ABSTRACT

LYNN, ASHLEY. Estimating Densities of White-tailed Deer along an Urban-Rural Gradient in Durham County, North Carolina. (Under the direction of Dr. Elizabeth Kierepka).

As urbanization increases across the globe, once continuous landscapes have shifted to gradients of human dominated and fragmented habitat. Urban-rural gradients have varying degrees of change, which creates novel habitats for species. As species adapt to these transitional areas, understanding populations becomes difficult with smaller parcel sizes and private land. The white-tailed deer (*Odocoileus virginianus*) is a commonly recognized species that use landscapes across gradients. The overall aim of this study was to estimate deer densities and how deer density changes across an urban-rural gradient around a metropolitan area in Durham County, North Carolina. In chapter 1, I conducted a study to test the feasibility of a cluster sampling design in a rural and suburban setting and to investigate if integrated fecal DNA (fDNA) and telemetry can work in parallel to produce density estimates. I concluded that the cluster design allowed sufficient flexibility for sampling in suburban areas where properties are smaller and often limited by private land access. Using integrated spatial capture-recapture (SCR) and telemetry methods, combined density estimate results were 54 deer/km^2 (95% CI = 35-84) in the rural site and 75 deer/km^2 (95% CI = 48-117) in the suburban site. In chapter 2, I expanded sampling spatially to include seven sites along an urban-rural gradient using imperviousness to define urbanization. I used fDNA-based SCR to estimate densities across seven sites with imperviousness ranging from 1% (rural) to 60% (urban) across public and private lands. Deer densities were negatively correlated with impervious surfaces with the highest densities in rural areas and declined as impervious surface increased. Estimated deer abundance in Durham County was 23,748 deer (95% CI = 18,384-30,678) over an area of 742 km^2, resulting in a county wide density of 32 deer/km^2 (95% CI = 25-41). These results report

areas along the urban-rural gradient where deer densities are higher, while simultaneously identifying spatial patterns in deer density within Durham County. Using fDNA-based SCR for further research into targeted areas can provide robust density estimates by considering animal behavior, season, and cost.

Estimating Densities of White-tailed Deer along an Urban-Rural Gradient in Durham County, North Carolina

by
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BIOGRAPHY

Ashley Lynn graduated with a B.S. in Biology with an Ecology and Evolution Concentration from Nevada State University. She immediately began working as a Wildlife Biologist with small mammals and birds. Ashley started her master's degree in Fall 2021 in the Kierepka lab working on using fecal DNA and spatial capture-recapture to estimate deer densities.

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LIST OF TERMS

Study Area: Durham County, North Carolina (Chapter 1 and 2), and Orange County, North Carolina (Chapter 1 only)

Sampling Site: the ~8 km^2 area that represents the gradient classification

Cluster: the formation of sampling plots within each sampling site

Sampling Plot: a 0.25-acre plot where fecal collection took place

Capture and Recapture: describes the identification of an individual deer through fecal DNA

secr: Spatially Explicit Capture-Recapture package used for data analysis

Space Use (σ): describes a deer's space use, and therefore detection probability. Specifically, the standard deviation of a bivariate-normal distribution

Detection Probability Intercept (λ_0 & g0): the probability of detecting a deer if the sampling plot was at a deer's activity center. Together, g0 and σ define the probability of detecting a deer given its activity center and distance to a sampling plot

Density (D): the density of deer reported in deer/km^2

CHAPTER 1: Methods for Integrating Fecal DNA and Telemetry into a Spatial Capture-Recapture Framework

Abstract

White-tailed deer (*Odocoileus virginianus*) are an important game species in the United States that often uuse anthropogenic landscapes including both suburban and highly urban areas. Deer use is well documented across gradients of urbanization; however, deer densities across such gradients are poorly understood. Challenges in estimating densities within anthropogenic landscapes include smaller property sizes, access to private lands, and fragmented habitats. To address these challenges, I conducted a study to test the ability of non-invasive genetic sampling and spatial capture-recapture to estimate deer densities in a suburban area in Durham County, North Carolina and rural area in Orange County, North Carolina. Fecal samples were collected in July and September 2022 over three weeks using a cluster sampling design in both suburban and rural areas. I collected 223 fecal samples and genotyped them at 9 microsatellite markers and one sex-determining marker. In total, I identified 157 unique deer (112F, 45M). Analyses integrated data from 31 GPS collared deer from the same study areas to supplement the fecal DNA due to low recaptures of male deer. I estimated rural densities at 54 deer/km^2 and suburban densities at 75 deer/km^2. Overall, this cluster design yielded high numbers of fecal pellets despite limited land access, but low fecal DNA recaptures of males necessitated the use of telemetry data to obtain density estimates. My findings demonstrate the potential of non-invasive genetic studies to estimate densities of wildlife populations across an urbanization gradient, but multiple study design considerations are needed when low recaptures are expected or encountered.

Introduction

Non-invasive genetic sampling has become an integral method for estimating densities of wildlife populations (Waits and Paetkau 2005, Ferreira et al. 2018). These methods rely on extracting DNA from shed materials such as feces, hair, or feathers to produce genotypic data that can track animals across space and time. Shed materials can be collected in high numbers compared to traditional (i.e., physical) capture methods and provide robust data suitable to estimate abundance and density using standard capture-recapture or spatially explicit capture-recapture (SCR) frameworks. Genotypic data is used in a continuous space and is reliant on multiple encounters (recaptures) of animals within a study area (Royle et al. 2011). Recaptures are essential to obtaining biologically relevant density estimates (Efford and Boulanger 2019), but SCR algorithms can be supplemented with independent datasets such as aerial photography (Gopalaswamy et al. 2012), wildlife cameras (Fisher and Bradbury 2014, Brommer et al. 2021), and telemetry (Bird et al. 2014, Chandler et al. 2022), if required. Therefore, considerable effort is needed to develop an appropriate sampling design that detects individuals repeatedly across space and time.

Non-invasive genetic studies may be particularly advantageous in cases where substantial challenges exist to physical capture methods. For example, anthropogenic habitats along urban-rural gradients (i.e., urban and suburban areas) contain fragmented landscapes, small property sizes, and a large number of private lands, all of which can greatly limit live capture and handling opportunities. In contrast, genetic material can be collected as points (e.g., hair snare) or small plots (e.g., searches for fecal material), which creates flexibility for sampling in a variety of landscapes (Gurney et al. 2020, Poutanen et al. 2019, Rounsville Jr. et al. 2022). Fecal DNA-based (fDNA-based) sampling has been used to identify individuals and estimate density

and abundance of wildlife populations (von Thaden et al. 2020, Zemanova 2019). Taken together, fDNA-based sampling and SCR provide opportunities to study wildlife across a matrix of landscapes, including anthropogenic landscapes where species are continuously adapting to novel habitats (Donihue and Lambert 2015, Miles et al. 2021).

The objective of this study was to evaluate the use of fDNA-based sampling to estimate white-tailed deer (hereafter 'deer'; *Odocoileus virginianus*) density in anthropogenic landscapes. Deer readily use suburban and urban areas, which has caused numerous problems including increased traffic collisions and damage to property (Decker and Richmond 1995, Potratz et al. 2019, Curtis 2020). Deer densities are mainly estimated via harvest information, but due to hunting regulations, such data is limited in more urbanized areas. This study had two specific objectives: 1) design a cluster sampling scheme to locate deer fecal pellets on both private and public lands and 2) estimate densities of deer in a suburban and rural area using integrated SCR. Since deer are highly abundant and mobile, I also incorporated telemetry data from an associated study to further refine density estimates. These results will provide critical information for science-based management of deer in more urbanized areas. Additionally, these methods describe important considerations for other studies interested in exploring wildlife populations in areas with fragmented and/or limited land access.

Study Area

My study areas were in Orange and Durham Counties, North Carolina (NC) in the Piedmont ecoregion. The study areas consist of two sampling sites (8.9 km^2 each): Eno River State Park (ER) and Umstead Road (UR). Eno River is a rural, 4,320-acre, forested park with 30 miles of trails and several campgrounds with an average elevation of 167 m. In contrast, UR is a suburban area (24 houses/km^2) with a mix of private and public lands with an average elevation

of 117 m. Public land within UR is used for hiking, biking, and fishing whereas private land is comprised of mostly single-family homes. Average annual precipitation in the region is 119 cm. Both areas contain mixed forests with hard mast trees and pine stands. The canopy of these forests includes white oak (*Quercus alba*), hickory (*Carya spp.*), and red maple (*Acer rubrum*), while the understory consists of American sweetgum (*Liquidambar styraciflua*), flowering dogwood (*Cornus florida*), American holly (*Ilex opaca*), and sugar maple (*Acer saccharum*). Common pines found throughout are the loblolly pine (*Pinus taeda),* Virginia pine (*Pinus virginiana*), and longleaf pine (*Pinus palustris*).

Methods

Sampling Design

I used a clustered sampling design that consisted of four plots arranged in a 2x2 formation where each plot was approximately 0.25 acres (~0.001 km^2) to mimic the average size of a suburban backyard. To create clusters within ER, I generated a fishnet of 0.25-acre plots using ArcGIS Pro (version 2.9) and randomly selected sets of four plots. Clusters were placed at least 400 m apart and plots were 60 m apart; this spacing allowed for multiple clusters and plots within an average deer home range (~1 km^2 based on deer telemetry in this area). Plots were then scouted to ensure their accessibility. Three selected plots were inaccessible, so new plots were chosen to create one additional cluster. In total, ER had 15 clusters (n = 54 plots) in an 8.9 km^2 sampling site (Figure 1.1).

Due to the restriction of homeowner access in UR, clusters consisted of 2-6 plots based on landowner permission. Prior to sampling in UR, I identified potential parcels of land via the Durham County Parcel Layer (NC OneMap 2022) based on the fishnet as described above. Once a set of parcels was chosen as a potential cluster, I conducted community outreach (e.g., door

knocking and flyers) to gain private landowner access. If a potential plot contained multiple parcels, all were contacted for access. Following outreach, 34 landowners permitted access to their parcels for sampling, resulting in 16 clusters (n = 52 plots) in an 8.9 km^2 sampling site (Figure 1.2). Landowners included single-family homeowners, city schools, churches, and a golf course.

Fecal Collection

Fecal collection occurred in July 2022 (UR) and September 2022 (ER). Sampling in both sites occurred during a three-week period and each site had three sampling occasions. We conducted area searches in each 0.25-acre plot with one to five technicians and collected all fecal samples in week one, but only fresh samples (e.g., mucus coating, green tint, soft to the touch) were collected in the subsequent weeks due to low amplification in older samples. Effort was consistent with each plot being surveyed for 20 person-minutes (i.e., person-specific survey time decreased with each technician in the plot). We collected three to four pellets from each pile and placed them in a 15 mL Falcon tube containing 100% molecular grade ethanol. All tubes were labeled with the cluster number, plot number, and occasion (1-3); each fecal pile within plots was given an additional identifier (A-Z). Samples were stored at 2°C until extraction.

DNA Extraction and Genotyping

Lab work was performed at the North Carolina Museum of Natural Sciences in Raleigh, NC. Fecal DNA extractions were completed using the NucleoSpin DNA Stool kits (Macherey-Nagel, Düren, Germany). I incubated 1-2 pellets at 70°C for 45 minutes (min) to remove all ethanol. Pellets were then placed into a 5 mL tube with ST1 lysis buffer, incubated for 10 min, and shaken on the vortex at 2800 rpm for 10 min. I then followed the published protocol (Macherey-Nagel, Düren, Germany) with a final elution volume of 50 µL.

Microsatellite amplification occurred on two multiplexes. The first multiplex was developed for mule deer (Lounsberry et al. 2015) and included six primers: SBT06, TGLA94, SBT05, ETH152, SBT07, and BM6506 (Table 1.1). The second multiplex contained a sex determining marker, SRY, and three primers (Ohe256, OheC273, and OarFCB; Table 1.1) developed for white-tailed deer (Hamlin 2021). All PCRs were carried out in 13 µL volumes containing 4 µL DNA, 6 µL of Qiagen Multiplex PCR Master Mix (Valencia, California, USA), and 3 µL of each primer plex (each primer concentration = 0.25-0.6 µM; Table 1.1). The touchdown PCR reaction conditions included an initial denaturation at 95°C for 5 min followed by 4 cycles of denaturation at 95°C for 45 seconds (s), touchdown annealing temperatures from 68°C to 60°C for 5 min, and extension at 75°C for 1 min. Next, a single touchdown cycle of annealing at 58°C to 56°C for 2 min, and extension at 72°C for 1 min occurred before a set of 31 cycles of denaturation at 95°C for 45 s, annealing 54°C for 2 min, and extension at 72°C for 1 min with a final extension of 72°C for 10 min. PCR products were analyzed on an Applied Biosystems Sequencer 3500 and sized via 600 LIZ size standard (Applied Biosystems Inc). I called allele sizes in the program Geneious Prime (Kearse et al. 2012). All samples were amplified in duplicate to ensure accurate calling, and any mismatches were amplified a third time. I calculated genotyping error rates (i.e., allelic dropout and false alleles) for each locus. An allelic dropout occurred when two duplicates mismatched, and the third amplification confirmed a heterozygote whereas the opposite was defined as a false allele.

Telemetry Data

Deer capture took place between January-May 2022. Each deer was fitted with a GPS collar (Model G5-2D; 4-hr mortality delay; Advanced Telemetry Systems, Isanti, MN, USA) that records locations every 2 hours for 1 year. For SCR analysis, one point per day was chosen at

random to reduce possible autocorrelation in locations. Deer were captured primarily in or around UR except one deer that was captured ~1 km away from ER (Figure 1.3). To ensure the telemetered deer were representative of summer behavior, I used data from 31 GPS collared adult deer (18 female, 13 male) during July and September 2022. Deer capture and handling was conducted under the authority of the North Carolina Wildlife Resources Commission. Deer handling followed all North Carolina State University guidelines and IACUC protocols (IACUC Protocol #21-370).

Spatial Capture-Recapture

I used the *secr* package v 4.2.0 (Efford 2020) in R version 4.2.1 (R Core Team 2021) to estimate densities of deer using fDNA and integrated fDNA + telemetry (hereafter; integrated). I created a detection history that included the plot, unique deer ID, occasion, and sex. Additionally, each sampling site had a file that notated spatial locations of each plot. I integrated telemetry data to inform the space use parameter (σ; Sollman et al. 2013; Figure 1.3; 'independent' telemetry in the *secr* package), under the assumption that telemetered individuals represent deer in the study area (Efford 2023).

I used a hazard half normal detection function with a 'proximity' detector for detection probability (i.e., individuals could be detected at multiple plots during a single occasion; Efford 2004, Efford 2011). Plots were buffered by 3000 m (>4σ), and I verified any further increase to the buffer did not change parameter estimates (Efford 2023). I used a hybrid mixture model to evaluate the sex-specific space use under the hypotheses that males may use larger areas compared to females. I assumed detection probability of an individual at its activity center (λ_0) was constant across sites. I allowed space use (σ) to vary by sex and density to vary by sampling

site. The full model was: (D~ site, λ_0~ 1, σ~ sex). I report results as estimates with 95% confidence intervals (95% CI).

Results

Genotyping

I collected a total of 223 fecal samples (n_{ER} = 83, n_{UR} = 140) but eliminated samples that amplified at <8 microsatellite markers (n = 26). After quality control, 197 genotypes were retained to calculate densities within ER and UR (Table 1.2). This dataset had high power to discern individuals ($P_{ID} = 6.30 \times 10^{-13}$) and siblings ($P_{Sib} = 7.70 \times 10^{-5}$). No false alleles were recorded, and allelic dropout was low (mean = 0.02% across all loci). There was a total of 177 spatial detections across both sampling sites. After eliminating repeat detections (individuals detected >1 time at the same plot on the same occasion), there were 157 individual deer (n_{ER} = 64, n_{UR} = 93; Table 1.2) used for SCR analysis.

Spatial Capture-Recapture

Based on fDNA only, estimates were not available for space use or sex-specific densities due to low recaptures. The integration of 31 GPS collared deer (18F:13M) provided enough data to estimate sex-specific space use. Female σ was estimated at 193 m (95% CI = 184-202; Table 1.3) while the estimate for males was 330 m (95% CI = 316-345; Table 1.3). Detection probability (λ_0) was equal across sexes and estimated at 0.03 (95% CI = 0.02-0.05; Table 1.3).

I identified 64 individuals (48F:16M) in ER during September 2022, two of which (2F) were recaptured (Table 1.2). Integrated analysis estimated density of females and males at 48 deer/km^2 (95% CI = 30-77) and 6 deer/km^2 (95% CI = 4-11), respectively (Table 1.3). Combined density across sexes was 54 deer/km^2 (95% CI = 35-84; Table 1.3; Figure 1.4). For UR, I identified 93 individual deer with 18 recaptures (2M:16F; Table 1.2). Integrated analysis

estimated density of females at 64 deer/km^2 (95% CI = 40-100) and male density at 10 deer/km^2 (95% CI = 6-17; Table 1.3). Combined density across sexes was 75 deer/km^2 (95% CI = 48-117, Table 1.3; Figure 1.4). Density estimates from fDNA only analysis (i.e., excluded telemetry) were >200 deer/km^2 at both study sites (Figure 1.4). Substantially higher density estimates from fDNA only analysis were largely driven by an inability to estimate reasonable space use parameters (σ) due to the lack of recaptures.

Discussion

In this study, I demonstrated the use of a cluster design, fDNA-based sampling, and integrated SCR to estimate densities of deer in anthropogenic landscapes. I found a cluster design allowed sufficient flexibility to account for sporadic private land access while still being able to detect individual deer on the landscape. Fecal DNA captured many individuals, but showed low recaptures in both sites, which hindered our ability to estimate sex-specific densities and male space use. Integration of telemetry data allowed estimation of those parameters and improved precision. Density estimates using integrated data were higher than regional deer estimates based on harvest but may reflect deer population dynamics in suburban areas with edge habitat (Urbanek and Nielsen 2013, Williams et al. 2013). Collectively, these methods provide a flexible framework that can overcome several challenges associated with density estimation in mobile, abundant wildlife like deer.

Both sampling sites had a large female bias, which suggests sex-specific behavior during the sampling period. In the summer, females and their offspring form herds in areas of high resources and protection (Grovenburg et al. 2012, Cherry et al. 2017). The female biased data could be a result of a late summer data collection or that females in general are more tolerant of suburban areas (Roden-Reynolds 2020, *MS Thesis*). Suburban areas often contain resources such

as vegetation, protection from predators, and less busy roadways, which females select when caring for young fawns (Kilpatrick and Spohr 2000, Karish 2022, *PhD Dissertation*). Conversely, male deer in summer months are segregated away from areas with females, forming bachelor herds around resources, which could reduce male presence within suburban sites (Henderson 2020, *MS Thesis*, Johnson et al. 2023). The low capture rate in males had a substantial impact on both space use and density estimates from fDNA, which was addressed by integrating sex-specific telemetry data.

Even with the inclusion of telemetry data, our space use estimates were lower than other non-invasive SCR deer studies (Goode et al. 2014, Beaver et al. 2016, Johnson 2019, *PhD Dissertation*), but similar to estimates derived from the local telemetry data. While sampling areas that are too small can result in restricted long-range movements leading to underestimates of space use (Goode et al. 2014), sampling sites (8.9 km^2) were designed based on telemetry data of deer across Durham County (personal communication). I also recaptured deer multiple times between clusters, but never across the total length of the sampling site. Therefore, a more likely explanation of small space use values was the short duration of our study (e.g., σ references a 3-week period) and possible behavioral differences of deer in our study areas. Recaptures are vital for parameter estimation; however, information on space use can also be acquired with telemetry data (Efford et al. 2004). Female space use was estimated with fDNA based on 12 recaptures, and when 18 GPS collared females were added, estimates remained consistent between the two analyses. Conversely, space use for males using three recaptures from fDNA was much smaller than the estimate produced from the addition of 13 GPS collared males. The addition of telemetry for the male deer increased precision for space use and produced more realistic results

based on the seasonal space use of male deer (Main et al. 1996, Webb et al. 2010, Dechen Quinn et al. 2013).

While telemetry greatly improved estimates of space use, low recaptures in the fDNA also impacted density estimation despite high genotyping success (ER = 82% and UR = 93%). Densities of mobile species can be difficult to estimate as movement may limit visitation to a sampling plot resulting in a low number of recaptures and possibly biased and imprecise density estimates (Keiter et al. 2017, Hostetter et al. 2022). Our estimated densities (n_{ER} = 54 deer/km^2 and n_{UR} = 75 deer/km^2) are substantially higher than county deer estimates based on hunter harvest (>19 deer/km^2 in Durham County; North Carolina Wildlife Resources Commission 2020). Our analysis was pre-hunt when fawns comprise 40%-50% of the population, and the separation of fawns and adults in SCR analysis may be a way to correct these high densities (Poutanen et al. 2019). In this study, ages were not available nor distinguishable via fecal size, so fawns are included in density estimates.

Overall, estimating biologically relevant space use is imperative to successfully calculate densities. The inclusion of telemetry helped overcome our low recaptures, demonstrating the benefits of independent datasets (e.g., cameras, telemetry) to supplement space use estimates. When independent datasets are not available, high sampling effort for non-invasive genetic sampling will likely be required to prevent low recaptures. Methods to improve recapture probability include an increase in sampling plots on the landscape, the number of sampling occasions, or both when possible. Despite the high effort needed for density estimates, results like this study fill in critical gaps for areas where agencies lack harvest data such as private lands in suburban and urban areas where wildlife may occur in high numbers (Curtis 2020). For deer, management agencies typically use hunter harvest information to report deer estimates, but this

information is usually male-biased and limited to hunting seasons and landscapes where hunting is permitted. With costs of non-invasive genetic work lowering, this sampling design, with sufficient effort to obtain recaptures, is a viable way to estimate densities within anthropogenic landscapes and complement existing data like harvest information.

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Table 1.1 Locus information from both multiplexes used in white-tailed deer amplification. Locus name and associated reference (superscript), final primer concentration in each PCR reaction (µM), multiplex set (1 or 2), dye-label, observed size range (bp), and total number of alleles per locus (N_A).

Locus	Primer Concentration (µM)	Multiplex Set	Dye	Size Range (bp)	N_A
SBT06[a]	0.4	1	6FAM	167-198	6
SBT05[a]	0.4	1	NED	90-155	15
BM6506[b]	0.6	1	PET	172-210	12
ETH152[c]	0.25	1	NED	194-224	10
TGLA94[c]	0.3	1	6FAM	126-148	7
SBT07[a]	0.4	1	VIC	142-200	15
Ohe256[d]	0.4	2	6FAM	90-110	8
OheC273[d]	0.4	2	6FAM	133-184	5
OarFCB193[e]	0.5	2	NED	84-134	9
SRY[f]	0.55	2	PET	177	1

a. Brinkman et al. (2010)
b. Bishop et al. (1994)
c. Steffen et al. (1993)
d. Jones et al. (2000)
e. Buchanan & Crawford (1993)
f. Wilson & White (1998)

Table 1.2 Number of deer fecal samples collected (samples), number of genotypes at >8 loci (genotypes), number of spatial detections used for spatially explicit capture-recapture inputs (detections), number of individuals identified (individuals), total males (M), total females (F), and number of total recaptures (recaptures) and recaptures by sex (M/F) from each sampling site, Eno River and Umstead Road, Durham and Orange Counties, North Carolina, summer 2022.

Sampling Site	Samples	Genotypes	Detections	Individuals	M	F	Recaptures (M/F)
Eno River	83	67	66	64	16	48	2 (0/2)
Umstead Road	140	130	111	93	29	64	18 (2/16)
Total	**223**	**197**	**177**	**157**	**45**	**112**	**20 (2/18)**

Table 1.3 Parameter estimates from white-tailed deer density analysis using spatially explicit capture-recapture (SCR) models for both sampling sites, Eno River and Umstead Road, Durham and Orange Counties, North Carolina, summer 2022. These estimates use integrated telemetry-SCR analysis and are based on the model (D~ site, λ0~1, σ~ sex). Included parameters are space use (σ) in meters, detection probability (λ_0), and density (D) in km^2. All parameters are reported with their 95% confidence intervals (lower, upper).

Parameters	**Eno River**			**Umstead Road**		
	Estimate	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
σ (male m)	330	316	345	330	316	345
σ (female m)	193	184	202	193	184	202
λ_0 (male & female)	0.03	0.02	0.05	0.03	0.02	0.05
D (male km^2)	6	4	11	10	6	17
D (female km^2)	48	30	77	64	40	100
D (combined km^2)	54	35	84	75	48	82

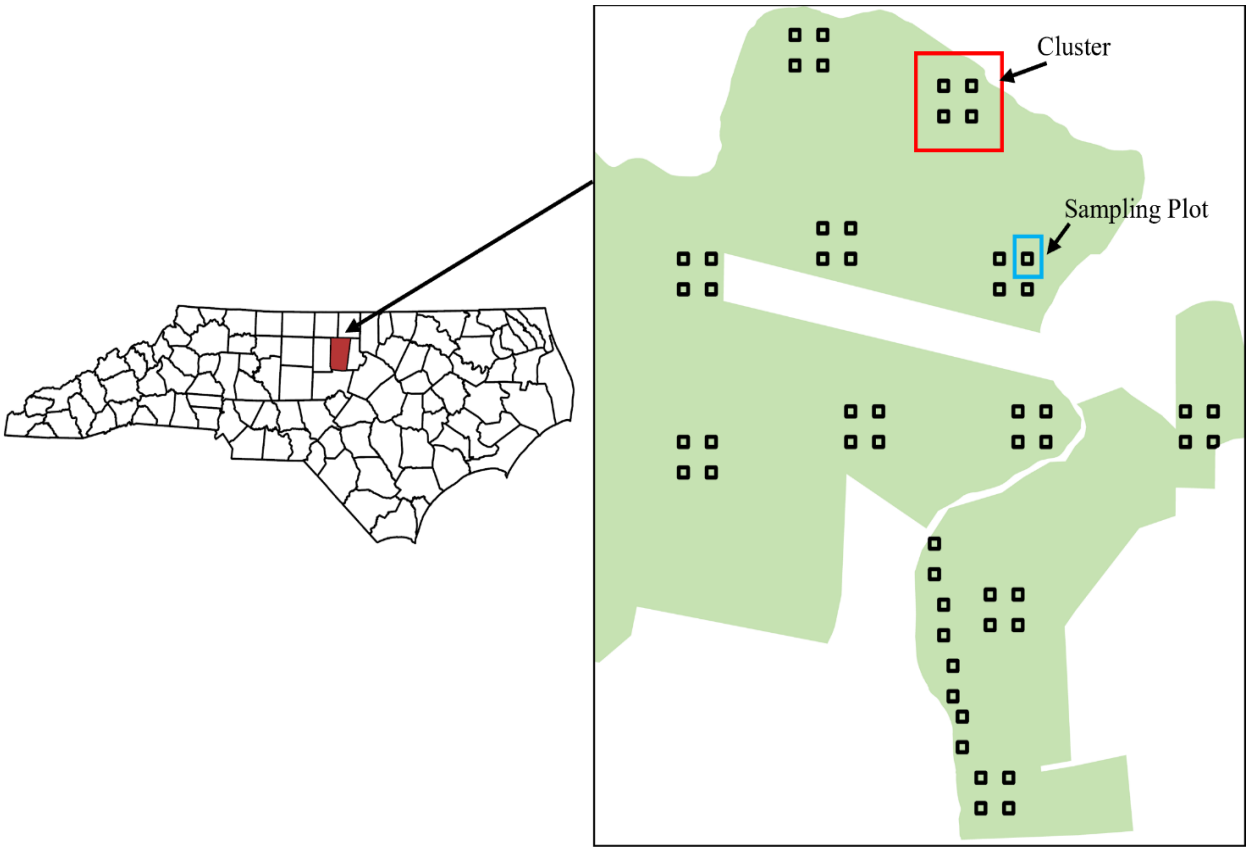

Figure 1.1 Rural sampling site (Eno River) in Orange County, North Carolina. The cluster sampling design (outlined in red) generally consisted of a 2x2 formation of the sampling plots (outlined in blue). In total, 60 plots were sampled at this site.

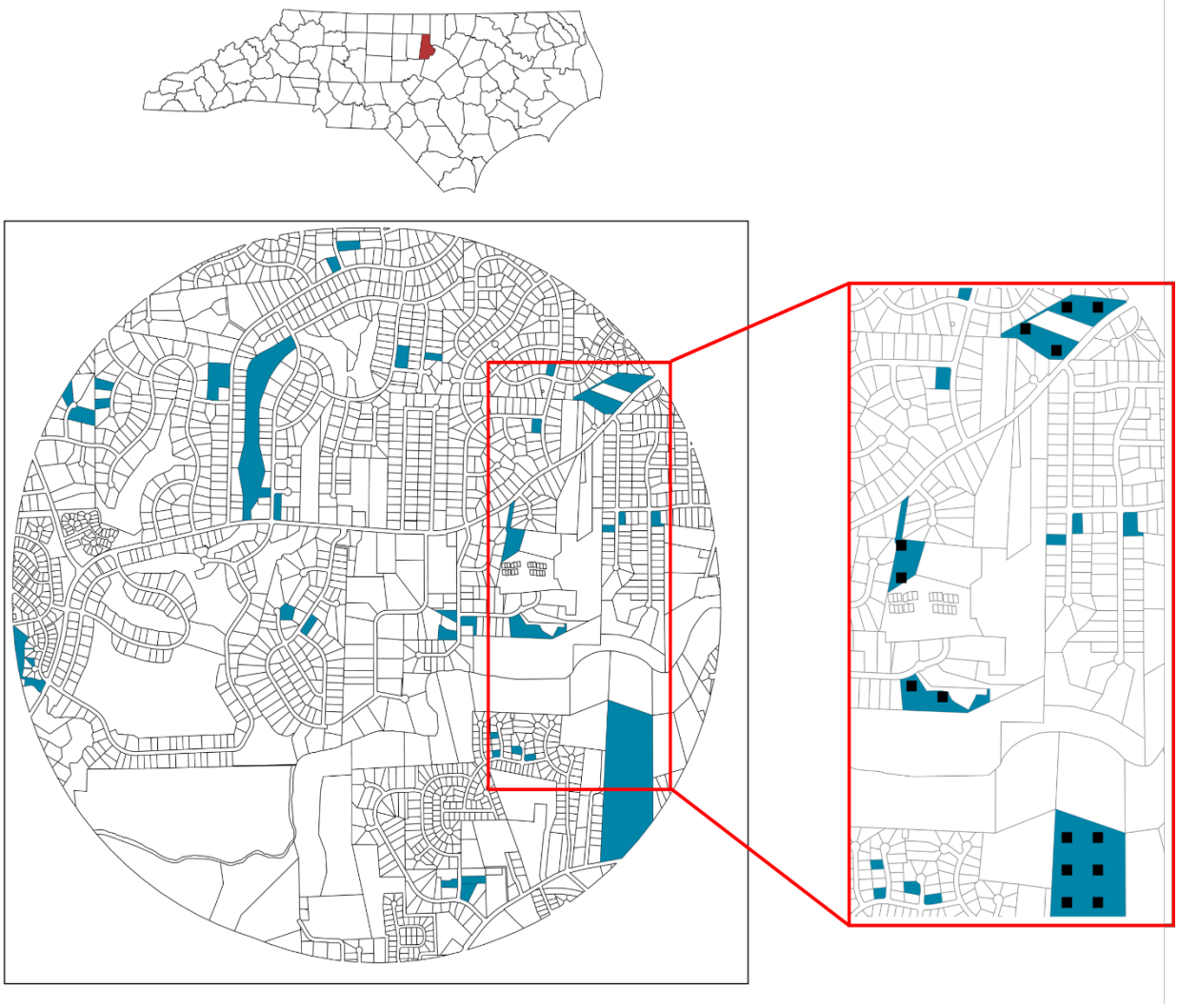

Figure 1.2 Suburban sampling site (Umstead Road) in Durham County, North Carolina. This site consists of 34 parcels (blue) which act as sampling plots in suburban sites. In the red box, it is shown how larger parcels incorporate multiple plots (black squares), while single-family parcels count as a singular plot. In total, 54 plots were sampled at this site.

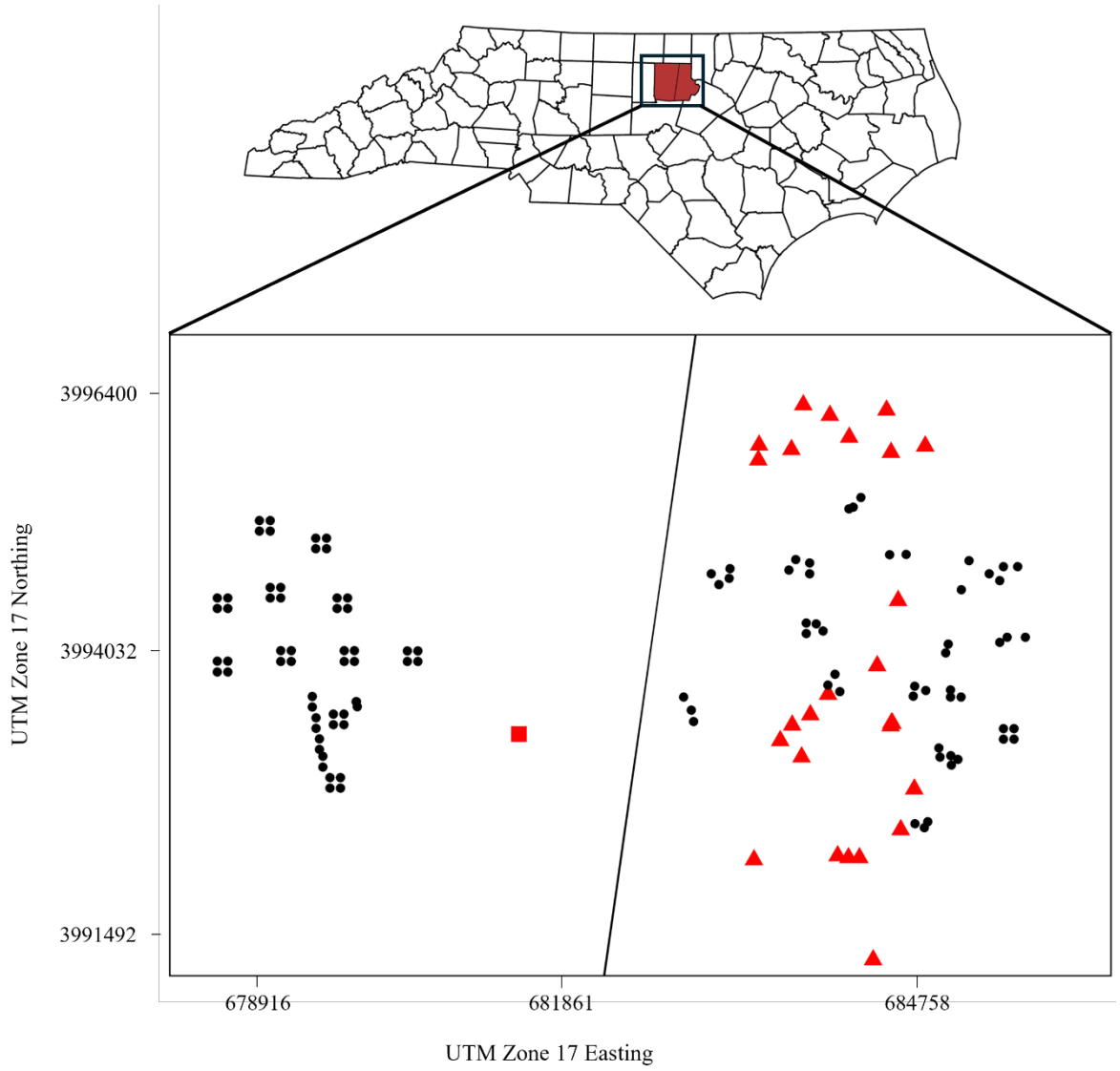

Figure 1.3 Locations of fecal DNA sampling plots (circle), average location of 1 GPS collared deer in site ER (square), and average locations of 25 GPS collared deer in site UR (triangle) used for integrated SCR modeling.

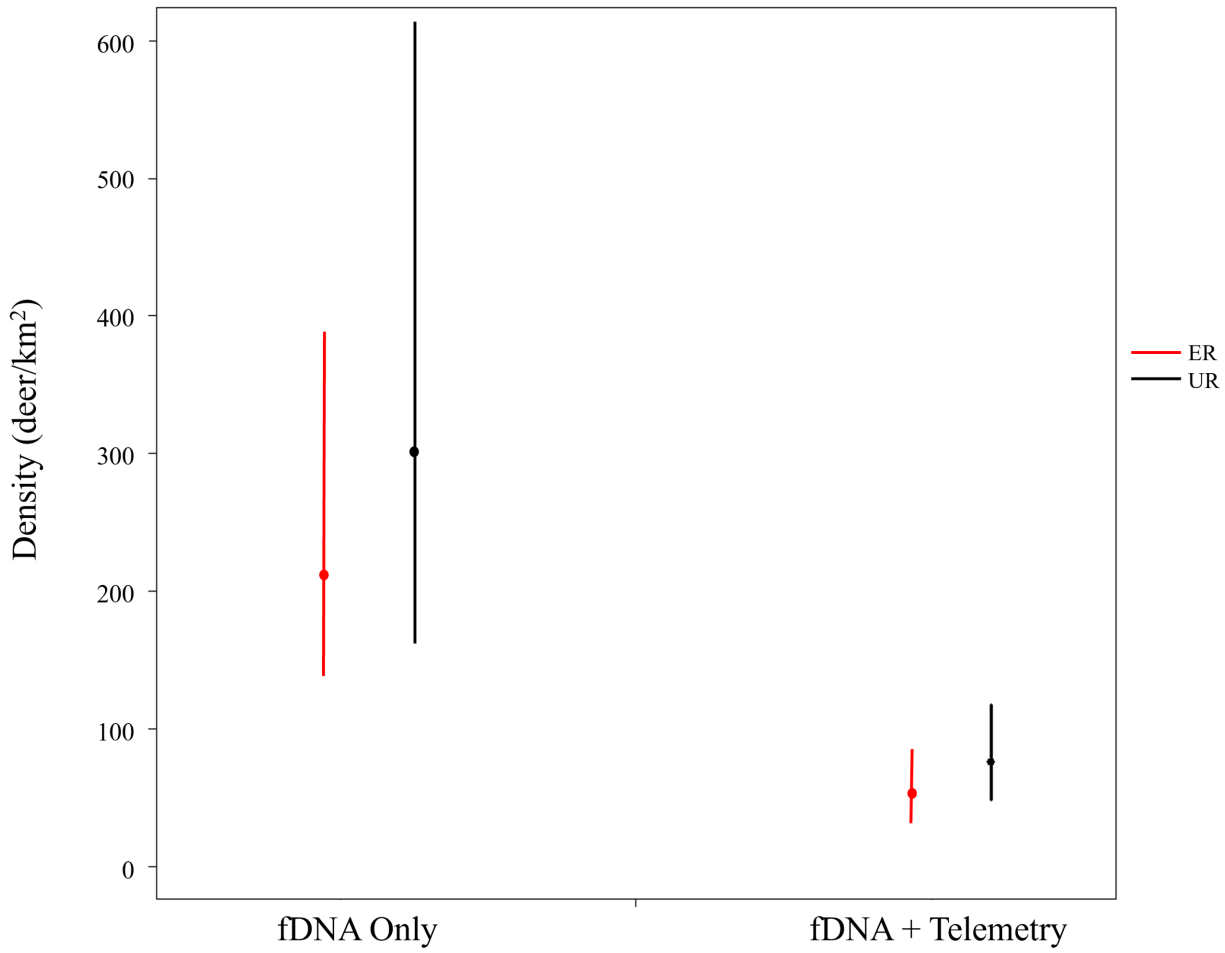

Figure 1.4 Fecal DNA only (fDNA only) and integrated analysis (fDNA + Telemetry) white-tailed deer density results in Eno River (ER; red) and Umstead Road (UR; black), Durham and Orange Counties, North Carolina, USA, summer 2022.

CHAPTER 2: White-tailed Deer Densities along an Urban-Rural Gradient

Abstract

Urbanization is expanding throughout the globe, creating urban-rural gradients and altering wildlife communities. While some wildlife populations decline as human presence increases, others such as white-tailed deer (*Odocoileus virginianus*) persist in areas with transitional habitats and increased urbanization. However, densities of white-tailed deer along urban-rural gradients are poorly understood. Consequently, there are often gaps in density estimates for deer in suburban and urban areas despite continued expansion of deer in these areas. I used a spatially explicit capture-recapture framework (SCR) with fecal DNA to estimate deer densities along an urban-rural gradient in Durham County, North Carolina. Sampling occurred at 356 plots across seven sites with percent impervious surface ranging from 1% (rural) to 60% (urban) and included public and private lands with 120 participating landowners. A total of 642 fecal samples were collected in February and March 2023 resulting in 380 unique deer. Based on SCR estimates, sampling site densities ranged from <1 deer/km^2 to 57 deer/km^2 with a negative relationship between density and percent impervious surface. Density for the entirety of Durham County was estimated at 32 deer/km^2 (95% CI = 25-41) with an abundance of 23,748 deer (95% CI = 18,384-30,678) over an area of 742 km^2. Our methods can be expanded to other species utilizing urban-rural gradients, providing managers with a reliable framework for density and abundance estimation.

Introduction

Globally, urbanization is a major cause of habitat loss, converting once continuous landscapes into smaller, fragmented green spaces surrounded by higher human density and impervious surfaces. Metropolitan areas show gradients from their urban centers through suburban developments to rural lands (Riem et al. 2012, DeStefano et al. 2005). Urban areas eliminate native habitats, and remaining vegetated areas become isolated and often heavily trafficked by humans (e.g., urban parks). Suburban areas experience the highest levels of development, creating large loss of open spaces that occur on rural fringe (Heimlich and Anderson 2001, DeStefano et al. 2005), while introducing new areas such as gardens and golf courses. While rural areas contain less humans, these areas also experience high levels of habitat conversion from agriculture and resource extraction that are interspersed with remaining native habitats. How wildlife respond to these gradients of habitat type and human pressure (hereby termed urban-rural gradient) are often species and landscape-specific (e.g., Randa et al. 2006, McKinney 2006, McKinney 2008, Parsons et al. 2018, Hansen et al. 2024), which complicates predictions of urbanization impacts on wildlife species.

Ultimately, wildlife responses to urban-rural gradients are an interaction between the life history of focal species and the underlying landscape. For example, urban areas are often unsuitable for large carnivores and ecological specialists due to insufficient amounts or an overall lack of suitable habitat (Riley et al. 2021, Parsons et al. 2018). In contrast, urban exploiters experience their highest densities and/or occupancy in highly urbanized areas by using structures for cover (Bateman and Fleming 2012), having broader diets (Palacio 2020), and changing their routines to avoid humans (Rodriguez et. al 2021). Between these two extremes exist species that can be highly adaptable across urban-rural gradients because they alter space

use and/or food sources based on the newly created habitat patches (Bateman and Fleming 2012, Ritzel and Galllo 2020). While these species may be rare in highly urbanized centers, they often thrive in suburban environments. Consequently, human-wildlife interactions occur in residential areas where food and cover are in abundance, drawing more individuals into areas frequently used by humans (Urbanek and Neilson 2013, Grade et al. 2022). Thus, suburban areas can contain diverse wildlife communities (Hansen et al. 2020, Bradfield et al. 2022, Johansson and DeGregorio 2024), but management in suburban areas can be difficult due to safety concerns, perception of animals, and private lands (Urbanek et al. 2011, Schell et al. 2021, Corbit and Hayes 2022). Therefore, greater understanding of how wildlife respond along urban-rural gradients is needed to develop management strategies to reduce negative impacts as urbanization continues to expand (Schell et al. 2021).

One species that presents a complex management challenge is the white-tailed deer (hereafter 'deer'; *Odocoileus virginianus*;), a commonly recognized generalist that exists along urban-rural gradients. Deer populations have increased in abundance and distribution since the 1970s due to a combination of factors including reduced hunting, directed reintroductions, predator removal, and a changing climate (Hanberry and Hanberry 2020, Perry et al. 2020, Laurent et al. 2021, Edelblutte et al. 2022). Deer are known to use space along the entirety of urban-rural gradients and have been well documented from camera trapping (Hansen et al. 2020, Hansen et al. 2024), but there is considerable variety in their ecologies, population densities, and hunting pressure across their North American range. For example, deer often prefer areas with intermediate housing density, open-woody habitat edges, residential yards, and anthropogenic food sources (Vogel 1989, Lovely et al. 2013, Van Helden et al. 2020), but several studies have also recorded a positive correlation between large land parcel size, a characteristic of rural areas,

and deer density (Roseberry et al. 1998, Lovely et al. 2013). Further complications in predicting how deer react to urbanization is that densities are difficult to estimate for deer due to their high numbers, large movements, and an inability to distinguish individuals. Thus, most density estimates are based on harvests, which may not be available within suburban and urban areas due to hunting regulations.

This study focused on deer within North Carolina (NC), where it is estimated that 1 million deer occupy the state (North Carolina Wildlife Resources Commission 2020). Like many states, management in NC largely focuses on harvest. Deer are primarily hunted on rural, large, parceled lands leading to a limited understanding of suburban and urban deer populations. Furthermore, previous studies that used camera trapping, distance sampling, or transect sampling to estimate deer density focused primarily on public lands (Urbanek and Nielsen 2013, Montague et al. 2017, Parsons et al. 2018, Laurent et al. 2021). I expect densities will vary across levels of urbanization, so this study quantified deer densities along an urban-rural gradient in Durham County, NC. Durham County deer densities are estimated at >19 deer/km^2 (North Carolina Wildlife Resources Commission 2020) based on hunter harvest, but no estimates are available where hunting is restricted. I predict that deer densities will be correlated with urbanization, with the lowest densities in the most urban areas of Durham County. Overall, this study will provide population densities along an urban-rural gradient and quantify the relationship between urbanization and changes in deer densities.

Study Area

My study occurred in Durham County, NC, within the Piedmont ecoregion. Durham County contains over 330,000 people (United States Census Bureau 2022) primarily concentrated within the central and southern parts of the county. This area is highly urban,

consisting of Durham and several unincorporated suburbs (Durham County Government). Private land makes up 95% of Durham County with the remainder comprised of large recreational parks, nature preserves, city parks, and game lands.

The climate in Durham County is classified as humid subtropical, with cool winters, hot, humid summers (Geiger 1954), and an annual rainfall of 119 cm. The topography is relatively flat with elevation ranging from 110 m to 218 m. Vegetation is a mix of forests with hard mast trees and pine stands. The canopy of these forests includes white oak (*Quercus alba*), hickory (*Carya spp*.), and red maple (*Acer rubrum*) while the understory consists of American sweetgum (*Liquidambar styraciflua*), flowering dogwood (*Cornus florida*), American holly (*Ilex opaca*), and sugar maple (*Acer saccharum*). Common pines found throughout are the loblolly pine (*Pinus taeda*), Virginia pine (*Pinus virginian*a), and longleaf pine (*Pinus palustris*). Notable wildlife includes the white-tailed deer (*Odocoileus virginianus*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), turkey (*Meleagris gallopavo*), eastern gray squirrel (*Sciurus carolinensis*), eastern chipmunk (*Tamias striatus*), and eastern cottontail (*Sylvilagus floridanu*s).

Methods

Sampling Sites

I focused on seven distinct sampling sites that spanned the urban-rural gradient in Durham County. Urbanization can be categorized based on different components that represent human presence on the landscape (McKinney 2002). In this study, impervious surface was used as a proxy of urbanization across Durham County, NC (Dewitz 2021). Average impervious surface for sites in rural areas ranged from (1%-14%) to (>50%) for the sites nearest to the City of Durham (Figure 2.1). The seven sampling sites included Hill Forest (HF), Treyburn (TB),

Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1) and Downtown 2 (DT2).

Hill Forest is a 2,690-acre forest in northern Durham County that is owned by North Carolina State University. This forest has public access for hunting, horseback riding, biking, hiking, and fishing, with over 10 miles of trails and roads throughout the forest. Like HF, Treyburn is in northern Durham County. Land consists of a mix of residential properties with a housing density of 5 houses/km^2 all situated along a golf course, nature preserves, and large private land parcels. Private land parcels are mostly used for recreation, hunting, and fishing. Umstead Road is in central Durham County and is primarily private land with a density of 24 houses/km^2 (Figure 2.1). Eno River State Park is the only public land in UR and is used for hiking, biking, camping, and fishing. Residential yards in this area are large with dense canopies. Hope Valley is in southern Durham County and consists of 91% private land with a housing density of 51 houses/km^2 (Figure 2.1). Finally, SD is in southern Durham County and has a housing density of 65 houses/km^2. This site contains large, master planned communities with areas of mature forest. Residential yards consist of manicured lawns with little to no canopy cover (Figure 2.1). The final two sites (DT1 and DT2) were in Durham city limits, a city with a population of 300,000 people (Durham County Government). Public lands are restricted to city parks, which are the main contributors of open green spaces within the city. Private lands here are mostly residential, but also include large shopping centers, churches, industrial parks, and schools (Figure 2.1).

Sampling Design

I used a cluster design as described in Chapter 1 (Methods; pg. 4). Briefly, clusters consisted of four to six plots arranged in a 2x2 formation where each plot was approximately

0.25 acres (~0.001 km^2) to mimic the average size of a suburban backyard. In total, HF had 12 clusters (n = 63 plots). Treyburn had 18 clusters (n = 56 plots; Table 2.1) and was a mix of public and private land. Through community outreach, I received access to 11 properties for sampling. Properties included one golf course, single-family homes, and several large, forested parcels.

Due to the restriction of homeowner access in UR, HV, and SD, clusters consisted of two to six plots based on landowner permission. Following outreach, 89 landowners permitted access to their parcels across all suburban sampling sites. Landowners included single-family homeowners, city schools, churches, one golf course, homeowners' association properties, County of Durham property, and City of Durham Parks. In UR there were 14 clusters (n = 51 plots), while HV and SD had 13 clusters (n = 65 plots) and 12 clusters (n = 54 plots), respectively (Table 2.1). Community outreach via phone or email yielded 10 private properties for sampling fecal pellets in DT1 and DT2. Properties included a museum, vacant lots, technical college, churches, and City of Durham schools. Additionally, a total of six City of Durham Parks were sampled throughout both sites. In total, DT1 had seven clusters (n = 23 plots) and DT2 had 11 clusters (n = 44 plots; Table 2.1)

Fecal Collection

Fecal sample collection occurred from 14 February to 30 March 2023. Sampling sites HF, UR, SD, and DT1 were sampled in February 2023 and TB, HV, and DT2 were sampled in March 2023 (Table 2.1). Each site had three sampling occasions except for HV and SD, which had four sampling occasions due to rain preventing two full days of collection (Table 2.1). Sampling plots included lawns, forests, golf courses, meadows, and marshy lands. Only fresh fecal samples were collected (e.g., mucus coating, green tint, soft to the touch) as older samples

failed to amplify in the pilot study (Chapter 1). Three to four pellets from each pile were placed in a 15 mL Falcon tube containing 100% molecular grade ethanol. We conducted area searches in each 0.25-acre plot with 1-5 technicians. Effort was consistent within each plot being surveyed for 20 person-minutes (i.e., person-specific survey time decreased with each technician in the plot). Fecal piles were destroyed (e.g., stepped on, covered, swept) to avoid repeat sampling. All tubes were labeled with the cluster number, plot number, and occasion (1-3); each fecal pile collected within plots was given an additional identifier (A-Z). Samples were stored at 2°C until extraction.

DNA Extraction and Genotyping

All laboratory work was performed at the North Carolina Museum of Natural Sciences in Raleigh, NC. Fecal DNA (fDNA) extractions were completed using the NucleoSpin DNA Stool kits (Macherey-Nagel, Düren, Germany). I incubated 1-2 pellets at 70°C for 45 minutes (min) to remove all ethanol. Pellets were then placed into a 5 mL tube with ST1 lysis buffer, incubated for 10 min, shaken on the vortex for 10 min, and placed back into incubation until the next steps. I then followed the published protocol (Macherey-Nagel, Düren, Germany) with a final extract volume of 50 µL. All DNA extracts were stored at 2°C until amplification.

I amplified 9 microsatellite loci in two multiplexes where the first multiplex contained six loci from a well-tested mule deer set (Lounsberry et al. 2015): SBT06, TGLA94, SBT05, ETH152, SBT07, and BM6506 (Table 1.1). The second set contained three loci (Ohe256, OheC273, and OarFCB; Table 1.1) from the published OdoPlex set (Hamlin 2021) and a sex-determining marker, SRY (Table 1.1). All PCRs were carried out in 13 µL volumes containing 4 µL DNA, 6 µL of Qiagen Multiplex PCR Master Mix (Valencia, California, USA), and 3 µL of each primer plex (each primer concentration = 0.25-0.6 µM; Table 1.1). The touchdown PCR

reaction conditions are as follows: initial denaturation at 95°C for 5 min followed by 4 cycles of denaturation at 95°C for 45 s, touchdown annealing temperatures at 68°C to 60°C for 5 min, and extension at 75°C for 1 min. Next, a single touchdown cycle of annealing at 58°C to 56°C for 2 min, and extension at 72°C for 1 min occurred before a set of 31 cycles of denaturation at 95°C for 45 seconds, annealing 54°C for 2 min, and extension at 72°C for 1 min with a final extension of 72°C for 10 min. PCR products were analyzed on an Applied Biosystems Sequencer 3500 and sized via LIZ size standard (Applied Biosystems Inc). I called allele sizes in the program Geneious Prime (Kearse et al. 2012). All samples were amplified in duplicate to ensure accurate calling, and any mismatches were amplified a third time. I calculated genotyping error rates (i.e., allelic dropout and false alleles) for each locus. An allelic dropout occurred when two duplicates mismatched, and the third amplification confirmed a heterozygote whereas the opposite was defined as a false allele.

Spatial Capture-Recapture

All spatial capture-recapture analyses were run in the R package *secr* v 4.2.0 (Efford 2020) in R version 4.2.1 (R Core Team 2021). I created an encounter history that included the plot, unique deer ID, occasion, and sex for each capture and recapture. Each sampling site had a trap layout with each sampling plot's centroid and covariates. Together, these files fit an observation model and a state model to estimate the probability of detection and density (D). I selected a half normal function with a 'proximity' detector for detection probability (i.e., individuals could be detected at multiple plots during a single occasion; Efford 2004, Efford 2011). However, I combined individuals detected multiple times at the same plot during the same occasion into one detection. I created a mask using a buffer of 2000 m with a grid spacing of 100 m and excluded areas of open water (e.g., lakes and rivers). I tested a series of larger buffers for

consistency and estimates did not change with larger buffers, so I retained 2000 m for all subsequent analyses.

I included covariates on each plot and each individual. I used the Percent Developed Imperviousness raster with a resolution of 30 m from the National Land Cover Database (NLCD; Dewitz 2021). This layer provides developed surfaces over 30x30 m pixels by considering buildings, parking areas, roads, and energy production sites. Moreover, this layer is widely available and consistently updated. I smoothed the imperviousness raster by moving a 1 km^2 (average home range of a deer in the study area) square across Durham County where all pixels had equal weights. This method helps better inform urbanization at a landscape level by considering the pixels around the sampling plot and weighing them together. I removed unsuitable deer habitat (open water) from the NLCD land cover raster with a spatial resolution of 30 m and a 16-class legend (Dewitz 2021). I projected both rasters to UTM Zone 17 and added them to the 2000 m mask made from the trap layout file. I tested two covariates (canopy cover and sex) that may influence the detection probability of deer. For canopy cover, I defined cover as open or closed via the NLCD land cover dataset (Dewitz 2021) as fecal pellets should be easier to find in open canopy areas. Space use (σ) of adult male and female deer likely differ; therefore, I used a hybrid mixture model, to allow g0 and σ to vary by sex. I derived sex-specific and combined densities over the area of the habitat mask (Borchers and Efford 2008).

Following sampling site density estimations, I predicted density across Durham County using the same 30x30 habitat mask. I estimated deer abundance by summing the density surface (Expected *N*) for each sampling site and Durham County. All estimates are reported with 95% confidence intervals (95% CI).

Results

Genotyping

In HF and TB, I collected 444 fecal piles from three sampling occasions (n_{HF} = 270, n_{TB} = 174). After quality control, 119 fecal samples were removed, leaving 325 total genotyped samples (n_{HF} = 166, n_{TB} = 159; Table 2.2). Genotyping in HF resulted in 124 spatial detections, which corresponded to 107 individual deer (53M:54F) and 18 recaptures (7M:11F; Table 2.2). Treyburn genotypes contained 151 spatial detections resulting in 125 individuals (95M:30F) and 26 recaptures (18M:8F; Table 2.2). Sites UR, HV, and SD yielded 167 fecal samples (n_{UR} = 74, n_{HV} = 50, n_{SD} = 43) across three (UR) and four (HV and SD) sampling occasions. In UR, I genotyped 56 samples resulting in 54 individual deer (49M:5F), and 2 recaptures (2M; Table 2.2). In HV, there were 45 genotypes representing 37 spatial detections, 30 individual deer (17M:13F), and 7 recaptures (6M:1F; Table 2.2). I genotyped 37 samples representing 42 spatial detections, 37 individuals (24M:13F), and 5 recaptures (3M:2F) in SD (Table 2.2). We collected the least number of fecal samples within the sites that had the higher impervious surface. One sample was collected in DT1, which resulted in 1 individual (1M). In DT2, I genotyped 29 samples out of the 30 samples collected representing 28 spatial detections, 26 individual deer (14M:12F), and 2 recaptures (2M; Table 2.2).

Across all seven sampling sites, there were no false alleles recorded and allelic dropout was low (mean = 0.06% across all loci). This dataset had high power to discern individuals ($P_{ID} = 1.40 \times 10^{-9}$) and siblings ($P_{Sib} = 4.0 \times 10^{-4}$). Genotyping success was 76% averaged across all seven sampling sites. The lowest amplification percentage was in HF (61%) while the highest was in DT2 (97%; Table 2.2).

Spatial Capture-Recapture

Overall, the model reported a decrease in density as impervious surface increased, (β = -0.08 95% CI = -0.10 to -0.07; Figure 2.2). There was an increase in detection probability in sampling plots with open canopy (β = 0.54 95% CI = 0.34 to 0.73). Sex as a mixture effect was supported on σ (β = 0.35 95% CI = 0.27 to 0.42). Additionally, σ estimates were forced to be constant across sampling sites; male σ estimates were 382 m (95% CI = 316-458) and female estimates were 261 m (95% CI = 206-326). Moreover, the estimated sex ratio was 64% males (95% CI = 54%-74%) and 36% females (95% CI = 26%-46%).

Both density and abundance were calculated for each sampling site, but I focus on densities and variation in densities across sampling sites (see Table 2.3 for abundances). The two least impervious sites, HF (53 deer/km^2 95% CI = 39-72) and TB (57 deer/km^2 95% CI = 43-77) had the highest estimated densities (Table 2.3; Figure 2.3).

There was a strong male bias in TB, estimating male densities at 43 deer/km^2 (95% CI = 31-59) and female density at 15 deer/km^2 (95% CI = 9-24; Figure 2.3). Like TB, UR had a strong male bias (male: 24 deer/km^2 (95% CI = 16-35), female: 3 deer/km^2 (95% CI = 1-6) but a lower combined density (26 deer/km^2 95% CI = 18-38) than HF and TB (Table 2.2; Figure 2.3).

The southern sampling sites (HV, SD, DT1, DT2) estimated the lowest densities (range = <1-16 deer/km^2 (95% CI = 0-24; Table 2.3; Figure 2.2; Figure 2.3). The lowest densities occurred in the most impervious sampling sites (DT1 and DT2) and no sex bias was observed (Figure 2.2; Figure 2.3). Only male densities were able to be estimated in DT1 due to the lack of female captures (Figure 2.3).

Based on the density surface model for Durham County, densities were highest in areas of low impervious surface, particularly in the northern and western parts of the county (Figure

2.4). However, even high urban areas in metropolitan Durham had patches of high deer densities in large areas with canopy cover. In total, Durham County deer density was estimated at 32 deer/km^2 (95% CI = 25-41) with an abundance of 23,748 deer (95% CI = 18,384-30,678) over an area of 742 km^2 (Figure 2.4).

Discussion

Based on estimated deer densities from seven sites across an urban-rural gradient, I found that density declined in areas with increasing impervious surface. While few density studies of deer have been conducted on urban-rural gradients, occupancy studies demonstrate varied responses to urbanization. For example, some studies found strong associations with suburban environments (Parsons et al. 2017, Hansen et al. 2024) while others report little response (Weiss et al. 2023) or avoidance (Magle et al. 2014). In our case, deer were found at all sites, but rural areas of northern and western Durham County showed consistent high densities due to large, continuous rural landscapes. Low densities were found near Durham's city center with high impervious surface, a similar result to deer in metropolitan Chicago (Magle et al. 2014). Therefore, deer in highly urbanized areas appear to be reduced to smaller pockets of habitat resulting in overall low densities but are not eliminated completely as observed in large carnivores or ecological specialists.

Four of our sampling sites (HV, SD, DT1, and DT2) were within the greater Durham metropolitan area that contains ~92% of the human population within the county. Estimated densities within these four sites were similar (<16 deer/km^2) and fecal samples were highly concentrated within city parks, forest edges, and residential yards. The remaining urbanized areas within these sites did not offer the tree cover, connectivity, or edges that deer require to safely move throughout the area (Blanchong et al. 2013, Dechen-Quinn 2013, Grund et al. 2022,

Hansen et al. 2024). Hope Valley and SD contained more "suburban" habitat than DT1 and DT2, but they were closer to the urbanized city center with higher human density and impervious surfaces than the remaining three sites. Magle et al. (2014) found that deer preferred areas with lower housing density and less humans within a large metropolitan area, so these factors could explain the relatively similar densities within the highly populated area.

Our three remaining sites represent the higher deer density sites that contained primarily residential areas (UR), a mix of residential and forest (TB), or majority forest (HF). Umstead Road's high density likely stems from large amounts of resources that are considered important for deer in suburban areas (Roseberry and Wolf 1998, Gorham and Porter 2011, Urbanek and Nielson 2013, Potapov et al. 2014). Residential yards, in particular, are important for deer, and my plots in UR focused on yards that had a mix of manicured lawns, mature growth, and human supplemented resources. Many of these yards were surrounded by natural areas (i.e., state park) and provided the edge habitat that deer prefer for movement. Thus, UR contained many features that would support the highest densities of deer along the urban-rural gradient. However, the enticing resources in suburban areas are not available year-round, which could force deer populations to move in and out of the areas depending on the season.

For TB and HF, it was unclear if densities would be high compared to more residential areas because these rural lands are hunted. Sampling was conducted one month after the hunt in NC ended (1 January), which is expected to reduce deer densities. However, deer populations have been observed reducing space use and/or increasing site fidelity within hunted lands (Little et al. 2016, Marantz et al. 2016). My results showed highest deer densities within rural areas (HF = 53 deer/km^2, TB = 57 deer/km^2) approximately 1-2 months post-hunt. Density estimates were significantly higher than regional deer densities based on harvest data (19 deer/km^2), a probable

result of several non-mutually exclusive factors. First, deer movement during winter is expected to be higher due to limited resources. When resources in other areas die off, deer increase their movements in search of forested understories, oak acorns, and agricultural crops (Brinkman et al. 2005, Little et al. 2016, Russell et al. 2017), which are common occurrences in more rural areas. In TB, there were several plots planted with soy, clover, and turnip to increase deer presence for hunting. Leftover crops are still readily available for consumption in winter, continuously drawing in large herds of deer. Another factor that may explain the high densities within rural sites is the male bias. Treyburn frequently had large groups of male pellets collected close to these planted plots, which is consistent with studies showing males have a higher probability of visiting "baited/planted" sites in winter (Newbolt et al. 2017, Johnson et al. 2021). Collectively, these factors likely caused a high number of individuals moving in the rural sites, which limited recaptures and likely inflated density estimates.

Further evidence for the sensitivity of density estimation to inflation occurred within HF where I identified an outlier plot. This plot was an open meadow with primarily clover vegetation while the other plots were highly forested with closed canopy. The outlier contained 31 individuals, 3 recaptures (2M:1F), and always had at least 20 fecal samples collected per occasion. Including this plot for estimates increased density from 53 deer/km^2 to 72 deer/km^2 (Appendix A). It should be noted that this removal also increased all sampling site densities by 2 deer/km^2, which suggests that this singular plot inflated densities along the gradient due to its high number of individuals (Appendix A). While this singular plot had the ability to inflate densities, multi-session modeling pools data from all sampling sites to inform parameters (Efford 2023); however, this is still contingent on having a large percentage of recaptures. Low recaptures are a known hindrance in SCR studies, reducing space use estimates and in turn,

overestimating densities (Greenspan et al. 2020, Tourani 2022). Studies that have accurately estimated densities in other ungulates typically have ≥80% of their individuals recaptured (Brazeal et al. 2017, McFarlane et al. 2020, Batter et al. 2021). In this study, only 16% of the individuals were recaptured, and while that was enough power to estimate space use, the discrepancy between number of individuals (380) and recaptures (60) was too large to prevent density overestimation.

Another important result from this study was seasonal differences (winter vs. summer) within one of the sampling sites (UR; see Chapter 1). With similar sampling intensities between seasons, summer surveys estimated more individual deer (n_{summer} = 93, n_{winter} = 54), female biased sex ratios (29M:64F), and a higher combined density (summer: 75 deer/km^2, winter: 26 deer/km^2). Conversely, in the winter, there was a large male bias in the area (49M:5F). This sampling site consists of older, established neighborhoods with large trees, forest, and canopy cover. Suburban areas in the summer often contain resources such as vegetation, protection from predators, and less busy roadways, which females select when caring for young fawns (Kilpatrick and Spohr 2000, Karish 2022, *PhD Dissertation*). In the winter, deer often seek protection on lands that prohibit harvest (Kays et al. 2017, Meier 2021, *MS Thesis*). These lands usually exist in suburban and urban landscapes, where parcel sizes are smaller and private land is dominant. The features of UR could be used seasonally by different sexes depending on protection needs, females with fawns or males during hunting season. Further study is needed to examine how seasonal differences may impact population dynamics of deer and corresponding management challenges along urban-rural gradients.

Overall, this study demonstrates the importance of urban-rural gradients on deer density, but applying methods like fDNA-based SCR requires additional study design considerations

when used in suburban and urban landscapes. First, defining an urban-rural gradient can include a multitude of factors (e.g., human and building density; Kaminski et al. 2021, Kent et al. 2021, Rodriguez et al. 2021); however, I chose impervious surface due to its ease of accessibility at large spatial scales. Additionally, impervious surfaces are not suitable deer habitat, so future studies should define a gradient based on the quality of spatial data as well as the life history of the focal organism(s). Second, landowner access likely explains the relative rarity of SCR studies along urban-rural gradients. I worked extensively with private landowners throughout my sampling sites, especially for access to residential yards, which was critical for estimations in areas with harvest restrictions. Finally, there is concern about overestimation using fDNA-based SCR due to lack of recaptures. If future work is to be done via this method, I suggest targeting smaller areas or increasing sampling occasions to maximize deer recaptures. We reported baseline density patterns at two different scales, sampling site and county, which will help future studies focus on areas of concern. By considering the animal behavior, the land access, and the season, creating robust fDNA-based SCR studies along gradients can be highly successful.

Management Implications

Robust density estimates for white-tailed deer are important for management as urbanization is increasing across their North American range. For Durham County, it is estimated that the human population will experience 7% increase by the year 2030 (Durham County Government). This process will inevitably change both deer behavior and the extent of urban-rural gradients, which will necessitate more management on private lands. With community outreach, fDNA-based SCR provides a robust framework to estimate density on private lands. Our model, based on impervious surface, can easily be translated to other metropolitan areas given the accessibility of raster files and geographic coverage, but we caution

that density estimates are highly sensitive to inflation in cases of low recaptures. Furthermore, deer density, space use, and sex ratio are all seasonal dependent and fDNA-based SCR can be designed for any season or landscape. Study design requires careful consideration, especially in highly mobile, abundant species like deer that are likely to exhibit low recaptures. To alleviate these challenges, small scale studies can validate models like ours or use simulations to evaluate sampling schemes based on landowner access or available funds. Non-invasive genetic sampling and SCR work well together to investigate wildlife along urban-rural gradients and can be an important complement to existing methods like hunter harvest where such data are limited.

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Table 2.1 Number of plots sampled on each occasion during fecal collection (plots), number of sampling occasions, and dates of fecal collection from each sampling site: Hill Forest (HF), Treyburn (TB), Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1), and Downtown 2 (DT2) in Durham County, North Carolina, winter 2023.

Site	Plots	Sampling Occasions	Collection Dates
HF	63	3	2/18/23 - 3/4/2023
TB	56	3	3/11/23 - 3/25/23
UR	51	3	2/14/23 - 2/28/23
HV	65	4	3/7/23 - 3/28/23
SD	54	4	2/16/23 - 3/2/23
DT1	23	3	2/16/23 - 3/2/23
DT2	44	3	3/9/23 - 3/23/23

Table 2.2 Summary of winter 2023 sample collection of deer in Durham County, North Carolina. Metrics include number of fecal samples collected, samples genotyped at >8 microsatellite loci (genotypes), total number of detections used in spatial capture-recapture analysis (detections), individuals identified (individuals), total males (M), total females (F), number of recaptures (recaptures), and genotyping success (genotypes/samples collected) from each sampling site: Hill Forest (HF), Treyburn (TB), Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1), and Downtown 2 (DT2).

Site	Samples	Genotypes	Detections	Individuals	M	F	Recaptures (M/F)	Genotyping Success
HF	270	166	124	107	53	54	18 (7/11)	61%
TB	174	159	151	125	95	30	26 (18/8)	91%
UR	74	56	56	54	49	5	2 (2/0)	73%
HV	50	45	37	30	17	13	7 (6/1)	90%
SD	43	37	42	37	24	13	5 (3/2)	86%
DT1	1	1	1	1	1	0	0	100%
DT2	30	29	28	26	14	12	2 (2/0)	97%
TOTAL	**642**	**491**	**439**	**380**	**252**	**127**	**60 (38/22)**	**76%**

Table 2.3 Estimated densities for white-tailed deer from each sampling site: Hill Forest (HF), Treyburn (TB), Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1), and Downtown 2 (DT2) in Durham County, North Carolina, winter 2023. Combined density includes both sexes (M and F), male and female densities are sex-specific. All density estimates are based on the final model of (D~ impervious surface, g0~ canopy + sex, σ~ sex). Density estimates are per km^2, expected N is the abundance of deer in each sampling site.

Site	Combined Density (95% CI)	Male Density (95% CI)	Female Density (95% CI)	Expected N (95% CI)
HF	53 (39-72)	25 (17-37)	28 (18-42)	1579 (1205-2068)
TB	57 (43-77)	43 (31-59)	15 (9-24)	1283 (988-1666)
UR	26 (18-38)	24 (16-35)	3 (1-6)	1311 (1015-1694)
HV	10 (7-15)	6 (3-9)	5 (3-9)	636 (488-829)
SD	14 (10-21)	9 (5-15)	5 (3-10)	600 (468-700)
DT1	1 (0-6)	1 (0-6)	NA*	195 (138-275)
DT2	16 (10-24)	8 (4-14)	8 (4-14)	319 (228-448)

* NAs represent parameters that could not be modeled due to lack of data.

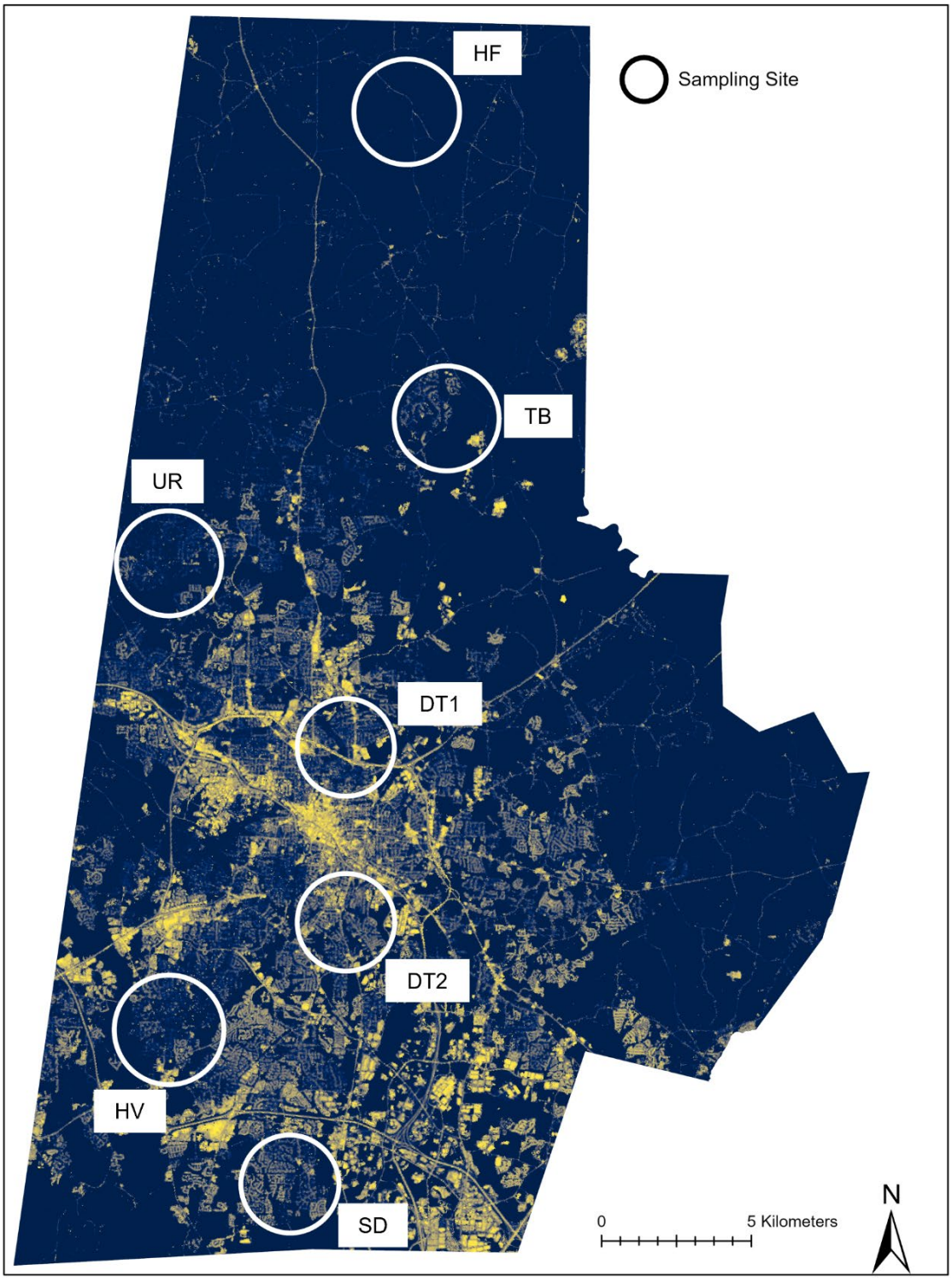

Figure 2.1 Seven sampling sites within Durham County, North Carolina, USA: Hill Forest (HF), Treyburn (TB), Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1), and Downtown 2 (DT2). Sampling sites are outlined in white and were created based on an average percent impervious surface within the site.

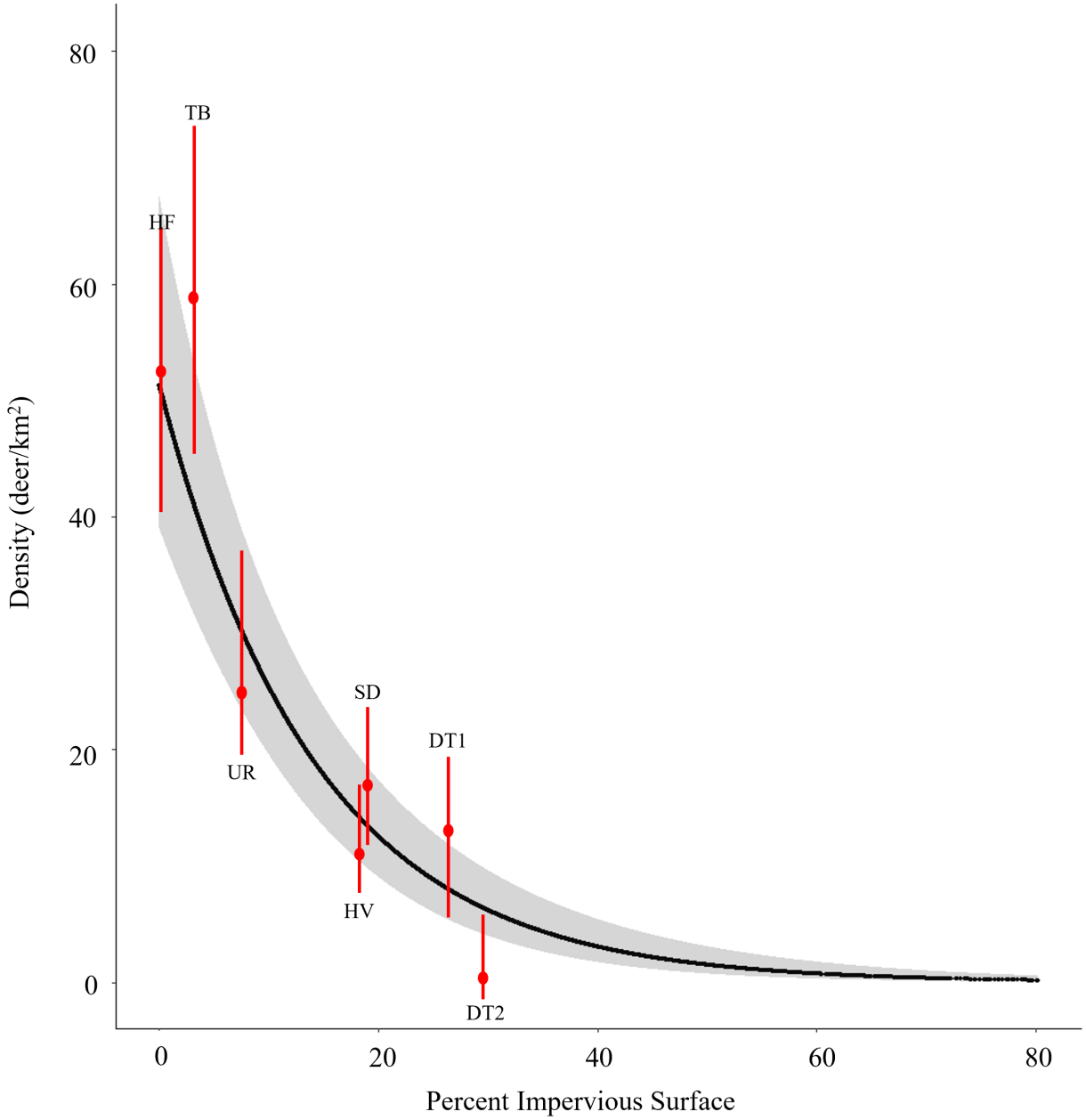

Figure 2.2 White-tailed deer density (deer/km^2) declines as percent impervious surface increases. Combined densities from the seven sampling sites are shown in red with their 95% confidence interval. Sites included are Hill Forest (HF), Treyburn (TB), Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1), and Downtown 2 (DT2), Durham County, North Carolina, USA, winter 2023.

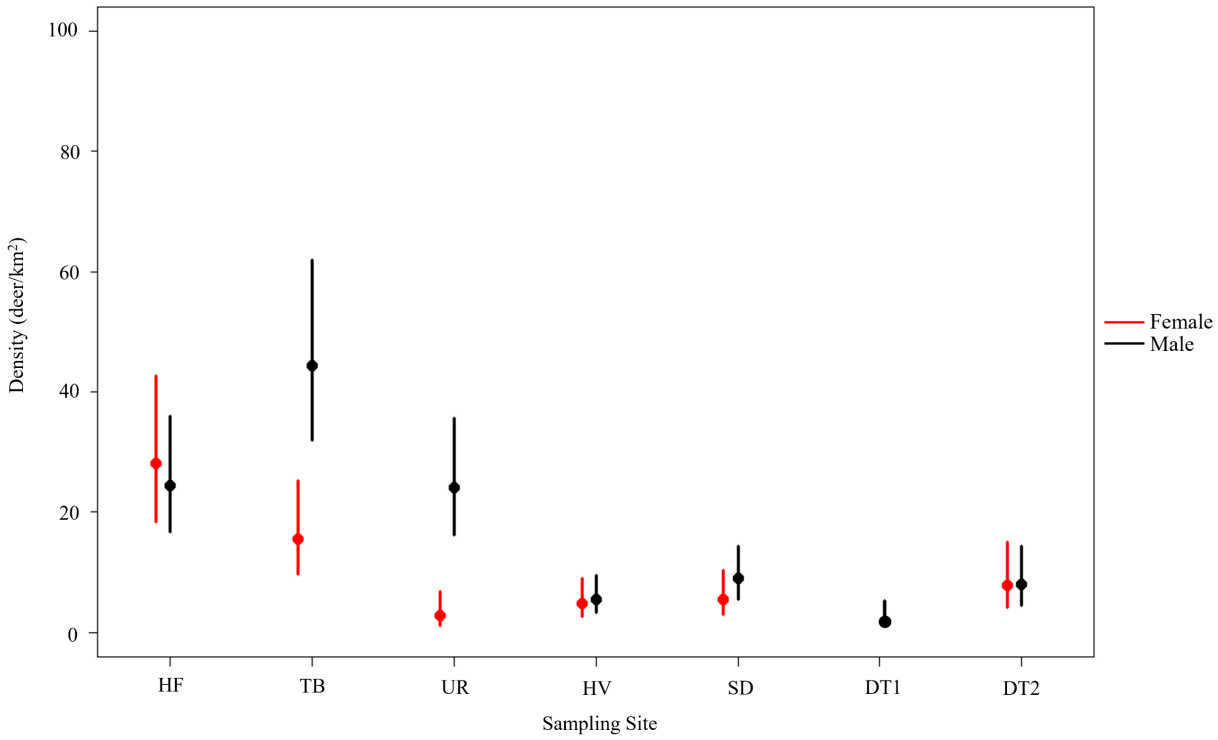

Figure 2.3 Estimated densities for white-tailed deer per sampling site Hill Forest (HF), Treyburn (TB), Umstead Road (UR), Hope Valley (HV), South Durham (SD), Downtown 1 (DT1), and Downtown 2 (DT2) in Durham County, North Carolina, winter 2023. Density estimates are reported in (deer/km^2), female specific (red), and male specific (black) with their 95% confidence interval.

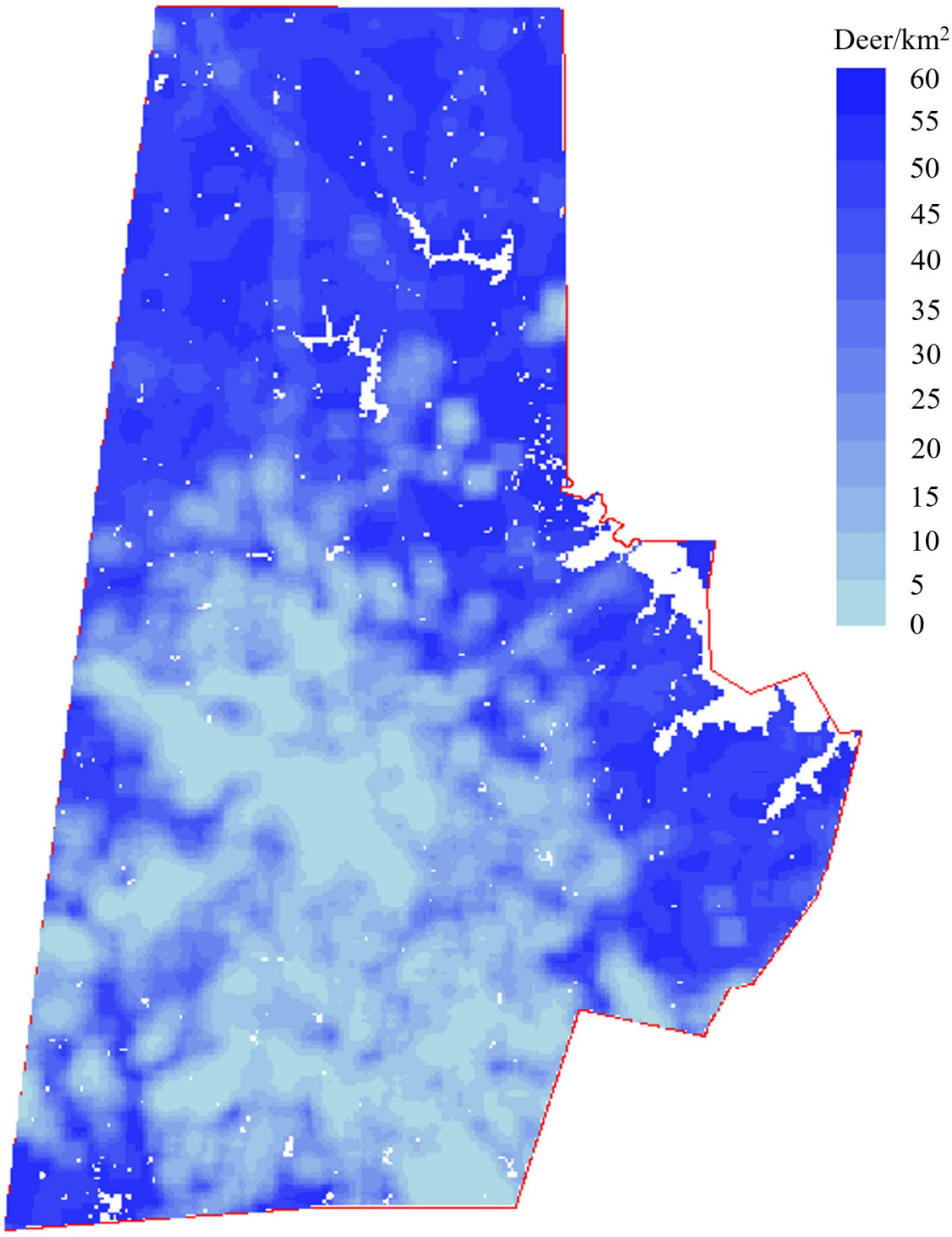

Figure 2.4 Density surface of white-tailed deer in Durham County, North Carolina, USA during winter 2023.

APPENDIX

Appendix A- Density Estimates including Plot 321

Site	Combined Density (95% CI)	Male Density (95% CI)	Female Density (95% CI)	Expected N (95% CI)	Male σ (95% CI)	Female σ (95% CI)
HF	72 (55-95)	35 (26-50)	37 (26-53)	1999 (1524-2599)	385 (367-403)	272 (256-289)
TB	59 (44-78)	45 (33-61)	14 (9-22)	1556 (1203-2011)	385 (367-403)	272 (256-289)
UR	28 (19-40)	25 (17-37)	3 (1-6)	1583 (1229-2038)	385 (367-403)	272 (256-289)
HV	11 (7-16)	6 (4-10)	5 (3-8)	663 (508-865)	385 (367-403)	272 (256-289)
SD	16 (9-21)	9 (6-15)	5 (3-10)	645 (497-836)	385 (367-403)	272 (256-289)
DT1	1.1 (0.2-5)	1.1 (0.2-5)	NA*	195 (138-275)	385 (367-403)	272 (256-289)
DT2	15 (10-25)	9 (5-15)	7 (4-13)	306 (217-432)	385 (367-403)	272 (256-289)

* NAs represent parameters that could not be modeled due to lack of data.