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# Wintering white-throated sparrows (Zonotrichia albicollis): home ranges, aggression and corticosterone

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WINTERING WHITE-THROATED SPARROWS (*ZONOTRICHIA ALBICOLLIS*):

HOME RANGES, AGGRESSION AND CORTICOSTERONE

By

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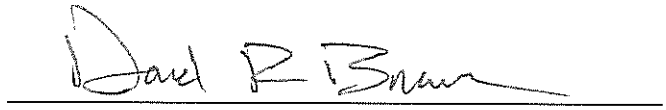

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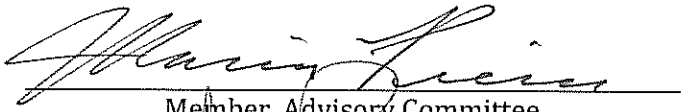

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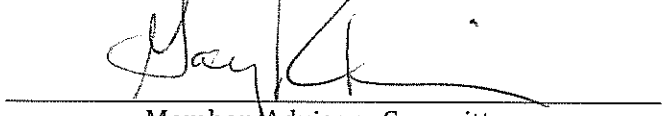

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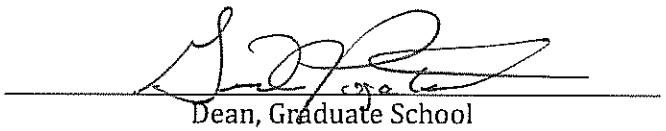

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# WINTERING WHITE-THROATED SPARROWS (*ZONOTRICHIA ALBICOLLIS*):

# HOME RANGES, AGGRESSION, AND CORTICOSTERONE

By

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Bachelor of Fine Arts
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Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
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DEDICATION

This thesis is dedicated to all my bird peeps, field crews, and mentors,

who have both tolerated and encouraged my obsession.

ACKNOWLEDGMENTS

I would like to thank Dr. David Brown for answering approximately three thousand questions and, slowly, painstakingly, helping me to figure it all out. I would also like to thank Dr. Gary Ritchison for knowing what's what in the bird world and teaching me how to string all my thoughts together most scientifically. Special props to Dr. Marcia Pierce, as well as Dr. Stephen Richter, for unending patience and revelations about lab work – a previously unknown and foreign world to me.

ABSTRACT

White-throated sparrows (*Zonotrichia albicollis*) are songbirds that spend the non-breeding season in southeastern North America, where they form philopatric territorial flocks. Flocks exhibit dominance hierarchies, with dominance rank associated with an individual's age and prior residence in the territory. Although social behaviors within flocks are well studied, few studies have described winter home ranges. I tagged white-throated sparrows (n = 12) in Madison County, Kentucky, with 0.9-g radio transmitters during the winter of 2010-2011. Locations were entered into ArcGIS and home range sizes were estimated with 50% and 95% kernel analysis for individuals with at least five locations. Mean core home ranges were 1.59 ± 0.3 (SD) ha, and 95% home ranges averaged 5.31 ± 0.8 ha. Core home ranges were significantly larger than estimates from previous studies. Differences among locations in food abundance and distribution may explain variation in home range sizes.

During the winter of 2011-2012, I focused on behavioral aspects of wintering sparrows. Flocks use distinct territories and maintain their ranked relationships throughout the winter, though familiarity lessens outright conflict as the season progresses. White-throated sparrows, unlike most songbirds, continue singing throughout winter and likely become accustomed to the songs of other flock members. I examined the responses of resident flocks to unfamiliar individuals and vocalizations throughout the winter, while also examining circulating levels of the stress hormone, corticosterone, to see if they paralleled behavioral changes. Concurrently, I validated identification of sparrow plumage morphs with genotype assays to determine reliability of field identification. I mounted study skins of white-throated sparrows 1 m above ground and played randomly selected 10-minute tracks of songs and chips interspersed with silence during three discrete periods during the (November, January, and March). Responses and agonistic behaviors were noted; plasma samples were collected concurrently to measure corticosterone. Accuracy of field identification of plumage morphs was 68.8%. Baseline corticosterone did not differ among sampling periods, suggesting these birds did not experience prolonged chronic stress throughout the winter. However, white-throated sparrows responded more aggressively to study skins and playback of conspecific calls and songs in November than in January and March. These results suggest that agonistic displays may be more important for defending winter territories and establishing dominance status in early winter.

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# PART 1

# HOME RANGE SIZES

# I. INTRODUCTION

White-throated sparrows (*Zonotrichia albicollis*) are small passerines whose range encompasses the eastern portion of North America. They spend the winter in the southeastern United States in site-faithful territorial flocks that exhibit dominance hierarchies (Falls and Kopachena 2010). Sparrows are habitat generalists during the non-breeding season, foraging near dense cover in overgrown pastures, hedgerows, forests, and cities (Falls and Kopachena 2010). This preference for cover limits the range and location of home ranges. Piper and Wiley (1990) estimated the size of the winter home ranges of white-throated sparrows based on recaptures along a linear trapline, and found that mean range sizes were 0.26 ± 0.02 (SE) ha for hatch-year (hereafter, HY) birds and 0.10 ± 0.02 ha for after-hatch-year (hereafter, AHY) birds. However, these measurements were extrapolated by estimating a 25-m range perpendicular to the trapline, which followed a hedgerow that included artificial feeding sites. This method of territory calculation, limited to one habitat type, gives an incomplete understanding of winter space use by white-throated sparrows, which has the potential to impact reproductive success the following breeding season (Norris 2005).

Using radio-telemetry, data can be collected that more accurately describes space use by birds over time periods ranging from weeks to months. White-throated sparrows are cryptically colored and typically remain in dense vegetation during the winter, so frequent re-sighting of individual birds can be difficult. With radio-telemetry, numerous locations can be obtained during a relatively short period of time. Those locations can then be compiled to create utilization-distribution maps that reflect space use based on the relative frequency of locations (Barg et al. 2004).

My objectives were to estimate the size of the winter home ranges of white-throated sparrows with utilization distribution maps generated by tracking radio-tagged sparrows in a variety of habitats in eastern Kentucky, and to compare these findings to previously published estimates of home range size. Determining home range sizes based on repeated relocation without use of food supplementation in multiple habitats can provide a better understanding of winter space use.

## II. METHODS

### *Study Sites*

My study was conducted at three locations in Madison County, Kentucky, including the Blue Grass Army Depot, Camp Catalpa, and Taylor Fork Ecological Area. The Blue Grass Army Depot (37.700121° N, -84.211063° W) is a 5906-ha site that contains pastures and woodlots of various sizes. Camp Catalpa (37.739261° N, -84.250159° W) is a 6-ha forested urban park on the eastern edge of Richmond, Kentucky, that is primarily wooded with brushy undergrowth; entry to the park is restricted for most of the winter and early spring, resulting in low pedestrian traffic. The site is surrounded by pasture and a lake. Taylor Fork Ecological Area (37.716757° N, -84.302001° W) is a 24-ha restoration site owned by Eastern Kentucky University containing old-fields and woody fencerows.

### *Mist-netting*

Sparrows were captured to attach radio-tags in a manner following standard mist-netting and banding techniques (Ralph et al. 1993). After visually or aurally locating a flock, a 6-m or 12-m mist net was erected at least 10 m from the flock to avoid disturbing the birds. Mist-nets were located to reduce net visibility to birds, but intercept natural flight paths through the habitat. Sparrows were captured both passively and using playback of recordings of white-throated sparrow songs and call notes to lure sparrows into nets. Nets were monitored continuously and birds were extracted immediately upon capture.

Radio-tags (0.9 g; Model BD-2, Holohil Systems Ltd., ON, Canada) were attached to 11 birds with leg-loop harnesses (Naef-Daenzer 2007). Radio-tag weight averaged 3.0% of sparrow body mass (mean mass = 29.8 ± 3.2[SE]g). Radio-tags were attached with elastic black thread looped around each leg such that the tag rested on the bird's synsacrum.

### *Radio-telemetry and Home Range Analysis*

White-throated sparrows (N = 11) were radio-tracked at the three study sites from November 2010 to March 2011. Birds were tracked with a Telonics TR-4 receiver and a Yagi three-element antenna (Telonics Inc., Mesa, AZ, USA). Tracking was conducted at varying times of day, five to seven days a week, and birds were located by triangulation, following the intersection of bearings from the strongest signal at multiple locations, and

subsequently resighting color band combinations from a distance to avoid influencing bird movements. Locations were recorded at least 30 min apart to insure independence (Kenward 2001) using Program ArcPad (Environmental Systems Research Institute, Redlands, CA, USA) on a handheld Juno SB (Trimble Navigation Ltd., Sunnyvale, CA, USA).

Locations were imported into ArcGIS 9 (ESRI, Redlands, CA, USA). Home range estimates were calculated using kernel density estimator with the Hawth's Tools extension (Bayer 2004). The kernel method, as it applies to home ranges, estimates intensity of space use, or the amount of time an individual might spend at any location based on the density of points on an overlaid grid (Seaman and Powell 1996). For each bird, a fixed kernel density estimator was run with a smoothing factor of 100 and a raster cell size of 10. Two volumetric values were used to estimate home range use, such that a certain percentage of the total density was contained within that area as defined by a contour polygon. Maximum home ranges were estimated with 95% volume contours, containing 95% of all estimated kernel density points, and core home ranges were estimated with 50% volume contours.

*Data Analyses*

Mean winter home range size was compared with previously published estimates (Piper and Wiley 1990) using a paired two-sample t-test. I compared the mean ranges of white-throated sparrows in my study (50% and 95% probability densities) with the average and maximum range sizes estimated by Piper and Wiley (1990). All data were tested for normality using the Shapiro-Wilk test. Statistical tests were run using SPSS version 20 (IBM Corp., Armonk, NY). Values are presented as means ± 1 SD.

## III. RESULTS

I radio-tagged 11 white-throated sparrows in 2010-2011 at three study sites. Two individuals were the sole flock members tracked at those locations (Appendix; Figures A-1 and A-2). At Catalpa (n = 4 birds) and the Blue Grass Army Depot (n = 5 birds), multiple birds captured over a period of several days at one location were tracked (Figures A-3 and A-4). Six birds were tracked for > 15 days (range = 7 – 43 days). The mean number of locations for each individual was 27.5 ± 20.2 (Table 1).

Table 1. Core and maximum home range sizes of white-throated sparrows radio-tagged in Madison County, Kentucky, during the 2010-2011 non-breeding season.

| USGS Band # | Study Site | # of Locations | # of Days Tracked | 50% (Core) Home Range (ha) | 95% (Maximum) Home Range (ha) |
|---|---|---|---|---|---|
| 2541-52685 | TFEA[1] | 15 | 26 | 0.71 | 2.89 |
| 2541-52695 | BGAD[2] | 5 | 16 | 1.77 | 6.75 |
| 2541-52697 | BGAD | 40 | 39 | 1.39 | 5.18 |
| 2541-52698 | BGAD | 24 | 19 | 3.48 | 20.69 |
| 2541-52699 | BGAD | 6 | 7 | 2.01 | 7.76 |
| 2541-52700 | Camp Catalpa | 25 | 11 | 1.19 | 5.05 |
| 2541-52751 | BGAD | 10 | 10 | 1.11 | 3.81 |
| 2541-52752[3] | BGAD | 16 | 8 | 2.93 | 10.33 |
| 2541-52754 | Camp Catalpa | 56 | 43 | 1.03 | 3.52 |
| 2541-52755[4] | BGAD | 64 | 43 | 0.84 | 4.07 |
| 2541-52756 | Camp Catalpa | 41 | 21 | 0.92 | 3.73 |

[1] Taylor Fork Ecological Area
[2] Blue Grass Army Depot
[3] Recaptured in Ontario, Canada, on 4 April 2011.
[4] Recaptured at original location in Madison County, Kentucky, in November 2011.

The average 50% core home range determined with kernel density estimation was $1.6 \pm 0.3$ ha (n = 11), and the average 95% home range was $5.3 \pm 0.8$ ha (range = 2.9 - 20.7 ha). Both the 50% core home ranges and 95% maximum home ranges of radio-tracked birds in my study were significantly larger ($t_{10} = 5.5$, $P < 0.001$ and $t_{10} = 6.9$, $P < 0.001$, respectively) than those reported by Piper and Wiley (1990) (Figure 1). Outer home range contours were projected into unusable habitat for two birds; in one, 95% home range borders included a nearby lake and, in the other, a mowed field, both of which are habitats that would be avoided by sparrows.

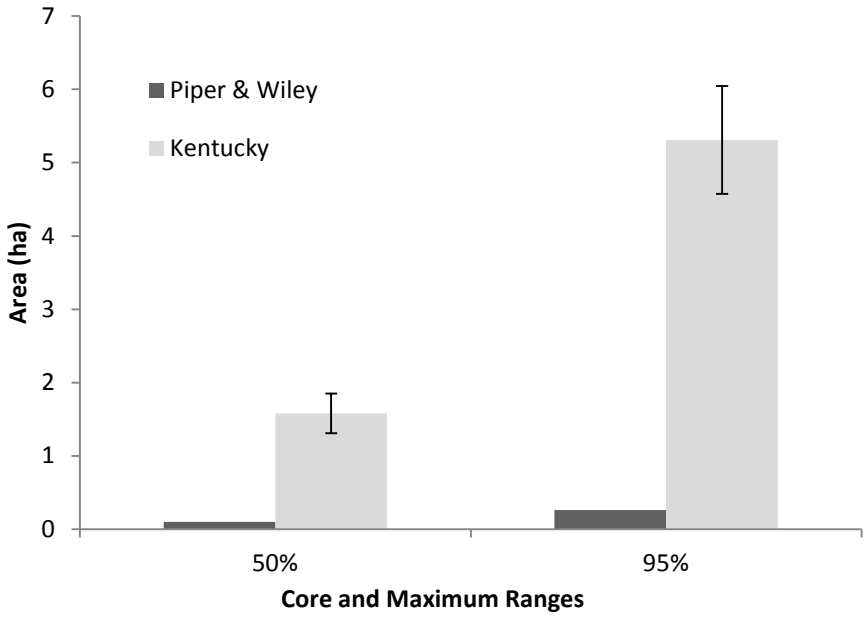


Figure 1. Core (50% ± SE) and maximum (95% ± SE) home range sizes of white-throated sparrows in 2010-2011 in Madison County, Kentucky, compared to conservative and maximum home range sizes reported by Piper and Wiley (1990) in North Carolina. *Source*: Piper, W.H. and R.H. Wiley. 1990. Correlates of range size in wintering white-throated sparrows (*Zonotrichia albicollis*). Animal Behaviour 40:545-552.

## IV. DISCUSSION

White-throated sparrows in my study in eastern Kentucky had significantly larger winter ranges than those studied by Piper and Wiley (1990) in North Carolina. The absence of artificial feeding sites may have accounted for some of this difference in range sizes. For example, in a study of dark-eyed juncos (*Junco hyemalis*), a similar-sized sparrow with comparable winter habits often found with white-throated sparrows, those with feeder access had significantly smaller home ranges (Roth and Vetter 2008). Average range size (1.5 ha) for juncos without feeder access was similar to the average core home range of white-throated sparrows in my study (Roth and Vetter 2008). Another possible explanation for the larger winter ranges of white-throated sparrows in my study was that I tracked individuals in a more heterogeneous habitat (i.e., woodlots interspersed with pastures) whereas Piper and Wiley (1990) studied birds located in linear hedgerows. The use of telemetry to track the movements of white-throated sparrows in my study likely

contributed to the larger estimates of range sizes because, in contrast to Piper and Wiley (1990), location data were not dependent on repeated captures in mist-nets along a linear hedgerow.

The results of previous studies suggest that a small number of locations may provide overestimates of territory and home range sizes when using kernel density estimators, with estimated territory sizes increasing with decreasing sample sizes (Barg et al. 2004). Seaman and Powell (1996) recommended that $\geq$ 30 locations be used to estimates territory and home range sizes. Because the maximum number of locations for one bird in my study was 64 (mean = 27.5), estimates of home range sizes in my study may be biased. Although a bird tracked for 11 days with few locations may have a comparable home range to a bird tracked for 43 days with almost three times as many locations, finer movements within that territory and the boundaries of intensive use may be inaccurate.

Utilization densities in my study tended to project home range boundaries beyond actual data points, which, in two instances, resulted in the inclusion of unused areas. A bird at my Catalpa study site was located 5 m from the perimeter of a neighboring 30-ha lake; this data point was factored into the 95% home range estimate and caused the inclusion of the edge of the lake in the range. Similarly, a bird tracked along a hedgerow was never located outside of the thick vegetation. Using the kernel method, however, the projected home range of this bird included areas extending 30 m from the hedgerow into mowed fields. In both of these cases, inclusion of unsuitable habitat in home range estimates likely led to inflated home range sizes. Others encountering this problem have recommended considering areas of unsuitable habitat as travel corridors, as individuals potentially may be found some distance from their preferred habitat (Blundell et al. 2001).

Several factors that can influence home range size may have contributed to the individual variation observed in my study. For example, the distribution and abundance of food is known to impact the size of home ranges and territories (Sherry et al. 2005, Brown and Long 2006). Breeding territory size has been found to be inversely correlated with food availability (Smith and Shugart 1987), and the same has been found for flocking winter birds (Roth and Vetter 2008). A shortage of food resources, caused either by habitat characteristics or adverse weather, could cause sparrows to range farther to forage. The size of habitat patches could also affect range size because white-throated sparrows preferentially forage in and near thick vegetation, and large stretches of open habitat may

restrict movement. Age and sex may influence range size on an individual level (Piper and Wiley 1990), but examination of the possible effects of these variables on range size in my study were not possible because of my small sample size.

Although white-throated sparrows are not a species of conservation concern, my results are useful for understanding space use by a winter flocking species. I found that white-throated sparrows have larger winter ranges than previously published. Habitat loss such as clearcutting may impact movements and winter success to a greater degree than already observed (Rousseau et al. 2012), particularly for philopatric individuals.

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# PART 2

# BEHAVIORAL AND CORTICOSTERONE CHANGES

# I. INTRODUCTION

White-throated sparrows (*Zonotrichia albicollis*) winter throughout southeastern North America and form territorial flocks that exhibit site fidelity and dominance hierarchies (Falls and Kopachena 2010). These sparrows have two plumage morphs, white-striped and tan-striped, that differ in the brightness of crown stripes and the throat patch. This phenotypic dimorphism is linked to behavioral differences in the breeding season, with white-striped birds tending to be more aggressive and pursuing more extra-pair copulations (Falls and Kopachena 2010). After the prebasic molt that occurs on the breeding grounds, the morphs of winter sparrows are difficult to discern. However, by factoring in age, sex, and crown brightness, Piper and Wiley (1989) developed a formula that accurately predicted a bird's morph 89% of the time, based on plumage determination by recapture after molting into breeding plumage. More recently, a genotyping assay was developed to determine morph (Michopoulos et al. 2007). I used blood samples collected in my study to validate field identification of morph with the genotyping assay to determine the degree to which morph identification may have been accurate in winter studies conducted prior to 2007.

In a captive population, Watt et al. (1984) found that some dominance relationships change among females from autumn to spring, perhaps due to spring hormonal changes. Behavioral changes during the non-breeding season were observed in another captive population: aggressive interactions were found to be more frequent in November than later in winter, which correlated with high androgen levels in November and, perhaps, the establishment of dominance relationships as flocks form (Schlinger 1987). During the 2010-2011 field season, I observed an apparent decrease in responsiveness of white-throated sparrows to playback of conspecific vocalizations from November to January, such that individuals no longer moved towards or investigated the source of playback, suggesting a decline in levels of aggression toward conspecifics. Schlinger (1987) reported a similar decline over the non-breeding season in aggressiveness between captive pairs competing for a food source. That study, however, was based on individual responses among 12 birds repeatedly paired with each other, so interactions were influenced by familiarity.

If flock formation entails frequent aggressive encounters, with hierarchical consequences that affect access to resources, October and November could be a period of high stress.

Corticosterone is a hormone released by the adrenal gland in response to stress, increasing locomotor activity and the release of energy stored in tissues, and high plasma levels can reflect chronic environmental stress due to factors such as risk of predation, weather, or resource availability (Angelier 2010). Baseline corticosterone levels have been found to increase in white-throated sparrows, particularly in mid-ranking birds, when food availability is reduced, which may indicate that dominance relationships among mid-rank birds are the most mutable (Schwabl et al. 1988). In song sparrows (*Melospiza melodia morphna*), individuals exposed to simulated territorial intrusions during the non-breeding season did not have a change in plasma corticosterone levels (Newman and Soma 2011). However, concentrations increased in the liver and muscle, which suggests a need for short-term energy release for aggressive interactions (Newman and Soma 2011). Variation in plasma corticosterone levels of white-throated sparrows could correspond with that of testosterone, with highest levels during flock formation in autumn if the associated aggression acts as an environmental stressor.

My objective was to examine intra-seasonal changes in wintering white-throated sparrows, both in behavioral responses to simulated intrusions and in baseline plasma corticosterone levels. I predicted that highest levels of aggression would correspond with the period of flock formation in October and November, and that the stress of flock formation and range establishment would cause higher baseline levels of corticosterone. Concurrently, genotype assays were used to estimate accuracy of morph field identification to determine if the results of morph-based studies conducted prior to the development of the assay in 2007 could have been affected.

## II. METHODS

### *Study Sites*

My study was conducted in Madison County, Kentucky, at the same sites used in 2010-2011 (see Chapter 1), including the Blue Grass Army Depot, Camp Catalpa, and Taylor Fork Ecological Area.

*Behavioral Trials*

Although white-throated sparrows begin to arrive in my study area in the first week of October, my study began on 1 November 2011. During October when individuals first appear in the region, birds may be transients that are still migrating and not members of local wintering flocks (Falls and Kopachena 2010).

Flocks were located visually and aurally within six hours of sunrise. Once located, a simulated intrusion was set up at least 15 m from the flock to minimize disturbance. A white-throated sparrow study skin was randomly selected from two plumage morph options to account for possible differences in response to morph, and mounted on a 1-m pole within habitat preferred by sparrows. A portable digital recording player and amplifier were used to broadcast white-throated sparrow vocalizations for 10 min from directly below the mounted study skin. Vocalizations consisted of randomly ordered songs (about 30 – 40) and chip calls (about 150-200) (Xeno-canto Foundation) arranged throughout a 10-min track. Fifteen different tracks were created and randomly selected for use to minimize pseudoreplication. After setup, I retreated at least 15 m from the study skin to monitor responses.

Agonistic behaviors in response to the playback were recorded for each flock member when possible, though the cryptic behavior of sparrows led to pooled and averaged scorings for response variables. The total number of birds in a flock and the percentage of responding individuals were recorded for each trial. Bird agonism was measured using metrics based on physical and vocal agitation. Latency to response, with response considered a vocalization or movement toward the study skin, was measured to the nearest minute after playback was initiated, with trials eliciting no agonistic individuals scored as maximum latency (10 min). Chip calls and songs were counted in the field, and later totaled and averaged based on the number of vocalizing individuals within an estimated 25 m of the study skin. Overall aggression was scored based on a scale of 0 to 5, with 0 = no response, 1 = some chipping in response, 2 = some chipping plus movement toward the playback or model, 3 = chipping and singing accompanied by investigative movement towards the study skin, 4 = persistent chipping and singing, with agitated movement such as wing flipping, and 5 = persistent chipping and singing, agitated movement, and aggressive flights at the study skin or playback speaker. Because of the secretive behavior of sparrows, scores and counts for individuals were tallied when possible, and were averaged by number of responding

individuals when not. Behavioral trials were conducted during three time periods throughout the non-breeding season of 2011-2012: November (n = 17), January (n = 19), and March (n = 11). Each trial was conducted in a new location at least 500 m from previous trial locations to avoid repeat testing and habituation with previously exposed flocks.

*Mist-Netting and Blood Sampling*

Sparrows were captured to obtain blood samples in a manner following standard mist-netting and banding techniques (Ralph et al. 1993). A 6-m or 12-m mist net was erected at least 10 m from flocks located within six hours of sunrise or following a behavioral experiment. Mist-nets were located to reduce net visibility to birds, but in likely flight paths throughout habitat. Sparrows were captured passively as well as with the assistance of playback recordings and flushing to help ensure a random sampling of both high- and low-ranking individuals. Nets were left open for 10 min to an hour, depending on weather conditions and capture success, and were monitored continuously. Birds were extracted from the net immediately upon capture, plumage morph was recorded, and blood was collected for genotype, sex, and hormone assays. Samples were taken within 3 min to provide for baseline corticosterone measurements (Angelier 2010). Samples were collected from each bird by extending the wing and puncturing the brachial vein with a sterile 18-gauge syringe needle held at an angle close to parallel to the vein. Approximately 50 to 100 μL of blood was collected from each bird in heparinized capillary tubes and stored on ice. Red blood cells and plasma samples were separated within four hours of collection after centrifuging (5 min x 14,000 rpm) and stored in a -20°C freezer.

*Hormone Assays*

Corticosterone assays were performed with a corticosterone enzyme immunoassay kit (Assay Designs No. 901-097, Ann Arbor, MI). Reagent preparation followed manufacturer's protocols immediately before each assay after reagents were brought to room temperature. Assay buffer 15 concentrate was diluted by pipetting 10 mL concentrate into 90 mL of deionized water. Wash buffer was prepared by diluting 5 mL of concentrate with 95 mL of deionized water. Five corticosterone standards were prepared: 100 μl of 200,000 pg/mL corticosterone standard solution was pipetted into 900 μl of assay buffer 15 and vortexed thoroughly; 200 μl of the vortexed solution was pipetted into 800 μl of assay buffer 15 and vortexed thoroughly; three subsequent dilutions were prepared in an identical fashion by

combining 200 µl of the prior solution with 800 µl of Assay buffer 15. The resulting solutions were used as standard dilutions for comparison with unknown samples (dilution values were 20,000 pg/mL, 4,000 pg/mL, 800 pg/mL, 160 pg/mL and 32 pg/mL, respectively).

Unknown plasma samples were brought to room temperature and vortexed thoroughly before preparation for assay. Manufacturer-provided steroid displacement reagent was diluted to 1:100 by vortexing 5 µl of concentrate in 495 µl of deionized water. Ten microliters of plasma sample was pipetted into 10 µl of diluted steroid displacement reagent, vortexed, and incubated at room temperature for 5 min. After incubation, 180 µl of Assay buffer 15 was added to the solution and vortexed, yielding a 1:40 concentration.

Samples and standards were run in duplicate in room-temperature Donkey anti-sheep IgG microtiter plates. Initial solutions were added to all wells except blank wells and total activity (TA) wells before a period of incubation. Non-specific binding (NSB) wells contained 150 µl of assay buffer 15 and 50 µl of corticosterone EIA conjugate (alkaline phosphatase conjugated with corticosterone). One hundred percent binding (Bo) wells contained 100 µl of Assay buffer 15, 50 µl of conjugate, and 50 µl of corticosterone EIA antibody (sheep polyclonal antibody to corticosterone). Wells assigned to corticosterone standards and samples contained 50 µl of conjugate, 50 µl of antibody, and 100 µl of sample; standard wells used the five dilutions of corticosterone standard, and unknown samples used the solutions of plasma and steroid displacement reagent. The plate was covered with a plate sealer and incubated at room temperature on a plate rocker for 2 hr.

After incubation, the contents of the wells were emptied and washed three times by adding 400 µl of wash buffer to each well and emptying after each wash. After the final wash, 5 µl of conjugate was pipetted into the TA wells, and 200 µl of p-nitrophenyl phosphate substrate solution was pipetted into every well; the plate was covered with a plate sealer and incubated for 1 hr without rocking. After incubation, 50 µl of trisodium phosphate stop solution was added to every well to stop the enzyme reaction.

Immediately after stopping the reaction, the plate's optical density was read at 405 nm in a GENios microplate spectrophotometer (Tecan Inc., Morrisville, NC, USA), using XFluor 4 software (Tecan Inc.). The blank wells' mean optical density was subtracted from all other wells' readings, and the mean values of the corticosterone standards' wells were used to

create a standard curve of percent bound versus corticosterone concentration. The curve was used to calculate unknown samples' mean concentrations from optical densities. Assays were replicated on samples with sufficient volume, but dehydration during freezer storage made many samples inviable. Dehydration could be minimized in future studies by storage in smaller Eppendorf vials, and by processing soon after collection. Kit assay sensitivity was 26.99 pg/mL.

*Genotype Assays*

DNA was extracted from blood with DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA). Samples were combined with proteinase K and phosphate-buffered saline to lyse the nucleated red blood cells. Lysed samples were mixed with salts and ethanol to precipitate DNA, then centrifuged through a filter.

Sex was determined by amplifying the CHD-W and CHD-Z genes on the W and the Z chromosomes, respectively; male birds possess two Z chromosomes (ZZ), whereas female birds have both the Z and W chromosomes (ZW) (Griffiths et al. 1998). Genes were amplified with polymerase chain reaction (PCR) in a thermal cycler using P8 and P2 sexing primers, adapting protocol from Griffiths et al. (1998). Samples were denatured at 94°C for 2 min, and annealed for 30 cycles (45 sec at 58°C, 45 sec at 72°C, and 30 sec at 94°C), with a final run of 1 min at 58°C and 5 min at 72°C. Gel electrophoresis was used to separate the PCR products; 2% agarose gels were run for 45-60 min to separate bands. A single CHD-Z band indicated a male bird, whereas two bands from the CHD-Z and CHD-W genes indicated female.

Plumage morphs of sparrows were determined with a restriction-fragment-length-polymorphism assay developed by Michopolous et al. (2007). Primers amplified the vasoactive intestinal peptide (VIP) gene during the PCR process. Denaturization was set at 94°C for 5 min, followed by annealing for 35 cycles (94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min), finished by 7 min at 72°C. A portion of the PCR product was digested with *Dra*I to determine the presence of the *Dra*I recognition site, and then run in a 2% agarose gel alongside undigested product. A single band in both digested and undigested product indicated a lack of *Dra*I recognition site, occurring in the TS VIP allele; three bands in digested product indicated the presence of that site, occurring only in the WS VIP allele.

*Data Analyses*

A contingency table Pearson's Chi-square test was used to compare the frequency of plumage morphs (WS or TS) visually identified in the field versus by genotyping. Principal components analysis (PCA) was used to create a composite agonism score, based on the linear combination of four agonistic responses (flock averages of aggression scored 0-5, chips, songs, and latency to response). Agonism scores (PC1) were analyzed as the response variable in a fixed-effects model ANOVA, with trial period (November, January, or March) and model morph (TS or WS) as fixed effects, and with a trial period*model morph interaction. Tukey's HSD post-hoc test was used to determine which trial periods differed. Estimated flock size was included as a covariate to account for variation in aggression depending on flock size. Fixed-effects ANOVAs were also used to examine each of the four agonistic responses. I used a contingency table Pearson's Chi-square test to compare the frequency of trials with any level of response by sparrows to trials with no response across the three sampling periods. Intraseasonal changes in baseline corticosterone were analyzed in a fixed-effects model ANOVA; trial period and sampled individuals' plumage morph and sex were fixed effects. Tukey's HSD post-hoc test was used to determine which trial periods differed. All tests were run using SPSS version 20 (IBM Corp., Armonk, NY).

## III. RESULTS

Of 77 White-throated sparrows captured and banded, the color morph of 61 was determined both in the field and by genotyping. Assuming that genotyping is 100% accurate, I accurately identified the morph of 42 white-throated sparrows (68.8%) in the field. Plumage morph frequency was independent of identification method ($\chi^2 = 0.04$, df = 1, n = 120, p = 0.85).

After excluding dehydrated plasma samples, 28 samples yielded corticosterone readings. One of these was excluded as an outlier because of exceedingly high values. Analysis revealed that baseline corticosterone did not differ among seasonal periods ($F_{2, 13} = 0.4$, p = 0.68; Fig. 2).

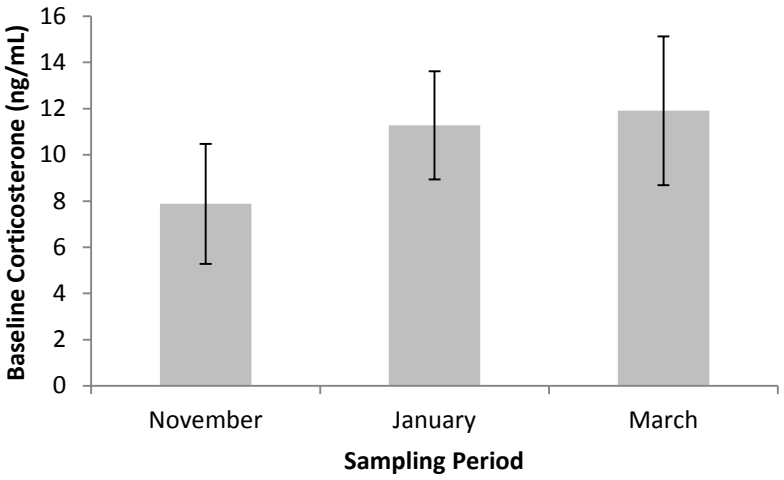


Figure 2. Mean baseline corticosterone levels ± SE (ng/mL) in White-throated sparrows captured in Madison County, Kentucky, 2010-2012.

I conducted 47 behavioral trials during the 2011-2012 non-breeding season. All flocks responded to simulated intrusions in 17 trials in November; there were no visible or audible responses to eight of 19 simulated intrusions in January and to six of 11 simulated intrusions in March. These differences in the percentage of trials with a flock response versus those with no response were significant (Pearson's chi-square test; $\chi^2 = 8.9$, df = 2, n = 47, p = 0.01). Analysis of agonistic responses showed that 59.3% of the variation between the three trial periods was explained by PC1, a composite score of all agonism variables (Table 2).

Table 2. Principal component factor loadings used to analyze non-breeding white-throated sparrows' agonistic responses to simulated intrusions.

| Agonistic Response | PC1 |
|---|---|
| Overall aggression | 0.93 |
| Song vocalizations | 0.70 |
| Chip vocalizations | 0.59 |
| Latency to response | -0.81 |

Overall response scores were significantly higher in November than in January and March and were not affected by either trial model morph or flock size (trial period main effect: $F_{2, 38} = 17.0$, $p < 0.01$; model main effect: $F_{2,38} = 0.4$, $p = 0.65$; flock size effect: $F_{1, 38} = 3.4$, $p = 0.07$; trial period*model interaction: $F_{2, 38} = 0.66$, $p = 0.52$). Post-hoc comparisons using the Tukey HSD test revealed that November response scores were higher than January ($q_{39} = 5.7$, $p < 0.01$) and March ($q_{39} = 5.2$, $p < 0.01$) scores, and January and March scores did not differ ($q_{39} = 0.2$, $p = 0.98$). Vocal responses followed the same pattern; flock members chipped more in response to intruders of both morphs in November than in later trial periods (trial period main effect: $F_{2, 39} = 8.8$, $p = 0.001$; model main effect: $F_{2, 39} = 0.1$, $p = 0.89$; flock size effect: $F_{1, 39} = 2.4$, $p = 0.13$; trial period*model interaction: $F_{2, 39} = 0.9$, $p = 0.40$; Fig. 3a), and also sang more (trial period main effect: $F_{2, 39} = 4.1$, $p = 0.02$; model main effect: $F_{2, 39} = 0.3$, $p = 0.73$; flock size effect: $F_{1, 39} = 0.2$, $p = 0.63$; trial period*model interaction: $F_{2, 39} = 3.4$, $p = 0.86$; Fig. 3b). Scores based on vocal and physical agitation similarly decreased between November and later trials (trial period main effect: $F_{2, 39} = 15.2$, $p < 0.01$; model main effect: $F_{2, 39} = 0.1$, $p = 0.92$; trial period*model interaction: $F_{2, 39} = 1.0$, $p = 0.37$; Fig. 3c). Latency to response increased throughout the non-breeding season, with November's response time significantly shorter than later trial periods (trial period main effect: $F_{2, 38} = 7.6$, $p = 0.002$; model main effect: $F_{2, 38} = 2.0$, $p = 0.16$; flock size effect: $F_{1, 38} = 3.0$, $p = 0.09$; trial period*model interaction: $F_{2, 38} = 0.4$, $p = 0.71$; Fig. 3d). Post-hoc comparisons with Tukey HSD showed that latency to response was shorter in November than in January ($q_{38} = -2.6$, $p = 0.03$) and March ($q_{38} = -3.9$, $p = 0.001$), but January and March response times did not differ ($q_{38} = -1.6$, $p = 0.25$).

## IV. DISCUSSION

My ability to accurately identify plumage morphs (68.8%) was lower than reported by Piper and Wiley (1990; 89%). Piper and Wiley (1990) created a formula that included age, sex, and crown brightness to identify white-striped and tan-striped morphs, but I did not use their formula in my study. My results indicate that white-throated sparrows with intermediate plumage are difficult to identify in winter, so incorrect identifications are to be expected. Previous studies of WS versus TS behaviors that purportedly demonstrate no

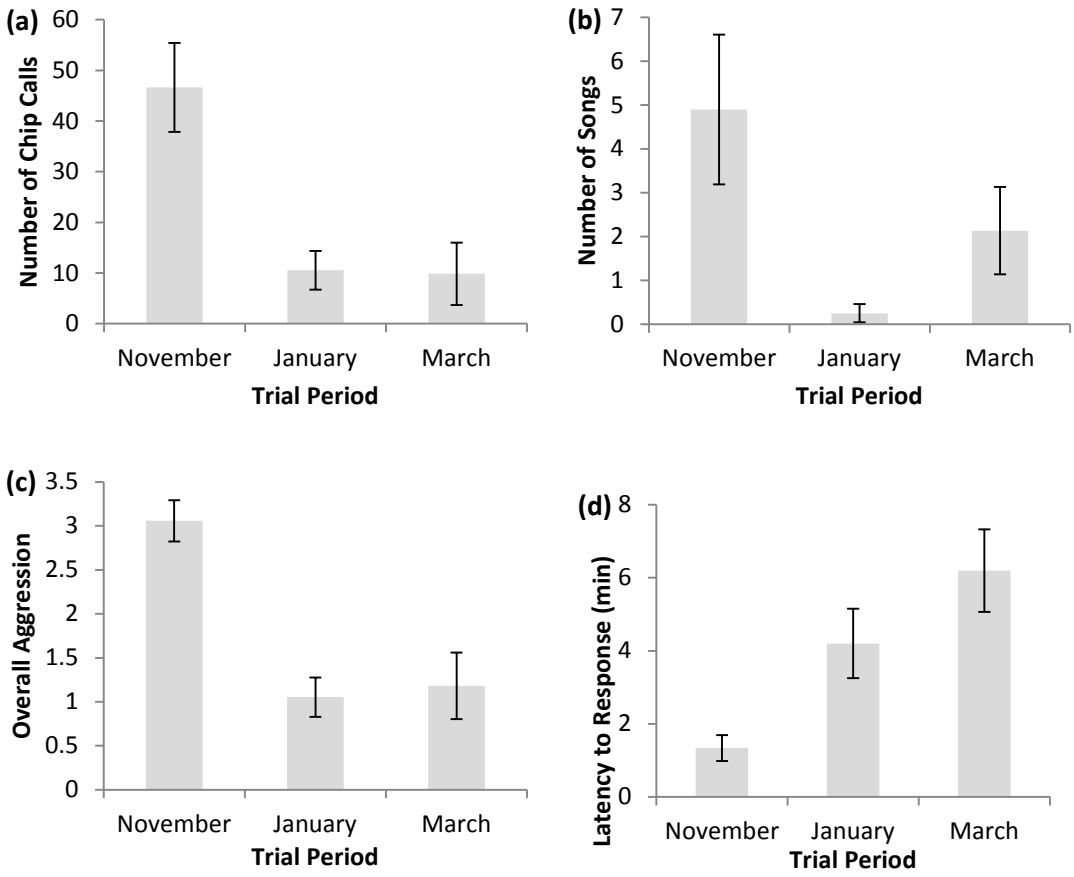


Figure 3. Behavioral responses of white-throated sparrows to simulated territorial intrusions (STIs) during the non-breeding season, averaged per individual per flock. (a-b) Vocal responses to intruders in November (n = 17), January (n = 19), and March (n = 11). (c) Overall aggression towards STIs and (d) latency to response in November (n = 17), January (n = 19), and March (n = 11). Values are means ± SE.

dominance differences between morphs in the winter season are potentially inaccurate if identification was not verified with genotyping (Schwabl et al. 1988, Piper and Wiley 1989), and the availability of the recently developed genotype assay will assist contemporary and future studies (Michopoulos et al. 2007).

Although based on a small sample size, baseline corticosterone levels of white-throated sparrows in my study did not change during the non-breeding season. Other investigators have examined the influence of dominance status on the corticosterone levels of white-throated sparrows during the non-breeding season. Schwabl et al. (1988) found that corticosterone levels varied with dominance rank, with lower-ranking HY females having higher baseline levels than higher-ranking sparrows of either sex. This difference may have been due to the need by lower-ranking HY females to forage further from cover (Schwabl et

al. 1988). In that study, captive birds were sampled from September through December, but possible variation in corticosterone levels of during the sampling period was not examined. Marra et al. (1995) examined baseline corticosterone levels of captive white-throated sparrows between 4 and 6 March, and found that the daily mean level was 16.45 ± 2.9 ng/mL, which was higher than the highest mean in March in my study (11.91 ± 3.2 ng/mL). The mean corticosterone level of free-living resident sparrows captured between 25 February and 4 March was 6.68 ± 1.0 ng/mL (Marra et al. 1995), which was comparable to that of birds sampled in November in my study (7.88 ± 2.6 ng/mL). Marra et al. (1995) cautioned that data from birds under one set of living conditions cannot be extrapolated to others, and, based on a comparison with my study, a similar caution might be applied to data collected from different times of year. Baseline corticosterone levels may have shown no variation because the aggressive interactions experienced in November are not sufficiently stressful to cause the release of energy-mobilizing hormones into the bloodstream. White-throated sparrows may instead store corticosterone in other organs, as song sparrows do (Newman and Soma 2011), for short-term energy and locomotor boosts. I only sampled plasma so do not have data to confirm or reject that hypothesis. My results do suggest, however, that periods during the non-breeding season with greater levels of agonism among white-throated sparrows (i.e., November) do not necessitate a chronic increase in either plasma corticosterone levels or energy expenditure.

I found that agonistic responses of white-throated sparrows were significantly greater in November than in January and March. Similarly, Schlinger (1987) reported that increased aggression between pairs of white-throated sparrows competing over food sources in November was correlated with increased levels of circulating androgens. This change in behavior suggests that aggressive displays toward unfamiliar conspecifics are strongest at a time of year when flock territories and dominance hierarchies within flocks are being established. Similarly, plasma androgen levels were higher in house sparrows (*Passer domesticus*) when hierarchies were being formed and decreased thereafter (Hegner and Wingfield 1987). Reduced aggression after hierarchy establishment may also be valuable in reducing predation risk. For example, Dunn et al. (2004) reported that response times to the appearance of a predator were longer when European robins (*Erithacus rubecula*) were defending winter territories against conspecific intruders. This change in the behavior of white-throated sparrows during the non-breeding season suggests that such changes are

not limited to transitions between breeding and non-breeding seasons, but can also occur within seasons.

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# APPENDIX


Figure A-1. Telemetry locations and core (50%) and 95% home ranges for a white-throated sparrow tracked from January to March 2011 at Blue Grass Army Depot, Madison County, Kentucky.

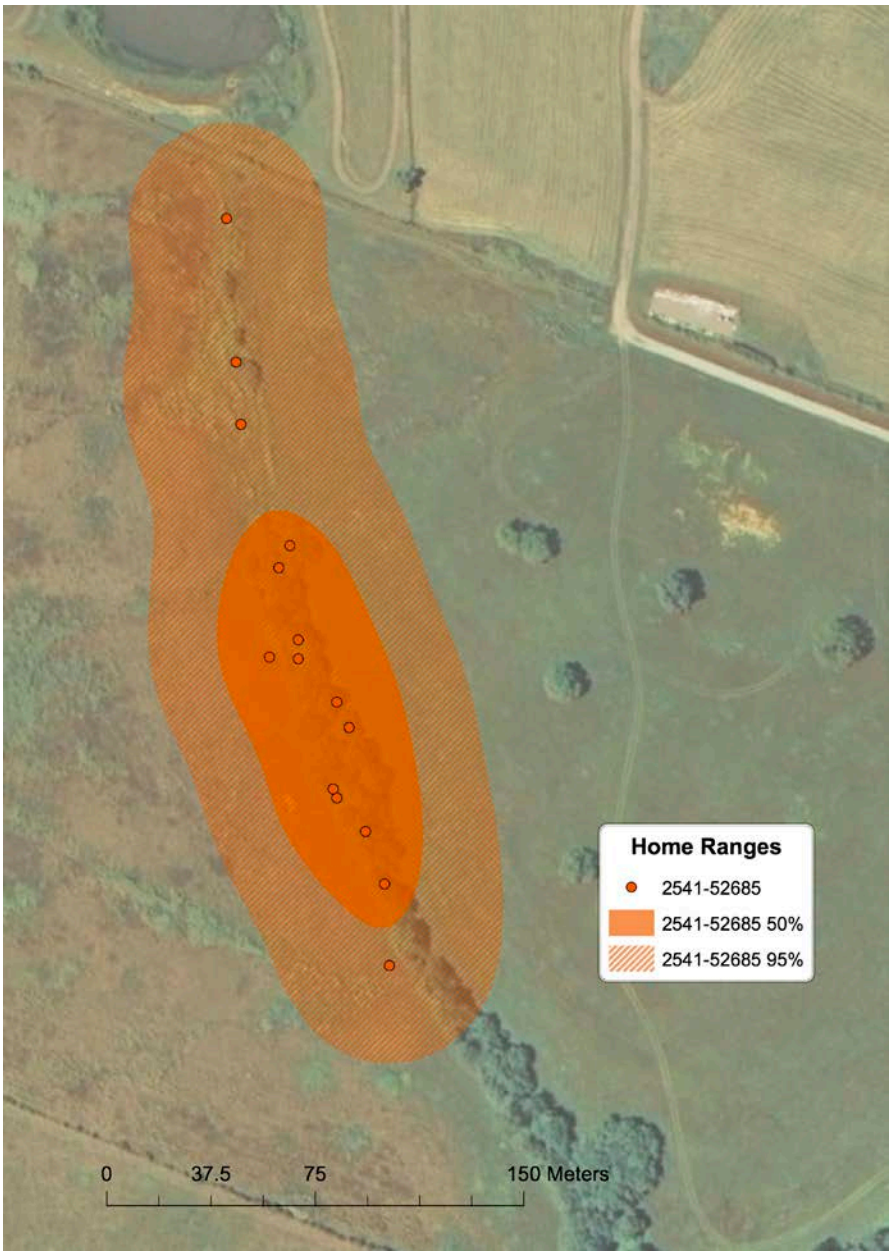


Figure A-2. Telemetry locations and core and 95% home ranges for a white-throated sparrow tracked during November and December 2010 at Taylor Fork Ecological Area, Madison County, Kentucky.

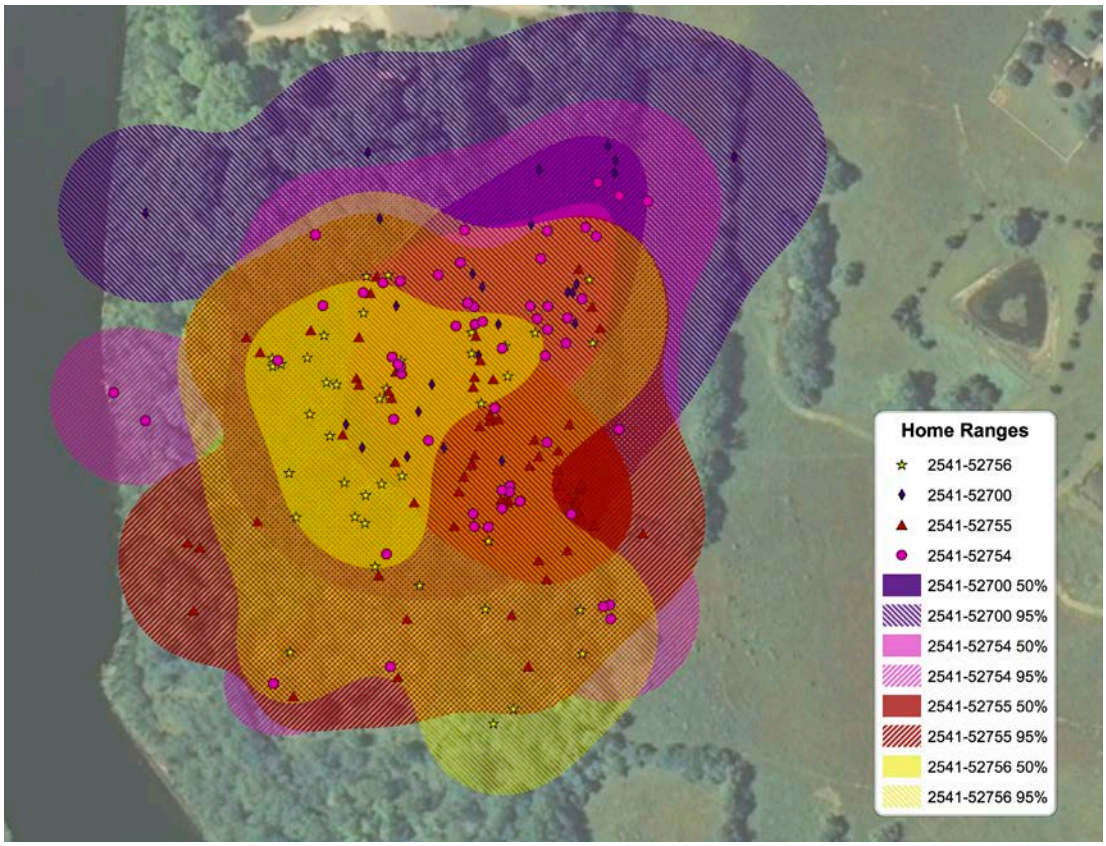


Figure A-3. Telemetry locations and core and 95% home ranges for four white-throated sparrows tracked from January to April 2011 at Camp Catalpa, Madison County, Kentucky.

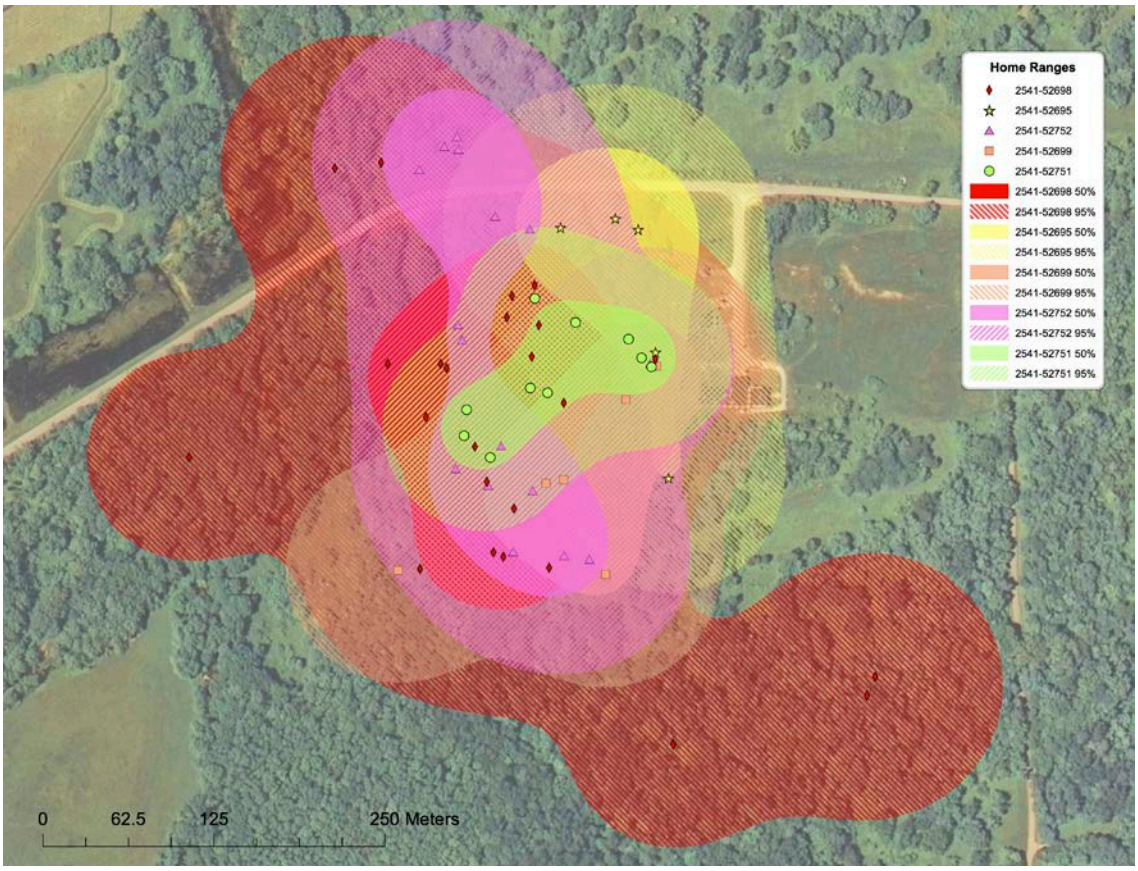


Figure A-4. Telemetry locations and core and 95% home ranges for five white-throated sparrows tracked during January and February 2011 at the Blue Grass Army Depot, Madison County, Kentucky.