DEVELOPMENT OF POPULATION MONITORING TECHNIQUES FOR WILD PIGS (*SUS SCROFA)*

by

DAVID A. KEITER

(Under the Direction of James C. Beasley)

ABSTRACT

Despite growing importance of wild pigs (*Sus scrofa*) as an invasive species, techniques to obtain estimates of demographic characteristics (e.g. population density, juvenile survival rates) are lacking. Information about these rates is critical to effective management of wild pig populations and assessment of risks to ecosystem health, necessitating development of techniques to measure these basic rates. In this thesis, I develop methods to facilitate genetic capture-mark-recapture of this and other social ungulate species (Chapter 2), compare a suite of common field and analytical techniques to estimate animal population density and evaluate their robustness to effects of common ecological and observational processes (Chapter 3), and pilot use of vaginal implant transmitters in wild pigs and evaluate techniques to monitor survival of juvenile wild pigs (Chapter 4). This work will aid in management of this invasive species and assessment of threats posed by wild pig populations to natural and anthropogenic ecosystems.

INDEX WORDS: Density estimation, Juvenile survival, Noninvasive genetic sampling, Population monitoring, Radiotransmitter attachment, Search protocols, *Sus scrofa*, Wild pigs

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B.S., SUNY College of Environmental Science and Forestry, 2013

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2016

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DEDICATION

To my loving parents Jim Keiter, Sandy Poole Keiter, Amy Keiter, and Robert Donatz, and my brothers Ken Keiter, Cooper Ayers, John Donatz, and Michael Donatz; your support kept me going. I would also like to dedicate this thesis to the memory of Guy Baldassarre, who believed more than anyone in the ability of his students to change the world.

ACKNOWLEDGEMENTS

This degree has truly taught me that it takes a village to raise a master's student. None of this work would be possible without the hard work of some truly dedicated individuals. Jim Beasley, thank you for allowing me to chase every hare-brained idea that arose during these projects and helping me develop as a scientist; I look forward a great deal to working with you as a peer, someday in the future. Thank you to my advisory committee: John Kilgo, Gene Rhodes, and Richard Chandler who constantly and consistently improved this work; your support was invaluable. Those who worked with me in the field collecting data for many of these projects deserve special recognition: E.J. Borchert, Ellen Bledsoe, Beckie Juarez, and especially Kevin Eckert (who saved my bacon by coming back for a second season); the hard work that you put in was incredible, and at no point could I have asked to work with better folks. Josh Smith, Peter Schlichting, and Liz Kierepka, thank you not only for your help in the field or lab, but for your sage advice in all matters. Thank you to the other members of the Beasley Lab and the techs, graduate students, and post-docs of the Savannah River Ecology Laboratory; truly you are what makes SREL a great place. Additional thanks to numerous members of the US Forest Service and USDA APHIS National Wildlife Research Center for their support, and T. Mims, M. Larsen, M. Vukovich,, A.J. Davis, and K.M. Pepin in particular. Finally, thanks to the US Forest Service, USDA APHIS National Wildlife Research Center, Savannah River Ecology Laboratory, and US Department of Energy Office of Environmental Management for funding and financial support of these projects.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In the past few decades, populations of wild pigs (*Sus scrofa*), and wild boar from which they descend, have rapidly grown in geographic distribution and size throughout their introduced and native ranges (Bevins et al. 2014, Massei et al. 2015). In many regions of the world, including North America, wild pigs are considered an invasive or pest species due to the broad scope of negative impacts they can have on native and anthropogenic ecosystems (Bengsen et al. 2014). These impacts have been reviewed frequently in the published literature and include damage to ecosystems, disease transmission, and competition with and predation of native wildlife species (Campbell and Long 2009, Barrios-Garcia and Ballari 2012, Bengsen et al. 2014, Bevins et al. 2014, Keiter and Beasley in press). Wild pigs are often considered 'ecosystem engineers' as a result of their substantive influence on environments to which they are introduced (Boughton and Boughton 2014). In particular, wild pigs can greatly modify habitat through rooting, a behavior in which they till the soil (Barrios-Garcia and Ballari 2012). Rooting can reduce growth and diversity of plant species and/or facilitate establishment of invasive plant species (Barrios-Garcia and Ballari 2012).

With increases in wild pig density, there are increases in 1) damage caused to ecosystems through habitat degradation (Krull et al. 2016), 2) risk of transmission of infectious diseases to humans, livestock, or wildlife (Gortazar et al. 2006), and 3) risks to human safety due to the involvement of pigs in animal-vehicle collisions (Beasley et al.

2013, Sáenz-de-Santa-María and Tellería 2015). As such, robust estimates of wild pig population density are necessary to assess the risks posed by this species to humans, wildlife, and native ecosystems, and to establish causal relationships between density and impacts to better guide development of management objectives. Overall, knowledge of wild pig population density will allow improved evaluation of the effectiveness of control measures and refinement of control campaigns in addition to facilitating monitoring of changes in pig populations. Despite the importance of population density estimates for management and research of wild pigs, further investigation of techniques to estimate density is necessary, as current methods may vary in accuracy, precision, and feasibility.

Catch-per-unit-effort (CPUE) and harvest metrics are used by many management agencies, including those in the U.S., as an index to the relative abundance of wild pigs (Fernandez-Llario et al. 2003, Acevedo et al. 2007, Engeman et al. 2013). Indices such as those derived from track plots (Engeman et al. 2001), counts of scat (Acevedo et al. 2007), remote camera surveys combined with lethal removal (Bengsen et al. 2011), and spotlight surveys (Choquenot et al. 1993) also have been evaluated as possible methods of monitoring population change in wild pigs. These indices are assumed to vary with population size consistently, but may not meet this assumption because of variation in observers, across space or time, and within populations of animals, all of which can affect detectability (Slade & Blair 2000, Anderson 2001).

Recently, a number of density estimation techniques have been developed for or applied to wild pigs beyond traditional capture-mark-recapture (CMR) methods based upon live-trapping data. These techniques include the use of remote camera systems within a CMR framework (Sweitzer et al. 2000, Hebeisen et al. 2007), use of biomarkers combined with lethal removal (Reidy et al. 2011), fecal pellet group counts with known defecation rates (Plhal et al. 2014), an estimator based on changes in survival probabilities following pig removal (Hanson et al. 2008), and use of genetic samples from feces in CMR analysis (Ebert et al. 2012). These methodologies include both invasive (animals are captured and handled) and non-invasive (animals are not handled) techniques and may differ in their susceptibility to the effects of ecological processes (e.g. movement) or underlying ecosystem characteristics (e.g. true population density). Thus, it is important that investigation into the performance of combinations of field and analytical techniques are conducted to contrast their ability to accurately estimate density, although such assessments are rarely performed (Bellemain et al. 2005, Rodgers et al. 2014). The lack of accurate and precise estimates of wild pig population density has hindered management of this invasive species (Bevins et al. 2014), necessitating comparison of density estimators for wild pigs, to provide researchers and managers with appropriate tools to monitor this invasive species.

Density estimation techniques may also require species or taxa-specific refinement to increase accuracy, precision, or both of estimates. In particular, while use of genetic CMR holds promise for wild pigs (Ebert et al. 2012), one pervasive issue in performing non-invasive genetic techniques is obtaining an adequate number of samples. It is generally recommended that the number of samples collected be 2.5-3 times greater than the number of animals suspected to be present in the sampling area to allow robust estimation of population size through genetic CMR (Solberg et al. 2006, Puechmaille & Petit 2007). Collection of larger sample sizes (e.g. greater numbers of scat samples) should allow more precise estimation of population size, better detection of heterogeneity, and determination of differing capture probabilities among groups (Ebert et al. 2010). Research designed to optimize sampling methods to allow robust estimation of population size in wild pigs by genetic CMR is, therefore, necessary and may additionally benefit other research using field-collected fecal samples (Kohn and Wayne 1997). Investigation of factors affecting sample detection may also allow greater refinement of sampling protocols.

Beyond the lack of information regarding wild pig population density in discrete locations, research has highlighted a need for greater understanding of wild pig demographic rates, and more specifically juvenile survival rates (Toïgo et al. 2008, Mellish et al. 2014). The lack of information about survival rates of juvenile wild pigs, or piglets, has resulted in the use of expert opinion (e.g. Servanty et al. 2011) or exclusion of animals of younger age classes (e.g. Hanson et al. 2009) in the creation of population models for this species. Robust estimates of piglet survival rates are particularly important to population models, as research suggests that juvenile survival strongly influences the population trajectory of wild pigs and wild boar (Bieber and Ruf 2005, Servanty et al. 2011, Mellish et al. 2014). Therefore, development of techniques to estimate piglet survival rates is necessary to better develop population models for this species.

The work of Baubet et al. (2009) highlights the difficulty in monitoring piglet survival and the necessity of techniques to 1) determine when parturition occurred to allow tagging of neonates, and 2) monitor survival of juveniles. In particular, success of known-fate monitoring will depend on the retention of radio-transmitters by tagged individuals. Comparison of techniques to attach radiotransmitters to juvenile wild pigs will benefit future studies of piglet survival and cause-specific mortality, and thereby allow refinement of models of wild pig population dynamics; this refinement is necessary to improve our understanding and management of this invasive species.

The goals of this thesis are to fill the aforementioned knowledge gaps and develop tools that can be employed to better understand the population dynamics of wild pigs and other appropriate candidate species. Specifically, the first portion of this thesis (Chapter 2) has two objectives: 1) to compare a previously employed scat collection protocol to a series of novel fixed-area radial search techniques in terms of the amount of scat samples detected, and 2) to evaluate effects of habitat, weather, and scat characteristics on the detectability of scat by observers (Chapter 2, Keiter et al. 2016). Chapter 3 builds upon our knowledge of the efficacy of methodologies to estimate pig density by addressing the following objectives: 1) to evaluate the robustness of a suite of common density estimators to the effects of ecological and observational processes, and 2) to provide recommendations as to the aptness of each estimator under varying conditions based upon simulation results and field implementation (Chapter 3, Keiter et al. in review). Finally, within this thesis I compare a suite of radiotransmitter attachment mechanisms for use on wild piglets, and pilot the use of vaginal implant transmitters (VITs) in wild pigs (Chapter 4). The results of Chapter 2 will allow refinement of scat sampling protocols for a number of candidate species (e.g., social ungulates), and, thereby, potentially improve estimates attained from research employing field-collected fecal samples. Chapter 3 will provide researchers and managers with valuable information to inform selection of an appropriate density estimation technique for wild pigs and other species. The results of Chapter 4 can be applied to begin to generate robust estimates of piglet survival and cause-specific mortality. Overall, the tools and techniques developed in this thesis will allow refinement of population monitoring programs for wild pigs, an invasive species of global importance.

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CHAPTER 2

OPTIMIZATION OF SCAT DETECTION METHODS FOR A SOCIAL UNGULATE, THE WILD PIG, AND EXPERIMENTAL EVALUATION OF FACTORS AFFECTING DETECTION OF SCAT[1]

[1] Keiter, D. A., F. L. Cunningham, O. E. Rhodes Jr., B. J. Irwin, and J. C. Beasley. 2016. Optimization of scat detection methods for a social ungulate, the wild pig, and experimental evaluation of factors affecting detection of scat. PLoS ONE 11(5):e0155615. doi:10.1371/journal.pone.0155615.
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Abstract

Collection of scat samples is common in wildlife research, particularly for genetic capture-mark-recapture applications. Due to high degradation rates of genetic material in scat, large numbers of samples must be collected to generate robust estimates. Optimization of sampling approaches to account for taxa-specific patterns of scat deposition is, therefore, necessary to ensure sufficient sample collection. While scat collection methods have been widely studied in carnivores, research to maximize scat collection and noninvasive sampling efficiency for social ungulates is lacking. Further, environmental factors or scat morphology may influence detection of scat by observers. We contrasted performance of novel radial search protocols with existing adaptive cluster sampling protocols to quantify differences in observed amounts of wild pig (*Sus scrofa*) scat. We also evaluated the effects of environmental (percentage of vegetative ground cover and occurrence of rain immediately prior to sampling) and scat characteristics (fecal pellet size and number) on the detectability of scat by observers. We found that 15- and 20-m radial search protocols resulted in greater numbers of scats encountered than the previously used adaptive cluster sampling approach across habitat types, and that fecal pellet size, number of fecal pellets, percent vegetative ground cover, and recent rain events were significant predictors of scat detection. Our results suggest that use of a fixed-width radial search protocol may increase the number of scats detected for wild pigs, or other social ungulates, allowing more robust estimation of population metrics using noninvasive genetic sampling methods. Further, as fecal pellet size affected scat detection, juvenile or smaller-sized animals may be less detectable than adult or large animals, which could introduce bias into abundance estimates. Knowledge of

relationships between environmental variables and scat detection may allow researchers to optimize sampling protocols to maximize utility of noninvasive sampling for wild pigs and other social ungulates.

Introduction

Noninvasive sampling, such as the collection of fecal material, by definition, allows wildlife researchers to obtain information about a population of interest while minimizing potential disturbances [1]. Collection of fecal samples is one of the most frequently used noninvasive sampling techniques, and has been applied extensively in studies of animal diet [2], disease prevalence [3], endocrinology [4], phylogeography, genetics, and population ecology [5]. In particular, noninvasive genetic sampling is increasingly being used in a capture-mark-recapture framework to estimate population size or density, due to reduced impacts to target species and logistical or financial benefits over traditional capture-recapture approaches [6,7]. However, genetic capture-mark-recapture studies typically require large sample sizes because samples can experience high levels of DNA degradation [8,9]. Therefore, it is generally recommended that researchers collect 2.5–3 times as many samples as the suspected number of animals present in the sampling area to yield robust estimates of population size [10]. The difficulty in obtaining sufficient sample sizes of feces to estimate abundance may be further exacerbated in species with relatively low defecation rates (e.g. wild boar *Sus scrofa*; [11]). For these reasons, optimization of sampling protocols to maximize the number of sampled scats and efficiency of collection methods is needed to allow sampling of large spatial areas.

In many types of research utilizing feces, collected scat samples should be representative of the population of scats as a whole, which supports the use of random sampling techniques, such as transects. However, these techniques can be inefficient at encountering sufficiently large numbers of samples due to the non-random distribution of scats on the landscape [2]. For this reason, researchers frequently collect scat in locations where density of scat is likely to be high based upon the behavior of the target taxa. For carnivores, this is often along roads and trails [12,13] or at communal latrines sites [14,15]. While much research has been performed to optimize fecal sampling methods for carnivores [16,17], further optimization is necessary for social ungulates, where transect sampling methods may be inadequate to obtain sufficient sample sizes of scat [9]. Unlike carnivores, many ungulate species avoid roads [18] and do not form latrine sites (but see [19,20]), requiring additional effort to acquire sufficient sample sizes of scats. The deposition of scat by many herbivores may, however, be greatly influenced by the presence of social structures, such as aggregative behaviors.

In an effort to account for the social behavior of some ungulate species, Ebert *et al*. [21] modified transect sampling to include elements of adaptive cluster sampling [22] to increase the number of scats encountered in a genetic capture-mark-recapture study of wild boar. In their methodology, a 5-m radius was searched around each scat encountered along a linear transect and a new 5-m radius was searched for each subsequent scat encountered, until no further scats were detected [21]. This protocol, hereafter referred to as ACS, is intended to take advantage of the deposition of scat by groups of animals; however, the effectiveness and efficiency of ACS depends on the distribution of scat on the landscape. For instance, ACS will not detect scat falling beyond the defined search radius, and its sequential search structure could lead researchers along highly directional search paths, suggesting that an adaptive cluster sampling approach incorporating a single larger search radius could be more effective.

Beyond accounting for the distribution of scat on the landscape through sampling techniques, knowledge of factors that may affect detection of scat can aid researchers in the design of sampling protocols to maximize sample collection. Because humans primarily detect scat visually, as opposed to scat detection dogs, which primarily use olfaction, it is likely that some environmental characteristics, such as percentage of vegetative ground cover, affect the detectability of scat by increasing visual obstruction. Further, characteristics of the scat itself, such as size and number of fecal pellets, could affect detection of scat samples. Knowledge of these relationships is necessary for the development of an appropriate sampling design and may aid researchers in assessing whether sufficient samples can be collected to meet research objectives.

We selected wild pigs (*Sus scrofa*) as a model organism to evaluate the effects of sampling protocol, scat characteristics, and environmental attributes on detection rates of scats because pigs exhibit numerous characteristics representative of a number of non-carnivorous species to which these methods could potentially be applied. For example, this species exhibits relatively low defecation rates [9,11], and, like many non-carnivorous mammals, infrequently deposits scats on roads. Thus, fecal sampling for wild pigs can be challenging because of the low encounter and detection probabilities associated with their scat. Furthermore, similar to many other ungulates, wild pigs often travel in small groups [23], and frequently deposit scat in clusters, though they do not create latrine sites. As a practical matter, wild pigs inflict tremendous damage on native ecosystems and pose a risk to humans, livestock, and wildlife populations as a reservoir for transmissible diseases (see [24-26] for review), necessitating the optimization of fecal sampling techniques for use in estimating abundance of this invasive species.

Our objectives in this study were 1) to compare scat detection rates using the 5-m radius ACS search protocol to a novel series of fixed-area radial search protocols (5, 10, 15, and 20 m) in two habitat types suspected to contain different densities of wild pigs to elucidate differences in the number of fecal samples encountered by each method, and 2) to evaluate the effects of habitat, weather, and scat characteristics on the detectability of scat by human observers. We hypothesized that larger-sized radial searches (15 or 20 m) would result in higher rates of scat encounters than the ACS sampling technique in both the high and low wild pig density study sites and that scat detectability would decrease as the percentage of vegetative ground cover increased and the number of fecal pellets present (single vs. group of three) and size of fecal pellets decreased. The characteristics affecting scat detection measured in this study are widely applicable across ecoregions and species, allowing broad application of these relationships. Thus, this information will provide researchers with a foundation to optimize sampling protocols for future studies requiring the collection of scats in social ungulates.

Methods

Study Area

We compared search protocols to detect scat at the Savannah River Site (SRS), a 78,000 ha United States Department of Energy facility on the Georgia-South Carolina border (33°20'N, 81°44W) in the Upper Coastal Plain physiographic region of the United States. Approximately 68% of habitat on the SRS consists of upland pine (Fig 2.1a.), mainly loblolly pine (*Pinus taeda*), long-leaf pine (*P. palustris*), and slash pine (*P. elliotii*) managed by the United States Forest Service [27,28]. Common understory plants in upland pine habitat at the SRS include broom sedge (*Andropogon virginicus*), bracken fern (*Pteridium aquilinum*), poison oak (*Toxicodendron pubescens*), deerberry (*Vaccinium stamineum*), sparkleberry (*Vaccinium arboreum*), wax myrtle (*Morella cerifera*), sweetgum (*Liquidambar styraciflua*), and scrub oaks (*Quercus* spp.) [28]. An additional 22% of the SRS consists of swamp and riparian bottomland habitat (Fig 2.1b.) dominated by water oak (*Quercus nigra*), tulip-poplar (*Liriodendron tulipifera*), sweetgum, and maple (*Acer* spp.; [28,29]). Understory plants in the bottomland hardwood area include switchcane (*Arundinaria tecta*), redbay (*Persea palustris*), shining fetterbush (*Lyonia lucida*), American holly (*Ilex opaca*), and dwarf palmetto (*Sabal minor*) [28]. Greater detail on the vegetative communities associated with these habitat types can be found in [28]. Elevation of the SRS ranges from 30–115 m above sea level. When the SRS was closed to the public in 1952 and resident farmers moved offsite, large numbers of domestic pigs remained. These animals since reverted to a feral state and expanded in abundance and distribution throughout the area [30], and today this population shows signs of introgression by wild boar genetics; for this reason we refer to them as wild pigs [31]. Previous research has suggested that wild pigs prefer bottomland hardwood habitat to upland pine habitat at the SRS [29,30], leading us to suspect that wild pig densities are different between these habitat types.

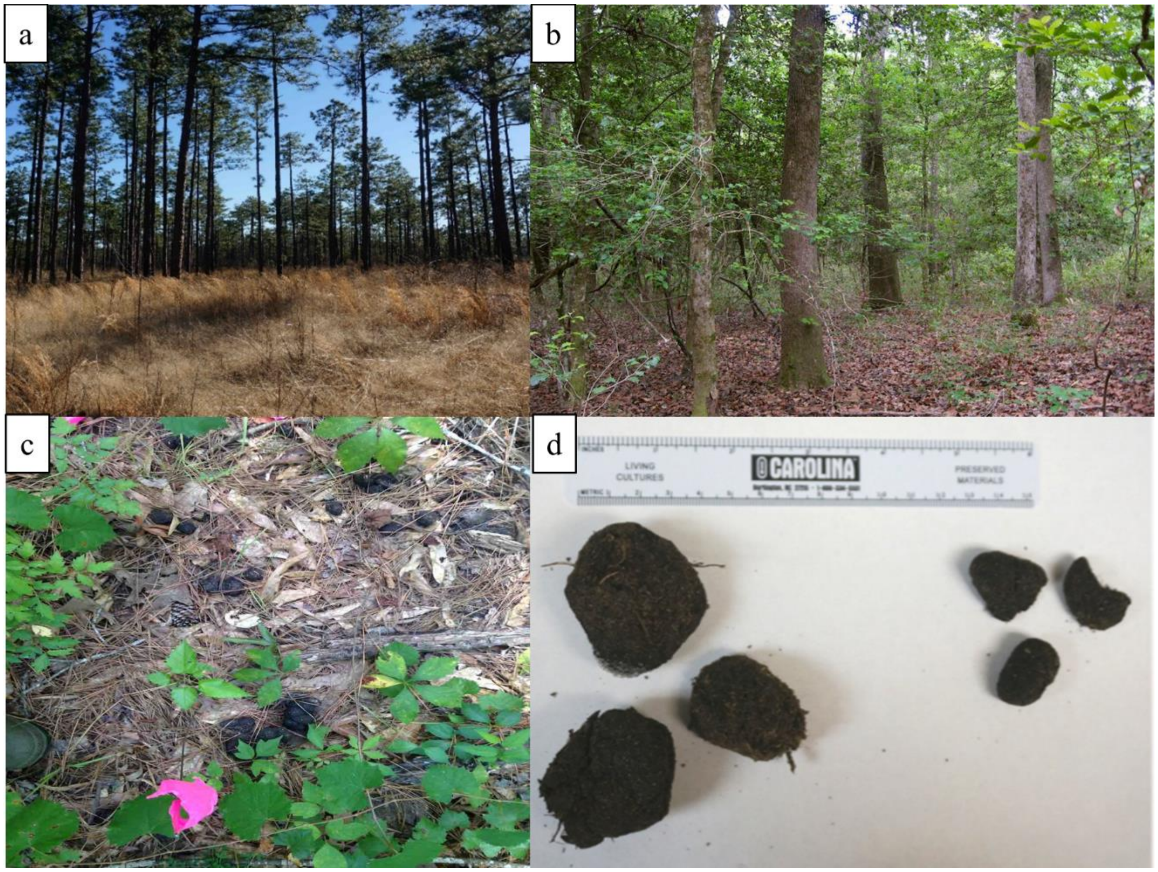

Fig 2.1 Examples of common habitat types of the Savannah River Site, South Carolina, USA, and wild pig feces. Examples show (**a.**) upland pine habitat, (**b.**) bottomland hardwood habitat, (**c.**) *in situ* wild pig (*Sus scrofa*) scat and vegetative ground cover, and (**d.**) wild pig scat distinguished into large (left) and small (right) size categories for experimental evaluation.

Additional experimental trials to evaluate detection rates of scat were conducted at Whitehall Forest, a 304 ha property owned by the University of Georgia, D.B. Warnell School of Forestry and Natural Resources. This property is near the city of Athens in the Piedmont region of Georgia (33°56’N, 83°24’W), USA. The upland portion of Whitehall forest where this research took place is characterized by loblolly pine, shortleaf pine (*P. echinata*), oak (*Quercus* spp.), sweetgum, and hickory (*Carya* spp., [32]). Understory vegetation in Whitehall Forest is similar to that described in [33] and includes sweetgum, oak, muscadine (*Vitis rotundifolia*), and wingstem (*Verbesina alternifolia*).

Data Collection

Search Protocol Comparisons

We conducted scat sampling along 22 transects at the SRS from 14 July – 7 August, 2014. Transects were spaced approximately 0.5 km apart and oriented roughly north to south. We used a handheld GPS unit to mark a beginning and ending point for each transect on a pair of parallel roads in the habitat type of interest, and searched the area between the two points for wild pig scat. Researchers were able to search a width of approximately 3 m: 1.5 m on either side of each transect. In bottomland hardwood habitat, we sampled 20.8 km of transects with an average length of 2.97 km per transect (SE = 0.60). In upland pine habitat, we sampled 36.9 km of transects with an average length of 2.46 km per transect (SE = 0.15).

Each time we encountered scat on a transect, we sequentially applied each of the five search protocols being compared. The first protocol used was the ACS method employed by Ebert *et al*. [21] in which we searched a 5-m radius around the initial scat encountered on the transect and then searched a 5-m radius around each additional scat encountered within the 5-m radius, and each subsequently encountered scat, until no further scat were found. The remaining protocols each consisted of a single search radius (either 5, 10, 15, or 20 m), centered around the scat detected within the original search window (approximately 3 m) along the main transect. These single search protocols did not include additional radii around scat subsequently found off-transect within the search radius. We marked each scat encountered with a survey flag to prevent double counting within each protocol and recorded the distance and bearing of each additional scat found to the initial scat encountered. As all five sampling protocols were applied each time a scat was found, we assumed that the vegetative cover would remain relatively constant across the protocols at each scat cluster (i.e. would not vary consistently between a 15 m and a 20 m radius centered on the same point), and therefore would not impact comparison of protocol effectiveness. For this reason we did not measure the percentage of vegetative ground cover at each encountered scat in this portion of the study.

Detection Probabilities of Scat

To estimate detection rates of scat, we created four 100 m transects in mixed pine-hardwood habitat at Whitehall Forest. Transects were spaced approximately 25 m apart, delineated by marked survey flags placed at 5 m intervals, and oriented roughly southeast to northwest. Wild pigs are not established at Whitehall Forest, so we added previously collected wild pig scat from the SRS to form experimental transects with known scat locations. The overall location for these transects was selected for its variable amounts of vegetative cover in order to test for an effect of percent vegetative ground cover on the probability of scat detection (Fig 2.1c.).

We visually separated previously collected wild pig scats into 2 size classes (SIZE): small and large (Fig 2.1d.), because previous research has demonstrated that scat from smaller species may be less detectable than that of larger species [34]. To ensure that our visual classification represented distinct size classes, we measured a sub-sample of 10 randomly selected fecal pellets from each to determine an average volume for fecal pellets of the small ($\bar{x}$ = 5.98 cm^3, *SE* = 1.17) and large size class ($\bar{x}$ = 27.79 cm^3, *SE* = 2.06). Experimental scat treatments were created by dividing fecal pellets of the 2 size classes into either single (consisting of one fecal pellet) or group categories (consisting of 3 fecal pellets; NUMB). We chose to use 3 fecal pellets for the group category, as this number was determined to reasonably represent conditions we observed in the field based on pilot studies conducted on the SRS. We placed 4–5 scats of each of the 4 treatments (small-single, small-group, large-single, large-group) at randomly generated distances along each transect for a total of 18-20 scat locations per transect. We randomly assigned each scat to be placed within 1 m to the left or right of each transect to ensure that all placements were within the estimated effective search distance for pig scat along the transect. We used a 1 m^2 area framed by PVC pipe and gridded into 100 equal-sized cells to estimate the percentage of vegetative ground cover (COVER) present at each location that a scat was placed.

From 17–20 November, 2014, 56 student volunteers, from a University of Georgia wildlife techniques course, sampled the four constructed transects. Each volunteer only walked an individual transect once and recorded each distance at which they detected a scat. This resulted in a binomial capture history for every known scat location consisting of its detection or non-detection by each observer that sampled each transect. Before sampling began each day, we walked transects and replaced any missing scats with scats of the same treatment type. Prior to sampling, volunteers were instructed that number and size of fecal pellets might vary and were shown examples of wild pig scat. None of the volunteers had prior experience searching transects for scat. One of the sampling periods occurred immediately following a rainstorm, so we incorporated rain prior to sampling (RAIN) as an additional predictor variable (event categorized as a 1 for rain and a 0 for non-rain). No permits were required for this work as researchers and volunteers did not come into contact with live or dead animals.

Data Analysis

Search Protocol Comparison

The negative binomial distribution is often appropriate for modeling non-negative, discrete count data [35], and thus, we developed a linear mixed model, in which the observed scat counts followed a negative binomial distribution. Sampling protocol (i.e., ACS and 5-, 10-, 15-, and 20-m radial searches) was included in the model as a categorical fixed-effect predictor variable to determine whether significant differences existed in the number of scat found among the different search protocols. We also evaluated a model incorporating the broad category of habitat type as an additional fixed effect to determine whether habitat type affected the number of scats found. Individual transects were treated as normally-distributed random effects in each of these models. Analyses were conducted using packages *lme4* and *glmmADMB* in R [36]. We judged the relative support of models using the second order Akaike's Information Criterion (AICc) and AIC weight ($AICw_i$), a measure of model likelihood [37]. If models were ≤ 2.0 AICc units from the best model, we considered them to be supported [37] unless they were judged to contain an uninformative parameter [38]. A model with an uninformative parameter is defined as being within 2.0 AICc units of the best model, with only one additional parameter and a similar model deviance [38].

Detection Probabilities of Scat

We created 5 *a priori* models (Table 2.1) to predict the probability of detecting or failing to detect scat, based on expected relationships between our predictor variables of interest (percent vegetative ground cover, scat pellet size, number of fecal pellets, and whether rain occurred immediately prior to sampling) and the observed response variable (detection or non-detection of wild pig scat). In these models, the predictor variables listed above were included as fixed effects, while observer and transect were held as random effects. The collected observations of "failure" and "success" of detecting scat were assumed to be binomially distributed. We used the same information-theoretic metrics as above to compare these 5 candidate models. All data and code are available online (S2.1-S2.4).

Table 2.1 Model selection results for *a priori* models relating probability of detecting wild pig scat to predictor variables, Whitehall Forest, Georgia, USA, 2014.

Model	K[a]	AICc	ΔAICc	w_i	-LL[b]
NUMB[c]+SIZE[d]+COVER[e]+RAIN[f]	7	4852.42	0.00	0.95	-2419.19
SIZE+COVER+RAIN	6	4858.57	6.15	0.04	-2423.27
NUMB+SIZE+COVER	6	4865.86	13.44	0.00	-2426.92
NUMB+SIZE	5	4875.76	23.34	0.00	-2432.87
NUMB	4	4982.13	129.71	0.00	-2487.06

Models are ranked by change in second order Akaike's Information Criterion (ΔAICc) and AIC weight (w_i),
[a] Number of parameters including two random-effect variances (Observer and Transect) and a global intercept term
[b] Negative log-likelihood
[c] Number of fecal pellets (single or group)
[d] Size of fecal pellets (small or large)
[e] Percentage of vegetative ground cover
[f] Rain event immediately prior to sampling (event or non-event)

Results

Search Protocol Comparison

In total, we encountered 35 scats in 8 clusters in the upland pine habitat and 467 scats in 33 clusters in the bottomland hardwood habitat of the SRS. Thus, we found a higher average scat density in the bottomland hardwood habitat ($\bar{x}$ = 73.71 scats/km, *SE* = 48.54) than in upland pine habitat ($\bar{x}$ = 0.94 scats/km, *SE* = 0.41). The negative binomial mixed model of the number of scats encountered that incorporated habitat type and sampling protocol as fixed effects was more supported (**Δ**AICc = 0.00, AICw_i. = 0.80) than the model that did not incorporate habitat type (**Δ**AICc = 2.58, AICw_i = 0.20), suggesting that the efficacy of sampling differs between habitat types. Our most supported model indicated that fewer scats were found in upland pine than bottomland hardwood habitat, as we hypothesized (Fig 2.2). We believe that this result was likely due to differing use of habitats by wild pigs (Kurz and Marchinton 1972), but it is possible that detectability of scat in the two habitat types differed as well.

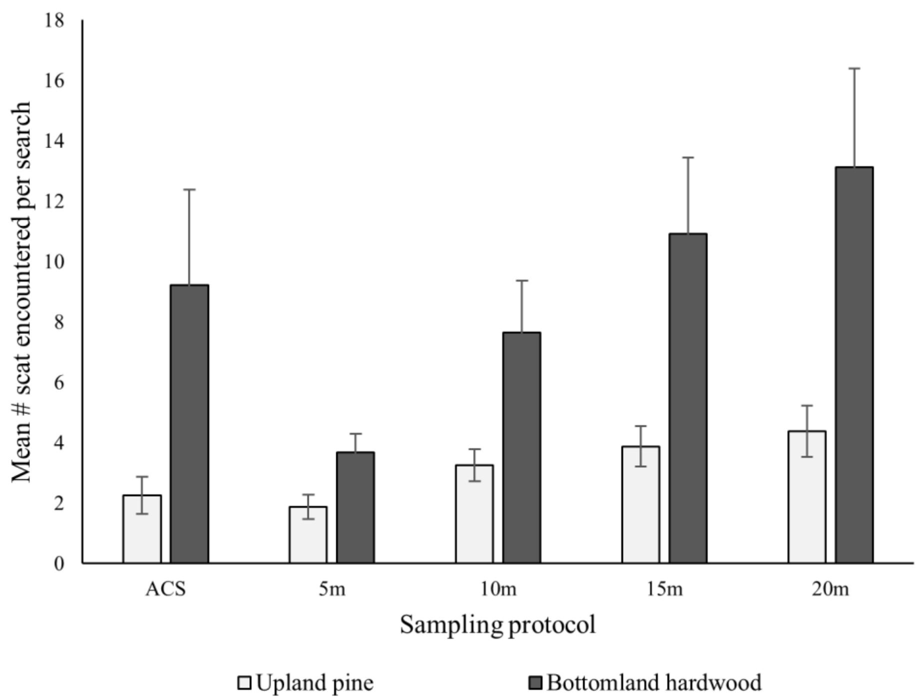

Fig 2.2 Amount of wild pig scat encountered by observers along transects at the Savannah River Site, South Carolina, USA, 2014. Depicts mean number of scats detected per cluster by the adaptive cluster sampling (ACS) scat sampling protocol and 5-, 10-, 15-, and 20-m radial search protocols in bottomland hardwood (BH) and upland pine (UP) habitat. Error bars represent one standard error.

The ACS method ($\bar{x}$ = 7.85, z = 6.79, SE = 2.58) resulted in a larger number of detected scats than a 5-m radial search ($\bar{x}$ = 3.32, SE = 0.52, z = -2.83, P < 0.010), but no difference in mean count was detected between ACS and the 10-m radius search ($\bar{x}$ = 6.78, *SE* = 1.41, *z* = 0.46, *P* = 0.648). Significantly more scats were encountered using the 15- and 20-m radius searches ($\bar{x}$ = 9.54, *SE* = 2.08, *z* = 2.33, *P* = 0.019 and $\bar{x}$ = 11.41, *SE* = 2.69, *z* = 3.20, *P* < 0.010 respectively, Fig 2.3, Table 2.2) than for ACS. As might be expected, the amount of time required to implement a single search increased with the amount of area searched (e.g. more time was needed to search a 10 m radius than a 5 m radius). When the amount of area searched is not fixed, as is the case with the ACS protocol we tested, it is not possible to compare the amount of time a search will take to fixed area protocols, as the time required varies depending on the distribution of scats and the amount of spatial overlap between searches.

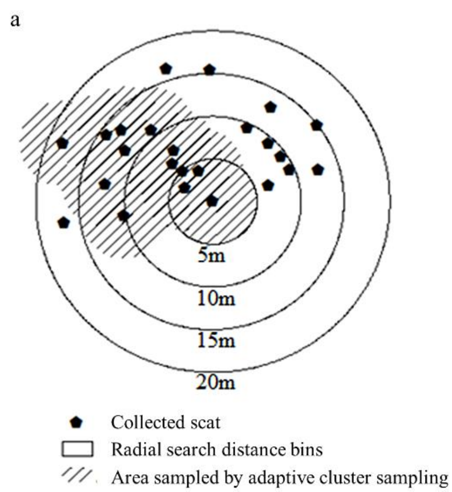

Fig 2.3 Illustration of tested scat sampling protocols. Diagrams illustrate differences in the number of wild pig scats encountered by the adaptive cluster sampling (ACS, shown as shaded area) protocol and 5-m, 10-m, 15-m, and 20-m radial search protocols in areas of high (a.) and low density (b.) of scat. Diagrams represent actual spatial distributions of scats observed in bottomland hardwood habitat at the Savannah River Site, South Carolina, USA, 2014.

Table 2.2 Parameter estimates for the best model comparing scat sampling protocols at the Savannah River Site, South Carolina, USA, 2014.

Parameter	β	SE	95% CI
Reference (adaptive cluster sampling, bottomland hardwood habitat)	1.845	0.272	1.32 to 2.38
5-m radius	-0.533	0.188	-0.90 to -0.16
10-m radius	0.080	0.176	-0.26 to 2.18
15-m radius	0.401	0.172	0.06 to 0.74
20-m radius	0.545	0.17	0.21 to 0.88
Upland pine habitat	-0.918	0.389	-1.68 to -0.16

Table reports parameter estimates (β), standard errors, and 95% confidence intervals for the parameters of a negative binomial mixed model of the effects of habitat type and sampling protocol on the number of wild pig scats detected.

Detection Probabilities of Scat

Each of the 4 experimental transects was sampled on average 31.3 times (SE = 0.18) by volunteers. Among the 56 volunteers, the percentage of scats detected on a single transect was highly variable, ranging from 5.2% – 85.0%. The most supported model of scat detectability revealed that the percentage of vegetative ground cover, scat pellet size (small or large), number of fecal pellets (single or group of three), and whether rain occurred prior to sampling (event or non-event) were important predictors of scat detection (ΔAICc = 0.00, AICw_i = 0.95, Table 2.1, Table 2.3). As percent ground cover increased, the probability of detecting scat decreased (Fig 2.4, Table 2.3). Smaller-sized scats had a lower detectability than larger-sized scats, and scat groups had higher detectability than single pellets (Table 2.3). Rain prior to sampling also noticeably reduced the probability of scat detection by observers (Fig 2.4, Table 2.3). As might be expected, the estimated remaining variability among the four replicate transects (0.034) was small relative to the estimated variability among volunteer observers (0.526).

Table 2.3 Parameter estimates for the best model of factors affecting detectability of wild pig scat at Whitehall Forest, Georgia, USA, 2014.

Parameter	β	SE	95% CI
Reference (large, group, ground cover = 0, no rain)	1.776	0.172	1.439 to 2.113
Single Pellet	-0.204	0.071	-0.343 to -0.065
Small Size	-0.755	0.072	-0.896 to -0.614
Ground Cover	-0.008	0.002	-0.012 to -0.004
Rain	-0.916	0.216	-1.339 to -0.493

Table reports parameter estimates (β), standard errors, and 95% confidence intervals for the best binomial mixed model of probability of scat detection as a function of fecal pellet number, pellet size, percent vegetative ground cover, and whether rain occurred prior to sampling.

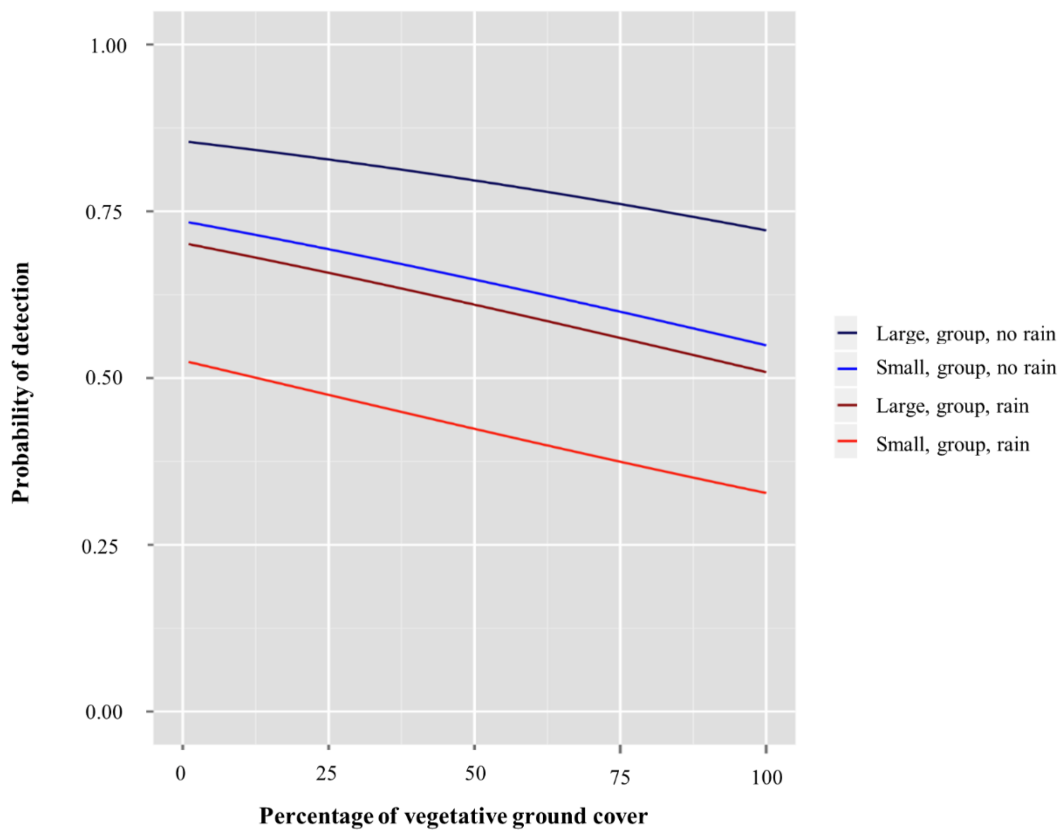

Fig 2.4 Relationships between environmental and scat characteristics and detectability of wild pig scat, Whitehall Forest, Georgia, USA, 2014. Graph depicts predicted probability of detection for groups of wild pig scat as a function of percent vegetative ground cover, fecal pellet size, and whether rain occurred immediately prior to sampling.

Discussion

In previous noninvasive genetic studies of *Sus scrofa* and other mammals, obtaining a sufficient sample size of feces has been a primary limiting factor to developing robust mark-recapture estimates [9, 39]. Sample size also can limit possible inferences of research on diet, disease prevalence, endocrinology, and other investigations utilizing scat. We demonstrated that use of a fixed radius (15 or 20 m) sampling area around the initial scat encountered along a transect was more effective in increasing sample size than the ACS protocol previously used for the collection of samples to estimate the population size of *Sus scrofa* [21]. In theory, ACS could encounter more scat than a fixed radial search in areas of uniformly high scat density, although such situations likely occur infrequently in nature. Moreover, the area searched by ACS is entirely dependent upon the spatial arrangement of scat present because every encountered scat prompts a new area to be searched, which could lead to an exceptionally large area sampled by this method, and, therefore, a large time required for a single search. Despite this, our fixed radial search method was more effective in encountering scat samples than ACS, even in areas of high scat density (i.e., 73.71 scats/km in bottomland hardwood habitat). Therefore, a radial search method as applied in this study appears to result in an increased sample size of collected scats for wild pigs, increasing the probability of successful estimation of abundance or density. Further exploration into these methods may be warranted for other social species.

Our research also revealed that both scat size and number of fecal pellets present affected detection of scat by observers. Juvenile wild pigs generally produce smaller scat than adults, and if smaller scats are less detectable, as demonstrated in this study, and the difference is not accounted for, biased detection rates could result in inaccurate estimates of population size. Though we tested only two specific size categories of scat, we expect that the relationship between probability of detection and scat size might vary more generally, similar to that of carnivore species [34]. Likewise, individual animals may generally be less detectable by scat surveys than those traveling in groups, as an individual animal will produce fewer fecal pellets than a group of animals. These effects of scat size and social structure on detectability could reduce accuracy in estimates of population size when using genetic capture-mark-recapture methods, but could be accounted for in the development of mark-recapture models.

As we hypothesized, increased vegetative ground cover resulted in decreased detection of scats by observers, most likely as a result of visual obstruction. This suggests that when possible, researchers may want to design surveys to take advantage of habitat types and seasons in which ground cover will be reduced to maximize the collection of fecal samples. Rain immediately prior to sampling also reduced the probability of an observer detecting scat. Research has demonstrated that the type of substrate present can affect detectability of animal sign by observers [34,40]. It seems likely that, in this study, rain reduced visual contrast between the scat and local substrate, diminishing the detectability of scats by observers. Exposure to rainfall and wet conditions also decreases the chances of successful genetic analysis of fecal samples [41,42]. Therefore, researchers might avoid sampling immediately following rain to maximize the number and quality of scats collected.

Overall, our fixed radius method was able to detect more fecal samples than ACS, when both methods were applied to the same locations. Increased sample collection may aid in improving abundance estimates of wild pigs and other social mammals through increased accuracy and precision and reduced bias [6,43]. Higher capture probabilities, which often result from increased sample sizes, also allow better detection of individual heterogeneity in abundance estimation [44]. Many species exhibit defecation patterns similar to wild pigs in that their defecation on roads is infrequent and they do not habitually create and use latrine sites, thereby eliminating two common sources of scat samples [2]. Therefore, search protocols such as those outlined in this paper should be useful for improving the performance of scat surveys for many social ungulates, in which sampling of roads or latrine sites is generally insufficient or infeasible. Knowledge of the relationships between scat detectability by observers and environmental and scat characteristics should be used in conjunction with information about the behavioral ecology of the taxa of interest to develop taxa-specific sampling protocols to meet targeted sample sizes.

Acknowledgments

We thank the volunteers in the fall 2014 Wildlife Techniques course at the University of Georgia who assisted with this research, R.B. Chandler for feedback, and L. Lee for her photograph of bottomland hardwood habitat at the Savannah River Site. All other photographs were provided by D. Keiter. The Georgia Cooperative Fish and Wildlife Research Unit is sponsored jointly by the U.S. Geological Survey, the Georgia Department of Natural Resources, the U.S. Fish and Wildlife Service, the University of Georgia, and the Wildlife Management Institute. This paper was prepared as an account of work sponsored by an agency of the United States Government. Neither the U.S. Department of Agriculture, nor any of its employees, makes any warranty, express or

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Supporting information and appendices

See version published in PLoS ONE, 2016

S2.1 Data for comparison of scat sampling protocols at the Savannah River Site, Aiken, South Carolina, USA, 2014.

S2.2 Data for evaluation of factors affecting detectability of wild pig scat, Whitehall Forest, Georgia, USA, 2014.

S2.3 R code for comparison of scat sampling protocols.

S2.4 R code for evaluation of factors affecting detectability of wild pig scat.

CHAPTER 3

IMPACT OF ANIMAL MOVEMENT ON COMMON METHODS OF ESTIMATING POPULATION DENSITY[2]

[2]Keiter, D. A., A. J. Davis, O. E. Rhodes Jr., F. L. Cunningham, J. C. Kilgo, K. M. Pepin, and J. C. Beasley. Submitted to *Scientific Reports, 09/06/16.*
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Abstract

Knowledge of population density is necessary for effective management and conservation of wildlife, yet rarely are estimators compared in their robustness to the effects of ecological and observational processes which can greatly influence accuracy and precision of density estimates. Field-based comparison of density estimators and assessment of conditions affecting their performance is necessary to provide managers and researchers with appropriate tools to monitor wildlife populations. In this study, we simulated data under different assumptions surrounding biological and observational processes using empirical data to assess effects of animal movement, true population density, and probability of detection on accuracy and precision of common density estimators. We also compare three common data collection techniques (camera traps, biomarker bait, and live-trapping and euthanasia) and a suite of analytical techniques (Lincoln-Petersen estimators, spatially explicit capture-recapture [SECR] models, and Bayesian hierarchical removal models) in their ability to estimate density of a globally widespread species *Sus scrofa*, the wild pig, at three study sites. Based upon our results, we provide recommendations on when each density estimator is most applicable and appropriate. We found that animal movement had the greatest impact on accuracy of density estimators, although all estimators suffered reduced performance when detection probability was low. Lincoln-Petersen estimators were relatively imprecise, particularly at low movement and detection rates. Data requirements were highest for SECR models, but when data were sufficient these techniques exhibited fairly high accuracy and precision. Removal models were most effective when population density was high and site-specific information was available to estimate the effective area sampled. Field

implementation of estimators revealed specific drawbacks and advantages to each, although simulations suggested that field estimates might be relatively accurate. The large influence of movement parameters on accuracy of estimators emphasizes the importance of effective post-hoc calculation of effective area sampled or use of methods that implicitly take spatial variation into account. In particular, low movement rates negatively affected estimators, emphasizing the necessity of appropriate sampling design to effectively measure movement parameters and thereby reduce bias in estimates. The field and analytical techniques tested herein can be broadly applied to a number of candidate species and sampling situations.

Introduction

Knowledge of population density is essential to the field of wildlife ecology, providing a foundation for effective planning of management and conservation and for basic ecological research. As such, numerous density estimators have been developed for broad and species- or situation-specific use (see Williams et al. 2001, Pierce et al. 2012 for review). These estimators are likely to differ in their sensitivity to the effects of 1) ecological processes, such as animal movement, 2) observational processes, such as baseline detection rates, 3) ecosystem characteristics, such as underlying population density, and 4) the interactions of these factors. For this reason, evaluation of potential impacts of these processes on accuracy and precision of estimators is necessary.

Density, or abundance per unit area, is often the parameter of interest in wildlife studies, as it allows comparison among studies which might not have sampled the same size area and provides spatial context to resulting information (Rich et al. 2014, Royle et al. 2014). However, there are many challenges inherent to estimating density of wildlife populations. A well-established issue is variability in the observational process resulting in a detection probability (p) of <1.0; thus, jointly estimating detection with density is necessary (Mills 2007). One of the simplest estimators to account for imperfect detection of animals is the 2-sample Lincoln-Petersen estimator (LPE) which uses a ratio of marked to unmarked animals to estimate $\hat{p}$ and abundance (Seber 1982). More complex capture-mark-recapture (CMR) estimators that explicitly model covariates affecting $\hat{p}$ and thereby refine estimates of density or abundance have since been developed (Pollock 1991, Mills 2007), as have non-CMR methods, such as Bayesian hierarchical removal models (e.g. Davis et al. 2016). Many of these techniques, however, do not explicitly account for movement of animals in estimating animal abundance. Conversion of resulting estimates of abundance to density, an inherently spatial metric, requires some knowledge of animal movement to determine the area to which inference about populations can be applied. Animal movement may greatly affect abundance estimates through changes in the availability of an animal to be sampled (i.e. an animal that is only present on a sampling grid for a short duration of the sampling period may not be detected as readily or frequently as one that is present on the sampling grid at all times; Lewis et al. 2015). This lack of geographic closure caused by animal movement is often addressed through ad-hoc estimation of the effective area sampled by a particular data collection method.

One common technique to determine the effective area sampled by abundance estimators is to buffer the convex polygon of the sampling grid by the mean maximum distance moved (MMDM) or half mean maximum distance moved (HMMDM) by animals during the study period (Wilson and Anderson 1985, Ivan et al. 2013). In contrast to post-hoc calculation of effective area sampled, spatially explicit capture-recapture (SECR) models allow for direct inference on effective sample area by accounting for spatial variability in the detection process, potentially resulting in improved inference about density of wildlife populations over traditional capture-recapture approaches (Royle et al. 2014). Recent research has compared spatially explicit density estimators with non-spatial estimators (i.e. those that use an ad-hoc approach to estimate the effective area sampled; Obbard et al. 2010, Sharma et al. 2010, Sollmann et al. 2011, Gerber et al. 2012, Noss et al. 2012, Blanc et al. 2013, Gerber and Parmenter 2015, Jůnek et al. 2015), but few studies have evaluated the accuracy and precision of tested estimators (but see Sharma et al. 2010, Gerber and Parmenter 2015, Jůnek et al. 2015). Further, while studies frequently compare analytical techniques in their ability to estimate population density without bias, a greater understanding of the effects of animal movement, an underlying mechanistic ecological process, on estimator performance is necessary to guide estimator choice. In addition to animal movement, population and environmental characteristics can affect overall detection rates, thereby influencing accuracy and precision of density estimators. Evaluation of both the effects of these processes and their interactions with animal movement on accuracy and precision of common density estimators and field-based comparison of density estimation techniques will allow researchers and managers to choose the most appropriate and applicable density estimator for their research conditions.

Wildlife population estimation methods have evolved over the last several decades to meet the challenges inherent in monitoring natural systems. Data collection for these methods may be noninvasive, in which capture and handling of animals is not required, potentially minimizing disturbance to populations (Taberlet et al. 1999); or invasive, in which animals are captured. Common noninvasive methods include use of camera traps (Noss et al. 2012), fecal pellet counts (Plhal et al. 2014), and collection of hair or scat for genetic testing (Taberlet et al. 1999). Analytical techniques such as mark-resight (Arnason et al. 1991) and genetic CMR (Lukacs and Burnham 2005) have been developed to estimate density from noninvasive data. Examples of invasive data gathering techniques include live-trapping and marking for analysis in a CMR framework (Pollock 1991), use of biomarkers and a recapture event (Reidy et al. 2011) for analysis by LPEs, and lethal removal or harvest of animals for analysis by removal models (Zippin 1958). Each data collection technique has unique advantages and disadvantages that may make it more or less susceptible to effects of ecological processes and underlying ecosystem characteristics, yet it is rare that multiple combinations of field and analytical techniques are compared in their ability to estimate density (Bellemain et al. 2005, Rodgers et al. 2014).

In this study, we had two objectives: 1) to evaluate the robustness of a suite of common density estimators to changes in animal movement, underlying population density, probability of detection, and the interactions between these processes, and 2) to provide recommendations as to when application of each estimator is appropriate based upon simulation results and the observed practicality and feasibility of field implementation of each. We accomplish these objectives by employing common invasive and noninvasive field techniques and a suite of analytical techniques to estimate population density of a globally widespread species *Sus scrofa*, the wild pig, at three study sites (Table 3.1), and using the gathered data to parameterize simulations for evaluation of estimator robustness to changes in ecological and observational processes.

Materials and Methods

Study Species

Wild pigs (*Sus scrofa*) and wild boar, from which they are descended, are found on every continent except Antarctica (Long 2003). This species is often harvested recreationally and lethally controlled in locations where it is invasive. There is well-recognized bias in capture probabilities of different demographic components of wild pigs through conventional trapping (Williams et al. 2011) and it is likely that movement rates of this species differ between habitat types.

Study Area

We conducted this research at the Savannah River Site (SRS), a 78,000 ha United States Department of Energy (DOE) facility on the border of South Carolina and Georgia (33°20’N, 81°44W; Fig. 3.1). Approximately 68% of habitat at the SRS consists of upland pine, while an additional 22% is comprised of swamp and riparian bottomland habitat (described in Imm and McLeod 2005). Additional areas, hereafter mixed habitat, are dominated by upland pine, but include riparian habitat. We selected a study site in each of these three broad habitat matrices (i.e. bottomland hardwood, upland pine, and mixed habitat) to test density estimators under varying field conditions. Populations of wild pigs on the SRS have grown recently as evidenced by increasing incidences of pig-vehicle collisions and numbers of individuals culled by U.S. Forest Service contractors (Beasley et al. 2013).

Field Methods

Within each study site (bottomland, mixed, upland), we applied three common field techniques to gather data. These techniques were 1) individual identification of animals using camera traps and natural marks, 2) use of a biomarker bait to mark individuals for capture-recapture analysis, and 3) application of trapping and lethal removal. Camera trapping and biomarkers were simultaneously applied in each study site prior to live-trapping. Each of the combined field and analytical methods we evaluated was self-contained (i.e. did not require capture and marking of individuals prior to implementation or gathering of external data, such as telemetry). Table 1 provides an overview of how field data fed into the analytical methods tested.

We established a 5 x 4 grid of white-flash trail cameras (Scoutguard SG565FV, HCO Outdoor Products, Norcross, USA; Fig. 3.1) in each study site. We placed cameras along transects 750 m apart (±75 m) in locations that would maximize the probability of animal detections based upon local habitat conditions or evidence of pig presence (e.g. rooting, scat, etc.). Cameras were set on motion triggers, with a 3-minute delay between trigger activation, and programed to take 3 pictures, 5 seconds apart, when triggered. We baited cameras with corn treated with Rhodamine B (RB), a biomarker that can be used for “batch-marking” individuals prior to removal efforts (described in Appendix S3.1 in Supporting Information; Beasley et al. 2015). Camera traps were active for 12 days in the upland and mixed study sites, and 13 days in the bottomland study site. We identified individual animals using unique combinations of pelage, scars, and association with other individuals from camera photos (Fig. 3.2). To create individual capture histories, each 24-hour period a camera was active defined a capture occasion. Using camera trap data, we determined whether each individual pig was likely to be marked by RB through evaluation of the amount of time it spent consuming treated bait and its estimated weight (Appendix S3.1). We assessed accuracy of our classifications of animals as “marked” or “unmarked” based upon their consumption of RB by determining how many individuals that were judged marked were not marked based upon whisker analysis (i.e. false positives), and how many animals thought to be unmarked were marked according to whisker analysis (i.e. false negatives).

Following camera trapping, we created a grid of 1 km^2 cells in each study site, and placed 10 corral traps (1 trap per grid cell) in areas with recent pig activity or in what was judged to be the best habitat if no fresh activity was found (Fig. 3.1). We pre-baited traps with whole corn for three days and live-trapping occurred for 14 days in each study site (i.e. 140 trap-nights per habitat type). To account for effort using traps, we recorded each occasion a trap was triggered without successfully catching a pig; these occasions were generally the result of a non-target species activating the trigger. Captured pigs were euthanized via cranial gunshot (University of Georgia IACUC permit A2015 05-004-Y). We collected 8-10 whiskers from each captured pig for use in analysis of RB consumption, and photographed each animal with a digital camera to allow identification of pigs that had previously visited camera traps. Whiskers collected from captured pigs were prepared for analysis according to the methods described in Beasley et al. (2015). Further detail on the implementation of field protocols is available in Supporting Information (Appendix S3.1).

Analytical Methods

Data sources for each analytical technique, method of implementation, and basic citations are in Table 3.1. We excluded individuals ≤ 20 kg from all analyses, as piglets travel with older individuals (as in Hebeisen et al. 2008), and would violate the independence assumption inherent in the estimation methods we used.

We assumed demographic and geographic closure existed among adult animals in each study site, as this study was conducted in a short time period (~1 month). Human harvest is frequently the largest source of adult mortality in wild pigs (e.g. Gabor et al. 1999), however, no hunting, vehicle deaths, or culling (outside our study design) occurred within ~2 km of the study sites during this project.

In the biomarker Lincoln-Petersen Estimator (LPE) and camera LPE, we calculated abundance of wild pigs using the Chapman correction for small sample size (Seber 1982, Pollock 1991). Marked animals for the biomarker LPE were those that consumed a sufficient amount of RB (described in Appendix S3.1), whereas in the camera LPE, marked animals were those that were individually identified by camera trap photographs. The recapture occasion for both LPEs consisted of corral trapping and lethal removal of animals.

For the camera SECR analysis, we created and compared 10 *a priori* SECR models of wild pig density, available in Appendix S3.2. These models included potential factors affecting density (D), the scale parameter (sigma), describing how detection declines with distance between an animal's home range center and a detector (i.e. camera), and the probability of detection (g0). These models assumed animals were distributed on the landscape following a homogenous Poisson point process, and that probability of detection was related to distance between an animal's activity center and detectors through a half-normal curve (Efford et al. 2004). We evaluated the level of support for each model using change in second order Akaike's Information Criterion (ΔAIC_C) and AIC weight (AIC_{wi}), measures of model likelihood (Burnham and Anderson 2002). We chose the results of the most supported model for comparison to the other population estimation techniques. If model selection uncertainty occurred, we used model averaging to estimate parameters.

For the trap SECR analysis, we used individual capture histories from the camera SECR method combined with live-trapping effort as additional potential capture occasions. We evaluated 10 *a priori* models of wild pig density using $\Delta AICc$ and AIC_{wi} (Appendix S3.2). Corral traps were considered a "proximity"-type detector to allow data analysis using R package *secr*. This implies that multiple individuals could be captured by the same detector during a time period, which was facilitated by bait placement and using continuous-catch gates on many of the traps (Appendix S3.1). Similar to Gerber and Parmenter (2015), this assumption likely did not influence our estimates, as mean trap saturation, or the occasion specific proportion of occupied traps, was low (<0.03, Appendix S3.3). We also tested models that included a categorical effect of trap type (i.e. camera trap vs. corral trap) on detection probabilities.

For the removal method, we used a Bayesian hierarchical removal model, accounting for variation in capture effort, to estimate abundance in each study site (Davis et al. 2016). The removal method was a standard removal model (Farnsworth et al. 2002) that jointly estimated capture rate and initial population size, and assumed changes in population size during the study were exclusively due to removals. Capture probability was dependent on the amount of effort (i.e. number of traps active in a given night). We implemented the model as in Davis et al. (2016).

Converting abundance to density

To compare the techniques employed, we converted abundance estimates (i.e. biomarker LPE, camera LPE, removal) to density, as estimates of density are scalable across studies. We estimated the effective area sampled by each method as the area encompassed by the sampling grid buffered by the mean maximum distance moved (MMDM; Wilson and Anderson 1985), as calculated using camera and corral trap capture data. We used the Delta method (Powell 2007) to calculate variances for the analytical techniques requiring a conversion from abundance to density (i.e. biomarker LPE, camera LPE, and removal). In addition, for the removal method we used a naïve buffer calculated from literature values for wild pig home range size (McClure et al. 2015) to determine how this estimator performed without site-specific movement data.

Simulation

We used simulations to evaluate the accuracy of each analytical method with varying densities, detection rates, and movement parameters. We excluded the biomarker LPE in simulations due to observational process uncertainty (see Results).

We simulated a homogenous landscape and added a camera grid as implemented in the field component of this study (i.e. 20 cameras spaced 750 m apart in a 5 x 4 grid). We used a similar method to simulate trap locations (i.e. 10 traps in a spatially balanced design within the camera array). We then simulated spatial distributions of animal home range centroids using a partial Poisson clustering algorithm (R function *PCP.sim,*package *splancs*) to account for the social dynamics of this species.

We created an observation process model in which detection of an animal depended upon the distance between its home range centroid and detectors. We assumed the probability of an animal being detected at a detector decreased with increased distance between that animal's home range centroid and the focal detector, similar to distance sampling (Buckland et al. 2001), which we implemented through a truncated Gaussian relationship. We simulated constant movement metrics by varying the standard deviation of the truncated Gaussian distribution to affect the potential that an animal would encounter a detector. The maximum distances at which animals might encounter corral traps were simulated as being greater than those of camera traps, as there would be fewer detectors and, therefore, bait, present on the landscape during the trapping period, potentially causing animals to move greater distances. We restricted the total number of traps an animal could visit in a single night using a multinomial process based upon our observed empirical distribution of trap attendance and capture rates. In addition, we included a behavioral effect that increased the chances of an individual returning to a camera in subsequent nights once it was detected (i.e. "trap-happiness"), as supported by SECR model results (Appendix S3.2). In simulating corral trapping, animals could only be detected at one trap ever, and then were removed from the population.

We simulated all combinations of a range of movement (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2), density (0.25, 0.50, 0.75, 1.25, 2.00, 2.50, 3.75, 5.00, 6.25, 7.50, 10.00, 15.00), and detection probabilities (0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 0.9), for a total of 588 combinations. We generated five sample datasets from each combination. We defined movement (sigma) as the standard deviation of a Gaussian distribution, based upon the average radius of two week home range sizes from collared animals at the SRS (Smith et al., Savannah River Ecology Laboratory, unpublished data). The detection probability was modeled as being 75% lower for corral traps than camera traps based upon field data, meaning that given the distance between its home range centroid and the detector was the same, the probability of a pig being captured in a corral trap was ¼ of its probability of being captured at a camera. We compared analytical methods in terms of scaled bias, the deviation of the estimated density from the true density used to create the simulation, and coefficients of variation, a scaled measure of variability representing relative precision. All data and code are available in Supporting Information (Appendices S3.4 and S3.5).

Results

Density Estimates

Density estimates from the five analytical techniques ranged from 0.91 - 2.60 adult animals/km^2 in the three study sites tested (Fig. 3.3). Camera SECR and trap SECR models generally produced higher estimates of density than other methods. Estimates across study sites were similar within a given method (Fig. 3.3). Numbers of animals marked or captured by each field method in the three study sites are presented in Appendix S3.3. In calculating false-positive and false negative rates of mark determination for the biomarker LPE, we found uncertainty in both the capture and recapture occasion. We, therefore, present a sensitivity analysis of the potential effects of this uncertainty on resulting density estimates in Appendix S3.6. Movement rates used to calculate buffer sizes for abundance estimators varied among habitat types (range of MMDM: 326 – 896 m), meaning the effective area sampled differed between study sites. Additional discussion of results specific to each field technique can be found in Appendix S3.3.

Simulation

Simulations showed movement parameters often influenced the scaled bias of estimators more than probability of detection or true density for all estimators (Fig. 3.4). We found that LPEs were biased high when movement rates were high, biased low when movement rates were low, and exhibited higher bias at low detection probabilities (Fig. 3.4). Camera SECR and trap SECR models exhibited similar patterns to each other. Under some simulated conditions SECR models were not able to estimate density based on the sparseness of encounters, although trap SECR models, which employed greater amounts of data, were less affected (Fig. 3.4). Camera SECR and trap SECR models tended to be biased high at high movement rates and were unable to produce results when movement rates were low (Fig. 3.4). The removal estimator was more affected by the density parameter than the movement or detection parameters; removal models were biased high at low densities and biased low when movement rates were low (Fig. 3.4). Buffer choice to estimate effective area sampled had a substantial impact when converting abundance estimates to density. In particular for removal models when a naïve buffer was employed models performed poorly, but when an appropriate buffer based upon prior information (e.g. MMDM) was employed, the bias caused by movement was minimized (Appendix S3.3). We found MMDM buffers performed better than other buffer choices (i.e. HMMDM, naïve) in converting estimates of abundance to density (Appendix S3.3).

In terms of precision, LPEs exhibited poor performance at low detection probabilities and low movement rates (Fig. 3.5). Further, when true variation was accounted for, inconsistent patterns in precision of LPEs emerged (Appendix S3.3). When data were sufficient to allow parameter estimation, camera SECR and trap SECR models were fairly consistent in estimating precision, and were most imprecise at low movement rates and low abundances (Fig. 3.5). The removal model was most imprecise at low densities and exhibited increased imprecision with increased movement rates when detection rates were low (Fig. 3.5; Appendix S3.3).

Discussion

We employed and compared five methods of density estimation under field conditions and used simulations of ecological (e.g. animal movement) and observational processes (e.g. baseline probability of detection) in lieu of known abundances to compare population density estimators under known conditions and evaluate their accuracy and relative strengths. Comparison of field methods to estimate animal density provides essential information for managers planning conservation or management programs and yet is infrequently conducted. Further, assessment of the accuracy of metrics is often impossible to perform in field conditions (but see Sharma et al. 2010, Gerber and Parmenter 2014, Jůnek et al. 2016), leading many studies to use indices or minimum population sizes as a metric of comparison (e.g. Bellemain et al. 2005, Hebeisen et al. 2008).

The range of our density estimates (0.91 – 2.60 adult pigs/km^2) is consistent with published estimates of wild pig density in the southeastern US (1.07 – 2.74 pigs/km^2; Hanson et al. 2009), suggesting that severe overestimation or underestimation by field application of the tested density estimators did not occur. Our results suggest that the ecological process of movement can have a large effect on density estimates, highlighting the importance of using effective post-hoc approaches to convert estimates of abundance to density (Ivan et al. 2013) or using techniques that implicitly consider spatial variation to estimate density. It should be noted, however, that the movement metrics observed from field data fell within a reasonable range for estimating density with fairly low bias from simulations (Fig.3.4). In general, estimators exhibited greater bias and imprecision when movement rates were low, which may partially be a result of fewer detections of individual animals at different detectors. Therefore, sampling design to maximize detections of individuals at multiple detectors, and thereby improve measurements of movement rates will likely improve accuracy of density estimates. When capture rates were low as a result of extremely low baseline probabilities of detection or densities, all estimators generally suffered reduced performance, regardless of movement parameter values. Our simulations also suggest that MMDM, rather than HMMDM, should be used to convert estimates of abundance to density for greatest accuracy, similar to previous research (Appendix S3.3; Obbard et al. 2010, Gerber et al. 2012). It should be restated, however, that there is no theoretical basis for use of MMDM as an appropriate buffer (Gerber et al. 2012), and that variation surrounding this estimate of an appropriate buffer size is not incorporated into the overall variation around the estimate of population density.

We found that LPEs generally estimated lower densities than other field techniques with relatively high precision; however, our simulations suggest that these estimates may be disputable. One assumption of LPEs is that marks are not lost or overlooked (Mills 2007), which was violated in the case of the biomarker LPE, and is likely to have affected the accuracy of density estimates (Appendix S3.3). It has previously been suggested that LPEs may be relatively unbiased when different methods of capture and recapture are implemented to reduce effects of individual heterogeneity

(Mills 2007), which may explain the apparent accuracy of LPEs in our simulations. The relative accuracy of LPEs in simulations may also be partially accounted for by the fact that LPEs are known to perform well when home ranges are circular (Ivan et al. 2013), as implemented in our simulations. However, LPEs often had poor ability to correctly estimate error (Fig. 3.5) and do not accommodate model selection approaches, which may limit their utility in determining effects of specific covariates on density estimates (Pollock 1991). LPEs were able to estimate densities even with low amounts of data, although the accuracy and precision of these estimates might be questionable. Overall, use of LPEs is likely most preferable when 1) a computationally simple method is necessary, 2) an assumption of circular home ranges is acceptable, 3) movement and detection rates are fairly high, and 4) the researcher or manager is comfortable with some degree of inaccuracy and/or imprecision.

Camera SECR and trap SECR methods resulted in the highest density estimates and performed similarly under field conditions and in simulations. It is not unexpected that this would be the case, as corral trap data did not significantly change the models, but simply represented additional data that could be used to estimate movement and detection parameters to better inform density estimates. Trap SECR models were generally more accurate and precise than camera SECR models as a result of this additional data. Spatially explicit models had the additional benefit of allowing incorporation of covariates to better account for underlying mechanisms that influenced the detection process (e.g. movement), although these models also required a greater amount of data than other methods tested, and failed to run when insufficient data were available. This implies additional effort may be necessary to implement SECR methods in the field compared to the other tested techniques, particularly when movement rates are low. Similar to other studies, SECR models were relatively imprecise under field conditions (Fig. 3.3), likely due to their incorporation of spatial variation into the estimation process (Gerber et al. 2012). Despite this, our simulations suggest that SECR models will be relatively precise when density, movement parameters, and/or detection rates are high, criteria that were not fully met by field data. Accuracy and precision of SECR models could be additionally improved by better tailoring the sampling grid design to reduce mismatches between perceived and actual movements, as discussed in Tobler and Powell (2013). In addition, individual heterogeneity has the potential to affect estimates, and one advancement to our models might include more explicit analysis of these effects. Based upon our results, we recommend SECR approaches be employed when 1) when recaptures at multiple spatial locations are likely, 2) fairly accurate and precise density estimates are required, and 3) mismatches between grid size and movement patterns of animals are unlikely or can be minimized.

Development of removal models suggests that they can generate robust estimates of abundance (Davis et al. 2016), however they do not inherently consider space, necessitating estimation of the effective area sampled by this technique through external data sources (e.g. use of remote cameras, telemetry) to allow density estimation. As expected, the buffer used for conversion to density must be realistic and preferably based on site-specific observations in order for good estimates to be obtained. While the removal models were somewhat biased when density and movement rates were low, they exhibited high accuracy when population density was large and capture rates were sufficiently high. As expected based upon simulations, this estimator performed poorly for the mixed study site, where capture rates were extremely low. This technique also had the lowest data requirements, needing only a simple count of animals removed during the study period and the effort required to remove them (here, trap nights). We believe that removal estimators will be most effectively employed when 1) population densities are fairly high and a reasonable capture rate can be attained (Davis et al. 2016), 2) a simple method of data collection is preferred, 3) the target population is already being managed by culling, and 4) data on movements of animals in the study area can be gathered or inferred.

Field Implementation

When choosing the most appropriate method to monitor populations, understanding the strengths and weaknesses of each technique is necessary. While we were able to individually identify animals of this species using photographs, the proportion of naturally marked and identifiable animals is likely to differ across regions and species. When unidentifiable individuals are present in the population, spatially explicit mark-resight methods that account for the proportion of unidentifiable individuals captured in photographs might offer a solution (e.g. Chandler and Royle 2013, Rich et al. 2014). Camera traps are already commonly used in many control programs for invasive and harvested species to assess presence and composition of populations prior to implementation of management strategies, suggesting camera-based methods could be an efficient technique for management applications.

A challenge with the biomarker-based method was that it was difficult to determine from camera-trap data whether individuals had consumed sufficient biomarker to be marked. This led to uncertainty in the number of marked animals within each study site, which could influence population estimates (Appendix S3.6). Using greater concentrations of biomarker, requiring less consumption by each individual to generate a mark, and/or a shorter marking period, as in Reidy et al. (2011), may improve results. However, higher concentration of some biomarkers could reduce palatability of bait or make consumption unsafe for non-target species, requiring further modifications of bait matrix for success. We also found uncertainty in the recapture occasion of this technique, which may be due to variation in biomarker consumption among animals. Thus, as currently implemented, the biomarker technique likely needs further development to reduce observational error for effective implementation in density estimation.

Trapping is a commonly used technique to manage invasive and harvested species (Williams et al. 2011), and use of trapping to estimate density, such as in the removal model, biomarker LPE, camera LPE, or trap SECR method we employed, is attractive as it may complement management programs (Davis et al. 2016). To better estimate the area sampled by detectors and further refine density estimates from methods using trapping data, researchers might consider collecting external telemetry data to estimate the amount of time spent by individuals in a sampling area (Ivan et al. 2013). This will allow improved density estimation by techniques that do not explicitly consider movement and space, and may improve estimates of those that do.

Although we conducted this study over a relatively short period of time, the age structure of populations differed dramatically between study sites (Appendix S3.3). Estimates of density that include young could change greatly within a few months in species that exhibit birth pulses, or across space in species that breed year round, necessitating careful interpretation of results or increased planning to account for temporal and spatial variation in births. We also believe future studies of social species, such as wild pigs, should investigate the independence of adult animals within the same group to ensure independence of samples or assess the necessity of modification to density estimation techniques. In addition, improved information about reproductive parameters, such as the proportion of animals reproducing and average litter size, could be used to incorporate non-independent juveniles into estimates. To our knowledge, no study has extensively evaluated the degree to which wild pigs of the same social group are spatially independent. Development of methods to incorporate spatial auto-correlation at the individual level might be valuable for future studies of this and other social species.

Acknowledgements

This study would not have been possible without the hard work of E. Bledsoe, E.J. Borchert, K. Eckert, and R. Juarez in collecting data, and R.B. Chandler for feedback on the manuscript. Additional thanks to the US Forest Service including T. Grazia, T. Mims, J. Nance, and M. Vukovich for logistical support of this work. This project was funded by the USDA Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center through APHIS agreement #15-7488-119-CA and the Department of Energy Office of Environmental Management through award number DE-FC09-07SR22506 to the University of Georgia Research Foundation. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of USDA-APHIS.

Data accessibility

All data will be available in Supporting Information (S2-S9) at time of publication.

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Tables

Table 3.1. Data sources, implementation, and references for tested density estimators, Savannah River Site, South Carolina, USA, 2015.

Analytical Technique	Field Data Used	Implementation	Citation
Biomarker LPE	Biomarker data, camera data, corral trap data	Simple function written in R (R Development Core Team 2014) or Excel	Seber 1982, Pollock 1991
Camera LPE	Camera data, corral trap data	Simple function written in R or Excel	Seber 1982, Pollock 1991
Camera SECR	Camera data	Package *secr* in R	Borchers and Efford 2008, Efford 2016
Trap SECR	Camera data, corral trap data	Package *secr* in R	Borchers and Efford 2008, Efford 2016
Removal	Corral trap data	Hierarchical Bayesian model using custom MCMC code written in R	Davis et al. 2016

Figures

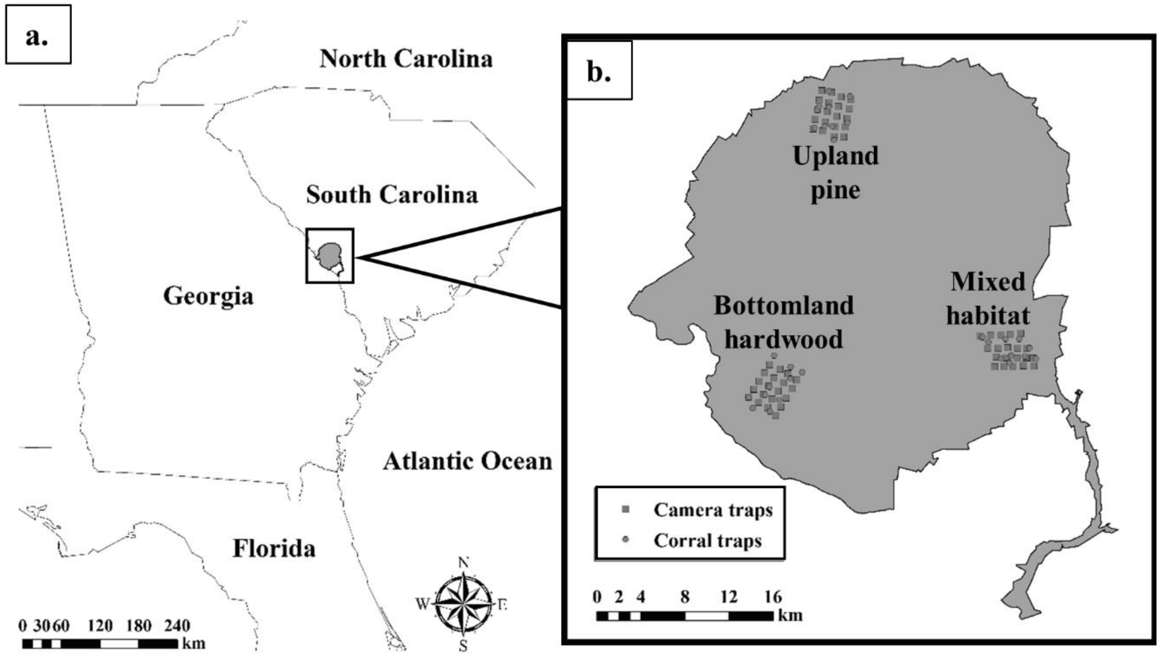

Fig. 3.1 Location of the Savannah River Site (**a.**) and distribution of detectors in the selected study sites (**b.**), South Carolina, USA, 2015.

a.

b.

Fig. 3.2 Example of a wild pig (*Sus scrofa*) individually identified by pelage patterns (**a.**) and a wild pig identified by scars (**b.**), Savannah River Site, South Carolina, USA, 2015.

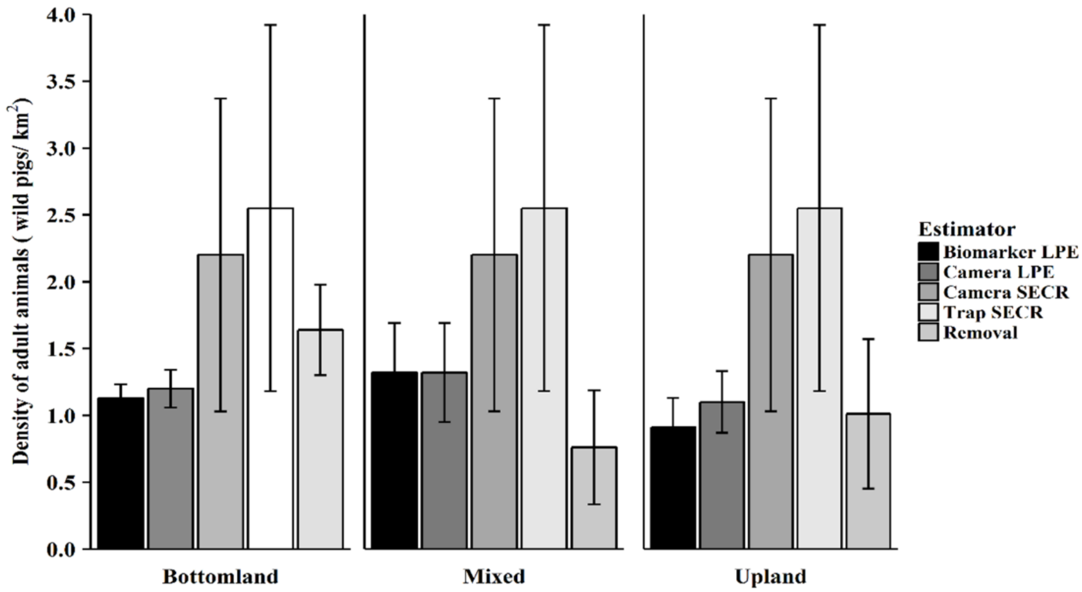

Fig. 3.3 Estimated densities of wild pigs (*Sus scrofa*) at three study sites at the Savannah River Site using five analytical techniques, South Carolina, USA, 2015.

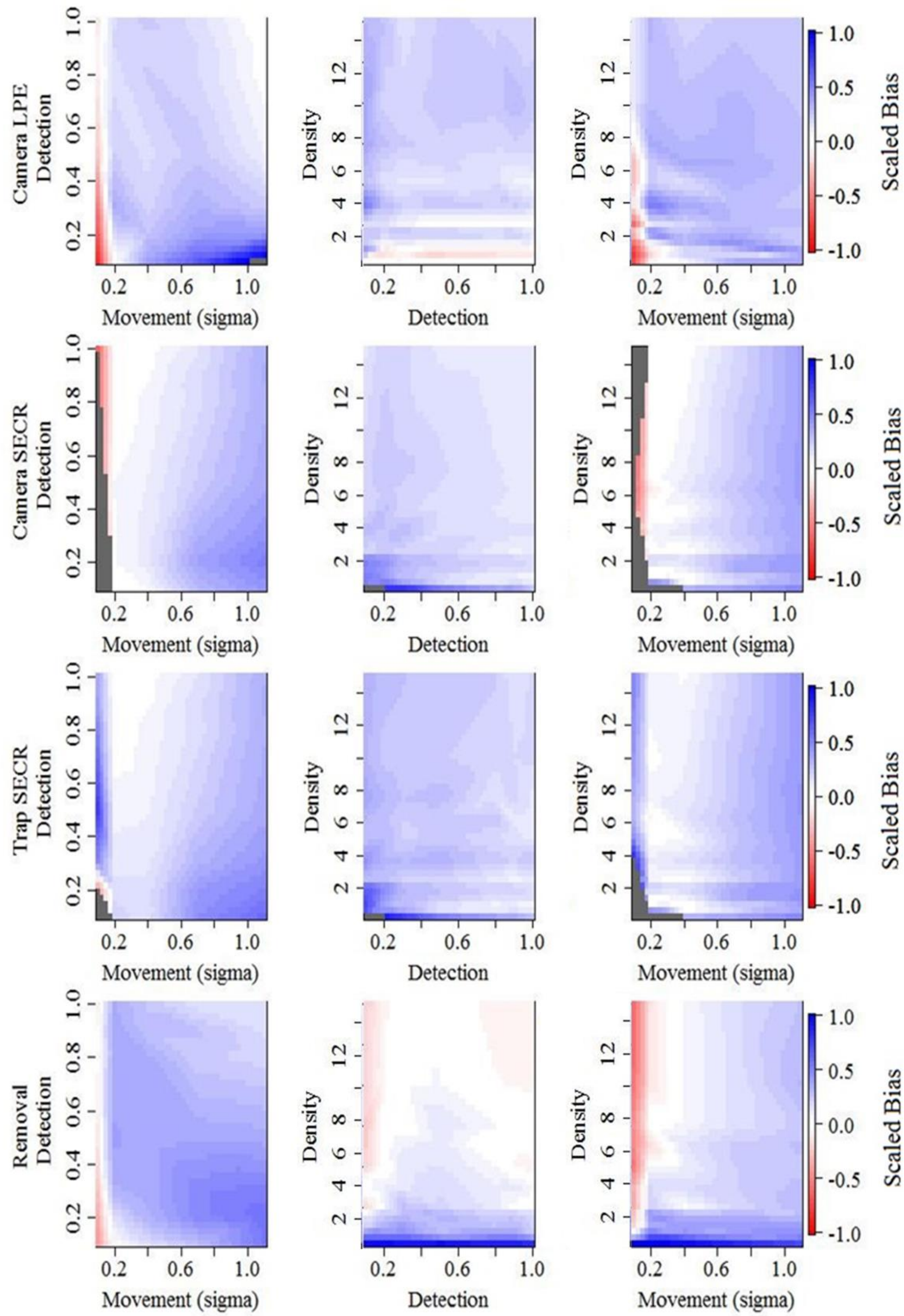

Fig. 3.4 Effects of movement, probability of detection, and density on scaled bias of tested analytical techniques from simulations. Parameter values at which models did not run are displayed in gray.

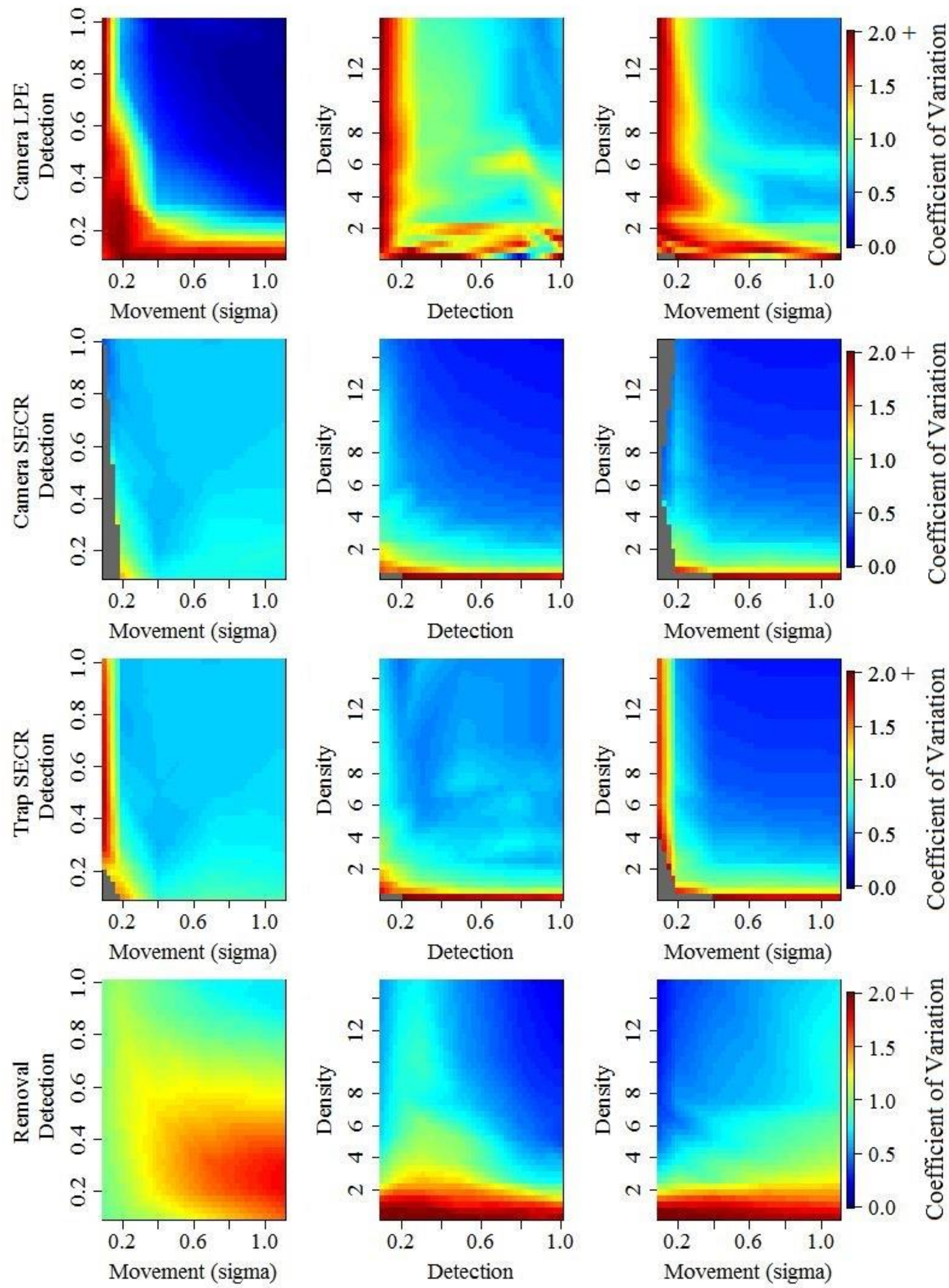

Fig. 3.5 Effects of movement, probability of detection, and density on the coefficients of variation (CV) of tested analytical techniques from simulations. The upper bound of CV values represents any values ≥2.0. Parameter values at which models did not run are displayed in gray.

Appendix S3.1

Impact of animal movement on common methods of estimating population density

Keiter, D. A., A. J. Davis, O. E. Rhodes Jr., F. L. Cunningham, J. C. Kilgo, K. M. Pepin, and J. C. Beasley.

<u>Additional description of field methods</u>

1. Camera traps

In addition to baiting each camera trap with 11.4 kg of RB-treated bait, we used 11.4 kg of whole corn to create bait trails to increase initial detection probabilities of animals at camera stations (e.g. Gerber et al. 2012). We replaced depleted bait piles with 11.4 kg of whole corn, 5-6 days following deployment of cameras. Additionally, following the camera trapping period we removed any remaining bait, biomarker-treated or otherwise, in order to avoid influencing the success of trapping efforts. When identifying individual pigs (*Sus scrofa*) to create capture histories, we created a separate computer folder for each individual animal containing representative photographs of that animal from different angles to facilitate re-identification throughout the analysis period.

2. Biomarker use

Rhodamine B (RB) is a fluorescent dye that can be used as a biomarker for studies of wildlife. The use of RB was recently assessed in wild pigs with the conclusion that it would be appropriate to use as a measure of bait consumption (Beasley et al. 2015). RB consumption leaves a fluorescing mark in the whiskers of animals that have consumed it, allowing their identification through whisker analysis. We created the RB-treated bait used in this experiment by thoroughly mixing 5.0 g of RB, 0.5 L of water and 11.4 kg of whole corn in an 18.9 L plastic bucket at each bait site. For RB marking to appear in the whiskers of a pig, the pig must consume a minimum dosage of 5 mg RB/kg mass (J. C. Beasley, University of Georgia, unpublished data). Therefore, at the dosage we used, a 100 kg pig must have consumed 1.14 kg of treated corn to exhibit a mark. 100 kg is far above the average weight of a wild pig on the Savannah River Site (36.6 kg; Mayer and Johns 2007), leading us to believe that pigs would likely consume sufficient amounts of RB-treated corn to be marked (Figure 1). Using camera imagery, we judged the amount of time that an animal spent at a bait pile consuming bait and its approximate weight to estimate whether it consumed a sufficient quantity of the treated bait to generate a mark. For example, if the pig weighed 50 kg, it must have consumed ~0.6 kg of bait to generate a mark; if we observed this 50 kg pig consuming bait for 15 minutes, it likely consumed more than 0.6 kg of bait, and therefore we considered it marked.

We conducted our whisker analysis as in Beasley et al. (2015). We cleaned whisker samples separately using distilled water, dried them, and then mounted each sample on a microscope slide using Fluoromount (Sigma-Aldrich, Missouri, USA). Three observers then independently evaluated each slide using a BX61 fluorescent microscope (Olympus Life Science Solutions, Pennsylvania, USA) and the consensus score was used to determine presence or absence of RB in each pig.

S3.1 Figure 1. Wild pigs at a bait pile of Rhodamine B-treated whole corn, Savannah River Site, South Carolina, USA, 2015.

3. Live-trapping and euthanasia

We constructed all corral traps from metal paneling supported either by angle iron frames or t-posts with either a continuous-catch or guillotine-style gate (Figure 2). Traps were triggered either by a root-stick or tripwire mechanism. We placed root-sticks and tripwires at a height greater than that estimated for piglets, in order to maximize capture success (as piglets frequently enter traps prior to adults and subadults). We baited traps in a manner to maximize capture success, according to the suggestions of West et al. (2009). In addition to pre-baiting corral traps, we created bait trails of whole corn leading to traps in order to improve pig detection of trap locations.

S3.1 Figure 2. Corral trap with continuous-catch or root gate (left) and wild pig (*Sus scrofa*) captured in a corral trap with a guillotine-style gate (right), Savannah River Site, South Carolina, USA, 2015.

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Appendix S3.2

Impact of animal movement on common methods of estimating population density

Keiter, D. A., A. J. Davis, O. E. Rhodes Jr., F. L. Cunningham, J. C. Kilgo, K. M. Pepin, and J. C. Beasley.

Tables presenting model comparison results for spatially explicit capture-recapture (SECR) models tested.

1. *Camera SECR*

S3.2 Table 1. Model selection results for *a priori* models of wild pig (*Sus scrofa*) density using camera trap data at the Savannah River Site, Aiken SC, 2015

Model	K[a]	AIC_C	ΔAIC_C	AIC_{wi}	-LL[b]
D[c] ~ 1 g0[d]~ b[e] + T[f] sigma[g] ~ session[h]	7	1843.24	0	0.9186	-913.3177
D ~ session g0 ~ b + T sigma ~ session	9	1848.087	4.847	0.0814	-912.8486
D~session g0~b sigma~session	8	1858.31	15.07	0	-919.4407
D~1 g0~b+T sigma~1	8	1873.453	30.213	0	-931.06
D~session g0~b+T sigma~1	5	1874.98	31.74	0	-929.1876
D~1 g0~b sigma~1	7	1889.185	45.945	0	-940.1577
D~session g0~b sigma~1	4	1890.742	47.502	0	-938.4166
D~1 g0~T sigma~1	6	2022.444	179.204	0	-1006.787
D~1 g0~1 sigma~1	4	2028.387	185.147	0	-1010.938

D~session g0~1 sigma~1	3	2029.311	186.071	0	-1008.9889
D~session g0~b sigma~session	5	1843.24	0	0.9186	-913.3177

[a] = Number of parameters
[b] = Negative log-likelihood
[c] = Density
[d] = Probability of Detection
[e]= Effect of behavior
[f] = Linear time trend in detection
[g] = Distance between activity center and a detector
[h] = Study site

2. Trap SECR

S3.2 Table 2. Model selection results for *a priori* models of wild pig (*Sus scrofa*) density using camera trap and corral trap data at the Savannah River Site, Aiken SC, 2015

Model	K[a]	AIC_C	ΔAIC_C	AIC_{wi}	-LL[b]
D[c] ~ 1 g0[d]~ b[e] + T[f] + Camera[g] sigma[h] ~ session[i]	8	2244.67	0	0.9408	-1112.835
D~session g0~b+T+Camera sigma~session	10	2250.2	5.53	0.0592	-1112.708
D~1 g0~b+T+Camera sigma~1	6	2267.739	23.069	0	-1127.03
D~session g0~b+T+Camera sigma~1	8	2268.641	23.971	0	-1124.821
D~1 g0~b+Camera sigma~1	5	2290.187	45.517	0	-1139.505
D~session g0~b+Camera sigma~1	7	2291.323	46.653	0	-1137.518
D~1 g0~T+Camera sigma~1	5	2455.39	210.72	0	-1222.107
D~1 g0~Camera sigma~1	4	2456.333	211.663	0	-1223.782
D~session g0~Camera sigma~1	6	2457.534	212.864	0	-1221.927
D~1 g0~1 sigma~1	3	2528.03	283.36	0	-1260.789

[a] = Number of parameters
[b] = Negative log-likelihood
[c] = Density
[d] = Probability of Detection
[e]= Effect of behavior
[f] = Linear time trend in detection
[g] = Detector type (i.e. camera trap or corral trap)
[h] = Distance between activity center and a detector
[i] = Session is the term used for study site within our models

Appendix S3.3

Impact of animal movement on common methods of estimating population density

Keiter, D. A., A. J. Davis, O. E. Rhodes Jr., F. L. Cunningham, J. C. Kilgo, K. M. Pepin, and J. C. Beasley.

Additional discussion of results

1. Camera trapping

We were able to identify each wild pig that encountered a camera trap in this study for the purpose of creating capture histories, based upon size, pelage, scarring, and associated group members, similar to Williams et al. (2011). We did not observe evidence of animal wariness regarding camera traps, resulting in high rates of identification, although a small proportion of photographs contained an individual that was most likely marked, but unidentifiable due to distance or angle of the camera relative to the pig. Similar to Sweitzer et al. (2000), we excluded these individuals from analysis. We identified a total of 51 individual adult and subadult pigs and 25 piglets across the three study sites (Table 1, Fig. 1). Greater numbers of piglets were identified in the upland site than the bottomland or mixed sites. Piglets were photographed with associated adult or subadult wild pigs at almost every capture occasion.

The most supported camera SECR model included an effect of behavior and a linear time trend on probability of detection, and differing movement rates between study sites (AIC_{wi} = 0.91; Supporting Information [S2]). The most supported trap SECR model included these same effects and an additional effect of detector type (i.e. camera trap vs. corral trap) on probability of detection (AIC_{wi} = 0.94; S2). These results provide evidence of “trap-happiness” in wild pigs visiting baited camera traps, and evidence of differing capture probabilities between camera and corral traps.

2. Biomarker use

Of the identified adult and subadult wild pigs, hereafter adults, we estimated that 46 individuals were marked through consumption of Rhodamine B (RB) based upon camera trap imagery (Fig.1). Analysis of whisker samples showed that of the 31 adults captured in corral traps, 26 (83.87%) were judged to have consumed RB. Of the animals captured in corral traps, 2 pigs were thought to have been marked based upon camera observations and were not marked based upon whisker analysis, giving a false positive rate of 6.45%, while 4 pigs were thought to have not consumed sufficient RB to be marked, but were positive by whisker analysis, giving a false negative rate of 12.90%. We present a file of R code which can be used in a sensitivity analysis to assess the potential effects of observer error on density estimates (Appendix S6).

3. Live-trapping and euthanasia

In corral traps, 32 adult pigs and 27 piglets were captured across the three study sites (Table 1). In total, 85 unique pigs were identified by camera traps, capture in corral traps, or both. Of the pigs captured in corral traps, the majority (84.74%) were identified on at least one camera trap prior to their capture in a corral trap. Mean trap saturation (occasion specific proportion of occupied traps) for the three study sites is presented in Table 2.

Buffer estimation

Movements as determined by camera and corral trap data were variable for pigs in all study sites, however, the highest mean maximum distance moved (MMDM) occurred in the bottomland site (897.37 m, SE = 151.09), while pigs in the upland study site (MMDM

= 647.32 m, SE = 161.83) and mixed site (MMDM = 327.81 m, SE = 116.99) appeared to move shorter distances. For this reason, the effective area sampled that was used to convert abundance to density was greater for the bottomland site than the upland or mixed site. Fig. 2 and Fig. 3 below present comparisons of the scaled bias in density estimates of the removal model using MMDM, HMMDM, and a naïve buffer from literature values (McClure et al. 2015).

Simulations

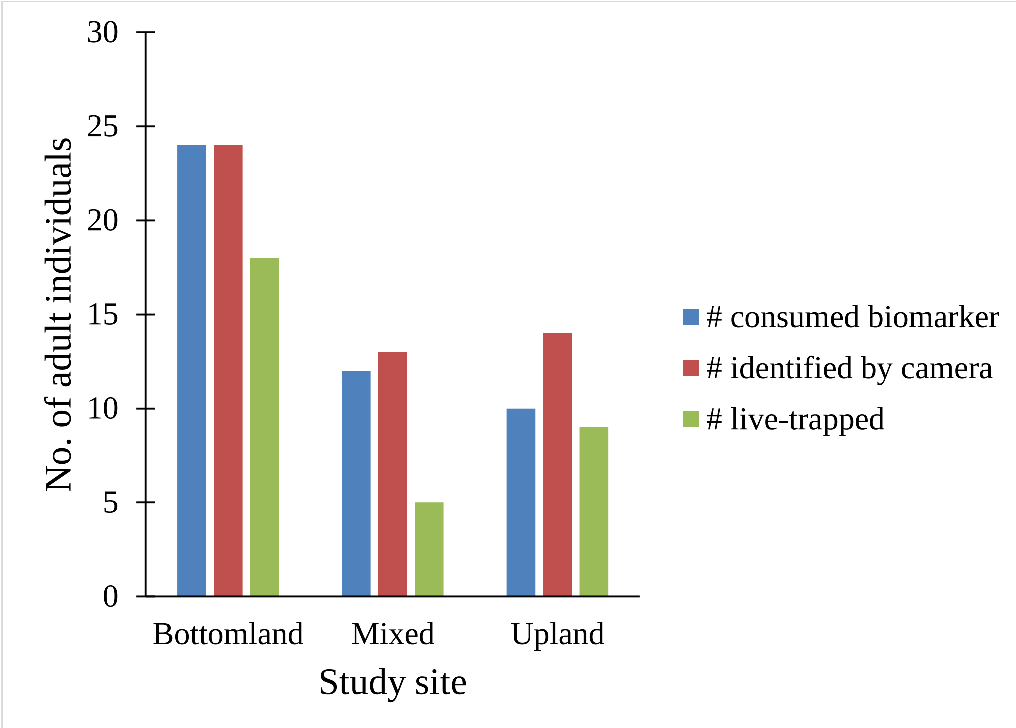
Fig. 4 below depicts the effects of movement parameters, probabilities of detection, and densities on the coefficients of variation of estimated densities by the tested analytical techniques, when the values of coefficients of variation are unconstrained.

S3.3 Table 1. Total captures of wild pigs (*Sus scrofa*) by study site and method, Savannah River Site, Aiken, SC, 2015.

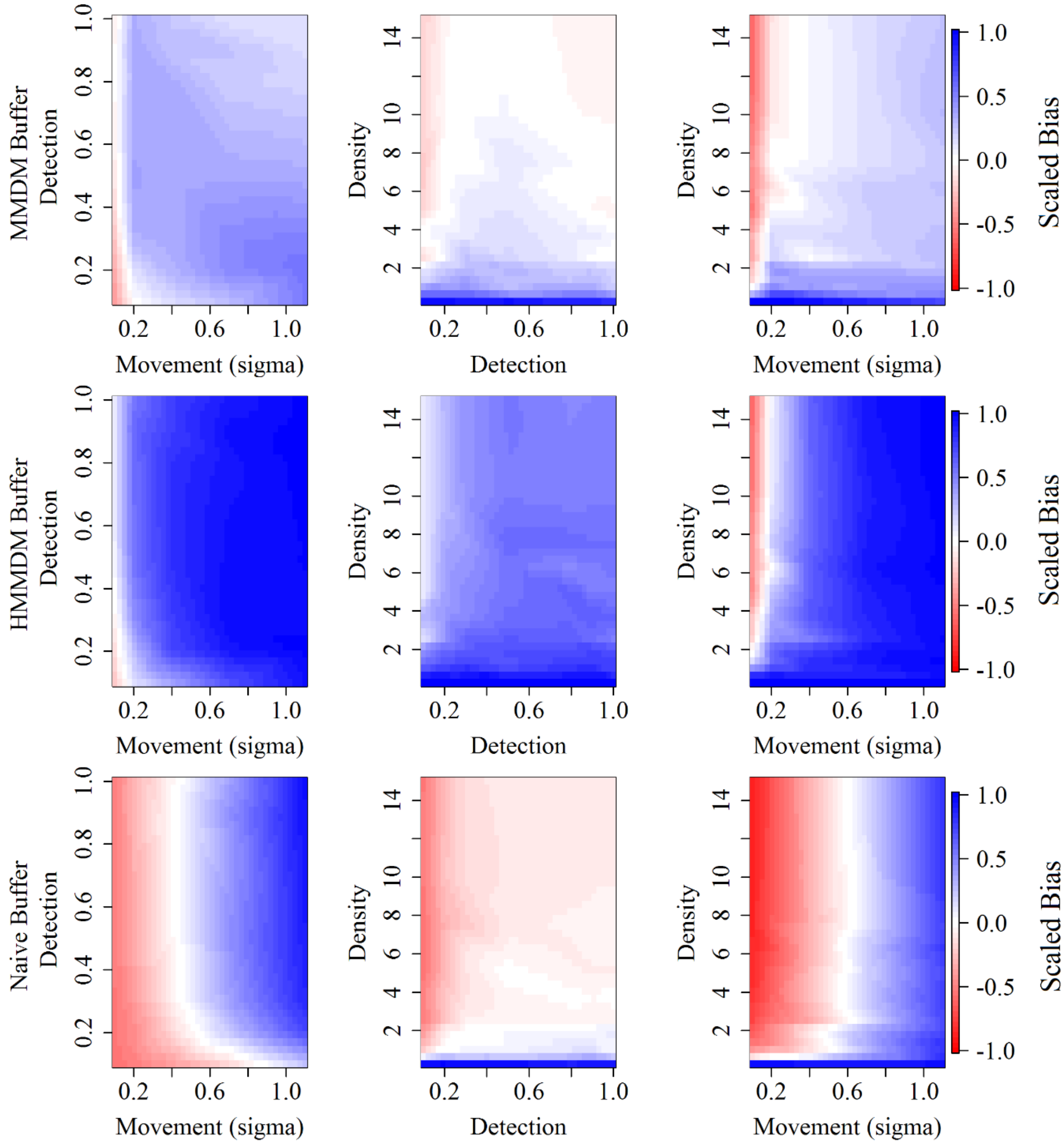
Habitat Type	# of Adults and Subadults (Camera)	# Piglets (Camera)	#of Adults and Subadults (Corral)	# Piglets (Corral)	# Trapped, not photographed
Bottomland hardwood	24	7	18	9	5
Mixed habitat	13	0	5	1	2
Upland pine	14	18	9	17	2
Total	51	25	32	27	9

S3.3 Table 2. Study-site specific mean trap saturation and standard error, Savannah River Site, Aiken, SC, 2015.

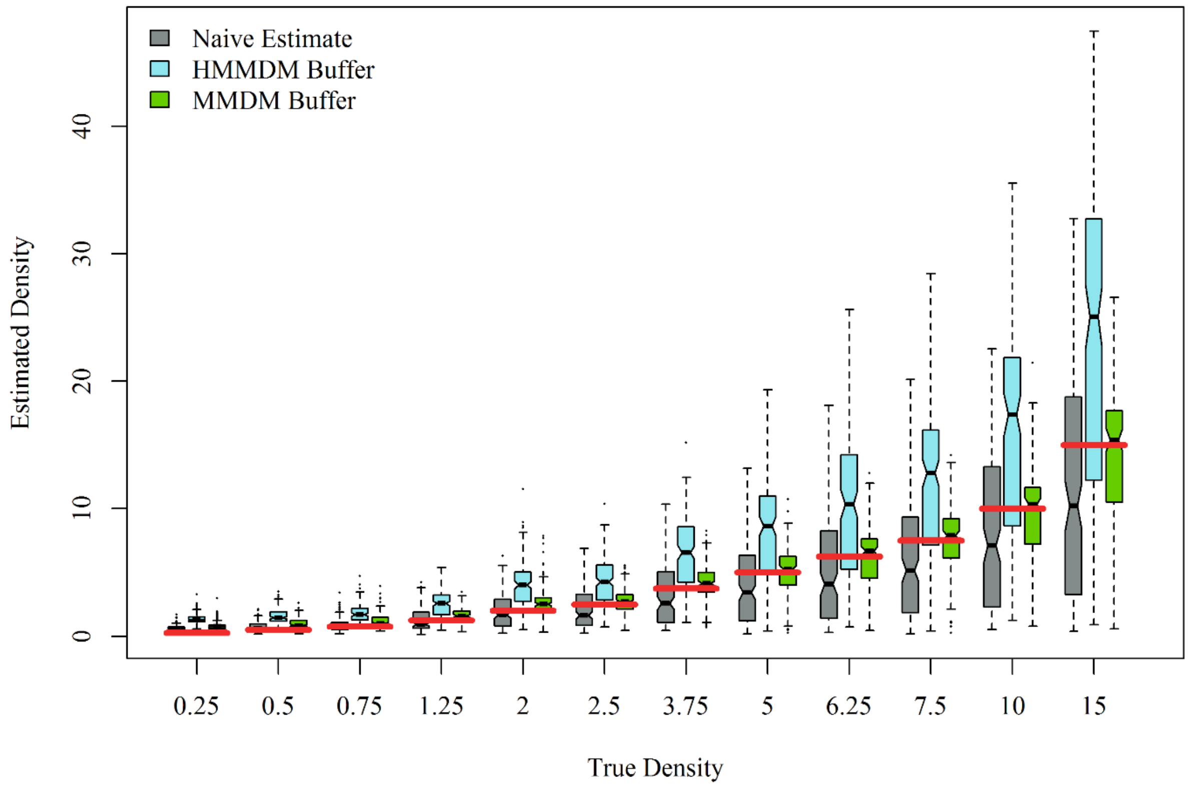
Study site	Mean trap saturation	Standard error
Bottomland hardwood	0.027	0.015
Mixed habitat	0.013	0.009
Upland pine	0.04	0.021
Total	0.027	0.015



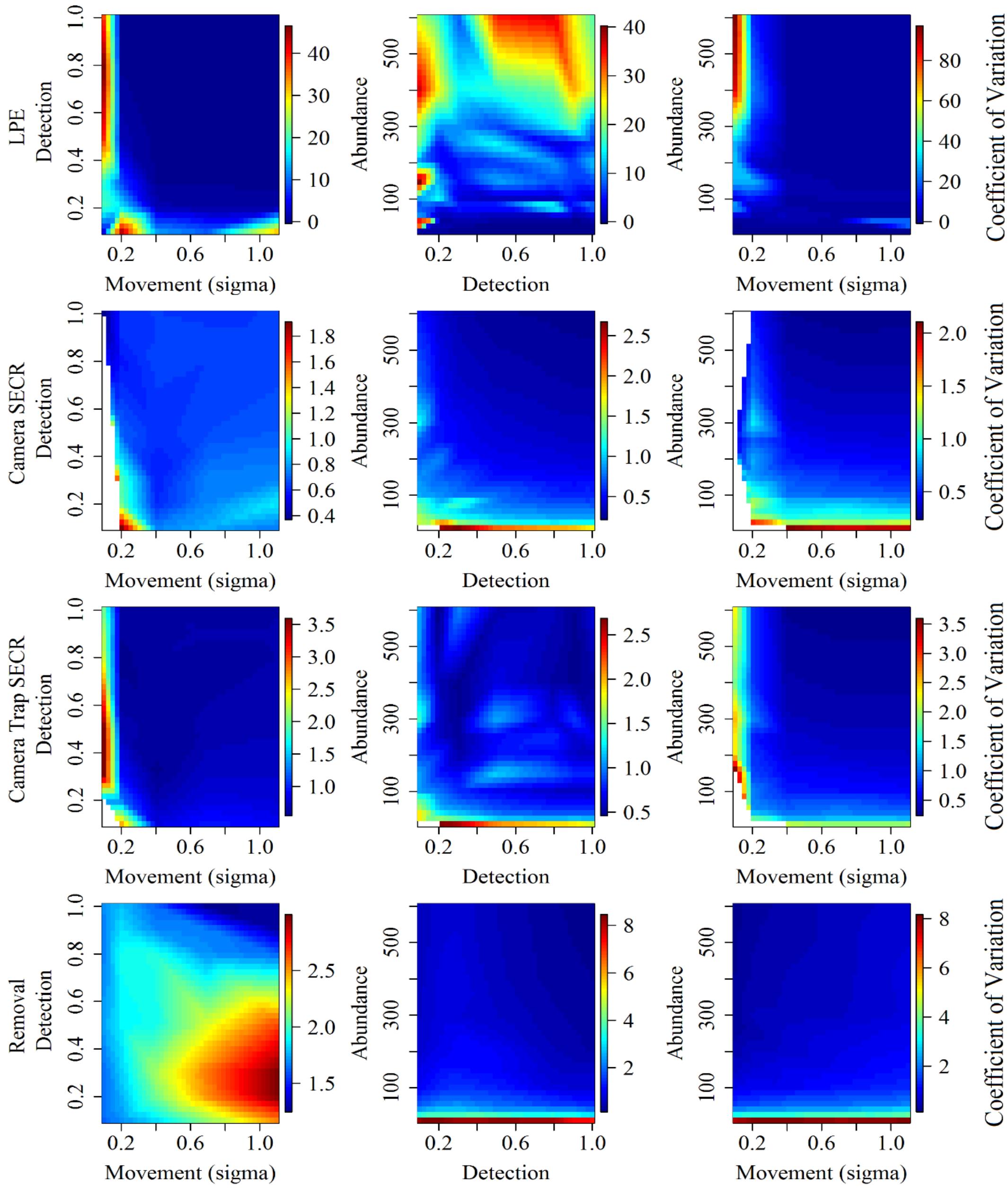
S3.3 Figure 1. Number of captured adult and subadult wild pigs (*Sus scrofa*) by field technique, Savannah River Site, South Carolina, USA, 2015.



S3.3 Figure 2. Scaled biased for the removal model using a mean maximum distance moved (MMDM) buffer (top row), half mean maximum distance moved (HMMDM), and a naïve buffer size (bottom row).



S3.3 Figure 3. Effects of buffer choice for the removal model. The red lines in the graph above depict the true density.



S3.3 Figure 4. Effects of movement parameters, probabilities of detection, and densities on unconstrained coefficients of variation of estimated densities by the tested analytical techniques: camera LPE, camera SECR, trap SECR, and removal models from simulations. The scale of coefficient of variation values varies. Combinations of parameters at which models did not run are displayed in white.

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Williams, B. L., R. W. Holtfreter, S. S. Ditchkoff, and J. B. Grand. 2011. Trap style influences wild pig behavior and trapping success. Journal of Wildlife Management 75:432-436.

Appendices S3.4, S3.5, S3.6, S3.7, and S3.8 will be included at time of publication

Appendix S3.4. R code for camera SECR and trap SECR models of wild pig density

Appendix S3.5. Sensitivity analysis of effects of uncertainty in the biomarker technique.

Appendix S3.6. Data for camera SECR analysis

Appendix S3.7. Data for trap SECR analysis

Appendix S3.8. Data for biomarker LPE, camera LPE, and removal models

CHAPTER 4

DEVELOPMENT OF KNOWN-FATE SURVIVAL MONITORING TECHNIQUES FOR JUVENILE WILD PIGS (*SUS SCROFA*)[3]

[3] Keiter, D. A, J. C. Kilgo, M. A. Vukovich, F. L. Cunningham, and J. C. Beasley. To be submitted to *Wildlife Research*.
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Abstract

Context. Despite the necessity of juvenile survival estimates to parameterize population models, to date no successful known-fate study of wild piglet survival ($\leq$ 5 months) has been conducted due to a lack of appropriate methodology for this species.

Aims. To aid in locating and tagging neonates, we piloted use of vaginal implant transmitters (VITs) in adult pigs, evaluated several combinations of transmitter types and attachment methods (i.e., stud ear-tag transmitters, clip ear-tag transmitters, sutured and epoxyed transmitters, harness transmitters, and surgically implanted transmitters) to monitor known-fate survival of piglets, and evaluated the effect of basic demographic factors (i.e., sex, weight) on transmitter retention.

Methods. We captured pregnant female pigs and implanted them with VITs. We tagged subsequently located neonates and piglets captured in traps with the aforementioned transmitter combinations and monitored them to determine retention times and feasibility of each method.

Key results. VITs were effectively used to determine the location and time of wild pig parturition allowing counting and tagging of neonates. Stud ear-tag and abdominal implant transmitters were well retained by piglets weighing 3 kg or greater, in contrast to the other tested transmitter types. Our model of factors affecting transmitter retention suggested a positive relationship between piglet weight at capture and transmitter retention time, although the effect size was not large.

Conclusions. Stud ear-tag and abdominal implant transmitters allowed known-fate monitoring of juvenile wild pigs, although, of these two types, stud ear-tag transmitters may be more practical as they do not require field surgery on piglets. Due to

their large size, the stud ear tag transmitters were infeasible for monitoring of true neonate piglets (~1 kg); however this application method may be suitable for neonates upon development of lighter-weight transmitters. The other transmitter attachment methods we tested were ineffective for monitoring of piglet survival due to poor retention of transmitters.

Implications. The techniques piloted in this study will facilitate research into the reproductive ecology of wild pigs and known-fate studies of piglet mortality to aid in population modeling and evaluation of factors affecting survival and cause-specific mortality of these often-invasive animals.

Introduction

Wild pig (*Sus scrofa*) populations, and Eurasian wild boar from which pigs descend, have been rapidly growing in abundance and geographic distribution over the past few decades globally (Bevins *et al*. 2014). This species has been introduced throughout numerous parts of the world (e.g., Africa, Australia, North America), and is frequently considered invasive, due to its negative impacts on native ecosystems (Barrios-Garcia and Ballari 2012; Bengsen *et al*. 2014). These impacts include deleterious effects on the local environment and wildlife through degradation of habitat, predation of and competition with native wildlife species, and transmission of infectious diseases (Campbell and Long 2009; Barrios-Garcia and Ballari 2012; Bevins *et al*. 2014; Keiter and Beasley in press). Beyond disease risk, wild pigs and wild boar also pose a threat to human health due to their increasing involvement in vehicle-collisions (Beasley *et al*. 2013; Sáenz-de-Santa-María and Tellaria 2015). Research has linked the population density of this species to the magnitude of their impacts on ecosystem and human health (Gortázar *et al*. 2006;

Beasley *et al*. 2013; Krull *et al*. 2016), necessitating improved knowledge of wild pig population dynamics to better evaluate risks posed by this species.

Wild pigs have the highest reproductive potential of any ungulate in North America (Taylor *et al*. 1998), and their life history traits more closely resemble those of small mammals or passerine birds than other ungulates (i.e., high reproduction, low survival; Servanty *et al*. 2011). Similar to other ungulates, however, adult survival of wild pigs and wild boar is generally higher and more constant than that of juveniles (Bieber and Ruf 2005; Hanson *et al*. 2009; but see Toïgo *et al*. 2008). For this reason, survival of juvenile wild pigs, or piglets, might be a strong driver of population dynamics in this species (Bieber and Ruf 2005; Servanty *et al*. 2011; Mellish *et al*. 2014). Despite the importance of this rate to population models, few studies have quantified piglet survival (Table 4.1); instead, studies of wild pig and wild boar population dynamics often base survival rates for piglets upon expert opinion (Servanty *et al*. 2011) or exclude animals of younger age classes (Hanson *et al*. 2009). Of the few conducted studies of piglet survival, the majority employed capture-mark-recapture techniques and harvest data (Table 4.1), despite a known bias in live-capture rates of wild pigs that also likely exists in harvest rates (Toïgo *et al*. 2008; Williams *et al*. 2011). Therefore, known-fate survival studies using radio-marked animals (e.g., Hayes *et al*. 2009) are needed to provide more robust estimates of juvenile survival. Previous research has, however, highlighted the difficulty in monitoring piglet survival and the necessity of developing techniques to 1) determine when and where parturition has occurred to allow tagging of piglets, and 2) subsequently monitor survival of juveniles (Baubet *et al*. 2009). Due to the lack of methodologies to accomplish these objectives, no successful known-fate study has been conducted on piglets less than approximately five months old (Keuling *et al*. 2013).

The success of a transmitter attachment mechanism to monitor animal survival depends greatly upon the morphology and behavior of the focal species. As such, numerous methods to attach transmitters have been developed and used in wildlife studies, including neck collars (Diefenbach *et al*. 2003), ear-tags (Keuling *et al*. 2010), suturing (Dreitz *et al*. 2011), epoxy (Fedak *et al*. 1983), harnesses (Hubbard *et al*. 1998), and surgical implantation inside the study animal (Hernandez *et al*. 2010). Neck collars, a frequently used attachment type, are infeasible in juvenile wild pigs due to their morphology (i.e., the head is approximately the same size as the neck) and rapid growth, necessitating evaluation of other potential attachment mechanisms. The overall size or weight of a transmitter must also be considered, as transmitters above a threshold size might affect animal behavior (Aldridge and Brigham 1988) or bias estimates of demographic rates (Warner and Etter 1983). Another potential hurdle to monitoring survival of juvenile wild pigs is the risk of researcher-induced abandonment of piglets by the mother; in France, Baubet *et al*. (2009) documented high rates of litter abandonment (50% of occasions) during or immediately following tagging of piglets with radiotransmitters. Natural abandonment (i.e., uninfluenced by human presence or activities) of piglets has also been documented and occurs when a piglet cannot keep up with the associated female and litter, resulting in its separation and subsequent mortality (Barrett 1978). Therefore, when evaluating the aptness of a technique to monitor known-fate survival of juvenile wild pigs, it is necessary to consider the attachment mechanism used, relative size of the transmitter, and the amount of time required for and invasiveness of the tagging procedure.

In this study, we present the first use of vaginal implant transmitters (VITs) in wild pigs to determine parturition date and location, and we evaluate the effectiveness of several combinations of transmitter units and mechanisms to attach these very high frequency (VHF) radiotransmitter units to wild piglets for known-fate monitoring. These methods include use of 1) stud ear-tag transmitters, 2) clip ear-tag transmitters, 3) sutured and epoxyed transmitters, 4) harness transmitters, and 5) surgically implanted transmitters. We compare these attachment techniques in terms of retention time and feasibility constraints affecting their success as a tool for monitoring survival of piglets and evaluate factors affecting retention time. Finally, we discuss implications and potential applications of the techniques.

Materials and methods

Study area

We conducted this research at the Savannah River Site (SRS), a 78,000 ha U.S. Department of Energy facility on the South Carolina-Georgia border, USA. Habitat on the SRS is managed by the USDA Forest Service (USFS) and is comprised mostly of upland pine forests with areas of bottomland hardwood and swamps characteristic of much of the Southeastern U.S. (Imm and McLeod 2005). Wild pigs on the SRS are the descendants of feralized domestic pigs that were released when the public, including farmers, were moved from the SRS in 1952, although the population shows morphological signs of genetic introgression by wild boar (Gaines *et al*. 2005; Mayer and

Brisbin 2008). Contractors of the USFS have controlled wild pigs lethally on the SRS since 1952 in an effort to reduce damage to habitat (Mayer and Brisbin 2008).

VIT Deployment

All capture and handling of animals was conducted in compliance with the University of Georgia's Animal Care and Use Committee (Permit: A2015 05-0004-Y2-A1). From December 2013 – July 2016, we captured wild pigs in corral or box traps baited with whole corn. We immobilized adult and subadult animals via dart rifle (X-CALIBER™, Pneudart, PA, USA) using a combination of Telazol® (4.4 mg/kg; MWI Veterinary Supply, Idaho, USA) and Xylazine (2.2 mg/kg; Wildlife Pharmaceuticals Inc., CO, USA). We determined age of captured individuals through examination of dentition (Mayer 2002*a*), recorded sex, and collected a tissue sample for future genetic analyses. We assessed whether captured females of ≥ 27 kg (Servanty *et al*. 2011) were pregnant using a portable ultrasound (SeeMore USB™, Interson Corporation, CA, USA). We implanted pregnant females with a 21-g VIT (M3930; Advanced Telemetry Systems [ATS], Isanti, Michigan, USA; Figure 1) in a manner similar to previous studies of white-tailed and mule deer (e.g. Bishop *et al*. 2011; Kilgo *et al*. 2012). In short, VITs were inserted into the vagina within a sterilized, rigid clear plastic tube and extruded using a metal plunger; we oriented the wings of the VIT laterally within the animal. We attached a VHF collar (Model M2520B, ATS) to females implanted with a VIT to facilitate monitoring; because the VHF collar is externally attached, unlike the VIT, it can transmit a signal greater distances which allowed us to locate the implanted female with greater ease. We monitored VITs 4-7 times weekly until the occurrence of parturition.

Tagging of Piglets

We captured juvenile wild pigs in two manners: 1) with adult or subadult wild pigs in box or corral traps baited with whole corn, or 2) by hand at the farrowing nest, which we located through use of VITs, shortly after parturition. *Sus scrofa* is one of few species known to create farrowing nests, and it is suspected that female movements are reduced during farrowing and for a short period of time following parturition (Mayer *et al*. 2002*b*). The only previous study to attempt to tag piglets at the farrowing nest encountered high rates of researcher-induced abandonment of piglets by females, resulting in rapid mortality of tagged animals (Baubet *et al*. 2009). In an attempt to avoid this outcome we waited 2-3 days following parturition to tag piglets at the farrowing nest, in the hope that greater bonding might occur between the female and piglets. We tracked VITs to farrowing nests and captured piglets by hand; in each case the female fled, allowing us to tag the piglets. During tagging, we attempted to limit disturbance that might attract predators or cause abandonment of piglets by minimizing noise and placing captured piglets in pillowcases with attached zippers. We assessed proximity of the female to the farrowing nest using telemetry during tagging of piglets, and found that in each case the female remained relatively close (estimated ≤ 300 m).

We tagged piglets captured at the farrowing nest with one of three techniques: sutured and epoxyed transmitters, harness transmitters, or surgically implanted transmitters (Figure 4.1). Piglets captured in corral or box traps were tagged with either stud ear-tag transmitters, clip ear-tag transmitters, surgically implanted transmitters, or sutured and epoxyed transmitters (Figure 4.1). The transmitters we attached to piglets via suturing and epoxy (9.0 g), harnesses (9.0 g), and clip ear-tags (8.4 g) were designed by

ATS according to our specifications and incorporated a mortality sensor set to activate following 12 hours without movement by the piglet. The surgically implanted transmitters weighed 11.0 g and incorporated a mortality sensor with the same settings (IMP100, Telonics, Inc., Mesa, AZ, USA). The transmitters we attached using the stud ear-tag mechanism were relatively large (20.0 g) and incorporated a mortality sensor set to activate following four hours without motion by the piglet (Model ZV2E 152, Lotek Wireless, Newmarket, Ontario, Canada; Model M3420, ATS). We attached a VHF collar (Model M2520B, ATS) to associated subadult or adult female pigs caught in corral or box traps with piglets to facilitate monitoring of tagged piglets.

Sutured transmitters

Prior to attaching sutured transmitters, we injected piglets with a local analgesic, Lidocaine (2%, MWI Veterinary Supply, ID, USA), at the site of attachment, but did not chemically immobilize the animals. We attached sutured transmitters dorsally, between the scapulae using dermal surgical sutures through anchor points on the transmitter body (Figure 4.1). We also applied a commercially available epoxy (the Gorilla Glue Company, Cincinnati, OH, USA) to the bottom of the transmitter and the site of attachment in an attempt to improve retention time for this transmitter type (Fedak *et al*. 1983). We injected captured piglets with penicillin (dosage: 1 mL/45.3 kg; 300,000 units/mL; Durvet Inc., Blue Springs, MO, USA) prior to release to decrease risk of infection. A licensed veterinarian trained field personnel in proper suturing techniques and use of analgesics prior to our implementation of this method.

Harness transmitters

Baubet *et al*. (2009) used harnesses constructed of elastic bands to attach transmitters to wild boar piglets, but found that piglets retained transmitters for only 2.5 days on average. In this study, we constructed harnesses from a Teflon ribbon (Bally Ribbon Mills, Bally, PA, USA) used in the attachment of harnesses to vultures (Holland 2015), that we believed might be more resistant to removal by associated females. We sized these harnesses based upon morphometric measurements of previously captured neonate piglets and sewed a 10-cm band of elastic material on either side of the harness to allow growth of the piglet. We mounted the radiotransmitter ventrally on the piglet in an additional attempt to make removal of this transmitter type by the associated female less likely (Figure 4.2). Chemical immobilization of captured piglets was not necessary to attach harnesses.

Surgically implanted transmitters

We immobilized captured piglets via intramuscular injection of a combination of Ketamine (10 mg/kg; MWI Veterinary Supply) and Xylazine (0.5 mg/kg; MWI Veterinary Supply) and administered Lidocaine via subcutaneous injection at the site of surgery. We sterilized transmitters and surgical tools in Nolvasan Solution (Zoetis Animal Health, Kalamazoo, MI, USA) and used surgical drapes to maximize sterility of field conditions. We created an incision in the abdomen of the immobilized piglet through the dermal layers and muscle tissue using a scalpel and inserted the sterilized transmitter into the exposed abdominal cavity. For males we located the incision anterior to the umbilicus and penis, whereas in females the incision was located posterior to the umbilicus. We injected additional Lidocaine directly into the muscle tissue of the abdomen. We closed the incision using one internal layer of surgical sutures through the muscle tissue and employed a second set of internal surgical sutures in the dermis (Figure 4.2). We used commercially available cyanoacrylate (‘super glue’) to further seal the incision, and then injected the piglet with penicillin to decrease risk of a post-operation infection. Finally, we used an intramuscular injection of Yohimbine (2mg/mL; MWI Veterinary Supply) to reverse the chemically immobilized animals. Surgery to implant transmitters into piglets was performed by field personnel trained by a licensed veterinarian.

Stud ear-tag transmitters

During attachment of stud ear-tag transmitters, piglets were chemically immobilized by intramuscular injection using the same dosages of Telazol and Xylazine as above to allow collection of biological samples (e.g. blood, tissue). It should be noted, however, that chemical immobilization to allow attachment of this transmitter is likely unnecessary, as a similar attachment technique (i.e., stud ear-tag) is approved for use on livestock without chemical immobilization. The larger size of these transmitters (20 g) precluded their use on piglets of approximately ≤ 3 kg, as the piglet’s ear was not large enough to adequately support the transmitter. We used the stud ear-tag mechanism to attach two transmitter body types (Model ZV2E 152 [Lotek Wireless] and Model M3420 [ATS]); we considered these two transmitter types to represent one category due to the fact that they were attached in the same manner and had similar specifications (e.g., weight, pulse rate, battery life, etc.; Figure 4.2).

Clip ear-tag transmitters

As with the stud ear-tag transmitters, piglets were chemically immobilized during attachment of clip ear-tag transmitters to allow collection of biological samples, although this may not be necessary for field application under all circumstances. Before attaching this transmitter type, we used a 5-mm biopsy punch to create a hole in the center of the captured piglet's ear, through which the clip could be threaded.

Monitoring

We located tagged piglets via radiotelemetry 3-7 times for the first week following capture, and 2-4 times weekly thereafter, with the exception of piglets tagged with stud ear-tag transmitters (monitored every 7-10 days). We monitored tagged piglets until mortality of the animal, detachment of the transmitter, hereafter failure, or a minimum of 3 months had passed. When we detected a mortality signal, we homed in on the transmitter and attempted to determine whether the signal was caused by mortality of the piglet or transmitter failure. In each case, we photographed the location of the transmitter and employed a thorough search of a 20-m radius circle surrounding the recovered transmitter for evidence of mortality. If there were no signs of mortality (e.g., carcass, bone fragments, signs of a struggle, bite marks on transmitters, etc.), we assumed that the transmitter attachment mechanism had failed, in order to be conservative in estimating piglet mortality rates. In cases of mortality, we determined the cause of mortality based upon carcass condition, presence of predator tracks, characteristics of cache sites, and patterns of piglet carcass consumption (Kilgo *et al*. 2012).

Data analysis

We compared average retention times of transmitters, excluding any animals that suffered mortality during the study. We also evaluated eight *a priori* models to determine the effect of animal weight at capture, sex, and transmitter type on the amount of time a transmitter was retained (Table 4.2). We used generalized linear mixed models in which the covariates of interest (weight, sex, transmitter type) were incorporated as fixed effects and litter was incorporated as a random effect. In these models, we assumed retention time would have a Poisson distribution, representing the count of days that the transmitter was attached to the tagged animal. We compared support for models using change in second order Akaike's Information Criterion (ΔAIC_C) and AIC_C weight (AIC_{Cw}), measures of model likelihood (Burnham and Anderson 2002). We considered a model to be most supported if the ΔAIC_C value of the next most supported model exceeded 2.0 (Burnham and Anderson 2002), and performed model averaging in the event of model selection uncertainty. We performed all analyses in R (R Core Team 2014) using packages *lme4* and *AICcmodavg*.

Results

We implanted 14 female pigs with VITs, resulting in the capture of 28 neonate piglets from seven females. Due to the handling time associated with each tagging technique, we did not radio-tag every neonate that we captured. On three occasions, we experienced failure of the VIT due to its expulsion from the pig prior to birth of piglets. On three additional occasions, we believe that VIT battery failure or misinterpretation of pregnancy status from ultrasound was responsible for failure to locate piglets. In one case, we tracked a VIT to a farrowing nest which had been flooded and contained carcasses of piglets. Results of the necropsy of piglets discovered in this nest were inconclusive, but ruled out their mortality due to drowning or stillbirth (UGA Veterinary Diagnostic Lab, personal communication). We found that piglets were relatively mobile at three days of age (i.e., able to walk or run) and we recorded one observation of a female and piglets > 300 m from the farrowing nest 3 days following parturition. When we approached the farrowing nest, piglets often exhibited a hiding strategy in which they did not move far from the nest (≤ 5 m), but held still when approached.

We captured, tagged, and monitored 71 piglets using the five combinations of transmitters and attachment mechanisms (Table 4.3). Of these piglets, 18 were captured by hand at the farrowing nest (25.4%), while 53 were captured in corral or box traps (74.6%). We discuss the cost, advantages, and disadvantages of each combination of transmitter and attachment mechanism in Table 4.4. We found that stud ear-tag transmitters were retained well by tagged piglets ($\bar{X}$ = 143.0 days, SE = 14.05), however, the large size of the transmitter's body precluded their use on true neonates or small-sized individuals (i.e., less than approximately 3 kg). Sutured transmitters, harness transmitters, and clip ear-tag transmitters exhibited poor retention times preventing successful monitoring of piglet survival using these attachment techniques (range of $\bar{X}$ = 2.6 - 20.6 days; Table 3). Whether the detachment of clip ear-tag and sutured transmitters was due to snagging of the transmitter on vegetation or behavior by conspecifics is unknown; however, we observed newly tagged piglets chewing and pulling on transmitters attached to other members of the litter. All harness transmitters failed 1-3 days after deployment due to removal of the Teflon straps and/or elastic bands via chewing. We attributed the removal of harnesses to the associated female pig rather than a predator, as there was no evidence of piglet mortality present at the sites where we recovered transmitters, and radiotelemetry suggested that harnessed piglets were travelling in company with the female immediately prior to harness removal. Given the near-immediate failure of all harness and sutured transmitters, we ceased trials of these attachment methods after deployment on four and ten piglets, respectively. Transmitters that were surgically implanted into piglets of ≥ 3 kg were successfully retained until battery failure occurred, approximately 3 months following deployment. In one case, an implanted 3-kg piglet suffered mortality within two days of release following surgery and was censored from analyses. We are uncertain whether this mortality was related to the surgical procedure. We attempted implantation of radiotransmitters into 2-day old and 3-day old neonates, but following failure of the surgery on two occasions, discontinued further attempts.

Due to small sample sizes of abdominal implant, sutured, and harness transmitters (Table 4.3), we excluded these transmitter types from analysis of factors affecting retention times and modeled retention of stud and clip ear-tag transmitters. We encountered model selection uncertainty and conducted model averaging (Table 4.2, Table 4.5). Model averaged estimates of coefficients demonstrated that stud ear-tag transmitters were retained significantly better than clip ear-tag transmitters (Table 4.5). In addition, the models suggested that as the weight at capture increased, the number of days a transmitter was retained also increased, although this effect may be relatively small, and that females tagged with clip ear-tag transmitters retained those transmitters better than males tagged with clip ear-tags (Table 4.5).

For all transmitter types, excluding mortality within a two-day censoring period, only three monitored piglets died throughout the study and were not included in models of retention times. Eleven tagged piglets of six litters were depredated or died and were scavenged within two days of tagging, and were thus censored. Although it is not possible to conclusively determine, in six of these cases, we believe that the mortalities resulted from separation of the piglet from the associated female, which may have been prompted by researcher activities. It is possible that separation occurred in some of these cases during the recovery of the tagged piglet from chemical immobilization. We only observed evidence suggesting researcher-induced abandonment by the female in one litter of piglets captured in a trap; despite being released at the same time as the female, all four tagged piglets were found depredated within two days, while the female was found alive within 1 km of the capture location. In addition to instances of observed separation, we encountered one situation in which five neonate piglets in a litter were tagged using harness transmitters and created a large amount of noise during the tagging process; three of these piglets were found depredated the following day, while the other two were determined to be alive and with the mother. Of the 11 observed scavenging or depredation events, caching evidence suggested that coyotes were responsible for the majority (90.1%; Figure 4.3), although further study is clearly necessary.

Discussion

No successful known-fate survival study of juvenile wild pigs or related wild boar has been conducted due to 1) difficulty in determining parturition date and location to allow tagging of piglets, and 2) lack of a technique to successfully monitor known fates of piglets. In this study, our use of VITs allowed us to determine with a high degree of accuracy when and where parturition by wild pigs took place. We did occasionally experience unsuccessful use of VITs as a result of premature expulsion from the female

(21.4%) or misreading of ultrasound results and equipment failures (21.4%), demonstrating that refinement of this technique for wild pigs is necessary. In addition to allowing the capture of neonates for tagging, VITs offer future avenues of research into the reproductive ecology of this species. Use of VITs in conjunction with GPS collars or triangulation might allow greater research into the natural history of wild pigs and how movement relates to parturition (Kurz and Marchinton 1972; Baubet *et al*. 2009).

We evaluated five combinations of potential attachment mechanisms and transmitter bodies and found that surgical implantation of a radiotransmitter into the abdominal cavity or attachment of stud ear-tag transmitters can be successfully employed to monitor survival of juvenile wild pigs (≥ 3 kg). Surgical implantation of transmitters has been employed successfully on other species (e.g. nine-banded armadillos [*Dasypus novemcinctus*]; Hernandez *et al*. 2010), but its use in juvenile wild pigs is novel. We believe the success of this transmitter type is due, in large part, to the internal placement of the transmitter which prevents potential loss due to snagging on vegetation or conspecific activity. Use of stud ear-tag transmitters to monitor survival of piglets is, however, likely advantageous over implant transmitters, in that this technique does not require surgery on captured piglets, is less costly, and may meet animal welfare requirements without chemical immobilization. Two-day old and three-day old piglets weighed approximately 1 kg, meaning that the piglets we tagged with these methods (≥ 3 kg) were likely a minimum of one month old (Barrett 1978). Therefore, further study is necessary to assess survival of piglets below this size threshold. In addition, miniaturization of the transmitter body that we attached via the stud ear-tag mechanism may allow monitoring of true neonate piglets.

The positive relationship we found between piglet weight at capture and transmitter retention, although small, suggests that, generally transmitters will be more effective for gathering data on larger-sized piglets, supporting previous observations of the difficulty in monitoring neonates of this species (Baubet *et al*. 2009). Clip ear-tag transmitters exhibited poor retention rates overall, but females retained these transmitters better than males; this relationship in clip ear-tag transmitters could potentially have its roots in social interactions among the litter (e.g. Newberry *et al*. 1988) or be an artifact of small sample sizes. In contrast to Baubet *et al*. (2009), we did not observe abandonment of any litter of neonates tagged at the farrowing nest (although we observed evidence of potential abandonment of a litter of piglets tagged in a trap), suggesting that the methods we tested will not result in highly-biased estimates of survival as a result of neonate abandonment. However, it is possible that some of the mortalities we observed throughout the study were due to the response of the female pig to researcher activities. Therefore, careful consideration and refinement of tagging and handling techniques is still necessary. We recommend that researchers minimize noise while in the vicinity of the farrowing nest, as excessive noise may attract predators or increase the chance of the female abandoning the litter. Similarly, minimizing time spent at the farrowing nest tagging piglets should reduce disturbance and possible olfactory cues that might be picked up by predators. Finally, situations in which piglets are chemically immobilized could potentially predispose them to separation from adults as a result of the physiological effects of recovery.

We observed that piglets, singly or in a litter, experienced high mortality rates when they were separated from the associated female. This implies that the adult pig associated with a litter might be effective at avoiding potential dangers or defending piglets against predators, allowing successful recruitment of offspring into the population (Vetter *et al*. 2016), and therefore, mortality of the female might also result in mortality of the offspring under certain circumstances. Adoption of ‘orphaned’ litter members by other female wild pigs could potentially reduce losses caused by abandonment or mortality of the mother, but more research is necessary to assess survival rates of orphaned or abandoned piglets and the frequency with which adoption events occur under natural circumstances. Additional research is also necessary to determine if independence exists in survival probabilities of piglets from the same litter. Our data suggest that, at least in cases of female abandonment, multiple members of the litter are likely to suffer mortality, suggesting non-independence of fates. Recent research in captive-holding facilities suggests that the personality traits of female wild boar influence litter survival in absence of predators (Vetter *et al*. 2016), and additional studies might investigate the influence of mother’s age, total litter size, and environmental conditions on the survival of neonate wild pigs.

Previous research reported crushing, conspecific aggression, depredation, exposure, and starvation as causes of death in wild piglets (Barrett 1978; Baubet *et al*. 2009). Although evidence suggested that the majority of mortality events we observed were depredation events, piglets in our study area are undoubtedly affected by these causes as well. Our discovery of non-stillborn, dead, two-day old piglets in the flooded farrowing nest confirms that piglets on site die due to causes other than depredation, despite the fact that cause of death was not conclusively determined. The discovery of these piglets also further highlights the need to monitor piglet survival as soon as feasible following birth in order to obtain the best possible data on survival rates for use in monitoring and modeling of population dynamics. Further development of attachment techniques to allow their use on true neonate animals is, therefore, necessary.

Management implications

Use of effectively retained stud ear-tag transmitters or surgically implanted transmitters will allow future studies to determine cause-specific mortality in wild piglets and evaluate the effects of environmental and demographic factors on mortality rates, thereby facilitating refinement of population models for this abundant invasive species. Use of VITs to determine litter sizes and allow tagging of neonates will also allow improvement in estimates of demographic rates in wild pigs. Our preliminary monitoring results suggest piglets may experience relatively low natural mortality rates, necessitating more effective lethal control of this age class to prevent population growth. Work by Bieber and Ruf (2005) and Mellish *et al*. (2014) has suggested the importance of management actions affecting juvenile pigs, but was largely unsupported by estimates of known-fate survival. Our data suggest that separation of piglets from the associated female, as might be caused by the female's mortality or abandonment of piglets, could predispose piglets to mortality, although more research is necessary. It is likely, however, that after piglets attain a threshold body size, they will be able to survive in absence of their mother, and therefore further known-fate study is also necessary to determine whether a threshold body size exists and when it is reached. Until an attachment technique is developed for true neonate piglets, use of VITs to count piglets immediately after parturition in conjunction with subsequent monitoring may allow coarse estimation of survival rates for piglets until they attain a size sufficient to allow tagging.

Acknowledgements

We would like to thank E. Bledsoe, E.J. Borchert, K. Eckert, R. Juarez, and P. Schlichting for their hard work and effort in tagging and monitoring piglets. We would also like to thank T. Grazia, M. Larsen, T. Mims, J. Nance, and various contractors of the US Forest Service for their support of this project, and O.E. Rhodes Jr. and R.B. Chandler for their feedback on this manuscript. This study was funded and supported by USDA-APHIS National Wildlife Research Center (grant number 14-7428-1087), by the US Department of Energy Office of Environmental Management (award number DE-FC09-07SR22506), and through Interagency Agreement number DE-AI09-00SR22188 with the USDA Forest Service - Savannah River, and by the USDA Forest Service Southern Research Station.

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Tables

Table 4.1. Basic descriptions of previous published studies estimating piglet (*Sus scrofa*) survival rates, 2016.

Citation	Population type; Country	Method
Jezierski 1977	Wild boar; Poland	Comparison of number of lactating teats on captured female versus number of piglets captured with female
Barrett 1978	Wild pigs; USA	Long-term visual observation
Singer and Ackerman 1981	Wild pigs, USA	Comparison of observed piglet number versus average pre-natal litter sizes from culled animals
Baubet *et al.* 1985	Wild boar; France	Capture-mark-recapture
Baber and Coblentz 1986	Wild pigs; USA	Comparison of observed piglet number versus average pre-natal litter sizes from culled animals
Toïgo *et al*. 2009	Wild boar; France	Capture-mark-recapture and harvest
Hanson *et al*. 2009	Wild pigs; USA	Capture-mark-recapture/resight, and harvest
Baubet *et al*. 2009	Wild boar; France	Known-fate using telemetry; unsuccessful due to poor transmitter retention

Table 4.2. Model selection results for *a priori* models of factors affecting retention of stud ear-tag (N = 17) and clip ear-tag transmitters (N = 22) by tagged wild piglets (*Sus scrofa*), Savannah River Site, South Carolina, USA, 2013-2016

[a], indicates the number of parameters including random effect variance (Litter) and a global intercept term. [b], is the negative log-likelihood value of the model. [c], represents the combination of transmitter and attachment mechanism (i.e. stud ear-tag or clip ear-tag transmitter).[d], is the sex of the tagged piglet. [e], is the weight of the tagged piglet at capture in kilograms.

Model	K[a]	AICc	ΔAICc	$AICc_{wt}$	-LL[b]
Type[c]*Sex[d]+Weight[e]	6	445.50	0.00	0.55	-215.20
Type*Sex	5	445.90	0.40	0.45	-216.88
Weight+Type	4	462.18	16.68	0.00	-226.40
Weight+Type+Sex	5	464.88	19.38	0.00	-226.37
Type	3	471.02	25.52	0.00	-232.11
Weight	3	479.75	34.24	0.00	-236.47
Null	2	486.80	41.30	0.00	-241.21
Sex	3	488.99	43.49	0.00	-241.09

Table 4.3. Summary data for performance of each transmitter and attachment mechanism combination tested on juvenile wild pigs (*Sus scrofa*), Savannah River Site, South Carolina, 2013-2016.
[a], indicates the combination of a transmitter unit and attachment mechanism.

Transmitter[a]	# Tagged (# litters)	# Transmitter failures	Mean transmitter retention (days)	SE
Large ear-tag	23 (6)	3	143.0	14.05
Small ear-tag	22 (6)	16	20.6	2.31
Sutured and epoxyed	10 (3)	10	5.0	1.03
Harness	7 (3)	4	2.6	0.4
Implant	9 (3)	0	98.3	3.25

Table 4.4. Practicality of transmitter types for monitoring survival of wild piglets (*Sus scrofa*), Savannah River Site, South Carolina, USA, 2013-2016

Transmitter	Cost (each)	Considerations
Stud ear-tag	\$179.55 - \$209.00	• High retention rates • ~5 month warranted battery lifespan • Large size disqualifies use on piglets ≤ 3.0 kg, but miniaturization may be possible • May not require chemical immobilization
Clip ear-tag	\$179.55	• Low retention rates • ~8 month warranted battery lifespan • May not require chemical immobilization
Sutured	\$179.55	• Low retention rates • ~8 month warranted battery lifespan • Requires basic surgical techniques (suturing) in field • Does not require chemical immobilization
Harness	\$179.55	• Low retention rates • ~8 month warranted battery lifespan • Does not require chemical immobilization • Requires manufacturing of harnesses
Implant	\$217.00	• High retention rates on piglets ≥ 3 kg • More testing necessary on neonates • ~2 month battery lifespan • Requires chemical immobilization • Requires performance of surgical techniques in field • May benefit from a holding period to allow recovery of piglets from surgery

Table 4.5. Model averaged parameter estimates (β) from generalized linear mixed effects models of factors affecting transmitter retention on wild piglets (*Sus scrofa*) at the Savannah River Site, South Carolina, USA, 2013-2015.

Parameter	β	SE	95% CI
Intercept (stud ear-tag transmitter, male)	4.59	0.17	4.25 to 4.93
Clip ear-tag transmitter	-1.8	0.21	-2.21 to -1.38
Weight	0.02	0.01	0.00 to 0.05
Female	0.01	0.04	-0.07 to 0.09
Clip ear-tag transmitter*Female	0.62	0.13	0.37 to 0.87

Figures

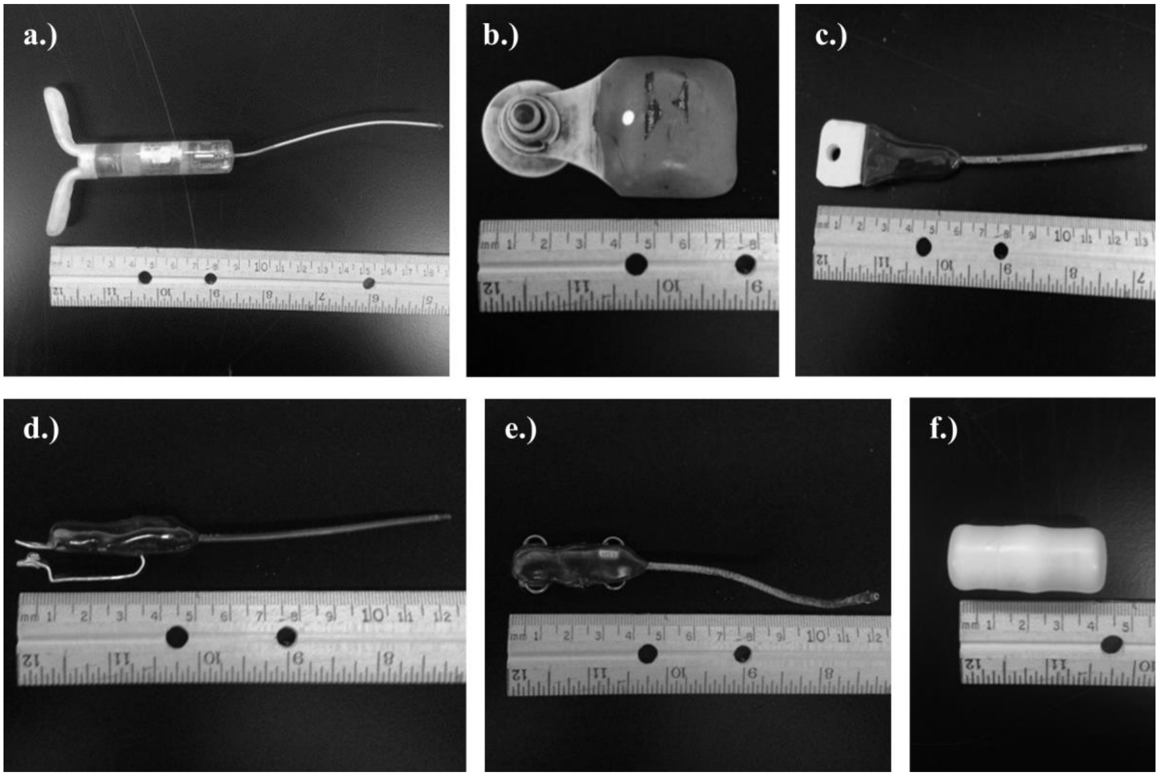

Figure 4.1. Tested combinations of transmitters and attachment mechanisms to monitor wild piglet (*Sus scrofa*) survival at the Savannah River Site, South Carolina, USA, 2013-2016. The above figure depicts a vaginal implant transmitter (VIT; a), stud ear-tag transmitters (b-c)), side-view of a clip ear-tag transmitter (e), a transmitter with anchor points for suture or harness material attachment (f), and a surgically implantable transmitter (g).

a.) b.) c.)

Figure 4.2. Selected examples of wild piglets (*Sus scrofa*) tagged with transmitters to monitor survival at the Savannah River Site, South Carolina, USA, 2013-2016. Photographs consist of a piglet tagged with a stud ear-tag transmitter (a), harness transmitter (b), and transmitter surgically implanted into the abdomen (c).

Figure 4.3. Depredated wild piglet (*Sus scrofa*) found cached in a manner suggesting that the piglet was killed by a coyote (*Canis latrans*), Savannah River Site, South Carolina, USA, 2015.

CHAPTER 5

CONCLUSIONS

Despite the growing global importance of wild pigs (*Sus scrofa*) as an invasive species, many questions remain surrounding the population ecology of this species. This is in large part due to a lack of methodologies required to monitor wild pig populations. Within this master's thesis, I evaluated and developed a number of tools that can be used to improve our understanding of wild pig population dynamics and act as a foundation for future research of this important species. Many of the techniques evaluated within are applicable to numerous species of interest and sampling situations, and should, therefore, represent a substantive contribution to the scientific literature of population ecology.

In Chapter 2, I compared a series of radial search protocols to a previously implemented adaptive cluster sampling protocol in terms of their effectiveness at encountering wild pig scats. In addition, I evaluated the effects of environmental conditions (i.e. ground cover, rain prior to sampling) and scat characteristics (i.e. size and number of fecal pellets) on the detectability of wild pig scats. My finding that radial search protocols of certain sizes (i.e. 15 m and 20 m radius) were more effective at encountering scats than the previously applied adaptive cluster sampling approach, indicates the potential utility of this technique in collection of scat belonging to this and other social ungulates. I also found that each of the potential factors we tested (listed above) influenced the detection of scat by observers. For this reason, in order to increase the amount of scat encountered by observers, it is likely most effective to sample at a time when levels of ground cover are lowest on the landscape, such as winter, and not sample immediately following rain. Further, the relationship we found between fecal pellet size and detectability may imply that smaller-sized or juvenile animals are less detectable than larger-sized or adult animals, potentially biasing estimates generated through scat collection. The relationships that I found between environmental and scat characteristics and detectability of samples can be applied to refine scat sampling protocols for many species.

In Chapter 3, I evaluated the robustness of a suite of analytical techniques to ecological and observational processes (i.e. underlying probability of detection, true population density, and movement rates) using simulations based upon empirical data. I also compared combinations of field and analytical techniques in their implementation at three study sites to estimate density of wild pigs. I found that density estimators generally tended to be most affected by the movement rates of the focal species, with the exception of the removal estimator which was most affected by the true population density. Each of the different techniques was most applicable under certain circumstances in terms of providing accurate and/or precise estimates, and in terms of field application. The large influence of movement rates on resulting estimates also emphasizes the importance of effectively calculating the area sampled by abundance estimators or employing a density estimation technique that implicitly accounts for spatial variation. The field techniques I tested (biomarker use, camera trapping, and live-trapping and euthanasia) can be applied to a variety of species to estimate population density.

In Chapter 4, I piloted use of vaginal implant transmitters in wild pigs to determine parturition date and location and facilitate tagging of neonate piglets. I also compared five combinations of attachment mechanisms and transmitter body types in their effectiveness in allowing monitoring of juvenile wild pigs. I found that VITs could be used effectively to locate the farrowing nest and capture neonate wild pigs. I found that two of the transmitters I tested, attached via stud ear-tag or implanted surgically into the abdominal cavity of the piglet, were effectively retained, allowing monitoring of tagged animals greater than approximately 3 kg in size. The other transmitter mechanisms I tested were not retained by tagged animals, preventing successful monitoring of those individuals. I also found evidence that transmitters were retained for longer periods of time on piglets that were larger (i.e. weighed more) at the time of capture, which further highlights the difficulty in monitoring true neonates of this species. I did not observe abandonment of piglets tagged at the farrowing nest, in contrast to previous research, which suggests that these methods may be effectively employed, although further development will be necessary to allow monitoring of true neonates.

Based upon the conclusions within this master's thesis, we can begin to undertake greater work to fill critical knowledge gaps regarding the ecology and management of wild pigs. The scat collection protocols within can be used to maximize scat collection from this or other social ungulate species, allowing improved inference as a result of greater sample sizes. The recommendations regarding selection of a density estimator should be valuable for providing causal links between wild pig populations and levels of damage to ecosystem and human health. In addition, accurate density estimation will facilitate assessment of how effective management strategies are at mitigating the risks posed by this species, allowing refinement of control campaigns. Finally, the techniques developed within to monitor survival of piglets will allow greater understanding of the population dynamics of this species and facilitate assessment of cause-specific mortality in juveniles. For all these reasons, this thesis represents an important contribution to the literature surrounding wild pigs and, more generally, population monitoring.