EFFECTS OF INTRINSIC AND ENVIRONMENTAL ATTRIBUTES ON THE REPRODUCTIVE ECOLOGY OF INVASIVE WILD PIGS (*SUS SCROFA*)

by

SARAH M. CHINN

(Under the Direction of James C. Beasley)

ABSTRACT

Population dynamics reflect the unique interactions between individual physiology and behavior with the environment and thus success, which regulates the number, spatial distribution, and genetic composition of populations. Identifying drivers of population dynamics of wild animals is important for determining why and how populations fluctuate spatially and temporally under changing conditions. Therefore, a comprehensive understanding of reproduction and the conditions contributing to successful reproductive events is critical, especially for invasive species because they can significantly alter ecosystems upon establishment in new habitats. Wild pigs are ecological generalists with the highest reproductive potential of any large ungulate, making them one of the most successful invasive species in North America. As populations continue to increase and expand there is a need to elucidate factors that influence reproduction will improve population models that can be used to inform management strategies. To address these gaps in knowledge, I conducted a broad assessment of the reproductive ecology of wild pigs across multiple seasons and years to identify individual and environmental attributes that contribute to female reproductive

success and juvenile recruitment. I found that reproduction occurred throughout the year, with peaks in conception followed by farrowing that coincided with seasonal food availability. Further, hormone quantification and fetal counts revealed many wild pigs were reproductively mature and contributed to population growth prior to one year of age, although increases in mass and age positively influenced litter size. I found that adjustment of litter size appeared to be the primary reproductive characteristic manipulated to increase fitness rather than adjustment of offspring sex ratio. Females decreased home range size from gestation to farrowing and gradually increased home range post-farrowing during the neonatal care period. Wild pigs selected for both upland and bottomland hardwood forests and avoided pine forests and developed areas during reproduction. I also present the first successful study of known-fate neonate survival, which revealed survival was dependent on neonate sex, pelage coloration, environmental attributes (temperature), and maternal traits (size). Together, these data provide a comprehensive picture of wild pig reproductive ecology in their invasive range that can be used to develop effective management policies that reduce the negative impacts of wild pigs.

INDEX WORDS:Wild pig (Sus scrofa); Invasive species; Reproductive ecology;Movement ecology; Habitat selection; Neonate; Sex ratio;Population dynamics

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DEDICATION

For my dad.

I think you would be proud of me, even though I am still working on being smarter than

what I touch.

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I am not sure my family or Ellie understand what I do, nonetheless they are my foundation, keep me upright, and moving forward – thank you. Mom, thanks for being my mom, and doing all the things to show me you love me and that I am smart.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Population dynamics – how populations change in size and structure over time – is driven by factors such as vital rates (e.g., births, deaths), stochastic environmental variation (e.g., food availability, habitat quality), density dependence (e.g., predation, immigration, emigration), and demographic variation (e.g., age structure, sex ratio; Sæther 1997, Gaillard et al. 1998). Population dynamics reflect the unique interactions between individual physiology and behavior with the environment and thus success, which regulates the number, spatial distribution, and genetic composition of populations. Understanding drivers that influence population dynamics of wild animals are important for determining why and how populations increase, decrease, and fluctuate spatially and temporally under changing conditions. In particular, reproduction, encompassing characteristics such as seasonality, age at maturation, fertility, energetic requirements, and the conditions that contribute to successful reproductive events, is a central component in the development of population models to inform management and conservation plans (Gaillard et al. 1998).

Mammalian reproduction is predominantly influenced by food availability and variation within the environment (e.g., the day/night cycle, temperature, humidity, and precipitation) (Bronson 1985). Energy acquisition, which is often dictated by local environmental conditions, is the most fundamental driver of reproduction in wild mammals. All mammals must expend energy to forage, convert food resources into

energy via various physiological processes, and then allocate the available energy among interacting and competing processes, including cellular maintenance, thermoregulation, and locomotion (Brody 1945, Hervey 1971, Trayhurn 1989, Zera & Harshman 2001, Speakman, 2008). Any additional energy can be secondarily allocated to growth, immune maintenance, physiological and behavioral costs of reproduction, or stored as fat reserves (Williams 1966, Stearns 1992, Peck et al. 2015). Species that exhibit phenotypic plasticity (the ability to alter physiology, morphology and/or behavior in response to environmental conditions) in relation to unpredictable fluctuations in environmental resources (e.g., bet-hedging) may have greater success in passing their genes to the subsequent generation, following abundant resources (Slatkin 1974). Species that exhibit such plasticity to environmental stressors may also be capable of breeding on a continuous cycle compared to being constrained by seasonal variation to opportunistically take advantage of any increases in resources that boost reproductive success and thus increase individual fitness (Bronson 1985, Beaumont et al. 2009).

A species' phenotypic characteristics may also frame its reproductive strategies and, under ideal conditions, increase reproductive success. Individual attributes, such as nutritional condition, at a given point in time influence future success and vital rates, which in turn may produce many different population-level responses (Hooten et al. 2017). Movement behavior decisions, largely driven by the need for food, are linked with space use, reproduction, and survival. Due to the physical and physiological constraints of mating, giving birth, and caring for young, an individual's reproductive state can have a substantive influence on their movement behavior, space use, and resource selection (Clutton-Brock et al. 1982, Berger 1991). Indeed, changes in movement behavior can signal timing of parturition events in ungulates (Carstensen et al. 2003, Ciuti et al. 2006, Long et al. 2009) and survival of neonates (DeMars et al. 2013, Bonar et al. 2018). Females also may shift home range sizes or habitat selection patterns to reflect changes in reproduction. For ungulates, it is not well understood how the interaction between habitat and reproductive stage affects movement behavior, space use, and habitat selection (Long et al. 2009). As such, determining which habitats are used by individuals can help determine structural and floristic components of the landscape that are essential for reproductive success.

Female condition also influences numerous aspects of reproduction, such as timing, age at first breeding, litter size, and offspring survival (Frisch 1984, Albon et al. 1986, Clutton-Brock et al. 1987). Maternal quality has also been proposed as a driver of offspring sex ratio (Trivers & Willard 1973). The Triver's Willard Model (TWM) is an ecological theory that predicts high-quality mothers should invest more into sons because males have higher variance in individual fitness (i.e., reproduction), provided: sons benefit more than daughters from this extra investment, offspring quality correlates to adult quality, and offspring quality is a good indicator of maternal quality. Thus, females should adjust offspring sex ratio in response to factors that could modify both their own lifetime reproductive success and the reproductive success of their progeny. Though well studied in vertebrate species, (Clutton-Brock et al. 1984, Hewison & Gaillard 1999, Kruuk et al. 1999, Rosenfeld et al. 2003, Sheldon & West 2004), it is not well defined if TWM applies to, or how it applies to polytocous species, those that produce several offspring per litter, because the trade-offs between size and number of offspring must also be considered (but see Servanty et al. 2007, Schindler et al. 2015). Additionally,

aside from the effect of food availability on maternal condition (for examples see Massei et al. 1996, Guinet et al. 1998), studies pertaining to the effect of the interaction between multiple environmental drivers and individual attributes on reproduction are limited for large, free-ranging mammals (Stewart et al. 2005, Servanty et al. 2007).

Survival is an integral component of population growth and is associated with fluctuations in a suite of intrinsic biological attributes (e.g., age, genetics, size; Coulson et al. 2006, Pelletier et al. 2007, Pettorelli et al. 2011) and extrinsic environmental factors (e.g., season, landscape characteristics, resource availability; Forchhammer et al. 2001, Owen-Smith et al. 2005). Survival can fluctuate with age, size, season, landscape characteristics, resource availability, and genetics. For long-lived vertebrate species, adult survival is relatively high and constant, while juvenile survival may be more variable (Gaillard et al. 1998, 2000), and thus can be one of the most important influences on recruitment. In addition to direct causes of mortality such as predation (Griffin et al. 2011, Kilgo et al. 2014), indirect factors such as body condition and mass at birth, as well as physiological condition have been associated with neonate survival across species (Coltman et al. 1998, Kolbe & Janzen 2001, Kissner et al. 2005, Carstensen et al. 2009). Availability of vegetative cover, food, water, and other resources critical to both neonates and the mother are also important for determining behavioral patterns that can influence survival (Forchhammer et al. 2001, Guttery et al. 2013, Duquette et al. 2014). Therefore, it is imperative to simultaneously assess the effects of individual characteristics, maternal attributes, and environmental factors when studying survival, particularly for neonates that tend to be especially susceptible to external factors.

Elucidating the underlying attributes driving reproduction, survival, and other vital rates is central to the development of appropriate strategies for the conservation and management of wildlife populations. Identifying factors influencing population dynamics is particularly critical for invasive species which can have significant ecological and economic impacts upon invading new habitats. Invasive species can substantially alter ecological interactions and ecosystem-level processes, and cost billions of dollars through impacts to agriculture, infrastructure, and human health. Wild pigs (Sus scrofa), which are of mixed ancestry of wild boar and domestic pigs (Keiter et al. 2016, Smyser et al. 2020), are one of the most successful and detrimental invasive species worldwide (Lowe et al. 2000). As ecological generalists with few natural predators, wild pigs quickly adapt to new environments within their introduced range (Bevins et al. 2014). Additionally, wild pigs have the highest reproductive rate compared to any other mammal of similar size (Taylor et al. 1998). The combination of early sexual maturity, large litter size, and frequent, year-round breeding characterize their high reproductive potential (Eisenberg 1981, Read & Harvey 1989, Fonseca et al. 2011). In their invasive range, many populations reproduce year-round, likely because they are able to exploit unclaimed niches or outcompete native species in newly invaded landscapes. Generalist behavior and high reproductive potential highlight why wild pigs are particularly well-suited for population expansion and invasion into novel habitats where they are now the most abundant and widespread medium-large size invasive vertebrate in North America (Mayer & Beasley 2018). Upon establishment in new areas, wild pigs exert disproportionately negative effects on the ecosystem, may outcompete native species, serve as reservoirs for diseases, and damage human infrastructure (Pitt et al. 2018,

Strickland et al. 2020). Because of these negative impacts, wild pigs are an ecological, economic, and epidemiological concern. Thus, management of this species is of particular ecological, economic, and epidemiological concern.

Despite their global distribution, wild pigs remain a highly understudied species (Beasley et al. 2018). Understanding the attributes that make wild pigs successful within a diversity of landscapes, both native and invasive, and the factors that influence population dynamics are important for management. Most management plans focus on lethal population control (Mayer & Brisbin 2009), often neglecting the study of the ecological and biological mechanisms that underlie reasons responsible for their abundance. For example, wild pigs have extremely high reproductive potential (Taylor et al. 1998), due to the introgression of domestic pig genes (Comer & Mayer 2009), and reproductive rates directly influence population dynamics. Therefore, there is a need to determine timing of reproduction, frequency of multiple reproductive events per year, and how biotic and abiotic factors contribute to variability within and between populations. Studies that have addressed reproductive traits are usually biased by sample availability and seasonality (Snow et al. 2020).

To address these gaps in knowledge, I present empirical data that address reproductive parameters (e.g., productivity, timing) and identify individual attributes and environmental factors that influence female reproductive success and juvenile recruitment across multiple seasons and years. In Chapter 2, I investigate phenotypic, genotypic, physiologic, and environmental factors that influence probability of pregnancy and litter size. To accomplish this, I sampled 515 sows and assessed reproductive status (pregnant, lactating, non-breeding) and collected body measurements and blood samples. From pregnant sows I counted, measured, and weighed fetuses to determine fetal age and thus conception and estimated farrowing dates. I quantified reproductive hormone concentrations for sows during pregnancy and a size threshold for physiological age at sexual maturity. I also present reproductive parameters such as average litter size in relation to population age structure.

In Chapter 3, I examine space use in relation to reproduction and which resources are used by individuals to determine landscape characteristics that are selected for during reproduction that likely increase success and offspring survival. To accomplish this, I captured and attached Global Positioning System (GPS) collars to 21 sows across 23 reproductive events. I quantified home range size during three physiologically distinct time periods in the reproductive cycle: late-gestation, parturition (farrowing), and the high neonatal-care period immediately post-parturition. I used resource selection functions to assess habitat selection during the three reproductive periods and used First Passage Time (FPT) to identify behavioral changes to reliably predict a farrowing event using GPS data.

In Chapter 4, I test sex ratio and reproductive investment theories in wild pigs. While these theories have been widely tested in vertebrates, it is not well-known how females modulate sex ratio and litter size to increase fitness for species that have multiple offspring. I accomplished this by sampling 89 pregnant sows from which I collected body measurements and counted, determined sex, weighed, and measured fetuses. I assessed whether older and larger females, as well as mothers with abundance food resources produce male-biased litters, whether high quality females invest more in producing larger litters and more male offspring, and if sex ratio varied relative to litter size. In Chapter 5, I investigate factors influencing survival of neonate wild pigs. I captured 24 sows to which I attached GPS collars and inserted Vaginal Implant transmitters (VIT) to determine time and location of farrowing. Within two days after farrowing, I captured neonates at the natal nest and attached Very High Frequency (VHF) ear transmitters for tracking real time survival until six weeks old, tag failure, or neonate mortality. I quantified survival in relation to individual (size, sex, color) and maternal (size) biological attributes, and environmental variables (season). Chapter 6 integrates the observed results from previous chapters to highlight the key biotic and abiotic factors influencing the reproductive success, sex ratio, space use, and offspring survival of invasive wild pigs. Finally, I draw conclusions about wild pig reproductive ecology and how my contribution to the field will advance management strategies for this highly adaptable and invasive species.

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CHAPTER 2

SIZE MATTERS: FACTORS INFLUENCING PREGNANCY, LITTER SIZE, AND REPRODUCTIVE PARAMETERS OF A HIGHLY FECUND INVASIVE MAMMAL

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Abstract

Reproduction is the most energetically expensive life-stage and must be balanced with existing resource, physiological, and environmental conditions. Wild pigs are genetic hybrids of feralized domestic pigs and wild boar and the introgression of domestic pig genes is a major contributing factor for having the highest reproductive potential of any ungulate. While reproduction in domestic pigs is well-studied, the timing of reproduction, extent of multiple reproductive events per year, and how individual and abiotic factors contribute to variability in productivity is not well understood in wild pigs. Because localized populations have distinct ancestral origins, there also may be reproductive differences both within and between populations. We quantified reproductive parameters in wild pigs relative to a suite of individual and environmental attributes across seasons and multiple years. We hypothesized sow mass, age class, number of teats, rump fat, and high yield mast years would increase probability of pregnancy and litter size, while increased boar ancestry would have the opposite effect. We measured reproductive hormones to establish profiles during pregnancy and hypothesized physiological sexual maturity would occur at < 1 year of age. We hypothesized adults would have the highest proportion of pregnant and lactating individuals. We predicted births would peak after a seasonal pulse in food availability. We expected litter size to be higher compared to wild boar but similar to prior wild pig studies and compared ovulation characteristics and in-utero mortality to other populations. Wild pigs produced offspring throughout all months with peaks in conception corresponding to a seasonal pulse in food availability. The likelihood of pregnancy increased with mass and age class and was greatest during years with

abundant resources. Litter size increased with sow mass and age, implying larger and older sows contribute most to recruitment. We present the first data relating genetic composition of wild pigs to reproductive output, and surprisingly, proportion of wild boar ancestry was not an important driver of productivity in our population. Through hormone analysis, we determined juveniles reach a physiological threshold of sexual maturity at approximately 30 kg. Average litter size was comparable to other populations, and despite in-utero mortality, wild pigs remain highly fecund. A thorough understanding of biotic and abiotic factors influencing reproduction are important for realistic population models which are critical for identifying areas to focus management needs and implementation.

Introduction

Animals must expend energy to forage, convert food resources into energy via various physiological processes, and allocate the available energy among interacting and competing processes, including cellular maintenance, thermoregulation, and locomotion (Brody 1945, Hervey 1971, Trayhurn 1989, Zera & Harshman 2001, Speakman 2008). Any additional energy can be secondarily allocated to growth, immune maintenance, physiological and behavioral costs of reproduction, or stored as fat reserves (Williams 1966, Stearns 1992). Among these secondary processes, reproduction, in terms of timing of breeding and age at sexual maturity, is predominantly influenced by food availability and environmental variation (e.g., the day/night cycle, temperature, humidity, and precipitation; Bronson 1985). Thus, species that exhibit phenotypic plasticity, the ability to alter physiology, morphology and/or behavior in response to unpredictable fluctuations in environmental resources (e.g., low food availability) may have greater success in passing their genes to the subsequent generation (i.e., bet-hedging; Slatkin, 1974). Species that exhibit such plasticity to environmental changes may also be capable of breeding on a continuous cycle compared to being constrained by seasonal exploitation of any increases in resources that boost reproductive success and thus increase individual fitness (Bronson 1985, Beaumont et al. 2009).

A species' phenotypic characteristics may also frame its reproductive strategies and, under ideal conditions, increase reproductive success. In mammals, females that are larger generally exhibit a lower basal metabolic rate (Kleiber 1975), which translates into lower thermoregulatory costs and a greater ability to store fat which can counter any acute fluctuations in available energy (Gittleman & Thompson 1988) within the species' thermal-neutral zone. Since fitness is determined by survival and reproductive success of an individual's offspring, it may benefit a female in good condition to breed as often as possible and produce large litters.

Successful reproduction must be balanced with existing resource, physiological, and environmental conditions. In mammals, reproduction (specifically lactation) is the most energetically expensive life-stage for females (Hanwell & Peaker 1977, Gittleman & Thompson 1988), which can be severely modulated by environmental factors and stressors (Sadleir 1969). Yet, the linkage between ecological and physiological factors driving reproductive success is not well understood. Thus, our ability to make generalizations about drivers that affect reproduction from species that are well-studied to those that are not, is limited and dependent on a suite of species-specific, individual, and abiotic factors and should be assessed accordingly.

Sus scrofa, which includes domestic pigs, wild boar, and wild pigs (Keiter et al. 2016) are ecological generalists with a high degree of behavioral plasticity (Bevins et al. 2014). Wild pigs are genetic hybrids of feralized domestic pigs and Eurasian wild boar (Keiter et al. 2016, Smyser et al. 2020) and exhibit reproductive parameters between domestic pigs (e.g., larger litter sizes and earlier sexual maturity) that were bred to maximize reproductive output for the livestock industry (Taylor et al. 1998) and wild boar depending on their ancestry (Comer & Mayer 2009). Thus, wild pigs have the highest reproductive potential of any ungulate species relative to size (Gaillard et al. 1993, Comer & Mayer 2009). Studies of wild boar in their native range have found that litter size has increased with introgression of domestic pig genes (Gethöffer et al. 2007, Fulgione et al. 2016). While reproduction and hormone variation during pregnancy in domestic pigs is widely studied, the extent to which domestic pig biology, physiology, and ecology apply to wild pig populations is not well known (Snow et al. 2020). Reproduction can occur year round in many populations and is characterized by short interbirth, gestation, and lactation periods, as well as large litter size and early sexual maturity (Eisenberg 1981, Read & Harvey 1989, Fonseca et al. 2011). Sow age and phenotypic traits such as mass also are positively associated with ovulation rate and litter size (Barrett 1978, Fonseca et al. 2004, 2011). However, timing of reproduction, the frequency of multiple reproductive events per year, and how individual attributes and abiotic factors contribute to variability within and between populations is not well understood, and studies that have addressed these reproductive parameters usually do not sample across multiple seasons or years (Snow et al. 2020). Because localized populations of wild pigs in North America have distinct ancestral origins, there may be

differences in reproductive ecology both within and between populations due to the genetic make-up of individuals within each population (Snow et al. 2020). Currently, there are no studies investigating the reproductive consequences of domestic pig gene introgression into the wild pig populations in North America (Snow et al. 2020). Further, exploitation of abiotic factors such as pulses in high quality forage (e.g., hard mast, agricultural crops) are associated with higher nutritional condition and increased litter sizes in both wild pigs and wild boar (boar: Massei et al. 1996, Frauendorf et al. 2016; pigs: Barrett 1978, Dissertation Ch 4).

Generalist behavior and high reproductive potential highlight why wild pigs are particularly well-suited for population expansion and invasion into novel habitats. As such, wild pigs are considered an ideal invasive species (Vercauteren et al. 2020) and regarded as one of the world's most detrimental large vertebrates (Lowe et al. 2000). Upon establishment in new areas, wild pigs exert disproportionately negative effects on the ecosystem, may outcompete native species, serve as reservoirs for diseases, and damage human infrastructure (Pitt et al. 2018, Strickland et al. 2020). Because of these negative impacts, wild pigs are an ecological, economic, and epidemiological concern. Therefore, understanding reproductive phenology and factors that influence reproductive output are vital for effective management of this invasive species.

The objectives of this study were to quantify reproductive parameters (e.g., phenology, physiological markers) in a population of wild pigs in the Southeastern U.S. relative to a suite of individual and environmental attributes. We hypothesized sow mass, age class, number of teats, rump fat, and high yield mast years would increase probability of pregnancy and litter size, while increased boar ancestry would decrease these reproductive attributes. We also measured reproductive hormones to establish concentration profiles during pregnancy and assessed physiological sexual maturity related to mass and age. We hypothesized age at sexual maturity would occur at less than one year of age and predicted reproductive hormone concentrations to increase, signaling sexual maturity, around the reported size threshold for breeding (i.e., ~30 kg). Since mass influences productivity, we hypothesized adults, which are typically larger, would have the highest proportion of pregnant and lactating individuals. We predicted births would peak after a seasonal pulse in food availability, the fall mast season, and peak in lactation would follow farrowing. We expected litter size to be higher than that of wild boar but similar to prior studies across their invasive range in North America (i.e., between 3.0-8.4). We also compared ovulation characteristics and embryonic and fetal mortality to other studies on wild pigs in North America.

Materials and Methods

Study site

Our study was conducted on the Savannah River Site (SRS), a federal property operated by the U.S. Department of Energy (DOE). The SRS is 803 km², located in the Upper Atlantic Coastal Plain region in western South Carolina, USA. The SRS is predominantly undeveloped, with habitat was dominated by managed upland pine forests (*Pinus* spp.) and riparian habitat characterized by cypress (*Taxodium distichum*), tupelo (*Nyssa* spp.), and oak (*Quercus* spp.) bottomland hardwood forests and forested swamp land. The population of wild pigs was descended from free-ranging domestic pigs that were released and became feral after the land was converted into a government facility in 1950 (Mayer et al. 2020). Introduction of wild boar and wild boar hybrids in the 1980s resulted in the introgression of wild boar genes into the population such that individuals exhibit a mix of wild boar and feral pig traits (e.g., morphology; Mayer et al. 2020). The wild pigs inhabiting the SRS are generally hybrids of Western heritage breeds of domestic pigs and wild boar, with percent ancestry varying substantially between individuals (Smyser et al. 2020). The wild pig population has been lethally managed since 1956, however the population continues to increase despite targeted trapping and hunting control programs, with >5,000 individuals at the time of this study (Keiter et al. 2017).

Data Collection

For this study, we collected samples from wild pigs in accordance with the University of Georgia Animal Care and Use Committee policies under protocols A2015 12-017, A2018 06-024, and A2019 01-012. We sampled female wild pigs (sows) that were both live-trapped and culled between March 2017 and May 2020. Most of the sampled sows were obtained from ongoing wild pig management on the SRS, although some individuals were culled as part of other research activities on the SRS and were also sampled for this research. Sows > \sim 20 kg were sampled for reproductive status and individual attributes. We weighed each sow (whole body mass to nearest 0.5 kg) and recorded the number of visible teats as well as the number of lactating teats by presence of visible active mammary tissue and palpation. We collected tissue (ear biopsy) for genetic analysis from all sampled pigs, as well as whole blood from a subset of pigs for use in hormonal assays. We collected whole blood via cardiocentesis using a 21 g needle

from dead pigs or from the jugular vein for live captured pigs (from other research studies, e.g., Chinn et al. 2021). Blood was centrifuged for 10-15 minutes at 2500 rpm and serum was stored at -80°C until analysis. As a metric of nutritional condition, we measured rump fat thickness between the ischium and ilium bones and ~3 cm lateral from the spine (to nearest 0.01 cm).

We then necropsied sows to remove reproductive tracts and determine pregnancy status (i.e., visibly pregnant, not visibly pregnant). We also included live captured sows where pregnancy was determined by ultrasound and sows were tracked via transmitters until farrowing and neonate sampling (Chinn et al. 2021). Data from live-captured individuals were included in reproductive hormone, phenology, age class, and litter size analyses. From culled pregnant sows, we removed fetuses and counted, weighed (whole body mass to nearest 0.1 g), and measured each for crown-rump length (CRL; to nearest 0.1 cm). We aged each fetus (in days) based on CRL and used the average gestation length of 115 days (Henry 1968), to estimate conception and farrowing dates. If present, the number of dead fetuses were recorded. Ovaries were excised, weighed (to nearest 0.01 g), and examined for number of corpora lutea (CL), structures that form on the ovary immediately after ovulation of follicles and remain on the ovary during pregnancy, secreting progesterone to prepare the uterus for implantation of the embryo and maintenance of pregnancy, and a smaller amount of estrogen (Bazer & Johnson 2014). If pregnant, the number of CL roughly correspond to the number of fetuses present in the uterus (Comer & Mayer 2009). If the ovum is not fertilized (i.e., the sow is not pregnant), CL regress into corpora albicantia after about 16 days (Geisert et al. 1990) and may or may not be visible on the surface of the ovary. Recent ovulation was determined by gross

examination of the ovaries. Specifically, within hours of ovulation, a CL develops and there is a noticeable distended region where the ovum erupted. Therefore, we could identify sows that had recently ovulated.

We assigned each sow into one of four age classes: adult (> 3 years old), subadult (1.5-3 years old), yearling (1-1.5 years old), and juvenile (< 1 year old) by tooth eruption and replacement patterns (Mastchke 1967; Mayer et al. 2002). It is important to note that wild pig age class designations are based on morphological traits and not by sexual maturity, therefore individuals in the juvenile and yearling age classes can be pregnant. To provide a more precise estimate of age for older sows, we extracted a complete tooth (full length and root tip, lower incisor 2 or 1) from sows field aged as older than the yearling age class to be aged by cementum analysis (Matson's Laboratory, MT). Briefly, processing at Matson's Laboratory entailed multiple cleanings, embedding in paraffin, cross-sectioning the tooth at 14 micron thickness, mounting onto slides, staining, and visualizing under high magnification to count cementum annuli which are formed annually. Thus, cementum aging is only appropriate for animals at least one year old.

We tested the effect of year on likelihood of pregnancy and litter size as a fixed variable. From field observations, our first sampling year (2017), followed a fall season of high mast production. Year 2018 was characterized by low mast productivity, and 2019-2020 were average (J.C.B. personal observation).

Hormone Analysis

For a subset of sows, we measured serum hormone concentrations in duplicate, using commercially available assay platforms according to the manufacturer's specifications. Progesterone and estradiol concentrations were measured using hormone specific I¹²⁵ radioimmunoassays (MP Biological, CA). Samples were run "neat" (undiluted) and both assay platforms were validated for use in wild pigs. Between 93% and 111% of added standards were recovered from samples. The mean coefficient of variation was < 5% for both hormones.

Ancestry Analysis

To quantify the percent wild boar ancestry of wild pigs included in our analysis, we used methods detailed in Smyser et al. (2020). Briefly, we extracted DNA from tissue using a magnetic bead-based (MagMax DNA, Thermo Fisher Scientific, MA) extraction protocol. We genotyped wild pigs using Illumina BeadChip microarrays developed for porcine (Genomic Profiler for Porcine HD, GeneSeek, a Neogen Corporation, MI; Ramos et al., 2009), which provide 29,375 biallelic single nucleotide polymorphism (SNP) loci for analysis. We then estimated the ancestry of individual wild pigs, using ADMIXTURE in a supervised framework to query an individual genotype against a comprehensive reference set assembled for *Sus scrofa* (comprised of 2,516 genotypes sampled from 105 domestic breeds, 23 wild boar populations and 4 sister taxa) and organized into 17 genetically cohesive ancestry groups. This analysis method proportionately associates the origin of individual wild pig genomes among the 17 ancestry groups that comprise the *Sus scrofa* wild-domestic species complex.

Statistical Analysis

Data analyses were performed using R version 4.0.5 (R Foundation for Statistical Computing 2020). We centered continuous variables (mass, number of teats, rump fat, percent boar ancestry) to a mean of 0 and a standard deviation of 1 for comparison. We tested for multicollinearity between continuous fixed effects and if variables were correlated, we did not include them in the same model. We used the glmmTMB package (Brooks et al. 2017a) in R to fit zero-inflated poisson (ZIP) regressions to assess the effects of *a priori* attributes we anticipated to have the greatest effect on our reproductive parameters of interest. Specifically, we evaluated the influence of phenotypic (mass, number of teats, rump fat), genotypic (percent wild boar ancestry), physiological (age class), and abiotic (year, mast year, month) attributes on litter size and the probability of being pregnant. We used a hierarchical approach to build a candidate set of ZIP models. First, we included all univariate models (n = 7). Any significant fixed effects (P < 0.05) for either the conditional regression (i.e., the regression model that addressed litter size) or the zero-inflated regression (i.e., the binomial regression model that addressed probability of pregnancy) were then used to create multivariate models (all two- and three-variable combinations) for further evaluation. Additionally, we tested the interactions terms between age class and mass, as well as percent wild boar ancestry with age class and year. If any of the interaction terms were significant, they were then included in the final candidate model set. Within each candidate model, we included all two- and three-variable combinations in the part of the model they were significant (i.e., univariate models significant in the conditional regression were used to build combinations for the conditional model only; Brooks et al. 2017b). We used Aikaike's

Information Criterion (AIC) and model weights to determine which candidate models provided the best support for the data. We used model weight to evaluate performance of competing models within $\Delta 2$ AIC (Burnham & Anderson 2002).

For the following analyses we assessed the continuous variables for normality with Shapiro-Wilk test and visually (gpplots), and variance with Bartlett's test. We transformed variables that were not normal. To characterize reproductive hormones of wild pigs, we used the *lme4* package (Bates et al. 2014) in R to fit linear regressions to assess if body mass influenced estradiol and progesterone concentrations (logtransformed) for juveniles compared to the remaining age classes (combined). We plotted average estradiol and progesterone concentrations by day to illustrate patterns and changes in levels during gestation. We also report estradiol and progesterone concentrations for each age class in relation to reproductive status (i.e., lactating, pregnant, ovulating, non-breeding). We used Analysis of Variance (ANOVA) to assess any differences in hormone concentrations (log-transformed) between age classes, nutritional condition (i.e., rump fat thickness, square-root transformed) between reproductive status (i.e., lactating, pregnant, non-breeding) and age class, and litter size between age classes and sampled month. If the ANOVA was significant (P < 0.05), we used Tukey's test to determine which contrasts differed.

Gross estimate of embryonic loss was measured by difference in mean number of CL and mean number of fetuses. Fetal mortality was calculated by the number of observed dead fetuses in relation to the total number of fetuses.

Results

We sampled 514 sows (126 adults, 113 subadults, 178 yearlings, 97 juveniles) between 2017-2020, of which 492 were culled and necropsied, and 22 were live captured and released. Among these, we found 160 pregnant sows (31.10%), of which there were 50 adults, 30 subadults, 53 yearlings, and 27 juveniles (Fig. 1). The age class with the highest proportion pregnant was adults (39.68%), followed by juveniles (30.00%), yearlings (28.19%), and subadults (26.55%; Fig. 1). There were pregnant sows in every month and the proportion of sows pregnant by month was highest between February-April, with ~50% of sampled sows pregnant, followed by a secondary peak from September-December (Fig. 2).

Among the 514 sampled sows, 93 were lactating (18.09%), of which there were 37 adults, 23 subadults, 30 yearlings, and 3 juveniles (Fig. 3). The average number of teats was 10.87 ± 1.19 (mean \pm SD). The age class with the highest proportion lactating were adults (29.37%), followed by subadults (20.35%), yearlings (16.85%), and juveniles (3.45%; Fig. 3). Proportion of sows lactating by month was greatest between April-July, with a decrease in August followed by a secondary peak from September-October (Fig. 4). Interestingly, four sows (0.78%) were simultaneously pregnant and lactating, and all were in early gestation.

We evaluated if sow mass, age class, number of teats, nutritional condition (rump fat), percent wild boar ancestry, month, and year influenced probability of being pregnant and litter size for 389 sows. We also included interactions between age class and mass, and boar ancestry with age class and year. The percent of wild boar ancestry for all individuals ranged from 6.03-47.28%, with a mean of $23.58 \pm 7.37\%$ (SD). From the

univariate models, mass and age class were significant for the conditional regression, and mass, rump fat, month, year, and the interaction term boar ancestry * age class were significant for the zero-inflated regression. We constructed 38 candidate models which resulted in four models within $\Delta 2$ AIC (Table 2). Our top model included mass + age class for the conditional regression (litter size) ($\beta_{mass} = 1.08 \pm 1.11$, estimate \pm SE; $\beta_{subadult}$ $= 0.99 \pm 1.14$; $\beta_{yearling} = 1.00 \pm 1.13$, $\beta_{juvenile} = 0.84 \pm 1.18$) and mass + fat + year for the zero-Inflated regression (probability of being pregnant; $\beta_{mass} = 0.41 \pm 0.54$, $\beta_{fat} = 0.37 \pm$ 0.54; $\beta_{2018} = 0.37 \pm 0.57$; $\beta_{2019} = 0.34 \pm 0.59$; model weight, $w_i = 0.32$). As mass increased probability of pregnancy decreased but litter size increased. Compared to the adult age class, juveniles were less likely to be pregnant and had smaller litter sizes. Subadults and yearlings had litter sizes comparable to adults. As rump fat increased, the probability of pregnancy decreased and sows sampled in 2018 and 2019 (low to moderate mast yield) were less likely to be pregnant compared to sows sampled in 2017 (high mast yield). The other supported models all had mass and age class as important factors influencing litter size. The other variables influencing probability of pregnancy in the remaining supported models were: mass + fat + month, mass + fat, and fat + month + year. Sows were less likely to be pregnant in August and July ($\beta_{August} = 0.78 \pm 0.65$; $\beta_{July} = 0.79 \pm 0.66$).

We measured estradiol and progesterone for 217 sows, of which 66 were pregnant. Average estradiol concentrations varied by age class ($F_{3,209} = 2.75$, P = 0.043; Table 1), with estradiol in adults being significantly higher compared to subadults (t = 2.66, P = 0.041). For juveniles, estradiol concentration had a positive relationship with increased mass ($r^2 = 0.06$, $F_{1,53} = 4.51$, P = 0.038; Fig. 5A). For all other age classes combined (adult, subadult, yearling), mass did not influence estradiol concentration (Fig. 5B). Progesterone concentration was positively associated with increased mass for juveniles ($r^2 = 0.34$, $F_{1,55} = 29.58$, P < 0.0001; Fig. 5C) and for all other age classes combined ($r^2 = 0.08$, $F_{1,163} = 15.98$, P < 0.0001; Fig. 5D). For pregnant sows, average progesterone peaked and remained stable between days 20-100, and substantially decreased upon farrowing (Fig. 6). Average estradiol concentration increased slightly around day 20 post-conception and markedly increased from day 60-80, where levels remained sustained until farrowing (Fig. 6).

Average rump fat thickness for all sows (n = 451), our index of nutritional condition, was 0.96 ± 0.03 cm (mean \pm SE). Nutritional condition varied between reproductive stage (F_{2,448} = 24.22, *P* < 0.0001). Pregnant sows were in the best condition (1.14 \pm 0.06 cm) compared to lactating (0.59 \pm 0.05 cm; *t* = -6.67, *P* < 0.0001) and non-breeding sows (0.80 \pm 0.04 cm; *t* = 5.06, *P* < 0.0001). Lactating sows were in the poorest condition of all pigs sampled and had significantly less rump fat compared to non-breeding sows (*t* = 3.00, *P* < 0.008). Nutritional condition did not vary between age classes.

Using CRL measurements averaged among individuals within each litter, we determined fetal age from 159 litters (one pregnant sow had an unknown number of fetuses) with 864 fetuses. Using the average gestation length of 115 days (Henry 1968), we calculated conception and farrowing dates to estimate peaks in reproductive phenology. Conception peaked between November-February, with a secondary peak between July-September (Fig. 7). Estimated peak in farrowing would have occurred March-June, with a smaller peak November-January (Fig. 7).

Average litter size from 159 pregnant sows (n = 864 offspring) was 5.43 ± 0.14 (SE) and ranged from 1 to 12 live fetuses. Average litter size differed by age class (F_{3,155} = 5.57, *P* = 0.0012). Adults had the largest litters (5.94 ± 0.25), followed by subadults (5.62 ± 0.33), yearlings (5.45 ± 0.24), and juveniles (4.26 ± 0.34 ; Fig. 8). Juveniles had significantly smaller litter sizes compared to adults (t = 4.03, *P* = 0.0005), subadults (t = -2.91, *P* = 0.021), and yearlings (t = -2.89, *P* = 0.023). Average litter sizes between adults, subadults, and yearlings did not differ (*P* > 0.05; Fig. 8). Litter size did not differ by month sampled (*P* = 0.41; Fig. 9). Matson age analysis was completed for 212 sows and resulted in 98 individuals ≤ 1 year old, 70 two-year olds, 24 three-year olds, 13 four-year olds, 2 five-year olds, 3 six-year olds, and a single nine-year old (Fig. 10). Since our sample size had a limited number of individuals in each adult age class (> 3 years old), we used our age classes determined through tooth eruption in the regression analyses (i.e., juveniles, yearlings, subadults, adults).

We obtained ovaries from 137 pregnant sows which had 751 fetuses. We recorded 118 more CL (n = 869) compared to the number of observed fetuses. Average number of total CL per sow was 6.34 ± 1.84 (SD), and ranged from 0-12 (two sows had 0 CL but both had 5 fetuses in utero). CL were distributed 48.91% in the right ovary (n = 425) and 51.09% in the left ovary (n = 444). Gross estimate of embryonic loss was 13.58%. We observed 14 dead fetuses in utero (from n = 864, 1.61% fetal mortality).

Discussion

Understanding reproductive patterns of wild pigs is challenging because of their generalist behavior and phenotypic plasticity, hence their ability to modulate fecundity

based on favorable conditions (Ahmad et al. 1995, Fernandez-Llario & Mateos-Quesada 2005). Further, because wild pigs are a genetic mix of wild boar and domestic pigs and percent of these lineages vary by subpopulation (Smyser et al. 2020), reproductive characteristics may differ dependent on these characteristics and their interaction with habitat, resources, age structure of the population, and environmental conditions. Through the integration of phenotypic, genotypic, physiologic, and environmental attributes, in this study we were able to quantify the influence of a broad suite of individual and extraneous factors on the reproductive ecology of invasive wild pigs. Our results revealed wild pigs produced offspring throughout all months of the year in our study population, with seasonal peaks in conception corresponding to the hard mast season (November-February) and farrowing approximately four months later. The likelihood of pregnancy also increased with sow mass and age class and was greatest in years with abundant hard mast resources. Litter size also increased with sow mass and age, indicating larger and older sows contribute most to recruitment in wild pig populations. We present the first data relating genetic composition of wild pigs to reproduction, and surprisingly the proportion of wild boar ancestry was not an important driver of productivity in our study population. We also present the first reproductive hormone data addressing physiological age of sexual maturity. Collectively, these data provide a comprehensive and quantitative assessment of reproductive ecology of invasive wild pigs.

Wild pig reproductive characteristics on the SRS were consistent with other areas in this species' invasive range. Under favorable conditions (i.e., ample forage), females may become sexually mature at less than one year old and reproduce within their first year (Briedermann 1990, Ahmad et al. 1995). While wild boar are reported to become sexually mature at ~30 kg (Fernandez-Llario & Mateos-Quesada 1998), younger and smaller females have been reported to breed (Bieber & Ruf 2005). In their invasive range, female wild pigs are reported to reach sexual maturity at variable ages and sizes, but ranges from 3-12 months (Giles 1980, Comer & Mayer 2009). In our study, 16.98% of juveniles (i.e., < 1 year old) were pregnant and a 20 kg juvenile sow was sampled with 37 day-old fetuses in utero. It is important to note that we primarily targeted sampling of sows > 30 kg, and thus the occurrence of pregnant sows below this weight is not well characterized by our data but anticipated to be infrequent in wild pigs.

The percent of reproducing individuals is reported to increase with age, with adults having the highest proportion pregnant, however most of these studies did not sample across seasons or over consecutive years (Snow et al. 2020). Given that wild pigs reproduce throughout the year across much of their invasive range, these studies may not capture spatio-temporal variability in reproduction in this species. Using data from year round sampling over three years, our results indicated that among age classes adults are likely to be pregnant at any given point in time. Similarly, percent of individuals lactating increased as age increased. About 40% of adults sampled in our study were pregnant, consistent with other estimates in the U.S., ranging from 12-100% (see Snow et al. 2020). Younger age classes in our dataset had slightly lower percentages of pregnant individuals, ranging from ~26-30%, which is on the lower end of what has been reported in the U.S. (30-75%; see Snow et al. 2020). However, most data from previous studies were taken during hunting seasons and did not represent year round totals. Therefore, the lower percent of younger pregnant individuals in our study may reflect a more inclusive

sampling design. The proportion of pregnant sows in our study peaked (~50%) for several months (February-April), and ~20-50% of sows were pregnant at any given time throughout the year. The highest percent of lactating sows occurred between April-October, where 20-35% of sows were lactating. Lactating sows were present throughout the year, indicating that there were dependent young on the ground year round.

Probability of pregnancy was influenced by several of our measured attributes, particularly sow mass, age class, month, and year. Larger sows were less likely to be pregnant. However, adults also were the age class with the largest proportion of lactating sows, so this relationship may reflect the fact that many larger sows already may have recently given birth. Surprisingly, sows in better nutritional condition were less likely to be pregnant. While our analysis of nutritional condition by reproductive stage showed that pregnant sows were in the highest nutritional condition, these results could be skewed by some individuals in very good condition that were not pregnant. For example, wild pigs that live near the SRS landfill had higher mass compared to individuals elsewhere on site due to the superabundant food source provided by the landfill (Mayer et al. 2021), and thus may have been in better condition. Sows in 2018 and 2019 were less likely to be pregnant compared to 2017, in which was preceded by a high hard mast production season (J.C.B. personal observation). Both wild boar and wild pigs which exploit pulsed resources and years of abundant resources (e.g., hard mast) are often associated with higher rates of pregnancy (Barrett 1978, Massei et al. 1996). Therefore, the increased proportion of pregnant females in 2017 following a high mast yield is consistent with previous studies (e.g., Barrett 1978, Massei et al. 1996, Frauendorf et al. 2016). Despite annual reproduction, our data revealed sows were less likely to be

pregnant during July and August, possibly because by the end of summer food availability may be declining and thus conditions may not be favorable for pregnancy. In addition, with a ~4-month gestation period, farrowing in the colder months of November and December could be less optimal for neonate survival (Chinn et al. 2021).

Consistent with other studies, litter size increased with sow mass (Bieber & Ruf 2005, Comer & Mayer 2009, Dissertation Ch 4). In fact, Chinn et al. (Dissertation Ch 4) found sow mass was a good predictor of investment (litter size and sex ratio) in litter production. Investment increased as sow size increased and in polytocous species like the wild pig, modulation of litter size appeared to be the primary reproductive parameter manipulated to increase reproductive success and fitness. Similar to the current study, Chinn et al. (Dissertation Ch 4) found juveniles invested significantly less in litter production compared to adults, subadults, and yearlings. Our data support several previous studies of wild pigs in North America that juveniles have smaller average litter sizes (Snow et a. 2020). Juveniles are still maturing and growing, thus the amount of energy they can allocate toward reproduction could be substantially less. Further, since lactation is energetically costly, juveniles may not be capable of successfully nursing larger litters. Rather, they may trade-off producing and caring for smaller litter sizes when they are younger in favor of allotting any additional energy reserves toward growth and development (Williams 1966, Stearns 1992). Nonetheless, our data indicate juveniles are pregnant and producing litters and therefore contributing to population growth.

While domestic pig reproductive physiology is well-documented (Hendricks et al. 1972, Parvizi et al. 1976, Ka et al. 2018), these parameters have not been investigated in wild pigs. Our hormonal data illustrate wild pig hormone profiles exhibit a similar pattern

in comparison to domestic pigs. Progesterone remained elevated during pregnancy because its primary functions are to prepare the uterus for implantation of embryos, maintain pregnancy, and fetal and mammary tissue development (Croy et al. 2009, Bazer 2013). Estradiol promotes placental and mammary tissue development (Bazer & Johnson 2014). We found that estradiol concentrations had two peaks, an initial smaller peak early in pregnancy (day 20) and a sustained peak at day 80 through farrowing. Domestic pigs have an initial peak a little earlier (day 12), a secondary spike between day 18-30, and a marked increase from day 90 until farrowing (Ka et al. 2018). Our data lack the second small peak, likely an artifact of small sample size. The difference in estradiol concentration between adults and subadults was also likely because of high variance between individuals. The difference in progesterone concentrations between adults and juveniles and yearlings, and subadults and juveniles could be from the difference in litter size. Adults had the highest progesterone concentrations and the largest litters. Progesterone is secreted by the CL in early pregnancy and then by the placenta in the latter part of gestation. The number of CL tends to be related to the number of embryos; hence more CL could produce higher levels of progesterone. Similarly, subadults had higher concentration of progesterone compared to juveniles, which may also be from having larger litter sizes. Both progesterone and estradiol concentrations for juveniles increased with mass. Concentrations were fairly low and uniform until 30 kg, suggesting this is likely a size threshold for reproductive maturity.

Nutritional condition varied by reproductive stage. Pregnant females were in the best condition, therefore implying condition may be a driver for determining reproductive status (i.e., pregnant, not-breeding; Schlichting et al. 2015). It may be important for

successful reproduction for pregnant sows to be in better nutritional condition to prepare for farrowing and neonatal care. Farrowing may require the sow stay close by the nest and likely decrease time spent foraging (Chinn et al. 2021), which may depend on condition during pregnancy. Lactation is the most energetically expensive stage of reproduction and requires increased food intake to support milk production (e.g., more time foraging) and increases movement to acquire food. Nutritional condition has been reported affecting age at sexual maturity and litter size, however we did not observe any difference between age classes.

We documented conception and farrowing in every month, highlighting wild pigs in the southeastern U.S. reproduce year round, regardless of individual attributes and environmental factors. However, conception in the SRS peaked in the late fall and early winter. This timing coincided with fall hard mast production. Farrowing peaked in spring (March-June) and was followed by a secondary peak in late fall/early winter, approximately four months later. The second peak may correspond to sows that lost their entire first litter during the peak farrowing season since timing coincided with the duration of gestation. This suggests that despite a total failure of one reproductive event, sows quickly became pregnant again and gave birth to a second litter during the same year (Barrett 1978). Conversely, there may be a second peak in farrowing independent of the outcome of a prior litter, highlighting year round reproduction. While uncommon and despite observations of wild sows breeding while lactating, Conley et al. (1972) found sows seldom conceived after these breeding events, and if they did, litter sizes were small. We observed a small number of sows that were both pregnant (with 5 and 7 fetuses) and lactating. Our data support other studies that show sows are physiologically

capable of producing at least two litters in a twelve-month period (Springer 1977, Johnson et al. 1982, Baber & Coblentz 1986, Taylor et al. 1998, Chinn et al. 2021) but litter size did not appear to be smaller than average, at least during the early gestation period.

In the U.S., average litter size of wild pigs is 5.3 (95% Confidence Interval = 4.8-5.7), typically ranging from 1 to 12 offspring (Snow et al. 2020). Litter size on the SRS during our study was slightly higher than average, but lower compared to previous studies in the same location (6.1-8.4; Sweeney 1979, Sweeney et al. 1979, Comer & Mayer 2009). Litter size of wild boar in their native range is smaller, ranging from 4.0-6.8 (Mauget et al 1991, Ahmad et al. 1995, Comer & Mayer 2009), likely because of lower ovulatory rates (Hagen et al. 1980). Our reported litter size on the SRS could be lower compared to previous reports due to changes in genetic composition of the population over time. Wild boar and wild boar hybrids were introduced to the SRS in the 1980s (Mayer et al. 2020) and proportion of wild boar genes in the SRS population could have increased or become more widespread over the past 40 years and resulted in lower average litter size presented in this study. However, wild boar ancestry was not an important predictor of litter size in our study. Litter size was comparable to the average size of litters in North America and higher compared to litter size in wild boar, suggesting other mechanisms may have contributed to variability in litter size among studies. Wild boar ancestry was highly variable in our population, and a majority of the ancestry was attributed to domestic heritage breeds (e.g., Piney woods rooters and Guinea hogs). Ancestry is variable among wild pig populations, therefore the extent of introgression of wild boar genes may differ and influence reproduction accordingly. Average litter size

increased with age class. Older sows are usually larger in size and may be able to invest more energy into gestation of larger litters and milk production for more piglets (Mayer et al. 2020). Litter size did not vary by month, suggesting that seasonal fluctuations in resource availability did not influence sow investment in the number of offspring produced.

Tooth cementum analysis is a reliable method for ageing wildlife and we are the first to present these data for wild pigs. The age structure of the SRS population was heavily skewed toward younger individuals, where cementum analysis and field-based ageing of yearlings and piglets represented 67.70% of our sample size. The wild pig population on the SRS was heavily harvested which was likely a strong driver of age structure. Thus, there were fewer older individuals in our population, in fact, of the samples sent for cementum analysis, only 9% were > 4 years old. Population age structure may be a result of hunting because larger individuals, that are often older, are targeted (Gamelon et al. 2011, Ditchkoff et al. 2017) and the proportion of younger individuals may be inflated (Servanty et al. 2011).

We observed lower embryonic losses than previously reported (25-34%, in Snow et al. 2020). While it is possible that the difference in the number of CL compared to the number of fetuses indicates embryonic loss, recruitment of additional CL during early pregnancy may also occur to support implantation of the embryo by secreting necessary amounts of the key hormones (e.g., progesterone) to maintain pregnancy. In fact, additional CL have been observed in species that have single offspring (e.g., sea otters: Chinn unpublished data). Therefore, estimates of embryonic losses may be overestimated. We reported lower percent of stillborn offspring compared to other studies (8-38%; Henry 1966, Baber & Coblentz 1986). Regardless, wild pigs maintain high productivity, bearing the largest litter sizes among all ungulates (Read & Harvey 1989) which may compensate for any embryonic or fetal mortality.

Since the 1990s, wild pigs in North America have rapidly expanded in number and distribution and have necessitated improved management strategies for controlling populations (Mayer & Beasley 2018). A thorough understanding reproductive phenology and parameters are important for realistic population models which are critical for identifying areas to focus management needs and implementation (Snow et al. 2020). From a robust dataset where we sampled across seasons and multiple years, we did not find a relationship between genetics and productivity; however, these are important data and warrant continued research because introgression of domestic pig genes may increase reproductive rates and output, thereby exacerbating the invasiveness of wild pigs and the impacts wild boar have on their native and invasive ecosystems. While our data are specific to a population of wild pigs in western South Carolina, application of our findings may be applied to other populations in similar habitats and could be used as a model of basic reproductive parameters for other areas.

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Tables

Table 2.1. Set of candidate models for influencing female wild pig (*Sus scrofa*) litter size and probability of pregnancy on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2019. First set of variables¹ corresponds to the conditional model (litter size) and the second set of variables (separated by "/") corresponds to the zero-inflated model (probability of pregnancy).

	log-				
Model Tested	likelihood	К	AIC	ΔΑΙϹ	Weight
age class + mass / mass + fat + year	-444.24	10	908.48	0	0.32
age class + mass / mass + fat + month	-435.74	19	909.49	1.01	0.19
age class + mass / mass + fat	-446.79	8	909.58	1.1	0.18
age class + mass / fat + month + year	-435.22	20	910.44	1.96	0.12
age class + mass / mass + fat + boar*age class	-440.9	15	911.79	3.31	0.06
mass / fat	-451.97	4	911.94	3.46	0.06
age class + mass / fat + month	-447.38	9	912.76	4.28	0.04
age class / fat	-451.9	6	915.81	7.33	0.01
age class + mass / fat + month	-440.16	18	916.31	7.83	0.01
mass / mass	-454.75	4	917.51	9.03	0
fat / fat	-454.77	4	917.55	9.07	0
age class + mass / mass + month + year	-439.83	20	919.67	11.19	0
age class + mass / mass + month	-441.89	18	919.78	11.3	0
age class + mass / fat + year + boar*age class	-443.96	16	919.92	11.44	0
age class*mass / age class*mass	-444.07	16	920.14	11.66	0
age class + mass / mass + boar*age class	-446.18	14	920.35	11.87	0
age class + mass / mass + month + boar*age class	-435.39	25	920.77	12.29	0
age class + mass / fat + month + boar*age class	-435.57	25	921.15	12.67	0
age class + mass / fat + boar*age class	-446.83	14	921.67	13.19	0
age class + mass / mass + year + boar*age class	-444.9	16	921.79	13.31	0
age class + mass / mass + year	-452.15	9	922.31	13.83	0
age class / boar*age class	-460.51	5	931.03	22.55	0
mass / year	-462.71	5	935.42	26.94	0
mass / month	-453.84	14	935.68	27.2	0

Model Selection for litter size and pregnancy in wild pigs, within $\Delta AIC \leq 2$

age class + mass / month + year	-449.97	19	937.93	29.45	0
age class / month	-453.8	16	939.61	31.13	0
age class + mass / month + year + boar*age class	-444.39	26	940.79	32.31	0
null	-468.41	2	940.83	32.35	0
age class + mass / month + boar*age class	-446.51	24	941.03	32.55	0
mass / boar*age class	-460.57	10	941.13	32.65	0
year / year	-465.24	6	942.47	33.99	0
boar / boar	-467.24	4	942.47	33.99	0
age class / age class	-463.59	8	943.19	34.71	0
teats / teats	-468.03	4	944.07	35.59	0
age class + mass / year + boar*age class	-457.36	15	944.71	36.23	0
boar*year / boar*year	-460.46	12	944.92	36.44	0
age class / year	-462.67	12	949.34	40.86	0
boar*age class / boar*age class	-459.14	16	950.29	41.81	0
month / month	-453.18	24	954.36	45.88	0

¹Age class: adult, subadult, yearling, juvenile

Boar: percent wild boar ancestry

Teats: total number of teats

Table 2.2. Estradiol and progesterone concentrations during overall and during each reproductive stage by age class for wild pigs (Sus scrofa) on the Savannah River Site, Aiken, SC USA, 2017-2020.

Reproductive Hormone Concentrations										
	Estradiol ± SE (pg/mL)			Progesterone ± SE (ng/mL)						
Age					Non-					Non-
Class	Average	Pregnant	Lactating	Ovulating	breeding	Average	Pregnant	Lactating	Ovulating	breeding
	677.70 ±	1374.46 ±	103.01 ±	165.21 ±	119.86 ±	20.02 ±	28.25 ±	8.06 ±	2.34 ±	19.81 ±
Adult	208.70	433.12	16.88	55.93	15.14	2.70	4.24	3.26	1.23	4.61
	263.86 ±	667.07 ±	76.63 ±	99.00 ±	104.81 ±	17.42 ±	17.72 ±	1.15 ±	2.25 ±	23.42 ±
Subadult	124.09	415.53	13.06	9.53	12.55	2.60	2.80	0.25	1.57	4.11
	571.47 ±	1920.19 ±	99.02 ±	195.75 ±	109.83 ±	13.47 ±	17.74 ±	1.37 ±	2.44 ±	18.16 ±
Yearling	47.00	628.81	21.89	28.93	16.01	2.35	3.62	0.37	0.31	4.23
	260.68 ±	1492.54 ±	79.40 ±	150.61 ±	109.12 ±	9.27 ±	24.25 ±	1.26 ±	14.95 ±	7.43 ±
Juvenile	126.41	1106.99	14.26	40.47	11.95	1.90	5.01	0.25	13.07	2.01

Figures



Figure 2.1. Percent of pregnant sows by age class for wild pigs (*Sus scrofa*, n=514) on the Savannah River Site, Aiken, SC USA, 2017-2020.



Figure 2.2. Percent of pregnant sows sampled by month for wild pigs (*Sus scrofa*, n=514) on the Savannah River Site, Aiken, SC USA, 2017-2020.



Figure 2.3. Percent of lactating sows by age class for wild pigs (*Sus scrofa*, n=514) on the Savannah River Site, Aiken, SC USA, 2017-2020.



Figure 2.4. Percent of lactating sows by month sampled for wild pigs (*Sus scrofa*, n=514) on the Savannah River Site, Aiken, SC USA, 2017-2020.



Figure 2.5. Estradiol and progesterone concentrations for juveniles (n=57) and adults + subadults + yearlings (n=120) in relation to mass for female wild pigs (*Sus scrofa*) on the Savannah River Site, Aiken, SC USA, 2017-2020. A) Estradiol vs. Mass for juveniles + piglets, $r^2 = 0.06$, $F_{1,53} = 4.51$, P = 0.038, B) Estradiol vs. Mass for adults + subadults + yearlings, P > 0.05, C) Progesterone vs Mass for juveniles + piglets, $r^2 = 0.34$, $F_{1,55} = 29.58$, P < 0.0001, D) Progesterone vs Mass for adults + subadults + yearlings, $r^2 = 0.06$, $F_{1,163} = 15.98$, P < 0.0001.



Figure 2.6. Estradiol (E) and progesterone (P) concentrations during gestation (n = 115 days) for female wild pigs (*Sus scrofa*, n = 66) on the Savannah River Site, Aiken, SC USA, 2017-2020.



Figure 2.7. Monthly conception and estimated farrowing dates for female wild pigs (*Sus scrofa*, n = 160) on the Savannah River Site, Aiken, SC USA from 2017-2020. Black bars indicate the number of fetal litters that were estimated to be conceived in each month. Gray bars indicate the number of fetal litters that were estimated to farrow in each month.



Figure 2.8. Average litter size (± SE) for wild pigs (*Sus scrofa*, n=159) on the Savannah River Site, Aiken, SC USA, 2017-2020.



Figure 2.9. Average litter size (\pm IQR) by month sampled for wild pigs (*Sus scrofa*, n=159) on the Savannah River Site, Aiken, SC USA, 2017-2020



Figure 2.10. Matson cementum age analysis for female wild pigs (*Sus scrofa*, n=211, plus 250 yearlings and piglets that were field-aged based on tooth eruption patterns) on the Savannah River Site, Aiken, SC USA, 2017-2020.

CHAPTER 3

REPRODUCTION DRIVES CHANGES IN SPACE USE AND HABITAT SELECTION OF AN ADAPTABLE INVASIVE MAMMAL

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Abstract

It is important to consider individual physiology and life-history events when asking what components contribute to animal movement decisions. For ungulates, it is not well understood how the interaction between habitat and reproductive stage affects movement behavior, space use, and habitat selection. We used known farrowing data to validate the use of First Passage Time (FPT) movement analysis as a tool for identifying wild pig reproductive events from animal GPS datasets. We examined home range size during three physiologically distinct time periods in the reproductive cycle: lategestation, parturition (farrowing), and the high neonatal-care period immediately postparturition and predicted home range would be largest during the late gestation period, smallest during the farrowing period, and gradually increase during the neonate care period. We also determined habitat selection during the three reproductive periods. Using FPT we found that sows exhibited a distinct behavioral change within 1-2 days prior to farrowing, therefore FPT can be a powerful tool for explicitly identifying farrowing events in sows fitted with GPS collars for other research purposes. We found a marked decrease in home range size during the farrowing period compared to late gestation and likely reflects nest building, the birth event, protection of neonates, and bonding. During the neonate care period we found home range size was intermediate between late gestation and the farrowing periods, reflective of caring for offspring which may restrict maternal movement. During all reproductive periods, sows avoided developed areas, which provided sparse canopy and ground cover, and were associated with human activities. During late gestation, sows avoided pine forests likely because of the open understory and less vegetative cover. During late gestation and neonate care, sows

selected bottomland hardwood forests likely because this habitat was characterized by ample food resources, cover, and proximity to water. During the farrowing and neonatal care periods, sows selected upland hardwood forests likely because they provided high quality food resources as well as cover for the neonates. Wild pigs exhibited behavioral changes in relation to space use and habitat selection associated with reproduction and parental care. The physiological requirements for reproduction provide the motivation by which females choose where and how large of an area they use as well as what habitats to concentrate their movements, and likely reflect choices that increase offspring survival and thus overall fitness.

Background

Animal movement is affected by physiological condition as well as environmental factors and can occur across multiple spatial and temporal scales. Consequently, movement decisions comprise a crucial component of individual fitness and thus population dynamics, community structure (Turchin 1998), and ultimately evolution (Nathan et al. 2008) and ecosystem function (Lundberg & Moberg 2003). Individual movement behavior decisions also reflect an organism's response to environmental perturbations and have downstream effects on demographics such as vital rates and offspring survival (Hooten et al. 2017). Animals respond to a dynamic environment through movement, and how an animal chooses to move is a function of the environment and behavioral state (e.g., foraging, traveling; Schick et al. 2018). Animals assess environmental conditions relative to their own behavior and energetic requirements, and

as such make state-specific choices (Schick et al. 2018). Thus, animal movement connects behavior, landscape ecology, and population dynamics.

Individual attributes, such as nutritional condition, at a given point in time influences future success and vital rates, which in turn may produce many different population-level responses (Hooten et al. 2017). For example, movement decisions relative to how an individual chooses habitat resources to meet life-history needs, effects energy acquisition and body condition (Hooten et al. 2017). Movement behavior decisions, largely driven by the need for food, are linked with space-use, reproduction, and survival. Therefore, it is important to consider individual physiology and life-history events when asking what components contribute to an animal's movement decisions, for example, a marked reduction in movement and home range size may signal a reproductive event (DeMars et al. 2013, VanBeest et al. 2013, Hooten et al. 2017).

Due to the physical and physiological constraints of mating, giving birth, and caring for young, an individual's reproductive state can have a substantive influence on their movement behavior, space use, and resource selection (Clutton-Brock et al. 1982, Berger 1991). Indeed, changes in movement behavior can signal timing of parturition events in ungulates (Carstensen et al. 2003, Ciuti et al. 2006, Long et al. 2009) and survival of neonates (DeMars et al. 2013, Bonar et al. 2018). Females also may shift home range sizes or resource selection patterns to reflect changes in reproduction. Females should select habitats with high quality food resources during late gestation and that serve as protection (i.e., dense vegetation) for neonates from predators immediately post-parturition (Bowyer et al. 1999, Barten et al. 2001) when mortality is highest (Chinn et al. 2021). Maternal energy requirements peak during lactation (Hanwell & Peaker 1977); therefore, females must balance energy acquisition with care of offspring that are not as mobile as adults for the first few weeks of life (Bowyer et al. 1999). For ungulates, it is not well understood how the interaction between habitat and reproductive stage affects movement behavior, space use, and resource selection (subsequently referred to as habitat selection; Long et al. 2009).

Despite their global distribution and impacts to natural and anthropogenic systems, wild pigs (*Sus scrofa*) are one of the most understudied ungulates regarding movement ecology (Morelle et al. 2014, Beasley et al. 2018). Wild pigs are a highly adaptable and ubiquitous invasive species that have detrimental impacts to native ecosystems, agriculture, and human and livestock health, thus, understanding movement patterns that influence population dynamics is crucial for effective management (Pimentel 2007, Pitt et al. 2018, Strickland et al. 2020). As ecological generalists with a high reproductive capacity (Taylor et al. 1998, Bevins et al. 2014), wild pigs thrive and rapidly expand in abundance in new environments. Consequently, it is critical to understand how habitat selection affects fitness (i.e., reproduction and offspring survival) and therefore population growth.

Habitat for a species is generally defined as encompassing a suite of resources and environmental conditions (biotic and abiotic) that determine the presence, reproduction, and survival of an individual or population (Gaillard et al. 2010). Determining which resources are used by individuals can help determine structural and floristic components of the landscape that are essential for reproductive success. Further, habitat selection and home range size are often a reflection of resource availability (Keuling et al. 2009, Wilber et al. 2020). Wild pig movement has been shown to be linked to changes in food availability (Keuling et al. 2008, Campbell & Long 2010). Like other ungulates, a decrease in movement and home range size is associated with parturition (Comer & Mayer 2009, Kay et al. 2017), and movement immediately post-parturition is not well-studied (Morelle et al. 2014, Gray et al. 2020). Moreover, reduction in movement and home range may only be apparent at certain spatial scales, thus it is important to identify the appropriate scale at which to test hypotheses (Kay et al. 2017). For example, habitat selection within a home range during important life-history events such as dispersal or gestation may give insights to which resources are important for survival and successful reproduction for individuals that determine population-level effects. Only recently has wild pig behavior been linked to landscape patterns and resources during different seasons and at various temporal and spatial scales (Wilber et al. 2020, Clontz et al. 2021).

Here, we examined space use and habitat selection of female wild pigs (sows) during three physiologically distinct time periods in the reproductive cycle: lategestation, parturition (farrowing), and the high neonatal-care period immediately postparturition. Determining timing of farrowing and understanding movement behavior is crucial for population modeling and management. However, deploying field instruments (e.g., Vaginal Implant Transmitters [VIT]) and tracking individuals daily until farrowing may be cost-prohibitive. Our objective was to use known farrowing data from VITs deployed in pregnant sows to validate the use of movement analysis as a tool for identifying wild pig reproductive events from animal GPS datasets in future studies. Therefore, we determined the reliability of using fine-scale movement data to identify abrupt changes in behavior (i.e., area restricted search [ARS] behavior) as a predictor of farrowing corroborated with known timing of births.

Greater energy acquisition during late gestation is associated with successful reproduction (i.e., higher maternal performance, offspring size; S-145 Committee on Nutritional Systems for Swine to Increase Reproductive Efficiency 1989, Butte & King 2005). As such, we hypothesized sows would have larger home ranges and employ a more generalist habitat selection strategy to increase their flexibility to exploit all available food resources within their home range. Typically, sows decrease their magnitude of movement and build nests ~24 h prior to farrowing (Kurz & Marchinton 1972, Boulton et al. 1997, Thodberg et al. 1999) and home range size is reported to decrease for ~2 weeks post-parturition (Kurz & Marchinton 1972, Kay et al. 2017). We hypothesized that during farrowing, home range size would be the smallest compared to the other periods and habitat selection would be reflective of areas that promote successful nest site placement (i.e., more cover). Upon farrowing, sows are constrained by neonates that depend on maternal provisioning (i.e., lactation), are less mobile, and are highly susceptible to predators (e.g., coyotes and bobcats; Keiter et al. 2017b, Chinn et al. 2021). Moreover, the sow must produce milk which requires considerably more energy compared to the non-lactation period (Hanwell & Peaker 1977), therefore she must maintain sufficient body condition to mobilize adipose for milk production as well as acquire increased energy from foraging (Theil et al. 2012). We hypothesized home range size would increase from the farrowing period to the neonatal care period, but that home range size would be more restricted compared to the late gestation period since neonates must be in close proximity to the sow for survival and they are typically less mobile for the first several weeks of life (Mayer & Brisbin 2009). Finally, we hypothesized sows

would select habitat that provides both protective cover for the neonates and ample food resources to support lactation and maintenance during the neonate care period.

Methods

Study Area

We conducted this study between 2017 and 2020 at the Savannah River Site (SRS), a ~800 km² U.S. Department of Energy (DOE) property located entirely within the Upper Coastal Plain physiographic region of western South Carolina, USA. About 95% of the site was undeveloped with the remainder established for industrial activity and facilities. The landscape of the SRS was composed of ~35% managed pine forests (Pinus spp.) and ~25% bottomland hardwood forest (Taxodium distichum, Nyssa spp.), including riparian habitat. The upland pine habitat was actively managed for timber harvest and wildlife (i.e., prescribed fire for red-cockaded woodpecker [Leuconotopicus *borealis*] conservation). Upland hardwood (including mixed pine-hardwood forest), Carolina Bay wetlands, and open canopy areas (e.g., grassland, shrub, herbaceous land cover, crop land) were also found throughout the SRS. The wild pig population on the SRS was descended from domestic pigs that free-ranged on the landscape and were released or abandoned after the private land was converted to a government facility in 1950 (Mayer et al. 2020). Later introduction of wild boar and wild boar x feral pig hybrids led to introgression of wild boar genes into the SRS wild pig population (Mayer et al. 2020, Smyser et al. 2020, Chinn et al. in prep). Since 1956, the wild pig population has been lethally managed, removing 24,980 individuals through 2018 (Mayer et al.

2020); nonetheless, the population has continued to increase over the past several decades to > 5,000 individuals (Keiter et al. 2017a).

Sow Captures

We captured pregnant sows on the SRS from September 2017 – February 2020 using corral traps baited with whole corn, which were monitored by remote cameras (Reconyx PC900, WI, USA) to determine reproductive status. We immobilized animals via dart rifle (X-CALIBER, Pneudart, PA, USA) using a combination of Telazol (4.4 mg kg⁻¹; MWI Veterinary Supply, ID, USA) and Xylazine hydrochloride (2.2 mg kg⁻¹; Wildlife Pharmaceuticals Inc., CO, USA) in 2017-2018, or with a combination of butorphanol (0.43 mg kg⁻¹), azaperone (0.36 mg kg⁻¹), and medetomidine (0.14 mg kg⁻¹) (BAM, 0.0064 ml kg⁻¹; Wildlife Pharmaceuticals Inc., CO, USA) and ketamine hydrochloride (2.2 mg kg⁻¹; Wildlife Pharmaceuticals Inc., CO, USA) in 2018–2020. Yohimbine (0.15 mg kg⁻¹; MWI Veterinary Supply, ID, USA) was injected intramuscularly to antagonize Xylazine hydrochloride and a combination of naltrexone (50 mg ml⁻¹; Wildlife Pharmaceuticals Inc., CO, USA) and atipamezole (25 mg ml⁻¹; Wildlife Pharmaceuticals Inc., CO, USA) was used to antagonize BAM. We released sows at the capture site and monitored them until they recovered from the anesthesia.

Under anesthesia, we weighed, measured body length and axillary girth, and aged sows through examination of molar eruption patterns (Mastchke 1967, Mayer et al. 2002). To determine if sows were pregnant, we used remote cameras at bait stations to monitor body condition prior to capture, and confirmed pregnancy using palpation and a portable ultrasound (SeeMore USB, Interson Corporation, CA, USA) upon immobilization. We implanted pregnant females with a 21g VIT (M3930; Advanced Telemetry Systems, MN, USA) via methods similar to white-tailed deer studies that previously have been employed for wild pigs (Keiter et al. 2017b, Chinn et al. 2021). A thermistor inside the VIT sensed a change in temperature upon expulsion during farrowing that tracked the number of 30-minute intervals elapsed since the change in temperature (i.e., the parturition event). We also collared sows with a Very High Frequency ([VHF], Model TGW-4501; Telonics Inc., AZ, USA) or GPS collar (Model TWG-4577; Telonics Inc. or Vertex Plus; Vectronic Aerospace, Berlin, Germany). Collars were equipped with a mortality sensor that activated after 12 h of inactivity.

We estimated location error of collars by placing a subset into open vegetation and dense forest for 5 days. We used the location data from these fixed locations to calculate the average distance of the fixes from the actual collar location. We used this error parameter to set the minimum distance between random available points for our habitat selection analysis.

Data processing

We obtained GPS data from pregnant sows at fix intervals of 30-min or 60-min. We subset the data for all sows with a 30-min GPS fix rate to maintain consistent temporal resolution of 60-min intervals for all individuals. We removed all 2-Dimensional fixes and further subset the data into the three physiologically distinct reproductive time periods described above (i.e., late gestation, farrowing, neonate care). Most sows were captured at least two weeks prior to farrowing; however, a subset of sows had less than two weeks of data prior to farrowing. For these sows we included data from the date they were collared, or after a brief censoring period characterized by movements that could be assumed as irregular immediately after capture (e.g., long distance movement, rapid movement to a destination).

Because sows may begin to restrict movements prior to farrowing, and the duration of time sows remain at the natal nest with neonates can vary among individuals, we developed moving window home range models to aid in delineating the initiation and duration of the farrowing period for each sow separately. We used the dynamic Brownian Bridge home range estimator (dBB, Kranstauber et al. 2012) with the *adehabitat* package (Calenge 2006) in R version 4.0.5 (R Foundation for Statistical Computing 2020) to quantify daily home ranges of sows beginning two weeks prior to farrowing through 55 days post-farrowing. We summarized changes in daily home range size using a three-day moving window average of daily range sizes and plotted 50% and 95% home range size through time (Fig. 1). For each sow, we used this quantitative diagnostic of space use to delineate the temporal scale for the three reproductive periods. Generally, the lategestation period began two weeks prior to the known farrowing event (from the VIT) and ended when home range size showed a marked and consistent decreasing trend. The farrowing period began when home range size began drastically decreasing and lasted until home range size showed an increasing trend for at least two days. Some sows lost their litter either by abandonment soon after farrowing or predation (neonate mortality was known from another study, Chinn et al. 2021) and their farrowing period was shortened to either the last day the sow was with the neonates or the day the neonates were preyed on. The neonatal-care period began once home range size consistently increased post-farrowing until two weeks from that date (i.e., 14 days) to approximate the

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timeline of the late gestation period. Since some sows lost their offspring during the farrowing period, they were not included in the neonatal-care period. Also, some sows lost their offspring during the two-week neonatal-care period, thus their time period was truncated to the last day their neonates were alive (as determined by Chinn et al. 2021). Each sow's GPS data was standardized with known farrowing date and time (as determined by VIT monitoring) as "Day 0" such that each sow's days (i.e., 24 h period) prior- and post-farrowing were relative to the actual time of farrowing. By calculating dBB home ranges for each sow, we were able to determine cut-off extents for our reproductive periods of interest that were optimized for each individual sow (Table 1). Hence, this method allowed the sow's behavior to determine the timespan of her reproductive periods, which we then used to estimate AKDE home ranges for each study period (Table 2).

Validation of farrowing event

We monitored sows with VITs 3–7 times/week until farrowing and recorded the time and date the VIT was extruded. We used First-passage Time (FPT) analysis (Fauchald & Tveraa 2003) to predict the timing of parturition from GPS data and validated it with the known timing of farrowing from the VIT. FPT is the time required for an individual to cross a circle of a given radius and measures how much time an individual spends in an area (Johnson 1992). It is a method by which to measure search effort, where the rate of increase of FPT for an increased radius of the circle becomes larger such that the mean FPT exponentially increases with an increase in the radius of the circle. The variance maximum occurs when the search area is clustered and the radius

is the scale at which an animal, in our case the sow, is intensively searching (Fauchald & Tveraa 2003, Byrne & Chamberlain 2012). Intensive search strategy is usually characterized by slow speed and high turn angles and tends to occur in areas with desired resources (i.e., preferred/high quality forage for farrowing, a nesting site). Area restricted search (ARS) is defined by the peaks in variance of log(FPT) as a function of the circle radius (at the peaks in variance). FPT is scale-dependent, thus the output represents the spatial scale at which the sow concentrated her movements. Using the *adehabitatLT* package (Calenge 2006) in R, we truncated the GPS data to two weeks prior- through two-weeks post-farrowing, calculated the highest peak in variance along the sow's path, which corresponded to the portion of the path with the highest FPT, the maximum amount of time it took for the sow to cross the circle at that point in time. We identified the point in time which signaled a distinct behavioral change (i.e., ARS, high FPT) that coincided with the farrowing event.

Home range analysis

To quantify home range sizes for each reproductive time period for each individual, we used autocorrelated kernel density estimation (AKDE, Fleming et al. 2015). Although previous studies have primarily used Minimum Convex Polygon (MCP) and kernel density estimators (KDE) to delineate wild pig home ranges (Gray et al. 2020), we used AKDE because it accounts for spatial autocorrelation inherent in GPS data with high fix rates (i.e., hourly). With short fix rate intervals, an individual's position and speed at one point in time are statistically correlated to their previous position and speed. Thus, AKDE is considered a better estimation of home range compared to KDE because KDE assumes independent and identically distributed data, which is violated by autocorrelation and nonstationarity (Silverman 2018), and often underestimates home range size (Fleming et al. 2014). We used the *ctmm* package (Calabrese et al. 2016) in R to fit a variogram and then parameter estimates to fit a model with a maximum likelihood estimate which was then used to generate 95% AKDE home range estimates. We removed outliers and locations indicative of traveling (i.e., subsequent locations in a linear trajectory between clustered locations) to meet the assumptions of AKDE. If the model did not converge, we subset the locations into ≥ 2 "home ranges" such that we obtained multiple 95% AKDE home range estimates and subsequently merged them together for total home range size and habitat selection analyses for each time period. For one sow's home range (P340 during the farrowing period) the AKDE model did not converge, we used the KDE function in the *adehabitat* package in R to calculate home range size for this one instance.

We used a linear mixed model (LMM) in the *lme4* package (Bates 2014) in R to determine whether AKDE home range estimates, with sow as a random effect, differed between the three reproductive time periods. Additionally, home range boundaries were used to place random available points within for the habitat selection analysis (see below).

Habitat selection analysis

Habitat covariates: From the 2016 National Land Cover Database (NLCD) raster layer (30 x 30 m-resolution), we reclassified landcover types into six individual raster layers in ArcGIS 10.7 (Environmental System Research Institute, Inc., CA, USA). The landcover layers included: (1) open water, (2) developed (i.e., buildings, paved areas), (3) open canopy (i.e., grassland, shrub, herbaceous, pasture, crops), (4) upland hardwood, (5) pine forest, and (6) bottomland hardwood (i.e., wetlands, riparian area). We then used a 3 x 3 pixel (i.e., 90 x 90 m) moving window to calculate the mean percent of each landcover type to assign to each used and random location (i.e., each location received an average percent from the 90 x 90 m buffer of each landcover that was it was observed in, or a "0" if the landcover was not present at that location). In ArcGIS, we separately calculated the Euclidean distance from streams, primary roads (paved and major gravel roads routinely used for transportation), secondary roads (minor gravel and dirt roads that were mostly unused), and buildings (facilities, buildings, parking lots) to used and random available locations. We used Light Detection and Ranging (LiDAR) data to estimate the percent canopy cover (30 x 30 m resolution), using a 3 x 3 pixel moving window to generate a mean percent canopy cover for each used and random available location. We used the SRS property border with a 5 km buffer as our covariate boundary for habitat selection analysis to account for long range movements outside of the SRS. All habitat covariates were centered and z-transformed to a mean of 0 and a standard deviation of 1 to allow for standardized comparison.

Habitat selection: We used resource selection functions (RSF; Boyce et al. 2002, Manly et al. 2007) to assess habitat selection during the three reproductive time periods. We quantified habitat availability for sows within their home range (i.e., third order selection, Johnson 1980) by comparing used GPS locations from the collar data to randomly sampled locations (i.e., available) within their home range for each habitat covariate. Available locations were randomly placed within the home range at a 1:1 ratio of used:available and at a minimum distance from one another determined by collar error (see above). By using resource selection functions during the three reproductive time periods, we could identify important habitats during each phase as well as discern if habitat selection changed between periods.

We calculated the RSFs with used-available logistic regression with random intercepts and included sow as a random effect to account for individual variation and repeated sampling across time periods. We fit generalized linear mixed models (GLMM) with the *lme4* package in R. RSFs with the used-available framework estimate the relative probability of selection as the disproportional use (i.e., selection) of a habitat type compared to its availability on the landscape (Manly et al. 2002). For each time period, we used a hierarchical approach to develop candidate models for determining selection by initially including all univariate models (n = 10). We then assessed strength of the fixed effect coefficient for each univariate variable for any statistically significant (P<0.05) models within each period (Table 3), and subsequently created multi-variable models for further evaluation. For the late-pregnancy period, univariate model coefficients with $\log\beta > |0.10|$ were then used in two-variable fixed effect models (all possible combinations), and coefficients with $\log\beta > |0.25|$ were included in threevariable fixed effect models (all possible combinations). For the farrowing period, univariate coefficients with $\log\beta > |0.05|$ were used for two-variable fixed effect models. We used a different $\log\beta$ cut-off for the farrowing period because the coefficient values were smaller, likely because of the fewer number of GPS locations during this period. No model coefficients were influential enough for us to warrant construction of threevariable fixed effect models for the farrowing period. Finally, for the neonatal-care period, coefficients with $\log\beta > |0.10|$ were used to build two-variable fixed effect models and a secondary cut-off of coefficients with $\log\beta \ge |0.25|$ was used for three-variable fixed effect models. We used Akaike's Information Criterion (AIC) and model weights to determine which candidate models provided the best support for the data for each reproductive time period. Model weight was used to evaluate the strength of influence among competing models (Burnham & Anderson 2002). We report back-transformed habitat coefficients in the top models for ease of interpretation.

We calculated variance inflation factors (VIF) between fixed effects in models with ≥ 2 variables. All VIF were < 3, indicating limited multicollinearity between our fixed effects. We assessed how well the top model fit the data using area under the curve (AUC) of the receiver operating characteristic (ROC), an evaluation metric for binary classification, using the *pROC* package (Robin et al. 2011) in R. An AUC value of 0.5 suggests the model is not able to distinguish between true or false positives, meaning the model predicts no better than random. However, when 0.5 < AUC < 1, the model predicts better than random and the higher the value, the better the model explains the data (Fawcett 2006).

Results

We deployed 22 VITs and had 23 reproductive events across 21 individuals from 2017-2020 (Table 1). One female farrowed twice in 2019 (P331; once with a VIT and once without) and again in 2020 (with a VIT). Sixteen sows were captured at least two weeks prior to farrowing. The remaining seven sows were captured between 3-12 days

prior to farrowing, thus GPS data for their late gestation period was abbreviated. One sow (P772) was captured and translocated approximately 6 km from her presumed home range and gave birth three days later. Because of atypical movement behavior due to translocation, we excluded this sow's movements from the late gestation period analyses (i.e., home range estimation and habitat selection). Of the 23 reproductive events (all included in the farrowing analyses), 16 were successful farrowing events such that there were live piglets at the nest and the sows subsequently exhibited parental care. An additional four sows had live piglets but abandoned their neonates (see Chinn et al. 2021) and were not included in the neonatal care period analyses. We observed no live piglets at two nests nor detected neonates with these sows after subsequent monitoring, and one litter was preyed on 1-2 days after farrowing (for details, see Chinn et al. 2021) and were not included in the neonatal care period analyses.

We used GPS data from all 23 sows to calculate both individual 50% and 95% dBB home ranges and a pooled-average home range for all sows two weeks before until 55 days post-farrowing (Fig. 1). We found home range size was largest during the late gestation period, and abruptly decreased immediately prior to the farrowing event (within 1-2 days of farrowing; Fig. 1). We used this abrupt shift in movement to delineate the end of the late gestation period and the beginning of the farrowing period. After farrowing, sow home range remained contracted, and ~1 week post-farrowing sows gradually increased home range size (Fig. 1). We identified the period at which sows began to expand movements away from the nesting area to signal the end of the farrowing period, and the beginning of the neonate care period.

Validation of farrowing event

Generally, we found FPT was a good predictor of farrowing events. There was an abrupt and apparent increase in FPT (i.e., longer time to move a certain distance) that coincided with the farrowing event. FPT analyses revealed ARS behavior indicative of farrowing for all sows generally within ~12-24 hours prior to the farrowing event. Some sows showed a second marked increase in FPT within days after farrowing, likely because the sow remained at or near the nest with the neonates (Fig. 2).

Home range analysis

Among the individuals monitored for this study, home range size varied between the three reproductive periods. The average 95% AKDE home range for all sows for the late gestation, farrowing, and neonatal care periods was 3.46 km² (95% Confidence Interval [CI]: 2.41-4.51), 0.44 km² (CI: -0.59-1.47), and 1.86 km² (CI:0.62-3.10), respectively (Fig. 3). Home ranges during the late gestation period were significantly larger than home ranges during farrowing (p = 0.0006); however, home range size during the neonate care period was highly variable and therefore not different from the late gestation or farrowing periods (Fig. 3). We calculated the 95% farrowing home range for sow P340 using KDE rather than AKDE because the AKDE model would not converge. The estimate was within the range of the other sow 95% AKDE home ranges.

It is important to note that the farrowing time period (average \pm SD, 7.83 \pm 4.91 days, n = 23) was shorter (Analysis of Variance [ANOVA], Tukey's test) compared to the late gestation and neonate care periods (12.63 \pm 3.22 days, n = 22, *p* = 0.0009 and

 13.44 ± 4.27 days, n = 16, p = 0.0004, respectively) which could affect home range calculations.

Habitat selection analysis

Late gestation: From the univariate GLMMs, sows selected to be closer to secondary roads and buildings, farther away from streams, and avoided areas with development, open canopy and pine forest, while selecting for upland hardwood and bottomland habitat (Fig. 4). We constructed a set of 24 candidate models (10 univariate + 10 two-variable + 4 three-variable) as described above (Table 4). Among these, there was a single top model, which indicated sows strongly selected bottomland habitat and avoided developed areas and pine forest (Table 4). The AUC value for this model was 0.68.

Farrowing: Univariate GLMM analysis indicated sows avoided developed areas, open canopy, and pine forest habitats and selected upland hardwood and bottomland habitats during the farrowing period (Fig. 5). We constructed a set of 20 candidate models (10 univariate + 10 two-variable models) as outlined above, resulting in a single supported model indicating sows selected upland hardwood and strongly avoided developed areas while farrowing (Table 5). The AUC value for the top model was 0.52.

Neonate care: The output from the univariate models revealed during the neonate care period sows selected upland hardwood and bottomland habitats, and to be closer to secondary roads, but avoided streams and areas with high canopy cover and developed, open canopy (i.e., grassland, shrub, herbaceous, pasture, crops), and pine forest habitats (Fig. 6). Using the β coefficient cut-off parameters outlined above, we constructed a set

of 20 candidate models (10 univariate + 6 two-variable + 4 three-variable; Table 6). This resulted in a single top model that indicated sows avoided developed areas and selected to be in or near upland hardwood and bottomland habitats (Table 6). The AUC value for this model was 0.63.

Discussion

Using fine-scale GPS data informed by known reproductive events for each individual, we found that physiological condition, centered around reproduction, influences movement and space use of invasive wild pigs, ultimately resulting in differential selection of habitats between the late gestation, farrowing, and neonatal-care periods. Wild pig movement ecology is not well-understood as most studies are focused on the outcomes of movement (e.g., disease transmission, damage, competition with native species; Morelle et al. 2014). Our study builds upon previous work on how spatial patterns of movement correlates with environmental features, and also fills a gap in knowledge about how an individual's life history requirements relative to reproduction (i.e., physiology and associated motivation) inform movement patterns. We provide a method using an existing movement analysis that identifies behavioral changes to reliably predict a farrowing event using GPS data. A better understanding of wild pig movement ecology is critical for effective management of this invasive species. Identifying habitats that are selected during reproduction informs managers about optimal temporal and spatial scales at which to concentrate management efforts to mitigate the negative effects of this destructive pest.

We employed a quantitative method informed by sow physiology and individual attributes to define three distinct reproductive time periods. Our results support that movement decreases during reproduction (Comer & Mayer 2002, Kay et al. 2017), and suggest the sow resides at the natal nest to be near the neonates. Neonates are fairly immobile, have poor thermoregulation, and are fully dependent on the sow for milk, protection, and warmth (Herpin et al. 2002, Comer & Mayer 2009). During this time, the sow may trade-off time spent foraging with attending the neonates, and the better condition she is in prior to farrowing, the longer she may be able to stay with the neonates during this critical period and thus increase offspring survival. Following the farrowing period, the neonates are more mobile and capable of following the sow during foraging bouts. Because lactation is the most energy intensive period of reproduction (Hanwell & Peaker 1977), it is necessary for the sow to obtain sufficient energy to convert to milk. Sows may concentrate foraging opportunities in areas with high-quality food that may increase the neonates' risk of detection by predators (i.e., if the sow forages in areas with sparse ground cover) or sows may choose to forage on lower quality resources in habitat that offers better concealment for neonates, which may also differ by seasonality of food resources (e.g., mast production). Hence, internal factors such as individual nutritional condition, parental experience, placement of home range, and external conditions such as season, habitat available within the home range, and predator density are factors that may contribute to the temporal scale of the three reproductive periods.

Wild pigs are amongst the most abundant and widely distributed ungulate globally, largely due to their high fecundity (Taylor et al. 1998); however, locating

farrowing nests and determining reproductive events in wild populations of this species is challenging and often impractical. We used an existing behavioral change analysis framework, first passage time, to identify farrowing events for individual wild pigs. By using FPT analysis to characterize ARS and VITs to validate farrowing events, we demonstrated sows exhibit a distinct behavioral change within 1-2 days prior to farrowing. This ARS behavior was sustained for a prolonged period (i.e., the ARS behavior during a farrowing event typically lasted several days), unlike ARS behavior exhibited during resting, foraging, wallowing that typically lasted on the scale of hours. While previous efforts have implemented rolling window MCP and behavioral change point analysis (BCPA; Nicholson et al. 2019), step length (DeMars et al. 2013, Bonar et al. 2018), clustered locations (Carstensen et al. 2003), and movement rates (Long et al. 2009) to infer reproductive events, our results reveal FPT can be a powerful tool for explicitly identifying farrowing events in sows fitted with GPS collars for other research purposes. Characterization of ARS behavior could give insights about sow movement and habitat selection during the distinct reproductive time periods, such that managers can target specific resources for management (i.e., traps, toxicant placement). This may be especially impactful during the late gestation period when sows should be spending more time foraging to build energy reserves to support the farrowing event and knowing areas of high use may facilitate trapping efforts, thereby removing the reproductive sow as well as any potential offspring she would have farrowed. For widespread invasive species like wild pigs, where numerous uncertainties remain regarding the frequency of reproductive events for individual sows, using movement data to quantify behavioral changes associated with parturition is useful for improving population dynamics models, which
ultimately are needed to better inform management and conservation strategies. Additional investigation of FPT analysis to discern a behavioral change for sows that abandon or lose their neonates would contribute to accurate offspring survival estimates and population modeling.

We predicted home range size would be largest during the late gestation period, markedly contracted during the farrowing period, and gradually increase during the neonate care period. Indeed, sows in our study generally exhibited a marked decrease in home range size during the farrowing period compared to their home ranges during the late gestation, as previously reported in other studies (Comer & Mayer, Kay et al. 2017). This substantial decrease in space use upon farrowing likely reflects nest building, the birth event, protection of neonates, and bonding, and thus is important for increasing reproductive success. Substantially larger home ranges during the neonatal-care period may negatively affect offspring survival because neonates would not be able to adequately keep up with the speed or distance traveled. During the neonate care period we found that home range size was intermediate between late gestation and the farrowing periods, reflective of caring for offspring which is reported to restrict maternal movement (Bowyer et al. 1999).

Although wild pigs are broadly considered ecological generalists, our results suggest that some habitats are important for reproductive success. During the last two weeks of the gestation period, when sows experience increased energetic demands in preparation for farrowing and lactation, sows selected bottomland hardwood forests and associated wetlands, likely because this habitat is characterized by ample food resources, cover, and proximity to water. On the SRS, bottomland hardwood forests are a mix of hardwood species found along riparian corridors and extend into the swamp lands and are frequently inundated with water (Workman & McLeod 1990), which is essential for pigs given their poor thermoregulatory ability (Gray et al. 2020). In addition to providing access to water, the mesic and hydric soils that occur throughout bottomland forest are optimal habitat for foraging (i.e., rooting) because they are more pliable for excavating subterranean food items (Mitchell & Mayer 1997, Welander 2000). In addition to the availability subterranean food items, bottomland habitats on the SRS are characterized by several hard mast producing species (i.e., oaks – *Quercus* spp.), which represent high quality forage that has been shown to increase reproductive output in pigs (Briedermann 1971, Barrett 1978, Servanty et al. 2009). Unflooded stands also typically have dense understory vegetation characterized by a diversity of herbaceous species including dog hobble (Leucothoe axillaris), which has been documented as a source of nesting material (Chinn unpublished data). Sows tended to avoid pine forests and developed areas, which encompassed buildings, paved areas, powerlines and areas adjacent to paved and gravel roads. Pine forests are the dominant habitat type on the SRS; however, sows avoided this habitat likely because of its open understory, lack of hard mast, and xeric soils which are less favorable for rooting behavior. Avoidance of pine is not unique to the late gestation period, rather it is a trend observed in other third order habitat selection studies (e.g., Clontz et al. 2021). Similar to other studies on wild pigs and wild boar, sows avoided developed areas, which provide sparse canopy and ground cover and are associated with human activities, (e.g., hunting pressure, vehicles; Gray et al. 2020). While avoidance of pine and developed habitats are reflective of general trends in habitat selection of wild pigs, our results indicate that they are avoided during reproduction. Our models revealed

variability among individuals in the extent of selection and avoidance of habitats, as has been widely observed in other studies of resource selection in wild pigs and wild boar (Thurfjell et al. 2009, Kay et al. 2017, Clontz et al. 2021), likely reflecting the generalist behavior and adaptability of this species.

During the farrowing period, when sows exhibited limited movement beyond the natal nest area, sows selected upland hardwood forests and avoided developed areas. Upland hardwood forests were likely favored during this period because they provide high quality food resources for the sow as well as cover for the neonates. On the SRS, this habitat is typically comprised of a suite of oak and hickory trees (*Carya* spp.) as well as a varied understory and ground cover that produce berries, grains, and seeds (Whipple et al. 1981, Workman & McLeod 1990). By placing farrowing nests in upland hardwood habitat, sows selected for areas that provide nesting material to construct farrowing nests, food resources close to the nest, and ground cover to provide protection for the neonates from the elements and predators. Sows also strongly avoided developed areas during the farrowing period, presumably due to the lack of ground cover and to avoid anthropogenic disturbance during the period when neonates are most vulnerable and susceptible to mortality (Chinn et al. 2021).

During the neonatal care period, sows selected for both upland and bottomland hardwood forests and avoided developed areas. In addition to the abundance of preferred food resources (i.e., mast, fruits) supported by hardwood habitats that are necessary to support the cost of lactation, upland and bottomland hardwoods provide more extensive understory and ground cover compared to pine forests. These vegetation features facilitate thermoregulation during the warmer months and provide cover for neonates to hide from potential predators. Increased ground cover and better access to food resources also may facilitate the sow leaving the neonates unattended while she forages in close proximity (S.M.C. personal observation) thus, selection for habitat that provides ample ground cover to camouflage offspring within proximity of quality food resources may increase reproductive success.

Wild pigs are highly adaptable and capable of exploiting a diversity of habitats (VerCauteren et al. 2020) provided they have access to food, cover, and water (Gray et al. 2020). Therefore, articulating patterns of landscape use are especially challenging given differential resource selection at different spatial and temporal scales (Adkins & Harveson 2007, Gray et al. 2020, Clontz et al. 2021). Collectively, our data suggest that despite being ecosystem generalists (Bevins et al. 2014), at the individual level wild pigs exhibit behavioral changes in relation to space use and habitat selection associated with reproduction and caring for young. The physiological requirements (i.e., internal state) associated with the various stages of reproduction (i.e., pregnancy, neonatal care, and lactation) provide the motivation (i.e., mechanism) by which females choose where and how large of an area they use as well as what habitats to concentrate their movements, in relation to what is available within their home range. Home range adjustment and habitat selection during the reproductive periods likely reflect choices made to increase offspring survival and thus overall fitness of the sow. Evaluating fine-scale habitat selection during a critical life-history event provides insight about important resources wild pigs depend on, or at least exploit, that influence population growth.

Conclusions

Movement analysis of fine scale GPS data during specific life-history events at the appropriate temporal and spatial scale provide a better understanding of environmental attributes integral for increasing individual fitness, population dynamics, and community structure. For a highly invasive species, it is critical to elucidate factors that contribute to their ability to invade, thrive, and expand in novel habitats to effectively manage populations. Our methodology also can be applied to sensitive/threatened species to identify biologically relevant temporal scales of critical life-history events leading to delineation of the appropriate scale at which to analyze movement data to provide insight about where individuals chose to place their home range, how much space to use, and how they use resources on the landscape to increase survival and reproductive success.

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Table 3.1. 95% Autocorrelated Kernel Density Estimate (AKDE) home range during three reproductive time periods for female wild pig (*Sus scrofa*) sows captured at the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC, USA from 2017–2020. Home range estimates include the 95% confidence intervals (low = 2.5%, high = 97.5%). *Home range calculated with KDE.

	Pre-Farrowing (km ²)			Farrowing (km ²)		Post-Farrowing (km ²)			
	low	95% estimate	high	low	95% estimate	high	low	95% estimate	high
P223	1.36	2.00	2.76	0.12	0.16	0.20	2.82	4.59	6.79
P321	2.04	3.69	5.82	0.17	0.25	0.34	0.98	1.25	1.54
P326	1.92	2.65	3.50	0.03	0.05	0.06	0.37	0.54	0.75
P328	2.17	3.00	3.96	0.94	1.65	2.55	0.14	0.39	0.75
P331a	2.43	3.39	4.50	0.48	0.80	1.20	1.29	1.76	2.30
P331B	2.58	4.97	8.14	0.09	0.15	0.24	0.19	0.30	0.43
P331C	4.21	9.12	15.90	0.23	0.50	0.87	1.87	2.90	4.17
P340	2.40	3.77	5.44	-	0.03*	-	-	-	-
P354	0.82	2.60	5.38	0.36	0.60	0.90	3.45	5.20	7.30
P355	0.09	0.30	0.64	0.00	0.01	0.03	-	-	-
P708	1.08	1.64	2.32	0.11	0.21	0.33	-	-	-
P749	0.29	0.59	0.99	0.30	0.42	0.55	1.44	2.21	3.15
P750	1.22	2.64	4.61	0.03	0.05	0.07	1.44	2.83	4.68
P758	1.43	2.74	4.48	0.01	0.03	0.04	-	-	-
P762	0.67	1.12	1.69	0.23	0.44	0.71	1.52	2.66	4.12
P769	3.37	5.86	9.04	0.49	1.36	2.66	-	-	-

Individual 95% AKDE Home Range Estimates

P772	-	-	-	0.01	0.01	0.01	0.11	0.15	0.19
P783	0.69	0.98	1.32	0.00	0.02	0.02	-	-	-
P784	0.78	1.14	1.56	0.37	0.52	0.69	0.53	1.06	1.78
P789	1.25	1.84	2.55	0.58	1.15	1.92	-	-	-
P795	0.22	0.29	0.40	0.18	0.40	0.71	0.22	0.30	0.39
P796	2.44	3.74	5.30	0.06	0.15	0.29	1.92	2.74	3.69
P797	7.71	18.06	32.75	0.72	1.16	1.72	0.69	0.89	1.12

Table 3.2. Data for female wild pig (*Sus scrofa*) captured at the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC, USA from 2017–2020. Reproductive time periods were determined for each individual using behavioral shifts in movement behavior and duration of time periods are listed below in relation to the farrowing date (standardized for all sows as day 14). Nest outcome: successful, sow exhibited parental care for the duration of the neonate care time period otherwise noted when offspring died, e.g., 1 wk success = piglets survived for 1 week and subsequent monitoring was discontinued; abandoned at nest, neonates tagged and sow abandoned nest; no piglets at nest, no live piglets observed at natal nest or later on with the sow; preyed on at nest, neonates born alive but killed by predator at natal nest.

	Late Gestation			
Sow	(days)	(days)	(days)	Litter Outcome
P223	6-12	13-21	22-36	successful
P321	0-14	15-20	21-35	successful
P326	0-14	15-20	21-35	successful
P328	0-13	14-18	19-20	1 wk success
P331A	0-14	15-24	25-39	successful
P331B	0-13	14-19	20-22	2 wk success
P331C	0-15	16-26	27-41	successful
P340	0-13	14-16	-	no piglets at nest
P354	4-13	14-24	25-39	successful
P355	6-12	13-16	-	no piglets at nest
P708	0-13	14-15	-	abandoned at nest
P749	12-14	15-32	33-47	successful
P750	1-13	14-20	21-35	successful
P758	0-14	15-16	-	abandoned at nest
P762	1-13	14-19	20-34	successful
P769	0-12	13-14	-	preyed on at nest
P772	-	13-22	23-37	successful
P783	0-13	14-16	-	abandoned at nest
P784	0-13	14-30	31-45	successful
P789	0-10	11-15	-	abandoned at nest
P795	0-14	15-19	20-34	successful
P796	0-13	14-30	31-45	successful
P797	0-12	15-28	29-43	successful

Reproductive Time Periods

Table 3.3. Univariate model landcover coefficients calculated from the Resource Selection Functions (GLMM) for each reproductive time period for female wild pig (*Sus scrofa*) habitat selection on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020. '*' indicates significant coefficient, covariate used for two-variable models, '**' indicates significant coefficient, covariate used for two- and three-variable models

		Farrowing	
Habitat covariate ¹	Late gestation log(β)	log(β)	Neonate care log(β)
road1	0.07	-0.01	-0.01
road2	0.14*	0.09*	0.06
building	0.34* ^a	0.01	-0.02
stream	-0.28**	-0.03	-0.05
canopy	-0.01	-0.05	-0.06
develop	-0.36**	-0.25*	-0.41**
open	-0.08	-0.05	-0.09
upland hardwood	0.08	0.14*	0.27**
pine	-0.37**	-0.07*	0.25**
bottomland	0.56**	0.07*	-0.27**

Coefficients from univariate models for three reproductive time periods

¹Variables included in model selection analyses: bottomland: bottomland

hardwood/swamp/wetland; develop: buildings, paved areas, powerlines, roadsides; stream: distance (m) to streams; pine: pine forest; road2: secondary roads – dirt/unpaved; building: distance to facilities; open: open canopy; upland hardwood: upland hardwood and mixed forests; road1: primary roads – paved and gravel; canopy: percent canopy cover.

^abuilding covariate was dropped from multivariate analyses because it was similar to the develop covariate

Table 3.4. Set of candidate models that included landcover covariates influencing female wild pig (*Sus scrofa*) habitat selection during the late gestation period on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020.

Model Tested ¹	К	AIC	ΔΑΙϹ	Weight
bottomland + develop + pine	5	14942.93	0.00	1.00
bottomland + develop + stream		15011.57	68.65	0.00
bottomland + develop		15011.67	68.74	0.00
bottomland + pine	4	15120.81	177.88	0.00
bottomland + pine + stream	5	15122.81	179.88	0.00
bottomland + road2	4	15147.89	204.96	0.00
bottomland + stream	4	15151.20	208.27	0.00
bottomland	4	15151.47	208.54	0.00
develop + pine + stream	5	15164.17	221.24	0.00
develop + pine	4	15183.30	240.37	0.00
pine + stream	4	15456.42	513.49	0.00
pine + road2	4	15498.80	555.87	0.00
pine	4	15505.17	562.25	0.00
develop + stream	4	15521.97	579.04	0.00
develop + road2	4	15540.81	597.88	0.00
develop	4	15571.53	628.61	0.00
stream + road2	4	15702.96	760.03	0.00
stream + road2	4	15742.65	799.73	0.00
road2	4	15787.60	844.67	0.00
building	4	15793.20	850.28	0.00
open	4	15806.35	863.42	0.00
upland hardwood	4	15806.51	863.58	0.00
null	3	15819.14	876.22	0.00
road1	4	15819.22	876.29	0.00
сапору	4	15820.94	878.02	0.00

Models analyzed for habitat selection during late gestation in wild pigs

¹Variables included in model selection analyses: bottomland: bottomland

hardwood/swamp/wetland; develop: buildings, paved areas, powerlines, roadsides; stream: distance (m) to streams; pine: pine forest; road2: secondary roads – dirt/unpaved; building: distance to facilities; open: open canopy; upland hardwood: upland hardwood and mixed forests; road1: primary roads – paved and gravel; canopy: percent canopy cover. Table 3.5. Set of candidate models that included landcover covariates influencing female wild pig (*Sus scrofa*) habitat selection during the farrowing period on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020.

Model Tested ¹	К	AIC	ΔΑΙϹ	Weight
develop + upland hardwood	4	10391.51	0.00	0.99
develop + road2		10401.20	9.68	0.01
develop + pine	4	10412.58	21.06	0.00
develop + bottomland	4	10418.94	27.43	0.00
upland hardwood + pine	4	10420.91	29.39	0.00
upland hardwood + road2	4	10420.91	29.39	0.00
develop	3	10422.54	31.02	0.00
upland hardwood + bottomland	4	10425.65	34.14	0.00
upland hardwood	3	10444.36	52.84	0.00
road2 + pine	4	10461.86	70.35	0.00
road2 + bottomland	4	10463.38	71.87	0.00
road2	3	10464.95	73.43	0.00
pine	3	10470.47	78.95	0.00
pine + bottomland	4	10470.80	79.29	0.00
bottomland	3	10471.30	79.78	0.00
open	3	10474.34	82.82	0.00
canopy	3	10475.12	83.60	0.00
null	2	10477.41	85.89	0.00
stream	3	10477.99	86.47	0.00
road2	3	10479.22	87.70	0.00
building	3	10479.34	87.82	0.00

Models analyzed for habitat selection during the farrowing period in wild pigs

¹Variables included in model selection analyses: bottomland: bottomland

hardwood/swamp/wetland; develop: buildings, paved areas, powerlines, roadsides; stream: distance (m) to streams; pine: pine forest; road2: secondary roads – dirt/unpaved; building: distance to facilities; open: open canopy; upland hardwood: upland hardwood and mixed forests; road1: primary roads – paved and gravel; canopy: percent canopy cover. Table 3.6. Set of candidate models that included landcover covariates influencing female wild pig (*Sus scrofa*) habitat selection during the neonate care period on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020.

Model Tested ¹	К	AIC	ΔΑΙϹ	Weight
develop + upland hardwood + bottomland	5	12957.08	0.00	1.00
develop + upland hardwood + pine	5	13033.57	76.48	0.00
upland hardwood + pine + bottomland	5	13069.54	112.46	0.00
upland hardwood + bottomland	4	13070.70	113.62	0.00
develop + pine + bottomland	5	13138.00	180.92	0.00
develop + pine + bottomland	4	13147.92	190.84	0.00
develop + upland hardwood	4	13158.09	201.00	0.00
upland hardwood + pine + bottomland	4	13185.17	228.09	0.00
develop + bottomland	4	13208.16	251.08	0.00
pine + bottomland	4	13290.22	333.14	0.00
develop	3	13295.83	338.75	0.00
upland hardwood	3	13301.27	344.18	0.00
pine	3	13311.24	354.16	0.00
bottomland	3	13344.00	386.92	0.00
open	3	13429.52	472.44	0.00
canopy	3	13439.38	482.30	0.00
road2	3	13441.27	484.19	0.00
stream	3	13442.24	485.16	0.00
null	2	13446.56	489.48	0.00
building	3	13447.78	490.70	0.00
road1	3	13448.51	491.42	0.00

Models analyzed for habitat selection during the neonatal care period in wild pigs

¹Variables included in model selection analyses: bottomland: bottomland hardwood/swamp/wetland; develop: buildings, paved areas, powerlines, roadsides; stream: distance (m) to streams; pine: pine forest; road2: secondary roads – dirt/unpaved; building: distance to facilities; open: open canopy; upland hardwood: upland hardwood and mixed forests; road1: primary roads – paved and gravel; canopy: percent canopy cover.

Figures



Figure 3.1. Moving window average (3 days) of dynamic Brownian Bridge (dBB) home range estimation 14 days prior to farrowing through 55 days post-farrowing for all female wild pigs (*Sus scrofa*), n = 23, on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020. Farrowing was standardized for all sows as day 14.



Figure 3.2. Example of First Passage Time (FPT) analysis used to identify area restricted search (ARS) behavior during the entire reproductive period (i.e., 14 days prior and post the farrowing event) for female wild pigs (*Sus scrofa*) on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020. Top panel: variance of log(FPT) corresponds to the spatial scale at which the sow exhibits ARS behavior. Arrow indicates peak in variance (350 m). Middle panel: FPT abruptly increased 1-2 days prior to

farrowing and remained high (sometimes increasing) immediately after the farrowing event for several days, while the sow attended the neonates. Arrow indicates farrowing day (September 27). Bottom panel: sow movement path during the entire reproductive period, warmer colors indicating ARS behavior. Nest site location was located in the red area (i.e., where there was ARS).



Figure 3.3. Average 95% Autocorrelated Kernel Density Estimate (AKDE) home range with 95% confidence intervals for each reproductive time period for all female wild pigs (*Sus scrofa*), n = 23, on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020.



Figure 3.4. Predictive odds with 95% confidence intervals for third order habitat selection during the late gestation period for female wild pigs (*Sus scrofa*), n = 22, on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020. Dashed line corresponds to value = 1 where if the confidence intervals overlap, the habitat variable is not disproportionately selected for in relation to its availability. Landcover variables > 1 indicates greater odd of association, distance variables < 1 indicates greater odds of being closer in proximity.



Figure 3.5. Predictive odds with 95% confidence intervals for third order habitat selection during the farrowing period for female wild pigs (*Sus scrofa*), n = 23, on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020. Dashed line corresponds to value = 1 where if the confidence intervals overlap, the habitat variable is not disproportionately selected for in relation to its availability. Landcover variables > 1 indicates greater odds of association, distance variables < 1 indicates greater off of being closer in proximity.



Figure 3.6. Predictive odds with 95% confidence intervals for third order habitat selection during the neonate care period for female wild pigs (*Sus scrofa*), n = 16, on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020. Dashed line corresponds to value = 1 where if the confidence intervals overlap, the habitat variable is not disproportionately selected for in relation to its availability. Landcover variables > 1 indicates greater odds of association, distance variables < 1 indicates greater off of being closer in proximity.

CHAPTER 4

MATERNAL INVESTMENT STRATEGIES AND SEX RATIO ADJUSTMENT IN A POLYTOCOUS LARGE MAMMAL

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Abstract

Skewed sex ratios at birth are widely reported, however the extent to which modulation of offspring sex is an adaptive strategy for parents to increase their own fitness remains unclear, particularly for polytocous species because trade-offs between size and number of offspring must be considered. In such cases, it may be adaptive for mothers to adjust both the number of offspring per litter and their sex to maximise reproductive returns. Using wild pigs (Sus scrofa) as a model, we tested whether (1) older and larger mothers produced male-biased litters, (2) females with abundant food resources produced male-biased litters, (3) high quality females invested more in litter size and more male offspring, and (4) sex ratio varied relative to litter size. Older females had male-biased litters, providing support for sex ratio adjustment, however neither maternal size nor food resources influenced offspring sex ratio. High quality mothers invested more in litter production, but this relationship was driven by adjustment of litter size, not sex ratio. There was no relationship between sex ratio and litter size. Collectively, our results emphasised adjustment of litter size appeared to be the primary reproductive characteristic manipulated to increase fitness rather than adjustment of offspring sex ratio.

Introduction

Sex allocation theory [1-2], particularly sex ratio variation (i.e., the proportion of male and female offspring produced), is a widely addressed concept in life-history theory [1-4]. While previous studies have documented skewed sex ratios favouring either males or females at birth, the question of whether modulation of offspring sex is an adaptive

strategy for parents to increase their own fitness remains unclear, particularly for vertebrates (for reviews see 4-6). Further, the physiological mechanism for sex ratio adjustment is also not well established, though there are several proposed processes that can occur either pre- (e.g., chromosomal control, motility/mortality of sperm, hormone concentration) or post-conception (e.g., differential implantation of male and female zygotes, differential mortality of male and female fetuses; 7-9). Recent studies on vertebrate species that employ either environmental (e.g., reptiles) or gonadal sex determination (e.g., birds and mammals) suggest mechanisms responsible for sex ratio variation are influenced by individual parental phenotype (i.e., maternal condition), genotype (i.e., genetic pathways and epigenetics) and physiology (i.e., corticosteroids), social changes (i.e., status and interactions), population density, and stochastic environmental conditions [10-12].

Parental condition, which can influence reproductive success [13-14], is one of the most commonly tested predictors of offspring sex ratio [15]. The Trivers Willard Model (TWM) is an ecological theory that predicts, in polygynous species in which males have greater variance in individual fitness (i.e., reproductive potential), highquality mothers should invest more into sons provided the following: sons benefit more than daughters from this extra investment, offspring quality correlates to adult quality, and offspring quality is a good indicator of maternal quality. Thus, females should be able to adjust their offspring sex ratio in response to factors that could modify both their own lifetime reproductive success and the reproductive success of their progeny. Though well studied in vertebrate species that produce a single offspring, [16-18], it is not well defined if TWM applies to, or how it applies to polytocous species, those that produce several offspring per litter, because the trade-offs between size and number of offspring must also be considered [5,19-20].

For polygynous polytocous species, it may be adaptive for the mother to adjust both the number of offspring per litter and their sex to maximise the reproductive returns of her offspring, according to her own fixed amount of resources [5]. Departing from Fisherian sex ratio that parental expenditure on both sexes should be equal [3], in most mammals the males are larger at birth and develop at a faster rate both in utero and during the post-parturition investment period [21-22]. Thus, for mammals it can be assumed there is a differential cost to producing male and female offspring [23]. As the number of offspring within a litter increases, reproductive returns from investing in male and female offspring will become equivalent and the mother may more greatly increase her fitness by adjustment of litter size rather than offspring sex ratio [2]. Thus, predictions of the TWM are complicated [5,20]. Studies of sex allocation exist in the literature for avian species where parental care is typically provided by both parents [24-26] and small mammals, especially in a laboratory setting [27-28], however it is not well tested for large mammalian species with multi-offspring litters (but see 29). Consideration of the effects of resource availability further complicate testing sex ratio theory, as the resources available to the mother can modulate investment in both litter size and sex ratio [5]. At one extreme, mothers with limited resources should have smaller and female-biased litters, while at the other extreme mothers with ample resources should have large and male-biased litters. Mothers should be selected to have intermediate-sized litters with more or less equal number of males and females under moderate resource availability [5].

Despite numerous studies assessing the influence of resource availability on maternal condition (for examples see 30-31), research evaluating the interaction between environmental drivers (abiotic) and individual attributes (biotic) on sex ratio theory is minimal for large, free-ranging mammals (but see 32-33). For example, stochastic climatic conditions and population density both can influence reproductive timing and offspring survival and have been identified as possible drivers of variance in sex ratio at both the individual and population levels [10,34-35]. Local resource competition varies depending on resource availability, population density, and social structure of the species in question, all of which can influence an individual's nutritional condition. Food availability, reflected by the mother's nutritional condition, can affect reproductive output in terms of timing, age at first breeding, litter size, and offspring survival [36-38]. Thus, if offspring sex ratio adjustment is an adaptive strategy, it is reasonable to expect the interaction between environmental factors (e.g., extreme climatic events) and individual phenotype (e.g., age) should predictably influence offspring sex ratio.

The wild pig (*Sus scrofa*) is an ideal species in which to study sex allocation theory, particularly offspring sex ratio where there are higher than expected reproductive returns when maternal investment is biased to the sex with the most reproductive potential, because of the trade-off between sex adjustment and the number of offspring. Wild pigs exhibit a polygynous mating strategy, are highly polytocous with mean litter size of 5.3 in North America [39], and have the highest reproductive rate compared to any other mammal of similar size [40]. Within North America wild pigs are a genetic mix of wild boar and domestic pig ancestry [41-42], which may influence litter size due to the introgression of domestic genes that were selected for by the livestock industry to
maximize reproductive output [40]. Studies have documented increased litter size and earlier sexual maturity of wild boar in their native range [43-45] and wild pigs in North America [46-48] relative to mast availability, as well as in response to pulses in other seasonal crops (wild boar [30,49]; wild pig [50-51]). Abundant resources may increase maternal condition and in turn, females in superior condition may have the physiologic resources to increase offspring size, number, and adjust sex ratio as means to increase lifetime reproductive success.

Using reproductive data collected over multiple years with varying resource availability, we applied broad sex allocation theory, specifically in terms of a suite of biotic and abiotic factors that have been proposed to drive adaptive adjustment of litter sex ratio and parental investment, to test the applicability of the TWM and Williams Models to wild pigs, a highly polytocous invasive species. First, we tested the hypothesis that maternal phenotype would influence litter sex ratio, a test of the TWM. If supported, we predicted older and larger females would produce male-biased litters. Second, we hypothesized environmental conditions would influence litter sex ratio and the interaction of these abiotic conditions and maternal attributes would increase the magnitude of any effects to litter sex ratio, a test if resources affected sex ratio as predicted by the TWM. We predicted in years with abundant resources (e.g., a pulsed food resources such as oak mast, *Quercus* spp.) females would be in better nutritional condition (i.e., more fat reserves) and produce male-biased litters. Third, to test the Williams Model, we hypothesised maternal age, size, and body condition would influence the relative investment in litter production cost, a metric reflecting litter size and the offspring sex ratio [5]. We predicted increased investment (i.e., larger litters and more males) in

females that were older, larger, and in better condition. Finally, we expected sex ratio would vary in relation to litter size, with smaller litters being male-biased and larger litters being female-biased, a trade-off in the Williams Model.

2. Materials and Methods

(a) Study Area

This study was conducted at the Savannah River Site (SRS), a 78,000 ha USA Department of Energy facility located in Aiken, Barnwell, and Allendale Counties, South Carolina, USA. Habitat on the SRS was mostly comprised of upland pine forests, bottomland hardwood forests, and swamps. Wild pigs on the SRS are descendants of feralised domestic pigs that were not recovered by farmers after the government purchased the land in 1950 [52]. These individuals found refuge and multiplied in the southern river-swamp habitat on the SRS [52]. A subsequent introduction of wild boar/feral pig hybrids occurred on the SRS in the 1980s, which have since expanded throughout the site. Despite being lethally managed since 1956, the wild pig population on the SRS has continued to increase over the last several decades [52-53]. Wild pigs are abundant throughout the entire SRS [52], with an estimated population size of >5,000 individuals at the time of this study [53]. Generally, this population is composed of hybrids of Western heritage breeds of domestic pigs and wild boar and tend to vary substantially in percent wild boar vs. heritage breed ancestry among individuals [42].

(b) Data Collection

We sampled from live-trapped and culled wild pigs throughout the year between March 2017 and July 2019. Wild pigs were primarily sampled from individuals obtained from SRS pig control contractors during ongoing wild pig management on the SRS, although additional individuals were culled as part of other wild pig research activities on the SRS. We collected fetal data and morphological measurements from sows > 27 kg captured across the SRS to quantify litter and maternal attributes. We weighed (whole body weight to nearest 0.5 kg) and measured each sow dorsally from snout to base of tail (to nearest 0.5 cm). We calculated a standardised size index for each individual as mass/length [54]. Our study encompassed years of differential mast availability, with 2017 as high availability and 2018 and 2019 having low to moderate mast availability (author, personal observation). We measured extraneous fat reserves (i.e., rump fat), a quantitative measurement of maternal nutritional condition, and used it as a proxy for local resource availability, namely hard mast production. We measured rump fat (to nearest 0.01 cm) between the ischium and ilium bones, approximately 3 cm lateral from the spine. We assessed age by tooth eruption and replacement patterns [55-56], and individuals were classified into 3 age classes: juvenile (between 6 months and 1 year), yearling (1-1.5 years), and adults (\geq 1.5 years old). If present, fetuses were removed, weighed (whole body mass to the nearest 0.1 g) and measured in a straight-line from crown to rump (to the nearest 0.1 cm). Fetuses ~40 days and older were reliably developed enough to visually determine sex.

(c) Data Analysis

To confirm rump fat was a suitable proxy for resource availability, we tested whether increased acorn mast production in 2017 resulted in differing rump fat deposits compared to the other years of our study using an ANOVA test. We calculated sex ratio for each litter as the proportion of males. We tested if the sex ratio differed significantly from parity using a one-sample t-test (i.e., the null hypothesis, $\mu = 0.5$).

Trivers Willard Model

We used the *lme4* package in R [57] to fit logistic regression models to test the influence of a suite of maternal quality attributes and environmental variables on litter sex ratio [58]. We included maternal age class, maternal size, resource availability (i.e., rump fat), litter size, average fetus mass, as well as the interactions between age and all the continuous variables as fixed effects, and sow and sample year as random effects in the global model, from which we created a candidate set of models using all possible combinations of fixed and random parameters (*MuMIn* package in R, [59]). We used Akaike's Information Criterion (AIC) corrected for small samples sizes (AICc) and model weights to determine which candidate models provided the best support for the data. Models within $\Delta AICc \leq 2$ of the top model were considered influential and reported. Model weight was used to evaluate the strength of influence among competing models [60]. Based on visualization of our data and published literature regarding the effects of maternal age class on sex ratio.

Williams Model

Since gestation duration differed among sampled pregnant females (i.e., individuals were in different stages of pregnancy), we used the proportional difference in mass between male and female fetuses within each litter to quantify the relative cost of producing a male or female fetus. On average, male and female mass was $350.82 \pm$ 280.03 g (\overline{X} ± standard deviation) and 345.11 ± 261.16 g, respectively. Male fetuses were 2% heavier than females, thus the direct cost of producing a male was slightly greater compared to producing a female (i.e., on average 1 male = 1.02 female). Using these averages, we calculated the investment cost of producing each litter, a function of the number of fetuses and their sex, to determine whether there was a trade-off between litter size and offspring sex ratio. For example, a litter with 2 females and 2 males would require an investment of $2 \ge 1 + 2 \ge 1.02 = 4.04$ units, whereas a litter of 4 females or 4 males would require an investment of 4.00 and 4.08 units, respectively. We tested if maternal attributes and food resource availability influenced investment in litter production. Using the same criteria as above for AIC model selection, we used linear mixed effects models (*lme4* package in R) to test if maternal age class, maternal size, resource availability (rump fat), and the age x size and age x rump fat interactions affected litter investment. We included sample year as a random effect in our models. In a post-hoc analysis we used a linear mixed effects model to test if litter size and sex ratio were related to maternal size to determine if either litter size or sex ratio were more influential in driving litter investment. Finally, we calculated the mean sex ratio for all observed litter sizes to test if sex ratio varied in relation to litter size, such that smaller litters would be male-biased and larger litters would be female-biased.

For all analyses we centered and z-transformed continuous variables to a mean of 0 and a standard deviation of 1 to allow for standardised comparison. Variance inflation factors (VIF) were < 3, indicating no multicollinearity between fixed effects. We checked for overdispersion and that the standardised residuals were randomly distributed around zero with respect to the fitted values [58]. We assessed model residuals for normality. All analyses were performed in R 3.5.3 (R Development Core Team 2004).

Results

Rump fat deposits were trending larger in 2017 (n =22, $\overline{X} \pm SE = 1.52 \pm 0.20$ cm), a high mast production year, compared to 2018 and 2019 (n = 43, 1.21 ± 0.10 cm and n =19, 1.30 ± 0.17 cm, respectively), low to moderate mast production years. While the difference in rump fat deposits between years was not statistically different for the pregnant sows used in the proceeding sex ratio analyses, there was a significant difference in rump fat between years when all reproductively mature sows sampled between 2017-2019 were analysed such that sows in 2017 had significantly more rump fat compared to 2018 or 2019 (n = 439, Chinn unpublished data). Thus, we believed that rump fat was a suitable proxy for resource availability for wild pigs on the SRS during our study period. We sampled 89 pregnant sows with fetuses that were old enough to visually determine sex. Litter size varied between 1 and 11 (n = 511, $\overline{X} \pm SE = 5.74 \pm$ 0.21, mode = 7). However, 13 litters were excluded from the logistic regression analysis because we did not have the complete set of morphometric variables. The average sex ratio across all litters (n=89) was 0.55, which differed significantly from parity (onesample t-test: $t_{88} = 2.19$, p = 0.03), and litters within our population were male-biased.

Trivers-Willard Model

Our analysis evaluating whether fetal sex ratio was influenced by maternal and environmental attributes resulted in six competitive models (Table 1). None of our predicted explanatory parameters: maternal age class, maternal size, litter size, average fetus mass, or resource availability influenced sex ratio (Table 1), as the null model was the highest ranked model ($w_i = 0.14$). The other candidate models within $\Delta 2$ AICc included average fetus mass, maternal age class, rump fat, maternal size + age + fat, and maternal size, respectively, all of which had low model weights (Table 1). Thus, none of our measured parameters provided support for the TWM. However, when we independently evaluated sex ratio relative to maternal age class, we found that adults and yearlings had litters that were significantly more male-biased compared to juveniles (ANOVA: $F_{2,86} = 4.01$, p = 0.02; Tukey's HSD: p = 0.02 and p = 0.03, respectively; Fig. 1).

Williams Model

The average mass of male and female fetuses was 350.82 ± 280.03 g ($\overline{X} \pm$ standard deviation) and 345.11 ± 261.16 g, respectively; however, mass was not significantly different between sexes (p = 0.82). Although mass did not statistically differ between males and females, males were on average 2% heavier compared to females, thus requiring differential investment by sows that is likely an additive cost and thus potentially biologically relevant because wild pigs produce multiple offspring of each sex per litter.

Model selection results pertaining to investment in litter production produced 4 competing models. Both maternal age and size, as well as their interaction, were included in all top performing models, indicating some support for Williams' hypothesis that the mother's attributes influenced her investment toward producing a larger litter with many males (Table 2). However, resource availability did not strongly influence sow investment in litter production (Table 2). The top ranked model included sow size, sow age, and their interaction term ($w_i = 0.23$). The second-ranked model only included age ($w_i = 0.23$). Litter investment increased as sow age increased ($X^2 = 10.24$, df = 2, p = 0.006), and juveniles invested the least compared to adults (p = 0.01, Fig. 2). The remaining models within $\Delta AIC \le 2$ included maternal age and maternal size ($w_i = 0.13$).

While sow investment was influenced by maternal size, we found that maternal size was positively related to litter size (t = 3.11, p = 0.003) but not to sex ratio (p = 0.71). The observed relationship between investment cost and maternal size was thus driven by litter size rather than sex ratio. Contrary to a similar study on wild boar sex ratio in their native range [27], we did not observe a relationship between litter size and sex ratio (Fig. 3).

Discussion

Using reproductive data for a globally distributed and highly polytocous large mammal, we tested sexual selection theory in relation to a suite of biotic and abiotic factors. The TWM predicts that to maximise their own reproductive success, high quality mothers should produce more sons, if males have higher individual fitness. Tests of the TWM are complicated for polytocous species since maternal resources must be spread across multiple offspring, therefore the number of offspring and the relative size difference (if any) between the sexes are important for determining allocation of resources toward offspring sex ratio [5]. When evaluated independently, we found support that maternal age may be associated with more male offspring, in accordance with the TWM. However, when analysed along with other biotic and abiotic attributes none of our measured attributes were found to contribute substantially to sex ratio variation among litters. We found that higher quality mothers produced more offspring, showing some preliminary support for the Williams Model. Collectively, our results emphasised that in polytocous species like the wild pig, preferential selection for adjustment of litter size appears to be the primary reproductive characteristic manipulated to increase reproductive success and fitness rather than adjustment of offspring sex ratio. Production of the largest litter size possible in which all offspring survive may provide the greatest reproductive returns, even if litter sex ratio is female-biased.

Female wild pigs produced litters with sex ratios that differed significantly from parity. Age appeared to be an important factor contributing to variance in litter sex ratios, as juveniles tended to have more female-biased litters than yearlings or adults, potentially supporting the TWM as adults and yearlings should be higher quality mothers. Presumably, older age classes had at least one previous litter and should have experience with farrowing behaviour such as nest site selection and construction to optimise cover, thermoregulation, and predator defence [64]. Knowledge of locations for reliable food resources [62,65] that could be vital during periods of the reproductive cycle, such as pregnancy and lactation [66], that require increased energy acquisition also undoubtedly increases with age. Older sows are usually larger which correlates with higher energy stores and ability to deposit fat reserves, relative to resource availability [67]. Thus, older sows may be able to invest more energy into gestation of larger litters, more male offspring, and milk production for more and larger male piglets.

Producing multiple offspring introduces a fitness trade-off between the number of offspring that can be invested in and their sex, if there is differential cost between the sexes [15,19-20]. While litter sex ratios become more male biased with increasing litter size in domestic pigs [68], the relationship between litter size and sex ratio in free ranging wild boar and wild pigs is less clear. Servanty et al. [33] found that in wild boar small litters tended to be biased towards males while larger litters were more female-biased. They suggested sows with larger litters might be limited in the additional investment required for males because males were larger and thus more costly to produce compared to females. While the authors reported sex ratio was male-biased in small litters and female-biased in large litters, the 95% confidence intervals in their analysis included equal sex ratio implying there was no statistical difference in sex ratios from parity for any litter size, except for litter size 4 (see Fig. 2 in Servanty et al. [33]). Similarly, Fernández-Llario et al. [69] also found no relationship between sex ratio and litter size in wild boar in Spain. While no significant relationship was apparent in our study (Fig. 3), intermediate litter sizes (n = 3, 4, 6, 7) had a higher proportion of males. However, sex ratio was not different from parity for the largest litter sizes (8-10) and was slightly female-biased for a litter size of two. Considering the average litter size of wild pigs is adapted to the largest number of offspring a sow can successfully care for, producing more males in the optimal range of litter size, that is the litter size where offspring

survival is highest, could be selected for to maximize reproductive returns and thus fitness. The sample sizes in our data for a given litter size were highest between 3-8 offspring, so any trends at the ends of the range should be interpreted with caution. However, expansion upon this notion by increasing sample sizes and sampling from different wild pig populations could provide more insight into this potential mechanism for optimizing inclusive fitness of a widely successful invasive species.

Within North America, wild pigs are genetically comprised of feralised domestic pigs and introduced Eurasian boar [41-42], with local variation in ancestral origins based on geography and the extent of human intervention to extend their distribution as a game species. On average, wild pigs have larger litters ($\overline{X} = 5.3$ piglets in North America [39]; 5.74 piglets in this study) than wild boar (4-5 piglets [70]), but smaller litters than domestic pigs (dependent on breed but range from an average of ~8-13 piglets [39,68]). Given the origins of wild pigs include domestic stock that were selectively bred to favour high reproductive rates, early sexual maturity and large litter sizes [40,61], there may be differences in reproductive ecology between populations due to the genetic make-up of individuals within each population [42]. Although age appeared to have some influence on litter sex ratios when examined independently, none of our selected parameters in the GLMM analysis influenced the sex ratio of wild pig litters in our study, suggesting additional attributes are likely contributing to variance in litter sex ratios that were not accounted for in our models. Our population is a mix of hybrids of Western heritage breeds of domestic pig and wild boar, where individuals vary considerably in ancestry [42]. Thus, it is plausible the genetic composition of individuals within our population could have contributed towards the variance in sex ratio that was unexplained in our

analyses. Further investigation is needed to determine genetic composition of localised wild pig populations and to include ancestry in modelling to better understand potential drivers of litter size and sex ratio.

Maternal social status has also been proposed to influence sex ratio. Specifically, females produce more male offspring in response to their ability to acquire greater resources because of higher rank [16,18,71]. Social rank can also be associated with differing nutritional stress, a mechanism to which sex ratio is adjusted, where subordinate females are susceptible to more stress because of decreased access to food and negative behaviour from dominant females and in return have smaller litters and fewer male offspring [28; but see 72]. In general, males are more susceptible to stressful conditions (e.g., to oxidative damage or high levels of maternal glucocorticoids in which effects are sex-specific at certain periods during gestation in pigs [74]) in utero leading to higher proportion of female offspring (for a review see [9]). In domestic pigs, Meikle et al. [74] found higher ranking sows produced more sons while Mendl et al. [72] found the opposite trend across multiple reproductive events. Interestingly, we found the number of male offspring increased as maternal age increased (Fig. 1). While we did not have a technique to measure maternal rank, it is a reasonable assumption that there is a hierarchical order according to age in wild pigs based on the matrilineal social structure that consists of multiple generations of related females [75-76]. We suggest further investigation of this potential mechanism as a driver of sex ratio in polytocous species.

Our environmental variable, food availability, measured as the amount of extraneous fat was not a significant parameter influencing sex ratio when considered with maternal attributes. While food resources are important for reproduction, it is not clear how food abundance and diet influence sex ratio for species that have multiple offspring per reproductive event [77-78]. While we found no influence of food availability on sex ratio, litter size increased as fat increased (Chinn unpublished data). Wild pigs exhibit a high degree of behavioural plasticity, exploiting environmental fluctuations such as increased food resources (i.e., good mast season) by increasing litter size. By taking advantage of pulses of high-quality resources, females may be able to bridge the energetic gap necessary to produce more offspring, and in concept support more piglets to successful weaning to increase lifetime fitness. Although we did not directly measure food availability, sex ratio was not influenced by extraneous fat, which should be reflective of resource availability and body condition. High quality mothers, those with more rump fat, did not alter offspring sex ratio, but instead modulated litter size. Our study supports evidence that altering litter size is selected for rather than adjusting offspring sex ratio [69]. Thus, it may be more beneficial to adjust litter size to increase reproductive success.

For polytocous species, Williams [5] predicted mothers maximise fitness by increasing investment in litter production, in relation to their condition. Wild pigs exhibited higher (~2%) maternal expenditure per individual offspring for males compared to females during pregnancy. The 2% increase in maternal investment may not be statistically different between the sexes when taken singularly, however, wild pigs produce multiple offspring per litter and the additive cost of producing multiple male offspring may result in a significant difference between the sexes as the litter sex ratio increases and becomes male-biased. We found maternal quality was a good predictor of sow investment in litter production such that investment increased as sow size increased. Since investment is a function of litter size and sex ratio, it is difficult to discern their influence on investment without looking at them separately. Servanty et al. [33] found no support for the Williams model because wild boar adjusted litter size rather than offspring sex ratio in relation to sow quality (i.e., mass), but did not directly test if investment was dependent on maternal mass. Fernández-Llario et al. [69] found investment cost was the best predictor of maternal quality for wild boar, supporting the Williams model. Sex allocation studies of other polytocous mammals are variable in support of TWM and the Williams Model, or support these predictions only under certain environmental conditions because the trade-offs between sex ratio and litter size complicate predictions of offspring sex in relation to maternal quality for polytocous species (e.g., marmot [79]; mice [80]; squirrel [81]).

To address any trade-off, we modeled how litter size and sex ratio were related to maternal condition. Litter size increased with maternal size, but there was no relationship between sex ratio and maternal size. Sows preferentially adjusted litter size and not sex ratio in relation to their condition and therefore under these specifications, the Williams model was not supported. If wild pigs adjusted litter size in relation to maternal condition, perhaps sex ratio was optimised so that the most offspring survived, dependent on maternal condition. Mothers in poor condition should have litters with more females because for mammals they tend to be cheaper to produce and the number of offspring depends on the maximum number of the cheaper sex that will be successfully weaned [20].

Polytocous species may employ a combination of sex allocation strategies: maximizing sex ratio in terms of the optimal litter size [20], adjusting litter size and sex ratio to optimize fitness [5], and a 'maternal coping strategy' where females in poor condition will maximise the number of males in the litter at the expense of their own condition (i.e., will increase the proportion of males in the litter with increased loss of maternal mass [80]). Since mechanisms of primary sex ratio adjustment are uncertain, true relationships may only be applicable in relation to measurements of maternal quality prior to conception, however the relative role of maternal attributes pre- and postconception remain unclear [18]. For *in situ* studies with wild animals, most measurements are limited to already pregnant individuals, with little or no prior knowledge of previous condition. Factors affecting primary and secondary sex ratio rates could differ and should be considered during the experimental design phase of a study to ensure relevant parameters are tested.

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Tables

Table 4.1. Generalized linear mixed model results (within $\Delta AICc \leq 2$ of the top model) evaluating the influence of maternal attributes and environmental variables on wild pig (*Sus scrofa*) sex ratio at the Savannah River Site, Aiken, SC USA.

Model Selection for fetal	sex ratio in wild pigs,	within $\Delta AICc \leq 2$
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Model Tested	df	AICc	ΔΑΙϹϲ	Weight
Logit(sex ratio) = null model	3	238.7	0	0.123
Logit(sex ratio) = Fetus Mass	4	240.1	1.3	0.062
Logit(sex ratio) = Age	5	240.2	1.5	0.058
Logit(sex ratio) = ResAvail	4	240.4	1.6	0.053
Logit(sex ratio) = Age + Size + ResAvail	7	240.4	1.7	0.052
Logit(sex ratio) = Size	4	240.6	1.9	0.047

Null, no covariates; Age, maternal age; ResAvail, resource availability (measured by rump fat); Size, maternal size index (mass/length)

Table 4.2. Linear mixed model results (within $\triangle AICc \le 2$ of the top model) evaluating the influence of maternal attributes on wild pig (*Sus scrofa*) investment in litter production at the Savannah River Site, Aiken, SC USA.

Model Selection for fetal sex ratio in wild pigs, within $\Delta AICc \leq 2$

Model Tested	df	AICc	ΔΑΙϹϲ	Weight
Investment = Age + Size + Age*Size	8	309.8	0	0.227
Investment = Age	5	309.9	0.02	0.225
Investment = Age + Size	6	310.5	0.64	0.165
Investment = Size	4	310.9	1.09	0.131

Age, maternal age; Size, maternal size index (mass/length)

Figures



Figure 4.1. Average sex ratio and SE for a given maternal age class for wild pigs (*Sus scrofa*) at the Savannah River Site, Aiken, SC, USA from 2017-2019. The dotted line denotes even sex ratio (equal number of males and females).



Figure 4.2. Average investment cost (calculated as the sum of the cost of each offspring according to sex) and SE in litter production for a given maternal age class for wild pigs (*Sus scrofa*) at the Savannah River Site, Aiken, SC, USA from 2017-2019.



Figure 4.3. Average sex ratio (mean proportion of males) and SE for a given litter size for wild pigs (*Sus scrofa*) at the Savannah River Site, Aiken, SC, USA from 2017-2019. The dotted line denotes even sex ratio (equal number of males and females).

CHAPTER 5

INFLUENCE OF INTRINSTIC AND EXTRINSIC ATTRIBUTES ON NEONATE SURVIVAL IN AN INVASIVE LARGE MAMMAL

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Abstract

Understanding factors influencing survival of neonates for wild species is important for successful management, particularly for determining drivers of population dynamics. Wild pigs (Sus scrofa) are invasive and populations are rapidly increasing in part due to high reproductive capacity. Survival of adults is generally high, however, survival of piglets, and particularly neonates, is largely unknown. We located neonates at the natal nest and quantified survival in relation to individual and maternal biological attributes, and environmental variables. During 2017-2020, we captured 50 neonates from 13 litters and documented 28 mortalities (56%) over six weeks. Survival was positively influenced by pelage coloration, likely as a form of camouflage from predators. Male neonates had higher survival. They were born larger than females, which could be beneficial for thermoregulation and competition for milk. Neonates born to larger sows had lower survival. Sow size was positively correlated with litter size, and this finding may reflect the increased nutritional demands of sustaining large litters, or difficulties in defending more neonates against predators. Neonates born in warmer months had higher survival than those born in cooler months. Neonates are inefficient thermoregulators, thus being born in warmer months could be beneficial for maintaining homeostasis as well as access to more food resources. These are the largest and most complete data for neonate wild pig survival and will inform population models for the development of management strategies to reduce negative impacts of this destructive invasive species on native ecosystems.

Introduction

Population dynamics – how populations change in size and structure over time – is driven by factors such as vital rates (births, deaths), stochastic environmental variation¹ (e.g., food availability, habitat quality), density dependence² (e.g., predation, disease, immigration, emigration), and demographic variation² (e.g., age structure, sex ratio). Consequently, population dynamics reflect the unique interactions among the environment, physiological and behavioral differences that culminate in individual success to determine the number, spatial distribution, and genetic composition of populations. Understanding drivers that influence populations of wild animals is important for determining why and how populations increase, decrease, and fluctuate spatially and temporally under changing conditions, and thus is vital to inform management and conservation initiatives.

Survival is an integral component of population growth and is associated with fluctuations in a suite of intrinsic biological attributes^{3,4,5} (e.g., age, genetics, size) and extrinsic environmental factors^{6,7} (e.g., season, landscape characteristics, resource availability). For long-lived vertebrates, juvenile survival is often lower and more variable compared to adults^{2,8}, and thus can be one of the most important influences on recruitment. In addition to direct causes of mortality such as predation^{9,10}, indirect factors such as body condition and mass at birth, as well as physiological condition have been associated with neonate survival across species^{11,12,13,14}. Availability of vegetative cover, food, water, and other resources critical to both neonates and the mother are also important for determining behavioral patterns that can influence survival^{6,15,16}. Thus, it is imperative to simultaneously assess the effects of individual characteristics, maternal

attributes, and environmental factors when studying neonate survival. Elucidating the underlying attributes driving survival is particularly critical in the management of invasive species, which can substantially alter ecosystem-level processes and have extensive economic impacts to agriculture, infrastructure, and human health^{17,18,19}.

Wild pigs (Sus scrofa) are among the most widely distributed and damaging invasive species worldwide²⁰. Typically comprised of mixed ancestry of wild boar and domestic pigs ^{21,22}, wild pigs are ecological generalists that are highly adaptable with the greatest reproductive capacity of any mammal of their size, and thus are able to thrive and expand quickly in new environments. Globally, wild pig populations have rapidly increased in abundance and distribution over the past few decades²³. Thus, understanding attributes that make wild pigs successful within a diversity of landscapes, and the factors that influence population dynamics are necessary to inform management of this invasive species. Although wild pigs are distributed worldwide and have extreme influence over ecosystems across their native and invasive range 24,25 , they remain significantly understudied²⁶. Most management plans focus on lethal population control²⁷, often neglecting the study of the ecological and biological mechanisms that underlie reasons responsible for their abundance. For example, juvenile survival is lower and more variable compared to adults^{28,29}, and plays a prominent role in population dynamics², but is widely ignored in most population models because it is difficult to quantify³⁰. Thus, an understanding of factors influencing survival of wild pig neonates is important for successful management, particularly for determining drivers of population dynamics.

While neonate survival assessments for many species of wild ungulates, such as white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), bighorn sheep (*Ovis*

canadensis), etc., are well-represented in the literature, there are few studies that assess survival for piglets of wild pigs or wild boar^{31, Table S1}, and none that have successfully quantified neonate survival rates using known-fate approaches³². Mortality among domestic piglets ranges between 12–30% and is most precarious during 1-3 days of life, with 50% of all neonate mortality occurring during this period³³, although it is unknown whether these rates are relevant to wild populations of this species. Baubet et al.³⁴ attempted to determine known-fate survival for neonate wild boar within its native range but were unsuccessful due to poor transmitter retention and high rates of abandonment by the sow. Keiter et al.³¹ piloted the use of vaginal implant transmitters (VITs) in invasive wild pigs to determine the location and time of parturition to facilitate capturing and radio-transmitter tagging of neonates at the natal nest. However, their attempt to determine known-fate survival of neonates was unsuccessful due to poor transmitter retention, largely due to transmitter size precluding use on small neonates (~1 kg) and removal of the transmitter by the sow or littermates.

Survival of wild pig neonates is likely variable, presumably influenced by a suite of neonate, sow, and environmental attributes. In particular, birth mass is thought to be the most important factor influencing piglet survival among domestic $pigs^{35,36}$. Neonates are born poorly insulated with < 2% body fat, lack brown adipose tissue, and must rely on shivering thermogenesis to maintain adequate body temperature^{37,38}. Neonates also have high surface area to volume ratio and must expend energy to maintain internal thermal homeostasis, thus thermoregulation and therefore survival are considered tied to birth mass. Wild pigs exhibit sexual dimorphism³⁹ and life-history theory predicts sexbiased mortality from the differential costs and benefits of raising each sex⁴⁰, such that
there may be higher maternal energetic requirements for the sex that has a faster growth rate⁴¹. Mothers in poor condition are predicted to terminate investment in the sex that requires higher energetic cost in the current reproductive event, but at a cost to their own future reproductive success if that sex has greater variance in individual fitness⁴⁰. However, the larger sex may also have intrinsic advantages that increase survival (i.e., larger body size facilitates thermoregulation). Wild pigs also can vary considerably in pelage coloration due to the introgression of domestic genes, which may play a role in camouflage from predators. Another factor that may be important for neonate survival is sow body condition and fat mobilization. Both of these factors are important for lactation⁴², particularly for initiating let down of colostrum, which is necessary for neonates to acquire immunoglobulins⁴³. For wild pigs, sow mass is positively associated with age^{32,44} and older sows should have more experience with rearing offspring.

We aimed to provide the first real-time survival monitoring of neonate wild pigs within their invasive range. Our objectives were to assess performance of a very high frequency (VHF) radio-transmitter and quantify neonate wild pig survival to six weeks of age in relation to individual biological attributes (mass, pelage color, sex), maternal characteristics (mass), and environmental factors (season). We hypothesized individual attributes would influence survival such that neonates born larger, male, and with the wild-type pelage coloration would have higher probability of survival. We hypothesized neonates born to larger sows would have higher probability of survival. Finally, we hypothesized environmental conditions at the time of birth would influence survival, with neonates born in the warm season having higher probability of survival. Further, among wild species, capture and tagging within the first days of life have been hypothesized to negatively affect neonate survival⁴⁵. Thus, we also aimed to explicitly test if neonates with ear tags had lower survival compared to untagged neonates to determine if tagging itself influenced survival.

Materials and Methods

Study Area

We conducted our study at the Savannah River Site (SRS) in Aiken, Allendale and Barnwell counties, South Carolina, USA. The SRS is an 80,000-ha U.S. Department of Energy property located in the Upper Coastal Plain physiographic region. The site is primarily composed of a mixture of upland pine habitat and bottomland hardwood swamps and riparian areas. Upland habitat was dominated by loblolly pine (*Pinus taeda*) and longleaf pine (*P. palustris*) that is actively managed for Red-cockaded woodpeckers with prescribed fire. Bottomland hardwood and cypress (Taxodium distichum)-tupelo (Nyssa aquatic and N. sylvatica var. biflora) forests characterized the floodplain. The wild pig population on the SRS was descended from feralized domestic pigs released after the private land was converted to a government facility in 1950⁴⁴. Later introduction of wild boar and wild boar and feral pig hybrids led to introgression of wild boar genes into the SRS wild pig population^{22,44}. Hybridization on the SRS led to high phenotypic variability, especially in pelage coloration within and between litters (Fig. 1). Despite lethal management, the population has continued to increase over the past several decades to > 5,000 individuals³⁰. Given the size of the SRS and lack of public access, potential predators of neonate wild pigs (e.g., coyotes, Canis latrans; bobcats, Lynx *rufus*) are abundant and widely distributed^{10,46}.

Adult Female Capture and VIT Deployment

All capture and handling of animals was conducted in compliance under approved protocol by the Institutional Animal Care and Use Committee under the University of Georgia (A2015 05-004; A2018 06-024) and the ARRIVE guidelines for the immobilization of animals for studies conducted in the field. From September 2017 to February 2020, we captured adult female wild pigs in corral traps baited with whole corn. We immobilized animals via dart rifle (X-CALIBER, Pneudart, PA) using a combination of Telazol (4.4 mg kg⁻¹; MWI Veterinary Supply, ID) and Xylazine hydrochloride (2.2 mg kg⁻¹; Wildlife Pharmaceuticals Inc., CO) in 2017-2018, or with a combination of butorphanol (0.43 mg kg⁻¹), azaperone (0.36 mg kg⁻¹), and medetomidine (0.14 mg kg⁻¹) (BAM, 0.0064 ml kg⁻¹; Wildlife Pharmaceuticals Inc., CO) and ketamine hydrochloride (2.2 mg kg⁻¹; Wildlife Pharmaceuticals Inc., CO) in 2018–2020. Xylazine hydrochloride was antagonized with yohimbine (0.15 mg kg⁻¹; MWI Veterinary Supply, ID) and BAM was antagonized with a combination of naltrexone (50 mg ml⁻¹; Wildlife Pharmaceuticals Inc., CO) and atipamezole (25 mg ml⁻¹; Wildlife Pharmaceuticals Inc., CO), and individuals were monitored until recovery.

Upon immobilization, individuals were weighed, measured, and age was determined through examination of molar eruption patterns^{47,48}. We assessed whether captured females were pregnant via serial remote camera images at bait stations, palpation, and a portable ultrasound (SeeMore USB, Interson Corporation, CA). Pregnant females were implanted with a 21g VIT (M3930; Advanced Telemetry Systems, MN) via methods similar to white-tailed deer studies that previously have been employed for wild pigs³¹. Briefly, the VIT was inserted into the vagina and placed against the cervix with the wings oriented laterally within the body. VITs included a thermistor that sensed a change in temperature upon expulsion during parturition that signaled the number of 30-minute intervals elapsed since the change in temperature. Females with VITs were also collared with a VHF (Model TGW-4501; Telonics Inc., AZ) or GPS collar (Model TWG-4577; Telonics Inc. or Vertex Plus; Vectronic Aerospace, Berlin, Germany) to facilitate tracking. Pregnant sows were monitored 3–7 times/week until parturition.

Neonate Capture and Handling

Because of the potential of abandonment, we waited 22-48 hours after parturition to capture and tag neonates to promote bonding and investment by the sow (e.g., ref. 34). We homed to the VIT signal at the farrowing nest, flushed the female, and captured piglets by hand. Once captured, we placed piglets in mesh bags, and subsequently weighed, photographed, and noted sex and pelage coloration of all individuals at the nest location. Within each litter, we tagged a subset of 1-3 piglets with a custom designed VHF transmitter ear tag (Model RI-2BMH; Holohil Systems Ltd., Ontario, Canada). Previous attempts to affix radio transmitters to neonate wild piglets resulted in poor retention or failure³¹. To address these issues, our modified design consisted of a stud ear tag with a nylon post that was approximately 2 cm in diameter and weighed 5 g. We customized it with an internal, coiled antenna to prevent damage or malfunction of the transmitter and included a mortality sensor that activated after 12 hours of inactivity. In the field we further modified the tag by using a metal fastener and a small and large neoprene washer on the front and backside of the ear, respectively, to prevent the tag from pulling out (Fig. 1). After these modifications, the tag weighed ~ 10 g at

deployment. Piglets were released simultaneously at the natal nest at the conclusion of processing. We deployed remote infrared motion sensor cameras (PC900 HyperFire Professional Series; Reconyx, WI) at the nest area to capture piglet activity post-tagging, document when the female reunited with her offspring, and when the female and piglets left the nest. We also were able to document presence of predators or offspring abandonment by the female through camera images.

Survival Monitoring

We relocated tagged neonates via radio telemetry 3–5 times per week until mortality, tag failure or detachment, or until they reached at least 6 weeks of age. When we detected a mortality signal, we tracked to the location of the transmitter and attempted to determine cause of mortality or whether the tag detached. If there was evidence of mortality, we attempted to determine the cause based upon carcass condition, presence of predator tracks, characteristics of cache sites if any were found, and patterns of carcass consumption⁴⁹. To supplement VHF tracking and allow for tracking of individuals that did not receive VHF tags, we also deployed remote cameras in locations where the sow exhibited localized movements post-parturition. Untagged neonates identifiable from unique pelage coloration patterns that were regularly photographed with remote cameras were included in survival analyses. This method also allowed us to confirm tag detachment versus mortality for any neonate transmitters recovered without adequate evidence of mortality. We assumed mortality occurred on the date the tag was heard in mortality status (12-hour delay) if the neonate was not detected with remote cameras. If tagged neonates were not detected during tracking, we homed in to the female signal to

confirm the absence of individuals. We attempted this at least twice if the tagged neonate was not detected, thereafter if the transmitter was never recovered and the neonate was not detected on remote cameras, it was assumed deceased either the day after it was last detected or the date it was first not detected (depending on the monitoring schedule). Neonates remain with and are dependent on the sow for the first several months of life⁵⁰.

Statistical Analysis

We used the *lme4* package⁵¹ in R 3.5.3⁵² to fit linear mixed models (LMM) to estimate if the number of days survived differed between years and tagging status (i.e., whether survival differed for tagged vs untagged neonates), with sow as a random effect since multiple neonates from each sow were monitored. The response variable, days survived, was centered to a mean of 0 and a standard deviation of 1. We checked that model residuals were randomly distributed around zero. If we found no differences in days survived as a function of year or tagging status, we pooled all data for subsequent multivariate survival models.

To evaluate factors that influenced neonate survival, we constructed a series of known-fate models in a Bayesian framework. We included multiple biotic factors such as neonate mass, sex, and pelage color, sow mass, and an environmental attribute (i.e., season). We used sow mass at capture as a proxy for sow condition at parturition and during the six weeks post-parturition. However, changes in mass and body condition could vary between sows between capture and parturition/post-parturition that we were unable to account for that could be important for neonate survival. Seasons (i.e., cold season and warm season) were based on average minimum, maximum, and daily

temperature (taken at 15-minute intervals) for each month for the timeframe of our study (September 2017-May 2020). Temperature data were recorded in an instrument shelter in the northwest region of the SRS, outfitted with an hygrothermograph (CS-500 Temperature/Relative humidity sensor, Campbell Scientific Inc., UT) to collect minimum and maximum temperature with a minimum accuracy of 1°C. Minimum accuracy was compared with an Vaiasala hand-held probes (Helsinki, Finland) used as reference standards once per year⁵³. Cold season was designated as having at least 5 days of minimum temperature ≤ 0 °C and daily average ≤ 12.78 °C (55 °F). The first and second half of March was split into cold and warm season, respectively, because the second half of the month was significantly warmer (12.67 °C and 15.29 °F, respectively; t-test: $F_{1,91}$ = 7.43, p = 0.008) and temperatures did not go below freezing in the latter half. We ran our models in Just Another Gibbs Sampler (JAGS⁵⁴) using the *runjags* package⁵⁵ in R. Each model was run for 2,000 iterations with an 8,000 burn in and a thin rate of 1. We checked for model convergence using the Gelman-Rubin diagnostic⁵⁶. We modeled daily survival for each neonate as a series of Bernoulli trials with a probability of success S_{it} = P(neonate *i* alive at time $t \mid$ alive at time t-1). Daily survival was modeled as logit(S_{it}) = $x_{it}\beta + Z_{\gamma}$, where x_{it} is the covariate, β is the estimate of the covariate, and Z_{γ} is the random effect (in our case, sow). We used a staggered entry modeling approach⁵⁷. Each neonate entered the study on its birthdate (time zero) and its endpoint corresponded to a mortality event or until six weeks old. The first neonates were born in September 2017 and neonates were monitored until May 2020. We chose six weeks because evidence suggests low mortality of wild piglets beyond this time period. A previous study at the same site captured and tagged 71 piglets from 23 litters, ranging in age from neonate (n = 28,

tagged 18 neonates) to older than six weeks. All neonate tags failed, however, piglets > 3 kg (approximately six weeks or older) had high tag retention and ~94% survival rate (D. Keiter, unpublished data).

We used a conservative approach such that we based our statistical power on the number of events (i.e., mortalities⁵⁸) rather than the number of neonates. Therefore, we only fit models with one to two variables to avoid overfitting. We excluded any neonates from litters that the sow had abandoned, which was ascertained from remote cameras. We accounted for non-independence of samples (e.g., multiple neonates were included from each litter) by nesting the data by sow⁵⁹. Using neonate sex, mass, pelage color (wild, i.e., brown striped, or not wild), sow mass, and season (cold: 1 November-15 March; warm: 16 March-October) we created a candidate set of intrinsic models to evaluate which factors influenced neonate survival. We centered the continuous variables (neonate mass and sow mass) to a mean of 0 and a standard deviation of 1 to allow for standardized comparison. We assessed the continuous variables for correlation using Pearson's correlation tests, and all pairwise comparisons were r < 0.7, indicating no strong relationships. All parameters were modeled using vague uniform priors.

We used Watanabe-Akaike's Information Criterion (WAIC), a Bayesian extension of AIC, for model selection to evaluate and rank candidate models⁶⁰. All models within Δ WAICc ≤ 2 of the top model were considered supported. Model weight was used to evaluate the strength of influence among competing models⁶¹. If a single model did not outperform competing models for best fit to the data, parameters from models with similar WAIC weights were considered influential and reported.

Results

We deployed 23 VITs across 22 individuals from 2017–2020. One female gave birth twice in 2019 (once with a VIT and once without a VIT but we were able to capture piglets through monitoring her movements with GPS data that showed extreme localization indicative of parturition behavior) and once again 2020 (with a VIT; Table 1). Two sows prematurely expelled the VIT (no signs of nesting behavior such as localized movement, nest construction or parturition) and we were unable to subsequently track parturition. Four sows abandoned nests after we tagged neonates (live piglets we found and tagged), and two sows appeared to have given birth (localized GPS data and constructed a nest) but neonates were never located nor were documented on camera and the sows' movement behavior was not reflective of caring for offspring (i.e., had wideranging movements after giving birth). We also were unable to locate one nest or neonates (but subsequently documented that sow with piglets on camera). We were unable to collect neonate data from these nine individuals, therefore, we obtained neonate data from 15 litters from 13 sows (one sow had three reproductive events as mentioned above; Table 1). From the successful litters, we captured 67 neonates $(4.67 \pm 1.85,$ average litter size \pm SD) and deployed VHF ear tags on 26 individuals. Of these, we were able to successfully track survival of 50 neonates, 24 tagged and 26 untagged, to mortality or at least 6 weeks old (Supplementary Table S1). The apparent survival rate was 44% (i.e., 22 neonates survived to six weeks), across the entire study duration. We excluded seven neonates from further analysis because of missing sex or mass data for those individuals. Thus, our sample for analysis assessing factors that influence survival

included 43 neonates from 13 litters and 12 sows (Table 1). We determined the survival fates of these 43 neonates and thus did not requiring censoring.

For the 50 neonates, survival was not significantly different across years, p = 0.56. Among the 26 untagged neonates, there were 13 mortalities (50% mortality), and among the 24 tagged neonates there were 15 mortalities (62.5% mortality). Of the 28 neonates that died before six weeks old, 16 mortalities occurred within 10 days after birth (57.14%). Average time to mortality for these neonates was 14.75 ± 2.13 days ($\overline{X} \pm SE$). For the neonates that survived to six weeks old, 13 were male (59.1%) and 9 were female (40.9%). Survival was not significantly different between tagged and non-tagged neonates (p = 0.81). Because year and tag status had no influence on survival rates, we pooled all neonates for subsequent analysis.

For the 43 neonates for which we had complete data, we found from the null model that only had the intercept and random effect (sow) as parameters, the mean daily survival probability of neonates within each litter ranged from 91.41-99.50% (Fig. 2) and cumulative survival probability to six weeks for neonates within each litter ranged from 16.68-82.14% (Fig. 3). Our analysis evaluating whether individual, maternal, and environmental attributes influenced neonate mortality resulted in 6 competitive models (Table 2). The top model included only season as being influential to survival. The top model did not carry substantial Watanabe-Akaike weight ($w_i = 0.21$), so we considered any competing models within $\Delta 2$ WAIC (Table 2). The other supported models included neonate sex, sow mass, neonate sex + sow mass, pelage color + sow mass, and pelage color. Neonates born in the colder season had lower survival ($\beta_{cold} = 2.48$, 95% credible interval [CI]: 1.52-3.00; $\beta_{warm} = 2.56$, 95% CI: 1.73–3.00) compared to neonates born in

the warmer season. Sow mass appeared in three of the top supported models, and as sow mass increased, neonate survival decreased ($\beta_{sow mass} = -0.77, 95\%$ CI: -3.08–1.38). Neonate sex occurred in two of the top supported models, with males having higher survival compared to females (β_{male} = 2.66, 95% CI: 2.00-2.76; β_{female} = 2.46, 95% CI: 1.61–2.57). Pelage coloration appeared in two of the models; neonates with wild pelage coloration had higher survival ($\beta_{wild} = 2.65, 95\%$ CI: 2.05-2.74; $\beta_{not wild} = 2.58, 95\%$ CI: 1.87–2.68) compared to other colors (e.g., black and white spotted, solid black; Fig. 1). While our objective was not to determine cause-specific mortality, we did observe one predation event of a litter (two neonates) prior to tagging. Carcass remains were present at the natal nest site and we observed hemorrhaging, leading us to conclude that the neonates were born alive and were preyed upon. The lack of caching of the remains suggested likely coyote predation. We also observed another event that we could not confidently discern between predation or scavenging of the litter (two neonates) at seven days old, approximately 800 m away from the natal nest. Additionally, we observed one 10-day-old neonate on camera that was emaciated, while the other two littermates appeared healthy. We did not subsequently detect this individual on camera images or with the sow and do not know whether it died from predation or other causes (e.g., abandonment, disease). The cause of the remaining mortalities could not be determined since we were unable to recover the carcasses.

Discussion

Using a combination of custom-designed VHF ear tag transmitters and remote camera images, here we present the results of the first known fate survival study for

neonate invasive wild pigs. Our data suggest survival of neonate wild pigs is relatively high (44%), underscoring the potential for this species to expand in population size and distribution upon establishment in novel environments. Survival for neonate/pre-weaned ungulate species span from 1-88%⁸. While wild pig neonate survival falls within the range of other ungulates, unlike other species, wild pigs are physiologically capable of producing multiple litters each year. Indeed, in our study, one sow gave birth to three litters in a span of 14 months (including three gestation periods). However, the extent to which a sow produces multiple litters within a single year across wild pig populations is unknown. Further, wild pigs are able to reproduce at several months of age and produce an average of 5.3 (range 1-12) offspring per litter³². These reproductive attributes of wild pigs often exceed those of wild boar due to their descent from feral domestic pigs that were bred for increased reproductive capacity^{32,62}, underscoring a critical mechanism by which this species is able to rapidly colonize and expand populations throughout much of their invasive range, even within populations with sustained management efforts³².

Parameter estimates in our models generally had overlapping CI's, likely due to limited sample sizes of neonates and extensive variability among individuals. Nonetheless, our results produced several supported models, suggesting despite generally overall high survival, several biotic and abiotic factors likely contribute to variability in survival of wild pig neonates. As predicted, neonates born in the warm season (n = 23 neonates from six litters) had higher survival compared to those born in colder months (n = 16 from seven litters), when temperatures often fell below freezing in our study area. Temperature is important for neonates because they are smaller compared to adults and lose body heat more quickly to the surrounding environment. Wild pig neonates may be particularly vulnerable to cold temperatures as they are born with little hair and are relatively immobile for the first few days post-parturition. Further, thermoregulation is difficult for neonates, as they are born without brown fat and must expend energy to shiver for thermogenesis³⁸. In cold environments neonates lose heat at a rapid rate because of high surface area to volume ratio and temperature difference between thermal homeostasis and the environment. Although the extent to which temperature may serve as a driver of recruitment is not well characterized in the literature, wild pigs occur across much of the globe and can reproduce year round in favorable environments³²; thus, survival of neonates may be subject to substantial spatial and temporal variability in weather across this species' range. For example, at the SRS, the primary birthing peak occurs from December-April (with some variation between years) demonstrating that wild pigs in our study do not avoid reproduction during the colder months⁴⁴ (Chinn unpublished data).

While not statistically significant given overlapping CI of the coefficients, likely due to limited sample size, our results also suggest several attributes of individual neonates likely influenced survival. In particular, male neonates had higher survival compared to females. On average, mammalian males are born larger than females⁶³, which results in a reduced surface area to volume ratio compared to smaller females. Historically⁴⁴ and in this study, wild pig males were 8.37% larger compared to females (1016.67 \pm 37.75 g and 938.16 \pm 38.33 g, $\overline{X} \pm$ SE, respectively). Consequently, males may be more efficient in thermoregulation, losing less body heat to the environment, requiring less energy to maintain thermal homeostasis (i.e., less shivering), and are at less risk of chilling and infection⁶⁴. Larger body size could increase male survival, but it is important to note that the sow is in attendance at the nest, potentially providing warmth to the offspring, and the neonates may also huddle together for thermoregulation 37 . In domestic pigs, smaller neonates tend to be the later born offspring in the litter⁶⁵, may take longer to initiate suckling⁶⁶, and consume less colostrum and milk⁶⁷, resulting in lower body condition. The higher birth weight associated with male neonates is associated with increased vigor and survival⁶⁸. However, in our study, neonate mass did not influence survival. Therefore, other traits associated with the male sex, aside from or in combination with mass, are important to survival. Because of sexual dimorphism in wild pigs, male offspring have higher energetic demands⁴¹ and may outcompete female siblings for high quality teats. Increased size and more energy available (from more milk consumed) could also make male offspring better equipped at traveling and keeping up with the sow when she moves locations. Life-history theory predicts sex-biased offspring mortality, dependent on maternal condition⁴⁰. Poor quality mothers are predicted to terminate investment in the sex that requires higher energetic cost, but at a cost to their future reproductive success if that sex has greater reproductive potential⁴⁰. However, it is not well known if this applies to species with multiple offspring per litter because the size and number of offspring present an investment trade-off⁶⁹. Studies in wild boar found fetal sex ratio (the proportion of males in the litter, i.e., survival of male offspring) was not affected by maternal condition but by litter size⁷⁰ and that sows modulated litter size and sex ratio to increase fitness⁷¹. Similarly, a study of wild pigs found no relationship between sex ratio and litter size and that sows adjusted litter size as the apparent primary method to increase fitness (Dissertation Ch 3). While studies of juvenile wild boar (less than 1 year old) show no difference in survival rates between sexes^{72,73}, it is possible that

survival may be affected by sex contingent on other factors such as sow quality⁴⁰, environmental condition, population density⁷², or hunting pressure⁷⁴.

Pelage coloration evolved from the needs for concealment from predators, communication, and physiological control and is thus vital for survival⁷⁵. Invasive wild pigs are somewhat unique among mammals as individuals can display extensive variability in phenotype depending on their ancestry. Indeed, piglets within our study area exhibit substantive variability in pelage, and this variability appeared to contribute to differences in survival among individuals. Even within litters individuals may range widely in coat coloration, including black, wild-type, spotted, or other variants in pelage. As predicted, offspring born with wild pelage coloration had higher survival compared to other pelage colors. The wild coloration is characterized by a striped pattern and may include spots (Fig. 1). The stripes break up the solid outline of the neonate and promote blending with the background environment in places with dappled light, promotes crypsis and may decrease detection by predators. This is typical of artiodactyls and species that tend to hide during the first weeks of life⁷⁵. On the SRS, potential predators have high visual acuity and rely primarily on visual cues for hunting^{76,77}, and neonates that are not wild patterned may be more conspicuous and at higher risk for predation. Interestingly, the two neonates that were preyed on at the natal nest had non-wild pelage coloration. It is not well understood if pelage coloration has physiological advantages such as thermoregulatory benefits⁷⁵. However, the brown color and black stripes/spots found in the wild pelage coloration could absorb more radiant heat from the environment and aid in conserving body temperature compared to the lighter colored neonates 78 .

In addition to individual characteristics, sow attributes appeared to be an important contributing factor for neonate survival. While not significant, most likely attributable to the limited sample size, neonates born to larger sows generally had lower survival. Larger sows are also associated with larger litter sizes³² (Chinn unpublished data) and these larger sows that bear larger litters may have more difficulty defending each neonate from predators. Further, there may be an optimal litter size that corresponds to the largest number of offspring that the sow can successfully provide parental care for (i.e., provide enough food for, keep alive), an extension of Lack's Principle (which applies to birds⁷⁹) to mammals. This may represent a trade-off where larger sows have more resources (i.e., higher body condition, fat reserves) to produce more offspring, yet these additional neonates have higher probability of mortality. Under optimal conditions, a larger sow may produce many offspring with high survival, thus increasing her reproductive success. A larger sow may bet hedge by producing a large litter and taking advantage of optimal conditions that promote high neonate survival⁸⁰. Similarly, sow mass in wild pigs is positively associated with age^{32,44}. Larger and older sows should have more experience rearing offspring and it is intuitive to hypothesize that neonates born to these sows would have higher survival. However, we found the opposite. Larger sows may employ a coin-flipping reproductive strategy by varying the phenotypic plasticity of their offspring⁸¹. For example, larger sows tend to bear more neonates but may have a mixture of larger and smaller sized neonates or produce neonates with different pelage coloration instead of all the same color. Diversifying phenotypic plasticity of neonates may be advantageous under certain environmental conditions and detrimental in others. Under optimal foraging or environmental conditions, small and

larger neonates may both survive, however under limited food conditions or inclement weather, smaller neonates may be more susceptible to mortality. Larger sows with larger litters could have higher neonate mortality if all or a majority of the smaller neonates perish. Depending on the habitat of the nest and where the sow establishes her home range during the first six weeks post-parturition, certain pelage coloration may be more effective camouflage from predators. Larger sows with high variability in neonate pelage color could have higher neonate mortality if the litter is composed of many non-wild pelage color neonates. Conversely, smaller sows are associated with smaller litters and may show more attentiveness over neonates because they are more anxious, thus neonates born to smaller sows may be better defended against predators⁸². These attributes could promote more time spent with neonates, less travel time or distance to resources and decreased risk of inadvertent abandonment during movement.

While survival probabilities for neonates were generally similar among sows, daily survival probabilities for neonates ranged from ~91–99% among individual sows, and cumulative neonate survival probability to six weeks old ranged from ~17–82%. Neonates born to four sows (P328, P749, P762, P784) had substantially more variation in daily survival, and the lowest cumulative survival probabilities across the duration of the study period. Thus, even with our small sample size, we demonstrate there is likely some variation in survival of neonates among individual sows that may be influenced by numerous biotic and abiotic factors. There did not appear to be any similar biological attributes among these four sows that we were able to quantify (i.e., age or mass); however, it is possible there were similar traits that we were not able to measure (i.e., foraging efficiency, parental care behavior, social status) that could have contributed to lower survival of their offspring. Resource availability and distribution, as well as predator density within an individual's home range likely also contributed to variability in neonate survival among sows, which we were unable to quantify for individuals tracked in this study. For example, the resources available during pregnancy and/or lactation may influence behavioral patterns such as time spent foraging and selection of habitat, as well as behavioral trade-offs related to concentrating foraging opportunities in areas with high-quality food resources that may present increased risk of contact with predators of neonates. Increased food intake during gestation has also been correlated with higher birth weight of neonates in domestic pigs⁸³.

Capturing and tagging neonates has the potential to decrease their survival⁴⁵. For example, collars or tags with VHF or Global Positioning System (GPS) capabilities may be cumbersome for neonates and may have negative effects on survival or behavior in individuals of any age class^{31,34,45}. While we could not control for capture-related impacts in our study, we found that survival of tagged neonates did not differ compared to untagged neonates. Thus, the use of small ear tag transmitters did not appear to have a significant effect on mobility, suckling, or foraging, nor did the tag increase neonate abandonment or rejection by the mother or introduce novel infection/physical disturbance. This suggests use of this style of ear tag may be more effective for tracking neonate movement and survival than alternative models that have been evaluated^{31,34}, but further improvements to the design of these transmitters would broaden their utility in studies of ungulate neonates. For example, use of an internal antenna and rubber washers eliminated issues related to transmitter retention experienced by Keiter et al.³¹, but in to overcome this limitation through tracking transmitters placed on sows but having to routinely home in within 100 m of neonates could increase the probability of disturbance, and greatly extends the amount of time needed to monitor neonates.

Wild pigs employ a suite of strategies such as high reproductive capacity and generalist foraging behavior to maintain high population numbers^{23,62}. Hence, wild pigs are among the most successful large-sized invasive mammals globally. Our data are the first quantification of neonate survival of wild pigs in their invasive range that will inform population models for the development of effective management strategies to ultimately reduce negative impacts of this destructive invasive species on native ecosystems, livestock, and human health. Future research should explore more factors influencing wild pig neonate survival such as resource selection by the sow, neonate genetics, wild pig and predator density in a larger sample as well as in other areas within their invasive range.

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Table 5.1. Data for all wild pig (*Sus scrofa*) sows captured for neonate survival study, n = 24 and number of VIT deployed, n = 23 at the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC, USA from 2017–2020. Nest outcome: successful, neonates tagged and sow returned to nest; failed, neonates tagged and sow abandoned nest; dropped VIT, VIT failure and could not determine parturition event; could not locate, sow gave birth but nest was not located. Note: sow P331 gave birth three times during the study.

	Capture		Sow Mass	Parturition	Nest
Sow	Date	Sow Age	(kg)	Season	Outcome
P223	9/19/2017	Subadult	86.18	warm	successful
P772	12/28/2017	Yearling	49.90	cold	successful
P708	11/22/2017	Adult	74.84	cold	failed
P783	3/23/2018	Subadult	74.84	warm	failed
P784	3/23/2018	Subadult	88.45	warm	successful
P789	8/9/2018	Adult	83.91	warm	failed
P758	10/24/2018	Subadult	58.97	cold	failed
P777	9/24/2018	Yearling	58.97	-	dropped VIT
					could not
P795	1/3/2019	Subadult	80.15	cold	locate
P797	1/13/2019	Adult	60.55	-	dropped VIT
P321	2/23/2019	Yearling	79.38	warm	successful
P326	3/11/2019	Subadult	61.23	warm	successful
P328	3/20/2019	Adult	83.65	warm	successful
P331	3/25/2019	Subadult	65.32	warm	successful
P340	4/9/2019	Adult	94.05	warm	no piglets
P331*	No VIT	Subadult	-	cold	successful
P750	12/13/2019	Yearling	47.63	cold	successful
P762	12/13/2019	Adult	81.65	cold	successful
P749	1/28/2020	Juvenile	33.11	warm	successful
P769	2/5/2020	Juvenile	52.16	cold	successful ^A
P796	2/5/2020	Juvenile	49.90	cold	successful

Reproductive Sow Data

P354	2/18/2020	Adult	56.25	cold	successful
P355	2/18/2020	Adult	71.62	cold	no piglets
P331 [#]	2/21/2020	Adult	81.65	warm	successful

Sow age class was determined in the field by molar eruption patterns^{47,48}. Age class categories were: Juveniles (< 1 year); Yearlings (1-1.5 years); Subadults (1.5-3 years); Adults (> 3 years). ^ALitter was successful because offspring were born alive, however, neonates were preyed on prior to tagging (see details below) and data were excluded from survival analysis using JAGS because sex data were missing.

Table 5.2. Set of competing models (within Δ WAIC ≤ 2 of the top model) that include neonate, sow and environmental covariates influencing wild pig (*Sus scrofa*) neonate survival to six weeks on the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC USA, 2017–2020.

	52		
Model Tested	WAIC	ΔWAIC	Weight
survival = season	219.80	0.00	0.21
survival = sex	220.60	0.80	0.14
survival = sow mass	220.99	1.19	0.11
survival = sex + sow mass	221.47	1.67	0.09
survival = wild + sow mass	221.64	1.84	0.08
survival = wild	221.65	1.85	0.08

Model Selection for neonate survival in wild pigs, within $\Delta AICc$

Variables included in model selection analyses: litter, litter size; season, season neonate was born (cold = December-15 March or warm = 16 March-September); sex, neonate sex (male or female); sow mass, neonate mass at capture; wild, wild pelage coloration.

Figures



Figure 5.1. Variation in pelage coloration of wild pig neonates (A-F) and VHF transmitter ear tag (G-H). Phenotypic variation in pelage coloration of wild pig (*Sus scrofa*) neonates and VHF transmitter ear tag (Model RI-2BMH; Holohil Systems Ltd.) designed for tracking real-time survival of neonate wild pigs on the Savannah River Site, Aiken, Allendale, and Barnwell counties, South Carolina, USA. A) Wild type, brown striped, B) wild type, brown striped and spotted, C) tri-colored (white, brown and black) spotted, D) solid black, E) black and white spotted, F) red/brown and spotted, G) front view of ear tag H) back view of ear tag.



Figure 5.2. Average daily survival probability of neonate wild pigs. Daily wild pig (*Sus scrofa*) neonate survival probability with 95% CI for each sow on the Savannah River Site, Aiken Allendale, and Barnwell counties, SC, USA from 2017–2020. Values are calculated from the null model with sow as a random effect. Note, sow P331 gave birth three times during the study and neonates from two litters were monitored for survival.



Figure 5.3. Cumulative survival probability of wild pig neonates to six weeks old. The cumulative survival probability for 13 litters of wild pig (*Sus scrofa*) neonates born to each sow over six weeks at the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC, USA., monitored via radio telemetry and remote camera images, and calculated from the differential daily survival rates for each sow (n = 12; note sow P331 had two litters).
CHAPTER 6

CONCLUSIONS

A comprehensive understanding of wild pig reproductive ecology, encompassing reproductive output, phenology, the biotic and abiotic drivers that impact probability of pregnancy, litter size, space use, habitat selection, reproductive success, and juvenile survival is essential for building realistic population models to advance management policies in their invasive range. Further, data collected from studies that span multiple seasons and years are crucial for capturing natural fluctuations, accounting for individual and environmental variation, as well as decreasing sampling bias. I studied reproductive ecology of wild pigs on the Savannah River Site (SRS), Aiken, SC USA, using a combination of biometrics, fetal counts, genetics, hormone quantification, and GPS data. I provide robust data centered around wild pig fecundity, highlighting important attributes that contribute to the invasiveness of this species.

In Chapter 2, I found that wild pigs produced offspring throughout all months of the year on the SRS, with seasonal peaks in conception (and subsequently farrowing) corresponding to fall hard mast, an important forage resource. The likelihood of a sow being pregnant increased with mass and age class and was greatest during years with abundant hard mast resources. Litter size also increased with sow mass and age, implying larger and older sows contributed most to recruitment in wild pig populations. I present the first data relating genetic composition of wild pigs to reproductive output, and surprisingly, proportion of wild boar ancestry was not an important driver of productivity in our population. I also present the first reproductive hormone data addressing physiological age of sexual maturity. Understanding reproductive phenology and parameters such as litter size, and the factors that influence productivity such as age structure and individual attributes provide a comprehensive assessment of wild pig reproductive ecology. High reproductive potential is a primary driver of wild pig invasion to non-native ecosystems; therefore, these data are important for robust population models which are critical for identifying areas to focus management needs and implementation.

In Chapter 3, I found that physiological condition, centered around reproduction, influences movement and space use of invasive wild pigs, ultimately resulting in differential selection of habitats between the late gestation, farrowing, and neonatal-care periods. Sow movement decreases from gestation to farrowing, then gradually increases during the neonatal care period. I also found that analytical methods for quantifying area restricted search movement behaviors from GPS data are able to identify reliably behavioral changes associated with a farrowing event. Home range adjustment and habitat selection during the reproductive periods likely reflect choices made to increase offspring survival and thus overall fitness of the sow. Evaluating fine-scale habitat selection during a critical life-history event provides insight about important resources wild pigs depend on, or at least exploit, that influence population growth. Identifying habitats that are selected during reproduction informs managers about optimal temporal and spatial scales at which to concentrate management efforts to mitigate the negative effects of this destructive pest. In Chapter 4, I found that litter size increases with maternal size, but there is no relationship between sex ratio and maternal size. Sows preferentially adjust litter size and not sex ratio in relation to their condition. If wild pigs adjust litter size in relation to maternal condition, perhaps sex ratio is optimized so that the most offspring survive, dependent on maternal condition. Collectively, my results emphasize adjustment of litter size appears to be the primary reproductive characteristic manipulated to increase fitness rather than adjustment of offspring sex ratio.

In Chapter 5, I found survival of neonate wild pigs is relatively high (44%), underscoring the potential for this species to expand in population size and distribution upon establishment in novel environments. While survival probabilities for neonates are generally similar among sows, daily survival probabilities for neonates range from ~ 91 – 99% among individual sows, and cumulative neonate survival probability to six weeks old ranges from $\sim 17-82\%$. These data demonstrate there is likely some variation in survival of neonates among individual sows that may be influenced by numerous biotic and abiotic factors. Of the parameters I tested, survival is positively influenced by pelage coloration, likely as a form of camouflage from predators. Neonates born in warmer months and male have higher survival, possibly a reflection of their poor thermoregulatory ability and traits associated with being male (e.g., dominance over siblings). Neonates born to larger sows have lower survival. Sow size is positively correlated with litter size, and this finding may reflect the increased nutritional demands of sustaining large litters, or difficulties in defending more neonates against predators. My data are the first quantification of neonate survival of wild pigs in their invasive

range and will inform population models for the development of effective management strategies to ultimately reduce negative impacts.

Despite being ecological generalists with high reproductive potential, I found a suite of individual attributes and environmental factors that exert influence over reproductive success in wild pigs. The cost of reproduction necessitates adjustment of space use, selection of specific habitats, and that sow size and age class appear to be two of the most important attributes that affect reproductive potential and neonate survival in wild pigs, and thus their ability to establish in new areas. While my data are specific to a population of wild pigs in western South Carolina, my findings may be applied to other populations in similar habitats and can be used as a model of reproductive parameters for other areas.

APPENDIX

Table S1. Data for wild pig (*Sus scrofa*) neonates captured for survival study, n = 50 at the Savannah River Site, Aiken, Allendale, and Barnwell counties, SC, USA from 2017–2020. Neonate ID: Sow ID + neonate number in order of processing during capture; Mass: neonate mass at capture (1-3 days old); Pelage Color: wild = brown with black stripes/spots; Litter size: number of live neonates captured at natal nest; VHF Tag: Y = neonate tagged with VHF radio transmitter tag, N = neonate not tagged, tracked by unique pelage coloration with remote camera images; JAGS Analysis: Y = neonate included in Bayesian survival analysis model, N = neonate missing data (sex, mass) and was not included in Bayesian survival analysis model; Days Survived: number of days neonate survived from birthdate.

neonate Data											
Neonate ID	Sex	Mass (g)	Pelage Color	Litter Size	VHF Tag	JAGS Analysis	Days Survived				
P223_P1	male	1200	black and white spotted	6	Ν	Y	24				
P223_P2	female	1100	wild	6	Ν	Y	36				
P223_P3	-	-	wild	6	Ν	Ν	36				
P321_P1	female	810	tri-color spotted	5	Y	Y	42				
P321_P2	male	1060	black and white spotted	5	Y	Y	42				
P321_P3	female	1000	black and white spotted	5	Y	Y	23				
P321_P4	male	1580	black and white spotted	5	Ν	Y	28				
P321_P5	female	910	black and white spotted	5	Ν	Y	42				
P326_P1	male	930	wild	4	Y	Y	16				
P326_P2	female	925	wild	4	Y	Y	42				
P326_P4	male	870	wild	4	Ν	Y	42				
P328 P1	female	980	wild	2	Y	Y	7				

Neonate Data

P328_P2	female	1230	red/brown and spotted	2	Y	Y	7	
P331_P1*	male	1020	tri-color spotted	6	Y	Y	42	
P331_P2*	female	1020	tri-color spotted	6	Y	Y	42	
P331_P3*	male	1020	black and white spotted	6	Ν	Y	42	
P331_P4*	male	1080	red/brown and spotted	6	Ν	Y	42	
P331 P5*	female	700	black and white spotted	6	Ν	Y	42	
P331 P6*	male	940	black and white spotted	6	Ν	Y	42	
P331 P1 [#]	female	1090	black and white spotted	5	Y	Y	6	
P331 P2 [#]	male	1130	black and white spotted	5	Y	Y	6	
P331 P3 [#]	female	820	black and white spotted	5	N	Ŷ	42	
P331_P4 [#]	female	1100	black and white spotted	5	N	Y	42	
P331_P5#	male	830	black and white spotted	5	N	v	42	
P331_P1\$	-		red/brown and spotted	3	N	N	6	
1331_11 D221_D2 [§]	famala	-	black and white spotted	2	N	N	0	
$F_{331}F_{2}$	formale	-	black and white spotted	2	IN NI	IN NI	0	
P331_P3*	remale	-	black and white spotted	3	IN V	N V	8 42	
P354_P1	famala	520	wild	2	I N	I V	42 10	
P354_P2	female	800	solid black	3	IN V	I V	19 34	
P749 P1	male	810	black and white spotted	6	I V	I V	8	
P749_P3	male	680	black and white spotted	6	N	Y	22	
P750 P14	male	1090	wild	7	N	Y	42	
P750 P4	male	930	wild	, 7	Y	Ŷ	6	
P750 P7	male	980	wild	7	N	Ŷ	42	
P750 P8	female	820	wild	7	Y	Y	5	
P762 P12	male	1070	wild	8	Y	Y	13	
P762 P2	female	930	tri-color spotted	8	Y	Y	23	
P762_P9	female	960	wild	8	Y	Y	6	
P769_P1	-	-	black and white spotted	2	Ν	Ν	3	
P769_P2	-	-	red/brown and spotted	2	Ν	Ν	3	
P772_P1	male	900	solid black	3	Y	Y	42	
P772_P2	male	1000	solid black	3	Ν	Y	42	
P772_P3	male	1100	solid black	3	Y	Y	42	
P784_P1	female	960	black and white spotted	4	Y	Y	4	
P784_P2	male	990	wild	4	Y	Y	37	
P784_P3	male	1340	wild	4	Ν	Y	10	
P784_P4	male	1050	black and white spotted	4	Ν	Y	9	
P796_P2	female	1140	wild	3	Y	Y	42	
P796P4	female	-	black and white spotted	3	Ν	Ν	42	-