authors have noted the influence of factors other than distance on the detection function, such as transect location, object size or time of day (Buckland et al., 2004; Marques, Thomas, Fancy, Buckland, & Handel, 2007; Royle & Dorazio, 2008), leading to the creation of distance sampling models involving one or more covariates.

The main role of covariates in statistical models, including models for LTDS data, is to improve inference about the response by accounting for sources of variability. For instance, properties of the object in a line transect survey, such as its size, color, or sound, may influence its detection by the observer. Because the role of the covariate is often limited to the singular purpose of variance reduction, inference about its distribution is not often a concern.

However, the distribution of a metric serving as a covariate may reflect important demographic properties of the population, such as distributions of age, breeding status, or physical condition. Successful conservation of animal populations may depend on accurate

assessment of these profiles in long-term or spatially extensive monitoring efforts. Obtaining reliable inference on these properties is challenging when the property itself is linked to detection of individuals, such that certain values associated with high detection probability are disproportionately seen during sampling. For example, for animals seen in clusters, individuals in larger clusters may have a higher rate of detection than those in smaller clusters and will be over-represented in the data, even if cluster sizes in the population are uniformly distributed. Thus, use of the object's property as a nuisance adjustment for valid inference about the population is key to making valid demographic inference about the property itself.

Successful modeling of covariate distributions, then, lies in resolving uncertainty about the covariate values of undetected individuals. Covariate distributions are often multi-modal and, thus, unrealistic to represent as a unimodal or non-modal distribution. Proposed techniques for working with partially-observed covariates include conditioning the likelihood on observed individuals (Huggins, 1989; Alho, 1990; Buckland et al., 2004), using pseudolikelihood estimation (Hu & Lawless, 1997), or estimating a mean value for use in calculations (Buckland et al., 2001; Thomas et al., 2010). Nevertheless, the best way to model uncertain covariate distributions remains an open question in the literature.

One example of this sampling scenario is the gopher tortoise (*Gopherus polyphemus*), a terrestrial species that digs its own burrows in a variety of habitats within the coastal plain of the southeastern United States. The gopher tortoise is often considered a keystone species due to the hundreds of invertebrate and vertebrate species documented using or occupying the tortoise's burrows (Eisenberg, 1983; Lips, 1991; Enge, Berish, Bolt, Dziergowski, & Mushinsky, 2006). Populations occur in parts of Louisiana, Mississippi, Alabama, Georgia, Florida, and South Carolina, but most are concentrated in southern Georgia and northern Florida (U.S. Fish and

Wildlife Service, 1987; Smith, Chambers, Berish, Tuberville, & Herpich, 2005). Gopher tortoise abundance has declined range-wide, largely as a result of habitat loss or degradation (McCoy & Mushinsky, 1992; Smith, Chambers, Berish, Tuberville, & Herpich, 2005). As a result, the gopher tortoise is threatened under the U.S. Endangered Species Act in sections of southwestern Alabama, Mississippi, and Louisiana (U.S. Fish and Wildlife Service, 1987) and is a candidate for listing in the remainder of the range (U.S. Fish and Wildlife Service, 2011). The U.S. Fish and Wildlife Service currently recommends LTDS as the standard method for estimating population size and monitoring population status across the range (U.S. Fish and Wildlife Service, 2013).

Based on the consensus of experts convened in July 2013, the Gopher Tortoise Council (2013) defined thresholds of 250 adult tortoises at a density of $\geq 0.4 \text{ ha}^{-1}$ as the minimum demographic attributes to maintain a viable gopher tortoise population (MVP). Additional minimum requirements include an equal adult sex ratio, evidence of active recruitment, representation of all size classes, and absence of major constraints to movement across the landscape. As gopher tortoises are rarely observed outside of burrows (Smith et al., 2009), surveys instead use gopher tortoise burrows as the object of interest. Burrow width roughly corresponds to the length of the resident tortoise's carapace (Martin & Layne, 1987; Doonan & Stout, 1994); however, burrow width sometimes exceeds the dimensions of the tortoise. Burrow widths range from 5 cm (hatchlings) to >35 cm (adults) (Martin & Layne, 1987), with detection probability increasing with greater widths (Ballou, 2013). Because conservation of the gopher tortoise is tied to the assessment of both population abundance and demography, a size class histogram of the widths of observed, occupied burrows is a conventional reporting outcome of a survey (Waddle, Mazzotti, and Rice, 2006; Smith, Linehan, Stober, Elliott, & Jensen, 2009). Size

scales with age, at least in younger gopher tortoises (Germano, 1994); thus, managers rely on these presentations to make inferences about the age structure of a population. Multiple modes observed in the histogram are interpreted as age pulses, and absence of modes in the smaller size classes are sometimes causes of concern. However, caveats about size-differentiated detection usually accompany these presentations, drawing attention to the fact that smaller burrows are underrepresented in the figure. Thus, it is difficult to differentiate whether underrepresentation of small burrows is due to low detection probability or due to their absence resulting from high mortality or low reproductive rates. Unadjusted frequencies are often used to make decisions about population status and conservation need despite the recognition that abundance estimates derived without considering burrow size could be underestimated (Ballou, 2013).

While density estimation of gopher tortoise LTDS data is conventionally carried out in a maximum likelihood framework under program DISTANCE (Thomas et al., 2010), we conducted analysis within a Bayesian framework using Markov chain Monte Carlo (MCMC) sampling (Brooks, 2011). We present a model for line-transect distance sampling in which a partially-observed covariate associated with the object of interest, in this case, gopher tortoise burrow width, helps to account for detection bias of all objects present and improves precision of estimated density. However, the size of undetected burrows is unknown, so a probability distribution is needed for the covariate. As with other individual covariate models, it is difficult to choose a probability distribution for burrow size. In this paper, we present a flexible approach for modeling the distribution of a partially-observed covariate, which could be multi-modal. We compare the raw distribution of widths of burrows detected during LTDS and the estimated posterior distribution produced when detection probabilities are accounted for. We compare the density point estimates and precision produced from DISTANCE v 6.2 and our own model for

both simulated and real datasets. Although we focus on individual size as a covariate, this method could be applied to any type of continuous covariate for which the underlying distribution may be of interest.

2. Methods

Traditional Distance Sampling Analysis

At the most basic level, conventional distance sampling calculates density as the total number of individuals detected during the survey divided by an estimate of the total area surveyed. In most cases, the total area surveyed is unknown because at some uncertain distance from the line, the observer's ability to detect individuals is substantially diminished and the area beyond is effectively non-surveyable. However, total survey area can be estimated as the product of total length of transects traversed and twice the "effective strip width" inferred by fitting a detection function to the distance data collected during the survey. The effective strip width, μ , is simply the distance from the line for which as many objects are detected beyond μ as missed within μ , and is entirely dependent on the fitted detection curve (Thomas et. al., 2010). Assuming all objects on the line are detected and probability of detection decreases with distance from the transect, then density can be estimated by:

$$\hat{D} = \frac{n}{2 * \mu * L} \quad (1)$$

where n represents the total number of detected individuals, L is the total length of transects, and μ is the effective strip width of the survey (Buckland et al., 2001; Williams, Nichols, & Conroy, 2002). Individual or site-level covariates can be incorporated in the detection model to account for variation in detection due to factors unrelated to distance (Royle and Dorazio, 2008).

Program DISTANCE implements maximum likelihood estimation to derive parameter estimates for some implementations of the distance sampling model and other quantities of interest.

Hierarchical Bayesian Approach

We chose a Bayesian approach over a maximum likelihood approach for the flexibility in model construction, including incorporation of hierarchical parameter structures and direct inference on latent (hidden) population parameters. Under our approach, each individual in the study area is associated with a distance to transect and a burrow width. These values are either known (because they were recorded for found individuals) or are latent (they exist but are unknown because the individual was missed). Following Royle and Dorazio (2008), we use a data-augmentation technique, where the known distances and covariate values for n_{ind} found individuals are augmented with a large number n_A of missing values representing potentially missed individuals. Assuming individuals are distributed at random on the survey site and transect lines are placed independently with respect to the individuals, we can assume distances are drawn from a uniform distribution such that:

$$x_i \sim \text{Uniform}(0, Bx) \quad (2)$$

where Bx is a given maximum distance of our choosing from the transect line. The model then evaluates each augmented individual and assigns a binary state indicating its existence in the population:

$$w_i \sim \text{Binomial}(1, \psi) \quad (3)$$

where ψ is a probability of inclusion in the population. All real individuals, both drawn from the augmented population (those in n_A with $w_i = 1$) plus those found during the survey, are then summed into a total population N which can be used to estimate population density:

$$\hat{D} = \frac{N}{2 * L * Bx} \quad (4)$$

with:

$$N = n_{ind} + \sum_{i=1}^{n_A} w_i \quad (5)$$

We model variation in individual detection through adjustments to the scale parameter, σ , of our detection function (Marques, Thomas, Fancy, Buckland, & Handel, 2007). We model the scale parameter as the log-link of an intercept and a burrow width predictor variable (z_i) with uninformative normal priors for the intercept and coefficient terms:

$$\ln(\sigma_i) = \beta_0 + \beta_\sigma * z_i \quad (6)$$

In addition to a distance value, a covariate value is drawn from a prior distribution for each augmented individual. As is the case with burrow widths in a gopher tortoise population, covariate distributions are often multi-modal and, thus, unrealistic to represent as a unimodal distribution. We model our covariate of interest as a mixture of a small number (K) of gamma distributions. Each missed individual in the population, $i = 1, \dots, (N - n_{ind})$, is assumed to belong to one of K gammas, $k = 1, \dots, K$, with shape and rate parameters α_k and β_k respectively:

$$z_{ik} \sim \text{Gamma}(\alpha_k, \beta_k) \quad (7)$$

The probability of belonging to group k can be modeled using a Dirichlet distribution:

$$p_k \sim \text{Dirichlet}(\omega_1, \dots, \omega_k) \quad (8)$$

$$\omega_k \sim \text{Gamma}(.001, .001)$$

By leveraging information about detection probability against the covariate values seen during the LTDS survey, we allow the model to minimize bias towards large covariate values when estimating the underlying covariate distribution.

The number of distributions comprising the mixture is unknown, so K must be set prior to modeling. Richardson and Green (1997) propose a ‘reverse jump’ Monte Carlo method (hereafter RJMCMC) for estimating both the number (K) and parameters of component Gaussian distributions that best fit a given dataset. The RJMCMC is a random sweep Metropolis-Hastings method (Metropolis et al., 1953; Hastings, 1970) adapted for general state spaces (Green, 1995;

Richardson and Green, 1997). At each sweep across the state space, a change in dimension ($K+1$ or $K-1$) is attempted, as well as within-model moves for all parameters to maximize likelihood equations. If a move type is accepted, the RJMCMC either combines two adjacent existing distributions or splits a single distribution into two contiguous distributions. We adapted this model to fit component gamma rather than Gaussian distributions. We drew the shape and rate (α_k, β_k) parameters of each gamma distribution from vague gamma hyperprior distributions and adjusted the likelihood formulas accordingly. We ran the RJMCMC for 80,000 iterations with a burn-in of 40,000 iterations. We calculated the frequency with which each value of K was selected and chose the K with the highest frequency. To account for the detection bias inherent in the data used to estimate the number of gamma distributions, we used $K+1$ distributions whenever the smallest expected covariate values (in our case, ≤ 20 units) were not seen in the raw data and K was > 1 . For instance, if the RJMCMC estimated a K of 2, but the raw data did not contain any small covariate values (≤ 20 units), we modeled the covariate distribution using 3 gamma distributions. Using $K+1$ distributions balanced the likelihood of underestimating the number of gammas distributions necessary to estimate the underlying covariate distribution with any potential reduction in precision associated with using too many distributions. For a more detailed description of the original RJMCMC model, see Richardson and Green (1997), Richardson and Green (1998), and Brooks and Giudici (1998).

In conventional distance sampling, probability of detection on the line ($g(0)$) is assumed to be 1. In practice, however, this assumption is often violated, leading to underestimation of the population (Thomas et al., 2006). We wanted our model to be able to account for imperfect detection on the line without the need for additional sampling effort. Therefore, we modeled detection on the line as a proportion (range 0 – 1) of the overall detection probability. We assume

the existence of a known threshold covariate value ($b.point$) above which detection on the line is perfect. However, we allow for uncertainty in the true detection probability of individuals below this threshold. For example, up to a certain burrow width, detecting gopher tortoise burrows that occur on the line is not guaranteed ($g(0) < 1$). For individuals with the smallest possible covariate value (z_{min}), we draw detection on the line from a uniform distribution bound between 0.2 and 1. Probability of detection of burrows on the transect line increases proportionally as burrow width varies between this minimum value and the threshold point:

$$\xi_i = \begin{cases} \left(\frac{1 - b}{b.point - z_{min}} \right) (z_i - z_{min}) + b, & z_{min} \leq z_i \leq b.point \\ 1, & z_i \geq b.point \end{cases} \quad (9)$$

$$b \sim \text{Uniform}(0.2, 1)$$

For the half-normal detection curve, probability of detection of burrow i at distance x from the transect line is described by:

$$g(x, z) = e^{\left(\frac{-x_i^2}{2\sigma_i^2} \right)} * \xi_i \quad (10)$$

Simulation

We considered two factors that could affect the performance of both conventional maximum likelihood methods and our new Bayesian hierarchical model: population density and the underlying distribution of a covariate affecting detection probability. We simulated line-transect distance sampling on a 100-ha² area, with parallel transects spaced 50 m apart. We also simulated 4 different covariate scenarios to evaluate model performance at different extremes: a negative linear distribution with a high concentration of low values, a unimodal distribution, a multi-modal distribution, and a uniform distribution (Distributions A, O, M and U respectively, see Figure 2.1.). We allowed the values of a generic covariate (with units unspecified) to range

from 5 to 100. For each of the covariate scenarios, individuals were randomly distributed across the area at a density of 2 and 6 individuals ha⁻¹.

We simulated the detection of individuals as probabilities from a half normal curve with $\log(\sigma_i) = 1 + 0.025 * z_i$ to allow detection to vary with size (Figure 2.1). Detection on the transect line was simulated as a broken stick regression with perfect detection on the line for any individual with a covariate value > 20. To avoid issues with parameter identifiability and convergence, we assumed this threshold value was known and fixed during analysis. For the smallest covariate value, detection on the line was set at 0.7.

We simulated each of our 8 covariate-density combinations 20 times, for a total of 160 simulations. We computed relative bias of point estimates and performance of credible intervals for overall density. We further compared the proportions of the population within selected intervals of the covariate for both our estimated distribution and the true covariate distribution. We tested for chain convergence using the Gelman–Rubin statistic (Gelman, Rubin, Carlin, & Stern, 2003) and extended simulations to meet the minimum iteration suggestion of the Raftery-Lewis diagnostic (Bernardo, Berger, Dawid, & Smith, 1992). Each model run took between 2-6 hours to complete on a 2.30 GHz processor using 3 cores. All models were run in the runjags package of R (<https://cran.r-project.org/web/packages/runjags/runjags.pdf>). For comparison to a conventional analytical approach, we estimated overall density under Program DISTANCE and computed the same performance measures. We followed the customary approach to estimating proportion of the population within selected intervals of the covariate by calculating corresponding proportions of detections from the raw LTDS data. For each simulated survey, we allowed Program DISTANCE to fit any of the detection curves available within the program (half-normal, hazard rate, negative exponential, uniform or gamma distributions).

Case Study

We analyzed data from gopher tortoise surveys conducted by the Georgia Department of Natural Resources 2009-2014 at three state-owned properties: Doerun Natural Area (Colquitt County), Holman Property (Taylor County), and Sansavilla Wildlife Management Area (WMA) (Brantley County). In most gopher tortoise surveys, size and distance information is collected on both occupied and unoccupied burrows, as occupancy is not thought to influence detection probability (Castellón, Rothermel, & Nomani, 2015; Stober, Prieto-Gonzalez, Smith, Marques, & Thomas, 2017). However, only the density of occupied burrows is usually of interest (Smith, Linehan, Stober, Elliott, & Jensen, 2009). Here, we assume that burrow occupancy status (o_i) is predicted by burrow size (larger burrows are more likely than smaller burrows to be encountered either temporarily unoccupied or abandoned). We assigned uninformative normal priors for an intercept and a burrow size coefficient:

$$o_i \sim \text{binomial}(1, \theta_i) \quad (11)$$

$$\text{logit}(\theta_i) = o.\text{int} + \beta_z * z_i$$

In general, this approach can be applied to any discrete characteristic that defines a population subset of interest. If the characteristic does not influence the individual's detection, then survey data collected from all individuals, with or without the characteristic, helps to inform detection probability.

We gave the breakpoint for perfect detection on the line a uniform prior bound between 15 and 20 cm wide burrows. Burrow density point estimates by maximum likelihood at the selected sites were 3.02 burrows ha⁻¹ at Doerun, 2.14 burrows ha⁻¹ at Holman, and 3.31 burrows ha⁻¹ at Sansavilla. Tortoise densities were 1.31, 0.28, and 1.19 tortoises ha⁻¹ respectively. For

comparison with our bias-adjusted model, we fit the raw size data available from the LTDS surveys to a mixture of gammas.

3. Results

Simulation

Figure. 2.3 summarizes the relative bias of point estimates of object density obtained from both Program DISTANCE and our bias-adjusted model for all simulations. Except for populations with a uniform covariate distribution at the lowest density (2 individuals ha^{-1}), the point estimates from our bias-adjusted model were more accurate (point estimate nearer the true value; smaller range of bias) than those from Program DISTANCE. The range of point estimate bias of both our bias-adjusted model and Program DISTANCE were dependent on population density, with a decrease in the range of bias at greater densities. Performance of both our bias-adjusted model and Program DISTANCE varied between simulations with differing underlying covariate distributions. The frequency with which each model's 95% confidence or credible interval captured the true density of the population is shown in Table 2.1. Across all simulations, Program DISTANCE captured the true density in the confidence interval 76% of the time compared to our bias-adjusted model's 94%. There were no obvious patterns in either model's credible or confidence interval performance among simulations with different covariate distributions.

Estimated proportion of the population with the smallest covariate values (≤ 20 units) from our model were closer to the true distribution than naïve summaries of the raw LTDS data in most cases (Figure 2.4). This pattern held true for all covariate distributions except the unimodal distribution. Our model had the most variation in estimation of the proportion of the population with the smallest covariate values when the true covariate values followed a uniform

distribution. Population proportion estimates for larger covariate values (≥ 80 units) were closer to the true generating values than were summaries of the raw LTDS data (Figure 2.5) in all cases tested. In contrast to the raw LTDS data, the accuracy of our bias-adjusted model's estimates of the proportion of the population with the smallest or largest covariate values (≤ 20 or ≥ 80 units) tended to improve with higher densities. For the linear and uniform covariate distributions, the RJMCMC tended to choose between 4 and 5 clusters to estimate the distribution despite the absence of modes in these distributions. For the multimodal and unimodal covariate distributions, the model consistently chose the correct values of 4 clusters and 1 cluster respectively.

Case Study

For all three sites providing real LTDS data, our bias-adjusted model estimated higher tortoise densities than did conventional distance sampling models (Table 2.2). The coefficients of variation (CVs) produced by our model were smaller for all sites in comparison to Program DISTANCE. Our bias-adjusted model also tended to estimate a greater proportion of smaller sized individuals than indicated by the raw LTDS data (Figure 2.6). This effect was more pronounced when we took occupancy into account and compared estimated tortoise (occupied burrow) sizes. At the Holman site, our bias-adjusted model also estimated a large proportion of medium sized tortoises (18 – 30 cm wide) that were available in the population but underrepresented in the raw data. Average occupancy of burrows differed between sites ranging from 22 % to 47 % observed occupancy (Table 2.3). For two of our three tested sites, our model estimated a positive slope for occupancy (occupancy probability increased with increasing burrow size), but all occupancy slope credible intervals included both positive and negative slope

values. For all three sites, our model estimated the probability of detecting a 5 cm wide tortoise burrow on the transect line was between 0.2 and 0.67.

4. Discussion

In some distance sampling applications, the distribution of a covariate that affects detection probability of individuals in a population may itself have relevance in understanding population status or demography. Our bias-adjusted model demonstrates the potential for estimating underlying covariate distributions in real data. In simulation, our model was able to partially account for survey bias against the smallest covariate values and provide a more accurate estimate of the underlying covariate distribution than indicated by the raw LTDS data. However, we saw this pattern breakdown for scenarios with only one cluster (Scenario O, unimodal distribution). For covariate distributions with more than one mode, our model accounts for bias by adjusting the probability of individual covariates being drawn from a specific gamma distribution. When only one mode is identified, the model simply fits the raw LTDS data to a single gamma distribution without adjustment. However, even in scenarios with an underlying unimodal distribution, our model still produced less biased density point estimates than did conventional distance analysis, suggesting that our model may still be a viable alternative to conventional distance sampling models even when we cannot fully account for covariate bias.

In conventional LTDS analysis, uncertainty in density estimation lies in estimating the area surveyed, with n , the number of individuals detected in that area, as a measurable quantity. Under a data augmentation framework, the survey area is known; uncertainty lies in estimation of the number of individuals within the surveyed region. As noted by Royle, Dorazio, and Link (2007), one of the main advantages of a data augmentation approach is that the size of the augmented dataset is fixed, permitting straightforward Bayesian implementation even as model

complexity increases. Moreover, prior information on N , the detection function, or the underlying covariate distribution can be easily included in the model, increasing model precision.

In most gopher tortoise surveys, only occupied burrows are subjected to analysis, though both occupied and unoccupied burrows are recorded during the survey process (Smith, Linehan, Stober, Elliott, & Jensen, 2009). Since small burrows are found infrequently regardless of occupancy status, a lack of occupied small burrows in the dataset is not necessarily indicative of a lack of small tortoises. Moreover, removal of unoccupied burrows from data analysis can often represent a significant loss of information on burrows below a certain size threshold, especially if no occupied small burrows were encountered during the survey. In our analysis of real datasets, the increased proportion of tortoises in the smallest size classes (<10 cm wide) predicted by our bias-adjusted model reinforces concerns that raw counts from surveys are frequently underestimating the proportion of juvenile tortoises in the population. When sample sizes are low, surveyors may repeatedly survey the same transects, sample additional transects, or increase the number of observers (Buckland et al., 2001; Glass et al., 2016; Stober & Smith, 2010; Stober et al., 2017). However, all these methods require additional survey effort, which can be costly and may not always improve precision of estimates (Castellón, Rothermel, & Nomani, 2015). Previous studies suggest that detection is not affected by occupancy status (Smith et al., 2005) and the detection curve may be estimated using all available burrows and separately accounting for occupancy with minimal additional uncertainty (Stober et al., 2017). Using data augmentation, we applied this same principle to our bias-adjusted model. However, our model permits occupancy rate to vary by covariates related to detection probability (e.g., burrow width) or any other covariates of interest. By including the full set of objects (in this case, burrows) when modeling detection, we can greatly increase sample size while still allowing for valid

inferences about a subset of our data. We anticipate that our bias-adjusted model may provide a powerful tool for gaining information about the true density and underlying demography in populations, even when low sample sizes do not permit accurate estimation of population density by conventional methods.

Though we compare our Bayesian model's performance to the frequentist approach under Program DISTANCE in simulation, we recognize this may not be a fair comparison. In our simulations, we incorporated imperfect detection on the transect line - a violation of one of the main assumptions of line transect distance sampling. Moreover, we simulated the detection probability on the transect line as varying with the covariate value of the surveyed individuals. Though the conventional approach allows for imperfect detection on the transect line, this value is assumed equal for all individuals in the population and must be specified during analysis. As a result, the improved performance of our Bayesian model relative to the conventional approach to LTDS may reflect our model's capacity to accommodate imperfect detection on the transect line rather than the effect of including covariate information. However, for surveys where detection on the line varies with a covariate, the decreased performance of Program DISTANCE in simulation suggests the conventional approach to LTDS may not be an appropriate tool for analysis.

Our model assumes knowledge of the threshold covariate value above which detection on the line is perfect. In practice, however, this value is likely unknown. We found that the model suffered parameter identifiability and would not converge if we provided weak or uninformative priors for the break point for detection on the line. However, the imposition of a mildly informative prior (for instance, a uniform distribution over a 10-unit range) was reasonable and led to successful model convergence. Previous studies have suggested either sampling at

multiple effort levels or employing double sampling techniques to account for imperfect detection on the line (Quang & Becker, 1997; Clement, Converse, & Royle, 2017). However, these methods are frequently specific to estimating group size and are not in widespread use for most LTDS surveys. Overly conservative estimation of the breakpoint for detection on the line (estimating the breakpoint as larger than the true value) may lead to overcompensation by the model for covariate values associated with low detectability, falsely inflating their relative density in the estimated covariate distribution. The addition of a double sampling strategy that focuses on harder to detect individuals may allow for more precise estimates of detection overall, ultimately improving both knowledge of the underlying covariate distribution and the true density of the population of interest.

Our bias-adjusted model may be readily adapted to other species surveys beyond the gopher tortoise, especially for species where detection is biased towards certain demographic subsets. Examples include: cetaceans such as the bottlenose dolphin (*Tursiops truncatus*) and the false killer whale (*Pseudorca crassidens*), which may travel in clusters of varying sizes (Dick & Hines, 2011; Bradford, Forney, Oleson, & Barlow, 2014); New Zealand's tuatara (*Sphenodon* sp), where males are easier to detect and probability of detection on the transect line is <1 (Cassey & Ussher, 1999); or whooping cranes (*Grus americana*), which build nests in clusters with multiple individuals (Strobel & Butler, 2014). In some instances, observations may be best modeled through detection functions other than the half-normal we use here. A natural extension of our model may be to include alternative detection functions or include outside information to better inform detection on the transect line.

Successful conservation of animal populations may depend on accurate assessment of wildlife population abundance and demographics. Without accurate baseline estimates, land

managers cannot confidently assess changes in populations over time or determine the efficacy of conservation efforts. We anticipate the use of bias-adjusting models, such as our Bayesian hierarchical model presented here, may provide an effective approach for accounting for and evaluating demographic covariates in populations, especially when accurate estimation of population density or demographics is not possible by conventional methods.

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Authors Contributions

H.E.G., B.L.N, and C.T.M designed the analytical approach and wrote the code for analysis. H.E.G. simulated the data and reformatted real site data for use in the study with guidance from C.T.M. All authors contributed to the drafting of the manuscript.

Data Accessibility

Simulated line-transect distance sampling observations, code for running the RJMCMC method, and R script for analyzing data in JAGS are available on Github:

<https://github.com/heathergaya/Bias-Adjusted-Model/releases/tag/v1.0-alpha>

5. Literature Cited

- Bernardo, J. M., Berger, J. O., Dawid, A. P., & Smith, A. F. M. (Eds.). (1992). *Bayesian Statistics 4*. Oxford: Clarendon Press.
- Bradford, A. L., Forney, K. A., Oleson, E. M., & Barlow, J. (2014). Accounting for subgroup structure in line-transect abundance estimates of false killer whales (*Pseudorca crassidens*) in Hawaiian waters. *PLOS ONE*, 9(2), e90464. doi:[10.1371/journal.pone.0090464](https://doi.org/10.1371/journal.pone.0090464)
- Brooks, S. (Ed.). (2011). *Handbook for Markov Chain Monte Carlo*. Boca Raton: Taylor & Francis.
- Brooks, S. & Giudici, P. (1998). Convergence assessment for reversible jump mcmc simulations. In *Bayesian Statistics 6* (pp. 733–742). Oxford: Oxford University Press.
- Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J., Borchers, D., & Thomas, L. (2004). *Advanced Distance Sampling* (Vol. 89). Oxford: Oxford University Press.
- Buckland, S. T., Anderson, D. R., Burnham, K. P., & Laake, J. L. (1993). *Distance Sampling: Estimating Abundance of Biological Populations*. New York: Springer.
- Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., & Thomas, L. (2001). *Introduction to Distance Sampling: Estimating Abundance of Biological Populations*. Oxford: Oxford University Press.
- Cassey, P., & T Ussher, G. (1999). Estimating abundance of tuatara. *Biological Conservation*, 88, 361–366. doi:[10.1016/S0006-3207\(98\)00114-1](https://doi.org/10.1016/S0006-3207(98)00114-1)
- Castellón, T. D., Rothermel, B. B., & Nomani, S. Z. (2015). A comparison of line-transect distance sampling methods for estimating gopher tortoise population densities. *Wildlife Society Bulletin*, 39(4), 804–812. doi:[10.1002/wsb.605](https://doi.org/10.1002/wsb.605)
- Dick, D. M., & Hines, E. M. (2011). Using distance sampling techniques to estimate bottlenose dolphin (*Tursiops truncatus*) abundance at Turneffe Atoll, Belize. *Marine Mammal Science*, 27(3), 606–621. doi:[10.1111/j.1748-7692.2010.00435.x](https://doi.org/10.1111/j.1748-7692.2010.00435.x)

- Eisenberg, J. F. (1983). The gopher tortoise as a keystone species. In *The Gopher Tortoise: A Keystone Species. Proceedings of the 4th Annual Meeting Of The Gopher Tortoise Council*. Florida State Museum, Gainesville, Florida.
- Enge, K, Berish, J. E., Bolt, R., Dziergowski, A., & Mushinsky, H. (2006). *Biological Status Report: Gopher tortoise*. Florida Fish and Wildlife Conservation Commission. Retrieved from http://myfwc.com/media/969926/Gopher_Tortoise_BSR.pdf
- Gelman, A., Rubin, D. B., Carlin, J. B., & Stern, H. S. (2003). *Bayesian Data Analysis* (2nd ed). Boca Raton: Chapman & Hall/CRC.
- Germano, D. J. (1994). Growth and age at maturity of North American tortoises in relation to regional climates. *Canadian Journal of Zoology*, 72(5), 918–931. doi:[10.1139/z94-125](https://doi.org/10.1139/z94-125)
- Glass, R., Forsyth, D., Coulson, G., & Festa-Bianchet, M. (2016). Precision, accuracy and bias of walked line-transect distance sampling to estimate eastern grey kangaroo population size. *Wildlife Research*, 42. doi:[10.1071/WR15029](https://doi.org/10.1071/WR15029)
- Gopher Tortoise Council. (2013, July 24). Gopher tortoise minimum viable population and minimum reserve size working group report. Retrieved from http://www.gophertortoisecouncil.org/conserv/MVP_Report_Final-1.2013.pdf
- Green, P. J. (1995). Reversible jump markov chain monte carlo computation and bayesian model determination. *Biometrika*, 82(4), 711–732. doi:[10.1093/biomet/82.4.711](https://doi.org/10.1093/biomet/82.4.711)
- Hastings, W. K. (1970). Monte carlo sampling methods using markov chains and their applications. *Biometrika*, 57(1), 97–109. doi:[10.2307/2334940](https://doi.org/10.2307/2334940)
- Hu, X. J., & Lawless, J. F. (1997). Pseudolikelihood estimation in a class of problems with response-related missing covariates. *The Canadian Journal of Statistics / La Revue Canadienne de Statistique*, 25(2), 125–142. doi:[10.2307/3315727](https://doi.org/10.2307/3315727)
- Lips, K. R. (1991). Vertebrates associated with tortoise (*Gopherus polyphemus*) burrows in four habitats in south-central Florida. *Journal of Herpetology*, 25(4), 477. doi:[10.2307/1564772](https://doi.org/10.2307/1564772)
- Marques, T. A., Thomas, L., Fancy, S. G., Buckland, S. T., & Handel, C. M. (2007). Improving estimates of bird density using multiple- covariate distance sampling. *The Auk*, 124(4), 1229–1243. doi:[10.1642/0004-8038\(2007\)124\[1229:IEOBDU\]2.0.CO;2](https://doi.org/10.1642/0004-8038(2007)124[1229:IEOBDU]2.0.CO;2)
- Martin, P. L., & Layne, J. N. (1987). Relationship of gopher tortoise body size to burrow size in a southcentral Florida population. *Florida Scientist*, 50(4), 264–267.
- McCoy, E. D., & Mushinsky, H. R. (1992). Studying a species in decline: gopher tortoises and the dilemma of ‘correction factors’. *Herpetologica*, 48(4), 402–407.

- Metropolis, N., Rosenbluth, A. W., Rosenbluth, M. N., Teller, A. H., & Teller, E. (1953). Equation of state calculations by fast computing machines. *The Journal of Chemical Physics*, 21(6), 1087–1092. doi:[10.1063/1.1699114](https://doi.org/10.1063/1.1699114)
- Nomani, S., Oli, M., & Carthy, R. (2008). Comparison of methods for estimating abundance of gopher tortoises. *Applied Herpetology*, 5(1), 13–31. doi:[10.1163/157075408783489167](https://doi.org/10.1163/157075408783489167)
- Richardson, S., & Green, P. J. (1998). Corrigendum: On bayesian analysis of mixtures with an unknown number of components. *Journal of the Royal Statistical Society. Series B- (Methodological)*, 59, U3–U3.
- Richardson, Sylvia, & Green, P. J. (1997). On bayesian analysis of mixtures with an unknown number of components. *Journal of the Royal Statistical Society. Series B (Methodological)*, 10(4), 62.
- Royle, J. A., & Dorazio, R. M. (2008). *Hierarchical Modeling and Inference in Ecology: The Analysis of Data From Populations, Metapopulations and Communities* (Reprint). Amsterdam: Acad. Press.
- Royle, J. A., Dorazio, R. M., & Link, W. A. (2007). Analysis of multinomial models with unknown index using data augmentation. *Journal of Computational and Graphical Statistics*, 16(1), 67–85. doi:[10.1198/106186007X181425](https://doi.org/10.1198/106186007X181425)
- Smith, L., Linehan, J., Stober, J., Elliott, M., & Jensen, J. (2009). An evaluation of distance sampling for large-scale gopher tortoise surveys in Georgia, USA. *Applied Herpetology*, 6(4), 355–368. doi:[10.1163/157075309X12470350858550](https://doi.org/10.1163/157075309X12470350858550)
- Smith, R., Chambers, A., Berish, J., Tuberville, T., & Herpich, K. (2005). Gopher tortoise burrow surveys: external characteristics, burrow cameras, and truth. *Applied Herpetology*, 2(2), 161–170. doi:[10.1163/1570754043492081](https://doi.org/10.1163/1570754043492081)
- Stober, J. M., Prieto-Gonzalez, R., Smith, L. L., Marques, T. A., & Thomas, L. (2017). Techniques for estimating the size of low-density gopher tortoise populations. *Journal of Fish and Wildlife Management*, 8(2), 377–386. doi:[10.3996/012017-JFWM-005](https://doi.org/10.3996/012017-JFWM-005)
- Stober, J. M., & Smith, L. L. (2010). Total counts versus line transects for estimating abundance of small gopher tortoise populations. *The Journal of Wildlife Management*, 74(7), 1595–1600.
- Strobel, B. N., & Butler, M. J. (2014). Monitoring whooping crane abundance using aerial surveys: influences on detectability. *Wildlife Society Bulletin*, 38(1), 188–195. doi:[10.1002/wsb.374](https://doi.org/10.1002/wsb.374)
- Thomas, L., Buckland, S. T., Burnham, K. P., Anderson, D. R., Laake, J. L., Borchers, D. L., & Strindberg, S. (2006). Distance sampling. In *Encyclopedia of Environmetrics*. Hoboken, NJ: John Wiley & Sons, Ltd. doi:[10.1002/9780470057339.vad033](https://doi.org/10.1002/9780470057339.vad033)

- Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L., ... Burnham, K. P. (2010). Distance software: design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology*, 47(1), 5–14. doi:[10.1111/j.1365-2664.2009.01737.x](https://doi.org/10.1111/j.1365-2664.2009.01737.x)
- U.S. Fish and Wildlife Service. (1987). Endangered and threatened wildlife and plants: determination of threatened status for the gopher tortoise (*Gopherus polyphemus*). Federal Register 52(129):25376-25380.
- U.S. Fish and Wildlife Service. (2011). Endangered and threatened wildlife and plants: 12-month finding on a petition to list the gopher tortoise as threatened in the eastern portion of its range; Final rule. Federal Register 76(144):45130-45162.
- U.S. Fish and Wildlife Service. (2013). *Range-wide conservation strategy for the gopher tortoise*. Retrieved from <https://www.fws.gov/southeast/pdf/strategy/gopher-tortoise-conservation-strategy-v2.pdf>
- Waddle, J. H., Mazzotti, F. J., & Rice, K. G. (2006). Changes in abundance of gopher tortoise burrows at Cape Sable, Florida. *Southeastern Naturalist*, 5(2), 277–284. doi:[10.1656/1528-7092\(2006\)5\[277:CIAOGT\]2.0.CO;2](https://doi.org/10.1656/1528-7092(2006)5[277:CIAOGT]2.0.CO;2)
- Williams, B. K., Nichols, J. D., & Conroy, M. J. (2002). *Analysis and Management of Animal Populations: Modeling, Estimation, and Decision Making*. San Diego: Academic Press.

Table 2.1 Confidence and Credible Interval Performance. The percentage of simulations ($n = 20$) for each scenario where the 95% confidence interval (Program DISTANCE) or credible interval (bias-adjusted model) included the true population density.

Simulation Scenario	Program DISTANCE		Bias-Adjusted Model	
	2 ind/ ha ⁻¹	6 ind/ ha ⁻¹	2 ind/ ha ⁻¹	6 ind/ ha ⁻¹
			1	1
Scenario A: Declining	85%	80%	95%	100%
Scenario O: Unimodal	70%	85%	100%	85%
Scenario M: Multi-modal	90%	80%	100%	90%
Scenario U: Uniform	55%	65%	90%	100%

Table 2.2 Density Estimate Comparisons for 3 Sites in Georgia. Gopher Tortoise (*Gopherus polyphemus*) burrow (D, burrows ha⁻¹) and tortoise (Dt, tortoises ha⁻¹) mean density estimates and coefficient of variation (SE/mean x 100%) from Line-Transect Distance Sampling (LTDS) data collected for 3 sites in Georgia and derived under Program DISTANCE and our bias-adjusted model. L = length of transect in km; #B Obs = number of burrows observed; #T Obs = number of occupied burrows observed.

Site	L	#B Obs	#T Obs	Model	D	Dt
Doerun Natural Area	28.09	143	62	DISTANCE	3.02 (14%)	1.23 (22%)
				Bias-adjusted model	3.13 (9%)	1.40 (12%)
Holman Property	85.95	517	115	DISTANCE	2.14 (8%)	0.45 (16%)
				Bias-adjusted model	1.87 (2%)	0.47 (6%)
Sansavilla WMA	25.33	248	90	DISTANCE	3.31 (11%)	1.19 (18%)
				Bias-adjusted model	3.91 (9%)	1.50 (12%)

Table 2.3. Burrow Occupancy And Detectability Estimates for 3 Sites in Georgia. Average occupancy of Gopher Tortoise (*Gopherus polyphemus*) burrows, estimated slope for burrow occupancy (95% credible interval) and estimated detection probability of 5-cm wide burrows on the line (95% credible interval) from Line-Transect Distance Sampling (LTDS) data collected for 3 sites in Georgia and derived under our bias-adjusted model.

Site	Average Burrow Occupancy	Estimated Burrow Occupancy Slope	Estimated Detection on the Line
Doerun Natural Area	0.43	0.015 (-0.025 – 0.071)	0.44 (0.20-0.67)
Holman Property	0.22	0.022 (-0.006 – 0.052)	0.45 (0.22 – 0.69)
Sansavilla WMA	0.36	-0.022 (-.047 – 0.003)	0.43 (0.20 – 0.67)

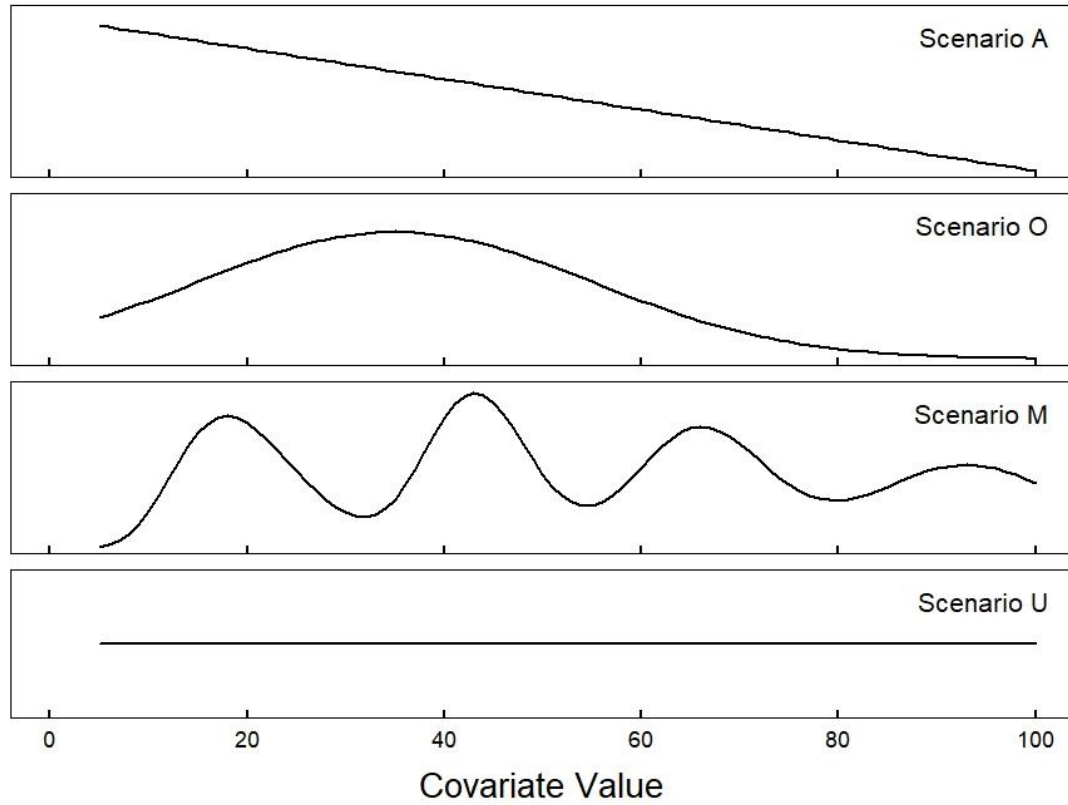


Figure 2.1. Alternative Covariate Distributions (A, O, M and U) Used in Simulations. Each Figure represents a scenario for the distribution of a generic individual-level covariate (range 5-100 units) occurring in the population. Each scenario was used in line-transect distance sampling (LTDS) simulations 40 times, for a total of 160 simulations.

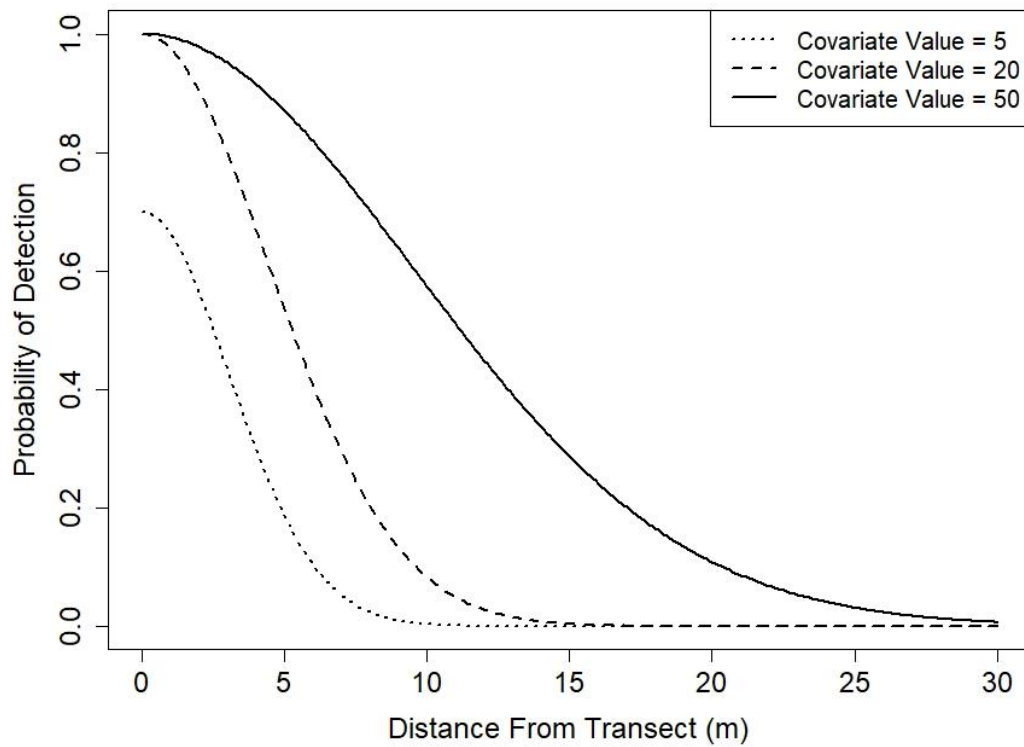


Figure 2.2 Example Detection Curves Used in Simulation. We used a half-normal detection curve to simulate detection, allowing the sigma parameter of the curve to vary with the value of a generic individual-level covariate. For objects with the minimum covariate value (5 units), probability of detection on the transect line (at distance 0 m) was set to 0.7. Objects with covariate values ≥ 20 units had perfect detection on the line (probability of detection at distance 0 m was 1).

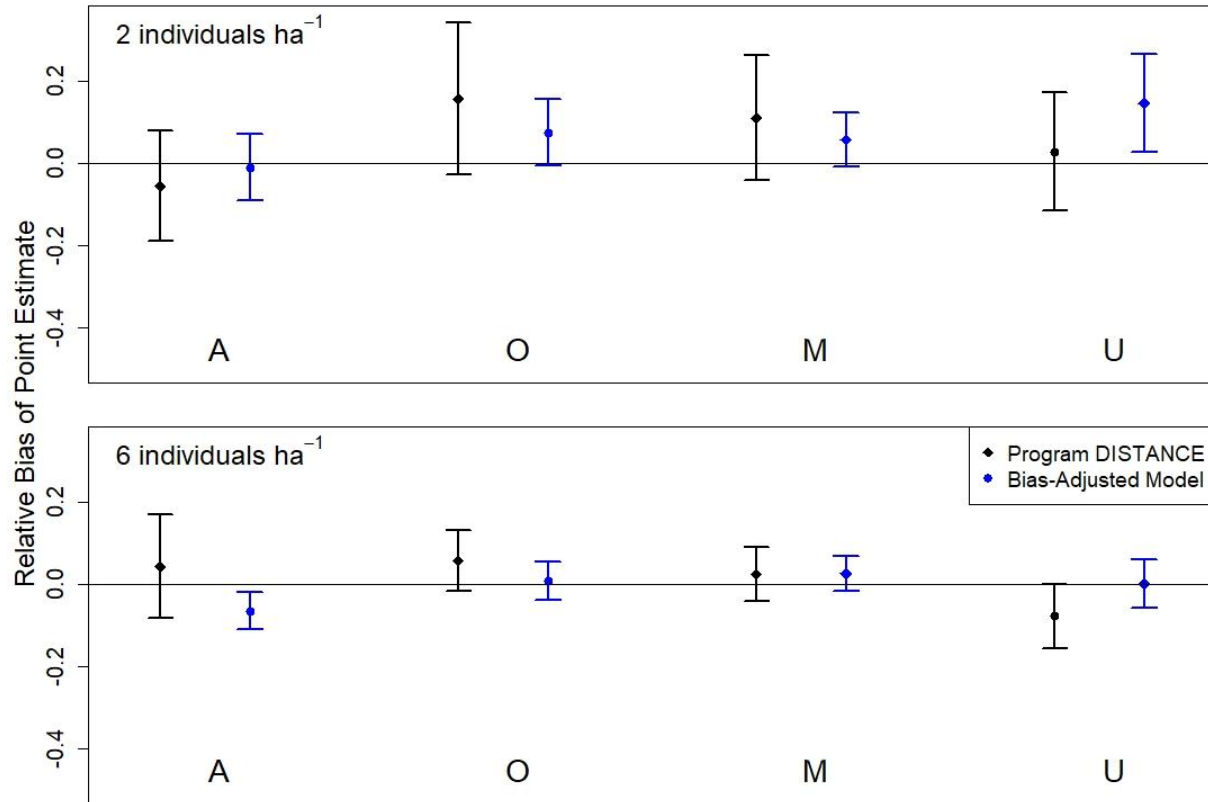


Figure 2.3 Relative Bias of Density Point Estimates. Here we show the mean ($n = 20$) and 95% CI interval for relative bias (estimate/true – 1.0) of the point estimates derived under program DISTANCE (95% confidence interval) and our bias-adjusted model (95% credible interval) for each of our 8 simulation scenarios. For each pair of means and ranges, the label below indicates shape of the covariate distribution (A=declining, O=unimodal, M = multi-modal, U=uniform). The horizontal line in each section of the Figure represents a relative bias of 0 (the point estimate was equal to the truth).

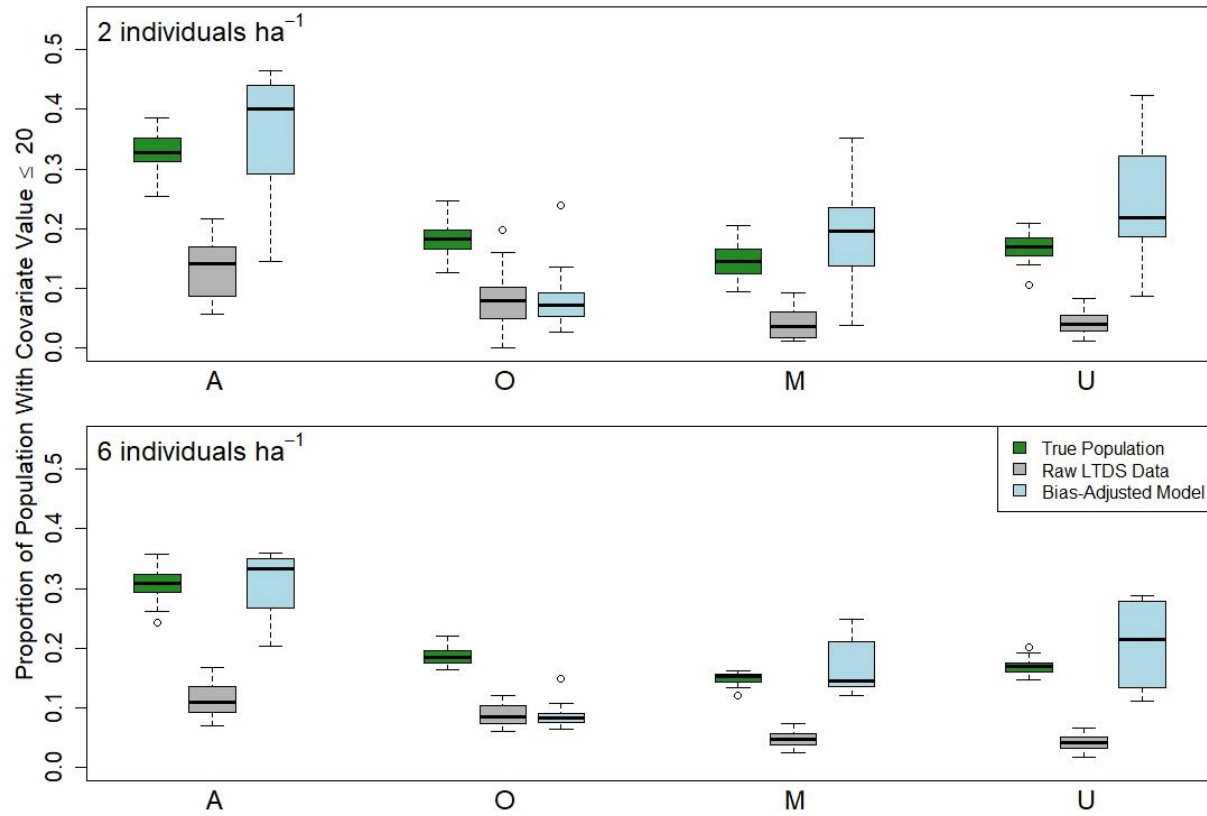


Figure 2.4. Estimated Proportion of Population with Smallest Covariate Values. The green boxplots in each scenario represent the true proportion of individuals in the population with value ≤ 20 for a generic individual-level covariate. Grey and blue boxplots display the proportion of individuals in the population with value ≤ 20 derived from raw LTDS data and our bias-adjusted model respectively. The labels below each set of boxplots indicate the shape of the covariate distribution (A=declining, O=unimodal, M = bi-modal, U=uniform).

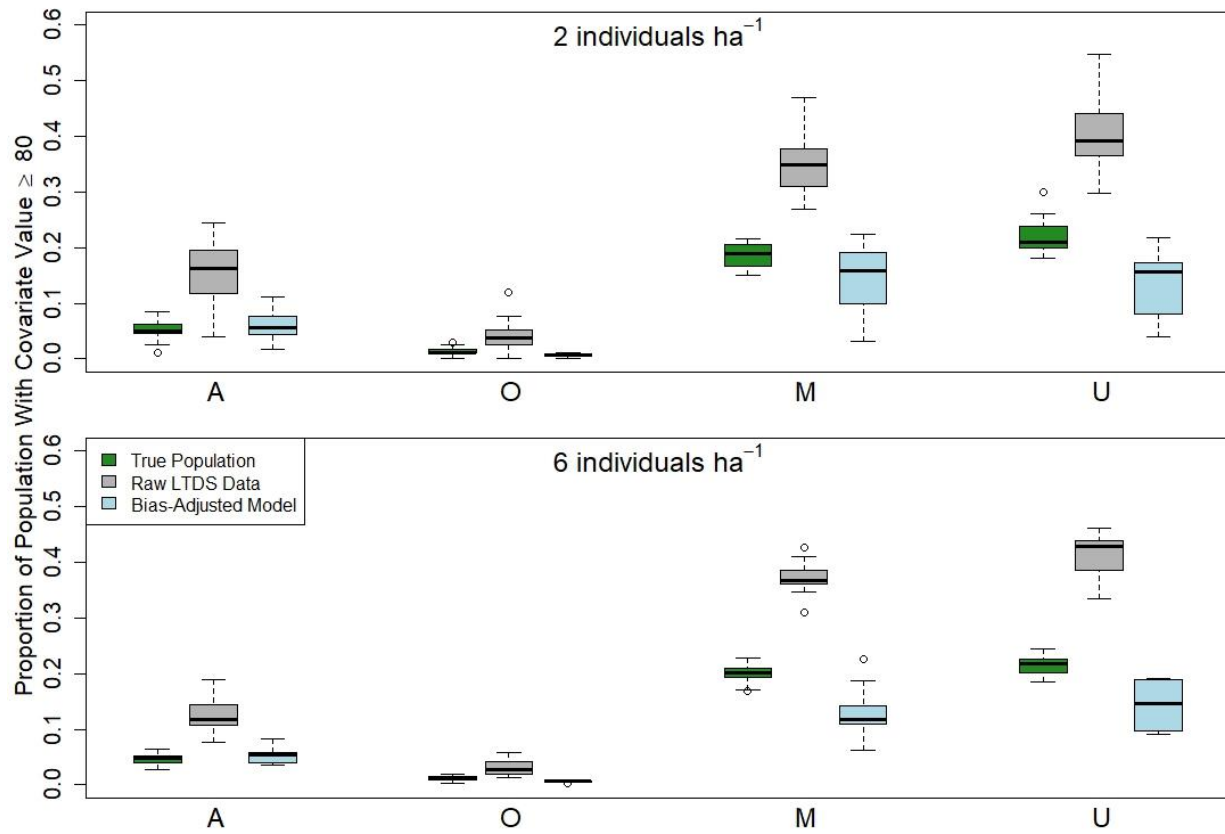


Figure 2.5. Estimated Proportion of Population with Largest Covariate. The green boxplots in each scenario represent the true percentage of individuals in the population with value ≥ 80 of a generic individual-level covariate. Grey and blue boxplots display the proportion of individuals in the population with value ≥ 80 derived from raw LTDS data and our bias-adjusted model respectively. The labels below each set of boxplots indicate the shape of the covariate distribution (A=declining, O=unimodal, M = bi-modal, U=uniform).

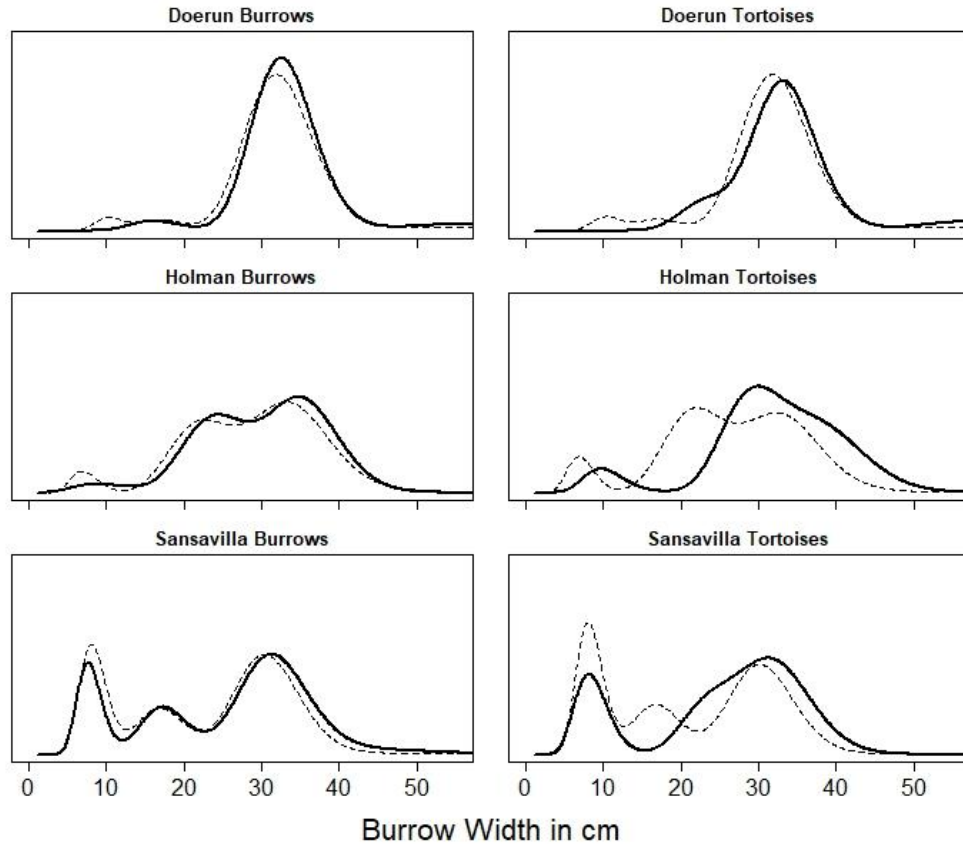


Figure. 2.6. Estimated Burrow (left panels) and Tortoise Size (right panels) Distributions (dashed) Overlaid on Smoothed Raw Size Frequencies at Three Sites in Georgia. For each site, the solid line is a smoothed representation of the raw LTDS data collected. The dotted line indicates the estimated true size distribution from our bias-adjusted model. Gopher tortoise burrow width roughly corresponds to tortoise length, though burrows may sometimes be larger than actual tortoise size.

CHAPTER 3

FINDING THE TORTOISE IN THE WIREGRASS: ACCOUNTING FOR VEGETATION VARIATION IN GOPHER TORTOISE SURVEYS ²

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Abstract

The presence of ground-level vegetation significantly decreases detection of tortoise burrows of all sizes during line-transect distance sampling (LTDS) surveys, but no field or analytical methods exist to control for vegetation obstruction as a source of variation in detection rate. Here we propose the addition of a simple measurement of ground-level vegetation to transect surveys that serves as a covariate for the detection function. We present a Bayesian hierarchical model in which 2 burrow covariates, burrow width and nearby vegetation height, help to account for detection bias of all burrows present and improve precision of estimated density. We investigate the performance of this covariate by simulation and by using real LTDS data collected at a site before and after application of prescribed fire. Across all simulations, our Bayesian hierarchical model produced the most accurate density point estimates (point estimates closer to the truth) of any of the models tested. For our pre- and post-burn surveys, Our Bayesian model tended to produce similar density estimates when vegetation covariates were included. Our study indicates that any level of spatial variation in vegetation obstruction decreases detection of burrows and may lead to large underestimation in population size when not considered during analysis.

The gopher tortoise (*Gopherus polyphemus*) is a burrowing land turtle occurring in upland scrub and longleaf pine (*Pinus palustris*) habitats of the southeastern United States. The species is currently declining range-wide, likely due to habitat loss and habitat degradation (McCoy and Mushinsky 1992, McCoy et al. 2006, Smith et al. 2006). As a result, the gopher tortoise is federally listed as threatened under the Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana, and it is listed as a species of special concern by the remaining states in its range (U.S. Fish and Wildlife Service 1987, Yager et al. 2007). Line-transect distance sampling (LTDS) is widely employed by land managers to obtain important demographic information (abundance, density, size distribution) about tortoise populations. Considering LTDS to be a reliable method of monitoring populations, the U.S. Fish and Wildlife Service currently promotes LTDS as the standard monitoring method for populations across the range (U.S. Fish and Wildlife Service 2013).

Gopher tortoise surveys use gopher tortoise burrows as the object of interest, as the tortoises themselves are rarely observed outside of burrows (Smith et al. 2009). Size and distance information is collected on both occupied and unoccupied burrows, though only the density of occupied burrows is usually of interest (Smith et al. 2009), as occupancy is not thought to influence detection probability (Castellón et al. 2015, Stober et al. 2017). Burrow widths range from 5 cm (hatchlings) to > 35 cm (adults) and roughly correspond to the length of the resident tortoise's carapace (Martin and Layne 1987, Doonan and Stout 1994).

However, studies indicate juvenile and adult burrows have different probabilities of detection during surveys, with juvenile burrows frequently missed regardless of distance to the transect (Ballou 2013). Juvenile tortoises also tend to burrow under objects such as dense shrubs or downed trees that mask the outward signs of burrow presence, increasing detection difficulties

(Alford 1980, Doonan and Stout 1994, Epperson and Heise 2003). Additionally, the presence of ground-level vegetation significantly decreases detection of tortoise burrows of all sizes, highlighting the importance of accounting for vegetation in gopher tortoise surveys (Berish and Leone 2014, Howze and Smith 2018). However, no field or analytical methods exist to control for vegetation obstruction as a source of variation in detection rate. Though covariates linked to the size of the tortoise (e.g., burrow width) are helpful in controlling for size-related detectability in LTDS models (Buckland et al. 2004, Marques et al. 2007, Thomas et al. 2010), it remains difficult to differentiate between a small proportion of juveniles in the population and low juvenile detection probability. As the gopher tortoise population continues to decline, improving the LTDS sampling design to account for both vegetation and size-biased detection will become increasingly important for making informed decisions about management and conservation practices throughout the tortoise's native range.

Here we propose the addition of a simple measurement of ground-level vegetation to transect surveys that serves as a covariate for the detection function. We investigate the performance of this covariate by simulation and by using real LTDS data collected at a site before (with obscuring vegetation cover) and after (vegetation cover mostly removed) application of prescribed fire. While density estimation of gopher tortoise LTDS data is conventionally carried out in a maximum likelihood framework under standalone Program DISTANCE (Thomas et al. 2010) or packages available in R (e.g., Rdistance (Miller et al. 2017)), we present an alternative formulation under a Bayesian framework using Markov chain Monte Carlo (MCMC) sampling (Brooks 2011). We find that the Bayesian approach permits great flexibility in model construction, including incorporation of hierarchical parameter structures and direct inference on latent (hidden) population parameters. We present a model for

LTDS in which 2 burrow covariates, burrow width and nearby vegetation height, help to account for detection bias of all burrows present and improve precision of estimated density. We also produce a detection-adjusted size frequency distribution of occupied burrows. We further compare the density point estimates and precision produced from DISTANCE v 6.2 and our own model for both a simulated and real dataset.

STUDY AREA

We conducted LTDS surveys on 76 ha distributed in 4 plots (A, B, C, and D) of upland habitat at the Joseph W. Jones Ecological Research Center in Newton, Georgia (Figure. 3.1). The plots consisted primarily of longleaf pine (*Pinus palustris*) or mixed longleaf pine/hardwood (*Quercus* spp.) with herbaceous cover dominated by wiregrass (*Aristida stricta*). We selected these plots because they contained gopher tortoise populations, they varied in groundcover density, and site managers had scheduled the plots to be burned in 2018 as part of routine habitat maintenance. Variation in pre-burn groundcover density was due to frequency of fire in the plot: plots C and D were last burned in 2017 (1 year prior) whereas plots A and B were last burned in 2016 (2-year rotations). Site managers burned the plots on 21 March 2018 (A), 23 March 2018 (B and C), and 25 April 2018 (D).

METHODS

Gopher Tortoise LTDS with Vegetation Measurements

Following conventional gopher tortoise LTDS survey guidelines (Smith et al. 2016), we surveyed transects using 3 observers. The central observer navigated the transect using a Trimble Juno T41 (1-2 m accuracy; Trimble Navigation, Ltd., Sunnyvale, CA) and searched for burrows on and near the transect. Additional observers searched for burrows on either side of the transect. We spaced transects spaced 50 m apart to maximize survey coverage of the site but also limit the

number of burrows that might be observed from adjacent transects (Smith and Howze 2016). At each burrow, we obtained a GPS location, measured burrow width at 50 cm inside the opening (Martin and Layne 1987), and determined occupancy status of non-collapsed burrows. We determined occupancy by scoping each burrow with a burrow camera and classifying the burrow as either (1) occupied (tortoise observed), (2) unoccupied or (3) unknown occupancy (Smith et al. 2016).

In addition to the conventional protocol, we recorded vegetation measurements at regular 15-m intervals from the beginning to the end of each transect line to estimate vegetation obstruction. We measured vegetation obstruction by placing a meter stick ~5 m off the transect and estimating the minimum height of vegetation (0 to 1m, 5 cm increments) where all markings were $\geq 50\%$ obscured. In the lab, we used inverse distance weighted interpolation of the point measurements in ArcMap v. 10.6.1 (Environmental Systems Research Institute, Redlands, California, USA) to create a smoothed layer of estimated vegetation density across the sampled landscape and extracted an estimate of vegetation obstruction (range 0-1 m) at the location of each detected burrow. The use of animals in this study was approved by the University of Georgia's Animal Care and Use Committee (permit A2017 03-016-Y2-A0).

Conventional Model for Distance Sampling

Under conventional distance sampling, population density is estimated as the total number of individuals detected during the survey divided by an estimate of the total area surveyed. Total area surveyed is estimated by fitting a detection function to the distance data (x) collected during the survey and inferring an “effective strip width” – an estimate of area surveyed on either side of the transects. Assuming all animals on the line are detected, density is estimated by:

$$\hat{D} = \frac{n}{2 * \mu * L}$$

where n represents the total number of detected individuals, L is the total length of transects, and μ is the effective strip width of the survey (Buckland et al. 2001). Parameter μ is interpreted as the distance from the line for which as many objects are detected beyond μ as missed within μ and is entirely dependent on the fitted detection curve (Thomas et al. 2010). Covariates may be incorporated in the detection function to account for variation in detection unrelated to distance (Buckland et al. 1993, Buckland et al. 2004, Royle and Dorazio 2008). For a half-normal or hazard-rate detection function, covariates are easily added to a linear model for the scale parameter, σ , such that:

$$\ln(\sigma_i) = \beta_0 + \beta_1 z_{1i} + \beta_2 z_{2i} \dots + \beta_j z_{ji}$$

where β_j is the beta coefficient for the j th covariate and z_{ji} is the measured value of covariate j for individual i . One difficulty of this approach, however, is deciding how best to assign covariate values to undetected individuals.

Implementation in a Bayesian Hierarchical Framework with Data Augmentation

Under an alternative Bayesian approach to modeling LTDS data, we associated each burrow in the study area with a distance to transect and burrow width. We used a data-augmentation technique (Royle and Dorazio 2008), where the known distances and covariate values for n_{ind} found burrows were augmented with a large number n_A of missing values representing potentially missed burrows. Assuming transect lines were placed independently with respect to the burrows, we drew distances for augmented burrows from a uniform distribution such that:

$$x_i \sim \text{Uniform}(0, Bx)$$

where Bx is a given maximum distance of our choosing from the transect line. The model then evaluated each augmented burrow and drew a binary value that proposed its existence in the population:

$$w_i \sim \text{Binomial}(1, \psi),$$

where ψ is a probability of inclusion in the population.

All real burrows, both drawn from the augmented population (those in n_A with $w_i = 1$) plus those found during the survey, were then summed into a total population N which was used to find the population density of burrows:

$$\hat{D} = \frac{N}{2 * L * Bx}$$

with:

$$N = n_{ind} + \sum_{i=1}^{n_A} w_i .$$

As in the frequentist approach, we modeled variation in burrow detection through adjustments to the scale parameter, σ , of our detection function (Buckland et al. 2004). For burrow i , we modeled the scale parameter as a log-linear function of an intercept, a burrow width predictor variable z_i , and a vegetation predictor variable v_i with uninformative normal priors for the intercept and coefficient terms:

$$\ln(\sigma_i) = \sigma.int + \beta_{\sigma z} * z_i + \beta_{\sigma v} * v_i .$$

Each missed burrow in the population $i = 1, \dots, (N - n_{ind})$ was assigned a vegetation measurement drawn from a beta distribution informed by all raw vegetation measurements (v_r) taken at the site:

$$v_r \sim \text{Beta}(d, e)$$

$$v_i \sim \text{Beta}(d, e)$$

The shape and rate parameters d and e for the beta distribution were given uniform priors bound between 0.1 and 50.

We modeled burrow width as a mixture of a small number (K) of gamma distributions to reflect the multi-modality of a size-structured tortoise population. We used a reverse-jump MCMC sampling method to estimate value K (Chapter 2). Each distribution was characterized by shape and rate parameters α_k and β_k respectively and was bound between reasonable limits for burrow widths. Each missed burrow in the population, $i = 1, \dots, (N - n_{ind})$, was assigned to 1 of the distributions, k , according to a categorical draw informed by a Dirichlet distribution:

$$k \sim \text{Multinomial}(1, \mathbf{p})$$

$$\mathbf{p} \sim \text{Dirichlet}(\omega_1, \dots, \omega_k) .$$

Finally, burrow width z_{ik} for missed burrow i in distribution k was drawn from the corresponding gamma distribution:

$$z_{ik} \sim \text{Gamma}(\alpha_k, \beta_k)$$

Parameters α_k and β_k for each gamma distribution were given uninformative priors, as were the ω_k :

$$\omega_k \sim \text{Gamma}(0.001, 0.001)$$

We wanted our model to account for imperfect detection on the transect line for smaller burrow sizes (Chapter 2). We assumed the existence of a threshold burrow width value ($b.point$) above which detection on the line was perfect. We drew this value from a uniform distribution bound between 15-22 cm, but another choice of prior could be appropriate if expert opinion or previous site knowledge suggests a different threshold of width for perfect detection on the line. For burrows of the smallest possible width (z_{min}), we drew detection on the line from a uniform distribution bound between 0.2-1.0. Probability of detection of burrows on the transect line

increased proportionally as burrow width varied between this minimum value and the threshold point; perfect detection beyond the threshold point thus produced a “broken stick” appearance for the function:

$$\xi_i = \begin{cases} \left(\frac{1-b}{b.point - z_{min}} \right) (z_i - z_{min}) + b, & z_{min} \leq z_i \leq b.point \\ 1, & z_i \geq b.point \end{cases}$$

$$b \sim \text{Uniform}(0.2, 1)$$

$$b.point \sim \text{Uniform}(15, 22) .$$

For the half-normal detection curve, probability of detection of burrow i at distance x from the transect line was described by:

$$g(x, z) = e^{\left(\frac{-x_i^2}{2\sigma_i^2} \right)} * \xi_i$$

Previous research suggests that larger tortoise burrows are less likely to be occupied than smaller burrows (Ballou 2013). We modeled burrow occupancy status (o_i) as linearly related to burrow width and assumed burrow occupancy had no effect on detection probability. We assigned uninformative normal priors for an intercept and a burrow width coefficient:

$$o_i \sim \text{Binomial}(1, \theta_i)$$

$$\text{logit}(\theta_i) = \theta.int + \beta_\theta * z_i$$

Simulation

We simulated line-transect distance sampling on a 100-ha² area, with parallel transects spaced 50 m apart. We considered 2 factors that might affect model performance: population density and the level of heterogeneity of vegetation on the landscape. We simulated vegetation at 3 levels of spatial variation (low, medium and high) using the landscapeR package in R (<https://cran.project.org/web/packages/landscapeR/landscapeR.pdf>) (Figure. 3.2). We created rasters with different size patches of vegetation, with patch size varying based on spatial

variation level. Patches of increasingly smaller sizes were added to the raster until all cells contained vegetation values. Maximum patch size was set at 700 m², 250 m² and 100 m² for low, medium and high spatial variation respectively. Each patch was randomly assigned a vegetation value drawn from a uniform distribution bound between 0.1 and 0.99. We simulated field-based measurements on the transect line every 15 m as the average vegetation value on the landscape within a 10-m radius of the measurement point. At the highest simulated spatial variation (100 m² maximum patch size), vegetation varied more frequently over space than the vegetation measurement interval. In the way described above for field data, we created a smoothed layer of estimated vegetation density across the simulated landscape and extracted an estimate of vegetation obstruction (range 0-1) at the location of each detected burrow.

For each vegetation scenario, burrows were randomly distributed across the area at a density of 2 and 6 burrows ha⁻¹. Individual burrow widths were drawn from a mixture of 4 gammas with possible burrow widths ranging from 5-65 cm (Figure. 3.3). We simulated the detection of burrows according to probabilities from a half normal curve with $\log(\sigma_i) = 1 + 0.025 * z_i - 1.5 * v_i$ to allow detection to vary with both burrow width and vegetation density at the burrow level (Figure. 3.4). Detection on the transect line was simulated as a broken stick regression with perfect detection on the line for any burrow width ≥ 20 cm. For the smallest covariate value, detection on the line was set at 0.7. For all simulations, the occupancy state, o , of all individuals in the population was set at 1, and we excluded the model component for θ .

We simulated each of our 6 scenarios (3 vegetation landscapes $\times$ 2 tortoise densities) 20 times, totaling 120 simulations. We compared 3 estimation methods for each simulation – Program DISTANCE without any covariates (hereafter: the base model), Program DISTANCE with vegetation and burrow width covariates, and our Bayesian hierarchical model with the same

covariates. We computed relative bias of point estimates and performance of credible intervals and confidence intervals for overall density of burrows. We compared the accuracy of estimates of the proportion of small burrows (≤ 20 cm width) from both the raw LTDS data and our Bayesian model using Welch's t-test (Welch 1938) for both simulated densities (2 and 6 burrows ha^{-1} ; pooled over vegetation landscapes). For each simulated survey, we allowed Program DISTANCE to fit any of the detection curves available within the program when covariates are used (half-normal, hazard rate, or uniform). For our Bayesian model, we tested for chain convergence (3 chains) using the Gelman–Rubin statistic (Gelman et al. 2003) and extended simulations ($\geq 300,000$ iterations per simulation scenario) to meet the minimum iteration suggestion of the Raftery-Lewis diagnostic (Bernardo et al. 1992). Each run of our Bayesian model took between 2-4 hr to complete using the runjags package of R (<https://cran.r-project.org/web/packages/runjags/runjags.pdf>) on a 2.30 GHz processor using 3 cores.

Pre- and Post-Burn Surveys

To further evaluate the performance of our 3 tested models, we surveyed plots A-D before and after a prescribed fire and compared pre- and post-burn density estimates. We conducted pre-burn surveys 16-18 March 2018 using LTDS sampling with systematic measurement of vegetation obstruction as described above. We resampled Plots A, B and C on 22-24 March 2018 using identical methodology from the pre-burn survey, except that we switched observer positions to reduce memory bias. Due to logistical constraints and time of burning, we were unable to resample Plot D until 24 June 2018.

We analyzed our field data with vegetation measurements using both Program DISTANCE v. 6.2 and our Bayesian hierarchical model. As described at the start of the methods, we assigned vegetation values to detected burrows using spatial interpolation of the point

measurements. For our post-burn surveys, we assumed perfect detection on the transect line for all burrows. As in our simulations, we explored results under Program DISTANCE with (1) both vegetation and burrow width covariates and (2) when no covariates were included. We allowed Program DISTANCE to fit any of the detection curves available within the program when covariates are used (half-normal, hazard rate, or uniform). We then used Akaike's Information Criterion (AIC) for model selection to choose the best fitting detection curve. Following Stober et al. (2017), we allowed Program DISTANCE to fit detection curves using all available burrows and then adjusted estimates by observed occupancy probability to separately estimate tortoise densities. For our Bayesian model, we tested for chain convergence (3 chains) using the Gelman–Rubin statistic (Gelman et al. 2003) and extended simulations to meet the minimum iteration suggestion of the Raftery-Lewis diagnostic (Bernardo et al. 1992). All simulations required $\geq 400,000$ iterations to meet these criteria. As true abundance on each plot was unknown, we looked at consistency in the point estimate of abundance for pre- and post-burn surveys as evidence of each model's success in adjusting for vegetation density. For all models, we compared estimated quantities derived from pre- and post-burn data, and we computed precision of density point estimates and select quantiles of the burrow distribution.

RESULTS

Simulation

Across all simulations, our Bayesian hierarchical model produced the most accurate density point estimates (point estimates closer to the truth) of any of the models tested (Figure. 3.5). Confidence and credible interval performance followed a similar pattern with intervals capturing the true density 87%, 89% and 94% of the time for the base model, Program DISTANCE with covariates, and the Bayesian model respectively. At the highest simulated level

of vegetation spatial variation, where vegetation varied more frequently than vegetation measurements were taken, the accuracy of all models' density point estimates decreased, reflected in greater variation in bias (2 individuals ha^{-1} ; Figure. 3.5A) or in greater mean absolute bias (6 individuals ha^{-1} ; Figure. 3.5B).

For both density scenarios, there was no significant difference between the true proportion of small burrows (≤ 20 cm width) in the population (2 individuals ha^{-1} : 0.26, SD = 0.03; 6 individuals ha^{-1} : 0.26, SD = 0.07) and the estimated proportion from our Bayesian model (2 individuals ha^{-1} : 0.28, SD = 0.09, $t = -1.32$ (63.4 df), $P = 0.19$; 6 individuals ha^{-1} : 0.26, SD = 0.02, $t = -0.28$ (66.3 df), $P = 0.78$). In contrast, summaries of the raw LTDS data consistently underestimated the true proportion of small burrows (≤ 20 cm width) in the population at both population densities (2 individuals ha^{-1} : 0.10, SD = 0.05, $t = 20.85$ (92.7 df), $P < 0.001$; 6 individuals ha^{-1} : 0.11, SD = 0.03, $t = 32.77$ (92.4 df), $P < 0.001$; Figure. 3.6).

Burn Surveys

We surveyed 15.41 km of transect for both pre-and post-burn surveys. Across all plots we detected 154 burrows during the pre-burn survey and 270 burrows during the post-burn. The base model (Program DISTANCE with no covariates) estimated fewer occupied burrows were present during the pre-burn surveys than the post-burn surveys at 3 of the 4 surveyed plots (Figure 3.7). Similarly, pre-burn estimates of occupied burrow abundance under Program DISTANCE with covariates were smaller than post-burn estimates at 2 of the 4 sites, but produced similar pre- and post-burn density estimates for Plot B. For both the base model and Program DISTANCE with covariates, the point estimate for the post-burn surveys was outside of the confidence interval for the pre-burn surveys at 2 plots (plot A and D). Our Bayesian model tended to produce similar density estimates for the pre- and post-burn surveys, though precision

was greater for post-burn surveys compared to pre-burn. At Plot D, where the post-burn survey followed prescribed fire by 2 months and followed the pre-burn survey by 3 months, all our tested models estimated a larger number of burrows present during the pre-burn survey relative to the post-burn survey. Across all plots, average estimated detection probability on the line during the pre-burn surveys was 0.38 (0.25 - 0.53) for the smallest burrow widths (5 cm wide), with perfect detection on the line not occurring until burrows were an average of 18 cm (13 – 21) wide (Table 3.1).

At all plots, more burrows were found post-burn than pre-burn across nearly all 5-cm burrow width categories (Figure. 3.8). This trend was especially noticeable for smaller burrows (≤ 20 cm wide) for which 3.7 $\times$ as many burrows were found post-burn ($n=37$) than pre-burn ($n=10$, Figure. 3.8). Estimated burrow width distributions from the Bayesian model were similar for the pre- and post-burn surveys, though the estimates of the proportion of individuals with smaller burrows (≤ 20 cm wide) differed slightly between surveys (Figure. 3.9).

DISCUSSION

Our study indicates that any level of spatial variation in vegetation obstruction decreases detection of burrows and may lead to large underestimation in population size when not considered during analysis. In simulation, our Bayesian model had the greatest accuracy of the density point estimate of any of the models tested, even when vegetation heterogeneity was very high. By sampling field sites before and after an intervening burn, we were able to evaluate each model's ability to analytically adjust for the effect of vegetation against a manipulated condition in which vegetation was effectively removed. Estimates from pre- and post-burn surveys were most in agreement under our Bayesian model, which made use of systematic vegetation measurements and accounted for imperfect detection on the line.

We recognize the inclusion of imperfect detection on the transect line likely impacted results of our simulation comparisons of our Bayesian model to both implementations of Program DISTANCE. In our study, we simulated imperfect detection on the transect line - a violation of 1 of the main assumptions of line transect distance sampling. Moreover, we simulated detection probability on the transect line as varying with the covariate value of the surveyed individuals. Though we equipped our Bayesian model to deal with this scenario, Program DISTANCE and other LTDS analysis packages are not designed to handle data violating this assumption. As a result, the decreased performance of Program DISTANCE relative to our Bayesian model reflects, to some degree, the capacity of each approach to accommodate imperfect detection on the transect line and is not solely attributable to the effect of modeling the underlying covariate distribution (for detected and undetected individuals). However, previous research has demonstrated that the assumption of 100% detection on the line is not being met in gopher tortoise surveys (Howze and Smith 2018), suggesting that use of conventional analytical approaches to LTDS may not be suitable for gopher tortoise survey analysis.

As suspected, estimated detection on the line for the smallest burrows was <1 for all our survey plots, reinforcing concerns that 1 of the basic assumptions of line-transect distance sampling is not being met in many gopher tortoise surveys (Buckland et al. 2001). Moreover, the proportion of small burrows (≤ 20 cm wide) found during the LTDS survey increased from 8% pre-burn to 14% post-burn, confirming previous research suggesting tortoise detections are strongly biased towards larger burrow sizes (Smith et al. 2009, Ballou 2013, Howze and Smith 2018). Unlike the raw count data, estimates of the burrow width distribution under the Bayesian model stayed roughly the same between surveys. However, our model assumes prior knowledge

of a reasonable range of values for both the threshold value of burrow width above which detection on the line is perfect and probability of detection on the line for the smallest burrows (5 cm wide). We based priors for both quantities on data from previous pre- and post-burn surveys performed at the Jones Center (Howze and Smith 2018). At many sites, however, these values are likely unknown. Vague priors for detection on the line may lead to low precision of parameter estimates. Overly conservative estimation of the probability of detection on the line (estimating detection probability as lower than the true value) may lead to overcompensation by the model for burrow widths associated with low detectability, falsely inflating their relative density in the estimated burrow width distribution. Previous studies have suggested either sampling at multiple effort levels or employing double sampling techniques to account for imperfect detection on the line (Quang and Becker 1997, Clement et al. 2017). The addition of a double sampling strategy that permits a more thorough search for cryptic individuals (such as juvenile burrows) may allow for more precise estimates of detection overall, ultimately improving both knowledge of the underlying burrow width distribution and the true density of the population of interest.

In comparison to either model implementation of Program DISTANCE, density estimates by the Bayesian model were relatively imprecise for pre-burn surveys. We suspect this imprecision arises from the added parametric structure that is not present in the model used by Program DISTANCE. Our model accommodates realistic sources of variation such as detection probability on the line, the threshold size for perfect detection on the line, and the underlying distribution for burrow width (expressed through a mixture of gamma distributions) through the inclusion of unfixed parameters, but uncertainty about their true values propagates into estimates of density and other demographic quantities. This effect is most obvious in data-poor situations,

such as the pre-burn surveys where reduced detectability of individuals rendered sample sizes of 20-50 individuals per plot. In such cases, the sparse data have limited ability to provide new information to the model priors. In situations where data are plentiful, such as the post-burn surveys, the priors are better informed, and precision under our model was roughly comparable to that of the other models. However, the added structure of our Bayesian model appears to reduce bias relative to the other models, even when sample size is limited, indicating a clear trade-off between accuracy and precision of estimates, particularly at low sample sizes.

One concern with incorporating vegetation covariates is trying to balance the survey effort of frequent vegetation measurements with the need to capture site vegetation heterogeneity. In simulation, when vegetation varied more frequently than obstruction measurements were taken (as seen in our high vegetation scenario), accuracy of the model's density estimates suffered, especially at higher population densities. For our field tests, we did not know the true level of heterogeneity at the site, but we believed that measuring vegetation obstruction at 15-m increments was a reasonable tradeoff between accuracy and survey effort (<1 minute per measurement (Gaya, personal observation)). The only exception was Plot D, where the pre-burn burrow density point estimate was almost double the post-burn estimate. Though the plot had been burned the previous year, we found that Plot D had multiple depressions with dense patches of young hardwoods surrounded by open wiregrass areas. Based on similarities to our high vegetation simulation scenario, we suspect these patches inflated average vegetation values in the wiregrass areas, resulting in over-compensation for vegetation obstruction and overestimation of pre-burn burrow density. For sites with thick patches interspersed with open areas, increasing the frequency of vegetation measurements is likely to help ensure vegetation heterogeneity is accurately accounted for during data analysis.

MANAGEMENT IMPLICATIONS

For gopher tortoise surveys, surveying after a burn is the best strategy to minimize the impact of vegetation and thus maximize detection of burrows. Burning significantly increased our detection of burrows at all distances, improving our sample size and allowing for more precise estimation of burrow density. However, in cases where burning prior to a survey is not feasible, the incorporation of vegetation measurements in the LTDS protocol may be a simple but powerful tool to improve the accuracy of population estimates. This protocol could be easily adapted for other surveys where vegetation is thought to influence detection such as burrow surveys for Southeastern pocket gophers (*Geomys pinetis*) or scat surveys for feral hogs (*Sus scrofa*). Moreover, the use of a model that accounts for underlying distributions of covariates associated with undetected individuals and for imperfect detection on the line increases the likelihood that estimates of demographic quantities needed for conservation decision making are more reflective of true population characteristics and less reflective of survey conditions.

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LITERATURE CITED

- Alford, R. A. 1980. Population structure of *Gopherus polyphemus* in northern Florida. *Journal of Herpetology* 14:177–182.
- Ballou, A. 2013. Aspects of gopher tortoise (*Gopherus polyphemus*) populations in Georgia: status, landscape predictors, and juvenile movements and burrow use. University of Georgia, Athens, GA.
- Berish, J. E., R. A. Kiltie, and T. M. Thomas. 2012. Long-term population dynamics of gopher tortoises (*Gopherus polyphemus*) in a pine plantation in northern Florida. *Chelonian Conservation and Biology* 11:50–58.
- Bernardo, J. M., J. O. Berger, A. P. Dawid, and A. F. Smith, editors. 1992. Bayesian statistics 4. Oxford science publications 4, Clarendon Press, Oxford.
- Brooks, S., editor. 2011. Handbook for markov chain monte carlo. Taylor & Francis, Boca Raton.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. Laake, D. Borchers, and L. Thomas. 2004. Advanced distance sampling. Volume 89. Oxford University Press, Oxford.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, and J. L. Laake. 1993. Distance sampling: estimating abundance of biological populations. Springer, New York.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. Introduction to distance sampling: estimating abundance of biological populations. Oxford University Press, Oxford.

- Clement, M. J., S. J. Converse, and J. A. Royle. 2017. Accounting for imperfect detection of groups and individuals when estimating abundance. *Ecology and Evolution* 7:7304–7310.
- Doonan, T. J., and I. J. Stout. 1994. Effects of gopher tortoise (*Gopherus polyphemus*) body size on burrow structure. *The American Midland Naturalist* 131:273–280.
- Epperson, D. M., and C. D. Heise. 2003. Nesting and hatchling ecology of gopher tortoises (*Gopherus polyphemus*) in southern Mississippi. *Journal of Herpetology* 37:315–324.
- Gelman, A., D. B. Rubin, J. B. Carlin, and H. S. Stern. 2003. Bayesian data analysis. 2nd ed. Texts in Statistical Science, Chapman & Hall/CRC, Boca Raton.
- Howze, J. M., and L. L. Smith. 2018. Detection of gopher tortoise burrows before and after a prescribed fire: implications for surveys. *Journal of Fish and Wildlife Management*.
- Marques, T. A., L. Thomas, S. G. Fancy, S. T. Buckland, and C. M. Handel. 2007. Improving estimates of bird density using multiple- covariate distance sampling. *The Auk* 124:1229–1243.
- Martin, P. L., and J. N. Layne. 1987. Relationship of gopher tortoise body size to burrow size in a southcentral Florida population. *Florida Scientist* 50:264–267.
- McCoy, E. D., and H. R. Mushinsky. 1992. Studying a species in decline: gopher tortoises and the dilemma of “correction factors.” *Herpetologica* 48:402–407.
- Miller, D. L., E. Rexstad, L. Thomas, L. Marshall, and J. Laake. 2017. Distance sampling in R. *bioRxiv* 063891.
- Quang, P. X., and E. F. Becker. 1997. Combining line transect and double count sampling techniques for aerial surveys. *Journal of Agricultural, Biological, and Environmental Statistics* 2:230–242.

- Royle, J. A., and R. M. Dorazio. 2008. Hierarchical modeling and inference in ecology: the analysis of data from populations, metapopulations and communities. Reprint. Acad. Press, Amsterdam.
- Smith, L., J. Howze, and J. W Jones. 2016. Sampling workbook gopher tortoise line transect distance. Jones Ecological Research Center.
<http://www.jonesctr.org/products/publications/Smith_2016_sampling_workbook.pdf>.
- Smith, L., J. Linehan, J. Stober, M. Elliott, and J. Jensen. 2009. An evaluation of distance sampling for large-scale gopher tortoise surveys in Georgia, USA. *Applied Herpetology* 6:355–368.
- Stober, J. M., R. Prieto-Gonzalez, L. L. Smith, T. A. Marques, and L. Thomas. 2017. Techniques for estimating the size of low-density gopher tortoise populations. *Journal of Fish and Wildlife Management* 8:377–386.
- U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants: determination of threatened status for the gopher tortoise (*Gopherus polyphemus*). Code of Federal Regulations. Volume 50 Part 17.
- U.S. Fish and Wildlife Service. 2013. Range-wide conservation strategy for the gopher tortoise. <<https://www.fws.gov/southeast/pdf/strategy/gopher-tortoise-conservation-strategy-v2.pdf>>. Accessed 30 Apr 2018.
- Welch, B. L. 1938. The significance of the difference between two means when the population variances are unequal. *Biometrika* 29:350–362.
- Yager, L. Y., M. G. Hinderliter, C. D. Heise, and D. M. Epperson. 2007. Gopher tortoise response to habitat management by prescribed burning. *Journal of Wildlife Management* 71:428–434.

Associate Editor:

Table 3.1. Burrow counts, estimated density (occupied burrows/ha), average burrow occupancy, estimated detection coefficient for size (95% credible interval), probability of detection on the transect line of the smallest (5 cm width) burrows (95% credible interval, pre-burn survey only), and estimated size threshold (cm) at which on-line detection is perfect (95% credible interval, pre-burn survey only) for pre- and post-burn surveys conducted on 4 plots at the Joseph W. Jones Ecological Research Center, Baker County, Georgia, in 2018. All plots were burned March-April 2018, and plots differed in number of years since previous burn. Pre-burn surveys were conducted March 16-18, 2018 with post-burn surveys taking place 22-24 March 2018 (Plots A, B, and C) and 24 June 2018 (Plot D).

	Burn Interval	Burrows Detected		Density Point Estimate (Occupied Burrows/ha)		Average Burrow Occupancy		Detection coeff. for size		Estimated Detection on the Line	Estimated Breakpoint
		Pre-burn	Post-burn	Pre-burn	Post-burn	Pre-burn	Post-burn	Pre-burn	Post-burn		
Plot A	2 yr	36	82	1.50	1.75	28%	41%	0.02 (0.00-0.04)	0.02 (0.00-0.03)	0.53 (0.34-0.70)	14 cm (10-17)
Plot B	2 yr	21	75	3.83	4.24	57%	50%	0.31 (0.00-1.14)	0.07 (0.04-0.11)	0.29 (0.14-0.40)	13 cm (10-17)
Plot C	1 yr	49	73	4.05	4.29	47%	44%	0.02 (0.00-0.03)	0.03 (0.01-0.04)	0.25 (0.10-0.60)	22 cm (20-25)
Plot D	1 yr	21	40	0.68	0.36	32%	33%	2.18 (0.50-6.29)	3.12 (0.00-7.4)	0.43 (0.33-0.50)	21 cm (18-22)

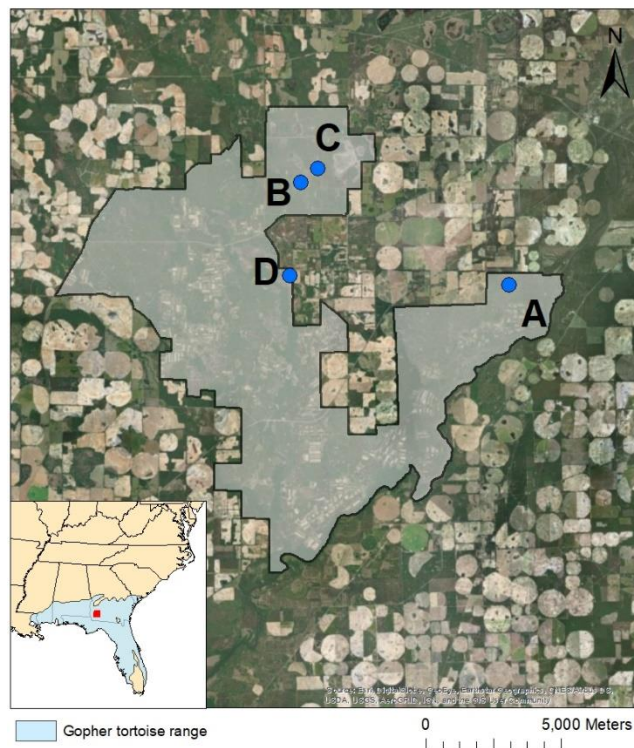


Figure 3.1. Location of 4 burn plots at the Joseph W. Jones Ecological Research Center, Baker County, Georgia. Plots were initially surveyed 16-18 March 2018 using line-transect distance sampling. The plots were burned on 21 March (Plot A), 23 March (Plots B and C), and 25 April 2018 (Plot D). We resampled Plots A, B and C on 22-24 March and Plot D on 24 June 2018 using identical methodology from the previous survey.

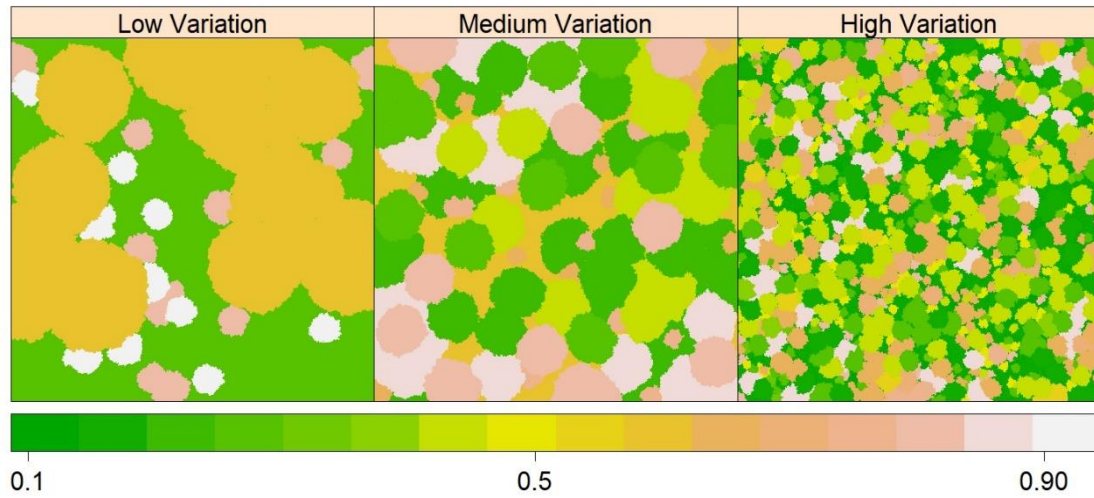


Figure 3.2. Example rasters used for low, medium and high scenarios of vegetation spatial variation in simulated gopher tortoise (*Gopherus polyphemus*) line-transect distance sampling surveys. To simulate the impact of vegetation obstruction on gopher tortoise burrow detection during line-transect distance sampling surveys, we randomly generated rasters across a 1-km² area with values varying at different spatial scales (low, medium or high) using the landscapeR package in R. Simulated values of vegetation obstruction ranged from 0.1 to 0.99. We set maximum patch size at 700 m², 250 m² and 100 m² for low, medium and high vegetation variation respectively. We simulated field-based measurements on the transect line every 15 m and incorporated these measurements into our distance model to account for vegetation obstruction during tortoise surveys.

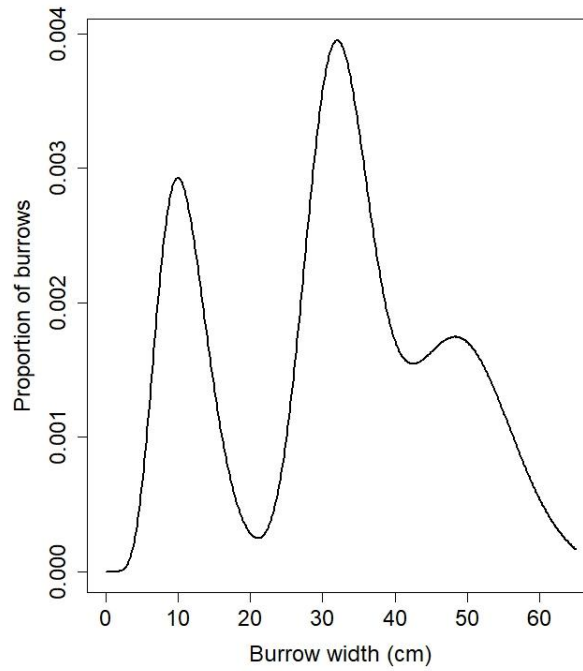


Figure 3.3. Burrow size distribution for simulated gopher tortoise (*Gopherus polyphemus*) populations. We simulated line-transect distance sampling on a 100-ha² area, with parallel transects spaced 50 m apart. We drew a burrow width for each individual in the simulated populations by sampling from the above distribution, a mixture of 4 gamma distributions. Burrow widths were bound between 5-65 cm.

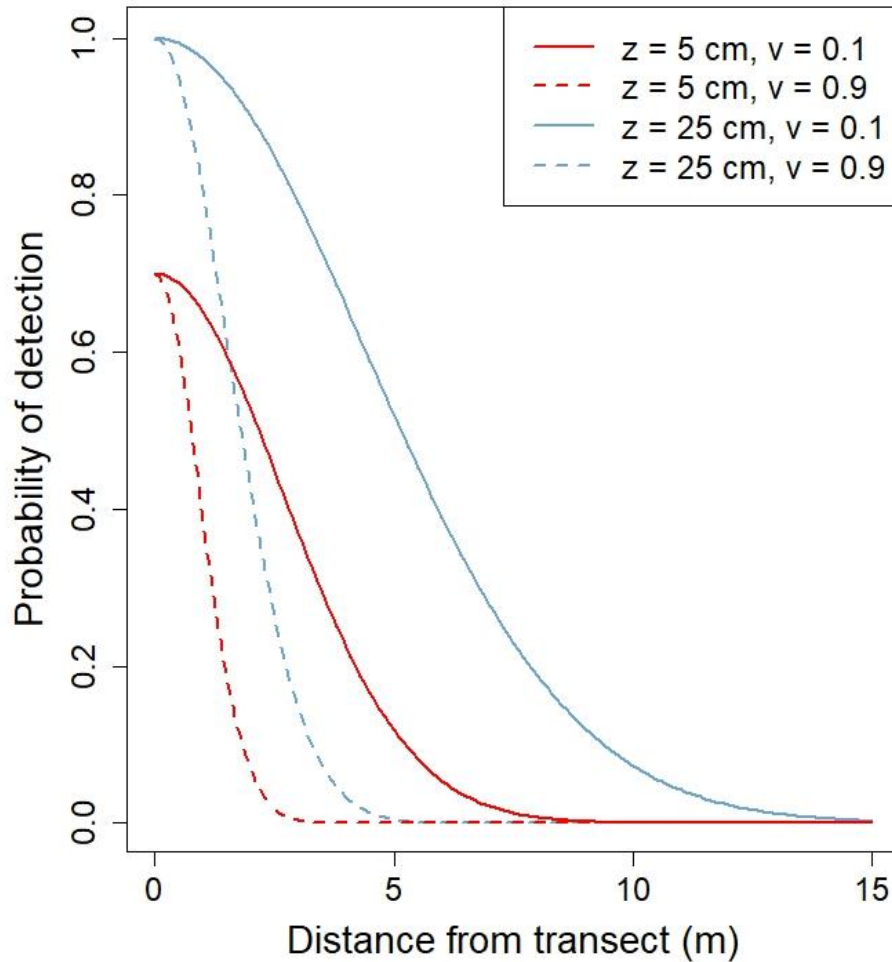


Figure 3.4. Example detection curves used in simulated gopher tortoise (*Gopherus polyphemus*) line-transect distance sampling surveys. We used a half-normal curve to simulate detection of gopher tortoise burrows, allowing the sigma parameter of the curve to vary with burrow width (z , cm) and vegetation obstruction (v). For the smallest simulated burrows (5 cm wide, red lines), probability of detection on the transect line (distance 0) was set to 0.7. Burrows ≥ 20 cm wide ($z = 25$ cm; blue lines) were detected perfectly on the line (probability of detection at distance 0 was 1). Increases in vegetation from sparse ($v = 0.1$, solid lines) to dense ($v = 0.9$, dashed lines) decreased the sigma parameter but did not change the probability of detection on the transect line.

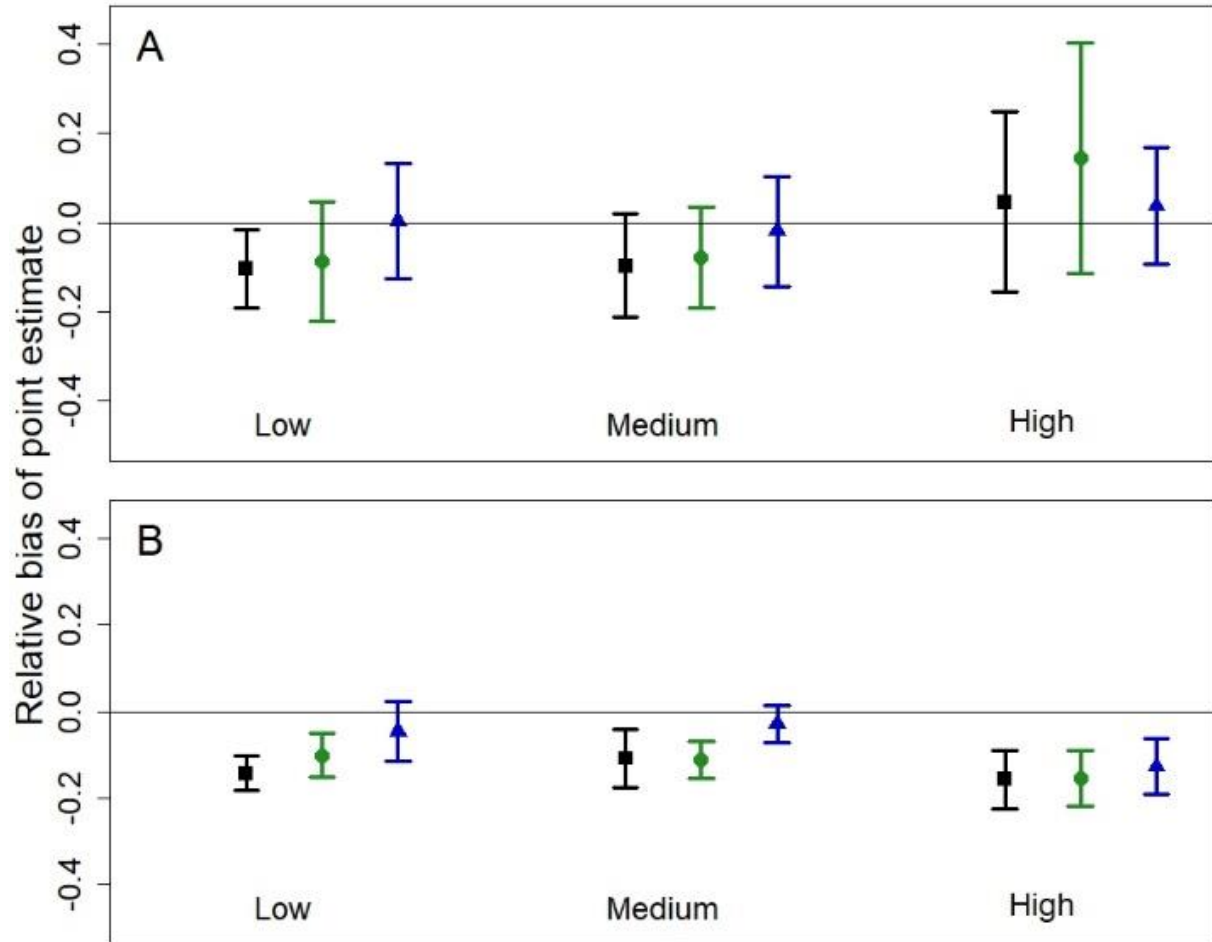


Figure 3.5. Relative bias of density point estimates for simulated gopher tortoise (*Gopherus polyphemus*) populations with A) 2 burrows ha⁻¹ or B) 6 burrows ha⁻¹. We show the mean ($n = 20$) and 95% CI for relative bias (estimate/true – 1.0) of the point estimates for each of 6 simulation scenarios. Each triplet of means and ranges is placed above a label indicating the level of spatial variation (“Low”, “Medium”, “High”) in vegetation obstruction (as seen in Figure 3.2). The horizontal reference line represents a relative bias of 0 (the point estimate was equal to the truth). In all scenarios, the Bayesian model (blue, triangles) tended to perform better than either the base model (black, squares) or covariate model run under program DISTANCE (green, dots), with mean bias closer to 0.

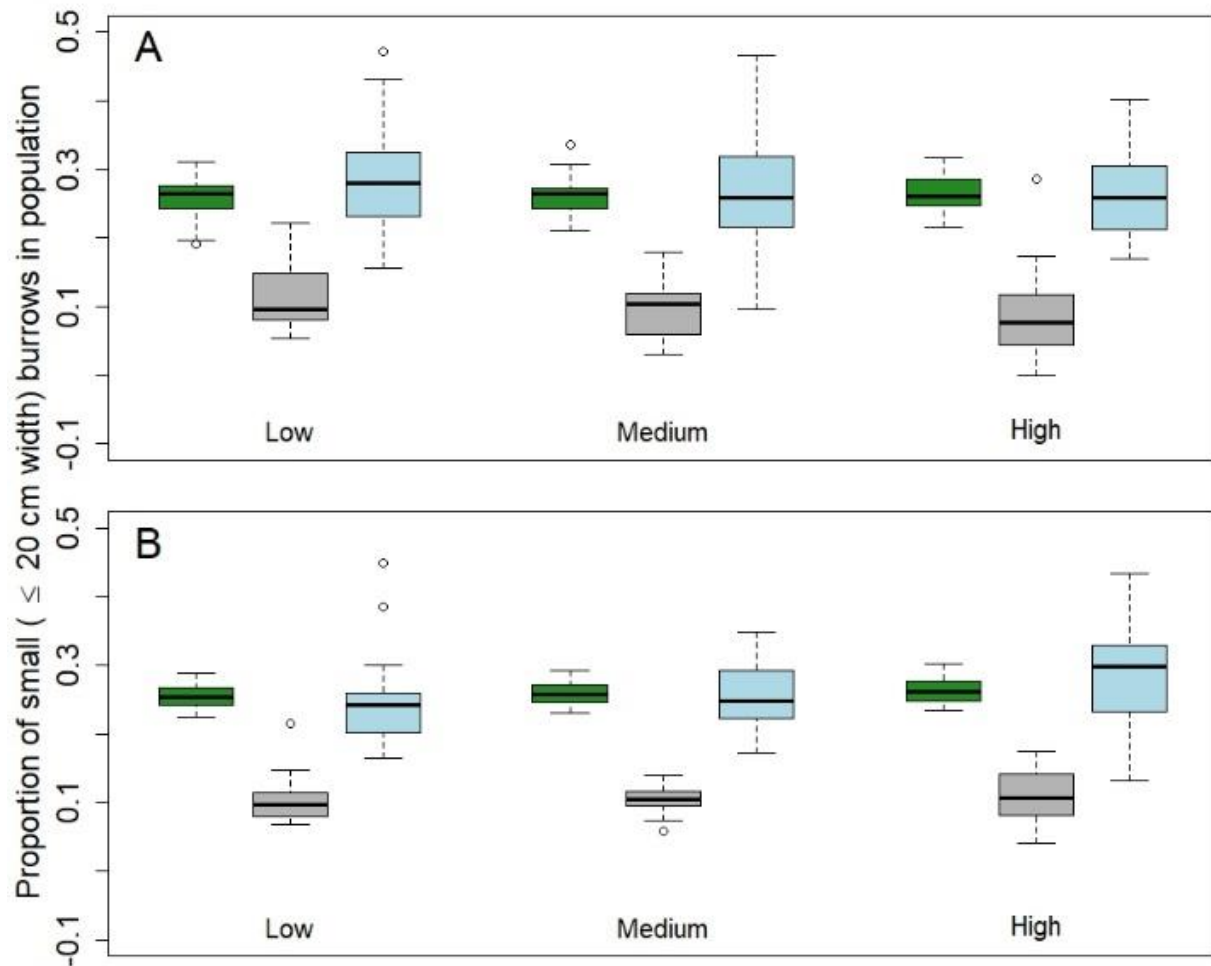


Figure 3.6. Estimated proportion of small (≤ 20 cm width) burrows in simulated populations of gopher tortoises (*Gopherus polyphemus*) at A) 2 burrows ha^{-1} and B) 6 burrows ha^{-1} . The green boxplots in each scenario represent the true (simulated) proportion of small individuals (burrow width ≤ 20 cm) in the population. Additional boxplots portray estimates of the same proportion computed from raw line-transect distance sampling data (gray) and from posterior summaries by the Bayesian model (blue). For all simulation scenarios, estimates by the Bayesian model were consistently closer to the true distribution than were averages of the raw line-transect distance sampling count data.

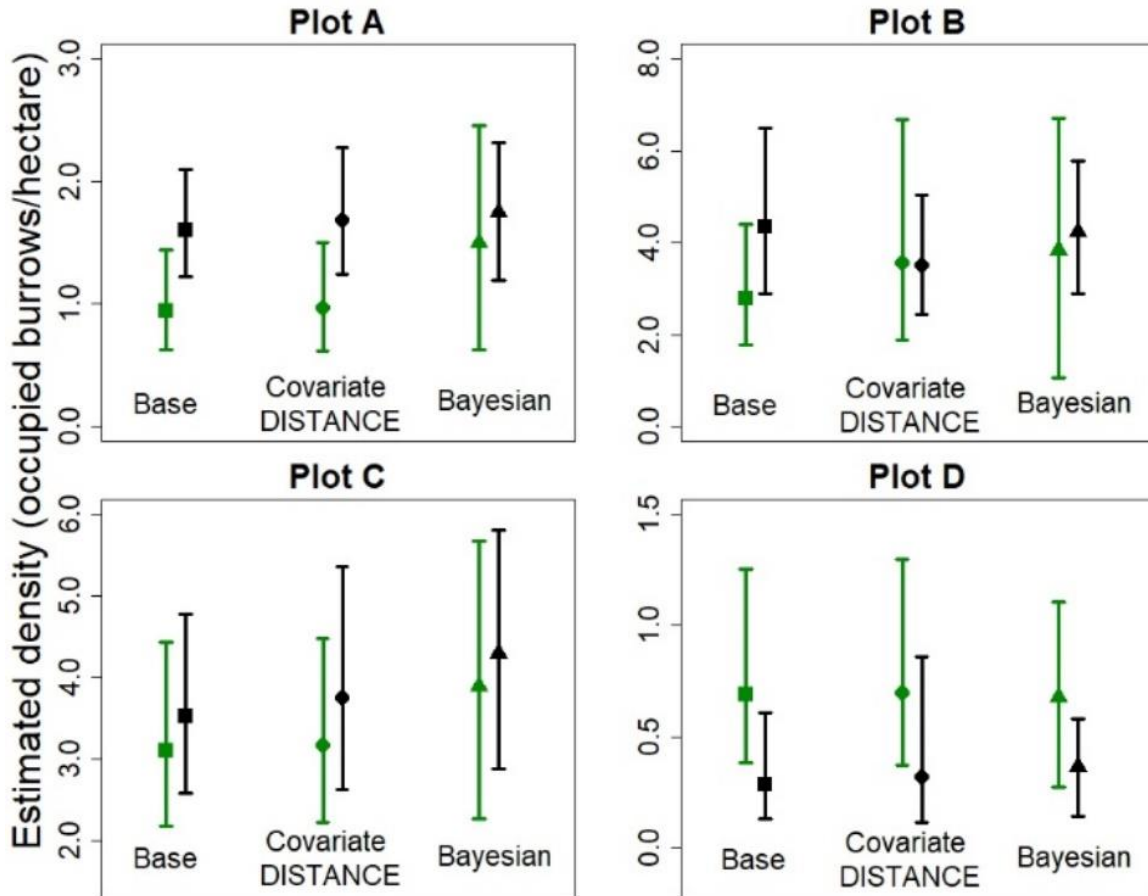


Figure 3.7. Pre- and post-burn occupied gopher tortoise (*Gopherus polyphemus*) burrow density estimates for 4 plots at the Joseph W. Jones Center, Baker County, Georgia. Pre-burn line-transect distance sampling surveys were conducted 16-18 March 2018 with post-burn surveys taking place 22-23 March 2018 (plot A, 1-2 days since burn), 24 March 2018 (plot B and C, 1 day since burn) and 24 June 2018 (plot D, 60 days since burn). The green bars in each box represent the pre-burn density estimates (occupied burrows ha^{-1}) for each of our 3 tested models (base model, Program DISTANCE with vegetation and burrow width covariates and the Bayesian model with the same covariates). Black bars represent the post-burn density estimate for each plot.

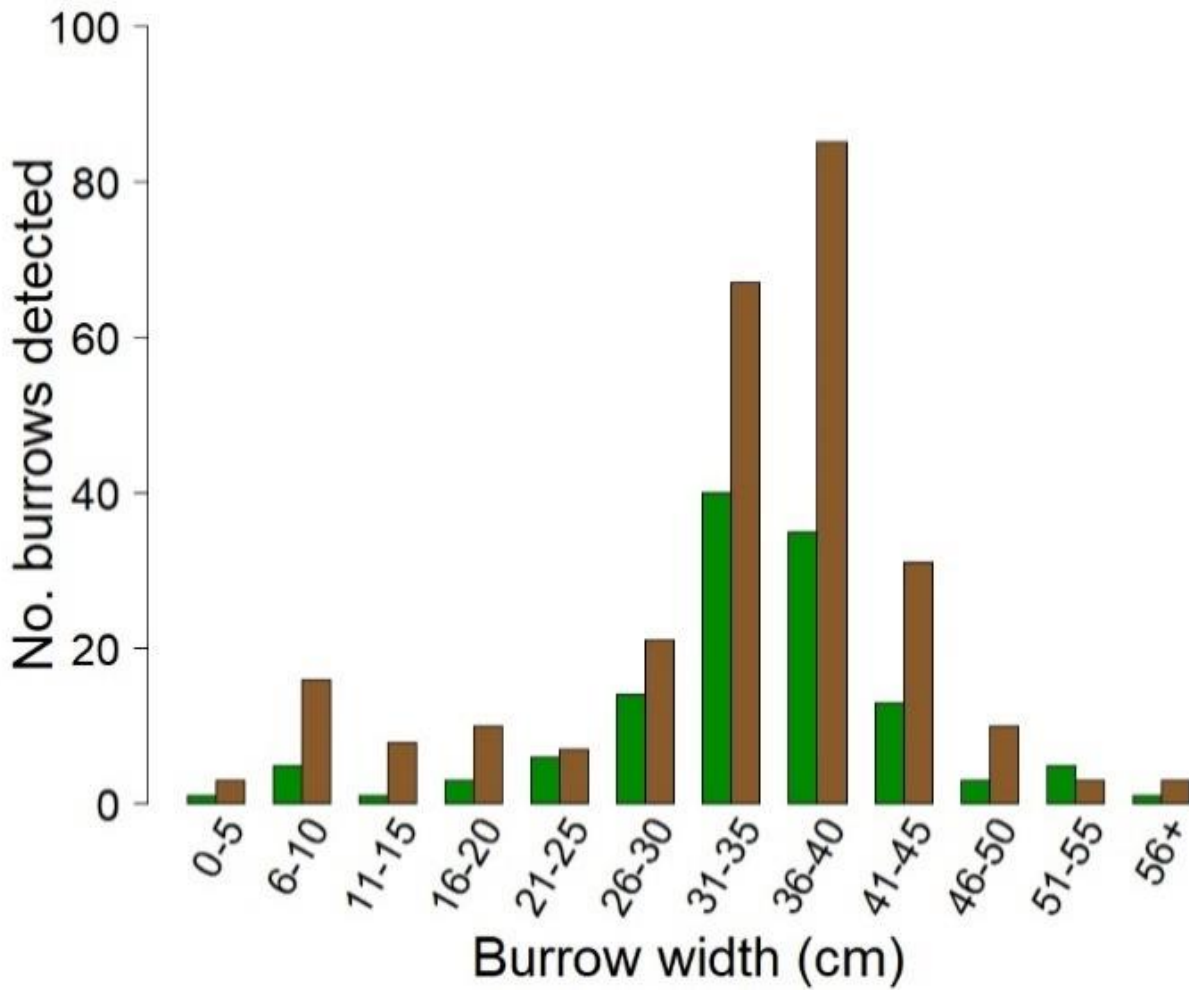


Figure 3.8. Distribution of width of gopher tortoise (*Gopherus polyphemus*) burrows detected pre-burn (March 2018, green) and post-burn (March and June 2018, brown) at 4 plots at the Joseph W. Jones Ecological Research Center, Baker County, Georgia.

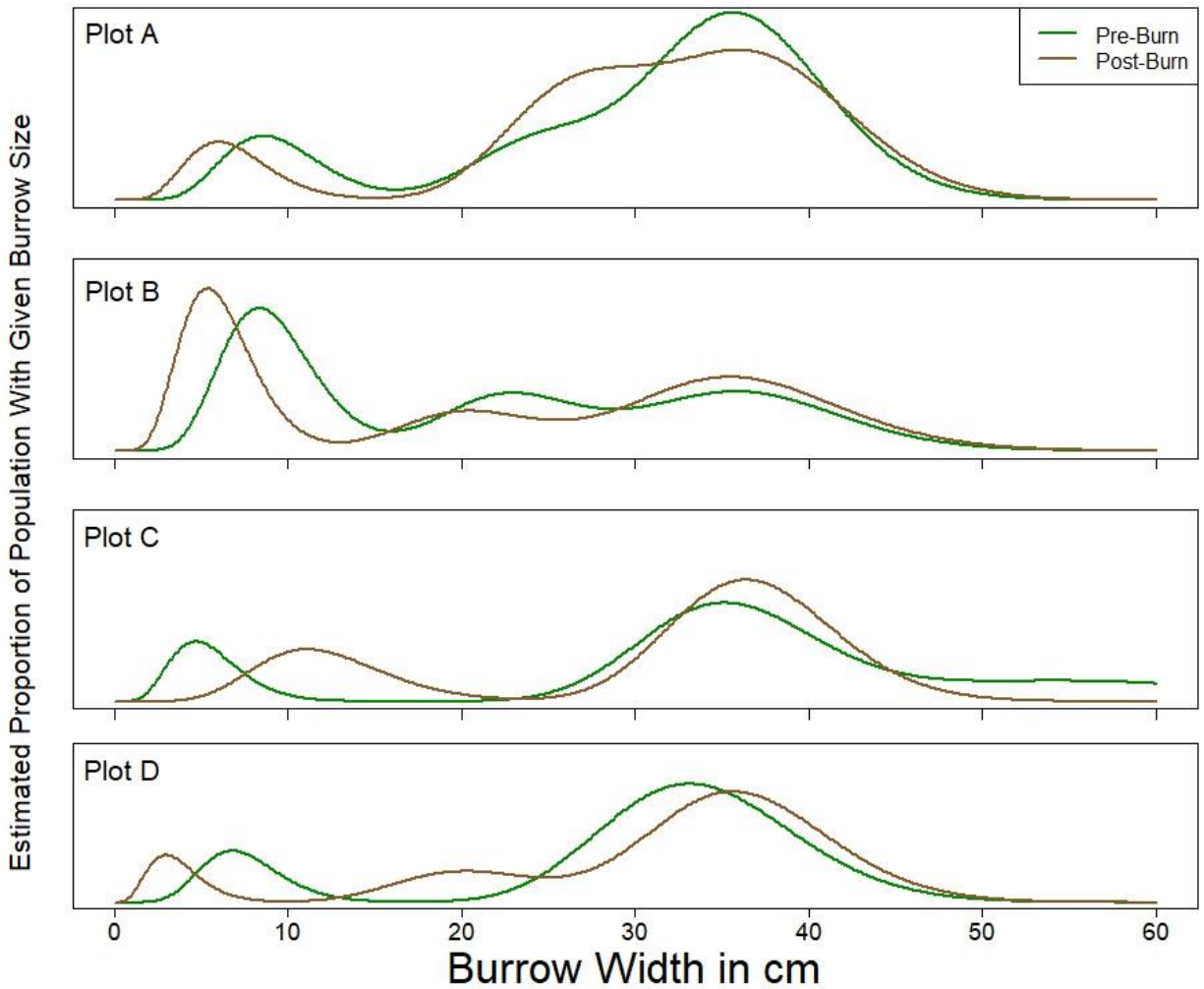


Figure 3.9. Estimated burrow size distributions for gopher tortoises at 4 burn plots at the Joseph W. Jones Center, Baker County, Georgia. Pre-burn line-transect distance sampling surveys were conducted 16-18 March 2018 with post-burn surveys taking place 22-24 March 2018 (plots A, B, C) and 24 June 2018 (plot D).

CHAPTER 4

AN ENHANCED LINE-TRANSECT DISTANCE SAMPLING DESIGN FOR ESTIMATING DENSITY OF CRYPTIC, SESSILE ORGANISMS ³

³ Gaya, H. E., L. L. Smith, and C. T. Moore. To be submitted to *Ecology*

Abstract

1. Line-transect distance sampling (LTDS) is a popular method for estimating abundance and density of animal populations. However, imperfect detection on the transect line negatively biases abundance estimates and may lead to faulty management decisions. Imperfect detection may be estimated and accounted for through double-sampling methods.
2. We examine a double-sampling technique to account for imperfect detection on the transect line through the example of the gopher tortoise (*Gopherus polyphemus*), a keystone species in the southeastern United States. Gopher tortoises are frequently surveyed using a standard line-transect distance sampling (LTDS) protocol, but juvenile populations are frequently underestimated due to size-related detectability, and accuracy of overall estimated population abundance is sensitive to variation in vegetation obstructions in the survey area. We present a Bayesian hierarchical model for double-sampling and compare the density and size-demography of burrows found using the standard LTDS methodology to burrows found with our double-sampling protocol for both a simulated and real dataset.
3. In simulation, we found that the addition of intensive searches led to more accurate burrow density and size-demography estimates for modest increase in search effort. For our real datasets, we saw increased juvenile gopher tortoise detection and a median 34% reduction in density point estimate CV when we included intensive searches.
4. *Synthesis.* Our results suggest that the addition of double-sampling to LTDS may prove an effective method for increasing the accuracy and precision of both density point estimates and the underlying burrow size distribution of gopher tortoise populations. We anticipate the use of double-sampling approaches, such as our enhanced line-transect distance protocol and model,

may prove a powerful tool to account for poor detection of cryptic individuals and imperfect detection on the transect line.

Keywords: distance sampling, double-sampling, *Gopherus polyphemus*, hierarchical models, population estimation

1. Introduction

Line-transect distance sampling (LTDS) is a widely-used method for estimating abundance and density of plant and animal populations (e.g., birds (Strobel and Butler 2014), marine mammals (Bradford et al. 2014), or vascular epiphytes (Gentry and Dodson 1987)), or their artifacts (e.g. deer dung (Marques et al. 2001) or monkey nests (Wich and Boyko 2011)). The method relies on observing objects from transect lines and measuring the perpendicular distance from the transect line to each detected object (Buckland et al. 2001). Imperfect detection of objects (i.e., inability to observe all objects in the survey field) is then accounted for during data analysis by modeling detection probability as a function of distance from the transect line (Buckland et al. 1993, Buckland et al. 2001). Assuming all objects on the transect line are detected and perpendicular distances are measured without error, perfect detection in the survey area is not necessary for inference on density, saving the researcher the time and energy necessary to complete a full population census (Buckland et al. 1993). Covariates other than distance that are thought to influence detection, such as habitat structure, object size, or object color, can also be included in the detection function to improve estimation (Buckland et al. 2004, Marques et al. 2007, Royle and Dorazio 2008).

For many sampling situations, however, the assumption of perfect detectability on the line is almost certainly violated, either because objects vary in their detectability (e.g., elk (Clement et al. 2017) or vascular plants (Chen et al. 2013)) or the observation platform does not permit an unobstructed view of the line (e.g., cetacean surveys (Bradford et al. 2014, Barlow 2015)). Failure to account for imperfect detection on the transect line negatively biases abundance estimates and may lead to faulty management decisions (Buckland et al. 2001, Chadès et al. 2008, Clement et al. 2017). One example of this sampling scenario is the gopher

tortoise (*Gopherus polyphemus*), a terrestrial reptile within the coastal plain of the southeastern United States that digs its own burrows. Gopher tortoises tend to occur at low densities, requiring considerable effort to obtain sample sizes large enough for precise estimation under conventional LTDS (Smith et al. 2009, Castellón et al. 2012). The gopher tortoise is threatened under the U.S. Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana (U.S. Fish and Wildlife Service 1987) and is a candidate for listing in the remainder of the range (U.S. Fish and Wildlife Service 2011). The U.S. Fish and Wildlife Service currently recommends LTDS as the standard method for estimating population size and monitoring population status across the range (Gaillard et al. 2017).

However, previous studies indicate juvenile gopher tortoise burrows may be missed directly on the transect line (Ballou 2013, Howze and Smith 2018, Chapter 2), violating a key assumption of LTDS (Buckland et al. 2001). Burrow width roughly corresponds to the resident tortoise's carapace length (Martin and Layne 1987, Doonan and Stout 1994), and tortoise detections are strongly biased towards larger (adult) burrow sizes (Smith et al. 2009). Smaller burrows experience low detection regardless of distance to the transect (Ballou 2013, Howze and Smith 2018, Chapter 2) making it difficult to differentiate whether underrepresentation of small burrows in surveys is an indicator of poor productivity and recruitment or merely reflective of low detection probability. The presence of ground-level vegetation may exacerbate this problem, significantly decreasing detection of tortoise burrows of all sizes (Howze and Smith 2018, Chapter 3). In Chapters 2 and 3, we presented models designed to account for varying detection probability of different-sized burrows using burrow width information and informative priors based on expert opinion of the site. Here we expand on this idea with the addition of double-

sampling plots to increase precision of population density and size distribution estimates and more accurately estimate the probability of detection on the transect line.

Traditional double-sampling surveys consist of two parts – a large sample using a rapid survey method followed by a subsample surveyed using intensive methods (Cochran 1977, Eberhardt and Simmons 1987, Bart et al. 2002, Collins 2007). The ratio of the mean detections in the subsamples using the rapid method to the mean detections using the intensive searches (assumed to represent the actual density) is then used to adjust results across the entire survey (Cochran 1977, Eberhardt and Simmons 1987). The rapid survey can take any number of forms including point-counts, distance sampling, or double-observer methods (Cochran 1977, Eberhardt and Simmons 1987, Bart et al. 2002, Bart et al. 2004, Collins 2007). As the intensive survey method is generally more expensive (economically or logistically), double-sampling survey design requires deciding how best to allocate resources between the rapid and intensive parts of the survey. Increasing the number of subsamples improves estimate precision and accuracy but requires additional time and money to collect data (Collins 2007) for the same spatial coverage of the rapid survey.

Current methods for combining distance sampling data with double-sampling tend to rely on linear regression, mark-resight data, or maximum likelihood estimation (MLE) models. Cochran (1977) proposed a technique using regression estimators for plot-based intensive survey methods where the counts from the rapid survey are compared to the intensive survey in each plot. The relationship between the counts from the two surveys is then used to adjust the initial estimate from the rapid survey method. Collins (2007) explored the cost-effectiveness of double-sampling strategies with intensive surveys, suggesting that with a sufficient number of detections during the rapid survey, double-sampling was an effective tool for reducing precision. However,

he noted that complexity in survey implementation, such as varying transect lengths or targeting multiple species in one survey, made analysis difficult. We wanted to investigate combining double-sampling surveys and LTDS in a framework that allowed for greater flexibility when accommodating multiple sources of variation. Bayesian hierarchical models allow for data to be shared between different sampling schemes to improve estimation of common data parameters and can accommodate data-poor situations (Royle and Dorazio 2006, Kéry and Royle 2016). We present a Bayesian model for line-transect distance sampling that incorporates randomly placed intensive-search plots to improve detection of gopher tortoise burrows for which size or amount of obstructing vegetation hampers their discovery. By using information from the intensive-search plots to account for detection bias against smaller burrows and burrows located in dense vegetation, we improve the precision of our population density estimates. We also use the intensive-search data to better inform our model about the burrow widths present in the population and produce a detection-adjusted size frequency distribution of all occupied burrows. We compare density point estimates and precision produced from our Bayesian double-sampling model to frequentist-based standard LTDS for both a simulated and real dataset. We further calculate the cost of surveying with and without intensive-search plots for our real data example. Although we focus on gopher tortoise surveys, this method could be applied to any type of line-transect distance survey of sessile organisms or objects for which increased confidence in population density or demographic estimates may be desired.

2. Methods

Survey Design

In both simulated and real applications, we followed conventional line-transect distance sampling design standards to systematically place (from a random starting location) parallel

transects Q meters apart throughout the study area (Buckland et al 2001, Thomas et al. 2007). We then systematically placed (from a random start) square double-sampling plots (intensive-search units, or ISUs hereafter) of size $2B \times$ meters every V meters along these transects such that a desired target percentage (e.g., 10%) of the study area was covered by S ISUs. All ISUs were centered on the transects and did not extend into non-suitable habitat (Figure 4.1). We allowed multiple ISUs to be placed on the same transect but did not allow any overlap between units.

Conducting the Survey

In our field application, we adapted the conventional gopher tortoise LTDS survey (Smith et al. 2016) for a survey with two observers, with each observer standing 2.5 m on either side of the transect. One observer navigated using a Trimble Juno T41 (1-2 m accuracy; Trimble Navigation, Ltd., Sunnyvale, CA) and searched for burrows on and near the transect. The other observer walked on the other side of the transect and searched for burrows on and near the transect. At each burrow, we obtained a GPS location, measured burrow width at 50 cm inside the opening (Martin and Layne 1987), and determined occupancy status of non-collapsed burrows. We determined occupancy by scoping each burrow with a burrow camera and classifying the burrow as either (1) occupied (tortoise observed), (2) unoccupied or (3) unknown occupancy (Smith et al. 2016).

In addition to the conventional protocol, we recorded vegetation measurements at regular intervals from the beginning to the end of each transect line to estimate vegetation obstruction (Chapter 3). Prior to data analysis, we used inverse distance weighted interpolation of the point measurements in ArcMap v. 10.6.1 (Environmental Systems Research Institute, Redlands, California, USA) to create a smoothed layer of estimated vegetation density across the sampled

landscape, and we extracted an estimate of vegetation obstruction (range 0-1 m) at the location of each detected burrow.

To survey each ISU, we first performed the conventional LTDS protocol on the transect that crossed the intensive-search unit. We then returned to a random corner of the ISU and began our intensive search. In a direction perpendicular to the LTDS transect, observers slowly walked in tandem close enough to each other such that the area between them was thoroughly scanned; in our case, we believed that separating observers by 4m was sufficient to achieve complete coverage. All burrows newly found by the intensive search were marked with a GPS location, measured, and scoped for occupancy. We relied on GPS locations and the presence of our own footprints on burrow aprons to differentiate between newly found burrows and previously recorded burrows. We did not re-measure or confirm the occupancy status of burrows that were already found during the conventional LTDS portion of the survey.

Estimation Model

Each burrow in the study area was associated with a distance to transect, burrow width, and vegetation obstruction measurement (Chapter 3). We used a data-augmentation technique (Royle and Dorazio 2008), where the known distances and covariate values for n_{ind} found burrows were augmented with a large number n_A of missing values representing potentially missed burrows. Assuming transect lines were placed independently with respect to the burrows, we used a uniform prior distribution for distances such that:

$$x_i \sim \text{Uniform}(0, Bx)$$

where Bx was a given maximum distance of our choosing from the transect line. The model then evaluated each augmented burrow and drew a binary value to represent its existence in the population:

$$w_i \sim \text{Binomial}(1, \psi),$$

where ψ was the probability of inclusion in the population.

To account for our two different sampling strategies, we subdivided the study area into sections corresponding to the sampling type – sections within S double-sampled intensive-search units (ISUs) and sections surveyed only by LTDS. We denoted these as Y' and T respectively.

The total length of transect surveyed was calculated as:

$$L_{total} = S * L_{Y'} + L_T$$

where L_T and $L_{Y'}$ were the length of transect surveyed by LTDS alone and by each individual ISU respectively. Each ISU $j = 1, \dots, S$ had a total width of $2*Bx$ and a length of $L_{Y'}$ such that the total area covered by ISUs equaled:

$$2 * Bx * S * L_{Y'}$$

When the ISUs are square, $L_{Y'} = 2 * Bx$ and the equation simplifies to:

$$4 * Bx^2 * S$$

In areas surveyed only by LTDS, all real individuals – both drawn from the augmented population (those in n_A with $w_i = 1$) plus those found during the survey (n_T) – were summed into a total population N_T such that:

$$N_T = n_T + \sum_{i=1}^{n_A} w_i$$

For the areas within intensive-search units, we assumed perfect detection of all individuals, such that $N_{Y'} = n_{Y'}$. To estimate the density across the entire site, we divided our two estimates of N over the total sampled area:

$$D = \frac{N_{Y'} + N_T}{2 * L_{total} * Bx}$$

We refer to the combined sampling of transects and intensive-search units matched to transect sections as Enhanced Line-Transect Distance Sampling (ELTDS).

We modeled variation in individual detection through adjustments to the scale parameter, σ , of our detection function (Buckland et al. 2004). We modeled the scale parameter in a log-linear model containing an intercept, a burrow width predictor variable, and a vegetation predictor variable with uninformative normal priors for the intercept and coefficient terms

$$\ln(\sigma_i) = \beta_0 + \beta_{\sigma z} * z_i + \beta_{\sigma v} * v_i.$$

Each missed individual in the population $i = 1, \dots, (N - (n_T + n_D))$, was assigned a vegetation measurement drawn from a beta distribution informed by all raw vegetation measurements (v_r) taken at the site:

$$v_r \sim \text{Beta}(d, e)$$

$$v_i \sim \text{Beta}(d, e).$$

The shape and rate parameters d and e for the beta distribution were drawn from uninformative uniform priors.

We modeled burrow width as a mixture of a small number (K) of gamma distributions to reflect multi-modality in a size-structured tortoise population. Value K was estimated using a reverse-jump Markov chain Monte Carlo (MCMC) method (Chapter 2). Each gamma distribution was characterized by shape and rate parameters α_k and β_k respectively. Each missed burrow in the population, $i = 1, \dots, (N - (n_T + n_Y))$, was assigned to one of the distributions according to a categorical draw informed by a Dirichlet distribution:

$$k \sim \text{Multinomial}(1, \mathbf{p})$$

$$\mathbf{p} \sim \text{Dirichlet}(\omega_1, \dots, \omega_k)$$

Burrow width z_{ik} for missed burrow i in distribution k was drawn from the corresponding gamma distribution:

$$z_{ik} \sim \text{Gamma}(\alpha_k, \beta_k)$$

Parameters α_k and β_k for each gamma distribution were given uninformative priors, as were the ω_k :

$$\omega_k \sim \text{Gamma}(0.001, 0.001)$$

Previous research suggests that larger tortoise burrows are less likely to be occupied than smaller burrows (Ballou 2013). We modeled burrow occupancy status (o_i) as linearly related to burrow width and assumed burrow occupancy had no effect on detection probability. We assigned uninformative normal priors for an intercept and a burrow width coefficient:

$$o_i \sim \text{Binomial}(1, \theta_i)$$

$$\text{logit}(\theta_i) = \theta.\text{int} + \beta_\theta * z_i$$

In conventional distance sampling, probability of detection on the line is assumed to be 1. However, probability of detection on the transect line for smaller burrow sizes is frequently <1 (Ballou 2013, Howze and Smith 2018, Chapter 2). We modeled detection on the line as a proportion (range 0 – 1) of the overall detection probability (Chapter 2). We assumed the existence of a threshold burrow width ($b.\text{point}$) above which detection on the line was perfect. We drew this value from a uniform distribution bound between 15 and 30 cm (the upper bound representing the largest conceivable size of a burrow escaping detection despite its occurrence directly on the line). For individuals with the smallest possible burrow width ($z_{\min}$), we drew probability of detection on the line from a uniform distribution bound between 0.2 - 1. Probability of detection of individuals on the transect line increased proportionally as burrow width varied between this minimum value and the threshold point; perfect detection beyond the threshold point thus produced a “broken stick” appearance for the function:

$$\xi_i = \begin{cases} \left(\frac{1 - b}{b.\text{point} - z_{\min}} \right) (z_i - z_{\min}) + b, & z_{\min} \leq z_i < b.\text{point} \\ 1, & z_i \geq b.\text{point} \end{cases}$$

$$b \sim \text{Uniform}(0.2, 1)$$

$$b.\text{point} \sim \text{Uniform}(15, 30)$$

For the half-normal detection curve, probability of detection of burrow i at distance x from the transect line was described by:

$$g(x, z) = e^{\left(\frac{-x_i^2}{2\sigma_i^2}\right)} * \xi_i$$

Simulation

We simulated Enhanced LTDS on a 100-ha area, with individuals randomly distributed across the area at either 2 or 4 individuals ha^{-1} . Parallel transects were spaced either $Q = 60$ m or $Q = 100$ m apart (for 2 or 4 individuals ha^{-1} respectively) to ensure adequate numbers of detections. We simulated 3 different frequencies of ISUs - 0%, 10% and 15% of the 100-ha area. Intensive-search units were simulated as 50m by 50m square plots centered on the transect line ($Bx = 25\text{m}$), and simulated intensive-searches were conducted with perfect detection inside each square.

We conducted all simulations using the same underlying covariate distribution for burrow width (Figure 4.2a). We allowed burrow width values to range from 5 cm to 65 cm. We simulated detection of individuals under two scenarios representing detection probability as either strongly or weakly related to burrow width (Figure 4.2b). For our scenario with detection weakly related to size, we simulated individual detection as a half normal curve with $\log(\sigma_i) = 1.5 + 0.05 * z_i$. For our alternative scenario of detection strongly related to size, we increased the effect of size on detection in the same model: $\log(\sigma_i) = 1.5 + 0.1 * z_i$. We used the broken stick regression model with $b.\text{point}$ and b fixed at values 20 cm and 0.5, respectively, to generate detection probabilities < 1 for burrows occurring on the line. We attempted to estimate these parameters from the simulated data by sampling $b.\text{point}$ from a uniform distribution bound

between 15 and 30 cm and by sampling b from a uniform distribution bound between 0.2 and 1. The probability of occupancy of each burrow (either detected or latent) was modeled with $\text{logit}(o_i) = 1 - 0.025 \cdot z_i$, such that occupancy decreased with burrow width. For all simulations, vegetation was set at 0 across the entire site.

We simulated each of our 12 sampling intensity $\times$ population density $\times$ size-related detection combinations 40 times, for a total of 480 simulations. We computed measures of bias and precision for estimates of density and select quantiles of the burrow width distribution (≤ 13 cm and ≥ 45 cm). We tested for convergence of 3 MCMC chains using the Gelman–Rubin statistic (Gelman et al. 2004) and extended simulations (≥ 300 K iterations per simulation scenario) to meet the minimum iteration suggestion of the Raftery-Lewis diagnostic (Bernardo et al. 1992). Each model run took between 3-4 hours to complete using the runjags package of R (<https://cran.r-project.org/web/packages/runjags/runjags.pdf>) on a 2.30 GHz processor using 3 cores. For comparison to a conventional analytical approach under a frequentist framework, we estimated the same quantities and computed the same performance measures via the Rdistance package in R (Miller et. al. 2017). For each simulated survey, we allowed Rdistance to fit any of the detection curves available within the package (half-normal, hazard rate, negative exponential, uniform or gamma distributions). Following Stober et al. (2017), we fit detection curves using all available burrows and then adjusted estimates by observed occupancy probability to separately estimate tortoise densities.

Case Study

We performed Enhanced LTDS surveys for gopher tortoises at 25 locations in Georgia (24) and Florida (1) during the summers of 2017 and 2018 (Figure 4.3). Sites were selected because they varied in vegetation structure, tortoise abundance, time since burning, and

management histories. Sites ranged in size from 33 ha to 235 ha. For all Georgia sites, we used transects created for previous gopher tortoise surveys by the GA DNR. Surveys were performed by 2 observers using 50m by 50m square ISUs ($Bx = 25m$) covering approximately 10% of the estimated suitable habitat at each site. We attempted to endoscopically examine all burrows to determine burrow occupancy, barring times of equipment failure. We recorded vegetation measurements at regular 15-m intervals from the beginning to the end of each transect line to estimate vegetation obstruction. We measured vegetation obstruction by placing a meter stick ~5 m off the transect and estimating the minimum height of vegetation (0 to 1m, 5 cm increments) where all markings were $\geq 50\%$ obscured. We compared the precision of density outputs and estimated size distributions under our hierarchical model with and without data collected by intensive-search.

We estimated the cost (in dollars) of adding ISUs as a function of length walked, time taken, and number of observers. We assumed that the walking pace of the LTDS part of the survey was $P1$ (hr/m) and that each observer costs W (\$/hr) in wages. We assumed the time spent examining burrows for occupancy was included in the walking pace. Assuming additional observers did not substantially reduce the time of surveying transects, the cost of surveying transects with combined length L_{Total} using n_{obs} observers was

$$C1 = W * P1 * L_{Total} * n_{obs}.$$

We further assumed that the walking pace in an ISU was $P2$ (hr/m) and that each observer could find all objects present within 2 m on either side of their position. Thus, the cost of surveying S square ISUs of width $2 * Bx$ and length L_Y , using n_{obs} observers was

$$\begin{aligned} C2 &= W * P2 * n_{obs} * \frac{(2 * Bx * S * L_Y)}{4 * n_{obs}} \\ &= 0.5 * W * P2 * Bx * S * L_Y. \end{aligned}$$

The total cost of a double-sampling survey with 2 observers and 50m by 50m square ISUs ($2 \times B \times L_Y = 50 \text{ m}$) was

$$C_{Total} = W * (2 * P1 * L_{Total} + 625 * P2 * S).$$

3. Results

Simulation

Across all scenarios, mean relative bias of density point estimates decreased with increasing frequency of ISUs, although only slightly so within the Bayesian models that accounted for imperfect detection on the line (Figure 4.4). In most scenarios in which detection on the line was imperfect, applying the conventional model resulted in severe underestimation of density (Figure 4.4). Bias was much less under the Bayesian models; however, all models tended to underestimate density, most noticeably for scenarios in which detection probability was strongly related to burrow width. Credible interval performance tended to improve with the addition of ISUs (Table 4.1). There was little difference in credible interval performance between 10% and 15% frequencies of ISUs.

Proportions of the population among the smallest ($\leq 13 \text{ cm}$ width) or the largest ($\geq 45 \text{ cm}$ width) burrows were more accurately estimated by models that accounted for imperfect detection on the line than by simple frequencies of the raw LTDS data (Figure 4.5, 4.6). The precision of the estimated proportion of burrows with the smallest widths ($\leq 13 \text{ cm}$) increased with increasing frequency of ISUs (Figure 4.5). However, in every scenario, all models slightly underestimated the true proportion of small burrows. For both small and large burrows, burrow width distribution was most accurately estimated when population density was high and when detection probability and burrow width were strongly related (Figure 4.5, 4.6). For the largest burrow widths ($\geq 45 \text{ cm}$), there was little difference in estimate performance between any of our

Bayesian models (Figure 4.6). For all burrow width proportion estimates (≤ 13 cm and ≥ 45 cm) frequencies derived from the raw LTDS data were farthest from the truth under the scenarios for which detection probability and burrow width were weakly related.

The coefficient of variation (CV) of density estimates was lowest for models with data from ISUs (Figure 4.7). Under scenarios where detection probability and burrow width were weakly related, CV was largest for our Bayesian model without intensive-search units (0% ISUs). Under scenarios with a strong relationship between burrow width and detection probability, estimates produced under the conventional frequentist-based model had the highest CV. Coefficient of variation decreased with increased ISU frequency and increased density.

Case Study

Burrow density estimates from our Enhanced LTDS model on data collected through double-sampling were more precise than estimates produced under the conventional LTDS design at 88% of sites analyzed (Table 4.2). Precision of burrow width distribution estimates for the smallest burrows (≤ 13 cm) followed a similar pattern, with 100% of sites producing more precise estimates when intensive-search data was considered (average percent difference in CV achieved by double-sampling = 68%; Figure 4.8). Estimate precision increased (percent reduction in CV between survey protocols increased) as more effort was expended in collecting double-sampling data (greater area coverage by ISUs) (Figure 4.9). Inclusion of ISUs led to a median 34% reduction in density point estimate CV for a median 14% (range 4% to 38%) increase in cost (Table 4.3). At 16 of our 25 surveyed sites, intensive searching detected at least one burrow on the transect line that was not detected during the standard portion of the LTDS survey. Estimated probability of detection on the line for the smallest (5 cm width) burrows ranged 0.44 – 0.78 for all sites (Table 4.4).

4. Discussion

Our results suggest that the addition of ISUs to LTDS may prove an effective method for increasing the accuracy and precision of both density point estimates and the underlying burrow size distribution of gopher tortoise populations. In simulation, our models with ISUs covering 10% and 15% of the survey area had the greatest accuracy of density point estimates of any of the models tested. By analyzing our field data with and without intensive-search data included, we were able to evaluate both the costs and benefits of including ISUs in gopher tortoise LTDS surveys. In agreement with our simulation results, estimates including ISUs had higher levels of precision for density point estimates and lower CVs than estimates produced without double-sampling.

Inclusion of ISUs in our simulated surveys yielded moderate increases in precision of burrow width distribution estimates as sampling intensity increased. Though the means of the distribution estimates were similar between levels of double-sampling intensity, estimates produced by our Enhanced LTDS model without incorporating data from intensive-searches had a much larger range in accuracy and were more prone to overestimating proportion of the smallest burrow sizes (≤ 13 cm) than those produced by incorporating intensive-search data. When ISUs were added, we saw more consistency in density point estimate accuracy, especially when detection probability was weakly affected by burrow width and density of the surveyed population was low. This reduction in false positives (more small burrows estimated than occurred) suggests the addition of ISUs may allow for greater confidence in the estimated proportion of harder to detect individuals in the population.

As seen in simulation, the addition of ISUs to our real LTDS surveys often resulted in smaller coefficients of variation for both burrow density and burrow width distribution estimates.

Much of this improved precision likely stems from reduced uncertainty of detection parameters, such as the probability of detection on the line for the smallest burrow sizes (5 cm) and the threshold point of burrow size for perfect detection on the line. If strong prior information can be provided prior to sampling, the addition of intensive-search units may not be necessary for accurate and precise estimation of tortoise populations. However, as is the case with most gopher tortoise samples, these values are generally unknown and may vary between surveys with different observers. Additional variation in detection on the transect line may stem from environmental variables such as soil characteristics, time of year, or the density of ground cover. Double-sampling techniques such as our Enhanced LTDS method may allow for greater ability to estimate population trends with high precision for repeated surveys.

At all our surveyed sites, we used two observers to perform both the standard and enhanced line transect sampling. When a previously missed burrow was found in an ISU, both observers stopped the search to help measure, scope and record the characteristics of the discovered burrow. With a third observer present, intensive-searching for additional burrows could have been carried out simultaneously, greatly reducing the amount of time spent in the ISU and improving the efficiency of the survey. For situations where total time available to survey may be a concern, we expect that ELTDS surveys carried out with three or more observers would increase the efficiency of surveying ISUs in the LTDS survey without sacrificing any of the improvements in precision or accuracy of population density estimates.

As suspected, estimated detection on the line for the smallest tortoise burrows (5 cm) was <1 for all our surveyed sites, confirming previous research suggesting that one of the basic assumptions of line-transect distance sampling is not being met in many gopher tortoise surveys (Buckland et al. 2001, Howze and Smith 2018, Chapter 2, Chapter 3). Imperfect and variable

detection on the transect line likely leads to underestimation of gopher tortoise populations (Thomas et al. 2006) and overrepresentation of larger tortoises in population demographic estimates. Stober et al. (2017) addressed concerns of imperfect detection in tortoise surveys by only analyzing LTDS data for burrows >12 cm. However, our models estimated perfect detection on the transect line was not reached until burrows were between 19-21 cm wide, suggesting this cutoff may not be sufficient to remove this source of bias from gopher tortoise density estimation. Moreover, accurate estimates of juvenile gopher tortoise survival and recruitment are necessary to assess the status and demographic trends of the species across the range. Low survival of juvenile gopher tortoises has been identified as a threat to the species (USFWS 2011); ignoring information about this vulnerable part of the population may lead to improper management or faulty conservation decisions.

Given the gopher tortoise's decline in recent years (McCoy et al. 2006, Smith et al. 2006), our ability to produce reliable population estimates and monitor changes in tortoise recruitment rate may be critical for making informed decisions about management throughout the gopher tortoise's native range. Uncertainty in juvenile population estimates limits our ability to understand the roles that fire suppression, disease, or anthropogenic habitat modifications may have on tortoise survival and population sustainability (Hermann et al. 2002, Seigel et al. 2003, McCoy et al. 2007). We anticipate the use of double-sampling approaches, such as our enhanced line-transect distance protocol and model, may provide a simple but powerful solution to account for low detection of juvenile gopher tortoise burrows or other cryptic, sessile survey objects, and imperfect detection on the transect line.

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5. Literature Cited

- Ballou, A. 2013. Aspects of gopher tortoise (*Gopherus polyphemus*) populations in Georgia: Status, landscape predictors, and juvenile movements and burrow use. University of Georgia, Athens, GA.
- Barlow, J. 2015. Inferring trackline detection probabilities, $g(0)$, for cetaceans from apparent densities in different survey conditions. *Marine Mammal Science* 31:923–943.
- Bart, J., S. Droege, P. Geissler, B. Peterjohn, and C. J. Ralph. 2004. Density estimation in wildlife surveys. *Wildlife Society Bulletin* 32:1242–1247.
- Bart, J., S. Earnst, and M. Murphy. 2002. Double sampling to estimate density and population trends in birds. *The Auk* 119:36–45.

- Bernardo, J. M., J. O. Berger, A. P. Dawid, and A. F. Smith, editors. 1992. Bayesian statistics 4. Clarendon Press, Oxford.
- Bradford, A. L., K. A. Forney, E. M. Oleson, and J. Barlow. 2014. Accounting for subgroup structure in line-transect abundance estimates of false killer whales (*Pseudorca crassidens*) in Hawaiian waters. PLOS ONE 9:e90464.
- Buckland, S. T., editor. 2004. Advanced distance sampling: Estimating abundance of biological populations. First edition. Oxford Univ. Press, New York.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, and J. L. Laake. 1993. Distance sampling: Estimating abundance of biological populations. Springer, New York.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. Introduction to distance sampling: Estimating abundance of biological populations. Oxford University Press, Oxford.
- Castellón, T. D., B. B. Rothermel, and S. Z. Nomani. 2012. Gopher tortoise (*Gopherus polyphemus*) burrow densities in scrub and flatwoods habitats of peninsular Florida. Chelonian Conservation and Biology 11:153–161.
- Chadès, I., E. McDonald-Madden, M. A. McCarthy, B. Wintle, M. Linkie, and H. P. Possingham. 2008. When to stop managing or surveying cryptic threatened species. Proceedings of the National Academy of Sciences 105:13936–13940.
- Chen, G., M. Kéry, M. Plattner, K. Ma, and B. Gardner. 2013. Imperfect detection is the rule rather than the exception in plant distribution studies. Journal of Ecology 101:183–191.
- Clement, M. J., S. J. Converse, and J. A. Royle. 2017. Accounting for imperfect detection of groups and individuals when estimating abundance. Ecology and Evolution 7:7304–7310.
- Cochran, W. G. 1977. Sampling techniques. 3d ed. Wiley, New York.

- Collins, B. T. 2007. Guidelines for using double sampling in avian population monitoring. *The Auk* 124:1373–1387.
- Eberhardt, L. L., and M. A. Simmons. 1987. Calibrating population indices by double sampling. *The Journal of Wildlife Management* 51:665–675.
- Gaillard, D., J. R. Ennen, B. R. Kreiser, C. P. Qualls, S. C. Sweat, R. Birkhead, T. D. Tuberville, M. Aresco, E. D. McCoy, H. R. Mushinsky, and T. W. Hentges. 2017. Range-wide and regional patterns of population structure and genetic diversity in the gopher tortoise. *Journal of Fish and Wildlife Management* 8:497–512.
- Gelman, A., editor. 2004. *Bayesian data analysis*. 2nd ed. Chapman & Hall/CRC, Boca Raton, Fla.
- Gentry, A. H., and C. H. Dodson. 1987. Diversity and biogeography of neotropical vascular epiphytes. *Annals of the Missouri Botanical Garden* 74:205–233.
- Howze, J. M., and L. L. Smith. 2018. Detection of gopher tortoise burrows before and after a prescribed fire: Implications for surveys. *Journal of Fish and Wildlife Management* X:xx–xx.
- Kéry, M., and J. A. Royle. 2016. *Applied hierarchical modeling in ecology: Analysis of distribution, abundance and species richness in R and BUGS*. Elsevier/AP, Academic Press is an imprint of Elsevier, Amsterdam; Boston.
- Marques, F. F. C., S. T. Buckland, D. Goffin, C. E. Dixon, D. L. Borchers, B. A. Mayle, and A. J. Peace. 2001. Estimating deer abundance from line transect surveys of dung: Sika deer in southern Scotland. *Journal of Applied Ecology* 38:349–363.

- Marques, T. A., L. Thomas, S. G. Fancy, S. T. Buckland, and C. M. Handel. 2007. Improving estimates of bird density using multiple- covariate distance sampling. *The Auk* 124:1229–1243.
- McCoy, E. D., and H. R. Mushinsky. 1992. Studying a species in decline: Gopher tortoises and the dilemma of “correction factors.” *Herpetologica* 48:402–407.
- Miller, D. L., E. Rexstad, L. Thomas, L. Marshall, and J. Laake. 2017. Distance sampling in R. [bioRxiv:063891](https://doi.org/10.1101/063891).
- Royle, J. A., and R. M. Dorazio. 2006. Hierarchical models of animal abundance and occurrence. *Journal of Agricultural, Biological, and Environmental Statistics* 11:249–263.
- Royle, J. A., and R. M. Dorazio. 2008. Hierarchical modeling and inference in ecology: The analysis of data from populations, metapopulations and communities. Reprint. Acad. Press, Amsterdam.
- Smith, L., J. Linehan, J. Stober, M. Elliott, and J. Jensen. 2009. An evaluation of distance sampling for large-scale gopher tortoise surveys in Georgia, USA. *Applied Herpetology* 6:355–368.
- Smith, R., A. Chambers, J. Berish, T. Tuberville, and K. Herpich. 2005. Gopher tortoise burrow surveys: external characteristics, burrow cameras, and truth. *Applied Herpetology* 2:161–170.
- Stober, J. M., R. Prieto-Gonzalez, L. L. Smith, T. A. Marques, and L. Thomas. 2017. Techniques for estimating the size of low-density gopher tortoise populations. *Journal of Fish and Wildlife Management* 8:377–386.
- Strobel, B. N., and M. J. Butler. 2014. Monitoring whooping crane abundance using aerial surveys: Influences on detectability. *Wildlife Society Bulletin* 38:188–195.

- Thomas, L., S. T. Buckland, K. P. Burnham, D. R. Anderson, J. L. Laake, D. L. Borchers, and S. Strindberg. 2006. Distance sampling. John Wiley & Sons, Ltd, Hoboken, NJ.
- Thomas, L., R. Williams, and D. Sandilands. 2007. Designing line transect surveys for complex survey regions. *Journal of Cetacean Research and Management* 9:1–13.
- U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants: Determination of threatened status for the gopher tortoise (*Gopherus polyphemus*). Federal Register 52(129):25376-25380
- U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the gopher tortoise as threatened in the eastern portion of its range. Federal Register 76(144):45130-45162.
- Wich, S. A., and R. H. Boyko. 2011. Which factors determine orangutan nests' detection probability along transects? *Tropical Conservation Science* 4:53–63.

Table 4.1. Confidence and Credible Interval Performance. Percentage of simulations (n = 40) for each simulation scenario (2 levels of strength of relationship between detection probability and burrow width $\times$ 2 population densities) where the 95% confidence interval (conventional approach under Rdistance) or credible interval (Bayesian models) included the true population density.

Analysis Approach	Strength of Relationship Between Detection Probability and Burrow Size			
	Weak		Strong	
	2 ind ha ⁻¹	4 ind ha ⁻¹	2 ind ha ⁻¹	4 ind ha ⁻¹
Rdistance	65%	58%	71%	60%
0% ISUs	78%	83%	73%	73%
10% ISUs	78%	85%	80%	75%
15% ISUs	80%	85%	73%	78%

Table 4.2. Burrow and Tortoise Density Estimates from 25 Site Surveys in Georgia and Florida. Gopher tortoise (*Gopherus polyphemus*) burrow (D, burrows ha⁻¹) and tortoise (Dt, burrows ha⁻¹) mean density estimates and coefficient of variation (SE/mean x 100%) from line-transect distance sampling data collected at 25 sites in Georgia and Florida and derived under our hierarchical model with and without intensive-search units (ISUs). L = length of transect in km; *n* = number of burrows observed without (No ISUs) and with (ISUs) intensive searches. NA represents sites where burrow occupancy information was not collected.

Site #	L	<i>n</i>		D		Dt	
		No ISUs	ISUs	No ISUs	ISUs	No ISUs	ISUs
1	11.58	42	50	1.19 (28%)	1.32 (14%)	0.26 (46%)	0.38 (21%)
2	8.99	42	45	0.97 (8%)	1.32 (14%)	0.23 (23%)	0.39 (23%)
3	54.44	133	140	0.53 (14%)	0.57 (9%)	0.23 (17%)	0.24 (11%)
4	14.83	70	82	1.27 (15%)	1.51 (11 %)	0.24 (27%)	0.29 (21%)
5	25.79	116	131	1.44 (12%)	1.58 (9%)	0.44 (19%)	0.51 (13%)
6	5.09	33	54	1.01 (37%)	3.14 (11%)	NA	NA
7	7.77	101	117	3.28 (15%)	4.12 (10%)	1.32 (19%)	1.62 (13%)
8	13.09	74	74	1.66 (16%)	1.67 (16%)	0.69 (20%)	0.70 (19%)
9	10.25	40	43	0.87 (34%)	1.03 (15%)	NA	NA
10	6.76	68	93	1.94 (29%)	2.66 (10%)	0.96 (35%)	1.40 (12%)
11	24.79	132	149	2.16 (12%)	2.45 (10%)	1.01 (16%)	1.09 (14%)
12	9.44	50	57	1.25 (25%)	1.33 (14%)	0.29 (41%)	0.33 (22%)
13	9.75	105	128	1.51 (15%)	1.73 (10%)	NA	NA
14	15.56	59	69	1.45 (48%)	1.83 (10%)	0.53 (33%)	0.68 (14%)
15	4.10	62	65	4.36 (21%)	4.75 (14%)	2.84 (22.5%)	3.08 (15%)
16	15.64	28	28	0.23 (49%)	0.20 (18%)	0.10 (51%)	0.09 (19%)
17	14.57	149	171	3.45 (8%)	3.39 (9%)	NA	NA
18	3.98	156	170	8.26 (14%)	8.95 (9%)	NA	NA
19	2.50	35	41	2.57 (43%)	4.61 (23%)	0.99 (68%)	2.22 (34%)
20	11.84	69	88	1.75 (21%)	2.05 (11%)	1.13 (24%)	1.22 (13%)
21	9.61	102	116	2.08 (17%)	2.10 (13%)	NA	NA
22	7.00	71	84	4.34 (16%)	5.44 (12%)	1.63 (21%)	1.92 (17%)
23	12.98	69	111	2.93 (17%)	3.92 (10%)	1.21 (25%)	1.58 (13%)
24	15.36	167	185	1.63 (4%)	1.80 (4%)	NA	NA
25	11.36	107	133	1.95 (7%)	2.19 (5%)	0.84 (13%)	0.96 (11%)

Table 4.3 Cost of Enhanced Line-Transect Distance Sampling. Cost of sampling (U.S. dollars) and CV (SE/mean x 100%) of gopher tortoise (*Gopherus polyphemus*) burrow density estimates for enhanced line-transect distance sampling (ELTDS) and conventional line-transect sampling (LTDS) for 25 sites in Georgia and Florida. Surveys were conducted in May – July of 2017 and 2018.

Site	Cost LTDS (\$)	LTDS Burrow Density CV	Cost ELTDS (\$)	ELTDS Burrow Density CV
1	\$ 347.41	28%	\$ 408.61	14%
2	\$ 269.62	8%	\$ 305.32	14%
3	\$ 1,633.25	14%	\$ 1,888.25	9%
4	\$ 444.80	15%	\$ 495.80	11%
5	\$ 773.58	12%	\$ 855.18	9%
6	\$ 152.81	37%	\$ 198.71	11%
7	\$ 233.14	15%	\$ 294.34	10%
8	\$ 392.57	16%	\$ 407.87	16%
9	\$ 307.65	34%	\$ 343.35	15%
10	\$ 202.92	29%	\$ 259.02	10%
11	\$ 736.53	12%	\$ 828.33	10%
12	\$ 283.07	25%	\$ 334.07	14%
13	\$ 292.63	15%	\$ 358.93	10%
14	\$ 466.85	48%	\$ 522.95	10%
15	\$ 123.11	21%	\$ 138.41	14%
16	\$ 469.06	49%	\$ 494.56	18%
17	\$ 437.09	8%	\$ 513.59	9%
18	\$ 119.38	14%	\$ 165.28	9%
19	\$ 75.15	43%	\$ 95.55	23%
20	\$ 355.23	21%	\$ 396.03	11%
21	\$ 288.41	17%	\$ 344.51	13%
22	\$ 209.99	16%	\$ 235.49	12%
23	\$ 389.30	17%	\$ 445.40	10%
24	\$ 460.75	4%	\$ 527.05	4%
25	\$ 340.92	7%	\$ 391.92	5%

Table 4.4 Enhanced Line-Transect Distance Sampling Detection and Occupancy Parameter Estimates from 25 Sites in Georgia and Florida. Estimated threshold burrow width (*b.point*) for perfect detection on the line (95% credible interval), detection probability of 5-cm wide burrows on the line (95% credible interval), and average occupancy, by site, for gopher tortoise (*Gopherus polyphemus*) surveys conducted at 25 sites in Georgia and Florida. Surveys were conducted in May – July of 2017 and 2018. Occupancy estimates with NA represent sites where burrow occupancy information was not collected.

Site	b. point (cm)	P(g(0)) for 5 cm burrow	Estimated Occupancy
1	21 (15-26)	0.73 (0.38-1.00)	0.29
2	21 (15-26)	0.69 (0.35-1.00)	0.29
3	21 (15-26)	0.64 (0.32-0.95)	0.42
4	22 (17-27)	0.52 (0.3-0.86)	0.19
5	21 (15-26)	0.66 (0.34-1.00)	0.32
6	21 (15-26)	0.65 (0.34-1.00)	NA
7	20 (15-26)	0.45 (0.30-0.70)	0.39
8	21 (15-26)	0.61 (0.30-0.96)	0.42
9	21 (15-26)	0.68 (0.35-1.00)	NA
10	21 (15-26)	0.62 (0.30-0.95)	0.53
11	21 (15-26)	0.50 (0.30-0.83)	0.44
12	20 (15-24)	0.57 (0.33-0.80)	0.25
13	21 (15-26)	0.73 (0.41-1.00)	NA
14	21 (15-26)	0.67 (0.34-1.00)	0.37
15	20 (15-26)	0.71 (0.36-1.00)	0.65
16	21 (16-27)	0.64 (0.31-0.97)	0.46
17	23 (19-27)	0.77 (0.53-0.99)	NA
18	21 (15-26)	0.72 (0.37-1.00)	NA
19	20 (15-24)	0.50 (0.30-0.75)	0.48
20	21 (16-27)	0.78 (0.47-1.00)	0.59
21	25 (22-27)	0.44 (0.30-0.65)	NA
22	22 (16-27)	0.57 (0.30-0.91)	0.35
23	22 (16-27)	0.55 (0.30-0.89)	0.40
24	19 (15-24)	0.55 (0.31-0.78)	NA
25	21 (15-26)	0.52 (0.30-0.84)	0.44

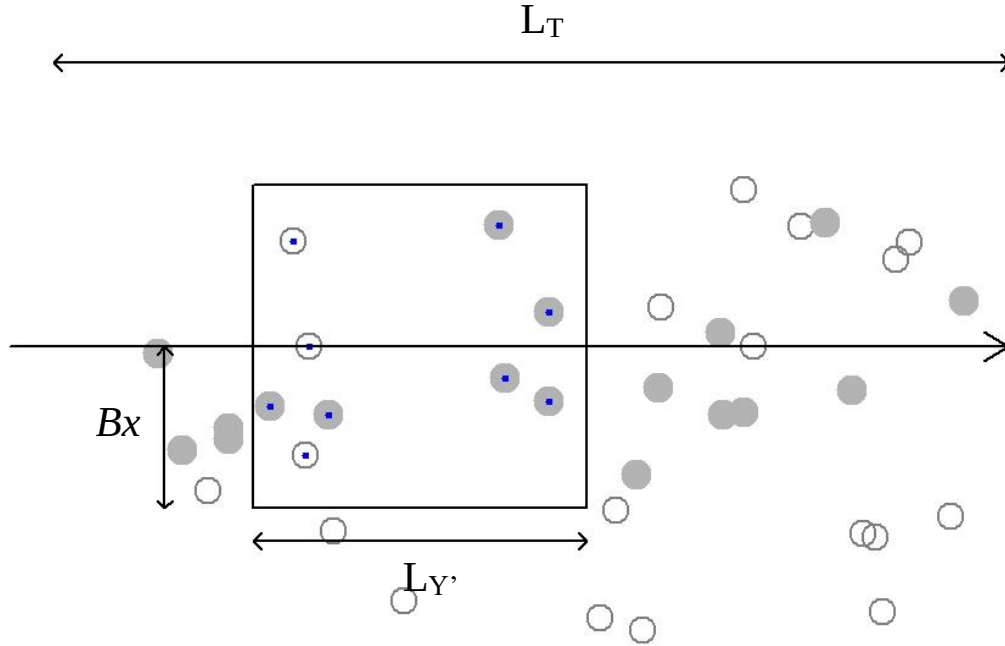


Figure 4.1 Schematic of Enhanced Line-Transect Distance Sampling. A conventional LTDS survey of the area takes place along the transect line (arrow), followed by an intensive search of the area in the black box. Each intensive-search unit (of size $2B_x$ by $L_{Y'}$) is centered on a transect (of total length L_T), and perfect detection is assumed during the search of the unit. Individuals in the population (shown as circles) can either be missed during the LTDS part of the survey (open circle), found only by the LTDS survey (grey-filled circle), found only by intensive searching (open circle with blue dot), or found during both surveys (grey-filled circle with blue dot). Individuals found in the intensive-search units but missed during the LTDS surveys can be used to inform a hierarchical model about the density and covariate values of missed individuals throughout the site.

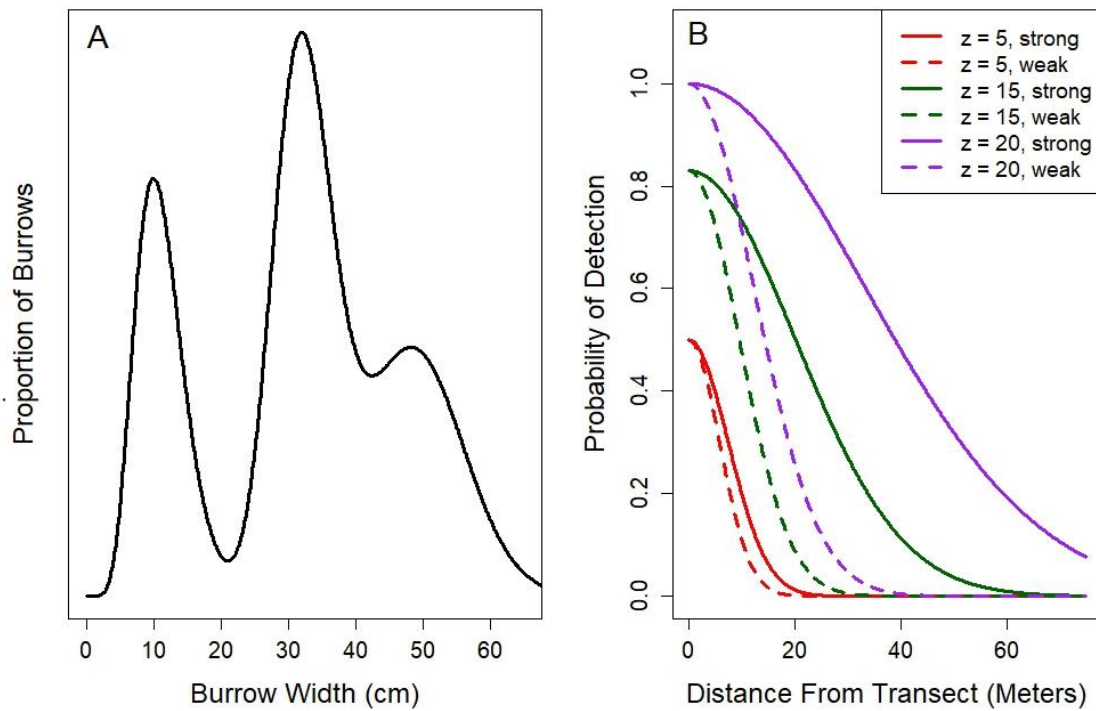


Figure 4.2 Burrow Width Distribution and Detection Curves Used in Simulation of Enhanced Line Transect Distance Sampling. A) underlying burrow width distribution of the gopher tortoise (*Gopherus polyphemus*) population used in simulation. B) detection curves used to simulate detection of individuals during the conventional line-transect distance sampling portion of the survey. Detection was simulated under two scenarios with burrow width (z) having a strong (solid lines) or weak (dashed lines) effect on the relationship between distance from transect and detection probability.

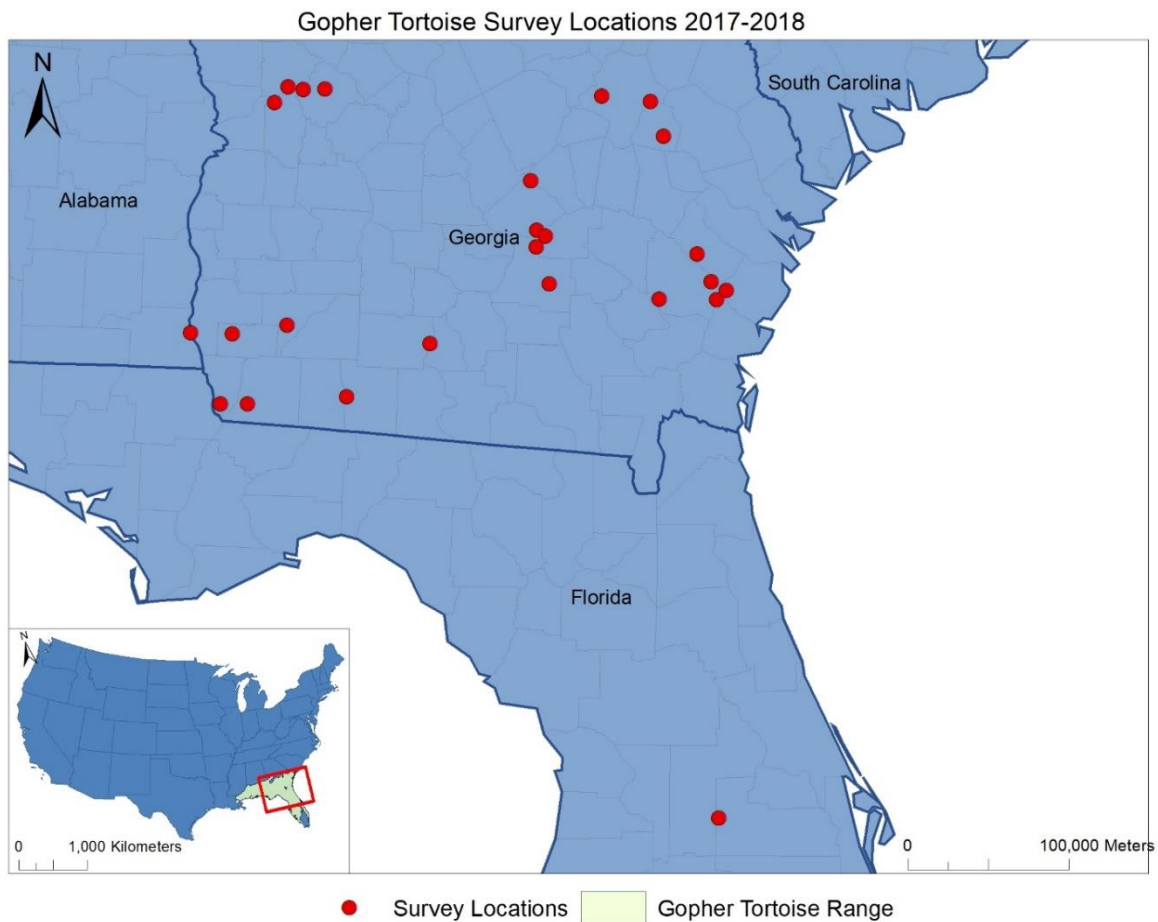


Figure 4.3 Locations of 25 Gopher Tortoise Populations Surveyed with Enhanced Line Transect Distance Sampling Between May 2017 and July 2018. All sites were surveyed for gopher tortoise (*Gopherus polyphemus*) using enhanced line-transect distance sampling with two observers in May-July of 2017 (11 sites) and 2018 (14 sites). Intensive-search units covered approximately 10% of the surveyable area at each site.

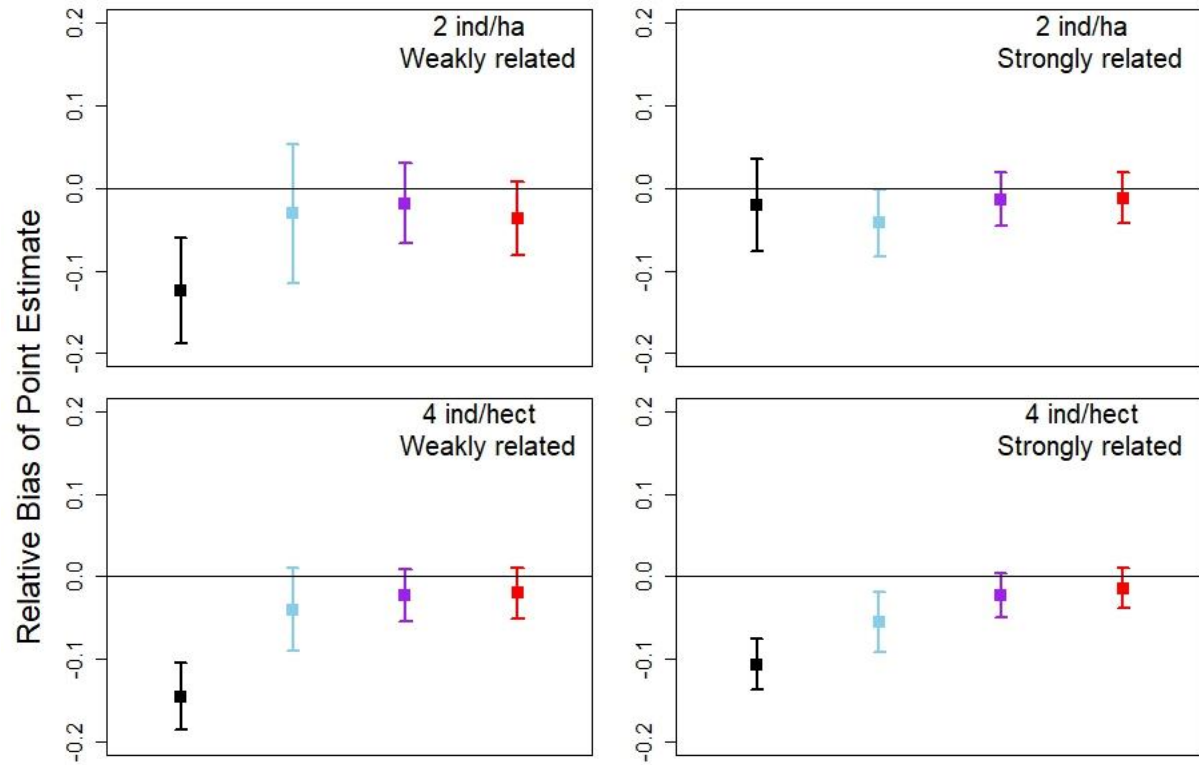


Figure 4.4 Relative Bias of Density Point Estimates for Simulated Gopher Tortoise Populations. The mean ($n = 40$) and 95% CI for relative bias (estimate/true – 1.0) of the density point estimate for each of 4 scenarios (2 population densities $\times$ 2 levels of strength of relationship between burrow width and detection probability) of simulated gopher tortoise (*Gopherus polyphemus*) population surveys and estimation models: conventional LTDS survey and estimation model (under Rdistance, shown in black) and a Bayesian model for the LTDS design paired with double-sampling of 0% (blue), 10% (purple), and 15% (red) of the survey area. In all scenarios, detection on the line was imperfect, with detection probability related to burrow size. The horizontal line in each section of the Figure represents a relative bias of 0 (the point estimate was equal to the truth).

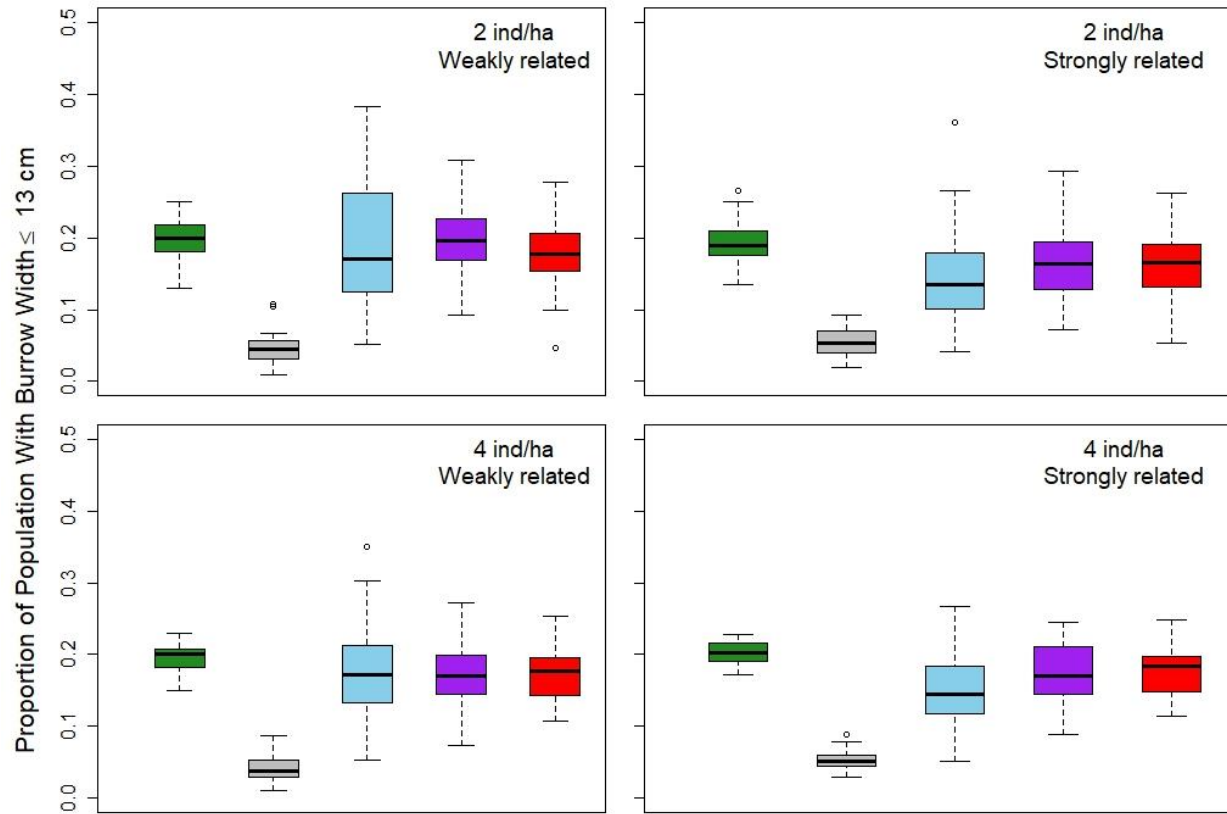


Figure 4.5 Estimated Proportion of Simulated Population with Burrow Width ≤ 13 cm. The green boxplots in each scenario (2 population densities $\times$ 2 levels of strength of relationship between burrow width and detection probability) represent the true proportion of gopher tortoise (*Gopherus polyphemus*) burrows in the simulated population ($n = 40$) with width ≤ 13 cm. Additional boxplots portray estimates of the same proportion computed as simple frequencies of raw line-transect distance sampling (LTDS) data (grey) and from posterior summaries by our Bayesian models with intensive-search areas (ISUs) at a frequency of 0% (blue), 10% (purple) and 15% (red) of the survey area.

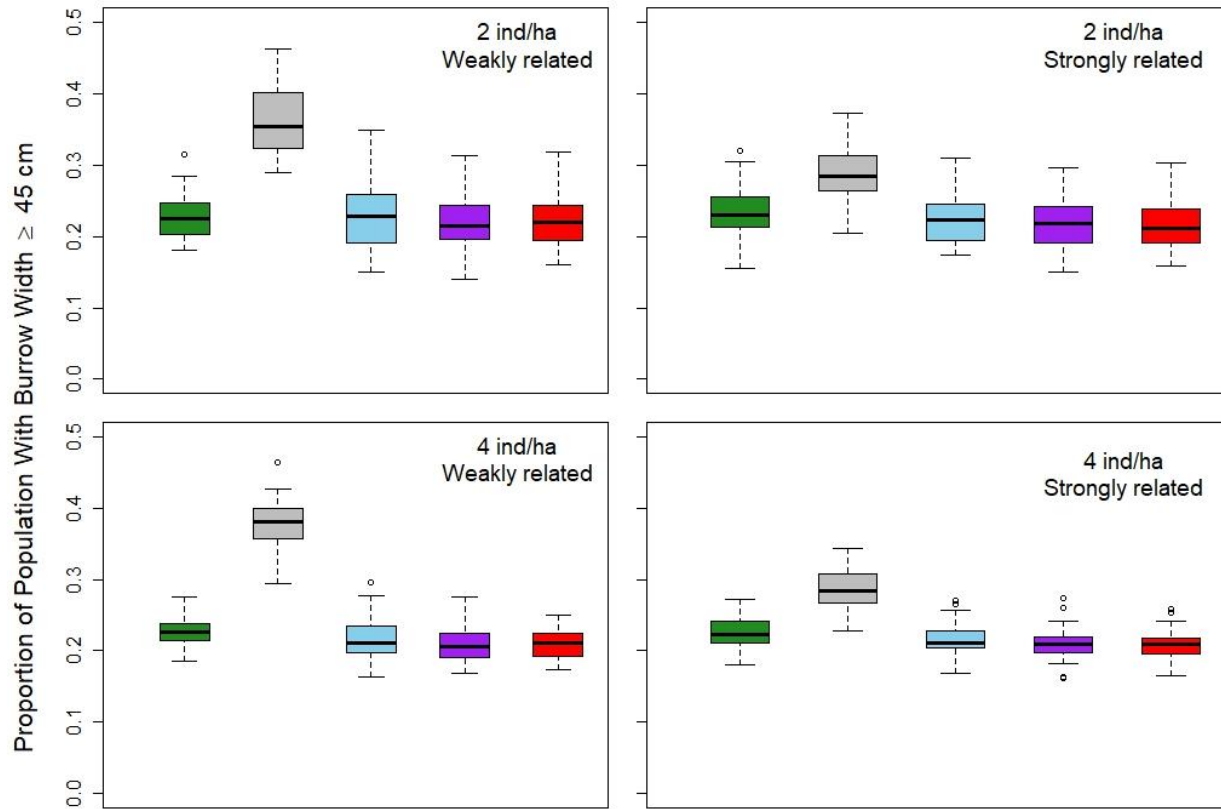


Figure 4.6 Estimated Proportion of Simulated Population with Burrow Width ≥ 45 cm. The green boxplots in each scenario (2 population densities $\times$ 2 levels of strength of relationship between burrow width and detection probability) represent the true proportion of gopher tortoise (*Gopherus polyphemus*) burrows in the simulated population ($n = 40$) with width ≥ 45 cm. Additional boxplots portray estimates of the same proportion computed as simple frequencies of raw line-transect distance sampling (LTDS) data (grey) and from posterior summaries by our Bayesian models with intensive-search units (ISUs) at a frequency of 0% (blue), 10% (purple) and 15% (red) of the survey area.

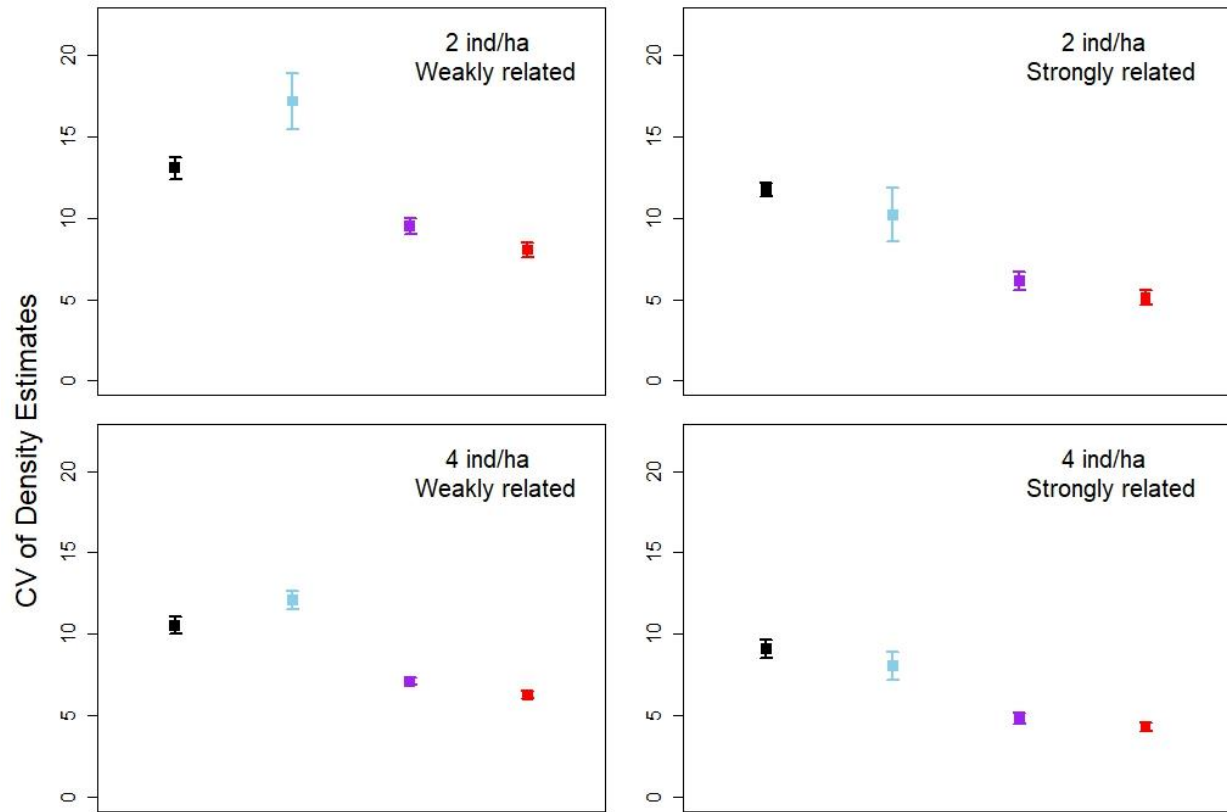


Figure 4.7. Mean Coefficient of Variation (CV) for Simulated Enhanced Line-Transect Distance Sampling Surveys of Gopher Tortoise Populations. Mean ($n = 40$) and 95% CI for coefficient of variation ($SE/\text{mean} \times 100\%$) of the density point estimates are displayed for each simulation scenario (2 population densities $\times$ 2 levels of strength of relationship between burrow width and detection probability). Gopher tortoise (*Gopherus polyphemus*) population surveys were simulated under 4 different scenarios and evaluated with a conventional frequentist model for LTDS data (under Rdistance; black) and 3 Bayesian models with intensive-search units (ISUs) distributed over 0% (blue), 10% (purple) or 15% (red) of the survey area.

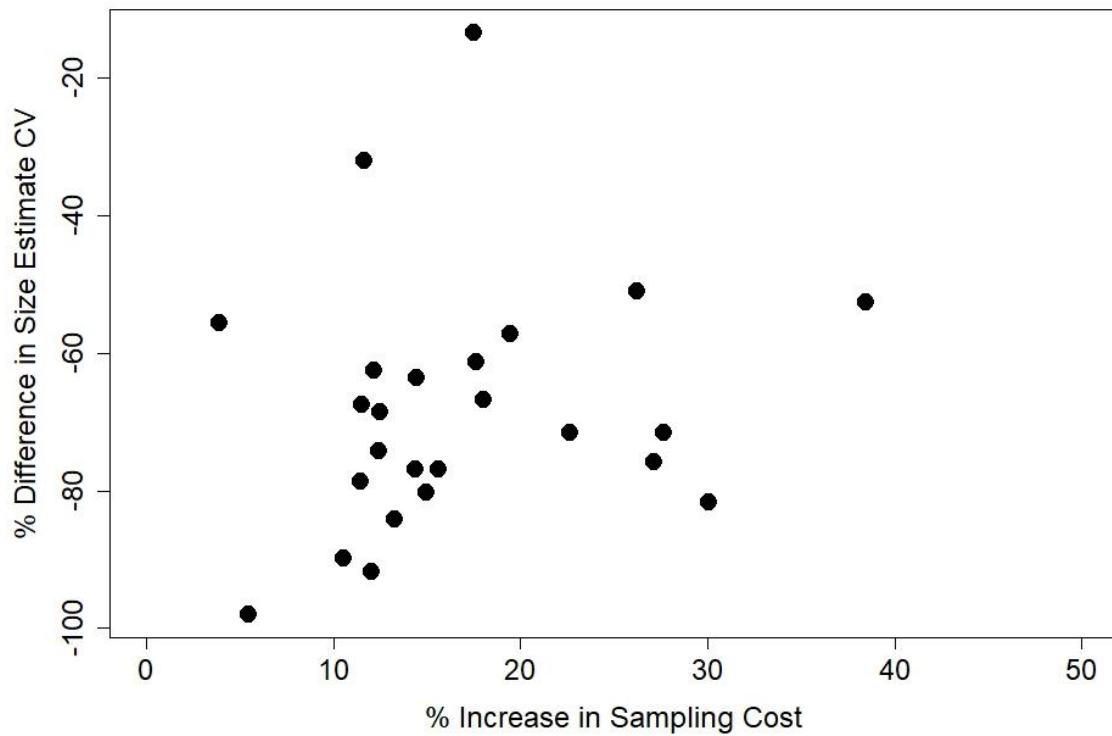


Figure 4.8. Relationship Between Sampling Cost and Precision of Burrow Width Distribution Estimates from Enhanced Line-Transect Distance Sampling at 25 Real Sites in Georgia and Florida. Percent difference in CV ($SE/\text{mean} \times 100\%$) of the proportion of burrows ≤ 13 cm wide between enhanced line-transect distance sampling and conventional line-transect distance sampling plotted against percent increase in sampling cost (U.S. dollars) for surveys of gopher tortoise (*Gopherus polyphemus*) at 25 sites in Georgia and Florida. Each black circle represents a different site.

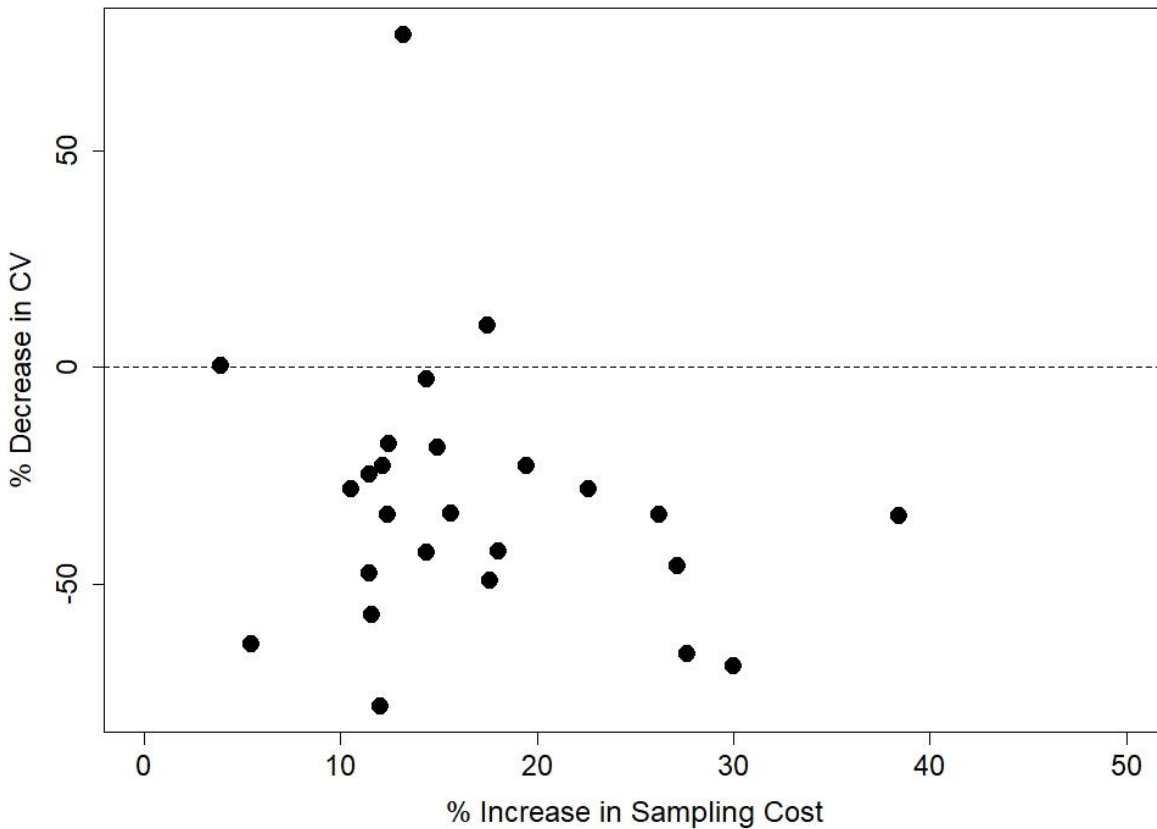


Figure 4.9. Relationship Between Sampling Cost and Coefficient of Variation (CV) for Density Estimates from Enhanced Line-Transect Distance Sampling at 25 Real Sites in Georgia and Florida. Percent difference in CV ($SE/mean \times 100\%$) between enhanced line-transect distance sampling and conventional line-transect distance sampling plotted against percent increase in sampling cost (U.S. dollars) for surveys of gopher tortoise (*Gopherus polyphemus*) at 25 sites in Georgia and Florida. The horizontal line represents no difference in CV between methods. Points below the line indicate the precision of estimates was greater when intensive-searches were included in data analysis.

CHAPTER 5

CONCLUSIONS

The gopher tortoise is federally listed as threatened under the Endangered Species Act in southwestern Alabama, Mississippi, and Louisiana, and it is listed as a species of special concern by the remaining states in its range (U.S. Fish and Wildlife Service 1987; Yager et al. 2007). Conservation of the gopher tortoise is tied to the assessment of both population abundance and size demography. The U.S. Fish and Wildlife Service currently recommends line-transect distance sampling (LTDS) as a standard method for monitoring gopher tortoise populations (U.S. Fish and Wildlife Service 2011).

As gopher tortoises are rarely observed outside of burrows (Smith et al., 2009), surveys instead use gopher tortoise burrows as the object of interest. Burrow size scales with age, at least in younger gopher tortoises (Germano, 1994), such that larger burrows tend to correspond with older resident tortoises. However, tortoise detections are strongly biased towards larger (adult) burrow sizes (Smith et al. 2009). Juvenile gopher tortoise burrows may be missed directly on the transect line (Ballou 2013; Howze and Smith 2018) and smaller burrows experience low detection regardless of distance to the transect (Ballou 2013; Howze and Smith 2018). Thus, it remains difficult to differentiate whether underrepresentation of small burrows in surveys is an indicator of low recruitment or merely reflective of low detection probability. The presence of ground-level vegetation may exacerbate this problem, significantly decreasing detection of tortoise burrows of all sizes (Howze and Smith 2018). The purpose of this study was to design

field methods and develop analytical models to reduce bias and increase precision of tortoise density and size-demography estimates.

To improve estimation from previously collected data, I developed a Bayesian hierarchical model (hereafter, bias-adjusted model) for line-transect distance sampling in which a covariate, in this case burrow width, helps to account for detection bias of all objects present and improves precision of estimated density. Since the covariate values of undetected objects is unknown, I modeled the distribution using a flexible gamma-mixture approach. In simulation, I found the point estimates for density from the bias-adjusted model were more accurate (point estimate nearer the true value; smaller range of bias) than those provided by a conventional analytical package, Program DISTANCE. Across all simulations, Program DISTANCE captured the true density in the confidence interval 76% of the time compared to my model's 94%. In real data collected at 3 sites, the bias-adjusted model estimated higher tortoise densities than did conventional distance sampling models. The coefficients of variation (CVs) produced by the new model were smaller for all sites in comparison to Program DISTANCE. The bias-adjusted model estimated the probability of detecting a 5 cm wide tortoise burrow on the transect line between 0.2 and 0.67, depending on site.

In gopher tortoise surveys, burrow size both impacts detection of tortoise burrows and reflects important demographic properties about the size and age distribution of the population. Managers rely on size class histograms of the widths of observed, occupied burrows to make inferences about the age structure of a population (Waddle et al. 2006; Smith et al. 2009). Multiple modes observed in burrow size histograms are interpreted as age pulses, and absence of modes in the smaller size classes are sometimes causes of concern, despite an understanding that poor detection of juvenile tortoises may influence these histograms (Smith et al. 2009; Ballou

2013). My bias-adjusted model addresses the duality of burrow size as both a detection nuisance and a demographic parameter by partially removing survey bias against small burrows when estimating the underlying burrow size distribution. In simulation, my model was able to partially account for survey bias against the smallest covariate values and provide a more accurate estimate of the underlying covariate distribution than indicated by the raw LTDS data. Estimated proportion of the population with the smallest covariate values (≤ 20 units) from the bias-adjusted model tended to be closer to the true distribution than naïve summaries of the raw LTDS. Population proportion estimates for larger covariate values (≥ 80 units) were closer to the true generating values than were summaries of the raw LTDS data in all cases tested.

In addition to accounting for size-biased detection during LTDS surveys, my bias-adjusting model allows for both occupied and unoccupied burrows to be used in analysis. Since small burrows are found infrequently regardless of occupancy status, a lack of occupied small burrows in the dataset is not necessarily indicative of a lack of small tortoises. In my analysis of real datasets, the increased proportion of tortoises in the smallest size classes (<10 cm wide) predicted by the bias-adjusted model reinforces concerns that raw counts from surveys are frequently underestimating the proportion of juvenile tortoises in the population. By including the full set of objects (in this case, burrows) when modeling detection, I was able to increase my sample size while still allowing for valid inferences about a subset (occupied burrows) of the data.

The presence of ground-level vegetation significantly decreases detection probability of tortoise burrows of all sizes (Berish and Leone 2014; Howze and Smith 2018), but no field or analytical methods exist to control for vegetation obstruction as a source of variation in detection rate. I proposed the addition of a simple, systematic measurement of ground-level vegetation to

transect surveys that serves as a covariate for the detection function. I investigated the performance of this covariate by simulation and by using real LTDS data collected at the Joseph W. Jones Center before (with obscuring vegetation cover) and after (vegetation cover mostly removed) application of prescribed fire. Across all simulations, my Bayesian hierarchical model produced more accurate density point estimates (point estimates closer to the truth) than any produced by a conventional analytical package (Program DISTANCE). Estimates of proportion of small burrows (≤ 20 cm width) in the population by my Bayesian model were consistent with truth. In the analysis of field data, my Bayesian model tended to produce similar density estimates for the pre- and post-burn surveys, whereas density estimates by Program DISTANCE were dissimilar between surveys. As expected, precision was poorer for pre-burn surveys compared to post-burn; however, consistency between pre- and post-burn density estimates demonstrated a degree of accuracy provided by my model that was not evident in Program DISTANCE. Estimated burrow width distributions from the Bayesian model were also similar for the pre- and post-burn surveys, though the estimates of the proportion of individuals with smaller burrows (≤ 20 cm wide) differed slightly between surveys. Across all plots, average estimated detection probability on the line during the pre-burn surveys was 0.38 (range 0.25 - 0.53) for the smallest burrow widths (5 cm wide), with perfect detection on the line not occurring until burrows were an average of 18 cm (range 13 – 21 cm) wide.

My vegetation study indicates that any level of spatial variation in vegetation obstruction decreases detection of burrows and may lead to large underestimation in population size when not considered during analysis. I found that surveying after a burn was a useful strategy to minimize the impact of vegetation and maximize detection of burrows. In cases where burning

prior to a survey is not practicable, the incorporation of vegetation measurements in the LTDS protocol may be a simple tool to improve the accuracy of population estimates.

In addition to improvements on the traditional line-transect distance estimation model, I proposed the addition of double-sampling plots to increase precision of population density and size distribution estimates and more accurately estimate the probability of detection on the transect line. Methods that combine line transect distance sampling with mark-recapture (e.g., double-observer sampling; Quang and Becker 1997) are single-pass approaches that permit the estimation of detection probability on the transect line. Such methods work efficiently where it is possible to logically “mark” and “recapture” objects and where survey logistics (e.g., many vehicle-based surveys) make it impractical or expensive to depart from the transect line to conduct more intensive searches. However, in some contexts, the survey team has logistic flexibility to periodically break from the transect portion of the survey and conduct an essentially complete count within a defined plot. To allow for greater flexibility when accommodating multiple sources of variation, I designed a Bayesian model for line-transect distance sampling that incorporates randomly placed intensive-search plots to improve detection of gopher tortoise burrows for which burrow size or levels of obstructing vegetation hinders their discovery. I compared density point estimates and precision produced from my Bayesian double-sampling model to frequentist-based standard LTDS for both a simulated and real dataset. I further calculated the cost of surveying with and without intensive-search plots for 25 plots in Georgia and Florida.

Across all scenarios, mean relative bias of density point estimates decreased with the addition of intensive-search units. Proportions of the population among the smallest (≤ 13 cm width) or the largest (≥ 45 cm width) burrows were more accurately estimated by models that

accounted for imperfect detection on the line than by simple frequencies of the raw LTDS data. Burrow density estimates from our Enhanced LTDS model on data collected through double-sampling were more precise than estimates produced under the conventional LTDS design at 88% of sites analyzed. The use of double-sampling led to a median 34% reduction in density point estimate CV for a median 14% (range 4% – 38%) increase in cost.

My results suggest that the addition of double-sampling to LTDS may prove an effective method for increasing the accuracy and precision of both density point estimates and the underlying burrow size distribution of gopher tortoise populations. I found that estimated detection probability on the line for the smallest tortoise burrows (5 cm) was <1 for all my surveyed sites, confirming previous research suggesting that one of the basic assumptions of line-transect distance sampling is not being met in many gopher tortoise surveys (Buckland et al. 2001, Howze and Smith 2018).

Successful conservation of gopher tortoise populations may depend on accurate assessment of population abundance and demographics. Given the gopher tortoise's decline in recent years (Hermann et al. 2002; McCoy et al. 2006; Waddle et al. 2006), our ability to produce reliable population estimates and monitor changes in tortoise recruitment rate may be critical for making informed decisions about management throughout the gopher tortoise's native range. Uncertainty in juvenile population estimates limits our ability to understand the roles that natural or anthropogenic change may have on tortoise survival and population sustainability (Hermann et al. 2002; Yager et al. 2007). Without accurate baseline estimates, land managers cannot confidently assess changes in populations over time or determine the efficacy of conservation efforts. I anticipate the use of bias-adjusting models or enhanced line-transect

distance sampling will provide an effective approach for accounting for low detection of juvenile gopher tortoises and imperfect detection on the transect line.

Literature Cited

- Ballou, A. 2013. Aspects of gopher tortoise (*Gopherus polyphemus*) populations in Georgia: Status, landscape predictors, and juvenile movements and burrow use. University of Georgia, Athens, GA.
- Berish, J. E. D., and E. H. Leone. 2014. Follow-up demographic survey of a Florida gopher tortoise population. *Southeastern Naturalist* 13:639–648.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. Introduction to distance sampling: Estimating abundance of biological populations. Oxford University Press, Oxford.
- Cochran, W. G. 1977. Sampling techniques. 3d ed. Wiley series in probability and mathematical statistics, Wiley, New York.
- Germano, D. J. 1994. Growth and age at maturity of North American tortoises in relation to regional climates. *Canadian Journal of Zoology* 72:918–931.
- Hermann, S. M., C. Guyer, J. Hardin Waddle, and M. Greg Nelms. 2002. Sampling on private property to evaluate population status and effects of land use practices on the gopher tortoise, *Gopherus polyphemus*. *Biological Conservation* 108:289–298.
- Howze, J. M., and L. L. Smith. 2018. Detection of gopher tortoise burrows before and after a prescribed fire: Implications for surveys. *Journal of Fish and Wildlife Management* X:xx–xx.
- McCoy, E. D., H. R. Mushinsky, and J. Lindzey. 2006. Declines of the gopher tortoise on protected lands. *Biological Conservation* 128:120–127.
- Quang, P. X., and E. F. Becker. 1997. Combining line transect and double count sampling techniques for aerial surveys. *Journal of Agricultural, Biological, and Environmental Statistics* 2:230–242.
- Smith, L., J. Linehan, J. Stober, M. Elliott, and J. Jensen. 2009. An evaluation of distance sampling for large-scale gopher tortoise surveys in Georgia, USA. *Applied Herpetology* 6:355–368.

- U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants: Determination of threatened status for the gopher tortoise (*Gopherus polyphemus*). Federal Register 52(129):25376-25380
- U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the gopher tortoise as threatened in the eastern portion of its range. Federal Register 76(144):45130-45162.
- Waddle, J. H., F. J. Mazzotti, and K. G. Rice. 2006. Changes in abundance of gopher tortoise burrows at Cape Sable, Florida. Southeastern Naturalist 5:277–284.
- Yager, L. Y., M. G. Hinderliter, C. D. Heise, and D. M. Epperson. 2007. Gopher tortoise response to habitat management by prescribed burning. Journal of Wildlife Management 71:428–434.