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## Towards understanding the interactions between Ospreys and human-made structures in the Tennessee River Valley

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Towards understanding the interactions between Ospreys and human-made structures in the  
Tennessee River Valley

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for the Degree of Doctor of Philosophy  
in Wildlife, Fisheries and Aquaculture  
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Raptor nests on human-built structures represent a significant source of conflict as they can result in bird mortality, fires, structure damage, service distribution, or power outages when falling nest materials or animals connect with energized conductors. Power companies, such as the Tennessee Valley Authority (TVA), wish to mitigate these conflicts to avoid service disruptions. In this dissertation, I present my work towards understanding and mitigating the interactions between Ospreys (*Pandion haliaetus*) and human-made structures. To achieve this, I explored multiple elements of conflict identification, monitoring, and basic ecology of the target species to better inform conflict mitigation. In Chapter I, I modelled the influence of suitable habitat and transmission infrastructure distribution on the potential risk of nest-infrastructure conflict within the TVA power service area. My integrative model would be most useful to utilities when adjacent (i.e., within 10 km) areas to ‘risky’ zones are also considered when looking at mitigation efforts and pre-development planning. Going forward, similar models for other species and for distribution structures may benefit utilities such as TVA. In Chapter II, I aimed to identify the most suitable nest monitoring technique for Ospreys, as nest information is required to make informed decisions for conflict mitigation (e.g., nest removal). I found that

unoccupied aircraft systems, or drones, are less disturbing and more time-efficient than other methods tested. In the last two chapters, I focused on obtaining detailed information on the basic ecology of Ospreys in the southeastern United States, which represent an understudied population. My findings in Chapter III contribute to general migration ecology theory, identify key stopover areas for Ospreys in Florida, provide valuable comparisons for further investigations, highlight areas for future research, and identify possible metrics relevant to conservation and management action. Lastly, I quantified the genetic diversity and structure of southeastern Ospreys to generate hypotheses for future testing and provide valuable comparisons for further investigations. While gene flow appears to be high and sampled regions show little genetic differentiation, more research is required to elucidate patterns of cryptic population structure and provide concrete implications for management decisions (e.g., source population for translocation programs).

## DEDICATION

I dedicate this work to my partner, Jake Mulholland, for years of steadfast support.

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CHAPTER I

IDENTIFYING PRIOROTY MITIGATION AREAS FOR HUMAN-OSPREY CONFLICT  
USING HABITAT SUITABILITY AND TRANSMISSION INFRASTRUCTURE  
INFORMATION

**Abstract**

Raptor nests on human-built structures represent a significant source of conflict as they can result in bird mortality, fires, or power outages due to falling nest materials or animals connecting with energized conductors. Power companies typically try to mitigate these conflicts to avoid service disruptions. However, performing mitigation measures across all potentially problematic power infrastructure is generally not a practical solution given logistical, time, or budgetary constraints. Therefore, there is a need for quantitative, landscape-scale tools to identify conflict risk and prioritize mitigation. I examined the influence of suitable habitat and transmission infrastructure distribution on the potential risk of Osprey (*Pandion haliaetus*) nesting-infrastructure conflict within the Tennessee Valley Authority (TVA) power service area. I modelled risk using a three-stage approach which intersected a nesting habitat suitability model and density of infrastructure to evaluate the potential risk of conflict. Habitat suitability was greater on or near open water and closer to developed areas with 10% of the TVA service area being highly suitable for breeding Ospreys. Transmission line density was low ( $0.14 \pm 0.29$  lines/km<sup>2</sup>) and heterogenous across the service area with more lines within urban centers and near power facilities. Integrating habitat suitability and transmission infrastructure information

revealed that very low and low-risk areas comprised 99% of the service area. Less than 1% comprised 1113.7 km<sup>2</sup> of moderate and 82.1 km<sup>2</sup> of high- or very high-risk, mostly concentrated along major rivers, lakes, and around urban centers. Risk was more prevalent in the northeastern portion of the service area. This work presents a top-down approach to mitigating Osprey-power infrastructure conflict, allowing for information about potential conflict risk across a given power service area to help inform the prioritization of mitigation actions and facilitate long-term coexistence with this protected species.

## **Introduction**

Human-built structures represent a source of anthropogenically-driven avian mortality (Martin, 2011). Power infrastructure, fences, offshore platforms, wind-turbines, and buildings have long been recognized as threats to a variety of bird species (Allen and Ramirez, 1990; Loss et al., 2015). For instance, up to 1.5 billion bird mortalities are annually attributed to collisions with such structures in the United States alone (Loss et al., 2015). Differences in avian behavior (Smallwood et al., 2010) and morphology (Janss, 2000; Bernardino et al., 2018), migration ecology (van Doren et al., 2021), structure type and placement (Klem et al., 2004; APLIC, 2006, 2012), surrounding landscape composition (Rollan et al., 2010; Mojica et al., 2018), and weather (Loss et al., 2020) can all influence avian susceptibility to various infrastructure-based threats.

Growing human populations and urbanization has led to the expansion of developed areas and an increased demand for power infrastructure. Globally, electricity demand has, on average, been increasing by >2% per year from 2015 – 2019 (IEA, 2022). In the United States, the national electric grid consists of >321,860 km of high-voltage transmission lines and approximately 8.8 million km of local distribution lines (U.S. Dept. of Energy, 2019). These power structures are a known source of avian mortality, linked to an estimated 12 – 64 million

bird mortalities (8 – 57 million by collision, 0.9 – 11.6 million by electrocution) annually (APLIC, 2006; Loss et al., 2014). Avian mortality caused specifically by nest-related conflicts on power infrastructure is rarely quantified; however, the use of power structures for nesting is well-documented (Washburn et al., 2014; Taylor et al., 2017). Nevertheless, nests on infrastructure can generate conflict with utility reliability and increased maintenance needs. For instance, nests may result in fires when debris falls on energized conductors or cause flashovers (i.e., unintended high voltage electric discharge) when large nests become wet (Brown and Lawson, 1989; APLIC, 2006). Further, the build-up of avian excrement can reduce the ability of insulators to prevent flashovers and reduce reliability of power infrastructure (APLIC, 2006; Washburn, 2014). For this reason, nests that are built on precarious locations often require management and mitigation by utility personnel.

Mitigation measures, such as installing nest deterrents (APLIC, 2006, 2012) or alternative nesting platforms (Guill and Forys, 2020; Parayko et al., 2021), to mitigate conflict with avian species across all potentially problematic power infrastructure is generally not affordable or practical. Nest removals for problematic nests (following permitting requirements set forth by federal and state governments) are often reactive responses to power outages or other safety-related issues (Slankard et al., 2020). Overall, nest removals and mitigation can present a challenge to utility companies. Therefore, there is a need for quantitative, landscape-scale tools to identify risk and to prioritize mitigation for high-risk areas (e.g., Pérez-García et al., 2017; Bedrosian et al., 2020; Gauld et al., 2022). This is particularly valuable in cases where further surveys to identify precise high-risk structures or exact nest conflicts are desirable, or where the distribution of a problem species varies across the landscape (Bedrosian et al., 2020). Accurate prediction of potential risk hotspots combines multiple types of information, including the

distribution of the problem species, the distribution of risk, and the occurrence of risk events (Pérez-García et al., 2017). Such quantification of potential risk can aid power utilities in proactively mitigating conflict between infrastructure and nesting birds, as many species that nest on infrastructure in the United States – such as a variety of corvids, woodpeckers, and raptors – are protected by the Migratory Bird Treaty Act (16 United States Code 703-71) and other state and federal law.

Ospreys (*Pandion haliaetus*) are widespread raptors, with North American populations having experienced an estimated 50 – 99% increase over the last 50 years (Pardieck et al., 2019). This rebound is primarily a result of improved environmental policy (e.g., banning of dichlorodiphenyltrichloroethane, DDT) and direct efforts to increase Osprey abundance (e.g., construction of nest platforms, translocations; Poole, 2019). Artificial structures, whether designed for Osprey use or not, are readily used by these raptors. Such structures are attractive to Ospreys because they are less likely to be climbed by a mammalian predator or destroyed by adverse weather, and, in treeless areas, tall human-made structures provide maximum visibility to minimize competition and predation (Vana-Miller, 1987; Poole, 2019). As Osprey populations increase and continue to exploit human infrastructure, there is an increasing potential for conflict. Typically, nests located on distribution poles or transmission towers are left in place unless they pose a risk (Liguori, 2009), such as a fire or power outage. However, there is currently little understanding on the spatial distribution of this risk for nesting Ospreys, which makes it difficult for utilities to proactively allocate resources to reduce and/or mitigate such risk. These considerations are particularly relevant in the pre-development stage of utility construction, where a lack of information on spatial distribution of Ospreys and associated risk may result in conflict post-construction.

Here, I examined the influence of broad-scale landscape attributes (i.e., suitable habitat and transmission line distribution) on the potential risk of Ospreys nesting and the associated conflict with utility infrastructure. I predicted that interactions between high transmission infrastructure density and high habitat suitability would lead to increased potential for conflict. By developing a top-down approach to mitigating Osprey-transmission infrastructure conflict, I aimed to generate an effective method of prioritizing mitigation action and facilitating long-term coexistence between utility distribution and Ospreys.

## **Methods**

### **Study area**

My study area encompassed the Tennessee Valley Authority (TVA) power service area, located in the southeastern United States. The service area spans 220 counties in Tennessee, Alabama, Georgia, Kentucky, Mississippi, North Carolina, and Virginia (approx. 265,860 km<sup>2</sup>; Figure 1.1). TVA operates five fossil plants, three nuclear plants, 29 hydro plants, one pumped storage hydroelectric plant, nine natural gas combustion turbine plants, eight natural gas combined cycle gas plants, one diesel generator site, and 14 solar energy sites, with another approx. 4000 small-scale power generating facilities contracted by TVA in the region.

Within this area Ospreys are migratory and typically found near freshwater waterways, lakes, and reservoirs during the breeding season (March – August; Bierregaard et al., 2020) given their diet consists almost entirely of fish. Ospreys can be found nesting on a variety of structures, including live and dead trees, stumps, power infrastructure, channel markers, lock and dam infrastructure, and communication towers. My study area is within the eastern temperate forest ecoregion (for detailed descriptions of flora and fauna see CEC, 1997) and is mostly defined by a humid subtropical climate with average summer (June – August) and winter

(November – January) temperatures ranges between 24 – 25.8 °C and 3.5 – 8.5 °C respectively (NOAA, 2020). Annual precipitation ranges from 129 cm in central Mississippi to 132 cm in central Kentucky (NOAA, 2020), and elevation across the service area ranges from 0 – 2,030 m above sea level (USGS, 2019).

### **Habitat suitability**

I built a habitat suitability model for nesting Osprey using an ensemble modelling approach (Araújo and New, 2007) that related Osprey nesting locations and environmental predictor variables presumed to influence the spatial distribution of breeding Ospreys. To identify nesting locations, I compiled 3,007,775 unique GPS locations for Osprey sightings within all parts of the states served by TVA from eBird (1932 – 2020; Sullivan et al., 2009) and an additional 13 confirmed nests from TVA records for the service area that were collected in 2019. Observations from eBird were then limited to the TVA service area, the Osprey breeding season (March – August), and to those observations that reported breeding codes (i.e., occupied nest, nest with young, or nest with eggs) resulting in 11,134 independent nesting locations identified between 1990 – 2020 (Figure A.1; Table A.1 for list of breeding codes included).

The environmental predictor variables used included: distance to seven landcover categories derived from the National Landcover Database (Dewitz, 2019; Table S2); open water (Vana-Miller, 1987); forest (Ewins, 1997; Bei et al., 2009); grasslands; cropland (Bei et al., 2009); wetlands (Vana-Miller, 1987); anthropogenic development (Martell et al., 2002; Washburn, 2014; Forys et al., 2021); and shrubland. I also included mean wind speed at 50 m above ground level (Global Wind Atlas 3.0, 2019), as wind speed can affect ability to forage and, thus, breeding site selection (Machmer and Ydenberg, 1990; Poole, 2019). Elevation and slope were included due to their variable influence on nest site selection and as water availability

varies with these landscape features (Henny et al., 1978; van Daele and van Daele, 1982).

Although I made inferences at the level of the TVA service area, all variables were obtained at a 30-m resolution and, due to the extensive range of this study, all occurrences and environmental variables were projected into a North America Albers Equal-Area Projection.

Given that absences of the target species are required but rarely recorded for distribution modelling, I used a 1:1 ratio to generate the same number of pseudo-absences to presences. Pseudo-absences were not located in cells with presences and were placed randomly across the service area to adequately sample the background environment (Vanderwal et al., 2009; Barbet-Massin et al., 2012). For each known occurrence and pseudo-absence location, I then extracted values associated with each of the ten environmental variables. Following evaluation using *stats* R package (*cor* function; R Core Team, 2022), I removed distance to shrublands from analysis due to high ( $r > 0.7$ ) correlation with distance to forest cover (Pearson's correlation coefficient,  $r = 0.72$ ). I then ran three distribution models, each of which was performed using 80% of locations and then checked using the remaining 20%, repeated five times for 5-fold validation (Dunford et al., 2014). My three approaches included: a maximum entropy model (Maxent; Phillips et al., 2006; R package *dismo* Hijmans et al., 2011; *rJava*, Urbanek, 2019); a gradient boosting model (GBM; R package *gbm*, Greenwell et al., 2019); and a generalized linear model (GLM; logistic regression). I ran each model using default settings and no interactions between scaled predictors. For the GBM, I first performed tuning before the final model was run (tree optimum = 4,450 trees; Elith and Leathwick 2017). Each of the three models were used to predict habitat suitability as a range of suitability values from 0 (not suitable) to 1 (highly suitable) across the entire service area. Continuous model maps (values ranging from 0 to 1) were transformed to a binary format (0 = absence, 1 = presence) using a likelihood threshold that



maximized model sensitivity plus specificity (Liu et al., 2013). I created binary maps for each individual model and used raster math to calculate the number of cells that were in agreement in ArcMap™ v. 10.7.1.

I then assessed model performance using three measures (Gantchoff et al., 2019; Boudreau et al., 2022): the area under the curve of a receiver operating characteristic plot (AUC-ROC); the true skill statistic (TSS; calculated as sensitivity + specificity - 1) using the *ROCR* R package (Sing et al., 2005); and the continuous Boyce Index (Hirzel et al., 2006) using the *modEvA* R package (Barbosa et al., 2013). Values for AUC-ROC range from 0 – 1, where 1 is perfect alignment and a value of 0.5 is equivalent to random predictions (Allouche et al., 2006). Values for TSS and the Boyce index range from 1 to -1, with a value of 1 indicating perfect agreement and values  $\leq 0$  representing random predictions (Allouche et al., 2006; Hirzel et al., 2006). I used the outputs of these three models, along with their AUC-ROC, to build an ensemble habitat suitability model (Araújo and New, 2007) using proportional AUC-ROC weight-averaging, a technique that reduces the uncertainty associated with individual models (Marmion et al., 2009; Dormann et al., 2018). My continuous ensemble model map (values ranging from 0 to 1) was also transformed to a binary format using a likelihood threshold that maximized model sensitivity plus specificity (Liu et al., 2013).

### **Transmission infrastructure density**

I used a power transmission line shapefile from the Homeland Infrastructure Foundation Level Database (HIFLD, 2020) to create a transmission infrastructure density layer for the TVA service area. This layer represented high voltage (69 kV – 765 kV) transmission structures within the United States. Then I excluded underground transmission lines and cropped the layer to the TVA service area, leaving 122,669 km of overhead line. As TVA operates approx. 26,070 km of

transmission line in the region (Tennessee Valley Authority, 2022), these data represent TVA lines and other regional transmission lines. Given that Ospreys nest on power poles and rarely interact with lines, I verified that transmission lines were an accurate representation of power pole distribution and density by manually identifying poles along 1 km of transmission lines for each of the transmission line bins (see risk assessment below) in Google Earth Pro (v. 7.3.6). Power line density can be correlated with structural complexity due to multiple connecting service lines, suggesting that high line density can also be correlated with additional nesting structures (i.e., power poles) and increased hazard (Dwyer et al., 2016; Bedrosian et al., 2020).

Movement data from 19 GPS-GSM tagged Ospreys (females = 15, males = 4; standardized fix rate of 15 minutes during the breeding season only) from within the TVA survey area indicated that the average daily distance travelled by Ospreys from their nests was 2 km (see Chapter III, this document). Therefore, I used this radius when calculating transmission infrastructure density (number of lines per km<sup>2</sup>) in ArcGIS, as this was the likely scale of influence for nesting birds in the TVA service area. Resulting transmission line density was at a 30-m resolution and scaled as a value from 0 – 1 to match the habitat suitability prediction.

### **Nesting conflict risk assessment**

Following the quantitative risk assessment framework used by Bedrosian et al. (2020), I summarized risk as an interaction between hazard and exposure, whereby hazard was the negative interaction between nesting Ospreys and power infrastructure (i.e., with transmission line density as a proxy for this hazard) and exposure was the degree of opportunity to encounter hazards (i.e., as reflected by the amount of suitable habitat for nesting Ospreys). I quantified risk as the interaction between exposure and hazard whereby the riskiest areas had both high exposure (i.e., Osprey nesting suitability) and high hazard (i.e., transmission line density). To

rank risk in relation to increasing suitability or line density, I first reclassified nesting suitability and line density (both with values from 0 – 1) into five equal bins (i.e., bin 1 contained values from 0 – 0.2, bin 2 values 0.2 – 0.4, and so on), with each raster pixel in the TVA service area being assigned an exposure value (ranking after binning; 1 – 5) and a hazard value (1 – 5). Thus, I created a risk matrix representing all possible combinations of exposure and hazard values (Tack and Fedy, 2015): (1) very low-risk included matrix cells that contained 1 on either axis; (2) low-risk included matrix cells with 2 as the lowest bin on either axis; (3) moderate-risk included matrix cells with 3 as the lowest bin on either axis; (4) high-risk included matrix cells with a 4 as the lowest bin on either axis; and (5) very high-risk was the single matrix cell with 5 on both axes. Area (km<sup>2</sup>) and percentage of the TVA service area within each of the exposure-by-hazard combinations, and within each of the five risk categories, was then calculated.

## **Model evaluation**

I used nest survey data (n = 277 nest records) collected by the Kentucky Department of Fish and Wildlife Resources (Taylor et al., 2017) to independently evaluate the habitat suitability model (i.e., the proxy for exposure). I compared nest data used in modelling and the independent nest observations by extracting values from the predicted habitat suitability model for both datasets. For both nest datasets, I then placed nests into five previously derived bins, and then divided the total number of nests in each bin by the amount of total area for that bin (i.e., area-adjusted frequencies; see Boyce et al., 2002). Nest density frequencies across bins for each dataset were compared using Spearman's rank correlation and Cohen's d. For Cohen's d, effect sizes are thought to be small when  $\leq 0.4$  and large when  $\geq 0.8$  (Cohen, 1988).

To independently evaluate risk, I used locations of known conflicts (n = 48; i.e., nest removals, nest fires, power outages, power interruptions) during the breeding season collected by

TVA and the U.S. Department of Agriculture Animal and Plant Health Inspection Service - Wildlife Services. From the area values previously calculated for each of the exposure-by-hazard combinations, I then calculated the proportion of the total area in each combination (i.e., each bin of habitat suitability, of transmission line density, and risk). Under the null hypothesis that conflicts were distributed proportional to area within the service area, I multiplied this proportion by the number of known conflicts (i.e., 48) to estimate the number of expected conflicts within each combination (per Bedrosian et al., 2020). Given that multiple bins had no conflicts, which does not allow for the computation of correlation coefficients, I dropped those bins, leaving me with 12 bins for comparison out of an original 25. Expected and observed conflicts in each bin were compared using Spearman's rank correlation and Cohen's d. If conflicts fell outside of moderate-, high- and very high-risk areas, I further quantified the number of conflicts within 10 km of these areas, which is one-half the maximum distance an Osprey may nest from the nearest water source (Greene et al., 1983; Hagen and Walters, 1990; Bierregaard et al., 2020).

## **Results**

I found that all three Osprey nesting habitat suitability models had strong predictive performance with AUC-ROC, TSS, and Boyce Index values being 0.92, 0.41, and 0.97 for the Maxent, 0.97, 0.50, and 0.99 for the GBM, and 0.90, 0.41 and 0.97 for the GLM model, respectively. Variables most influential to breeding Osprey were distance to open water and development, with breeding Osprey having a higher likelihood of occurrence on or near open water and closer to developed areas (Table 1.1, Figure A.2). Mean windspeed influenced habitat suitability only for the GLM, suggesting Ospreys had a higher likelihood of occurrence when windspeeds were <10 m/s (Table 1.1, Figure A.2). Although elevation and slope were in the top rankings in some models, they had little influence on Osprey occurrence (Table 1.1). Distance to

forest cover, grasslands, crops, and wetlands did not influence Osprey distribution across all three models (Table 1.1). Using likelihood thresholds (Maxent = 0.27; GBM = 0.52; GLM = 0.58), I created binary maps from the three individual models which showed <12% disagreement (Figure A.3).

Proportional AUC-ROC weights were 0.33, 0.34, and 0.32, for the Maxent, GBM, and GLM, respectively. When the three models were combined for the ensemble, the final model (Figure 1.2A) had an AUC-ROC score of 0.97, a TSS score of 0.49, and a Boyce Index of 0.99. Approximately 10% (or 24,010 km<sup>2</sup>) of the TVA service area appeared to be highly suitable for Ospreys (suitable area above the likelihood threshold of 0.512 for the ensemble model; Figure 1.2B). Within areas of suitability, distance to development and open water ranged from 0 – 7.8 km (mean = 217.6 m) and 0 – 4.2 km (mean = 169.9 m), respectively, compared to 0 – 10.1 km (mean = 1.1 km) and 0 – 11.6 (mean = 344.6 m) across the entire service area. Windspeed ranged from 1.1 – 12.2 m/s (mean = 4.9 m/s) within areas of suitability and 0.8 – 15.4 m/s (mean = 5.0 m/s) across the entire service area. My model evaluation indicated that bins for predicted model values and observed nests from the independent dataset were similar (Spearman's rank,  $r_s = 1$ ,  $P = 0.02$ ; Figure A.4) with a small effect size ( $d = 0.4$ ), suggesting good model predictive performance.

Transmission line density ( $0.14 \pm 0.29$  lines/km<sup>2</sup>; range = 0 – 5.8 lines/km<sup>2</sup>) was not homogenous across the TVA service area (Figure 1.2C), with density being highest in urban centers and developed areas. In the TVA service area, transmission lines appear to be an accurate representation of pole distribution, as pole density in urban areas was also highest in urban areas (Figure A.5). Given habitat suitability and transmission line density was so variable, conflict risk was also unevenly distributed across the landscape (Figure 1.3). Overall, very low-risk areas

dominated and were broadly distributed, comprising 99% (263,507.8 km<sup>2</sup>) of the TVA service area while areas of low- (1,157 km<sup>2</sup>), moderate- (1,114 km<sup>2</sup>), high- (76 km<sup>2</sup>) and very high-risk (6 km<sup>2</sup>) represented approx. 1% (Figure 1.3). Moderate-, high-, and very high-risk areas were concentrated along major rivers, lakes, and around urban centers (Figure 1.3). Risk was also more prevalent in the northeastern part of the service area (Figure 1.3). My evaluation exercise indicated that expected and observed conflicts were highly correlated (Spearman's rank,  $r_s = 0.91$ ,  $P < 0.01$ ; Figure A.6) with a small effect size ( $d = 0.001$ ), suggesting conflicts were distributed proportional to area within the service area. The number of conflicts located within 10 km of moderate-, high- and very high-risk areas was 43 (i.e., 89.6%) and the proportion of area within 10 km of risk was 28.6% of the service area (75,992 km<sup>2</sup>).

## **Discussion**

Risk of negative interactions between Ospreys and transmission infrastructure appears low throughout much of the TVA service area. However, hotspots of risk occurred, including the northeast (Figure 1.3), a region that hosts numerous fossil, renewable, and nuclear facilities in addition to highly populated urban centers (i.e., Knoxville, Tennessee) and suitable Osprey habitat in the form of dammed river systems. While high-risk hotspots appear limited to localized areas of high infrastructure density, this study does not suggest that the risk of potential conflict is nonexistent throughout the rest of the service area. As Ospreys are known to nest up to 20 km (Greene et al., 1983; Hagen and Walters, 1990; Bierregaard et al., 2020) from the nearest water source, vigilance may be required on any suitable nesting structure within these distances from lentic systems. This is validated by my conflict information, as the conflict distribution was almost identical to what would be expected under the null expectation of conflicts being distributed by area of each risk level. While this is not what I expected, when I examined the

spatial distribution of observed conflicts, they are predominately clustered around waterbodies near urban spaces, and while a low number of these conflicts fell within the comparatively small area comprising moderate- to very high-risk areas, the remainder were adjacent to areas (87.5% within 10 km; 5.8 km average across all conflicts outside these areas) identified as moderate- to very high-risk. Due to this, I again stress that the risk of potential conflict is not constrained to the areas identified as ‘risky,’ but that adjacent areas also be considered when looking at mitigation efforts. I also do not know the exact impact of being in these areas to the birds themselves. While records of Osprey electrocutions remain infrequent (e.g., Ospreys comprised <1% of confirmed raptor electrocutions in the US between 1986 – 1996 and 3.8% between 2000 – 2015; Harness and Wilson, 2001; Kagen, 2016), there is little documentation on how other aspects related to these structures contribute to Osprey mortality, such as collisions with overhead wires or mortality from nest fires. Finally, the narrow distribution of risk suggests that more localized risk models (e.g., models for a specific coastal or lake-side city or watershed) may be beneficial. I also recommend periodically reassessing risk as both Osprey populations and infrastructure density continue to increase.

Ospreys’ association with human development is unsurprising, given their common use of man-made structures for nesting and the concentration development near water across the service area (Martell et al., 2002; Washburn, 2014). Human infrastructure, such as communication towers and utility poles, provide tall, stable nest sites that are far above human disturbances, and easily defensible from predators, resulting in nests that are productive contributors to the population (Vana-Miller, 1987; Bierregaard et al., 2020; Barnes et al. in press). Association with water and low wind speeds is also unsurprising given known Osprey ecology. Since Ospreys hunt by plunging into the water and their diet consists almost entirely of

fish, they are closely associated with lakes and rivers (Bierregaard et al., 2020). Augmentation of supporting habitat through the creation of lentic systems via the damming of riverine systems may have greatly benefitted Osprey in the southeastern United States, with many artificial lakes and reservoirs appearing highly suitable for nesting Ospreys. These areas become even more appealing when average windspeeds are low, as Osprey foraging efficiency is known to be reduced at windspeeds  $>7$  m/s (Machmer and Ydenberg, 1990; Strandberg et al., 2006).

Previous studies suggested that Ospreys prefer forested landscapes (Ewins, 1997; Bei et al., 2009). However, distance to forest cover did not rank highly in any of the models. Comparatively long wings and poor maneuverability of Ospreys can limit their ability to navigate within heavily forested environments (Ewins, 1997) and may drive selection of nest sites with open surroundings (Bierregaard et al., 2020). As a result, Ospreys may prefer to nest in areas without significant tree cover (Petersen et al., 2020) to avoid nest predation from arboreal predators. As such, it is likely that Ospreys in the TVA service area are making use of convenient human infrastructure adjacent to waterways that open previously densely forested areas when available.

My habitat suitability model exhibited good model performance (Swets, 1988), and the risk model also has use if potentially conflicting infrastructure is considered in areas adjacent to risky areas, and so I believe this model will still be helpful for managers to identify core areas within which to focus monitoring and mitigation. However, I recognize that this model also had other limitations. While costly to obtain for large service areas, predictions may be improved at local scales by including other variables that likely influence Osprey ecology including water depth, water clarity, prey abundance, nest predator abundance, and competitor distributions (Wisz et al., 2013). Future work should weigh the costs and benefits of including such elements,



in addition to incorporating Osprey nesting behavior. As Ospreys are semi-colonial nesters and frequently nest at high densities in the TVA service area, it is likely that these dynamics also influence risk (Hagen and Walters, 1990; Bierregaard et al., 2020). Variability in use of various power structure types (transmission, distribution, etc.) and nest placement on these structures should be accounted for in future assessments of nest-related risk. Not all nests on power structures lead to conflicts and some nests may present higher risk than others due to their placement on or near energized parts.

Finally, I used eBird data to create these models. Citizen science data are often biased, with problems in uneven sampling, such as increased concentrations of observations around more developed or urban areas (Callaghan et al., 2019). While these potential biases may affect the results, suggesting a stronger than expected relationship between Ospreys and development, eBird suggests models be validated against reliable survey data (Walker and Taylor, 2017) and I was able to achieve this using an independent, systematic survey-based data source that included nest surveys in lesser developed areas (Taylor et al., 2017). As a result of a high amount of agreement between the model and independent nest data, it is unlikely that the use of citizen science data influenced the outcome. Further, while this has not been empirically tested, detection probability for Osprey nests is unlikely to differ across environmental predictor variables due to conspicuous nesting habits (e.g., frequent calling, sky dances, prey delivery) and the large size of Osprey nests (i.e., 3 – 4 m deep, 1 – 2 m diameter; Bierregaard et al., 2020). However, these presence-only data lack information regarding absence and prevalence, meaning that these data cannot be used to estimate probability of occurrence (Kent and Carmel, 2011; Valavi et al., 2022). I would recommend structured sampling of Osprey nests that incorporates detection/non-detection data for use in future models to allow for probability estimates.

Similarly, transmission infrastructure density was low across the TVA service area and not homogenous, with more infrastructure within urban centers and near power facilities. As I used publicly available data on power infrastructure location, it is possible that some information is not in the public database and actual density may differ from what I applied in this model. Despite this, the information I did have for power infrastructure (i.e., transmission lines) was related to the number of poles, which is the infrastructure Osprey typically interact with, allowing for the assessment of risk across the landscape.

Ospreys are widespread and experiencing population increases (Pardieck et al., 2019) concurrently with power infrastructure expansion in the United States. Consequently, managers can expect continued conflicts between these raptors and infrastructure. While I recommend that local assessments continue to be used to mitigate human-Osprey conflicts, my work provides opportunities for effective landscape-scale mitigation efforts, monitoring, and planning. I present a top-down approach to mitigating Osprey-power infrastructure conflict, allowing for prioritizing mitigation action and facilitating long-term coexistence between utility distribution and Ospreys. Further, this model can be applied in the pre-development stage to allow for informed utility construction outside of areas within and adjacent to high-risk or within areas of high nest suitability. I recommend that these efforts be applied proactively to nest conflict mitigation to allow for the effective use of limited resources. Specifically, I recommend that areas within and adjacent to high- or very high-risk areas be targeted for proactive monitoring for active Osprey nests and for proactive mitigation, such deployment of nest deterrents, installation of artificial nest platforms, structure reconfiguration, or avoiding construction of new lines in these areas. I further suggest that this approach may be expanded to other protected species that exhibit similar nesting behavior to Ospreys, such as other raptors.

## Tables & Figures

### Tables

Table 1.1 Ranking of each variable\* from the nesting suitability model developed for Osprey within the Tennessee Valley Authority service area, USA. Relative influence is provided for the GBM, percent contribution is provided for Maxent, and coefficient strength is provided for the GLM.

Variable	Maxent	GBM	GLM
Open water	<b>61.9</b>	<b>57.40</b>	-0.002
Development	<b>34.5</b>	<b>13.67</b>	<b>-0.003</b>
Forest Cover	0.7	2.89	0.0003
Grassland	0.4	1.86	0.00001
Crops	0.7	4.55	0.0002
Wetlands	0.3	2.73	-0.0003
Windspeed	0.7	5.16	<b>-0.36</b>
Slope	<b>0.8</b>	5.23	<b>-0.07</b>
Elevation	0.4	<b>6.52</b>	0.0007

\*Top three contributing variables are bolded.

## Figures

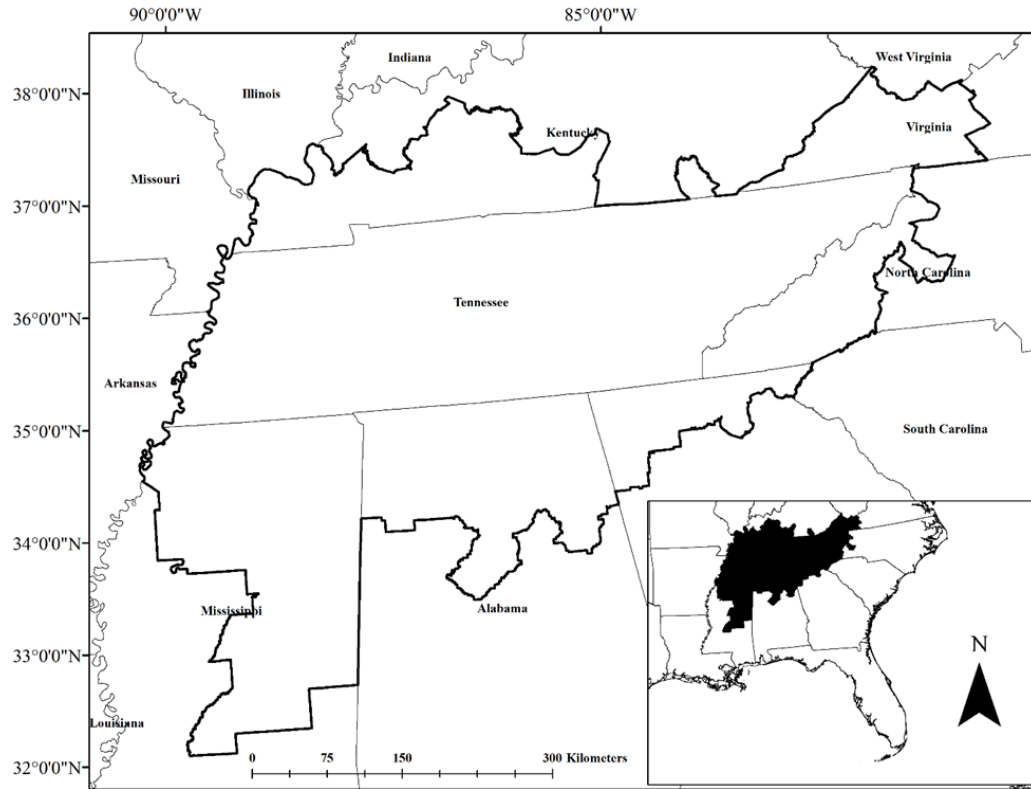


Figure 1.1 The Tennessee Valley Authority power service territory (approx. 265,860 km<sup>2</sup>) in the United States. The border highlights all counties serviced in Tennessee, Alabama, Georgia, Kentucky, Mississippi, North Carolina, and Virginia.

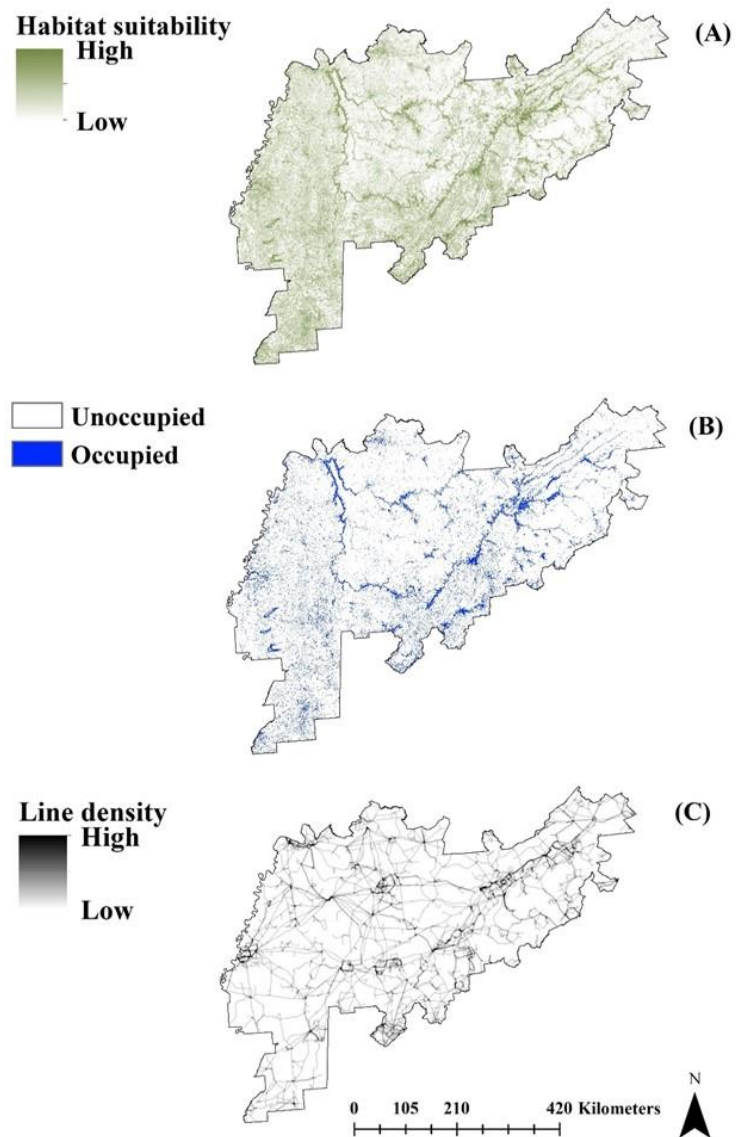


Figure 1.2 (A) Continuous and (B) binary (presence/absence) ensemble species distribution model for breeding Ospreys based on data collected from 1990 – 2020, and (C) density of power transmission structures in the Tennessee Valley Authority service area, USA.

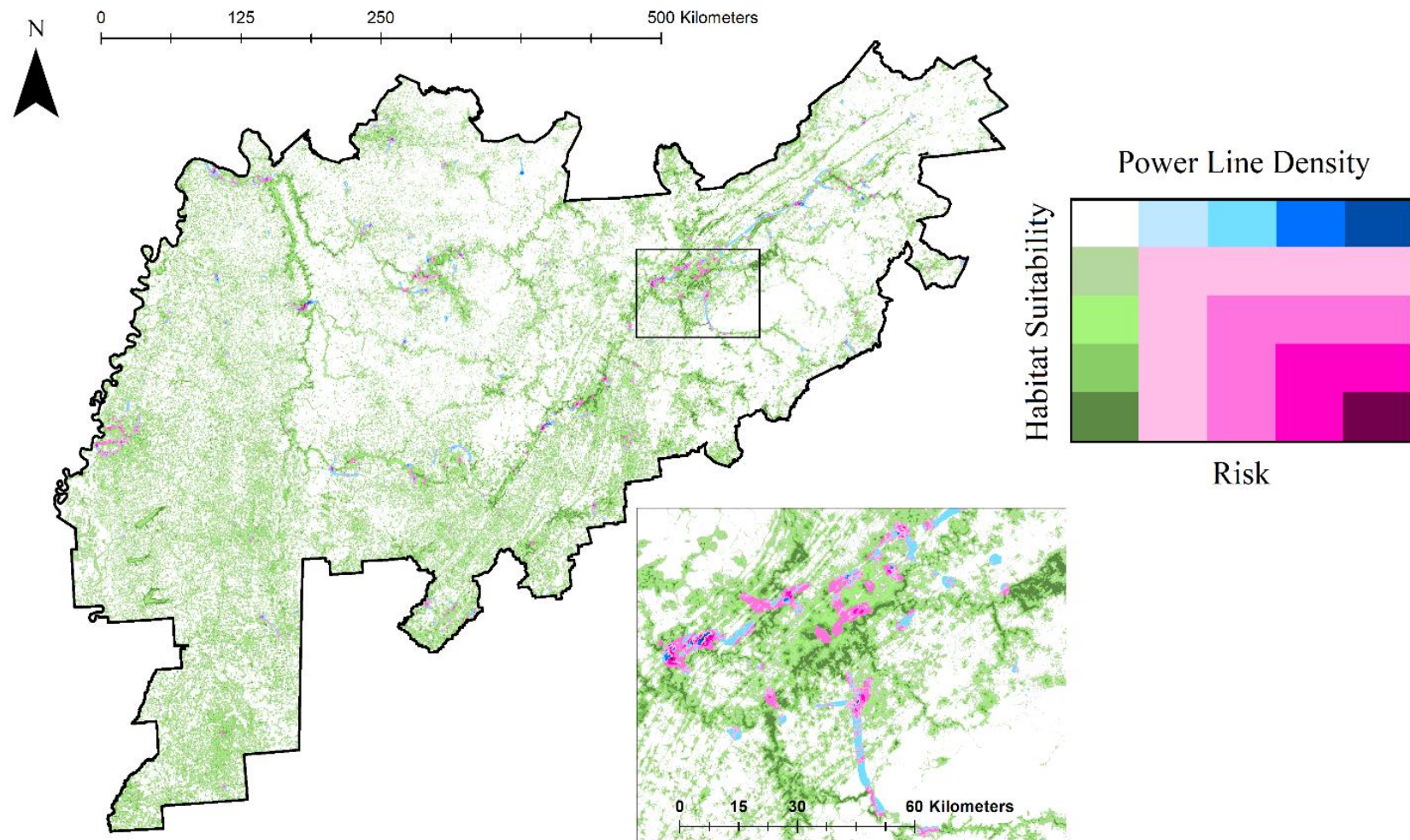


Figure 1.3 Risk of conflict with nesting Ospreys and transmission infrastructure in the Tennessee Valley Authority service area, USA. Risk matrix legend shows increasing risk from left to right in increasing purple hues, increasing habitat suitability top to bottom in green hues, and increasing line density left to right in blue hues. Inset highlights northeastern part of the service area where risk was more prevalent

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CHAPTER II

BEHAVIORAL RESPONSE OF BREEDING OSPREYS TO DIFFERENT NEST SURVEY  
TECHNIQUES

**Abstract**

Unoccupied aircraft systems (UAS) are commonly used to determine nest occupancy, clutch size, and nestling development at inaccessible bird nests, such as on cliffs, human infrastructure, or within the tree canopy. While there are a growing number of studies documenting avian behavioral responses to various UAS, there is a continued need to monitor taxa-specific responses to different UAS models. I explored both the impacts of different nest survey methods (UAS, nest climbing, and observations from a bucket truck) and different UAS model sizes (small, medium, large) on the nest defense behavior of breeding Ospreys. I conducted 166 surveys (126 UAS, 25 climbing, 15 bucket truck) at 85 Osprey nests across three nesting stages. Of the seven behavioral groups examined, I found variation in calling, flying, at nest, and perching behaviors with survey method, sex, and nest stage. Agitated calling and flying were less frequent during UAS surveys compared to other approaches. Ospreys spent greater time at their nests engaging in nest-protection behaviors during UAS surveys compared to other methods tested. Females were more responsive to all survey methods compared to males and engaged in nest-protection behaviors most frequently during incubation. I recorded few defensive behaviors (i.e., diving, or defensive posturing) across all survey types and recorded no strikes on researchers or UAS. Size of UAS used also appeared to influence behavior, as female

Ospreys spent, on average, 19 seconds more calling when surveyed with medium-sized UAS. Based on these findings and methods tested, UAS appear to be the best choice for monitoring Osprey nests as they are adaptable to multiple nest types, time-efficient, and less disturbing to nesting Ospreys than other methods tested. This research aids in setting best practices, optimizing UAS size, and developing evidence-driven approaches for monitoring avian nests across a variety of landscapes and contexts.

## **Introduction**

Unoccupied aircraft systems (UAS, otherwise known as drones) are used for a wide variety of industrial, environmental, and ecological applications, including animal monitoring situations (Christie et al., 2016; Śledź et al., 2021; Mandirola et al., 2022; Elmore et al., 2023). For example, UAS have been deployed successfully for monitoring domestic livestock (Barbedo and Koenigkan, 2018), tracking animal movement (Saunders et al., 2022), and population assessments for a wide variety of taxa, such as terrestrial mammals (Wich et al., 2016; Hu et al., 2020), marine mammals (Verfuss et al., 2019), fishes (Harris et al., 2019), amphibians (Dufresnes et al., 2020), reptiles (Monks et al., 2022), and birds (Junda et al., 2015; Kuhlmann et al., 2022). For avian species, rotary-winged UAS have been used to determine nest occupancy, clutch size, and nestling development at inaccessible bird nests, such as on cliffs (e.g., sea-cliff nesting Eleanora's Falcon, *Falco eleonora*; Hadjikyriakou et al., 2020), human infrastructure (e.g., urban-nesting gulls; Blight et al., 2019), or within the tree canopy (e.g., canopy-nesting Hooded Crow, *Corvus cornix*; Weissensteiner et al., 2015).

When applied to the above scenarios, UAS represent relatively inexpensive, efficient, and safe alternatives for monitoring avian species compared to traditional methods, such as nest climbing or the use of occupied aircraft (Sasse, 2003; Weissensteiner et al., 2015; Christie et al.,

2016; Gallego and Sarasola, 2021). Further benefits include increased survey accuracy, decreased surveyor bias, and, potentially, reduced impacts for wildlife (Koh and Wich, 2012; Christie et al., 2016; Borrelle and Fletcher, 2017; Horton et al., 2019). Response by wildlife can vary, with UAS simultaneously possessing potential to modify bird behavior in a way that reduces human-wildlife conflict (i.e., increase wildlife disturbance; Wandrie et al., 2018; Egan et al., 2020; Pfeiffer et al., 2021), but also have minimal to no impact on bird behavior to allow for sensitive monitoring of endangered species (Gallego and Sarasola, 2021). Relevant constraints, aside from wildlife disturbance, include short battery life, technical difficulties in the field (e.g., sensor calibration, signal interference), and lack of concrete best practices (Elmore et al., 2023). While there are a growing number of studies documenting avian behavioral (e.g., Vas et al., 2015; Egan et al., 2020) and physiological (e.g., Scholten et al., 2020) responses to various UAS, avian responses are complex, varied, and often species-specific regarding various elements of UAS operations (e.g., flight pattern, UAS model, various onboard sensors). For example, avian response may vary with size of UAS model because, for example, noise generated tends to increase with size (Mulero-Pázmány et al., 2017; Kuhlmann et al., 2022).

Deepening our understanding of how wildlife respond to interactions with UAS remains particularly important in the United States as UAS operators must stay in compliance with the federal regulations surrounding disturbance to wildlife, such as the Airborne Hunting Act (16 United States Code 742j1), the Migratory Bird Treaty Act (16 United States Code 703-71), and the Bald and Golden Eagle Protection Act (16 United States Code 668-668d). In this chapter, my objectives were to explore the impacts of different survey methods, and different UAS models, on the nest defense behavior of breeding Osprey (*Pandion haliaetus*), a species protected under the Migratory Bird Treaty Act in North America. Ospreys are, like many birds of prey,



aggressive nest defenders (Morrison et al., 2006; Bierregaard et al., 2020) and adaptable to urbanization (Boal and Dykstra, 2018; Forys et al., 2021). Ospreys readily construct their large, bowl-shaped stick nests on anthropogenic structures, such as billboards, power structures, cranes, communication towers, and spotlights (Ellis et al., 2009; Bierregaard et al., 2014; Poole, 2019). Inevitably, managers are faced with the challenge of coexistence and mitigating Osprey-human conflicts as nests on anthropogenic structures, such as power infrastructure, can pose safety (e.g., fire, electrocution) and reliability (e.g., power outage) risks, as well as legal violations with species protections if people disturb or attempt to remove them (APLIC, 2005; Liguori, 2009). Addressing challenges in Osprey-human conflicts requires monitoring of nests across different environments to provide landowners, biologists, and industry personnel with information that can assist with conflict mitigation and legal compliance (APLIC, 2005; Washburn, 2014; Pollack et al., 2017). Thus, the Osprey represents an important, protected species where the utility of UAS for nest monitoring, along with its behavioral impact and efficiency compared to traditional survey methods, needs to be further explored.

## **Methods**

### **Study area**

I surveyed Osprey nests in Alabama, Kentucky, Mississippi, and Tennessee in the southeastern United States (Figure 1) during breeding seasons of 2021 and 2022. Using nest records from state wildlife agencies, utility companies, and eBird (eBird, 2020), I identified clusters of Osprey nests on five waterbodies (i.e., Tennessee-Tombigbee waterway, Watts Bar reservoir, Cumberland River, Guntersville reservoir, and the Alabama coast). Ospreys are typically found near these freshwater waterways, lakes, reservoirs, and coastal areas during the breeding season (March – August; Bierregaard et al., 2020). I identified accessible nests in

navigable airspace on artificial (e.g., communication towers, spotlights, cranes, barge ties, day markers, navigation lights, and purpose-built Osprey nesting platforms) and natural substrates (e.g., snags and trees) which were suitable for surveys. I only selected nests for surveys that were in open landscapes, with clear flight paths and limited obstruction by adjacent trees, buildings, or structures.

### **Nest surveys**

I conducted diurnal surveys across three nesting stages, defined based on existing literature (Bierregaard et al., 2020), to allow comparison of parental behavioral response as the young develop. I defined nesting stages as: (i) occupancy (i.e., adults at nest without eggs or young present); (ii) incubation (i.e., adults at nest with eggs present; adults engage in incubation and nest defense); and (iii) young in nest (i.e., adults at nest with young present; adults engage in brooding and nest defense). Not all nests were surveyed within each nesting stage as occasionally a nest did not progress to the next stage, or the nest could not be visited during that stage for logistic reasons (e.g., weather, access, pilot availability). As other studies of raptor response to disturbance found no short-term habituation through repeated trials (Carrete and Tella, 2010), I did not consider this a factor in my short-term study with three nest surveys per year per nest.

I used three survey methods to identify nest status and contents – climbing, observations from a bucket truck, and UAS imagery. All three methods required a visual observer to document parental Osprey behavior from the ground while other personnel, either a climber, bucket truck operator, or remote pilot, conducted the survey and recorded nest contents. All nesting structures selected for climbing surveys were accessible to the climber using ladders, rungs, or steps, and basic climbing equipment. Bucket truck surveys raised surveyors to nest

height in a large bucket mounted on an extendable, hydraulic boom. Dry conditions in open areas with road access directly beneath the nest were always required, allowing for quick set-up and withdrawal of the bucket truck.

All UAS models were small UAS according to Federal Aviation Administration weight specifications (i.e., <25 kg). However, to test behavioral response of Ospreys to different model sizes, I grouped six rotary-wing UAS models into three comparative size categories based on wheelbase size (Table B1 for specifics): 1) small (350 – 400 mm), 2) medium (400 – 600 mm), and 3) large (600 – 900 mm). Rotary-winged models were selected as they are less likely to disturb birds compared to other types of UAS and due to their ability to ascend or descend vertically, allowing for timelapse images to be taken directly above the nests while hovering in place (Chabot and Bird, 2015; Egan et al., 2020).

Behavioral surveys began as both the visual observer and the climber, bucket truck, or UAS moved to a starting position  $\geq 30$  m of the nest. In cases where the only suitable UAS starting position (i.e., take-off site) was  $> 30$  m from the nest (range 30 m – 1.5 km), surveys began as the visual observer reached  $\geq 30$  m from the nest and the UAS powered on at the starting position. This starting distance of  $\geq 30$  m from nests was selected as UAS presence at this distance from nests does not appear to generate negative behavioral responses from nesting raptors (Junda et al., 2015; Radiansyah et al., 2017; Hadjikyriakou et al., 2020). Surveys ended when the climber returned to the starting position, the bucket truck had returned to the starting position and shut off the engine, or when the UAS returned to the starting position and powered down or moved to a new starting position in the air for the next nest, which occurred when landing between nests was not possible (e.g., surveying nests over water, nests  $<100$  m apart).

For UAS surveys, a Part 107 certified remote pilot (FAA, 2016) completed a preflight assessment to ensure adequate flight conditions (e.g., wind gusts <32 km per hr, no heavy precipitation, and temperature between 5 and 35 °C), and to ensure the UAS was fully operational. Following all Part 107 regulations, the remote pilot manually ascended the UAS vertically to at least 30 m above nest height, moved horizontally until directly above the nest, then descended vertically to 10 – 20 m above nest height (a close-up flight; Chabot and Bird, 2015). Upon descent, image acquisition began at timed intervals of 3 – 5 seconds, and the UAS hovered above the nest for >10 seconds to ensure clear images of nest contents were obtained. Once suitable images were obtained, or if Ospreys displayed excessive defensive behavior towards the UAS, the UAS either returned along the path it took to the nest to a designated landing site or continued to next nest for monitoring.

The visual observer remained at the starting position for the duration of each survey. Visual observers recorded the start and end time, number and sex of adult Ospreys present along with the nest type (natural or artificial substrate), nest height, nest status, and the number of eggs/young present. Sex of adults was determined based on plumage and behavior (Bierregaard et al., 2020). I excluded adults of unknown sex (n = 5) from analyses. Visual and auditory cues allowed the visual observer to distinguish between adult Osprey behaviors, which were recorded sequentially on a digital voice recorder. Behaviors were recorded in relation to an ethogram (Table B.2) that was developed prior to the start of the study based on behavioral response to test flights carried out in 2020 and on the known repertoire of Osprey behavior (Bretagnolle and Thibault, 1993). This ethogram included various forms of calling (i.e., alarm, guard, and scream calls), flying and flushing, perching, defensive, location-related behaviors (e.g., at nest, out of sight) and other behaviors, such as feeding (Table B.2). For purposes of understanding Osprey

response to survey methods, I selected certain behaviors as proxies for ‘less disturbed’ (e.g., remaining at the nest, feeding, perching) and ‘more disturbed’ (e.g., calling, flying, diving attacks, out of sight) behavior.

## **Behavior analysis**

My final dataset comprised of both counts and duration for each of the behaviors described in the ethogram (Table B.2). To reduce the number of parameters in analyses, Osprey behaviors were condensed into seven behavioral groups, based on similarities in behavior action (e.g., flying), function (e.g., defense), or location (e.g., at the nest; Table B.2). For behavioral counts, I built a generalized linear mixed model (GLMM) to examine the effects of survey method on specific Osprey behavioral groups (R package *glmmTMB*; Brooks et al., 2017), except for ‘other’ behaviors (i.e., copulation, feeding) as the model did not converge due to low sample size. For most behavioral groups, based on an overdispersion test (R package *performance*; Lüdtke, et al. 2021), I selected a negative binomial distribution, and I used a Poisson distribution in cases where overdispersion was not an issue. I modeled total count of each behavior as the response variable against predictor variables of nest type (artificial or natural substrate), an interaction between survey method and sex, and an interaction between survey method and nest stage. These interactions were expected as Ospreys display both sex-specific and nesting stage-specific differences in nest defense behavior (Bretagnolle and Thibault, 1993; Morrison et al., 2006). Nest type was later dropped as a predictor variable due to lack of contribution to any of the models. For all models I included a nested random effect of individual nest within waterbody to account for variation associated with behaviors of nesting pairs and regional similarities. As survey length varied and could therefore influence behavioral measures, I included log-transformed survey length as an offset variable to account for varying survey

effort. If there was an effect of the UAS survey on a behavioral group, I then explored the influence of UAS model size on the given nesting Ospreys' behavior, using only UAS data and an interaction between UAS size and sex, and an interaction between nest stage and UAS size. Models again had the same nested random effect of individual nest within waterbody an offset for survey length as above.

The proportion of time spent in each behavior category per bird per survey was calculated by dividing time spent displaying a behavior by total survey length. As my data represent >2 categories (Douma and Weedon, 2018), I used a Dirichlet regression (R package *DirichletReg*; Maier, 2014) to model the continuous proportion data without transformations. I modeled proportion of time spent exhibiting each behavior as the response variable against predictor variables of nest type, an interaction between survey method and sex, and an interaction between nesting stage and survey method. Nest type was again dropped as a predictor variable due to lack of contribution to models. If there was an effect of the UAS survey method, I again further explored the effect of UAS size on the proportion of behaviors using an interaction between UAS size and sex, and an interaction between nest stage and UAS. Across all models and analyses, an alpha-value of  $\leq 0.05$  was used to evaluate the significance of beta coefficients. Fixed and random effects were treated as degrees of freedom in reporting. A Kruskal-Wallis rank sum test was conducted to test the difference in total time taken to conduct a survey across the three survey methods and UAS size categories.

## **Results**

I surveyed a total of 85 active Osprey nests, of which 66 were on artificial substrates and 19 on natural substrates. Nests averaged 11.9 m in height (range = 1 – 55 m) on artificial substrates and 8.3 m in height (range = 1 – 16.7 m) on natural substrates. I carried out a total of

166 nest surveys across three nesting stages, including 126 UAS surveys (large UAS = 44, medium = 22, small = 60), 25 traditional climbing surveys, and 15 bucket truck surveys. In total, all surveys combined represented 143 adult Ospreys (females = 79; males = 64), assuming site fidelity and recording the same individuals at each nest per survey. This resulted in a total of 257 observations and 3,593 individual behaviors recorded. On average, Ospreys displayed  $14 \pm 18$  unique instances of behaviors during surveys. Out of 126 UAS surveys, nest contents could not be determined for 30% ( $n = 38$ ) of nests due to female Ospreys obstructing views of eggs or young. If adult Ospreys were flushed from the nest during surveys (48% of all surveys), they either returned to the nest during surveys (24.7%) or immediately post-survey (75%). However, one pair surveyed during occupation (i.e., no eggs or young) flushed from the nest and left the area for >10 minutes post-survey.

Calling was the most common behavior (33% of all behaviors recorded) and alarm calls comprised 75% of all calls, followed by guard calls (20%), and screams (6%). Calling frequency and duration were influenced by sex (Table 2.1) with female Ospreys typically calling 11.8 times more frequently and spending 24 additional seconds calling compared to males (Figures 2.2A and 2.4A). The proportion of time spent calling was also influenced by an interaction between sex and survey method (Table 2.1), with females spending 1 minute  $\pm$  53 seconds calling during climbing surveys compared to  $20 \pm 28$  seconds during UAS surveys; calling during climbing and bucket truck (1 minute  $\pm$  33 seconds) surveys was similar (Figure 2.4A). Using only UAS survey data for the proportion of time spent calling, I found that call duration was also influenced by an interaction between sex and UAS model size (Table 2.3). Female Ospreys spent an average of  $30 \pm 35$  seconds (range = 0 – 139 seconds) calling during surveys using a medium-sized UAS, compared to  $9 \pm 21$  seconds (0 – 110 seconds) for large-sized and  $13 \pm 20$  seconds (0 – 83

seconds) for small-sized UAS (Figure 2.4B). A higher proportion of calls were screams (6%) or guard calls (11%) during surveys with medium-sized UAS compared to large (screams = 0%, guard calls = 7%) and small (screams = 1%, guard calls = 9%) models.

I recorded 80 initial flushes from the nest and 18 additional flushes during surveys. Ospreys initially flushed 36 times and additionally flushed 12 times during UAS surveys, compared to 30 initial flushes and 2 additional flushes for climbs and 14 initial flushes and 2 additional flushes during bucket truck surveys. Frequency and duration of all flying behaviors, including flushes, varied by survey type and nest stage (Table 2.1), as Ospreys flew 1.5 times more frequently during climbing and spent an average of  $3 \pm 2$  minutes flying during climbing surveys in the occupation stage compared to  $25 \pm 59$  seconds for UAS surveys (Figure 2.3A). Additionally, Ospreys flew 1.9 times more frequently and spent  $5 \pm 3$  minutes flying during bucket truck surveys when young were in the nest, compared to  $21 \pm 44$  seconds for UAS surveys (Figure 2.3A). Flying frequency was also influenced by sex and survey type (Table 2.1), as females were recorded flying 6.7 times more frequently during bucket truck, 5.8 times more frequently during climbing, and 1.5 times more during UAS surveys compared to males. Flying behaviors were not influenced by UAS size (Table 2.3).

Perching frequency was influenced by survey method and an interaction between nesting stage and survey method (Table 2.2). Ospreys were recorded perching only once during bucket truck surveys when young were in the nest, compared to 110 instances of perching during UAS surveys in the same nesting stage (Figure 2.3A). Further, perching frequency was also influenced by an interaction between nesting stage and UAS size (Table 2.3). Ospreys typically perched 1.3 times more when young were in the nest, especially during surveys with large-sized UAS (Figure 2.3B). Adults also perched more frequently during surveys with medium-sized UAS models



during the occupation stage (Figure 2.3B). There was no influence of any variables on perching duration (Table 2.2).

Ospreys returned to their nests after flushing 56 times during surveys, with 43 returns during UAS, 9 during climbing, and 4 during bucket truck surveys. Frequency and duration of Ospreys being at the nest varied by sex (Table 2.2), with female Ospreys typically being recorded at the nest 11.2 times more frequently and spending 1 additional minute at the nest compared to males. Survey method influenced both frequency and duration of at nest behaviors (Table 2.2). Ospreys were recorded at the nest 19 times and 7.9 times more frequently during UAS surveys compared to bucket truck surveys and climbing surveys, respectively. On average, Ospreys spent 2 minutes at the nest during UAS surveys, but this decreased to 51 seconds and 38 seconds during bucket truck and climbing surveys, respectively. The proportion of time spent at the nest also varied with an interaction between nesting stage and survey method (Table 2.2), as Ospreys spent at greater amount of time ( $2 \pm 2$  minutes) at the nest during the incubation stage across all survey types (Figure 2.5A). For UAS data only, the proportion of time spent at the nest was influenced by interactions between sex and UAS size category (Table 2.3). Male Ospreys spent the least amount of time ( $21 \pm 31$  seconds) at the nest during surveys with medium-sized UAS (Figure 2.4B). Ospreys also spent an additional  $3 \pm 2$  minutes at the nest during the incubation stage when surveyed with a medium-sized UAS compared to larger ( $2 \pm 2$  minutes) or smaller ( $2 \pm 2$  minutes) models (Figure 2.5B).

Frequency of recording Ospreys leaving the study area varied by sex, as males were 2.7 times more likely to be recorded out of sight than females (Table 2.2; Figure 2.2A). Proportion of time Ospreys spent away from the survey area varied with an interaction between nesting stage and survey method (Table 2.2). Ospreys spent, on average,  $10 \pm 2$  minutes out of sight

during bucket truck surveys that were carried out during the occupation stage, compared to  $48 \pm 131$  seconds for climbing surveys and  $40 \pm 77$  seconds for UAS surveys conducted in the same nesting stage (Figure 2.5A). There was no influence of UAS size on duration of time spent out of sight (Table 2.3).

There was no effect of any variables of interest on the frequency or duration of defensive behaviors (Table 2.1). Defensive behavior was rare ( $n = 44$ ; 39 diving attacks and 5 defensive poses; 1.2% of all behaviors recorded), especially in males. I recorded only one instance of a male engaging in defensive behavior, which was a 3 second defensive pose during a large-sized UAS survey. Only 7 dives were recorded across all UAS surveys, compared to 21 during climbing and 11 during bucket truck surveys. Dives and poses are rapid behaviors, and I recorded a maximum dive duration of 10 seconds. I was unable to estimate any effects between my variables of interest and the frequency and duration of ‘other’ behaviors due to low sample sizes. These rare behaviors, such as copulation and feeding ( $n = 24$ ; 0.6% of all behaviors recorded), were only recorded during small and large UAS surveys, but not during surveys with medium-sized UAS.

Time to carry out a survey varied by survey method ( $H_2 = 40.825$ ;  $P < 0.001$ ). Bucket truck surveys were more time intensive than the other methods, averaging  $8 \pm 4$  minutes (range = 3 – 15 minutes) to complete. In comparison, climbing surveys took  $5 \pm 2$  minutes (2 – 13 minutes) and surveys with UAS took  $4 \pm 1$  minutes (1 – 7 minutes) to complete. Nests surveyed by climbing were typically shorter in height ( $\bar{x} = 7$  m; range 1 – 30 m) than those surveyed with bucket trucks (11 m; 8 – 20 m) or with UASs (10 m; 1 – 55 m). In comparison, there was no difference ( $H_2 = 3.6$ ;  $P = 0.16$ ) in survey time between the three UAS size categories (large  $\bar{x} = 4$

$\pm 2$  minutes; range = 1 – 7 minutes, medium =  $4 \pm 1$  minutes; 1 – 7 minutes, small =  $4 \pm 1$  minutes; 2 – 5 minutes).

## **Discussion**

With the increase in available technology for wildlife monitoring, it is important to critically evaluate the use of new survey approaches to develop best practices that lead to lower count bias and disturbance to animals. This study supports the use of one such emergent monitoring approach, the use of UAS for monitoring the nests of Ospreys and potentially other raptors. I found that, in obtaining nesting information needed for monitoring or management, UAS were not only more time-efficient than bucket truck and climbing surveys, but also less disturbing to breeding Ospreys. Ospreys called less, returned to their nests more frequently, and remained at their nests more frequently and for longer periods of time when surveyed with UAS compared to other approaches. Additionally, UAS surveys elicited few defensive behaviors when flown safely by experienced remote pilots, and UAS were adaptable across numerous nesting substrates, including monitoring nests at heights up to 55 m, over water, on human infrastructure, and in the tree canopy. Further, UAS size can be optimized to reduce defensive behavioral response of Ospreys to nest surveys. However, there appears to be a trade-off between reducing disturbance and obtaining nest information.

Ospreys are considered aggressive nest defenders who defend their nests by diving and striking at nest intruders (Morrison et al., 2006; Bierregaard et al., 2020). In contrast, I recorded few defensive behaviors (i.e., diving, or defensive posturing) across all surveys and recorded no strikes on researchers or UAS. Of four raptor species surveyed by Junda et al. (2015, 2016), Ospreys displayed the strongest nest defense behavior towards UAS, but their behavioral response did not differ between focal observations from the ground and UAS surveys (Junda et

al., 2015). Other large raptors have also been reported to respond similarly to UAS surveys as to ground-based surveys (e.g., Steller's Sea Eagle, *Haliaeetus pelagicus*; Potapov et al., 2013). Junda et al. (2016) reported one strike (i.e., a bird hit the UAS) across 51 UAS surveys and Ospreys have been reported chasing UAS flown by the public (Rebolo-Ifrán et al., 2019). To avoid such negative interactions with the UAS, I followed best practice guidelines from previous raptor surveys using UAS, by starting ( $\geq 30$  m) and maintaining ( $> 10$  m) the UAS at safe distances from the nest. Unlike other studies (e.g., Junda et al., 2015, 2016), I did not force flush Ospreys during UAS surveys. While force flushing (e.g., shaking the base of the nest to illicit a flush) allows for unimpeded images of the nest contents, this action may result in birds being more defensive and prone to strike the UAS, as raptors usually engage in active nest defense behaviors following a flush from the nest (Keeley and Bechard, 2011). Flushing can result in egg damage, accidental ejection of young from the nest, nest predation, nest exposure, or nest abandonment (Cantú de Leija et al., 2023; Zink et al., 2023). Consequently, flushing behavior should be viewed as the strongest disturbance response to nest surveys. I therefore suggest that force flushing, while allowing for nest content information to be collected, should be avoided if the goals of the survey are to obtain nest status information only and to reduce the likelihood of negative interactions with raptors (e.g., Gallego and Sarasola, 2021). However, as force flushing is often required to obtain more detailed nest data (e.g., Junda et al., 2015, 2016), there is a need for future research to explore the taxa-specific behavioral and physiological responses to force flushing by UAS for these purposes.

I observed Ospreys at the nest more frequently and for longer periods of time during UAS surveys compared to other methods. As nest defense in response to a flying intruder often takes the form of nest-protection in Ospreys (not necessarily aggressive action), especially for

females (Bretagnolle and Thibault, 1993), this suggests that UAS are seen as flying intruders and thus disturbing. Nest-protection behavior can be considered more intense nest defense behavior, as it allows potential threats more time to reach the nest and may be followed by a direct attack if the intruder enters the nest (Bretagnolle and Thibault, 1993; Sergio and Bogliani, 2001). However, other methods tested (i.e., climbing and bucket truck observations) were more likely to elicit flushing from the nest and, once flushed, a raptor is more likely to engage in direct aggressive action, such as diving attacks (Keeley and Bechard, 2011). My observations of Ospreys calling less and continuing normal nesting activities (i.e., incubation or brooding while nest-protecting, copulation, feeding of young) during UAS surveys also suggests these types of surveys are less disturbing compared to other methods tested. However, it is important to consider that what is being described at a behavioral level may not reflect the physiological stress response to disturbance (Wich and Koh, 2018). While both costly and logistically difficult in real-world scenarios, I suggest future studies incorporate both behavioral and physiological responses (e.g., heart rate) when evaluating the impacts of disturbance.

I compared behavioral response across three size classes of multi-rotor UAS models and found differences in response to model size. The medium-sized UAS appeared to generate more disturbance to nesting Ospreys than larger or smaller models. Males spent less time at the nest and incubating adults engaged in increased nest-protection behavior when medium-sized UAS overhead. Further, no copulation or feeding behaviors were recorded during surveys with the medium-sized UAS but were recorded when using large- or small-sized UAS. Based on my study and supporting literature (Sergio and Bogliani, 2001; Morrison et al., 2006; Shah et al., 2015), I believe that perceived threat influences call type, call duration, and time spent at the nest. My interpretation is illustrated in the differing responses to the UAS model size classes;

small-sized UAS are a perceived non-threat (i.e., elicit lower call rates and durations), large-sized UAS are a ‘risky’ threat (i.e., elicit fewer high intensity calls, such as screams, to avoid drawing attention to the nest location), and medium-sized UAS are threatening enough to increase call intensity, call duration, and exhibit stronger nest-protection behavior. My results for medium-sized UAS could also be an artifact of small sample size (surveys = 22; representing observations of 11 males and 20 females), having only one model within the medium-size category (i.e., potentially this model was perceived as especially threatening), or that all medium-sized UAS surveys were carried out at the same location (Cumberland River). However, defensive responses are unlikely to reflect local conditions for nesting Ospreys as the same birds did not respond with increased calling or nest defense to other sized UAS in the same location. While my results suggest that small- and large-sized UAS models are less disturbing to Ospreys, more research is needed to fully explore the consistency of this relationship across other UAS models.

My results are concordant with previous research on Osprey nest defense, which suggested no differences in nest defense behavior between artificial and natural nest types (Braga, 2017). Although defensive behaviors, such as calling, diving, and striking, did not vary with nesting stage, Ospreys generally behaved differently as nests progressed. For example, Ospreys perched near the nest more frequently when young were in the nest and required less direct incubation/brooding by the adults. As both sexes incubate, but typically females spend more time incubating (Levenson, 1979), I observed Ospreys spending more time at the nest during incubation. In comparison, adults, especially males, were more likely to leave the area during surveys (out of sight) in the occupation stage. Some raptors, including Ospreys, are known to invest less into nest guarding at this time and are more likely to leave the nest during

nest surveys when no eggs or young are present (Morrison et al., 2006; Gallego and Sarasola, 2021). Ospreys also tended to spend more time flying during the occupation stage, but only during bucket truck surveys, which may reflect the increased instances of Ospreys leaving the nest area when surveyed with bucket trucks, a method that is seemingly more disturbing. Based on my results, I suggest that incubation is an ideal time to survey nests as Ospreys are more likely to remain at the nest, especially when surveyed with UAS. While using UAS instead of more invasive methods during incubation will assist in reducing disturbance and possible nest abandonment, obtaining accurate nest content estimates may be difficult with UAS as the birds are less likely to flush from the nest.

Time efficiency is always a priority during nest surveys to reduce overall disturbance. Overall, my surveys were highly time efficient (range = 1 – 15 minutes), and this is most clearly seen in the short climbing surveys. Generally, traditional climbs of tree nests can take > 15 minutes (Grier, 1969; Gallego and Sarasola, 2021). These much shorter traditional climbing surveys were due to the accessibility of nests (i.e., short heights and/or the presence of ladders at infrastructure nests). The maximum survey lengths recorded for traditional climbs in my study are more representative of what to expect when surveying natural nests in trees that require extensive climbing (Gallego and Sarasola, 2021). Further, I found the use of professional and experienced UAS pilots, bucket truck operators, and climbers assisted in decreasing survey time, in addition to decreasing disturbance to nesting birds and increasing human safety (Mulero-Pázmány et al., 2017; Gallego and Sarasola, 2021). Comparing across survey methods, bucket truck surveys were not only more time intensive than other methods, but also involved more personnel and costly equipment. While climbing surveys were only 54 seconds longer on average than UAS surveys, UAS generated much less disturbance to the breeding Ospreys.

Additionally, UAS can survey taller nests in similar amounts of time due to their ability to ascend rapidly, whereas the time spent climbing a nest will increase with nest height. These results again indicate UAS as a preferred survey method for this type of monitoring.

Sensitivity to interactions with UAS is likely a species-specific trait (Vas et al., 2015; Weston et al., 2020). As UAS are increasingly used in animal monitoring situations, deepening our understanding of how specific taxa respond to UAS will remain critical to ensuring their effective use. Here, I explored the use of UAS in an applied setting to provide industry personnel and biologists an effective, low-disturbance monitoring tool. Intervention with an Osprey nest may be necessary when avian mortality, human safety, structure or service reliability, or legal compliance become concerns and details on nest status are required to make informed mitigation decisions (APLIC, 2005). Based on my findings and methods tested, UAS appear to be the best choice for monitoring Osprey nests. I found UAS to be adaptable to multiple nest types, time-efficient, and less disturbing to nesting Ospreys than other methods tested. Further, UAS present a cost-saving opportunity as these survey methods require fewer personnel and comparatively inexpensive equipment. Lastly, UAS are safer for human observers than methods such as climbing or manned aircraft. This research aids in setting best practices and evidence-driven approaches for monitoring raptor nests across a variety of landscapes and contexts.



## Tables & Figures

### Tables

Table 2.1 The effect of survey type (unoccupied aerial systems = UAS), and nest stage on the frequency and duration of calling, flying and defensive behavior of adult Ospreys surveyed in 2021 – 2022 across four southeastern US states.

	<i>Calling</i>		<i>Flying</i>		<i>Defensive</i>	
	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>
<i>Frequency</i>						
Survey (climbing)	0.49 ± 0.62	0.42	0.06 ± 0.50	0.90	1.88 ± 1.17	0.11
Survey (UAS)	-0.77 ± 0.53	0.14	-2.76 ± 0.44	<b>&lt;0.001</b>	-0.53 ± 0.11	0.62
Sex (male)	-2.50 ± 0.56	<b>&lt;0.01</b>	-1.50 ± 0.40	<b>&lt;0.001</b>	-0.23 ± <0.001	1
Stage (incubation)	-0.15 ± 0.56	0.77	-0.29 ± 0.43	0.50	-0.16 ± 1.21	0.88
Stage (occupation)	-18.97 ± 1498	0.99	-2.52 ± 1.12	0.02	-0.18 ± <0.001	0.99
Incubation*Climbing	-0.41 ± 0.76	0.59	0.16 ± 0.60	0.78	-0.18 ± 1.57	0.90
Occupation*Climbing	17.9 ± 1498	0.99	2.01 ± 1.22	0.10	-0.16 ± <0.001	1.0
Incubation*UAS	-0.72 ± 0.62	0.25	-0.10 ± 0.51	0.83	-0.665 ± 1.39	0.64
Occupation*UAS	18.1 ± 1498	0.99	2.83 ± 1.15	<b>0.014</b>	0.17 ± <0.001	0.99
Sex*Climbing	0.67 ± 0.73	0.34	0.30 ± 0.51	0.54	-0.10 ± <0.001	1.0
Sex*UAS	0.59 ± 0.61	0.33	1.47 ± 0.45	<b>0.001</b>	0.21 ± <0.001	1.0
<i>Duration</i>						
Survey (climbing)	0.68 ± 0.47	0.15	-0.50 ± 0.53	0.34	0.28 ± 0.45	0.53
Survey (UAS)	-1.63 ± 0.40	<b>&lt;0.001</b>	-3.40 ± 0.48	<b>&lt;0.001</b>	-0.29 ± 0.36	0.43
Sex (male)	-1.31 ± 0.40	<b>0.001</b>	-0.58 ± 0.44	0.19	-0.11 ± 0.39	0.76
Stage (incubation)	-0.75 ± 0.40	0.06	-1.14 ± 0.43	<b>0.02</b>	-0.14 ± 0.40	0.72
Stage (occupation)	-0.97 ± 0.84	0.25	-1.14 ± 0.43	<b>0.008</b>	0.84 ± 0.80	0.29
Incubation*Climbing	-0.45 ± 0.51	0.38	0.71 ± 0.54	0.18	-0.11 ± 0.54	0.83
Occupation*Climbing	-0.79 ± 0.93	0.39	-3.14 ± 1.20	<b>0.009</b>	-1.26 ± 0.90	0.16
Incubation*UAS	0.39 ± 0.44	0.37	1.09 ± 0.46	<b>0.02</b>	0.14 ± 0.44	0.75
Occupation*UAS	0.53 ± 0.86	0.53	-2.57 ± 1.15	<b>0.02</b>	-0.89 ± 0.82	0.28
Sex*Climbing	-0.40 ± 0.50	0.41	-0.50 ± 0.53	0.34	-0.23 ± 0.50	0.63
Sex*UAS	0.82 ± 0.43	<b>0.05</b>	0.74 ± 0.47	0.11	0.04 ± 0.41	0.90

*P* values less than 0.001 are reported as <0.001, otherwise exact values are provided. Any *P* values <0.05 are in **bold**. Number of observations: 257. GLMM random effects = 85 (nests), 5 (waterbodies).

Table 2.2 The effect of survey type, nest stage and on the frequency and duration of calling, flying and defensive behavior of adult Ospreys surveyed in 2021 – 2022 across four southeastern US states. Unoccupied aerial systems = UAS.

	<i>Perching</i>		<i>At Nest</i>		<i>Out of Sight</i>	
	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>
<i>Frequency</i>						
Survey (climbing)	-8.50 $\pm$ 1.24	0.17	1.55 $\pm$ 0.57	<b>0.04</b>	1.28 $\pm$ 1.03	0.21
Survey (UAS)	2.85 $\pm$ 1.26	<b>0.02</b>	1.79 $\pm$ 0.48	<b>&lt;0.001</b>	0.31 $\pm$ 0.91	0.72
Sex (male)	-0.10 $\pm$ 1.04	0.91	-1.35 $\pm$ 0.62	<b>0.02</b>	2.01 $\pm$ 0.77	<b>0.008</b>
Stage (incubation)	2.25 $\pm$ 1.30	0.08	0.06 $\pm$ 0.57	0.91	0.14 $\pm$ 0.68	0.83
Stage (occupation)	-16.6 $\pm$ 7003.26	0.99	-1.57 $\pm$ 1.34	0.24	0.94 $\pm$ 0.89	0.28
Incubation*Climbing	-2.49 $\pm$ 1.61	0.12	0.005 $\pm$ 0.75	0.99	-0.32 $\pm$ 0.94	0.72
Occupation*Climbing	16.2 $\pm$ 7003.2	0.99	0.81 $\pm$ 1.47	0.58	-1.21 $\pm$ 1.17	0.30
Incubation*UAS	-3.25 $\pm$ 1.36	<b>0.017</b>	-0.19 $\pm$ 0.61	0.75	-0.08 $\pm$ 0.83	0.92
Occupation*UAS	16.2 $\pm$ 7003.2	0.99	1.22 $\pm$ 1.36	0.36	0.32 $\pm$ 0.97	0.74
Sex*Climbing	0.96 $\pm$ 1.29	0.45	-0.55 $\pm$ 0.79	0.49	-0.70 $\pm$ 0.94	0.45
Sex*UAS	1.10 $\pm$ 1.29	0.30	-0.69 $\pm$ 0.66	0.29	-0.89 $\pm$ 0.84	0.28
<i>Duration</i>						
Survey (climbing)	0.04 $\pm$ 0.46	0.92	0.84 $\pm$ 0.44	<b>0.05</b>	0.09 $\pm$ 0.42	0.83
Survey (UAS)	0.007 $\pm$ 0.37	0.98	0.29 $\pm$ 0.35	0.40	-0.52 $\pm$ 0.34	0.12
Sex (male)	0.03 $\pm$ 0.39	0.93	-1.45 $\pm$ 0.59	<b>0.01</b>	0.22 $\pm$ 0.34	0.52
Stage (incubation)	0.007 $\pm$ 0.41	0.32	-0.03 $\pm$ 0.40	0.92	-0.09 $\pm$ 0.40	0.81
Stage (occupation)	0.79 $\pm$ 0.80	0.32	2.47 $\pm$ 1.17	<b>0.03</b>	6.10 $\pm$ 1.03	<b>&lt;0.001</b>
Incubation*Climbing	-0.05 $\pm$ 0.54	0.92	-0.55 $\pm$ 0.52	0.29	-0.33 $\pm$ 0.58	0.57
Occupation*Climbing	-0.67 $\pm$ 0.91	0.46	-3.60 $\pm$ 1.24	<b>0.003</b>	-6.87 $\pm$ 1.14	<b>&lt;0.001</b>
Incubation*UAS	-0.16 $\pm$ 0.44	0.71	0.21 $\pm$ 0.44	0.62	0.13 $\pm$ 0.44	0.75
Occupation*UAS	-1.08 $\pm$ 0.83	0.19	-2.78 $\pm$ 1.18	<b>0.01</b>	-5.93 $\pm$ 1.05	<b>&lt;0.001</b>
Sex*Climbing	0.03 $\pm$ 0.50	0.95	0.08 $\pm$ 0.67	0.89	0.45 $\pm$ 0.52	0.38
Sex*UAS	0.67 $\pm$ 0.42	0.10	0.14 $\pm$ 0.61	0.81	-0.06 $\pm$ 0.38	0.86

*P* values less than 0.001 are reported as 0 <0.001, otherwise exact values are provided. Any *P* values <0.05 are in **bold**. Number of observations: 257. GLMM random effects = 85 (nests), 5 (waterbodies).

Table 2.3 The effect of unoccupied aerial systems (UAS) size, nest stage and sex on the frequency and duration of various behaviors of adult Ospreys surveyed in 2021 – 2022 as influenced by the UAS survey method.

	<i>Flying</i>		<i>Perching</i>		<i>At nest</i>			
<i>UAS data only</i>	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>		
<i>Frequency</i>								
Size (medium)	0.06 ± 1.18	0.95	-1.68 ± 0.90	0.06	1.11 ± 0.60	0.06		
Size (small)	1.12 ± 0.98	0.25	-1.56 ± 0.74	<b>0.03</b>	0.44 ± 0.53	0.40		
Sex (male)	0.11 ± 0.50	0.82	0.82 ± 0.48	0.08	-1.64 ± 0.36	<b>&lt;0.001</b>		
Stage (incubation)	0.41 ± 0.94	0.66	-1.85 ± 0.74	<b>0.01</b>	0.05 ± 0.56	0.91		
Stage (occupation)	1.28 ± 0.96	0.18	-1.49 ± 0.74	<b>0.04</b>	-0.27 ± 0.59	0.64		
Incubation*Medium	-0.41 ± 1.24	0.74	0.26 ± 1.19	0.82	-0.12 ± 0.72	0.86		
Occupation*Medium	0.51 ± 1.39	0.71	2.13 ± 1.13	<b>0.05</b>	-0.49 ± 0.84	0.55		
Incubation*Small	-1.07 ± 1.21	0.37	0.90 ± 0.99	0.36	-0.41 ± 0.68	0.54		
Occupation*Small	-1.23 ± 1.14	0.27	0.61 ± 0.97	0.53	0.19 ± 0.70	0.78		
Sex*Medium	0.23 ± 0.86	0.78	0.55 ± 0.92	0.54	-1.03 ± 0.65	0.10		
Sex*Small	-0.11 ± 0.63	0.85	0.32 ± 0.66	0.61	-0.41 ± 0.48	0.38		
<i>Duration</i>								
	<i>Calling</i>		<i>Flying</i>		<i>At Nest</i>		<i>Out of Sight</i>	
Size (medium)	1.34 ± 0.55	<b>0.01</b>	-0.009 ± 0.55	0.98	0.87 ± 0.54	0.10	-0.05 ± 0.57	0.92
Size (small)	0.36 ± 0.47	0.44	0.18 ± 0.47	0.69	0.57 ± 0.48	0.24	0.12 ± 0.47	0.78
Sex (male)	-0.14 ± 0.25	0.56	0.08 ± 0.25	0.73	-0.90 ± 0.25	<b>&lt;0.001</b>	0.11 ± 0.25	0.65
Stage (incubation)	-0.28 ± 0.49	0.56	0.003 ± 0.49	0.99	0.10 ± 0.49	0.83	0.07 ± 0.48	0.87
Stage (occupation)	-0.23 ± 0.49	0.62	0.21 ± 0.49	0.66	-0.07 ± 0.49	0.88	0.29 ± 0.48	0.54
Incubation*Medium	0.08 ± 0.64	0.89	0.63 ± 0.64	0.33	1.80 ± 0.63	<b>0.004</b>	0.63 ± 0.66	0.34
Occupation*Medium	-0.33 ± 0.67	0.62	-0.09 ± 0.70	0.89	-0.59 ± 0.67	0.78	-0.33 ± 0.69	0.62
Incubation*Small	-0.10 ± 0.56	0.85	-0.03 ± 0.56	0.95	0.15 ± 0.56	0.78	-0.02 ± 0.56	0.95
Occupation*Small	-0.27 ± 0.55	0.62	-0.25 ± 0.55	0.64	-0.20 ± 0.55	0.71	-0.14 ± 0.55	0.78
Sex*Medium	-1.26 ± 0.46	<b>0.006</b>	0.38 ± 0.49	0.44	-1.47 ± 0.45	<b>0.001</b>	0.57 ± 0.53	0.27
Sex*Small	-0.43 ± 0.33	0.19	0.07 ± 0.33	0.82	-0.62 ± 0.33	0.06	0.02 ± 0.33	0.94

*P* values less than 0.001 are reported as <0.001, otherwise exact values are provided. Any *P* values <0.05 are in **bold**. Number of observations: 188. GLMM random effects = 63 (nests), 3 (waterbodies).

## Figures

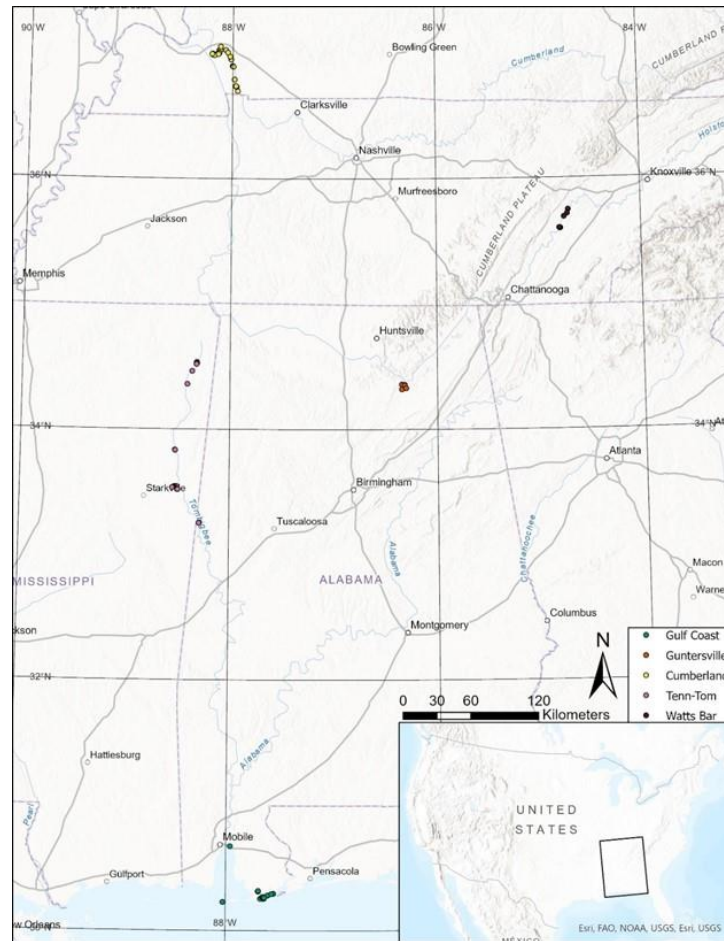


Figure 2.1 Nest locations surveyed (2021 – 2022) across five major waterbodies in the southeastern United States: The Gulf Coast (Alabama), Guntersville Reservoir (Alabama), Cumberland River (Kentucky), Tennessee-Tombigbee Waterway (Mississippi/Alabama), and Watts Bar Reservoir (Tennessee). Inset shows study area in context of the United States.

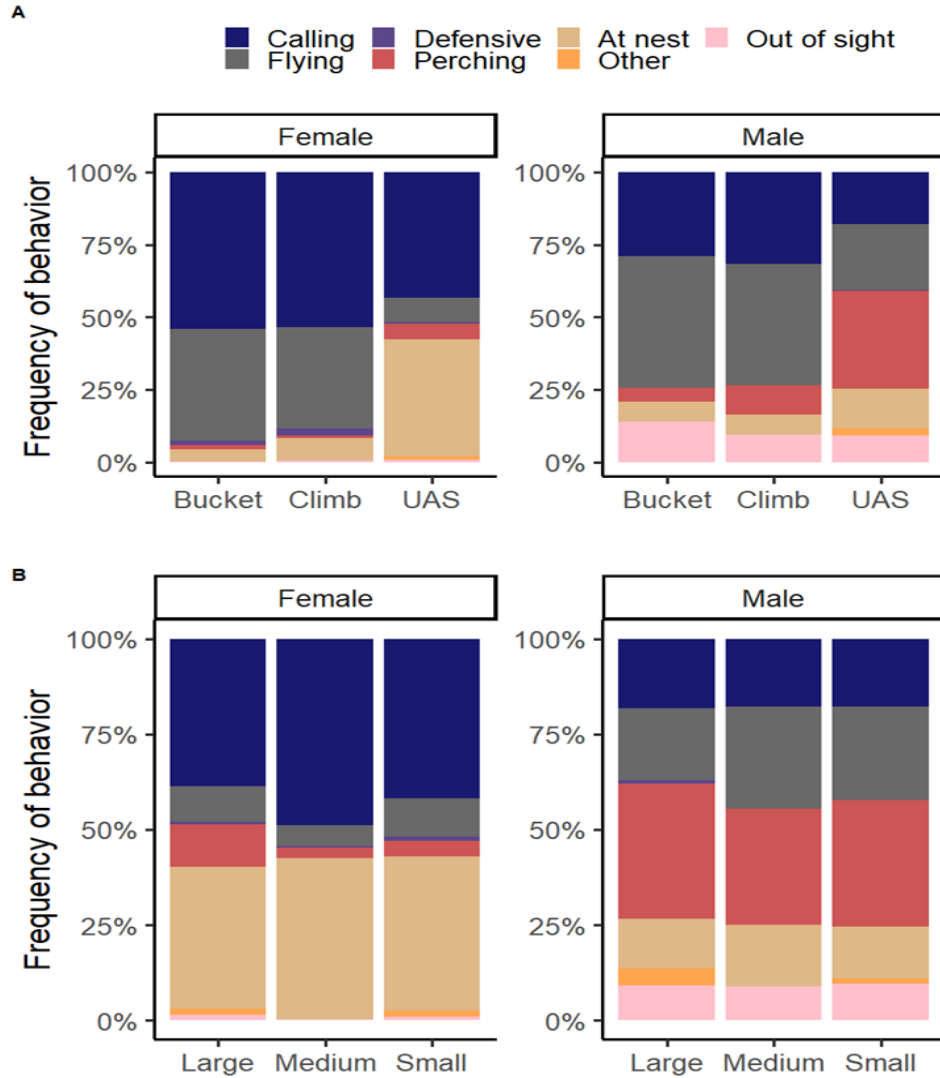


Figure 2.2 Panel-A (top), the frequency (as percentage, not offset for survey duration) of behaviors across the three survey methods for female (n=79) and male (n = 64) Ospreys, surveyed in 2021 – 2022 across four southeastern US states. Panel-B (bottom), the frequency of behaviors for the three model sizes: small (350 – 400 mm), 2) medium (400 – 600 mm), and 3) large (600 – 900 mm) unoccupied aerial systems (UAS) for female and male Ospreys.

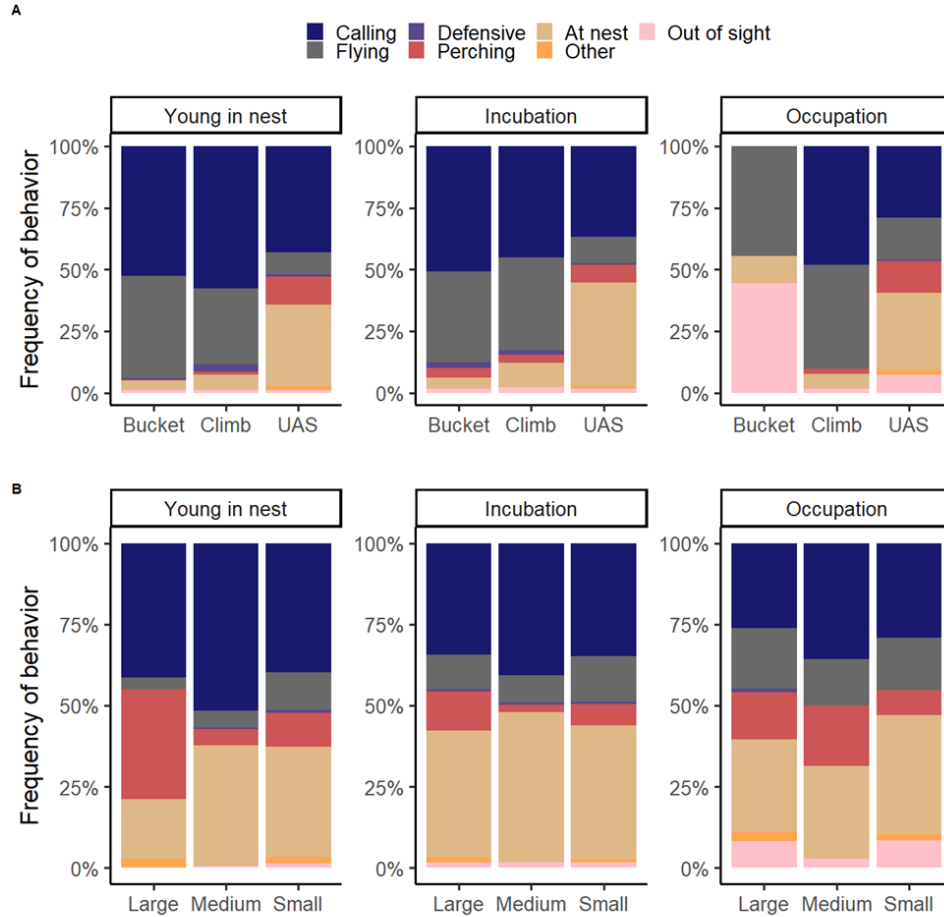


Figure 2.3 Panel-**A** (top) the frequency (as percentage, not offset for survey duration) of behaviors across the three survey methods for the three nesting stages (young in nest = 66, incubation = 52, occupation = 48). Panel-**B** (bottom) the frequency of behaviors for the three model sizes: small (350 – 400 mm), 2) medium (400 – 600 mm), and 3) large (600 – 900 mm) of unoccupied aerial systems (UAS) for the three nesting stages.

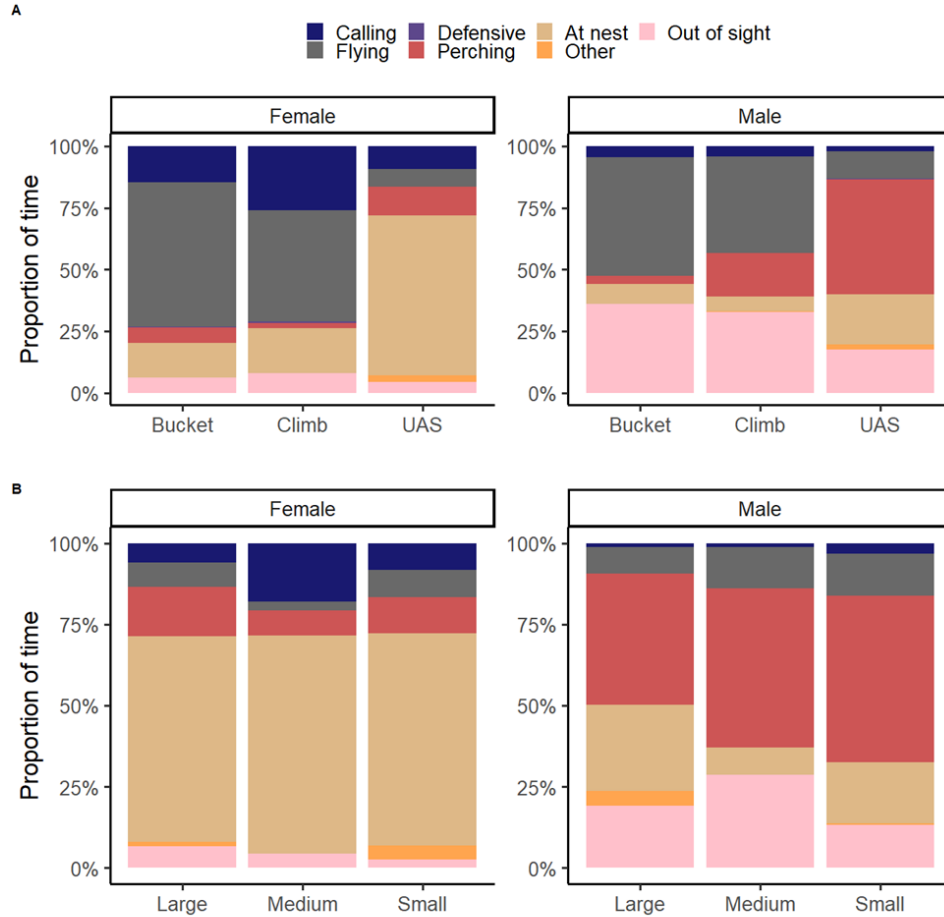


Figure 2.4 Panel-A (top), the proportion of time adult male ( $n = 64$ ) and female ( $n = 79$ ) Ospreys surveyed in 2021–2022 across four southeastern US states spent in each behavior during the three types of nest survey and Panel-B (bottom), surveys with the three model sizes: small (350 – 400 mm), 2) medium (400 – 600 mm), and 3) large (600 – 900 mm) of unoccupied aerial systems (UAS).

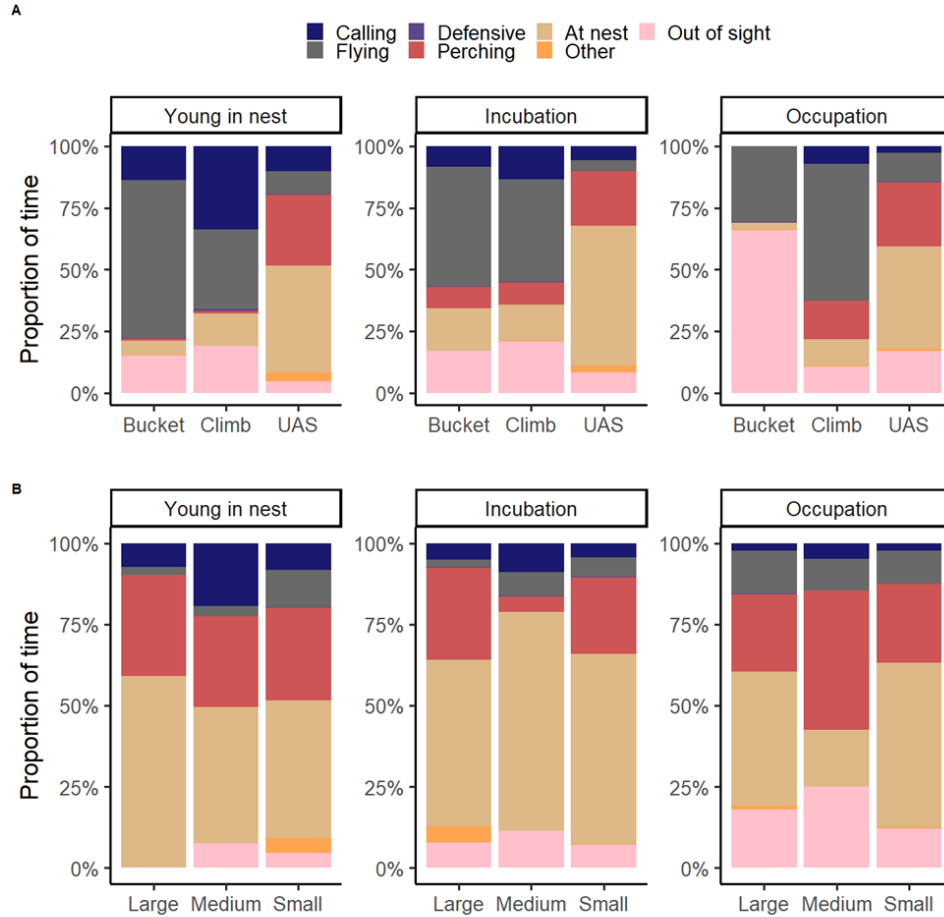


Figure 2.5 Panel-A (top), the proportion of time adult Ospreys surveyed in 2021 – 2022 across four southeastern US states spent in each behavior during each of the three nesting stages (young in nest = 66, incubation = 52, occupation = 48) for each of the three types of nest survey and Panel-B (bottom), the three model sizes; small (350 – 400 mm), 2) medium (400 – 600 mm), and 3) large (600 – 900 mm) unoccupied aerial systems (UAS).



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CHAPTER III

BREEDING SPACE USE AND MIGRATORY MOVEMENTS OF OSPREYS IN THE  
SOUTHEASTERN UNITED STATES

**Abstract**

Conservation and management of migratory species often requires a detailed understanding of their movement and space use across the annual life cycle. Various metrics from all stages, such as home range size during the breeding period and migratory stopover use, are important in developing conservation management for migratory taxa. I explored the breeding and post-breeding movements of 27 adult Ospreys in the southeastern United States (i.e., Alabama, Kentucky, Mississippi, Tennessee) to obtain such metrics. Average breeding Osprey home-range size was 248 km<sup>2</sup> (95% kernel density estimates) with an average core (50% kernel density estimates) home range of 38 km<sup>2</sup>. Home range size was influenced by sex and geographic region, with female Ospreys in Tennessee having much larger home ranges than birds of either sex from other areas. Ospreys travelled 2.4 km from their nests daily and this metric may be useful for nest relocation efforts. I followed the migration of 14 adult individuals and identified the first record of a resident Osprey remaining on the breeding range during the winter on the Alabama Gulf Coast. During migration within the United States, meteorological conditions (e.g., thermal uplift potential, precipitation, wind speed) appear to have little impact on the migration status of Ospreys, highlighting the adaptable migration strategies of this raptor. Ospreys stopped most frequently and for longer periods of time at night and stopover sites were

adjacent to open water and wetlands. I identified key conservation areas and waterbodies used by Ospreys during stopovers in Florida, a migration convergence corridor for migrating Ospreys. Southern Florida, including the Everglades National Park, appeared to be particularly important for staging prior to travelling across the Florida Straits. My findings contribute to general migration ecology theory, highlight areas for future research, and identify possible metrics relevant to conservation and management action.

## **Introduction**

Birds use a variety of landscapes and experience dynamic conditions and access to resources throughout their annual life cycle stages (i.e., reproductive, non-breeding, and migration; Marra et al., 2015; Tonra et al., 2019; Krøgenes, 2021; Stanley et al., 2021; Lalla et al., 2022). During the reproductive period, for instance, individuals require access to nest sites and plentiful food resources to feed themselves and their young. In northern latitudes, temperature changes and reduced prey availability in the winter drive individuals to exploit alternative resources in the non-breeding season, either by prey-switching within the breeding area or migrating to resource-rich, warmer regions (Panzeri et al., 2014; Kassara et al., 2017; Bühler et al., 2023). Migratory species may travel thousands of kilometers from their reproductive range to find suitable non-breeding sites to overwinter, such as the Amur Falcon (*Falco amurensis*), which breeds in eastern Siberia and winters in southern Africa (Orta et al., 2020). Multiple migration strategies can exist within a species (e.g., migrants or non-migrants, complete or partial migrants), varying geographically or within populations, resulting in the need to follow individuals from various populations throughout the annual cycle to fully describe migratory behavior (Martell et al., 2004; Monti et al., 2018a; Bossu et al., 2022).

To conserve and manage migratory species appropriately, biologists and managers require detailed understanding of their movement and space use patterns across annual life cycle stages (Runge et al., 2014; Marra et al., 2015). In both the breeding and non-breeding seasons, home range size and daily distances travelled are relevant metrics for conservation or management actions, such as evaluating the utility of protected areas (Limiñana et al., 2015; Montillo et al., 2022), mitigating human-wildlife conflict (Ng et al., 2022), informing nest relocation (Parayko et al., 2021), or creating supplemental habitat (Arroyo et al., 2014). Information on migration initiation dates will also identify when it may be best to engage in management actions, such as structure maintenance or habitat restoration, without disturbing protected species. Identifying populations as full or partial migrants will also influence the timing and applicability of management decisions (Monti et al., 2018b). Finally, during the migratory period, details on stopover use and migratory routes are critical to ensuring key stopover locations and migratory corridors are managed and infrastructure developments, such as wind farms, are avoided (Villegas-Patraca et al., 2014; Pearse et al., 2021; Watson, 2021)

Ospreys (*Pandion haliaetus*) are cosmopolitan raptors that have resident, partial migrant, and long-distance migrant populations across the globe (Monti, 2021; Washburn et al., 2014; Poole, 2019). Ospreys typically migrate from northern latitudes (between 40° and 70°N) to the global south (Washburn et al., 2014; Poole, 2019) although resident populations can be found in Florida, the Caribbean, the Mediterranean, Australia, and southeast Asia (Martell et al., 2004; Dennis and Clancy, 2014; Wiley et al., 2014; Monti et al., 2018b), with some partial migrants being recorded in Florida, the Mediterranean, and Japan (Martell et al., 2004; Shoji et al., 2011; Monti, 2021). In the Southeastern United States, it is unclear if Ospreys are migratory, partial migrants, or residents and therefore, how management must tailor to these populations is also



uncertain. Throughout their annual cycle, Ospreys display a high degree of fidelity to individual-specific breeding sites, stopovers, and wintering areas, so understanding their space use across the entire annual cycle has direct implications for the management of the landscapes they occupy (Vardanis et al., 2016; Meyburg and Holte, 2023).

For the breeding season, few data are available on home range size for Osprey and home range estimates vary widely (e.g., 0.0008 – 4.17 km<sup>2</sup>), although there seems to be some evidence for larger home ranges in male Osprey while female activity is centered at the nest (Bedrosian et al., 2015; Bierregaard et al., 2020). While migration route fidelity in Ospreys is low, populations in the southeastern United States are mostly migratory and routes tend to converge at geographic bottlenecks (e.g., Straits of Gibraltar; Alerstam et al., 2006) offering possible areas for targeted management actions. Ospreys are also flexible migrants as they use both soaring-gliding and energy-demanding flapping flight when necessary. During migration, Ospreys may fast, engage in lengthy refueling stopovers, or use a fly-and-forage strategy (Monti, 2021; Strandberg and Alerstam, 2007; Mellone et al., 2015; Mackrill, 2017; Duriez et al., 2018). As such, identifying important stopover sites for Ospreys may be difficult. Previous studies have explored Osprey stopover during migration on a daily scale (Hake et al., 2001; Crawford and Long, 2017; Monti et al., 2018a), but this may result in the underestimation of stopover use, especially when stopping for short foraging bouts or if immediate weather conditions influence stopover (e.g., Mallon et al, 2021).

Here, I explored movements of Ospreys while breeding in the southeastern United States and migrating southbound over land. I expected nesting outcome and sex to influence home range size and daily movement distances during the breeding season. I also examined the timing of migration onset, stopover use, potential route bottlenecks, and potential wintering sites of

Ospreys in the southeastern United States. During migration within the United States, I explored the influence of immediate meteorological conditions (e.g., temperature, precipitation, thermal uplift potential, windspeed) and landcover variables (e.g., distance to open water) on stopover initiation and duration. I expected that Ospreys in the southeastern United States would be mainly migratory, and that they would respond to unfavorable weather conditions (e.g., high precipitation, low thermal uplift potential) by pausing their migration and having longer stopovers.

## **Methods**

### **Study area**

I sampled Ospreys from five waterbodies representing distinct populations in the southeastern United States (Figure 3.1): Tennessee-Tombigbee Waterway (Mississippi), Guntersville Reservoir (north Alabama), Gulf State Park (coastal Alabama), Kentucky Reservoir (Kentucky), and Watts Bar Reservoir (Tennessee). The Tennessee-Tombigbee Waterway is a canal that completed construction in 1984 and dams constructed in the 1930s – 1940s impound the three reservoirs. These waterbodies had high nest site accessibility for capture of adults and were located within the Eastern temperate forest ecoregion (CEC, 1997). This ecoregion is characterized by broadleaf, deciduous trees, and needle-leaf conifers with elevation ranging from 0 – 2,000 m (CEC, 1997; USGS, 2019). Within the timeframe for adult captures (April – May), average daily maximum temperatures range from 19°C – 29°C, and yearly precipitation ranges from 1,000 – 1,500 mm (CEC, 1997; NOAA, 2020). Across this area, Ospreys are found near the coast, freshwater waterways, lakes, and reservoirs during the breeding season (March – August; Bierregaard et al., 2020).

## **Capture and transmitter deployment**

Between 2019 – 2022 a total of 27 (21 ♀, 6♂; Table C.1) adult Ospreys were captured on the nest during the occupation or incubation breeding stage using a bow-net or noose carpet placed directly over the nest. Each captured bird was fitted with GPS-GSM solar-powered telemetry units (ES-400-50-BKP, Americas Version, Cellular Tracking Technologies LLC, Rio Grande, NJ, USA) secured with Teflon™ ribbon using the backpack method (Kenward, 1987). All birds were also banded with standard U S. Geological Survey aluminum leg bands and alpha-numeric leg bands. Of the 27 individuals tagged, eight (7♀, 1♂) were on Guntersville reservoir, two (1♀, 1♂) in coastal Alabama, six (4♀, 2♂) on the Cumberland River, six (6♀) on Watts Bar reservoir, and five (3♀, 2♂) on the Tennessee-Tombigbee waterway. Mean body mass for adult Ospreys was  $1,812.8 \pm 168.4$  g for females and  $1,475 \pm 93.8$  g for males. Based on these measures, and total package mass of 45g, the GPS-GSM transmitters were within recommended weight limits (<3% body mass; Kenward, 2001). Location fixes (nominal accuracy  $\pm 15$  m) were taken every five minutes to an hour and upload cycles were scheduled daily. Trapping and tagging efforts were carried out under associated IACUC (#21-168) and banding permits (#23835).

## **Daily distances travelled and home ranges during the breeding season**

To determine individual departure dates of tagged Ospreys from their breeding grounds as well as segment movement paths into sedentary and southbound migration, I used net squared displacement (NSD; Singh et al., 2016; Soriano-Redondo et al., 2020). I identified migration initiation as the first point at which NSD continuously increased away from the breeding range and the end of migration as the first point at which NSD values plateaued as individuals reached the wintering grounds (Buechley et al., 2018; Phipps et al., 2019). Sedentary breeding range data

were resampled (R package *amt*; Signer et al., 2019) to exclude fixes within 15 m of the nest site and to a standardized fix rate of 1 hour (with a tolerance of  $\pm 15$  minutes) to mitigate spatial bias in subsequent analyses.

I used minimum convex polygons (MCPs; total using 100%) and kernel density estimates (KDEs; core using 50% and total using 95%) to determine breeding space use using R package *adehabitatHR* (Calenge, 2006). While MCPs are heavily biased by extremes in an animal's movements (Laver and Kelly, 2005), I included this method to allow direct comparisons to previous work on Osprey space use (e.g., Galarza and Dennis, 2009; Bedrosian et al., 2015; Krøgenes, 2021; Meyburg et al., 2023). As there was substantial variance in the size of KDEs, I log-transformed home range areas and used simple linear regression to test for differences in home range size in relation to the sampled population as well as an interaction between sex and nest success (i.e., successfully fledged young, failed to fledge young, or outcome unknown). Nest success was determined via nest monitoring post-transmitter deployment as described in Chapter II. For breeding season data, I also calculated average daily distances from nest sites (distCosine R package *geosphere*; Hijmans, 2022) and I compared these distances using simple linear regression with population and an interaction between sex and nest success as predictor variables.

### **Initiation, daily distances travelled, and stopover during migration**

To examine migratory stopover behavior, movements outside the United States were excluded so that movements over open ocean or areas without detailed landcover data were not considered (resulting in the exclusion of data from three Ospreys: Figure C.1). These data were then resampled (R package *amt*; Signer et al., 2019) to a standardized fix rate of 15 minutes (with a tolerance of  $\pm 5$  minutes) to mitigate spatial bias in subsequent analyses. While other

studies define an individual as stopped when moving <100 km within a 24-h interval during migration (Hake et al., 2001; Crawford and Long, 2017; Monti et al., 2018a), this daily scale of identification may result in the underestimation of stopover use in response to fine-scale weather changes. For instance, soaring raptors have been shown to use short duration stopovers frequently in response to their immediate environment (e.g., Mallon et al., 2021). Therefore, I identified stopovers by distance travelled, as there was a clear breakpoint between stationary and migratory states based on NSD (Figure C2). Based on this clear breakpoint, I defined a stopover as any period where the bird travelled <500 m. To ensure distinction between these states, I removed a total of 155 locations that fell between 499 - 500 m. As migratory routes seemed to converge through Florida (see Results), for each stopover location in Florida I identified key conservation areas and waterbodies used by Ospreys during stopovers (n = 113) using Florida Natural Areas Inventory (FNAI, 2023) and National Landcover Database (Dewitz, 2019) data.

Across all stopover locations, I examined stopover occurrence and duration in relation to environmental predictor variables thought to be related to Osprey behavior including distance to various landcover types and a variety of weather variables. I included distance to eight landcover categories derived from the National Landcover Database (Dewitz, 2019; Table C.2): open water, wetlands (woody and emergent herbaceous), forest (deciduous, evergreen, and mixed), barren, shrub, development (developed open space and low to high intensity), cultivated cropland (hereafter, agriculture), and grassland (pasture/hay and all herbaceous landcover types). However, distances to six landcover types (shrubs, agriculture, development, barren, forest, and grassland) were highly positively correlated (Pearson's correlation coefficient,  $r > 0.7$ ) with one another for both occurrence and duration datasets. As a result, I conducted a principal component analysis (PCA; R package *factoMineR*; Lê et al., 2008) using the centered and scaled correlated

landcover types to reduce dimensionality and the number of variables included in my models. I retained the first axis as this axis explained >80% of the variance across variables and this was the only axis with an eigen value >1 (Tables C.3 and C.4; Figure C.5). I then used this information in subsequent models in place of the six landcover types.

I obtained weather data associated with thermal soaring conditions or inclement weather (see Table C.5 for definitions of weather variables) from Movebank and the European Centre for Medium-Range Weather Forecasts using Movebank's Env-data annotation feature with bilinear interpolation (Dodge et al., 2013). I removed boundary height due to positive correlation with temperature ( $r = 0.72$ ) and negative correlation with surface sensible heat flux ( $r = -0.81$ ). To identify differences in stopover occurrence and duration at different times of day, I created a period of day variable which defined morning (0530 h – 1059 h), afternoon (1100 h – 1659 h), evening (1700 h – 1959 h), and night (2000 h – 0529 h) periods.

To examine stopover occurrence, I used a generalized linear model (GLM) with a binomial distribution and logit link. I included migratory state as a binary response variable whereby stopover was coded as '1' and active migration was coded as '0'. I modeled the binary response of stopover occurrence against the predictor variables of distance to landcover types that were not water (i.e., PC1), distance to open water, distance to wetlands, and weather variables (including surface sensible heat flux, air pressure, orographic updraft velocity, precipitation fraction, temperature, wind speed; see Table C.5 for definitions of weather variables), and period of day.

To examine stopover duration, I used a generalized linear mixed model (GLMM) with a negative binomial distribution (R package *glmmTMB*; Brooks et al., 2017) given that the response variable was over dispersed (using R package *performance*; Lüdtke et al., 2021;

dispersion ratio = 195.6). I modeled the duration of stopover (in minutes) as the response variable against the same predictors included in our stopover occurrence model, except predictors were now averaged over the length of the stopover duration, while time of day was defined by the time at the start of the stopover. I included a nested random effect of individual tagged Osprey within waterbody to account for variation associated with behaviors of individuals and similarities within locations where the birds were initially tagged. For both analyses, all numeric variables were scaled, and effects plots were created using R package *effects* (Fox and Weisberg, 2019). Finally, sex was excluded from both models as a predictor variable because of the lack of data for males ( $n = 4$ ). As sex does not appear to influence southbound stopover initiation or duration in Ospreys (Bierregaard et al., 2020; Anderwald et al., 2021), I believe this omission does not bias the results I present below.

## Results

On average, Ospreys (21 ♀, 6♂) were tracked for  $110 \pm 72$  days (range = 10 – 352 days), with overall tracks representing a total of 397,551 GPS location fixes, with an average of 121 fixes per bird per day (range = 18 – 279; Table C.1). I had breeding season information on all 27 individuals, with transmitter failure before migration initiation on 12 birds. As a result, I had information during the migration period for 15 individuals (11♀, 4♂; Figure C.1), including one male who did not migrate. During the breeding season, adult Ospreys travelled an average of  $2.4 \pm 6.9$  km from their nests per day. I found no influence of population, sex, or nesting success on distance travelled per day from the nest location (Table 3.1). Average breeding Osprey home-range size was 731 km<sup>2</sup> using 100% MCP (range = 0.06 – 8,915 km<sup>2</sup>) and 248 km<sup>2</sup> (range = 0.03 – 4,971 km<sup>2</sup>) using 95% KDE (Figure C.3). Average core (50% KDE) home range size was 38 km<sup>2</sup> (range = 0.003 – 777 km<sup>2</sup>). While I had a low sample size of males ( $n = 6$ ), there seemed to

be sex-specific differences in home range size (Table 3.1). Typically, males had smaller home ranges than females (95%; males = 201 km<sup>2</sup>, females = 261 km<sup>2</sup>; 50%; males = 24.8 km<sup>2</sup>, females = 41 km<sup>2</sup>). I also found that Ospreys (6 females) at Watts Bar Reservoir (Tennessee) had larger home ranges than birds from other locations (Table 3.1). Core home ranges in Tennessee were typically 20 times larger ( $142 \pm 312$  km<sup>2</sup>) than birds from other populations ( $7 \pm 31$  km<sup>2</sup>) and 95% home ranges were 14 times larger ( $897 \pm 2,001$  km<sup>2</sup>) than other populations ( $62 \pm 237$  km<sup>2</sup>). While there was variability in nesting outcome, with a total of 17 nests successfully fledging young following transmitter deployment, seven nests experiencing breeding failure, and three with unknown outcomes, nest outcome did not influence home range size (Table 3.1). Average 95% home range size for successful breeders was 325 km<sup>2</sup> (0.03 – 4,971 km<sup>2</sup>) and 164 km<sup>2</sup> (0.09 – 1095.8 km<sup>2</sup>) for failed breeders.

Of the 15 birds that had information during the migration period, the single male in coastal Alabama that did not migrate remained associated with the nest location within Gulf State Park, Alabama throughout the annual cycle. All 14 Ospreys that did migrate, migrated in a southeastern direction, travelling through Florida (Figure C.1). I was able to track only three adults outside the United States, each of which travelled to Cuba (Figure C.1). One male originating from north Alabama remained in Cuba during the wintering period and one female originating from the same reservoir travelled from Cuba to Honduras before continuing to her wintering site in Peru (Figure C.1). Another male, originating from north Mississippi, travelled from Cuba to the Dominican Republic then across to Venezuela to his wintering site in Brazil (Figure C.1). Migrants had an average departure date from the breeding areas of August 19, with migration starting between July 26 – September 8. While migrating through the United States, Ospreys travelled an average of  $3.6 \pm 4.8$  km per day (range = 590 m – 15 km per day).



During migration, Ospreys stopped an average of  $2.6 \pm 1.3$  times per day and I identified a total of 273 stopover sites. Time of day influenced migration status with Ospreys more likely to stopover at night and in the morning (Table 3.2, Figure 3.2). Ospreys most frequently initiated stopovers at night (typically between 2100 – 0000 h), resuming migration the following afternoon (typically between 1100 – 1300 h; Figure C.4). Only 12% of migration movements began after 2000 h, with nine Ospreys migrating nocturnally for an average of  $23.2 \pm 21.2$  minutes (range = 10 – 93 minutes). Stopovers were highly associated with water as Ospreys typically stopped within 504 m of open water (range = 0 – 5.1 km) and within 376 m (range = 0 – 4 km) of wetlands (Table 3.2, Figure 3.2). In comparison, active migration over land typically occurred 1.9 times further from water than did stopovers. Stopover occurrence was influenced by temperature, precipitation fraction, and heat flux (Table 3.2, Figure 3.2). Average temperatures during stopovers were 16% lower (mean = 25 °C, range = 14 – 33 °C) than those recorded during active migration (mean = 30 °C, range = 20 – 38 °C; Figure 3.2). Precipitation fraction was 1.6 times higher during stopovers (706, range = 0 – 3600) compared to active migration (430, range = 0 – 3586). Ospreys were also more likely to pause migration when heat flux was close to zero or positive (Figure 3.2) indicating little to no thermal uplift potential. Other weather variables were not associated with stopover occurrence (Table 3.2, Figure 3.2). Stopover length was 12.2 hours on average (range = 10 minutes – 40 hours) and this duration was influenced by time of day at stopover initiation (Table 3.2). Stopovers which began at night were typically 2.2 times longer than stopovers initiated during the rest of the day (Figure 3.3). Duration of stopover was influenced by temperature and heat flux, but not the composition of the surrounding landscape (Table 3.2). Stopovers were typically longer when temperatures were lower and heat flux values were negative (Figure 3.3).

Within Florida, I identified 113 stopovers (41.3% of total stopovers) and found that 56.6% (n = 64) of stopovers occurred inside Florida's conservation lands (Figure 3.4). Conservation lands which were important for stopovers included the Everglades National Park, Withlacoochee State Forest, and Apalachicola River Management Area (Figure 3.4). While very large lakes, such as Lake Okeechobee, were not used during stopovers, Ospreys made use of rivers with 64 stopovers adjacent to a major river (Figure 3.4). Overall, 91 stopovers occurred either within conservation lands, adjacent to a major river, or both, with the tip of Florida being a major stopover point before heading across the Florida Straits.

## **Discussion**

My research provided information on Osprey movements and space use during the breeding season and on their southbound migration from their breeding grounds in the southeastern United States. I found home range estimates for Ospreys which were smaller than those recorded in Norway (i.e., median 95% MCP = 87.20 km<sup>2</sup>; Krøgenes, 2021), but larger than estimates from Wyoming (i.e., median 95% MCP = 1.75 km<sup>2</sup>). Regional and sex differences I identified in home range size were likely influenced by female Ospreys in Tennessee leaving the core home range prior to the young dispersing, a behavior frequently recorded in Ospreys in response to competition for resources with young (Martell et al., 2001; Bedrosian et al., 2015; Meyburg et al., 2023). For example, one female in this study travelled approximately 220 km from her nest site to a reservoir in Georgia and another extended her range to Nantahala River, North Carolina, approximately 108 km from her nest site. As no females at other locations in my study engaged in these movements and had comparatively small home ranges, this behavior may be tied to resource availability at Watts Bar reservoir, Tennessee. Like many raptors, home range size for Ospreys is likely resource-dependent and range may expand or contract in response to

prey availability (Marquiss and Newton, 1981; Peery, 2000). I also noted that nest density was high at the Tennessee site due to the installation of artificial nest platforms ( $n = 14$ ) that were close together (mean distance = 99.5 m, range = 37 – 232 m), which may have caused an ecological trap by placing attractive nesting opportunities in a low resource area or increasing disturbance from adjacent pairs, each of which may contribute to increased female movements before young disperse. While I was unable to account for prey abundance or local impacts on resource availability (e.g., competition, reservoir drawdown operations), I suggest future studies focusing on breeding movement at this site would help elucidate the reason for this variation in female movements.

Buffers placed around nests to act as impact zones or areas in which nest relocation may be most effective are often based on core home range size (e.g., Parayko et al., 2021). In this study I found that even core home range estimates were very large (i.e., 38 km<sup>2</sup>), and that this occurred even though Ospreys typically only travelled 2.4 km from their nests per day. This suggests that home range sizes were biased by rare long-distance movements, such as those seen in female Ospreys nesting in Tennessee. As such, it may not be practical to use Osprey core home range size as a basis for creating impact zones or deciding where to provide artificial nesting opportunities when original nests must be moved. Commonly, Osprey nests are relocated within 20 – 100 m of the original nest (APLIC, 2006) but, based on my findings, I would recommend continuing to relocate Osprey nests within line of sight of the original nest or placing 1 – 2 artificial nesting opportunities within a 2 km buffer (based on daily distances travelled) of the original nest.

Departure dates (August 19, range = July 26 – September 8) for southbound migration were like those recorded in previous studies of Ospreys on the east coast of North America. For

example, Martell et al. (2001) found a median date of August 19 (range = July 12 – September 9) for southbound migration. Ospreys in my study also followed a similar path to that described for east coast Ospreys (Martell et al., 2001), with all individuals travelling through Florida, and the three adults tracked outside the US travelling onwards to Cuba. However, one female tracked in my study travelled through Honduras before continuing to Peru which deviates from most migratory patterns (i.e., east to Haiti and south over the Caribbean; Martell et al., 2001; Martell et al., 2014), but has been documented before (Martell and Douglas, 2019). I also identified a non-migratory resident male and migratory female at the same location (i.e., Gulf State Park, Alabama). In Florida, Ospreys can be migrants, partial migrants, or non-migrants (Martell et al., 2004) and information from this study may suggest similar migratory patterns in Ospreys along the Gulf Coast, with a mix of resident and migrant individuals within the same population. Given my low sample size, it is uncertain the exact proportion of the population that may reside in either category, although previous satellite tracking work suggests that these populations may follow a 50:50 split between migrants and residents (Martell et al., 2004). I recommend further study of Osprey populations in the Gulf Coast region to fully elucidate these patterns. Considering ongoing conservation issues on the Gulf Coast, such as land loss, increasing human populations, and pollution events, e.g., oil spills (Gulf Coast Restoration Council, 2013; Sesser et al., 2022), this type of basic ecological knowledge can inform best practices for managing populations. For example, the close association of the resident male with Gulf State Park suggests these conservation lands are important to Ospreys in all parts of the annual cycle.

Over one-half of stopovers identified in Florida occurred within conservation lands, also highlighting the importance of these areas for migratory birds. Notably, the Everglades National Park was frequently used for staging prior to crossing the Straits of Florida to Cuba. While

Ospreys appear to be selecting stopovers near water and wetlands, which suggests stopover locations were selected for foraging opportunities, large lakes were avoided. As Florida's lakes support some of the largest concentrations of nesting Ospreys in the world (Martell et al., 2004; McMillian, 2013), these areas may be avoided by migrant Ospreys due to competition with large numbers of resident individuals and potential migrants wintering in Florida. Instead, migrant Ospreys seem to be using major rivers during stopovers in Florida and many of these rivers, such as the Apalachicola River, are within conservation areas. This further emphasizes the importance of maintaining healthy river systems for both resident and migrating birds, such as Ospreys.

Meteorological conditions frequently impact soaring migrants, such as raptors, as they depend on uplift to assist in soaring or gliding (Richardson, 1990; Bohrer et al., 2012; Lanzone et al., 2012). However, Ospreys are flexible migrants who engage in a variety of flight patterns and migration strategies (Monti, 2021; Strandberg and Alerstam, 2007; Mellone et al., 2015; Mackrill, 2017; Duriez et al., 2018). As such, Ospreys may not be strongly dependent on thermal uplift during migration. The mixed response to sensible surface heat flux, my proxy for thermal uplift potential, illustrates this as Ospreys were more likely to pause migration when uplift potential was low (i.e., values were close to zero or positive) but tended to stop for longer when uplift potential was high (i.e., values were negative). This mixed response may suggest that Ospreys are not entirely dependent on thermal uplift potential during migration and this selection for stopovers when uplift potential was low may simply reflect the need for Ospreys to stopover at night to sleep, when there are no thermals (Thorup et al., 2006). Ospreys in my study also migrated at night (12% of active migration), which is not uncommon for the species (Alerstam et al., 2006), and again suggests a lack of dependency on thermal uplift during migration. I also found a lack of response to orographic uplift velocity, suggesting that orographic uplift may not

be important for Ospreys migrating through this area. Indeed, orographic soaring is correlated with windy weather conditions and mountainous terrain slopes, which are not characteristic of the southeastern United States (CEC, 1997; Murgatroyd et al., 2018).

My findings show that Ospreys do not pause migration for great lengths of time due to changes in weather conditions, but it must be noted that these data represent only southbound migrations and birds may respond differently to weather conditions on northbound migrations as they are presented with an urgency to return to and defend their nest for the breeding season (Kokko, 1999; Dodge et al., 2014). Temperature influenced migration status and stopover duration, with Ospreys stopping more frequently and for longer periods of time when temperatures were among the lower end of those values recorded in my study. This reduced mobility of migrating Ospreys during periods of relatively low temperatures likely reflects the temperature decreases that occur at night when Ospreys were more likely to pause migration and rest for longer periods. Like Thorup et al. (2006), I found no influence of wind speed on Osprey movements, suggesting that winds may not directly impact stopover decisions in Ospreys. I did however find that precipitation influenced migration status as Ospreys were more likely to stopover when precipitation was relatively high, but precipitation did not influence stopover duration. While other soaring migrants will experience unfavorable conditions (i.e., low thermal uplift potential) in rain (Richardson, 1990; Mallon et al., 2021), the fact that Ospreys here were more likely to stopover during precipitation events is curious as Ospreys have been seen migrating during periods of precipitation (Thorup et al., 2006). However, rain has been shown to decrease the number of actively foraging Ospreys while not influencing foraging performance (Flemming and Smith, 1990; Machmer and Ydenberg, 2011). This known relationship between Ospreys and precipitation may help explain why precipitation influenced migration status but not

stopover duration; Ospreys may avoid flying in rain but may not require lengthy stops to effectively refuel during rain events and can resume migration during rain if needed, compared to other raptors who experience reduced foraging performance in rain or must wait for thermal conditions to improve prior to resuming migration (Rijnsdorp et al., 1981; Dawson and Bortolotti, 2000; Mallon et al., 2021).

In this chapter, I used a clear breakpoint between stationary and migratory states based on NSD to define a stopover as any period where the bird travelled <500 m. Compared to classical work, this definition is more quantitative. Although this definition allowed for standardized classification of behavioral segments, it is limited in it is not possible to directly compare my results to previous work (e.g., Hake et al., 2001; Monti et al., 2018a). I recognize that this approach also had other limitations. For instance, a small percentage of migratory movements occurred at night over land, which may be an artifact of the method oversimplifying behavioral segmentation. While nocturnal migration over water is not uncommon for Ospreys (Alerstam et al., 2006), it is unlikely that Osprey migrate at night over land (R. Bierregaard, pers comm). In these instances, manual quality control could be applied to identify points that were incorrectly classified. However, that approach also removes the benefits of standardized classification. Overall, I believe this method is useful and could be refined by the inclusion of additional measures, such as turn angles and travel speed, to better define the behavioral switch. Future research should aim to model multiple movement metrics for defining behavioral segments into ‘migration’ and ‘stopover’ (e.g., a two-state hidden Markov model; Patterson et al., 2009; Klappstein et al., 2023) to improve our confidence in the automated classification of stopovers.

Through this research, I aimed to inform conservation and management of Ospreys in general by deepening scientific understanding of their movement and space use patterns across

annual life cycle stages. I also identified key aspects of the ecology of these raptors in the southeastern United States, including breeding home range size, migration initiation dates, migration status, and how Osprey move in relation to stopover sites and migration routes. To the extent of my knowledge, I also reported the first instance of a resident Osprey wintering in coastal Alabama. Further investigations into the migration status of Ospreys in the Gulf Coast region would be required to inform best practices for managing potential resident Osprey populations in these areas. In addition, I believe future research should aim to compare response to meteorological conditions and stopover use across years, sexes, and between south- and northbound migrations. My findings contribute to general migration ecology information for this protected raptor species, provides valuable comparisons for these recommended further investigations, and offers insight into potential management action.



## Tables & Figures

### Tables

Table 3.1 The effect of tagged location, sex, and nest success on the daily distance travelled (m) from the nest and total and core home range size of 27 adult Ospreys (km<sup>2</sup>) in the southeastern United States (2019 – 2022).

<i>Predictor Variable</i>	<i>Daily Distances</i>		<i>Total Home Range Size</i>		<i>Core Home Range Size</i>	
	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>
Location (coastal)	260.9 ± 6867.9	0.97	2.57 ± 2.47	0.31	2.49 ± 2.29	0.29
Location (Kentucky)	-508.7 ± 4829.8	0.91	0.96 ± 1.73	0.58	1.39 ± 1.61	0.39
Location (Mississippi)	2288.4 ± 5312.7	0.67	0.63 ± 1.91	0.74	1.46 ± 1.77	0.41
Location (Tennessee)	6634.6 ± 4588.2	0.16	4.30 ± 1.65	<b>0.01</b>	4.94 ± 1.53	<b>&lt;0.001</b>
Sex (male)	8313.6 ± 6514	0.21	5.34 ± 2.34	<b>0.03</b>	5.35 ± 2.17	<b>0.02</b>
Nest success (successful)	2621.6 ± 4210.6	0.54	0.04 ± 1.51	0.97	0.52 ± 1.40	0.71
Nest success (unknown)	2501.6 ± 7176.2	0.73	2.70 ± 2.58	0.31	3.31 ± 2.40	0.18
Sex:Nest Success (successful)	-8826.2 ± 8172.8	0.29	-2.92 ± 2.94	0.33	-3.74 ± 2.73	0.18
Sex:Nest Success (unknown)	-8426.3 ± 11332.5	0.46	-6.99 ± 4.08	0.10	-7.43 ± 3.79	0.06

*P* values <0.001 are reported as <0.001, otherwise exact values are provided. Any *P* values <0.05 are in **bold**. Degrees of freedom for all three models = 9.

Table 3.2 The effect of tagged location, sex, and nest success on the daily distance travelled (m) from the nest and total and core home range size of 27 adult Ospreys (km<sup>2</sup>) in the southeastern United States (2019 – 2022).

<i>Predictor Variable</i>	<i>Daily Distances</i>		<i>Total Home Range Size</i>		<i>Core Home Range Size</i>	
	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>	$\beta \pm SE$	<i>P</i>
Location (coastal)	260.9 ± 6867.9	0.97	2.57 ± 2.47	0.31	2.49 ± 2.29	0.29
Location (Kentucky)	-508.7 ± 4829.8	0.91	0.96 ± 1.73	0.58	1.39 ± 1.61	0.39
Location (Mississippi)	2288.4 ± 5312.7	0.67	0.63 ± 1.91	0.74	1.46 ± 1.77	0.41
Location (Tennessee)	6634.6 ± 4588.2	0.16	4.30 ± 1.65	<b>0.01</b>	4.94 ± 1.53	<b>&lt;0.001</b>
Sex (male)	8313.6 ± 6514	0.21	5.34 ± 2.34	<b>0.03</b>	5.35 ± 2.17	<b>0.02</b>
Nest success (successful)	2621.6 ± 4210.6	0.54	0.04 ± 1.51	0.97	0.52 ± 1.40	0.71
Nest success (unknown)	2501.6 ± 7176.2	0.73	2.70 ± 2.58	0.31	3.31 ± 2.40	0.18
Sex:Nest Success (successful)	-8826.2 ± 8172.8	0.29	-2.92 ± 2.94	0.33	-3.74 ± 2.73	0.18
Sex:Nest Success (unknown)	-8426.3 ± 11332.5	0.46	-6.99 ± 4.08	0.10	-7.43 ± 3.79	0.06

*P* values <0.001 are reported as <0.001, otherwise exact values are provided. Any *P* values <0.05 are in **bold**. Degrees of freedom for all three models = 9.

Table 3.3 The effect of distance (m) to landcover (open water, wetland, and all other landcover types captured by PC1 where PC1 explained >80% of the variation in the data set), weather, and time of day on stopover occurrence and duration of adult Ospreys.

Stopover Occurrence	$\beta \pm SE$	<i>P</i>	Stopover Duration	$\beta \pm SE$	<i>P</i>
Precipitation Fraction	-0.11 $\pm$ 0.04	<b>0.011</b>	Precipitation Fraction	-0.11 $\pm$ 0.06	0.08
PC1	-0.09 $\pm$ 0.04	<b>0.014</b>	PC1	0.05 $\pm$ 0.05	0.34
Sensible Heat Flux	0.72 $\pm$ 0.06	<b>&lt;0.001</b>	Sensible Heat Flux	-0.56 $\pm$ 0.10	<b>&lt;0.001</b>
Air Pressure	-0.09 $\pm$ 0.06	0.15	Air Pressure	0.05 $\pm$ 0.08	0.50
Orographic Updraft	-0.002 $\pm$	0.94	Orographic Updraft	0.10 $\pm$ 0.06	0.09
Velocity	0.03		Velocity		
Temperature	-1.83 $\pm$ 0.07	<b>&lt;0.001</b>	Temperature	-0.80 $\pm$ 0.07	<b>&lt;0.001</b>
Wind Speed	-0.01 $\pm$ 0.04	0.74	Wind Speed	0.12 $\pm$ 0.07	0.10
Distance to Water	-0.51 $\pm$ 0.03	<b>&lt;0.001</b>	Distance to Water	-0.07 $\pm$ 0.06	0.24
Distance to Wetlands	-0.07 $\pm$ 0.03	0.07	Distance to Wetlands	0.10 $\pm$ 0.06	0.14
Time of Day (evening)	0.80 $\pm$ 0.13	<b>&lt;0.001</b>	Time of Day (evening)	0.73 $\pm$ 0.29	<b>0.011</b>
Time of Day (morning)	2.81 $\pm$ 0.26	<b>&lt;0.001</b>	Time of Day (morning)	0.50 $\pm$ 0.48	0.13
Time of Day (night)	1.56 $\pm$ 0.11	<b>&lt;0.001</b>	Time of Day (night)	1.20 $\pm$ 0.23	<b>&lt;0.001</b>

*P* values <0.001 are reported as <0.001, otherwise exact values are provided. Any *P* values <0.05 are in **bold**. Degrees of freedom for GLM are 8603 (number of GPS locations used). Number of observations for GLMM: 273 (number of stopovers). GLMM random effects = 14 (individuals), 5 (waterbodies). Random effect contributed 2% variance.

## Figures

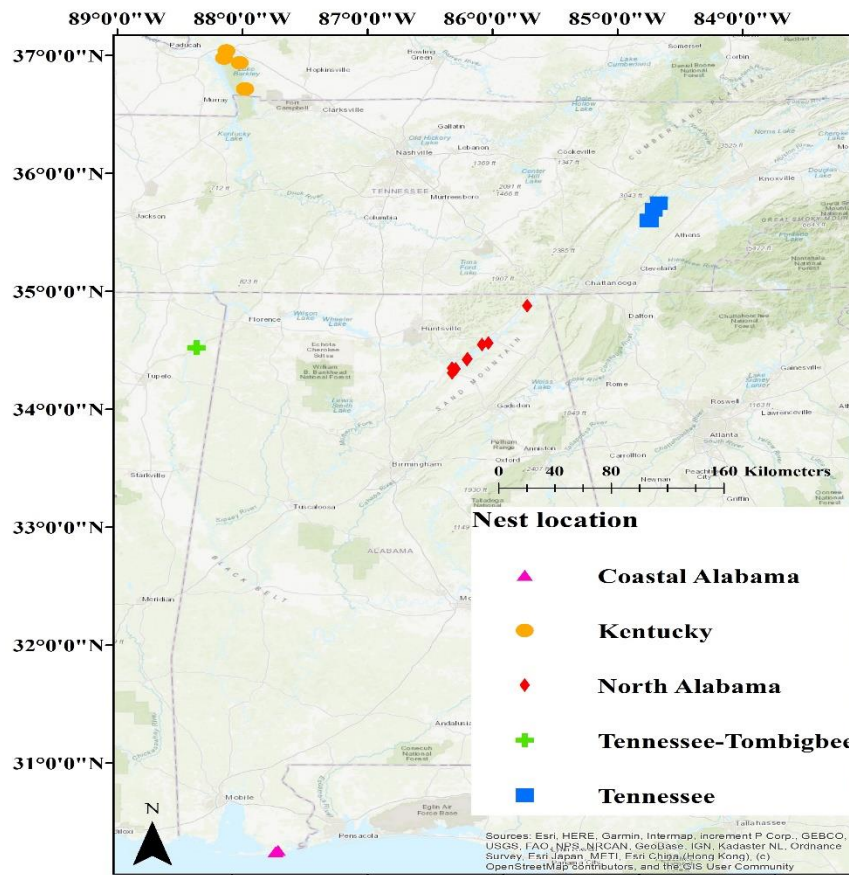


Figure 3.1 Locations (i.e., nests) where 27 individual adult Ospreys were captured and fitted with GPS transmitters across five waterbodies (Gulf Coast, Guntersville reservoir, Cumberland River, Tennessee-Tombigbee waterway, and Watts Bar reservoir) in the states of Alabama, Kentucky, Mississippi, and Tennessee, respectively. Nesting locations are imposed on a base map from OpenStreetMaps for context. Inset map shows the location of the four states in relation to the continental United States.

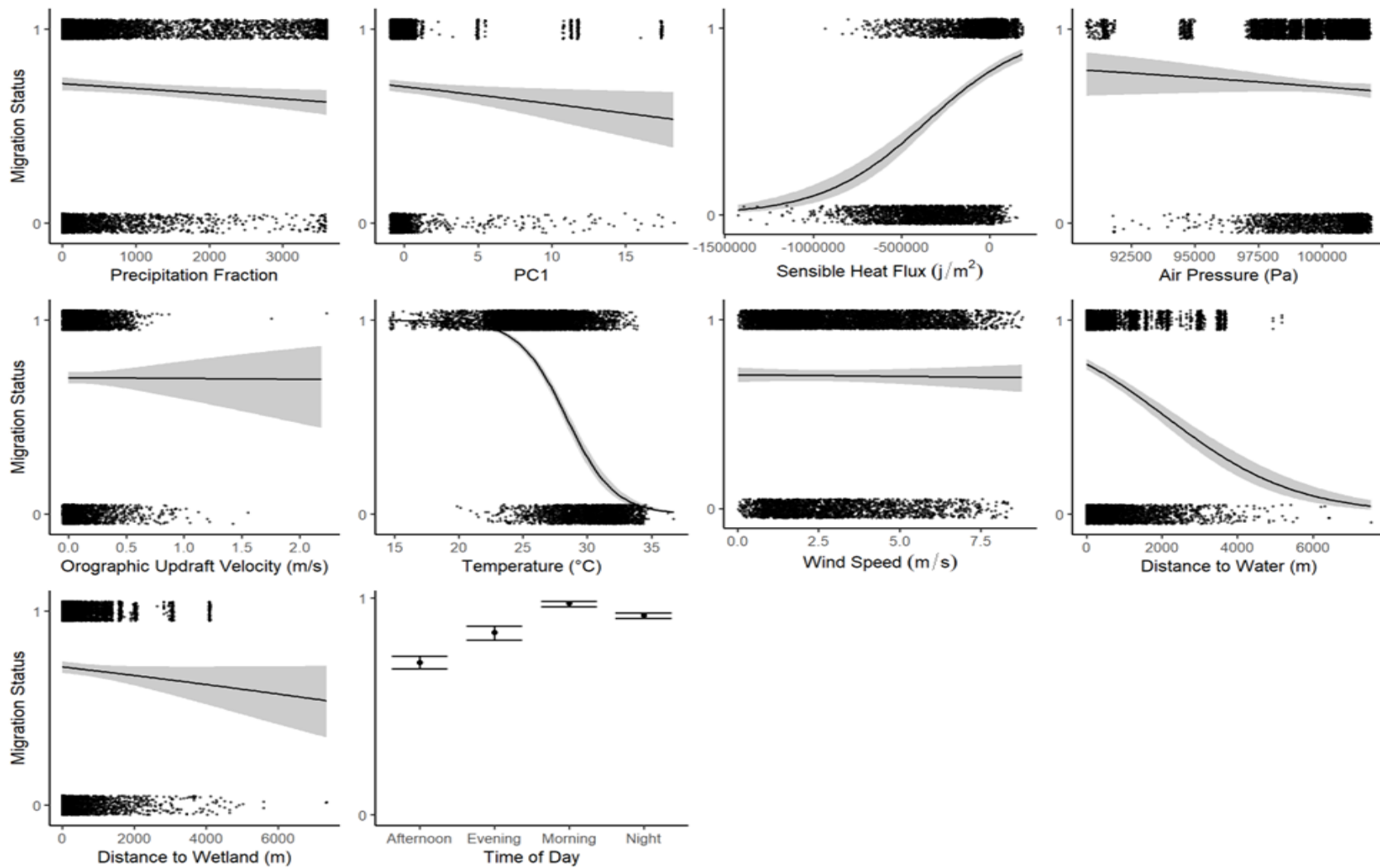


Figure 3.2 Effect of distance to landcover (open water, wetland, and all other landcover types captured by PC1), weather, and time of day on the occurrence of stopovers whereby ‘1’ is when the Osprey are within a stopover and ‘0’ is when they are in active migration.

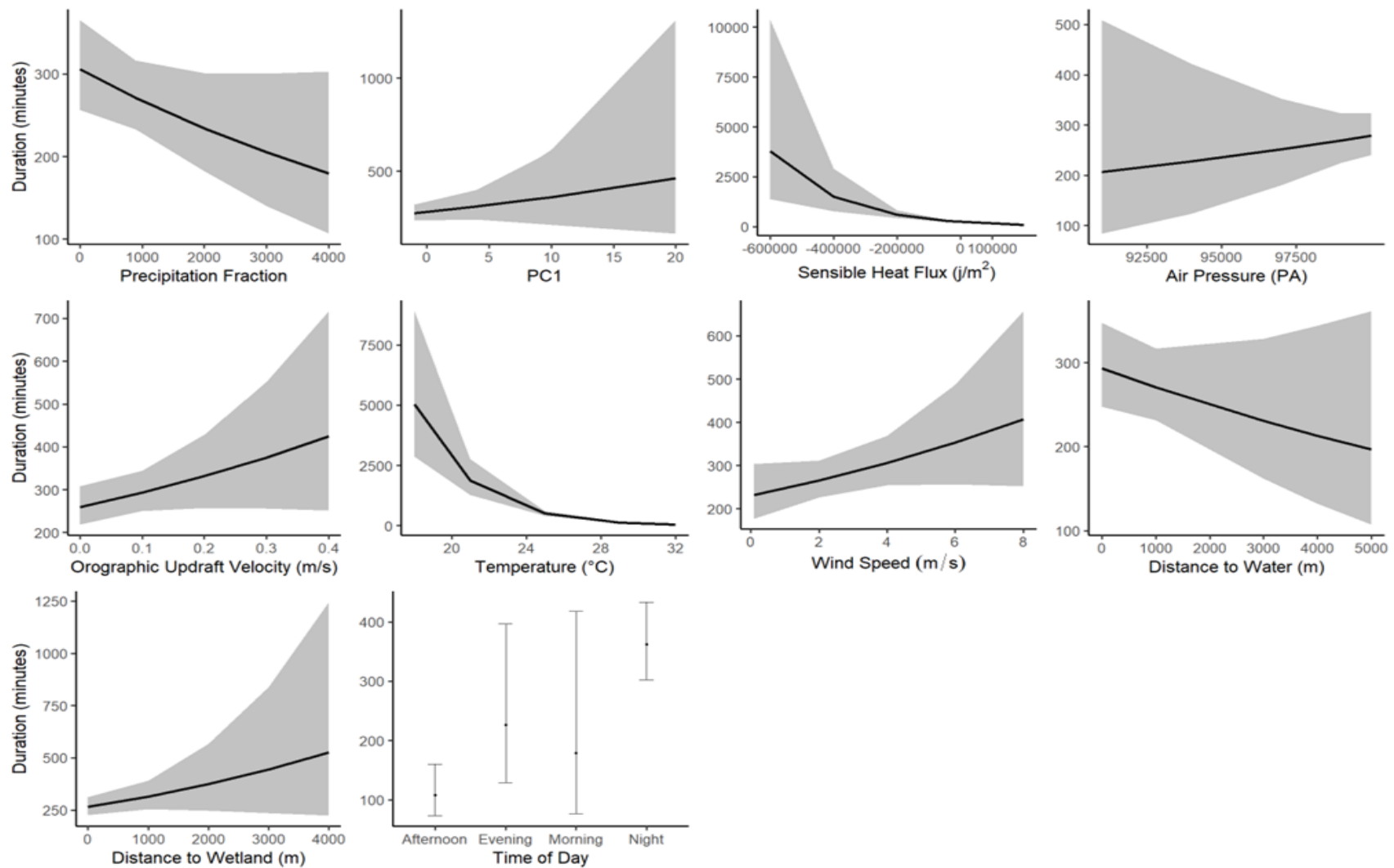


Figure 3.3 The effect of distance to landcover (open water, wetland, and all other landcover types captured by PC1), weather, and time of day on the duration of Osprey stopovers (in minutes) during southbound migration.

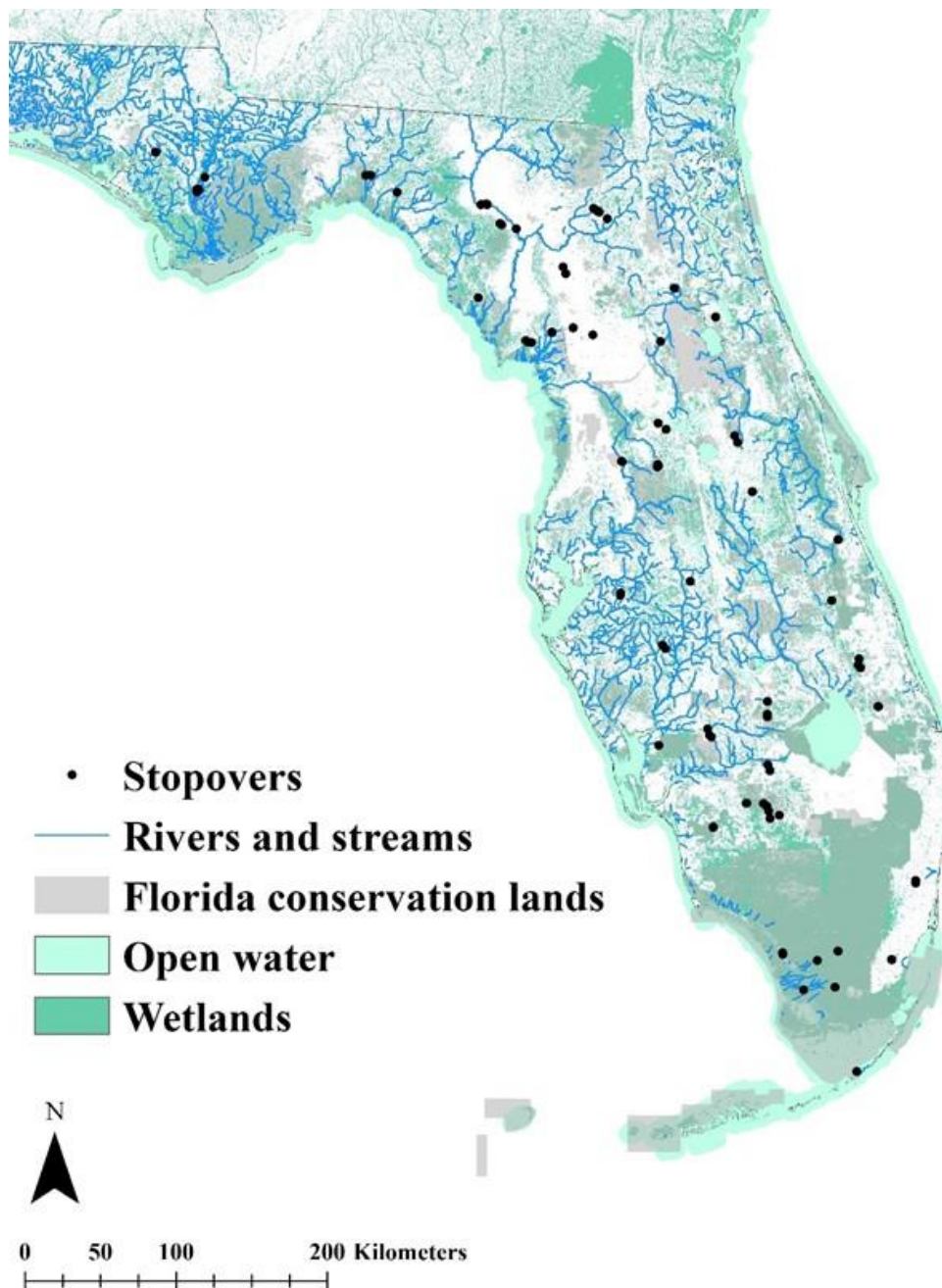


Figure 3.4 Stopover locations ( $n = 113$ ) of 14 adult Ospreys on southbound migration in Florida, US, with respect to waterbodies (including wetlands) and conservation lands, including federal, state, local, and private managed areas.

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CHAPTER IV

GENETIC VARIABILITY AND POPULATION STRUCTURE OF OSPREYS IN THE  
SOUTHEASTERN UNITED STATES

**Abstract**

Recovering populations present opportunities to assess the genetic processes and mechanisms involved in population response and expansion. However, it can be difficult to develop and test alternative hypotheses about the impact of historic or current events on contemporary genetic structure without a well-documented demographic history. In this chapter, I genotyped 79 individual Ospreys (*Pandion haliaetus*) distributed across six regions using 13 microsatellite loci to quantify genetic variation within the southeastern United States. A lack of follow-up monitoring and genetically informed translocation measures have resulted in a population of unknown admixture and unclear origin. As such, my objectives were to quantify admixture and population structure of southeastern Ospreys and compare the genetic diversity of southeastern Ospreys to the east coast source populations used for translocation efforts. I expected genetic differentiation between sampling sites based on known translocation histories, geographic distance, and differences in migratory behavior. Fixation indices ( $F_{ST}$ ) revealed low levels of genetic differentiation and Bayesian assignment tests revealed no genetic differentiation between the regions sampled. As pairwise  $F_{ST}$  does suggest weak differentiation between the east coast sampling site and more geographically distant locations, further work incorporating continuous geographic coverage is required to understand the implications of the on-going

translocations. Patterns of low genetic differentiation in southeastern Ospreys appear to be the result of extensive gene flow between the sampling sites, which is not uncommon in migratory birds. However, further research is required to quantify gene flow and identify if any other variables or processes contribute to the patterns observed in my results. Gene flow and current population structure may be the result of multiple non-mutually exclusive hypotheses that require further investigation, including the influence of rare long-distance or sex-biased dispersal, historic management action, contemporary range expansion, and the limited ability of certain genetic markers to detect population structure.

## **Introduction**

The Osprey (*Pandion haliaetus*) is a cosmopolitan, migratory raptor considered an indicator species for aquatic systems (Grove et al., 2009; Poole, 2019). Osprey global populations declined during 1950 – 1970 due to organochloride pesticides, habitat loss, and persecution (Hammer and Hatcher, 1983; Poole, 2019; BirdLife International, 2023). While the Osprey is still listed as threatened locally in some areas (e.g., Illinois, USA), North American populations have experienced an estimated 50 – 99% increase over the last 50 years (Houghton and Rymon, 1997; Pardieck et al., 2019). Today, the breeding range in North America is widespread and expanding (Bierregaard et al., 2020). Similar patterns of recovery are also true for other raptor species, such as Peregrine Falcons (*Falco peregrinus*; Cade et al., 1988) and Bald Eagles (*Haliaeetus leucocephalus*; Zimmerman et al., 2021). These recovering populations present opportunities to examine the genetic processes involved in population recovery and subsequent expansion (Hagen et al., 2015; Munclinger et al., 2022). In addition, estimates of genetic diversity, gene flow, and population admixture can provide vital information for future management decisions to sustain population levels (Allendorf et al., 2013).



Translocation (i.e., the intentional movement and release of wild individuals) is an important management tool in the recovery of many raptor species (Love and Ball, 1979; Martell et al., 2002). In the southeastern United States, translocation efforts for Ospreys were led by the Tennessee Valley Authority in both Kentucky and Tennessee, where newly impounded lentic systems (e.g., Watts Bar Reservoir) presented ideal locations to establish breeding Osprey populations (Bent, 1961; Henny, 1983; Beddow, 1990; Ray et al., 2009). Efforts in Tennessee involved the translocation of 42 birds from Maryland and Virginia to 15 sites in east Tennessee from 1978 – 1981 (Hammer and Hatcher, 1983; Beddow, 1990). Reintroductions began in Kentucky in 1981 and continued until 1991 with approximately 97 individuals translocated from the Chesapeake and Delaware Bays to 13 sites in central and western Kentucky (Ray et al., 2009). However, post-reintroduction monitoring was poor across both states, and it is unclear how many pairs were established as a direct result of these programs (Houghton and Rymon, 1997). This is particularly relevant as the dispersal distances of Ospreys tend to be greater for translocated individuals and there is no clear evidence that translocated birds in the southeast did not disperse out to other locations (Houghton and Rymon, 1997; Martell et al., 2002; Stout et al., 2009).

A general lack of genetically informed conservation measures and follow-up monitoring across translocation programs can result in unknown degrees of mixture and population origin (Weeks et al., 2011; Novak et al., 2021; Munclinger et al., 2022). This is further compounded when historic population trends are unknown. Population dynamics of Ospreys in the southeastern United States have long been overlooked, especially when compared to extensively studied populations such as in Chesapeake Bay (Stinson and Byrd, 1976; Academia and Watts, 2023), and little information on past population numbers is available (Henny, 1983; Beddow,

1990). For example, only coastal Osprey populations are shown in a detailed range map of Ospreys in North America by Henny (1983) for Mississippi and Alabama. While the Tennessee-Tombigbee Waterway had not yet completed construction in 1983, there were many suitable reservoirs in Mississippi (e.g., Sardis, Arkabutla, Enid, Grenada) that were flooded in the 1940s – 1950s and represent suitable Osprey habitat (see Chapter I). Similarly, distribution maps by Houghton and Rymon (1997) and Poole et al. (2002) excluded Osprey populations on the Tennessee-Tombigbee Waterway and Mississippi reservoirs. Population estimates for the region (Mississippi, Alabama, Kentucky, and Tennessee) increased from 47 pairs in 1981 to 165 pairs in 1994, but range did not appear to expand (Houghton and Rymon, 1997). Today, Osprey populations in these four states are estimated at 11,340 individuals and their range has expanded considerably (Bierregaard et al., 2020; Partners in Flight Science Committee, 2023). Without a well-documented demographic history, it can be difficult to develop and test alternative hypotheses about the impact of historic (e.g., translocations) or current (e.g., range expansion) events on contemporary genetic structure.

In this chapter, I use microsatellite loci developed by Dawson et al. (2015) from a single male European Osprey (*P. h. haliaetus*) for the estimation of genetic variation in North American Ospreys (*P. h. carolinensis*). My objectives were to 1) quantify genetic diversity and population structure of southeastern Ospreys, 2) compare the genetic diversity of expanding southeastern Ospreys to an outgroup representing the east coast source populations used for translocation efforts, and 3) generate hypotheses for future testing and provide comparisons for further investigations. While range expansions, dispersal, and translocations can result in genetically homogenous populations (Excoffier et al., 2009; Finnegan et al., 2013; Hagen et al., 2015), behavioral factors (e.g., natal philopatry, differences in breeding phenology) may limit gene flow

between populations (Lecomte et al., 2009; Monti et al., 2018). As such, I expected genetic differentiation between sampling sites based on known translocation histories, geographic distance, and differences in migratory behavior (i.e., variation in the patterns of movement from one geographic region to another).

## **Methods**

### **Study area**

Based on nest location data collected from eBird, local utilities, and state agencies, I selected five sites across the southeastern United States for sampling (Figure 4.1). These included nesting clusters with a history of translocations including Kentucky Reservoir (Kentucky), and Watts Bar Reservoir (Tennessee) and without a history of translocations, including the Tennessee-Tombigbee Waterway (Mississippi/Alabama), Guntersville Reservoir (north Alabama), and the Alabama Gulf Coast (coastal Alabama). The Tennessee-Tombigbee Waterway consists of a canal that links multiple impounded reservoirs. Dams constructed in the 1930s – 1940s impound the Guntersville, Kentucky, and Watts Bar reservoirs. All nesting clusters host Ospreys that are migrants, except for the Alabama coast, which may hold a resident population (see Chapter III).

Once sites were identified for my study, I then selected individual nests for sampling based on nest site accessibility. In addition, I included samples collected from Osprey chicks that were translocated from Massachusetts to Illinois in 2022 as part of an ongoing reintroduction program in the state of Illinois. To ensure unrelated founders for reintroduction, a single chick was collected from 12 individual nests in Westport, Massachusetts. As polyandrous mating behavior is considered extremely rare among Ospreys (0.4% of 248 copulations; Mougeot et al.,

2002), it is unlikely these individuals were related. Osprey populations in the state of Massachusetts are estimated at 940 individuals (Partners in Flight Science Committee, 2023).

### **Sample collection**

From 2019 – 2022, I visited 84 unique nests during the Osprey breeding season (March – August) and collected 230 blood samples from Ospreys across the five waterbodies sampled: Tennessee-Tombigbee Waterway (n = 43), Guntersville Reservoir (n = 68), Alabama Gulf Coast (n = 18), Kentucky Reservoir (n = 43), and Watts Bar Reservoir (n = 58). Adult Ospreys (n = 63; 49♀, 14♂) were captured on the nest during the occupation or incubation breeding stage using a bow-net or noose carpet placed directly over the nest. Chicks (n = 167) were hand-captured from the nests at approximately 30 – 35 days old. Blood samples (1 ml) were collected from the cutaneous ulnar vein by 25-gauge needle with 1 ml manual syringe. I also included blood samples from 12 chicks collected from Massachusetts in 2022. Samples were stored in heparinized or EDTA treated vials at -80°C until DNA extraction. An additional drop of blood was also collected on filter paper and sent to Animal Genetics Inc. (Tallahassee, FL, USA) for sexing. Trapping and sampling efforts were carried out under IACUC protocol #21-168 and banding permit #23835.

### **Genetic analyses**

I extracted DNA from 25 µl of blood using a Maxwell® 16 Tissue DNA Purification Kit (Promega Corporation, WI, USA) according to the manufacturer's instructions. Individuals were genotyped at 27 microsatellite loci developed by Dawson et al. (2015): *Pha13*, *Pha15*, *Pha16*, *Pha17*, *Pha19*, *Pha23*, *Pha27*, *Pha28*, *Pha30*, *Pha03*, *Pha12*, *Pha18*, *Pha29*, *Pha31*, *Pha36*, *Pha02*, *Pha05*, *Pha10*, *Pha11*, *Pha14*, *Pha35*, *Pha04*, *Pha09*, *Pha20*, *Pha25*, *Pha33*, and *Pha37*.

However, only 13 loci were used in the final analyses (Table D.1; see Results). Loci were amplified by polymerase chain reaction (PCR) with fluorescently labelled primers according to recommend conditions (Dawson et al., 2015) using an Applied Biosystems 2720 Thermal Cycler (Life Technologies, CA, USA). These conditions included: Initial denature at 95 °C for 5 minutes, 35 cycles [95 °C for 30 seconds (denature), 54 – 58 °C (Table D.2) for 90 seconds (annealing), and 72 °C for 1 minute (extension)] and a final extension step (30 minutes at 60 °C). PCRs included negative controls and were repeated multiple times to ensure consistency. MicroAmp 96-well reaction plates containing multiplexes of four loci were sent to Cornell Institute of Biotechnology (Cornell University) for fragment analysis (capillary electrophoresis). I manually scored alleles using Peak Scanner™ Software v1.0 (Applied Biosystems™) and expected base pair ranges from Dawson et al. (2015). Alleles were scored independently by two researchers to reduce genotyping errors.

As the presence of related individuals (e.g., full siblings) can bias allele frequencies (Goldberg and Waits, 2010), I included only adults ( $n = 63$ ; coastal Alabama = 2; northern Alabama = 21, Mississippi/Alabama = 11, Tennessee = 11, Kentucky = 18) and all unrelated chicks in Massachusetts ( $n = 12$ ) and coastal Alabama ( $n = 4$ ) in genetic analyses. First, I performed tests for conformance of genotype proportions to the expectations of Hardy-Weinberg Equilibrium (HWE) using R package *pegas* (Paradis, 2010) with a Bonferroni correction for 114 tests (i.e., significance level 0.05 divided by the number of tests; Cooper, 1968). I assessed linkage disequilibrium using R package *poppr* and the correlation metric  $\bar{r}_d$  (Agapow and Burt, 2001; Kamvar et al., 2014). Finally, I used the program *FreeNa* (Chapuis and Estoup, 2007) to estimate the frequency of null alleles where a null allele frequency  $>0.5$  resulted in that locus being excluded from any further analyses (Huang et al., 2016).

I calculated a variety of genetic diversity indices using *GenAlEx* (Peakall and Smouse 2006, 2012), including the number of alleles per locus ( $N_a$ ), expected and observed heterozygosity ( $H_e$  and  $H_o$ , respectively), number of private alleles ( $A_p$ ), and fixation index ( $F$ , or  $F_{ST}$ ). I also calculated Rarefied (i.e., corrected for sample size) allelic richness ( $A_r$ ) in R using the *PopGenReport* package (Adamack and Gruber, 2014). I calculated  $F_{IS}$  (inbreeding coefficient; Weir and Cockerham 1984) in R using the *hierfstat* package (Goudet and Jombart, 2022).

I measured population structure using two approaches. First, I quantified genetic differentiation among the six sampling regions using  $F_{ST}$  values with significance between values assessed following 9,999 permutations and I also visualized pairwise  $F_{ST}$  using principal coordinate analysis (PCoA) in *GenAlEx*. Then, I further investigated genetic structure using the individual based Bayesian clustering method implemented in the program *STRUCTURE* (Pritchard et al., 2000) to identify the most probable number of genetic clusters ( $K$ ) within the data. To identify subtle population structure, I ran the analysis with default settings (i.e., admixture permitted, assumed correlated allele frequencies, and allowed degree of admixture  $\alpha$  to be inferred from the data; Falush et al., 2003; Evanno et al., 2005; Porras-Hurtado et al., 2013). For each  $K$  model tested I performed 10 runs with a burn-in of  $10^6$  steps followed by  $5 \times 10^6$  Markov chain Monte Carlo steps to allow for convergence. I tested a range of  $K$  from 1 to the assumed number of populations plus three (i.e., maximum of nine, for six regions plus three; Evanno et al., 2005). Optimal models were assessed using mean likelihood,  $L(K)$ , and  $\Delta K$  in the post-processing web-based program *STRUCTURE Harvester* (Evanno et al., 2005; Earl and vonHoldt, 2012).

## Results

Out of the 27 microsatellite loci from Dawson et al. (2015), six failed to amplify in any PCR (Table D.2) across my samples: *Pha13*, *Pha15*, *Pha31*, *Pha35*, *Pha37*, and *Pha33*. A further two loci, *Pha23* and *Pha02*, amplified but were discarded due to polymerase slippage during PCR (termed “stutter”) resulting in low confidence in allele scores and excessive ( $>0.5$ ) null alleles. Each locus was polymorphic globally, except for *Pha17* which was monomorphic across all samples and was removed from further analyses. Within populations, *Pha03* was monomorphic in coastal Alabama ( $n = 6$ ) and Massachusetts ( $n = 12$ ), while *Pha25* was monomorphic in Massachusetts and Mississippi/Alabama ( $n = 11$ ). Tests indicated no linkage disequilibrium between any loci ( $prD = 0.395$ ; see Figure D.2). I found that six loci departed significantly (Bonferroni corrected  $P < 0.0004$ ) from Hardy-Weinberg proportions (HWP) globally and were subsequently removed from my analysis (Table D.3): *Pha19*, *Pha12*, *Pha20*, *Pha04*, *Pha30*, *Pha05*. These deviations from HWP varied between sampling sites at the locus level and no locus was out of HWP across all populations (Table D.4). As a result of a failure to amplify, presence of null alleles, a nonpolymorphic locus, and departures from HWP, I was left with 13 viable microsatellite loci for analyses.

Genotyping of 13 microsatellite loci in 79 samples revealed a total of 64 alleles (average: 7 alleles per locus; range = 2 – 7). Across all six sampling regions, the numbers of alleles ranged from 3.15 – 4.30 and allelic richness ranged from 2.80 – 3.17 (Table 4.1). I identified a low number of private alleles for all sampling sites (0 – 0.15; Table 4.1). Overall levels of genetic variability were broadly similar across all regions, with observed heterozygosity values ranging from 0.45 – 0.55. Observed values were lower than expected in Tennessee and Kentucky (Table 4.1). Observed heterozygosity was higher than expected in both coastal Alabama and north

Alabama (Table 4.1). Measured inbreeding coefficients ( $F_{IS}$ ) were negative in the samples from coastal Alabama and Massachusetts (Table 4.1), suggesting that individuals may be outbred relative to what is expected under the HWE. This appears to be in line with the excess of heterozygotes seen in these sampling sites (i.e.,  $H_o > H_e$ ,  $H_e = H_o$ ). Fixation index values were close to zero across all regions (Table 4.1) suggesting very little genetic differentiation between sites.

Genetic differentiation across all sampling sites measured as  $F_{ST}$  indicated three distinct population clusters: Mississippi/Alabama, coastal Alabama, and Massachusetts, with Kentucky-Tennessee-North Alabama clustering together (Figure 4.2). The Mississippi/Alabama cluster appeared differentiated from coastal Alabama and Massachusetts clusters, while coastal Alabama and Massachusetts clusters were differentiated from one another (Table 4.3; Figure 4.2). These  $F_{ST}$  comparisons indicated low ( $F_{ST} = 0.055$ ) but significant genetic differentiation, suggesting only weak genetic structure (Ramos et al., 2016). Results of *STRUCTURE* analyses also provided the most support for  $K = 4$  (Figures D.2, D.3; Table D.6). In *STRUCTURE* analysis, membership coefficients ( $q$ ) represent the proportion of an individual's ancestry that is derived from the associated  $K$  (Porrás-Hurtado et al., 2013). I found that individual cluster  $q$  values were generally in the order of  $\sim 0.2$  in the  $K = 4$  model (Figure 4.3). This even distribution and ambiguous assignment of membership may suggest that the  $K = 4$  model is unsuitable. Further, a lack of substructure is indicated by the unstructured geographic distribution of individuals assigned to each  $K$  based on maximum probability (Figure D.4). It is also important to note that the method of Evanno et al. (2005) (i.e.,  $\Delta K$ ) cannot detect a  $K = 1$  scenario. Based on Figure D.3, I also examined the  $K = 2$  model (Figure 4.4) and I found that individual cluster membership coefficients were generally in the order of  $\sim 0.5$  (Figure 4.4), which again suggests



$K = 1$ , i.e., an overall lack of detectable genetic structure (Miller et al., 2012; O'Reilly et al., 2021).

## Discussion

With information on genetic variability and genetic structure, scientists can examine, for example, microevolutionary responses to past human activities or the ability of populations to respond to change. Scientists can also identify distinct population segments, such as those defined under the United States Endangered Species Act (Barrett and Schluter, 2008; Pigeon et al., 2016; Major et al., 2021; Davis et al., 2021). In this chapter, I quantified the genetic diversity and population structure of southeastern Ospreys to set the basis for understanding gene flow in the region given the history of translocation and contemporary population expansion. I found that gene flow appears to be high, and sampled regions show little genetic differentiation. While I have identified the presence of gene flow, future work to directly assess and quantify the direction of gene flow both past and present, investigate potential hybridization levels between geographic regions, and identify migratory genotypes would be beneficial.

I screened 27 microsatellite markers developed by Dawson et al. (2015) from a single European Osprey and successfully used 13 loci for the estimation of genetic variation among Ospreys in North America. These results can assist other researchers when selecting markers for use in Ospreys from this geographic region. I included 11 of the same loci as Viverette (2016), who tested 20 microsatellite markers from Dawson et al. (2015) with samples from American Ospreys. In both studies, *Pha02* was unreliable, producing stutter and false alleles. Also, *Pha02* was found to have a heterozygote deficit by Dawson et al. (2015). However, it is difficult to make direct comparisons between this study and Viverette (2016) without standardizing methods. Differences in laboratory techniques, equipment, and sample type may also explain

variations in results from the shared markers tested across the two studies (Ellis et al., 2011). For instance, when compared to blood samples, feather samples, as used by Viverette (2016), can produce reduced quality microsatellite alleles and result in low signal, stutter, and allele dropout (Presti et al., 2013).

Observed genetic variation was generally similar to that previously reported for Ospreys in North America using the same genetic markers ( $H_e = 0.44$ ,  $A_r = 2.3$ ; Viverette, 2016). Monti et al. (2018) reported lower levels of genetic variation in North American Ospreys with a different marker set ( $H_e = 0.239$ ,  $A_r = 2$ ). However, Skujina et al. (2021) later identified the markers used by Monti et al. (2018) to be less polymorphic than the markers published by Dawson et al. (2015), which may have resulted in an underestimation of genetic variation. Comparing to other global populations, European Ospreys appear to have higher levels of genetic variation than North American Ospreys (Skujina et al. (2021);  $H_e$  0.55 – 0.57,  $A_r = 3.42$  – 3.76; Dawson et al. (2015):  $H_e$  0.24 – 0.94). Higher levels of genetic variation among European Ospreys may reflect multiple genetically differentiated populations (Helbig et al., 1998; Monti et al., 2015; Monti et al., 2018; Skujina et al., 2021; Monti et al., 2022).

Comparisons of  $F_{ST}$  values revealed low levels of genetic differentiation between sample regions, and the *STRUCTURE* analysis approach revealed no genetic differentiation. This lack of structure suggests no contemporary mixing of genetically distinct groups and implies a lack of structure may extend beyond the sampled range. Other results, such as  $F_{IS}$ , fixation index, and few private alleles, also support outbreeding and low levels of genetic differentiation across the sampled regions. Little genetic structure has been reported in many highly mobile species (Purcell et al. 2006; Ball et al. 2010; Finnegan et al., 2013; Ramos et al., 2016), including raptors with strong natal philopatry (Arshad et al., 2009; Miller et al., 2012). These results are echoed in

other studies. In a global study of Ospreys, Monti et al. (2015) also found no evidence of substructure across North America based on 25 samples while Viverette (2016) found genetic differentiation among populations to be low using 433 historic and contemporary samples from Ospreys across the United States.

My results suggest high gene flow and possible outbreeding across the sampled range and this pattern may be due to a variety of mechanisms. For example, two non-mutually exclusive hypotheses may explain the lack of genetic structure: population expansion and historical translocations. A lack of clear genetic structure may be caused by population expansion via natural colonization; this can be difficult to disentangle from historic influences, such as translocations (Hagen et al., 2015; Chafin et al., 2021). Osprey populations have been increasing over the last 50 years (Pardieck et al., 2019) and with on-going population expansion across North America, some parts of the range may be experiencing influxes of immigrants from surrounding populations. My results indicate a lack of latitudinal pattern in genetic diversity and from this I hypothesize that expansion may be occurring from multiple sources. Observed heterozygosity was higher than expected in both coastal and northern Alabama, which may imply an isolate-breaking effect. The effect describes the mixing of two previously isolated populations causing heterozygote excess ( $H_o > H_e$ ; Wahlund, 1928). This can be caused by sampling immigrants or the offspring of immigrants in a population (Šnjegota et al., 2021). However, heterozygote excess can also be a result of genotyping errors. Most adult Ospreys sampled in my study were females ( $n = 51$ , 65% of samples), which is relevant as mean dispersal distances are greater in females (114 km to 23 km for females and males in North America, respectively; Spitzer et al., 1983; Kinkead, 1985; Postupalsky, 1989; Martell et al., 2002; Poole, 2019), thus increasing the probability of sampling an immigrant due to increased dispersal

capabilities. It would be important to investigate these dispersals and their impact on range expansion via genetic recapture techniques (e.g., Cross et al., 2017) as occasional long-distance (> 1,000 km) dispersal has been recorded in Ospreys, particularly females and translocated individuals (Martell et al., 2002; Stout et al., 2009). For example, in my study, one wild female banded as a chick in coastal Alabama dispersed 1,240 km to Richmond, Virginia. These movements can disproportionality impact gene flow during range expansion, thus warranting detailed consideration when identifying variables contributing to genetic structure (Nichols and Hewitt, 1994).

Low genetic variation may reflect the known history of overwhelmingly successful translocation programs, which date to the 1970s and continue today (Hammer and Hatcher, 1983; Schaadt and Rymon, 1983; Houghton and Rymon, 1997; Illinois Department of Natural Resources, 2023). These human-mediated movements can decrease genetic differentiation (e.g., as seen in Canada Geese (*Branta canadensis*); Finnegan et al., 2013). While multiple studies have found evidence of genetic differentiation among European Ospreys (Helbig et al., 1998; Monti et al., 2015; Monti et al., 2018; Skujina et al., 2021; Monti et al., 2022), translocations programs in Europe have only been widely implemented since 1996 (Monti et al., 2022). These patterns suggest that long-term translocations may result in genetically homogenized Osprey populations in North America. However, as Ospreys in the southeastern United States have not been systematically monitored or studied over the last 50 years, scientists are left with uncertain and complex demographic histories which create difficulties when attempting to disentangle potential effects from contemporary processes (Viverette, 2016; Chafin et al., 2021). Next-generation molecular techniques may allow for disentangling these effects, particularly if historical samples (i.e., museum specimens) can be incorporated (Jacobsen et al., 2007; Chafin et

al., 2021). Sampling contemporary geographic intermediates between translocation sites and source populations may also elucidate these patterns (Hagen et al., 2015). As pairwise  $F_{ST}$  does suggest weak differentiation between the east coast sampling site and more geographically distant locations (i.e., coastal Alabama, the Tennessee-Tombigbee Waterway), further work incorporating continuous geographic coverage is required to understand the implications of the on-going relocation of east coast Ospreys to interior locations, such as translocation Massachusetts birds to Illinois (Monti et al., 2022).

I did not find any genetic differentiation between coastal non-migratory and northern migratory populations despite known differences in phenology (i.e., earlier breeding in non-migrants) that may result in reproductive isolation (Bierregaard et al., 2020). However, my sample sizes were low ( $n = 6$ ) for coastal Alabama, where partial and non-migrants are likely to occur (see Chapter III). Viverette (2016) also found no relationship between genetic population assignment and phenology for Florida Ospreys, which comprise both migratory and non-migratory individuals (Martell et al., 2004). While this result may appear curious when considering the known differences in breeding phenology associated with migration timing and associated reproductive isolation, it may be indicative of within-population variation in migratory genotypes. For example, Bossu et al. (2022) found that distinct early and late migratory genotypes exist within panmictic populations of American Kestrels (*Falco sparverius*) and are not associated with distinct geographic regions. Identifying if such within-population variation in migratory genotypes exists in Ospreys would be relevant for managing ongoing translocations programs, identifying management units, developing movement ecology theory, and quantifying the adaptive potential of existing populations.

In this chapter, I have taken the first steps in quantifying genetic variation and structure in Ospreys in the southeastern United States. While my results indicate little genetic differentiation between the sampled regions, I suggest further exploration of population structure as other techniques (e.g., fine-scale spatial autocorrelation analysis, mitochondrial DNA haplotype diversity, whole-genome sequencing) may reveal structure at varying scales and assist in disentangling past and present genetic processes (Finnegan et al., 2009; Miller et al., 2012; Chafin et al., 2021). This is particularly important for informing management decisions that require detailed understanding of population delineations, such as translocations and conservation management plans. This work sets the stage for future investigation that work to identify the underlying mechanisms driving the patterns described herein, as management implications will likely differ depending on the variables contributing to contemporary gene flow and population structure in North American Ospreys.

## Tables & Figures

### Tables

Table 4.1 Summary diversity indices for each population for 79 individual Ospreys including average (across 13 loci) numbers of alleles, rarefied allelic richness, private alleles, observed expected heterozygosity,  $F_{IS}$  (inbreeding coefficient), and fixation index.

Genetic Parameter (average across loci)	Osprey populations					
	MS/AL	N AL	C AL	TN	KY	MA
Number of alleles	3.61	4.30	3.07	3.46	4.07	3.76
Rarified allelic richness	2.86	3.17	2.74	2.85	3.08	2.82
Private alleles	0.15	0.07	0.15	0	0.15	0.07
Expected heterozygosity ( $H_E$ )	0.48	0.54	0.47	0.49	0.52	0.47
Observed heterozygosity ( $H_O$ )	0.48	0.56	0.56	0.46	0.50	0.47
$F_{IS}$	0.11	0.02	-0.13	0.30	0.05	-0.07
Fixation index	-0.01	0	-0.19	0.01	0.02	-0.02

Abbreviations: C AL = coastal Alabama, N AL = north Alabama, KY = Kentucky, TN = Tennessee, MS = Mississippi, MA = Massachusetts.

Table 4.2 Pairwise  $F_{ST}$  values (below the diagonal) based on 13 microsatellite loci for 79 individuals, with exact P values provided above the diagonal. Any P values <0.05 are in **bold**.

C AL	N AL	KY	TN	MS/AL	MA	
-	0.180	0.290	0.337	<b>0.021</b>	<b>0.045</b>	C AL
0.035	-	0.222	0.888	0.184	0.168	N AL
0.036	0.017	-	0.541	0.324	0.113	KY
0.042	0.014	0.021	-	0.442	0.204	TN
0.055	0.023	0.024	0.028	-	<b>0.012</b>	MS/AL
0.053	0.022	0.027	0.032	0.044	-	MA

Abbreviations: C AL = coastal Alabama, N AL = north Alabama, KY = Kentucky, TN = Tennessee, MS = Mississippi, MA = Massachusetts.

## Figures

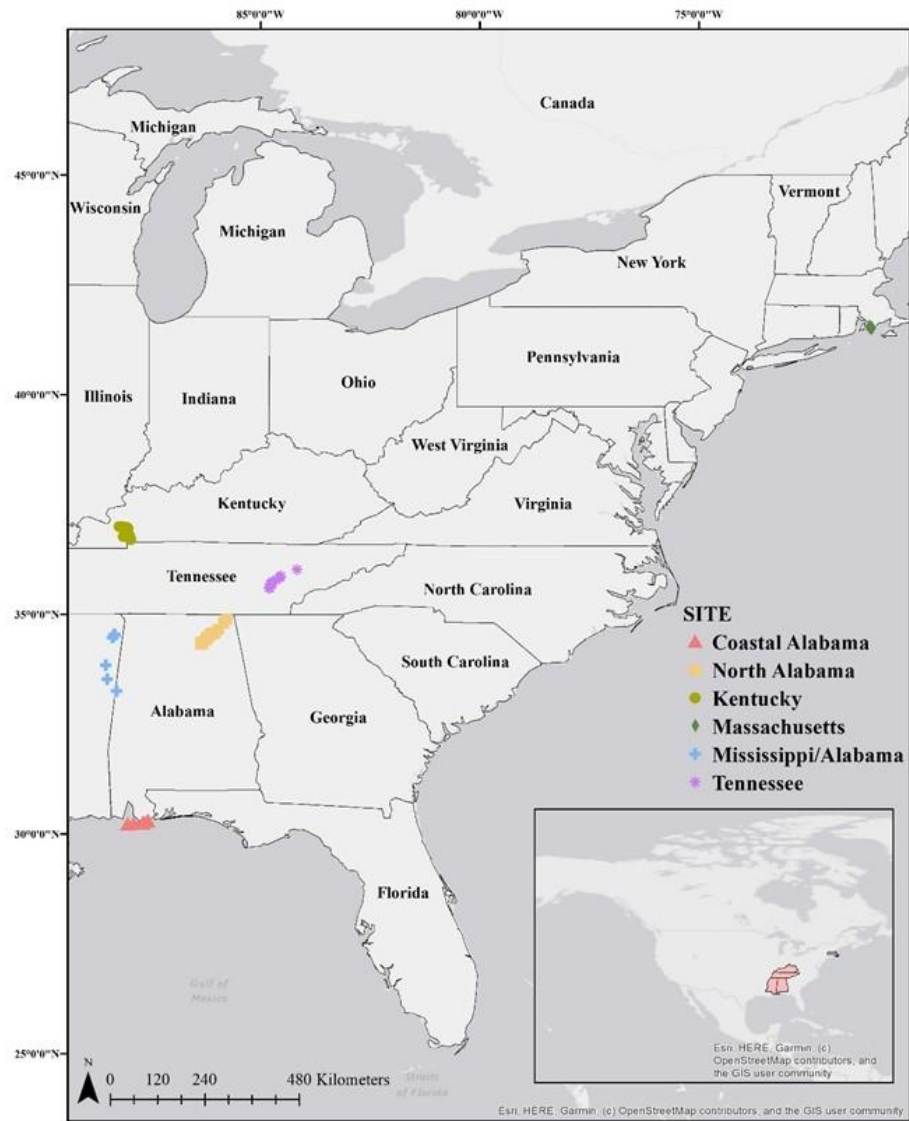


Figure 4.1 Locations (i.e., nests,  $n = 96$ ) in the United States where 242 individual Ospreys were sampled between 2019 – 2022 for genetic analysis: Coastal Alabama, North Alabama, Kentucky, Massachusetts, Mississippi/Alabama, and Tennessee. Nesting locations are imposed on a base map from Esri (Redlands, CA, USA) for context. Inset map shows the location of the five states in relation to the continental United States.



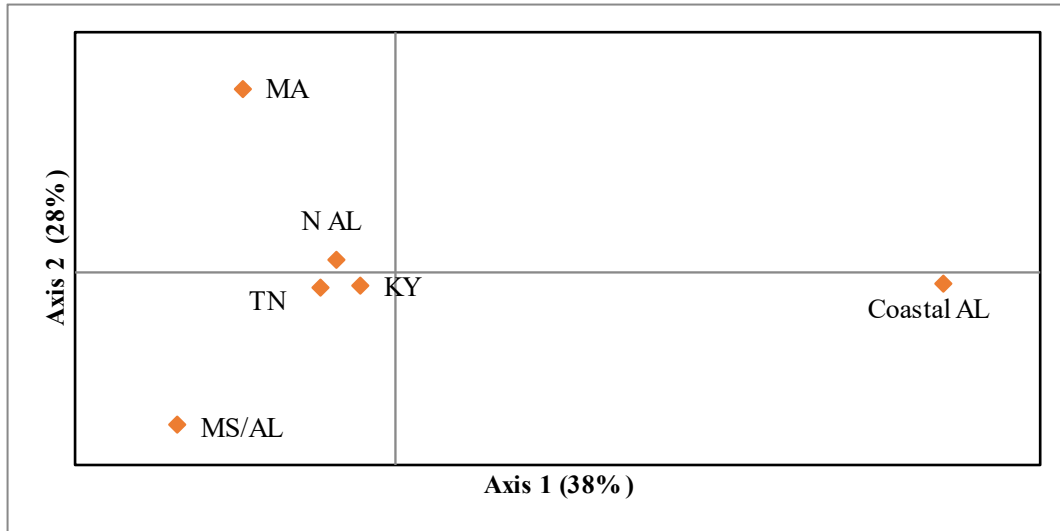


Figure 4.2 Principal coordinates analysis (PCoA) of pairwise  $F_{ST}$  values (see Table 4.2) for six Osprey populations: Coastal Alabama, North Alabama, Kentucky, Massachusetts, Mississippi/Alabama, and Tennessee. The first two axes explain 66% of the variance. See Table D.5 for associated eigen values.

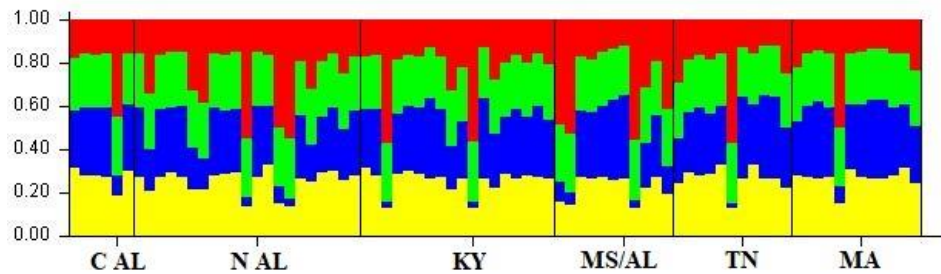


Figure 4.3 Stacked bar chart from the results of *STRUCTURE* analysis with  $K = 4$  (probable number of unique genetic clusters), as indicated by  $L(K)$  and  $\Delta K$ . Each bird is represented by a single bar, broken into  $K$  colored segments, with the length of each segment being proportional to the membership of each  $K$ . Individual cluster membership coefficients were  $\sim 0.2$ .

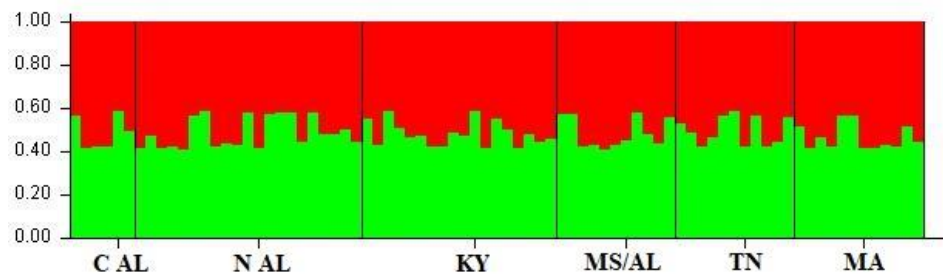


Figure 4.4 Stacked bar chart from the results of *STRUCTURE* analysis with  $K = 2$  (probable number of unique genetic clusters). Each bird is represented by a single bar, broken into  $K$  colored segments, with the length of each segment being proportional to the membership of each  $K$ . Individual cluster membership coefficients were  $\sim 0.5$ .

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## APPENDIX A

### SUPPLEMENTARY MATERIAL: CHAPTER I

## Tables

Table A.1 Descriptions of eBird breeding codes used to select citizen science data for analysis.

Code	Definition
NY Nest with Young (Confirmed)	Nest with young seen or heard.
NE Nest with Eggs (Confirmed)	Nest with eggs.
NB Nest Building (Confirmed/Probable)	Nest building at apparent nest site.
FL Recently Fledged Young (Confirmed)	Recently fledged or downy young observed while still dependent upon adults.
ON Occupied Nest (Confirmed)	Occupied nest presumed by parent entering and remaining, exchanging incubation duties, etc.
UN Used Nest (enter 0 if no birds seen) (Confirmed)	Nest is present, but not active. Use only if you are certain of the species that built the nest.

Table A.2 Descriptions of the nine environmental variables used in the Osprey nesting habitat suitability models with a citation for the data source, description (including NLCD legend codes for landcover data), and modifications performed for each variable.

Source	Variable	Description	Details
National Landcover Database (Dewitz, J., 2019)	Open Water	Areas of open water (11).	
	Forest Cover	Areas dominated by trees generally > 5 m tall (41, 42, 43).	Composed of mixed, evergreen, and deciduous landcover classes.
	Grassland	Areas dominated by graminoid or herbaceous vegetation, legumes, or grass-legume mixtures; ranging from unmanaged to intensively managed (81, 71).	Composed of pasture/hay, grassland and herbaceous landcover classes.
	Crops	Areas used for the cultivation of annual crops (82).	
	Development	Areas dominated by constructed material and impervious surface, ranging from low to high intensity (21, 22, 23, 24).	Composed of developed open space, low intensity, medium intensity, and high intensity development landcover classes.
	Wetlands	Areas where the soil or substrate is periodically saturated with or covered with water (90, 95).	Composed of woody wetlands and emergent herbaceous wetlands landcover classes.
	Shrubs	Areas containing shrubs <5 m tall and with shrub canopy >20% of total vegetation. Includes true shrubs, young trees in an early successional stage or trees stunted from environmental conditions (52).	Removed due to correlation with forest cover.
Global Wind Atlas	Windspeed	Mean windspeed at 50 m above sea level	
USGS, 2019	Slope	Rate of change of elevation	Slope was determined from the elevation layer using the Slope function in ArcGIS.
USGS, 2019	Elevation	Height of land above sea level	

## Figures

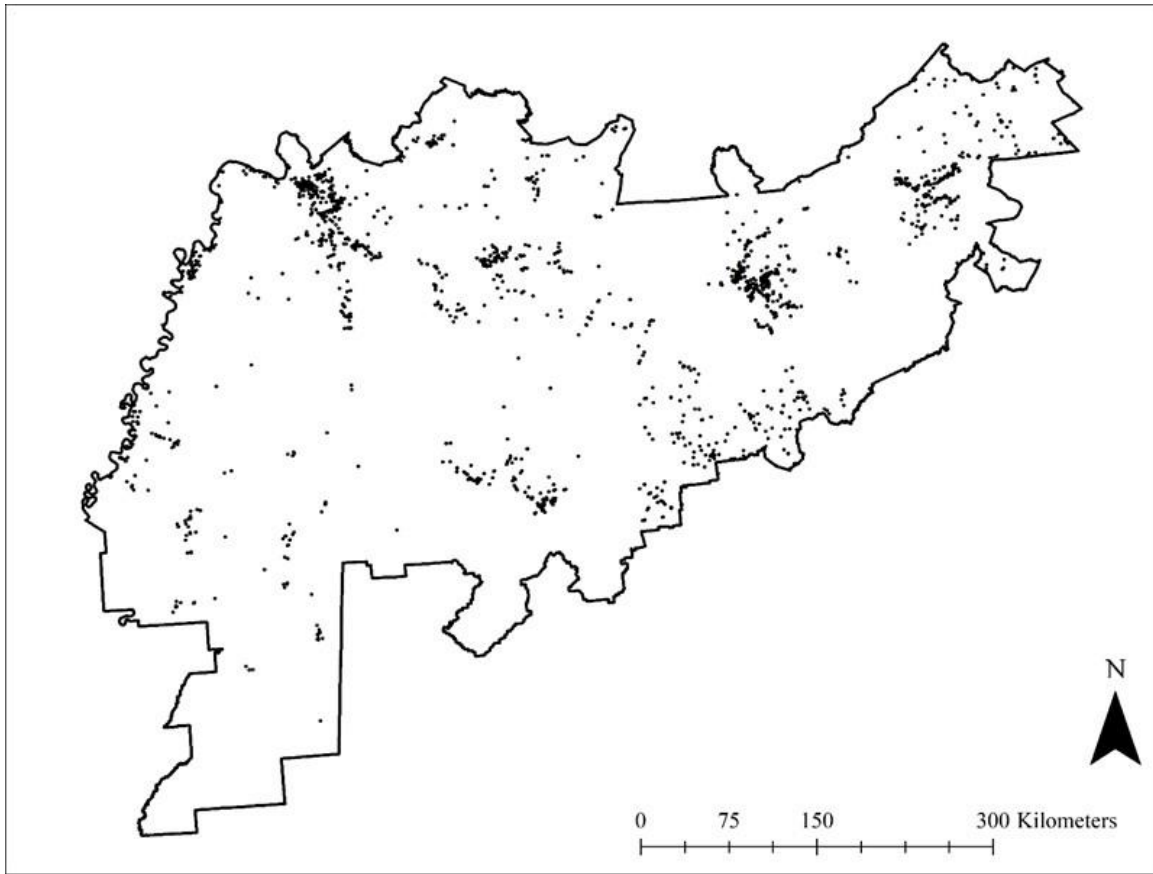


Figure A.1 Osprey nests ( $n = 11,134$ ) obtained from eBird and a Tennessee Valley Authority (TVA) database, across the TVA service area in the southeastern United States.

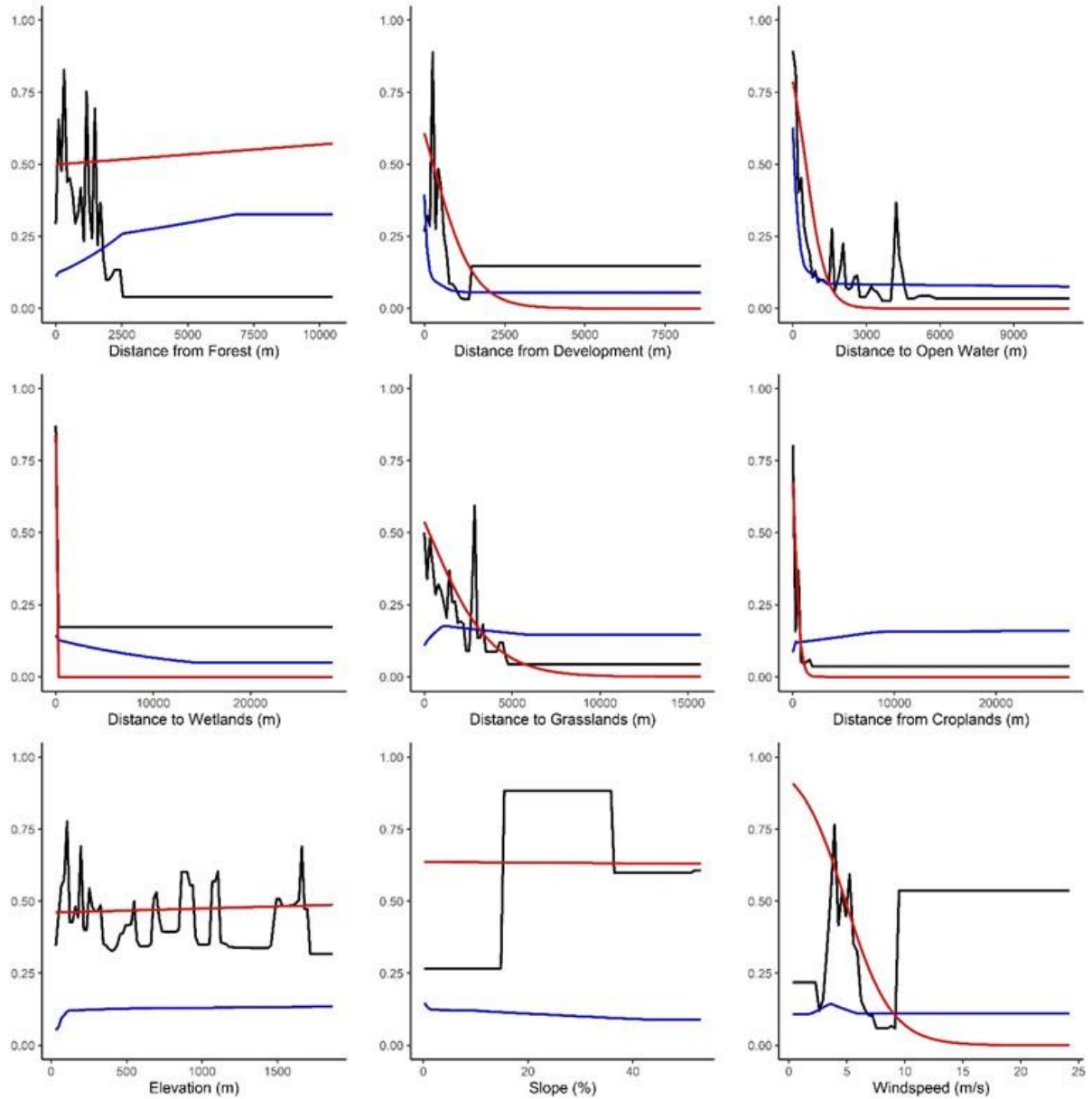


Figure A.2 Response curves for the 9 environmental variables used to develop Osprey nesting habitat suitability models based on data collected from 1990 - 2020 in the Tennessee Valley Authority service territory, USA. Maxent model response in blue, gradient boosted model in black, and generalized linear model response in red.

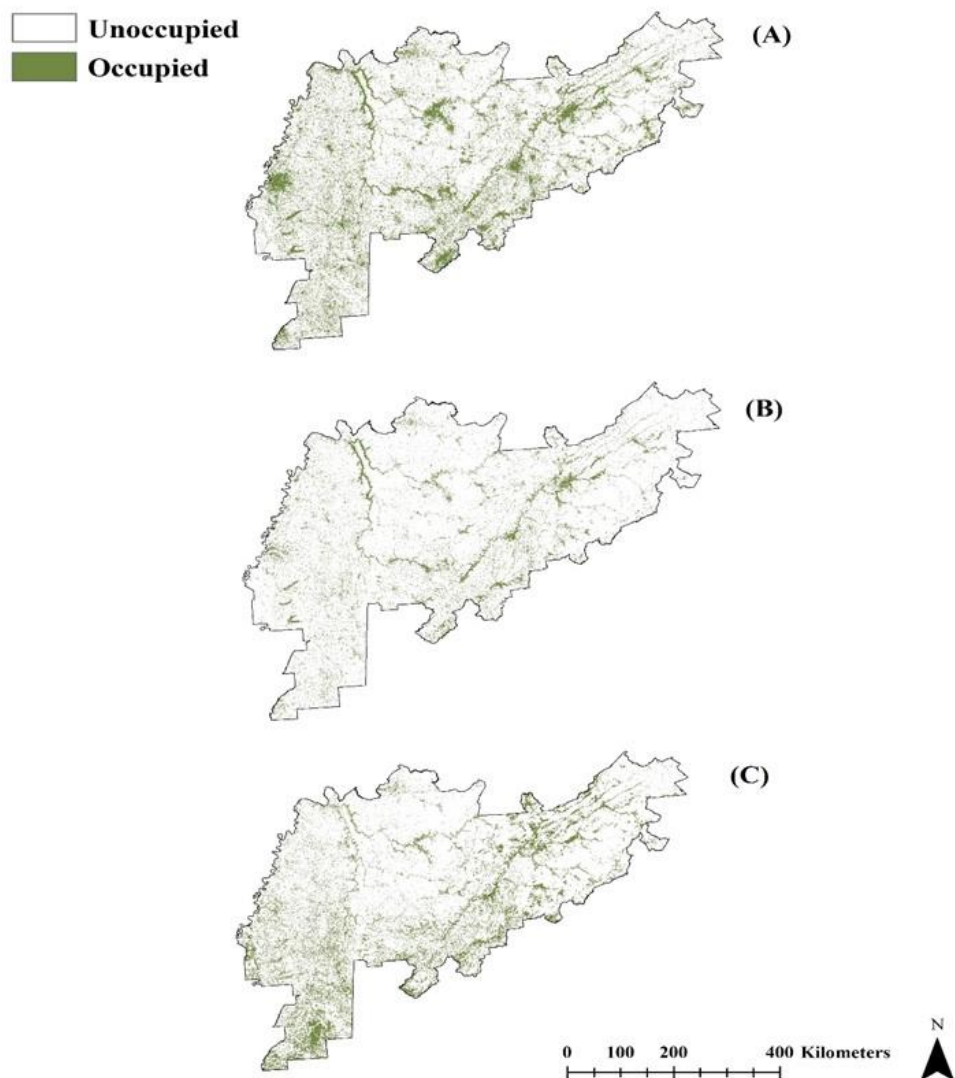


Figure A.3 Binary a) Maxent, b) gradient boosted, and c) generalized linear habitat suitability models for breeding Osprey (*Pandion haliaetus*) in the Tennessee Valley Authority service area in the southeastern United states, created using likelihood thresholds that maximized individual model sensitivity plus specificity. When compared, the binary maps from the three individual models showed <12% disagreement.

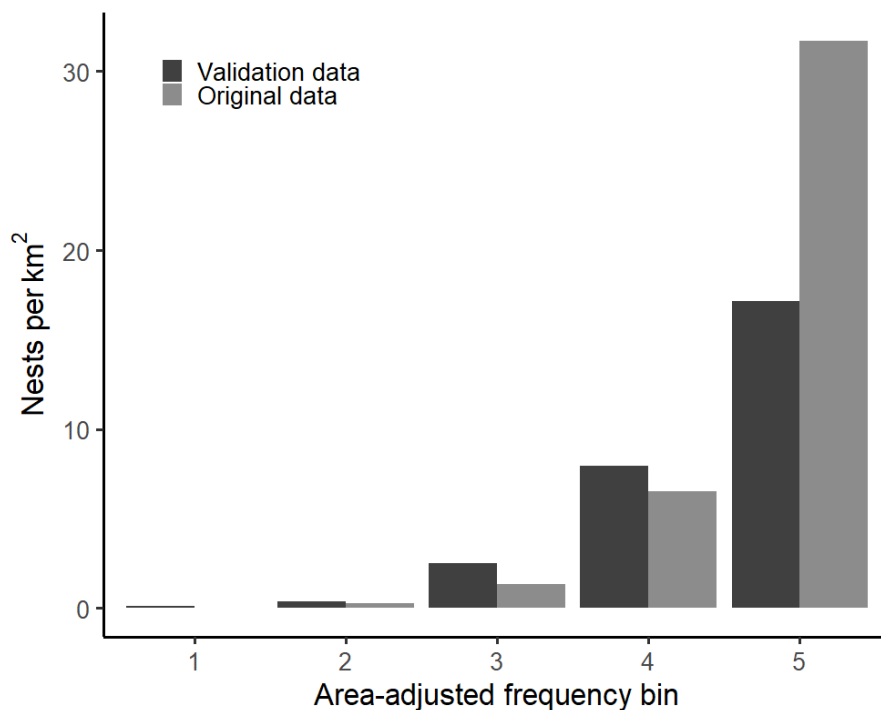


Figure A.4 Number of nests per km<sup>2</sup> across five equal bins (1 = 0 – 0.2, 2 = 0.2 – 0.4, etc.) for both the original nest locations used in the habitat suitability model and the validation data.

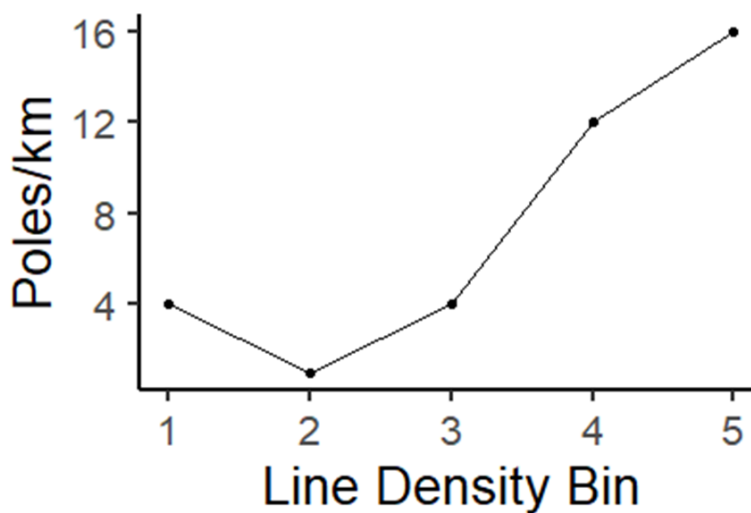


Figure A.5 Poles per km when poles were examined along 1 km of line in each of the line density bins for the TVA service area in the southeastern United States.



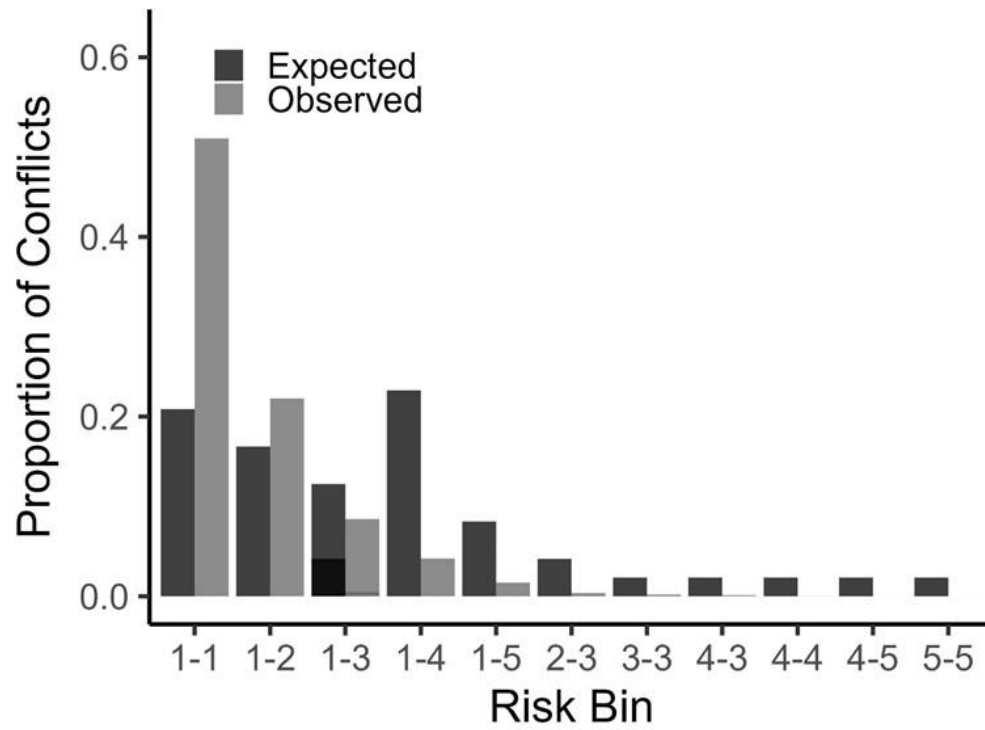


Figure A.6 Proportion of expected and observed nest conflicts across rank bins with conflicts  $>0$ .

## APPENDIX B

### SUPPLEMENTARY MATERIAL: CHAPTER II

## Tables

Table B.1 Based on diagonal wheelbase, six small UAS (unoccupied aerial systems) were placed into three categories for this study. Details on mass and measurements from respective manufacturer websites.

Category	Model	Manufacturer	Camera/sensor	Primary color	Mass (g)	Diagonal wheelbase (mm)	# Surveys
Small	MavicPro	SZ DJI Technology Co. Ltd	Built-in	Black	743	353.6	14
Small	Evo II Dual	Autel Robotics	Built-in	Orange	1150	397	8
Small	Phantom 3	SZ DJI Technology Co. Ltd	Built-in	White	1216	350	38
Medium	3DR SOLO	UAV Systems International	GoProHero3+	Black	1800	460	22
Large	Matrice 200	SZ DJI Technology Co. Ltd	DJI Zenmuse XT2 and X7	Black	3800	643	37
Large	Matrice 300	SZ DJI Technology Co. Ltd	DJI Zenmuse H20T	Black	3600	895	7

Table B.2 Ethogram describing the recorded behaviors and groups used for analysis. Groups combine like behaviors with similar actions (e.g., flying), functions (e.g., defensive), or location (e.g., at nest). Some behaviors were observed but not recorded, such as drinking.

Group	Code	Description
Calling	CA	Alarm call; usually in response to predator/nest intruder
	CS	Scream call; increased intensity from guard call
	GC	Guard call; usually in response to conspecific intruder
Flying	IF	Initial flush from nest, if on nest at start time
	AF	Additional flush, if returned to and landed at nest during survey
	FY	Flying, circling
Perching	PR	Perched away from nest, relaxed, may preen
	PU	Perched away from nest, upright, crest erect, vigilant
Defensive	DP	Defense pose: Body horizontal, tail may be spread
	D	Diving, rapid attack flight
At nest	RN	Returned to nest after other behavior (e.g., flying)
	ON	Incubating, brooding, or standing on the nest
Out of Sight	OS	Focus Osprey leaves the area and cannot be seen
Other	FE	Feeding, self or young
	CP	Copulating or attempting to copulate

APPENDIX C

SUPPLEMENTARY MATERIAL: CHAPTER III

## Tables

Table C.1 Summary of the raw tracking data for 27 Ospreys (21 females, 6 males) tracked between 2019–2022.

<b>ID</b>	<b>Sex</b>	<b>Location</b>	<b>Dates tracked</b>	<b># Days tracked</b>	<b>Mean fixes per day</b>	<b>Total Fixes</b>
75710	Female	Alabama	17/05/19 – 04/05/20	353	18	6216
74432	Female	Alabama	16/05/19 – 09/08/19	93	18	1755
73673	Female	Mississippi	09/05/20 – 15/06/20	37	18	666
75777	Female	Mississippi	09/05/20 – 15/06/20	37	18	668
72840	Male	Mississippi	09/05/20 – 14/08/20	151	18	15908
47817	Female	Alabama	13/04/21 – 02/05/21	20	96	6065
46850	Female	Alabama	15/04/21 – 06/08/21	55	96	7028
51314	Male	Kentucky	27/04/21 – 07/05/21	10	142	1491
48294	Female	Tennessee	15/04/21 – 26/07/21	134	95	15812
48716	Female	Tennessee	17/04/21 – 26/07/21	106	96	10752
50647	Female	Kentucky	28/04/21 – 08/09/21	135	279	37386
50332	Female	Kentucky	28/04/21 – 14/08/21	108	142	15363
48724	Female	Tennessee	17/04/21 – 17/08/21	128	96	12888
50316	Female	Kentucky	28/04/21 – 19/08/21	122	277	33925
48187	Female	Tennessee	17/04/21 – 22/08/21	135	95	15943
50308	Male	Mississippi	30/04/21 – 22/08/21	122	279	34095
46884	Female	Alabama	14/04/21 – 23/08/21	132	95	15563
47098	Female	Tennessee	17/04/21 – 24/08/21	140	95	15091
76445	Male	Kentucky	14/05/21 – 10/09/21	135	142	19130
47973	Female	Alabama	11/04/22 – 02/08/22	113	56	13416
43841	Female	Mississippi	12/05/22 – 03/06/22	22	233	5728
49706	Female	Coastal	04/05/22 – 05/09/22	131	95	14006
22667	Female	Alabama	09/05/22 – 23/05/22	14	279	3826
43593	Female	Kentucky	27/04/22 – 16/06/22	50	24	6306
47486	Female	Tennessee	15/04/22 – 31/07/22	115	95	16926
43486	Male	Alabama	11/04/22 – 28/08/22	190	95	23583
51165	Male	Coastal	04/04/22 – 22/09/22	171	279	48015

Table C.2 Summary of the raw tracking data for 27 Ospreys (21 females, 6 males) tracked between 2019 – 2022.

Source	Variable	Description	Modifications
National Landcover Database, Dewitz, 2019	Open Water	Areas of open water.	
	Forest Cover	Areas dominated by trees generally > 5 m tall.	Composed of mixed, evergreen, and deciduous landcover classes.
	Grassland	Areas dominated by graminoid or herbaceous vegetation, legumes, or grass-legume mixtures; ranging from unmanaged to intensively managed.	Composed of pasture/hay, grassland, and herbaceous landcover classes.
	Agriculture	Cultivated cropland. Areas used for the cultivation of annual crops.	
	Development	Areas dominated by constructed material and impervious surface, ranging from low to high intensity.	Composed of developed open space, low intensity, medium intensity, and high intensity development landcover classes.
	Wetlands	Areas where the soil or substrate is periodically saturated with or covered with water.	Composed of woody wetlands and emergent herbaceous wetlands landcover classes.
	Shrubs	Areas containing shrubs <5 m tall and with shrub canopy >20% of total vegetation. Includes true shrubs, young trees in an early successional stage or trees stunted from environmental conditions.	
	Barren	Areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material. Generally, vegetation accounts for less than 15% of total cover.	

Table C.3 Eigen values, percentage contribution to variance, and correlations of dimensions from two principal components analyses on distance to six landcover variables (development, forest, barren, shrub, agriculture, and grassland).

PCA: Stopover occurrence			PCA: Stopover duration		
	Eigen value	Variance (%)		Eigen value	Variance (%)
1	5.022	83.7	1	5.008	83.5
2	0.429	7.2	2	0.368	6.1
3	0.263	4.4	3	0.326	5.5
4	0.141	2.4	4	0.154	2.6
5	0.083	1.4	5	0.097	1.6
6	0.059	0.9	6	0.043	0.7

Table C.4 Correlations of dimensions from two principal components analyses on distance to six landcover variables (development, forest, barren, shrub, agriculture, and grassland).

PC1: Stopover occurrence		PC1: Stopover duration	
Variable	Dimension 1	Variable	Dimension 1
Agriculture	0.9374	Agriculture	0.9272
Barren	0.8103	Barren	0.8571
Developed	0.9223	Developed	0.8930
Forest	0.9566	Forest	0.9598
Grassland	0.9492	Grassland	0.9538
Shrub	0.9055	Shrub	0.8863



Table C.5 Definitions of weather variables obtained from Movebank's Env-data annotation using bilinear interpolation. Rationale for including these variables is listed with citation. \*Denotes inclusion in final models.

Weather variable	Definition	Rationale
*Air temperature (°C)	Air temperature 2 m above the ground, converted from Kelvin to Celsius.	Positive correlation with thermal soaring (1)
Boundary height (m)	The depth of air next to the earth's surface which is most affected by the resistance to the transfer of momentum, heat, or moisture across the surface.	Influence thermal development (5)
*Orographic updraft velocity (m/s)	The velocity of upward air movement caused when rising terrain forces air to higher elevations.	Uplift source (1,2)
*Surface sensible heat flux (j/m <sup>2</sup> )	Exchange of heat between the earth's surface and the atmosphere through turbulent air motion, excluding any heat transfer resulting from condensation or evaporation. Downward fluxes are positive values.	Influence thermal development (2,5)
*Surface Air Pressure (Pa)	Atmospheric pressure at the earth's surface.	Influences passage rates (8)
*Precipitation fraction	The accumulated fraction of the model grid cell that was covered by large-scale precipitation.	Influences flight speed (3), stopovers (6)
*Wind speed (m/s)	Calculated from wind u and v components.	Inhibit the development of thermals (1,2), migration speed (3), source of uplift (4)

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## Figures

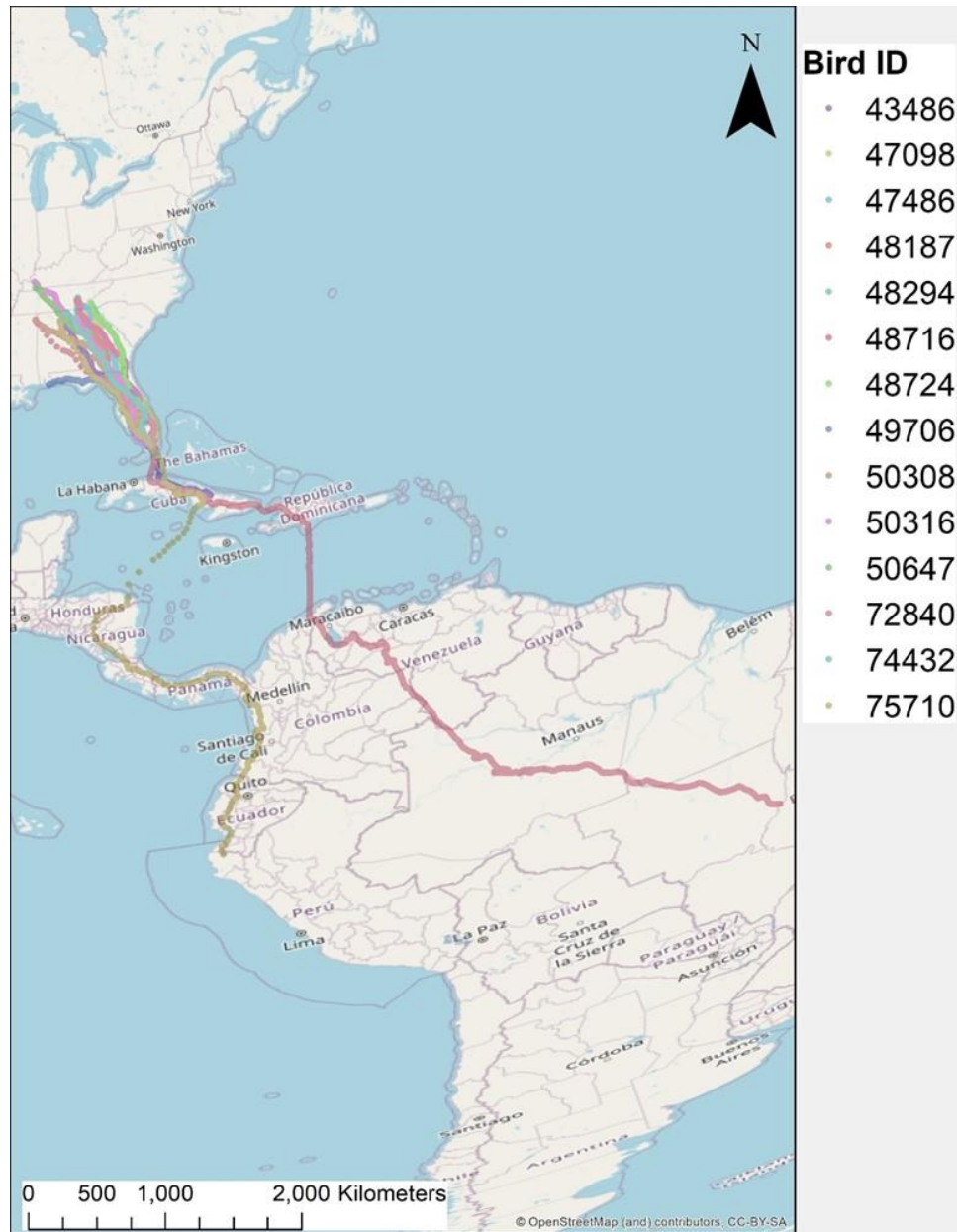


Figure C.1 Southbound migration (2019 – 2022) routes of 14 adult Ospreys (10♀, 4♂), showing the migratory movements outside the United States for three individuals. Base map from OpenStreetMaps.

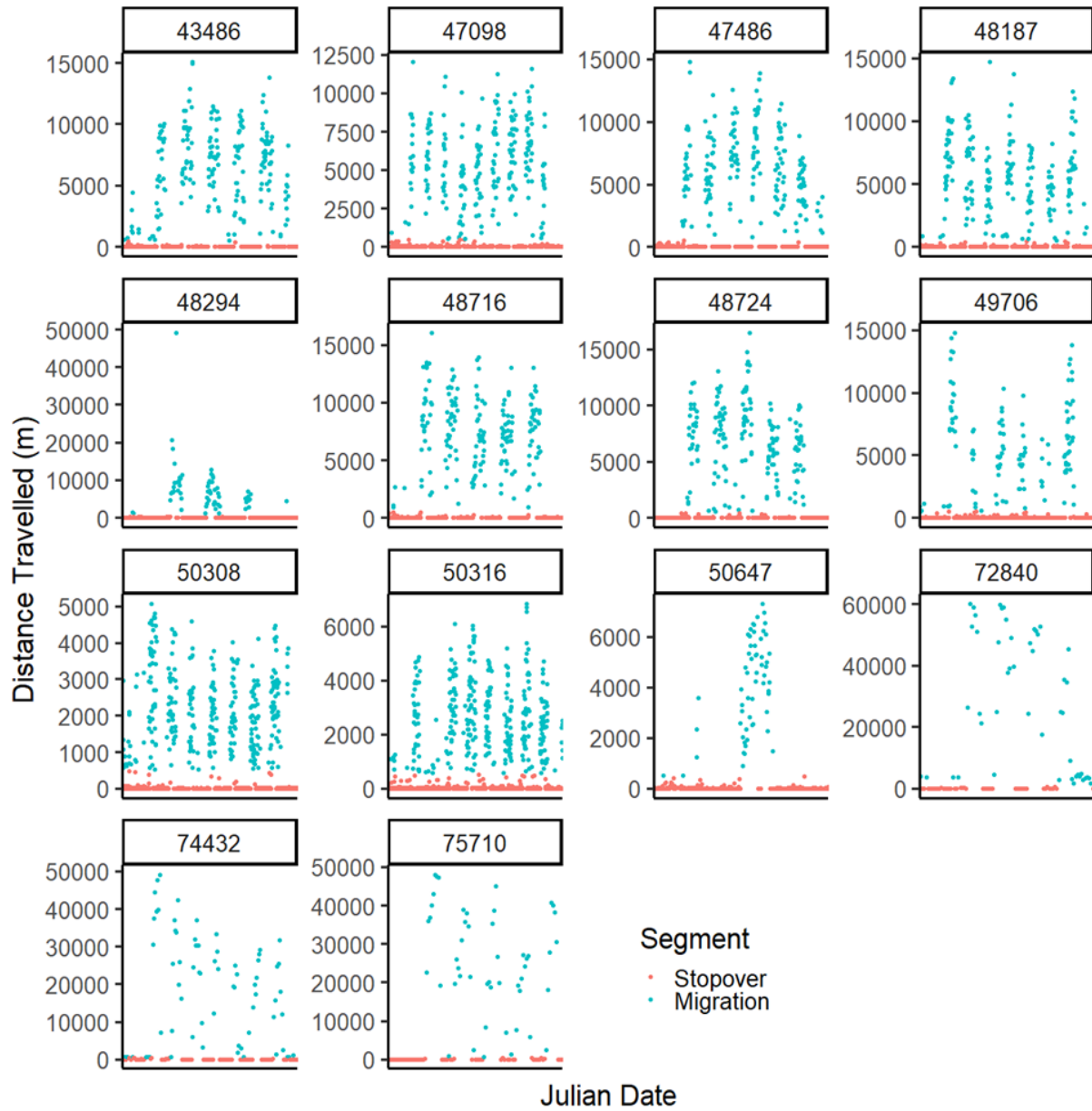


Figure C.2 Migration status segments (active migration and stopover) for 14 adult Ospreys in the southeastern US as determined using net squared displacement. I identified a stopover as any period where the bird travelled <500 m based on net squared displacement.

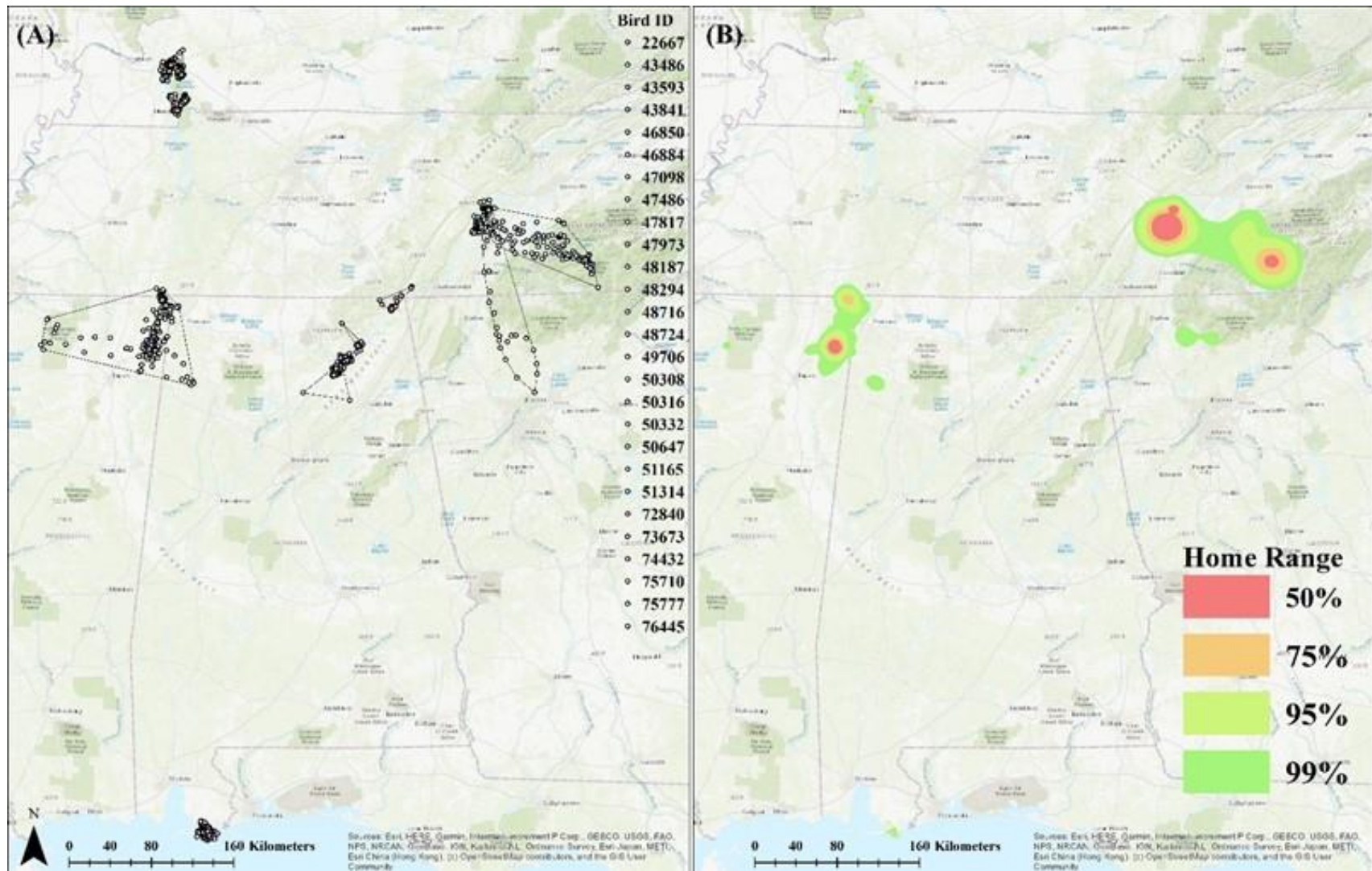


Figure C.3 Home range estimates for 27 Ospreys in the southeastern United States (2019 – 2022), including (A) 100% minimum convex polygons and (B) kernel density estimates from 50 – 99%. Base map from OpenStreetMaps.

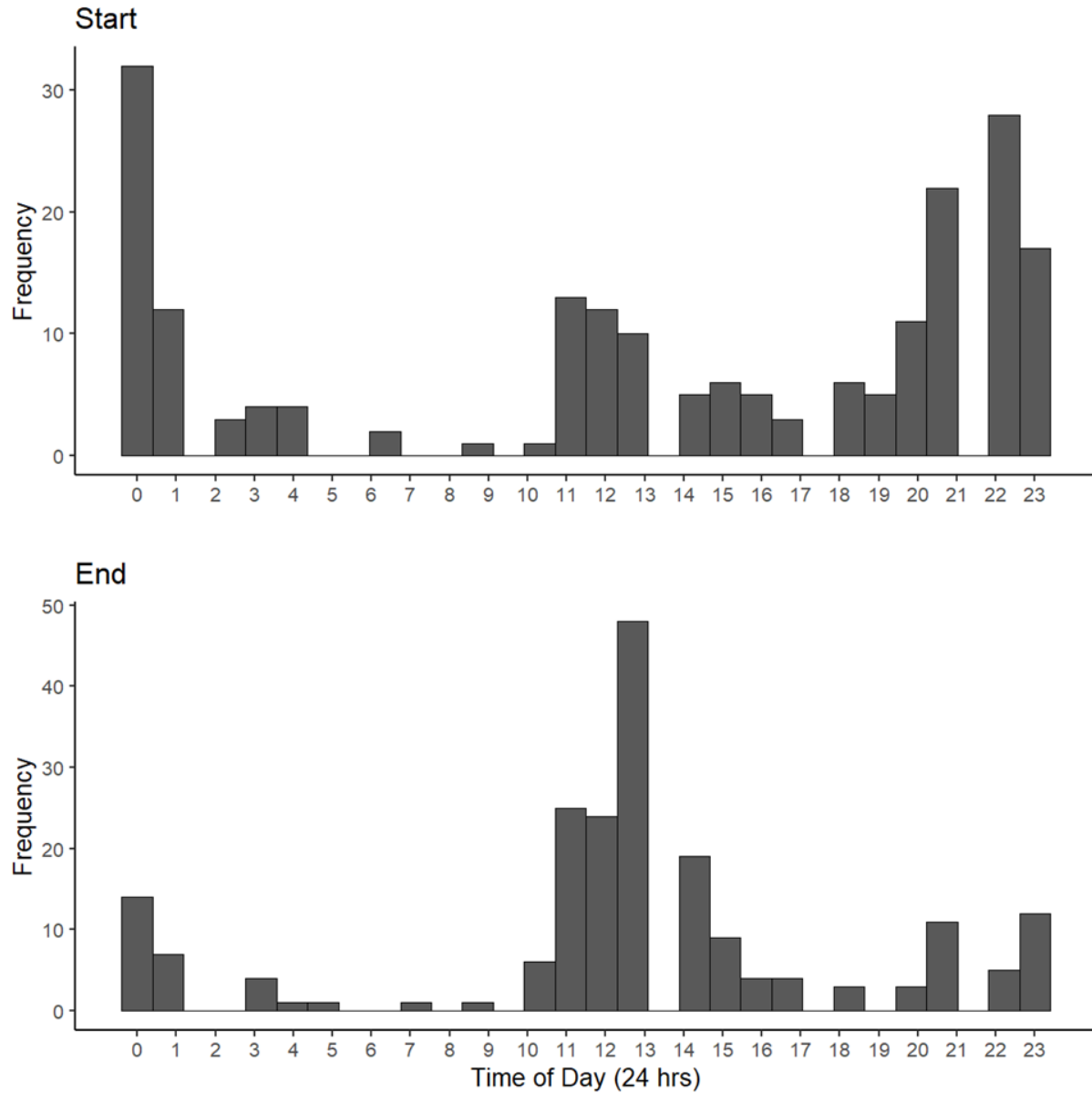


Figure C.4 Frequencies of times of day when 14 adult Ospreys start (top panel) or end (bottom panel) their stopovers during autumn migration (2019 – 2022).

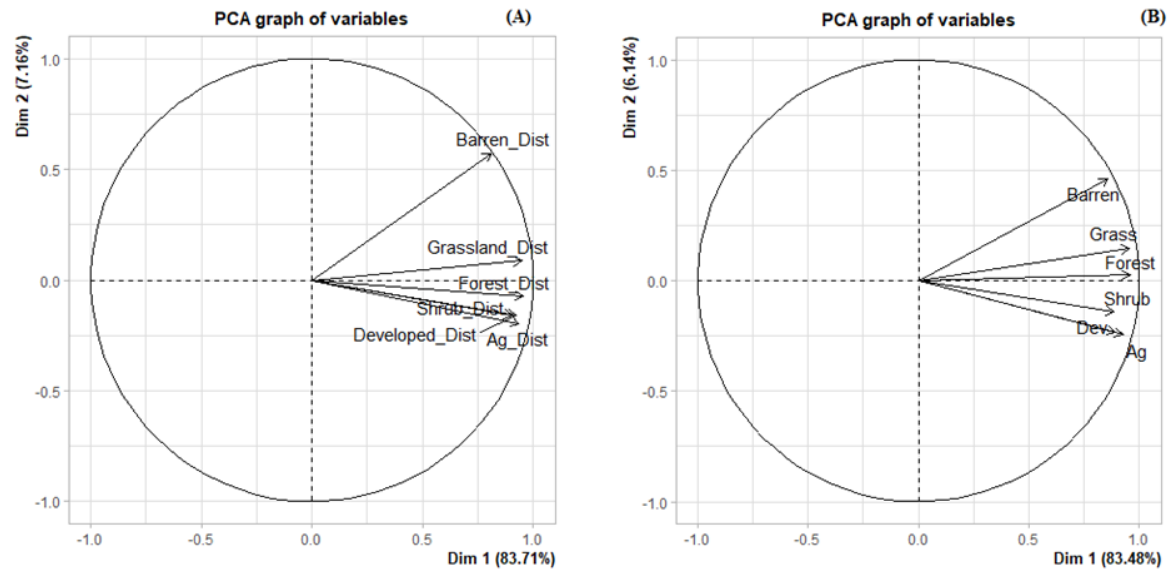


Figure C.5 Variables graphs from two principal components analyses on distance to six landcover variables (development, forest, barren, shrub, agriculture, and grassland), where (A) PC1 used for stopover occurrence and (B) is PC1 used for stopover duration.

APPENDIX D

SUPPLEMENTARY MATERIAL: CHAPTER IV



## Tables

Table D.1 Results of screening 27 microsatellite loci from Dawson et al. (2015) on 242 Ospreys sampled in the southeastern United States, with comparisons to Viverette (2016). Loci used in final analyses are in **bold**.

<b>Locus</b>	<b>Result</b>	<b>Viverette (2016) result</b>	<b># Alleles</b>	<b>Observed bp* range</b>
Pha02	Unreliable; stutter	Tremendous stutter & signal varies	-	-
<b>Pha03</b>	Low variation	Not tested	3	109 – 113
Pha04	Good	Good	4	154 – 160
Pha05	Good	Low variation	4	255 – 261
<b>Pha09</b>	Good	Good	6	245 – 255
<b>Pha10</b>	Good	Stutter peaks, difficult to call	5	163 – 173
<b>Pha11</b>	Good	Low signal	5	366 – 374
Pha12	Good	Very low signal	9	300 – 316
Pha13	No amplification	Not tested	-	-
<b>Pha14</b>	Good	Stutter peaks, difficult to call	6	161 – 179
Pha15	No amplification	No variation	-	-
<b>Pha16</b>	Good	Good	4	298 – 306
Pha17	Monomorphic	Split peaks	2	259 – 262
<b>Pha18</b>	Low variation	Low variation	2	203 – 205
Pha19	Low variation	Low variation, high error rate	3	89 – 93
Pha20	Low variation	Not tested	2	111 – 113
Pha23	Unreliable; stutter	Not tested	-	-
Pha25	Good	Not tested	4	168 – 180
<b>Pha27</b>	Good	Not tested	8	152 – 180
<b>Pha28</b>	Good	Not tested	6	113 – 133
<b>Pha29</b>	Good	Not tested	7	131 – 151
Pha30	Good	Not tested	4	240 – 252
Pha31	No amplification	Not tested	-	-
<b>Pha33</b>	Good	Not tested	5	117 – 133
Pha35	No amplification	Not tested	-	-
<b>Pha36</b>	Good	Not tested	7	150 – 174
Pha37	No amplification	Not tested	-	-

\*bp = base pair

Table D.2 Annealing temperatures used in this study for 27 microsatellite loci described by Dawson et al. (2015). Recommended temperatures were 57 – 58 °C (Dawson et al., 2015). Each locus was first tested with an annealing temperature of 58 °C and then progressively 1 °C lower until amplification was achieved. Pha13, Pha15, Pha31, Pha35, Pha37, and Pha33 did not amplify at the temperatures tested (54 – 58 °C).

Locus	Annealing temperature (°C)	Locus	Annealing temperature (°C)
Pha02	57 – 58	Pha19	56
Pha03	55	Pha20	58
Pha04	57 – 58	Pha23	57 – 58
Pha05	54	Pha25	57 – 58
Pha09	57	Pha27	56 – 57
Pha10	54	Pha28	57 – 58
Pha11	54	Pha29	58
Pha12	56	Pha30	55 – 58
Pha13	No amplification	Pha31	No amplification
Pha14	57 – 58	Pha33	54
Pha15	No amplification	Pha35	No amplification
Pha16	57	Pha36	57 – 58
Pha17	57	Pha37	No amplification
Pha18	54	-	-

Table D.3 Tests for Hardy-Weinberg proportions with a Bonferroni correction for 114 tests (i.e., <0.0004; significance level 0.05 divided by the number of tests). Exact P values are provided and P values <0.0004 are in **bold**. Exact tests based on Monte Carlo permutation (n = 1000).

Loci	$\chi^2$	Degrees of freedom	$\chi^2$ P	Exact Test P
<b>PHA19</b>	<b>56.9</b>	<b>3</b>	<b>&lt;0.0004</b>	<b>&lt;0.0004</b>
PHA27	118.6	21	0.001	0.016
PHA18	7.5	1	0.006	0.004
PHA16	11.7	6	0.067	0.05
<b>PHA12</b>	<b>153.6</b>	<b>36</b>	<b>&lt;0.0004</b>	<b>&lt;0.0004</b>
PHA14	14.1	15	0.518	0.183
<b>PHA20</b>	<b>81.3</b>	<b>1</b>	<b>&lt;0.0004</b>	<b>&lt;0.0004</b>
PHA29	12.6	21	0.920	0.671
PHA11	11.1	10	0.350	0.361
PHA10	15.1	10	0.127	0.075
<b>PHA04</b>	<b>21.4</b>	<b>6</b>	<b>0.001</b>	<b>&lt;0.0004</b>
PHA09	14.7	15	0.4689	0.36
<b>PHA30</b>	<b>59.7</b>	<b>3</b>	<b>&lt;0.0004</b>	<b>&lt;0.0004</b>
PHA25	15.3	1	0.001	0.015
PHA33	17.1	10	0.072	0.144
PHA03	4.7	3	0.194	0.279
PHA36	15.1	21	0.817	0.629
PHA28	9.9	15	0.822	0.418
<b>PHA05</b>	<b>60.7</b>	<b>6</b>	<b>&lt;0.0004</b>	<b>&lt;0.0004</b>

Table D.4 Test for Hardy-Weinberg proportions with a Bonferroni correction for 114 tests (i.e.,  $<0.0004$ ; significance level 0.05 divided by the number of tests) using exact tests based on Monte Carlo permutation ( $n = 1000$ ). Exact P values are provided, however P values  $<0.0004$  are reported as 0 and in **bold**.

Population	19	27	18	16	12	14	20	29	11	10	04	09	30	25	33	03	36	28	05
C AL	0.153	0.296	1	1	0.067	1	0.093	1	0.217	1	1	0.644	0.085	1	1	1	1	0.231	0.652
N AL	0.001	0.12	1	0.618	<b>0</b>	0.785	<b>0</b>	0.56	0.754	0.412	0.054	0.381	0.001	0.046	0.111	1	0.218	0.24	0.016
KY	0.001	0.566	0.262	0.33	<b>0</b>	0.228	0.001	0.597	0.375	0.375	0.015	0.301	0.001	1	0.291	1	0.846	0.605	0.001
MS/AL	0.013	0.361	1	0.039	0.004	0.063	0.003	1	0.635	0.285	0.605	0.437	0.01	1	0.099	1	0.355	0.605	0.64
TN	0.005	0.138	0.28	0.033	0.006	0.506	0.002	1	0.092	0.802	0.336	0.514	0.004	0.041	0.505	0.215	0.051	0.367	<b>0</b>
MA	1	0.53	1	1	0.95	0.443	1	0.885	0.841	0.729	0.053	0.08	0.001	1	0.914	1	0.486	0.937	0.001

Table D.5 Eigen values by axis and sample eigen vectors for the principal components analysis (PCoA) of pairwise  $F_{ST}$  values (see Table 4.2) for the six populations.

Axis No.	1	2	3	4	5
<b>EigenValue</b>	0.013	0.010	0.006	0.004	0.002
<b>Coastal AL</b>	0.102	-0.005	0.008	-0.010	-0.005
<b>North AL</b>	-0.011	0.005	-0.019	-0.014	0.041
<b>KY</b>	-0.007	-0.006	0.020	0.054	0.005
<b>TN</b>	-0.014	-0.007	-0.060	0.006	-0.020
<b>MS/AL</b>	-0.041	-0.064	0.028	-0.022	-0.009
<b>MA</b>	-0.029	0.076	0.022	-0.014	-0.012

Abbreviations: AL = Alabama, KY = Kentucky, TN = Tennessee, MS = Mississippi, MA = Massachusetts.

Table D.6 STRUCTURE analysis and STRUCTURE Harvester results identifying  $K = 4$ , which are in **bold**.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	10	-2524.72	0.1549	-	-	-
2	10	-2500.87	2.0849	23.8500	20.080000	9.631191
3	10	-2497.10	4.2040	3.7700	23.260000	5.532870
<b>4</b>	<b>10</b>	<b>-2470.07</b>	<b>3.2297</b>	<b>27.0300</b>	<b>46.890000</b>	<b>14.518195</b>
5	10	-2489.93	13.1202	-19.8600	39.970000	3.046443
6	10	-2549.76	27.2886	-59.8300	41.120000	1.506857
7	10	-2568.47	18.1614	-18.7100	30.770000	1.694255
8	10	-2617.95	13.7328	-49.4800	36.320000	2.644765
9	10	-2631.11	19.1768	-13.1600	-	-

Figures

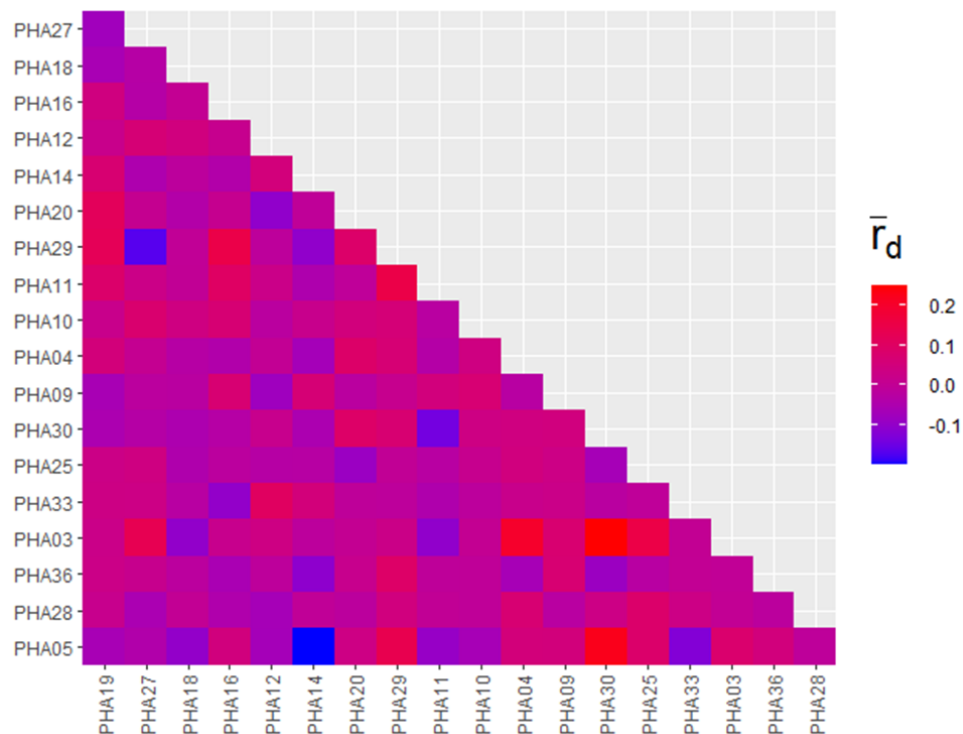


Figure D.1 Pairwise associations of 19 loci illustrating the measure of correlation,  $\bar{r}_d$ ; less than 5% of the variation in one marker is shared with the other marker.

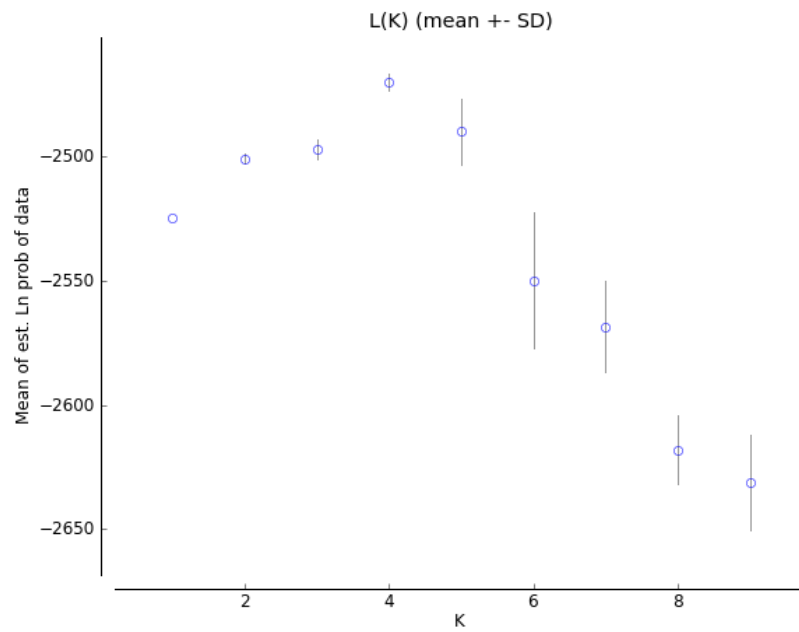


Figure D.2 Likelihood plot from *STRUCTURE* analyses of microsatellite data from 79 individual Ospreys, created by *STRUCTURE Harvester*. Where the mean L(K) is maximized, K (probable number of unique genetic clusters) is most likely (K = 4).

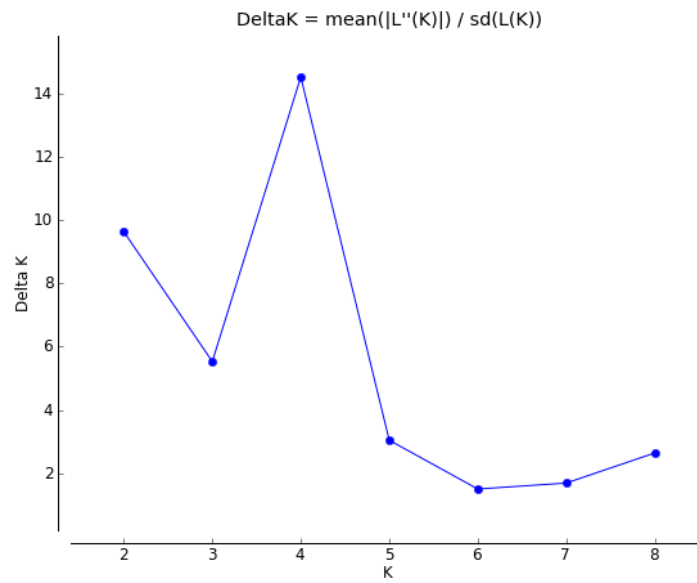


Figure D.3 *STRUCTURE Harvester* plot showing rate of change in log-likelihood values,  $\Delta K$ . The maximum  $\Delta K$  indicates the most likely number of unique genetic clusters ( $K = 4$ ).

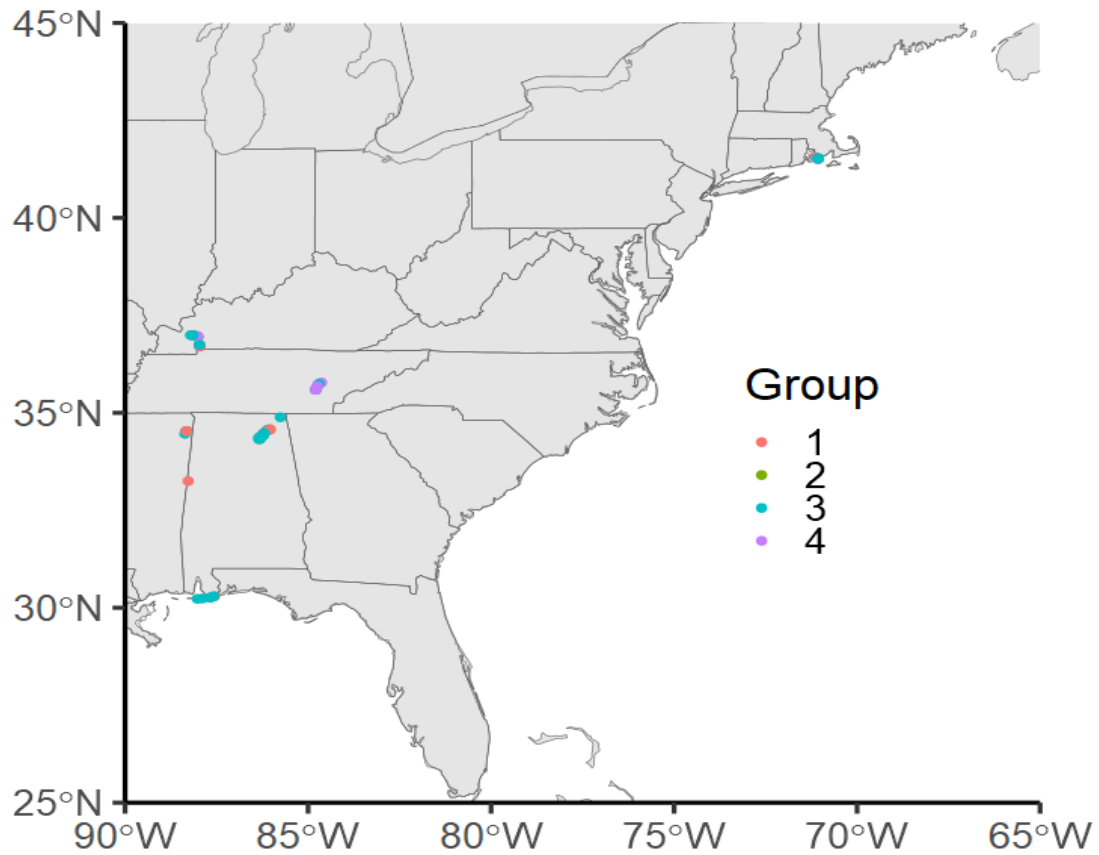


Figure D.4 Assignment of 79 individual Ospreys to one of  $K = 4$  clusters identified in program *STRUCTURE* based on 13 microsatellite loci. Birds are assigned to each population based on maximum probability. 57% ( $n = 45$ ) of birds were assigned to group 3.