CLAPPER RAIL DEMOGRAPHY AND POPULATION GENOMICS

by

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A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Wildlife Ecology

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POPULATION GENOMICS

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ABSTRACT

Understanding ecological and evolutionary dynamics in globally rare tidal marsh systems is important in the face of anthropogenically accelerated climate change. In particular, the north Atlantic coast of the U.S. is considered a hotspot for accelerated sea level rise, resulting in an urgent need for full annual cycle data to manage wildlife populations in this region. Secretive, migratory birds pose a challenge as they are difficult to track throughout their various life stages and their population connectivity is largely unknown.

My dissertation focuses on one such species, the Clapper Rail (*Rallus crepitans*), which is a cryptic salt marsh specialist endemic to North America. Although historical observations documented various aspects of Clapper Rail life history, virtually no quantitative research or contemporary data exist across most of its range. Data needs are particularly critical for the migratory subspecies, the declining Northern Clapper Rail (*R. c. crepitans*). I therefore focused my efforts on estimating important breeding season vital rates (nest and chick survival), quantifying space use and migratory patterns, developing a reference genome to support genomic work, and evaluating population genetic structure of Clapper Rail throughout their U.S. range.

I produced the first quantitative nest survival estimates for Clapper Rail in this region, revealing that site, nest visual obscurity, and time of season influence nest survival. Additionally, I provide here the first data directly tracking *Rallus* chick survival. Using tag technology, I produced the first home range estimates for the Northern Clapper Rail, assessed brood movement patterns by tracking chicks, and

discovered a novel migratory pathway taken by Clapper Rail in Delaware. After assembling a high-quality Clapper Rail reference genome, I used ddRAD sequencing and determined that Clapper Rail along the U.S. Atlantic and Gulf coasts are panmictic and hybridize extensively with King Rail throughout this range. My results will serve as baseline data for future work with Clapper Rail and the King/Clapper Rail species complex and provide a foundation for conservation and management of this declining species.

Chapter 1

CLAPPER RAIL NEST AND CHICK SURVIVAL

Introduction

Understanding the underlying biological processes regulating wildlife populations is crucial to wildlife management. Having accurate vital rate estimates is necessary when status and trends data suggest that a given species, subspecies, or population is declining. Without an understanding of a species' vital rates across the full annual cycle, it is extremely difficult to establish the potential causes of population declines or to identify what factors may be limiting populations. Nest survival is one demographic parameter needed for understanding bird population dynamics as it is directly related to fecundity and therefore, productivity (Newton 1998). For populations to replace adults lost to mortality or to increase, enough offspring must hatch and survive to the following breeding season.

The Clapper Rail (*Rallus crepitans*) is a salt marsh specialist species that evolved to persist in a dynamic ecosystem (Meanley 1985). Endemic to North American salt marshes, Clapper Rail are adapted to tolerate high salinity environments and dynamic hydrology characterized by tidal fluctuations. During the breeding season, their loud territorial calls allow their populations to be monitored through auditory surveys; however, their overall secretive nature and the inaccessibility of

their habitat introduces complexity in collecting the basic vital rate data necessary to understand Clapper Rail population dynamics. Clapper Rail spend much of their time obscured by marsh vegetation and are not regularly observed visually, making them unsuitable candidates for band resighting data collection.

The Northern Clapper Rail (*R. c. crepitans*, hereafter Clapper Rail) is the only migratory subspecies with a breeding range that extends from North Carolina north to southern New England (Figure 4.1, Rush et al. 2020). Population trend data revealed that Clapper Rail populations in the Northeast US declined 4.5% annually from 2000 – 2015 (Correll et al. 2017), but causes of this decline are unknown and vital rate information does not exist. The population size of Northern Clapper Rail was estimated at 110,000 and 151,000 with two-thirds of the population residing in Delaware Bay and Coastal Delmarva (Wiest et al. 2016, 2019). Delaware lost nearly 4,000 acres of wetland habitats between 1992 and 2007 with 83% of estuarine wetland loss attributed to conversion to open water (Tiner et al. 2011). In addition to direct flooding and submersion of terrestrial habitats, sea level rise is altering salinity gradients, tidal regimes, and storm impacts in the mid-Atlantic (Sallenger Jr et al. 2012, Lee et al. 2017). The Northern Clapper Rail range occurs entirely within a sea level rise hotspot stretching from Cape Hatteras, North Carolina northward to Cape Cod, Massachusetts. Sea level rise within this hot spot is occurring at a rate 3 - 4 times greater than the global average (Sallenger Jr et al. 2012) thereby increasing the magnitude of impact that sea level poses on the declining Northern Clapper Rail population.

Clapper Rail in the mid-Atlantic have been the focus of limited research since the 1940s when rails were both more abundant on the landscape and a more popular game species. Even so, no data on migration survival rates, juvenile survival, chick survival, or overwintering survival exists. Fortunately, records of nesting habits and nest survival from New Jersey and Virginia during this timeframe exist. Apparent survival (i.e. the raw amount of nests observed to succeed) in the 1940s was observed to be 89.3% near Ocean City, New Jersey (Kozicky and Schmidt 1949) and >90% in Chincoteague, Virginia (Stewart 1951). Nest survival was more variable in the longest-term nest survival study in the mid-Atlantic, which occurred in New Jersey from 1955-1967. Nest survival during this period ranged from 97.5% in 1962 to 50% in 1967; however, changes in mosquito spraying policies in 1967 may have been a contributing factor on low nest survival years (Ferrigno 1969). These historical data provide an opportunity to compare present-day nest survival estimates to historical baseline estimates. Predation and flooding, due to high tide or storm events, are the two primary causes of nest failure in Clapper Rail range wide (Kozicky and Schmidt 1949, Ferrigno 1969, Rush et al. 2010, 2020). Clapper Rail typically construct nests above the surface of the marsh and weave at least a partial nest canopy that they maintain throughout the nest period, but it is unclear if this serves to avoid detection by predators, prevent egg loss during floods, or both (Kosten 1984, Meanley 1985, Rush et al. 2020). Nesting habitat within a territory is likely based on elevation (Valdes et al. 2016), vegetation height (Kozicky and Schmidt 1949, Valdes et al. 2016), and the “grass height above tide” (Andrews 1980).

Currently, there are no estimates of Clapper Rail chick survival, information which is critical as the time from hatching to independence is a vulnerable life stage that can influence population dynamics (Newton 1998). Clapper Rail chicks are considered semi-precocial because they are ambulatory within a few hours of hatching but are unable to forage effectively, likely due to their small size (Meanley 1985). Both adults provision the chicks after hatching and construct woven brooding platforms to brood the chicks overnight (Adams and Quay 1958, Meanley 1985), but it is unclear how far they may travel with the brood. Adult Clapper Rails may also split the brood, especially if one adult is still tending the nest during an asynchronous hatch (Rush et al. 2020). Brood counts are frequently used to estimate productivity for precocial breeding birds (Rice 2003, Dahlgren et al. 2010) but the difficultly in spotting small, black Clapper Rail chicks among the marsh shadows coupled with the uncertainty about brood completeness makes brood counts untenable for this species. Previous work focused on King Rail (*Rallus elegans*) attempted to quantify chick survival by observing broods, but the authors note that some broods were not located until more than 5 weeks of age limiting the ability to estimate chick survival (Darrah and Krementz 2011). Given the significant logistical challenges associated with estimating chick survival, radio-marking and tracking is likely the most reliable way to directly monitor chick survival at this time.

To address these knowledge gaps in Clapper Rail nest and chick survival, we sought to locate and monitor rail nests in tidal marshes along Delaware Bay. When possible, we also captured chicks and tagged them using a novel adhesive approach to

monitor their survival immediately post-hatch. This is the first attempt to date to directly follow the survival of individual Clapper Rail chicks and, to the knowledge of the authors, the first attempt to tag any *Rallus* chick. Our objectives were to quantify nest survival across both the laying and incubation period of Clapper Rail nests and to provide the first estimates of Clapper Rail chick survival immediately post-hatch.

Methods

Study area

We selected three tidal marsh study sites in Delaware along a salinity gradient in Delaware Bay (Figure 1.1). Salinity is the primary diver in determining tidal marsh vegetation structure and composition (Crain et al. 2004) and is greatest at the mouth of the Delaware River (Gay and O'Donnell 2009). Our up-river site with lowest salinity, Woodland Beach Wildlife Area, was dominated by *Spartina alterniflora* and *S. cynosuroides* with *Bolboschoenus* sedges and *Phragmites australis*. Our mid-river site, the St. Jones National Estuarine Research Reserve, was dominated by *Spartina alterniflora*, *Spartina patens,* and *Iva frutescens*. Our site closest to the mouth of the Delaware River, Mispillion Harbor, was dominated by short-form *S. alterniflora*, *S. patens*, and *Disticulus spicata*. All three sites had evidence of human-altered hydrology, with the Mispillion River site having the most extensive grid-ditching (Figure 1.2).

Nest searching and monitoring

We conducted nest searching and monitoring from May through July of 2019-2021 (AUP 1157). Nests were located using three methods: systematic nest searching (i.e. walking transects in the marsh), Unmanned Aerial Vehicle (UAV) flights, and incidental discovery while traversing the marsh. Systematic nest searching transects were selected based on observed bird activity in the area and were typically conducted with three to four people. UAV flights were conducted in a subset of these areas using a forward-looking infrared (FLIR) thermal imaging camera mounted on a Matrice 210 V1 quadcopter. The pilot monitored the thermal footage in real time to identify potential nest heat signatures. The coordinates for those detections were recorded and ground truthed as soon as possible to determine if a nest was present.

Once we located a nest, we monitored each nest using the SHARP demographic protocols (tidalmarshbirds.org, Ruskin et al. 2017). Specifically, we recorded nest contents (eggs and chicks), parental behavior, nest status, and any information that might be pertinent for later nest fate classifications. We checked each nest every 3-4 days unless it was not possible to access the site (due to weather or equipment constraints) or if the nest was approaching hatch. When nests were near their predicted hatch date, we checked them daily to capture/observe chicks. Adult rails were often not observed during the nest visits, but we considered nests to be actively incubated if the eggs were warm. Nests were classified as abandoned when eggs past the laying stage were cold and no adults were present for two consecutive visits. If nest failure was suspected, photos and detailed notes were taken to document

the nest failure. We considered nests depredated if clear signs of predators were present at the nest check. These signs included caved-in eggs, eggs with holes in the side, disturbance to the nest structure, and predator sign (scat, tracks, etc.). We considered nests failed due to flooding if a failed nest coincided with a flood event and had signs of flooding (such as damage to the nest structure). See Appendix B for nest fate assignment dichotomous key.

Generally, Clapper Rail clutch sizes range from 4-14 eggs, incubation lasts 18-22 days, and chicks can leave the nest within one day of hatching (Kozicky and Schmidt 1949). Consequently, verifying nest age upon discovery is necessary to correctly categorize nest fates. To determine nest age and to monitor nests as they progressed through incubation, we marked and floated the same four eggs at each nest visit to establish the nest age and monitor nest progress. If egg marks were not present at a later visit, we marked new eggs and floated the newly marked eggs at subsequent visits. We modified the nest aging approach used by Rush et al. (2007) using data from nests found during the laying period (n = 24) to account for shorter incubation periods in the northeast relative to the Gulf coast (Figure 1.3). Using these egg float data and hatch dates when available, we back calculated the first egg date of the nests. In cases when the nests were found during the laying period, we back calculated to the first egg day by assuming one egg was laid daily (Kozicky and Schmidt 1949).

Directly observing the chicks following a successful hatch is difficult as the chicks are ambulatory within the first day post-hatch. However, nests typically hatch asynchronously over several days, permitting us to detect hatch events by increasing

visit frequency when float data suggested hatch was imminent. When nests were confirmed to have hatched, we estimated the number of successful chicks based on the number of chicks observed, progression of the nest, and the number of clearly failed/abandoned eggs remaining in the nest after the adults left with the brood. We considered a nest successful if at least one chick hatched and left the nest. We classified a nest as unknown fate in cases where the nest was within the predicted hatch window based on floating data, but insufficient information was available to definitively confirm a hatch event.

Chick capturing and monitoring

We captured chicks by hand when we encountered or checked a nest during hatching. Each captured chick was marked with a paint marker to identify it if it was recaptured in subsequent nest checks. The paint served as a short term mark and was distinguishable for several days, but the majority washed off within the first day resulting in a mark that was not visible from a distance. Chicks >12 g were tagged with a 0.31 g VHF radio tag using a clear-drying eyelash adhesive (Duo Strip Lash Adhesive, Figure 1.4). Tags were applied to the back of the chicks without any feather removal or mesh that would add additional weight. We attempted to locate each chick daily until they reached one week of age then reduced tracking frequency to every 2-3 days to monitor their survival immediately following a hatch event.

Covariate sampling

We measured nest height (cm; measured from the lip of the nest cup to the marsh surface) and nest canopy classification (either no canopy, a partial canopy, or a full canopy) at the time the nest was discovered. As soon as the nest was no longer active, we measured the greatest height at which visual obscurity was 100% (cm) and average vegetation crown height (cm) at the nest and at 4 additional points 5 m from the nest in each cardinal direction using a Robel pole (Robel et al. 1970). We also quantified ground cover (the percentage of coverage for each plant species around the nest) using a 1 m^2 quadrat at the nest location per Saltmarsh Habitat Avian Research Program protocols (tidalmarshbirds.org). Ground cover was classified as unvegetated, *S. alterniflorus*, *S. pumilis*, *Distichlis*, wrack (i.e. debris washed up by the tides), *Juncus*, or other. We used ArcGIS (ESRI) to measure the distance (m) between nests and tidally influenced waterways including man-made ditches, natural creeks, and rivers.

Statistical analyses

Nests with uncertain ages or that may have been discovered after hatch initiation were excluded from the analysis. This included nests discovered during the hatching process and nests that may have been abandoned prior to being located. Nests with unknown fates (see criteria above) were censored after the last day it had a known fate (i.e. the last nest check where the nest was active). Before the analysis, we assessed our covariates for multicollinearity using the cor() function in R (R Core Team, stats package, version 4.3.0). We excluded average vegetation crown height in

our candidate models because of its high correlation (>0.6) with obscurity measurements and nest heights (Table 1.1).

We used a logistic exposure model within a Bayesian framework (Schmidt et al. 2010, Darrah et al. 2018, Sinnott et al. 2022) to model Clapper Rail daily nest survival. The logistic exposure model allowed us to account for varying exposure time between nest visits and to model individual nest as a random effect to account for unmeasured variance at the nest level (for example, the varying degrees of defensiveness between different parents). We selected explanatory variables pertaining to the physical nest structure that could influence predation or flooding, the nest location (such as site or distance from a tidal creek), and temporal factors (time of season, year), We then used these explanatory variables to develop 12 candidate models (Table 1.2).

We fit our Bayesian hierarchical model using the rstan package in R (Stan Development Team 2022, R package version 4.2.1). We visually evaluated chain convergence and verified via the Gelman-Rubin statistic ($\widehat{R}$, Gelman et al. 2013), both measures indicating a reasonable assumption of convergence and by inspecting trace plots to ensure that chains were well mixed (Link and Barker 2010). We evaluated support for our predictions using leave-one-out cross-validation (LOO-CV) via the R package loo (Vehtari et al. 2018). We compared expected predictive accuracy of our competing models relative to the top model by estimating the difference in their theoretical expected log pointwise predictive density (elpd diff) and the standard error (se) of that difference (Vehtari et al. 2017, 2022). If the top ranked models differed in

elpd values by <2 when considering the standard error of elpd, we considered them to be competitive models and evaluated them further. We used stacking of predictive distributions to evaluate which of these models had the highest probability to be the most accurate model (Yao et al. 2018, Medeiros et al. 2022, Thiel et al. 2022) and selected the model with the highest weight as the top model. We estimated the effect of our top model on period survival by varying each variable of interest across its range of observed values and exponentiating estimates to 28 days to represent the exposure duration of a full nesting cycle. We began period predictions involving ordinal day at the earliest known nest initiation and cut them off at the latest known nest initiation. Credible intervals for our predictions were calculated using the Highest Density Interval method with the bayestestR package in R (Makowski et al. 2019). Unless otherwise noted, all values are presented as means ± SE.

Results

We found 205 Clapper Rail nests and were able to use 165 to model nest survival (see Table 1.3 for more information on excluded nests). We documented 26 nest failures due to depredation (18 at Mispillion River, 8 at Woodland Beach), 5 failures due to flooding (4 at St. Jones, 1 at Mispillion River), and an additional 17 nests that failed due to unknown causes. The majority (74%) of nests were located at the Mispillion River site (n = 122) with lower numbers of nests found at St. Jones (n = 8) and Woodland Beach (n = 35). The closest simultaneously active nests we found were 5 m apart at the Mispillion River site. Of the nests used in the analysis, 115 (69.7

%) were successful, 37 (22.4 %) failed, and 13 (7.9 %) were classified as having an unknown fate. Thirty-one nests (18.8%) were found during the laying period and 134 (81.2%) were found during the incubation period. The average visits per nests was 5.5 (± 0.09) and the average duration of time between visits was 2.27 days (± 0.03). Average clutch size was 9.16 (± 0.165) and decreased as the season progressed (Figure 1.5). The average estimated chicks per successful nest was 7.92 (± 0.23). Mean nest height was 21.43 cm (± 0.84) overall, but nests were higher on average at Woodland Beach (Figure 1.6). Nest height did not vary across the season at St. Jones or Mispillion River. At Woodland Beach, however, nest height increased over the season (Figure 1.6).

The global model had the greatest stacking weight among the competitive models (Table 1.4). Within the global model, three linear effects (two site effects and visual obscurity) and one quadratic effect had 90% credible intervals that did not overlap zero (Figure 1.7, Table 1.5). Obscurity had a positive relationship with nest survival (Table 1.5). Both the St. Jones and Woodland Beach site effects had negative relationships with nest survival. Nests at the Mispillion River site had the greatest daily survival rate (peak period survival was 0.96), followed by Woodland Beach (peak period survival was 0.82) and St. Jones (peak period survival was 0.74, Figure 1.8, Table 1.6).

The ordinal day quadratic effect indicated that nest survival was lowest in the early season, highest in the middle of the season, and declined toward the end of the nesting season (Figures 1.9, 1.10, 1.11). The highest probability of nest success for a

full 28-day nesting period occurred with a nest initiation day of 160 (8 June; Figures 1.12, 1.13, 1.14). Nests initiated on 8 June had a predicted period survival probability of 0.96, 0.80, and 0.71 for the Mispillion River, Woodland Beach, and St. Jones sites respectively (Table 1.6). Nests initiated on the earliest day we documented a rail nest initiation (23 April) had very low chances of survival; at Woodland Beach and St. Jones period survival across this time frame was nearly zero and at Mispillion River the probability of survival was 0.07.

We captured 299 chicks from 92 successful nests with an average of 3.25 chicks caught per nest (range 1-8, SE = 0.21). We tagged 81 chicks from 33 nests with VHF radio transmitters. On average, we tracked each chick for 4 days (range 0-22 days). Many tags were located off the chick in the nest or on brood platforms and were presumably removed by the adults. We tracked 19 chicks for >9 days or until a mortality event and 10 chicks went missing (i.e. had unknown fates). We documented four chick mortalities and apparent survival for the known-fate chicks across the 9-day period was 78.9%. One chick died of unknown causes, one chick appeared to have drowned, one chick appeared depredated by an unknown predator, and one chick was found in a crab burrow.

Discussion

Clapper Rail nest survival varied temporally within the breeding season and spatially across our study sites, and chick survival was high during the days immediately after hatching. Our high nest survival was consistent with that in the Gulf

Coast (Rush et al. 2010) and considerably higher than nest survival documented in South Carolina where period survival was estimated to be 43.5% (Ricketts 2011). Clapper Rail nest survival rates were high during the peak nesting season and nests initiated during the early season had much lower survival. The temporal effect of nest initiation time on nest survival could be driven by temperature, within season vegetation growth, migration phenology, or local density dependent effects. Temperatures across the season vary and the closely related King Rail (*Rallus elegans*) has been shown to exhibit plastic incubation behavior in response to temperature changes (Clauser and McRae 2017). Shifts in behavior or an inability to maintain the desired incubation temperature may help to account for seasonal variability. Ricketts (2011) found a small positive effect of ordinal day on Clapper Rail nest survival in South Carolina, but only modeled the linear relationship. They determined that the maximum high-tide water levels had a quadratic effect on nest survival, with nests at extremely low high-tide heights having a very low probability of success and nests at extremely high high-tide heights having a lower probability of success than at mean high-tide height. This finding suggests that despite a risk of flooding from the tides, nesting in areas with regular tidal flooding diminishes the probability of a nest being depredated (Ricketts 2011). Our results indicated that visual obscurity had a positive effect on nest survival, therefore it is possible that sparser vegetation characteristics earlier in the season may have allowed predators to locate nests more easily. Other potential explanations for the patterns observed in the seasonal variation in nest survival may be attributed to rails arriving early on the

breeding grounds may have lower energetic investment in early nests. Also, as nest density increases towards the peak breeding season, the additional nests on the landscape may diminish the odds of any one nest being located by a predator via predator satiation (Afton et al. 1992, Eckrich and Owens 1995).

Chick survival for the chicks we were able to sample was high, but there is considerable uncertainty given the number of chicks we did not monitor for which fates could not be determined. These missing chicks may have had tags that fell off in creeks or rivers or may have had their tags damaged by the parents. Predators could also have carried the tags out of range. Gulls are a known predator of Clapper Rail chicks (Segrè et al. 1968) that are prevalent in the area and may fly off with tagged chicks. Additionally, rail chicks are small enough to be consumed even by large turtles or fish.

Our data indicated that study site was a driver of nest survival. One potential underlying cause of a site effect may be related to variation in Clapper Rail nesting densities among sites. As the breeding season peaks in June, nest density increases which may lead to predator satiation (Afton et al. 1992, Ringelman and Stupaczuk 2013). Of the three study sites in this project, the Mispillion River site had the greatest nest survival and nest density compared to the other two sites despite apparently abundant food sources across all sites. Some nests at the Mispillion River site were in close proximity (< 5-10 m apart) and we documented one bird with five nests in his overall home range (see Chapter 2) suggesting a tolerance to high conspecific

densities. The Mispillion River site also has greater Clapper Rail abundances based on auditory survey data (Tymkiw et al. 2021) and had extensive mosquito ditching which Clapper Rail are known to utilize for nesting (Kozicky and Schmidt 1949, Ferrigno 1969, Meanley 1985). Density dependent effects are known to exist in waterfowl, with closely clustered nests correlating with increased nest success (Ringelman et al. 2012, 2014) , thus the higher nest density at the Mispillion River site may have played a role in nest success. However, this effect can vary across the season as predators key in on nest clusters (Ringelman et al. 2018). Nonetheless, Clapper Rail at the very least do not appear to have diminished fecundity or nest survival due to crowding that is observed in some bird species (Martin 1988) and may even exhibit adaptive nest clustering. Nest survival was lowest at the St. Jones site, where the fewest number of nests were found and the fewest rails were detected on auditory surveys (Tymkiw et al. 2021). Although we did not quantify nest-searching effort in this study, on-going work at these sites has located fewer nests per people-hour when searching at St. Jones despite similar habitat composition as the Mispillion River site (Glasko pers. comm.). The discrepancy in nest detection suggests that despite similar habitat structure between the Mispillion River and St. Jones sites, nest density was much lower at St. Jones. The Woodland Beach site had more Clapper Rails based on auditory surveys than St. Jones, but still had lower rail abundance when compared to the Mispillion River. The lower sample sizes at these two sites may also have made the estimates more sensitive to mortality events, such as storm events, which could have biased estimates low.

Our primary cause of nest failure was depredation. The three sites appeared to differ in predator communities and future work should seek to quantify these differences. Anecdotally, the Mispillion River site had greater gull densities than the other sites, which could potentially depredate nests (Segrè et al. 1968). We regularly detected North American river otters (*Lontra canadensis*) at the St. Jones site and have sighted rat snakes (*Pantherophis alleghaniensis*). We also detected mink (*Neogale vison*) at Woodland Beach, a known rail predator (Roth et al. 1972). Although foxes and raccoons were seen regularly at all three sites, these mesocarnivores likely had varying access to the marsh depending on the number of roads, boardwalks, and proximity to forested habitat. All sites were partially accessible from roads, but the marsh was on average closer to forested edges at St. Jones. Few nests failed due to flooding, with four out of five (80 %) flooding failures occurring at the St. Jones site during storm events. Although nests may experience partial nest failures during flooding events if the tides push eggs from the nest, Clapper Rail eggs can be inundated during high tide events and still hatch successfully (Kozicky and Schmidt 1949, Rush et al. 2020). Woodland Beach, the site with the highest tidal inundation, showed a pattern of increased nest height as the season progressed, possibly in response to these tidal patterns. Clapper Rail exhibit a suite of behavior to reduce nest failure due to flooding. During nest construction, most rails weave a domed canopy or arch structure that can prevent eggs from flooding from the nest (Kozicky and Schmidt 1949, Meanley 1985). The adults can also actively raise nests during unusually high

tide events (Meanley 1985) and displaced eggs can be retrieved by the adults and placed back into the nest (Pettingill 1938*a*).

Environmental contaminants are an additional factor that may contribute to the variation we observed in nest survival rates among sites. Pollution from agriculture, shipping vessels, and other anthropogenic activities has led to the bioaccumulation of contaminants in birds in Delaware Bay across diverse avian taxa (Rattner et al. 2000, Toschik et al. 2005, Warner et al. 2010). Environmental contaminants are known to reduce nest viability in the closely related Ridgway Rail (*Rallus obsoletus*, Schwarzbach et al. 2006). In Clapper Rail, contaminants accumulate in both the eggs and chicks (Cumbee et al. 2008) and may reduce egg thickness (Rodriguez-Navarro et al. 2002), thus potentially reducing nest success. The St. Jones site, which had the lowest survival rate, is also known to be highly contaminated due to its proximity to agricultural operations, the Dover urban area, and the Dover Airforce base (Delaware National Estuarine Research Reserve 1999). Environmental pollutants have also been suggested to negatively influence adult survival in response to storm events (Ferrigno 1969).

Our apparent Clapper Rail nest survival rate was lower than mid-Atlantic rates reported in the 1940s and 1950s (Kozicky and Schmidt 1949, Stewart 1951), but similar to rates reported in New Jersey in the 1960s (Ferrigno 1969). Both 1940s/1950s New Jersey and Virginia were reported to have apparent nest survival of ~90% while we observed ~70% apparent survival. However, both studies started relatively late in the breeding season. Nest searching efforts did not begin until May

26 in New Jersey (Kozicky and Schmidt 1949) or May 25 in Virginia (Stewart 1951) and both studies were conducted across a single year. These previous monitoring efforts may therefore have missed the first nesting attempts leading to estimates that were biased high. Research in New Jersey in the 1960s conducted across 13 nesting seasons showed a lower apparent survival rate of ~74% and was conducted beginning in April, thereby including early nests that are more prone to failure based on our findings (Ferrigno 1969). Although nest survival rates reported in the Gulf Coast were slightly higher at ~79% apparent survival (Rush et al. 2010), our survival rates appear to be much higher than those reported in the southeastern portion of the Clapper Rail range (Ricketts 2011). Apparent nest survival was reported to be as low at ~8% in Georgia (Valdes et al. 2016) and ~42% in South Carolina. There is no consensus among these studies on the greatest cause of nest failure; some studies observed much higher depredation rates, some observed failure due almost exclusively to flooding further supporting site level characteristics as key drivers of survival across the range of this species and not just within Delaware Bay. Additional work range wide is necessary to determine regional survival variation vs site level survival variation.

Summary and conclusions

Our results have important implications for targeted Clapper Rail management as well as tidal marshes as a whole. Our results join a growing body of work that suggests that multiple salt marsh obligate bird species have lower nest success in the early season (Roberts et al. 2017) and helps to inform management strategies and

prioritize the timing of protections. We found no influence of distance to tidal creek or ditch on nest survival and our site with the highest survival was heavily ditched, suggesting that these landscape features provide important habitat for rail nests. A preference for tidal creeks and ditches is well documented in the mid-Atlantic (Kozicky and Schmidt 1949, Stewart 1951, Meanley 1985) and our results suggest that, similar to Saltmarsh and Seaside sparrows (Roberts et al. 2017) attempts to plug or remove ditches could negatively impact nesting Clapper Rail.

TABLES

Table 1.1. Correlation matrix for continuous variables measured from 2019-2021 on Clapper Rail nests at the focal demographic sites in Delaware. "% alterniflora" refers to the percent of the ground cover sampling quadrat that was occupied by *Spartina alterniflora*. Nest height (cm) is the distance from the lip of the nest to the ground, obscurity (cm) is height of vegetative cover that provided 100% obscurity, crown height (cm) is height of the coverall vegetation near the nest, distance to creek (m) was the distance of the nest to the closest tidal creek/ditch/river, and ordinal day was the day of the year.

	Nest height	Obscurity	Crown height	Dist. to creek	Ordinal day
Obscurity	0.58	--	--	--	--
Crown height	0.44	0.69	--	--	--
Dist. to creek	0.11	0.25	0.19	--	--
Ordinal day	0.06	-0.02	-0.06	-0.01	--
% *S. alterniflora*	-0.16	-0.26	-0.25	-0.19	-0.07

Table 1.2. Candidate models and justifications for determining the factors that influence Clapper Rail nest survival in Delaware, 2018 - 2021. For candidate models containing linear ordinal day, a second model was included replacing linear ordinal day with quadratic ordinal day to account for both linear and quadratic time relationships.

Model Name	Model Justification
Global model (including linear ordinal day)	--
Global model (including ordinal day^2)	--
Null	--
Year + Site	Unmeasured variation from year or site
Height + Creek	Variables influencing flooding probability
Alterniflora + Obscurity + Canopy + Nest height	Variables influencing predator detection
Ordinal day	Linear seasonal variation
Ordinal day^2	Quadratic seasonal variation
Nest stage	Parental behavior influence after laying
Ordinal day + Nest stage	Combined season variation and parental behavior
Ordinal day^2 + Nest stage	Combined season variation and parental behavior

Table 1.3. The reasons Clapper Rail nests monitored in Delaware from 2019-2021 were excluded from analysis and how many nests were excluded by reason. Nests were excluded when uncertainty existed in whether the nest had already reached an ultimate nest fate prior to discovery.

Reason for exclusion	Number of nests excluded
Found during a hatching event	10
Possibly located after failure from unknown causes	9
Possibly located after a depredation event	6
Possibly located after a flooding event	1
Unknown if active when located and had unknown fates	14

Table 1.4. Model selection information for the candidate models of Clapper Rail nest survival in Delaware. The top model was selected based on the theoretical expected log pointwise predictive density (elpd) and Bayesian stacking weights using the loo package in R.

Model	elpd_diff	se_diff	stacking weight
Ordinal date^2	0	0	0.358
Ordinal date^2 + Nest stage	-0.5	1	0.000
Global (Ordinal date^2)	-2	4.3	0.404
Ordinal date	-2.2	2.3	0.000
Ordinal date + Nest stage	-2.7	2.7	0.000
Nest stage	-4.2	4	0.238
Null	-4.8	3.9	0.000
Obscurity	-5.5	4.2	0.0000
Year + Site	-6	4.7	--
Dist. to creek + Nest height	-6.7	4.1	--
Global (linear Ordinal date)	-7.3	4.7	--

Alterniflora + Obscurity + Canopy + Nest height	-7.8	4.5	--

Table 1.5. The median beta value estimates for the three significant linear effects on Clapper Rail nest survival in Delaware with 90% credible intervals.

1 Covariate	Median	Upper CI	Lower CI
St. Jones	-2.14	-0.89	-3.33
Woodland Beach	-1.66	-0.37	-2.97
Obscurity	0.92	1.61	0.27

Table 1.6. The predicted Clapper Rail nest daily and period survival rate by site under the best-case scenario temporally (i.e. if the nest was initiated on the day with the highest probability of period survival).

Site	DSR	Upper CI	Lower CI	28 day
Mispillion River	0.999	0.999	0.995	0.964
St. Jones	0.989	0.998	0.952	0.736
Woodland Beach	0.993	0.999	0.973	0.824

FIGURES

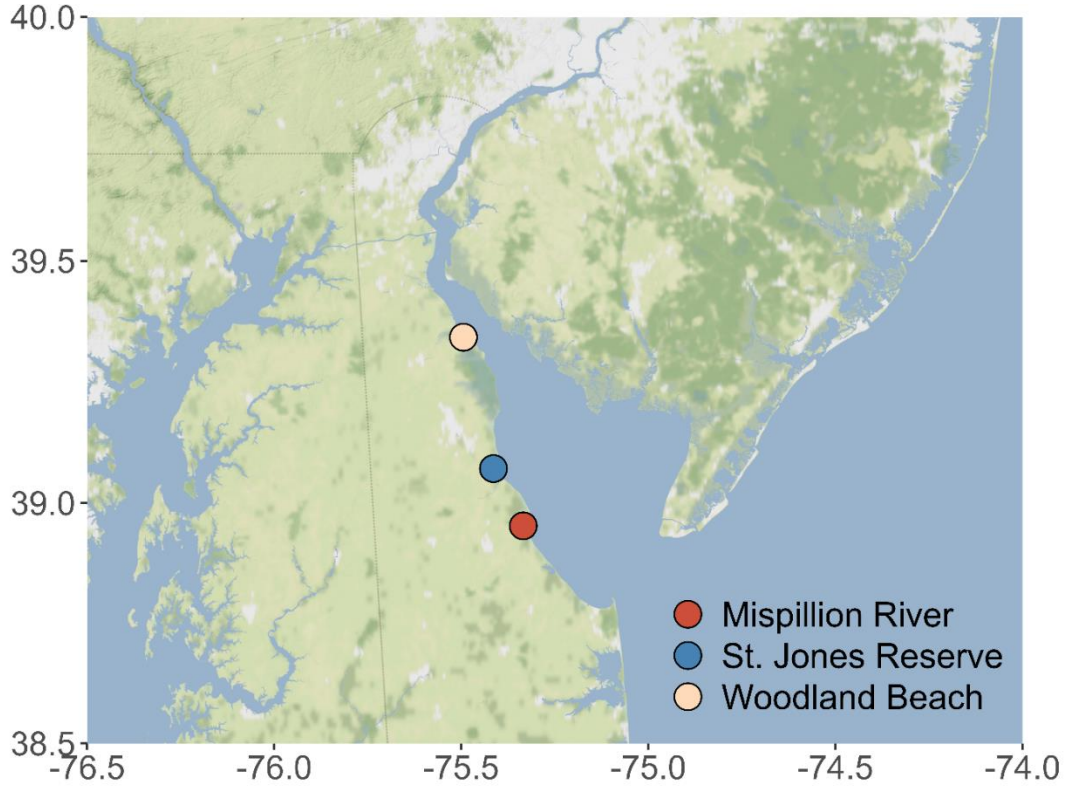

Figure 1.1 Clapper Rail demographic study sites where nests were located and monitored from 2019-2021 at the Mispillion River (including Milford Neck Wildlife Area), the St. Jones National Estuarine Research Reserve, and Woodland Beach Wildlife Area along Delaware Bay.

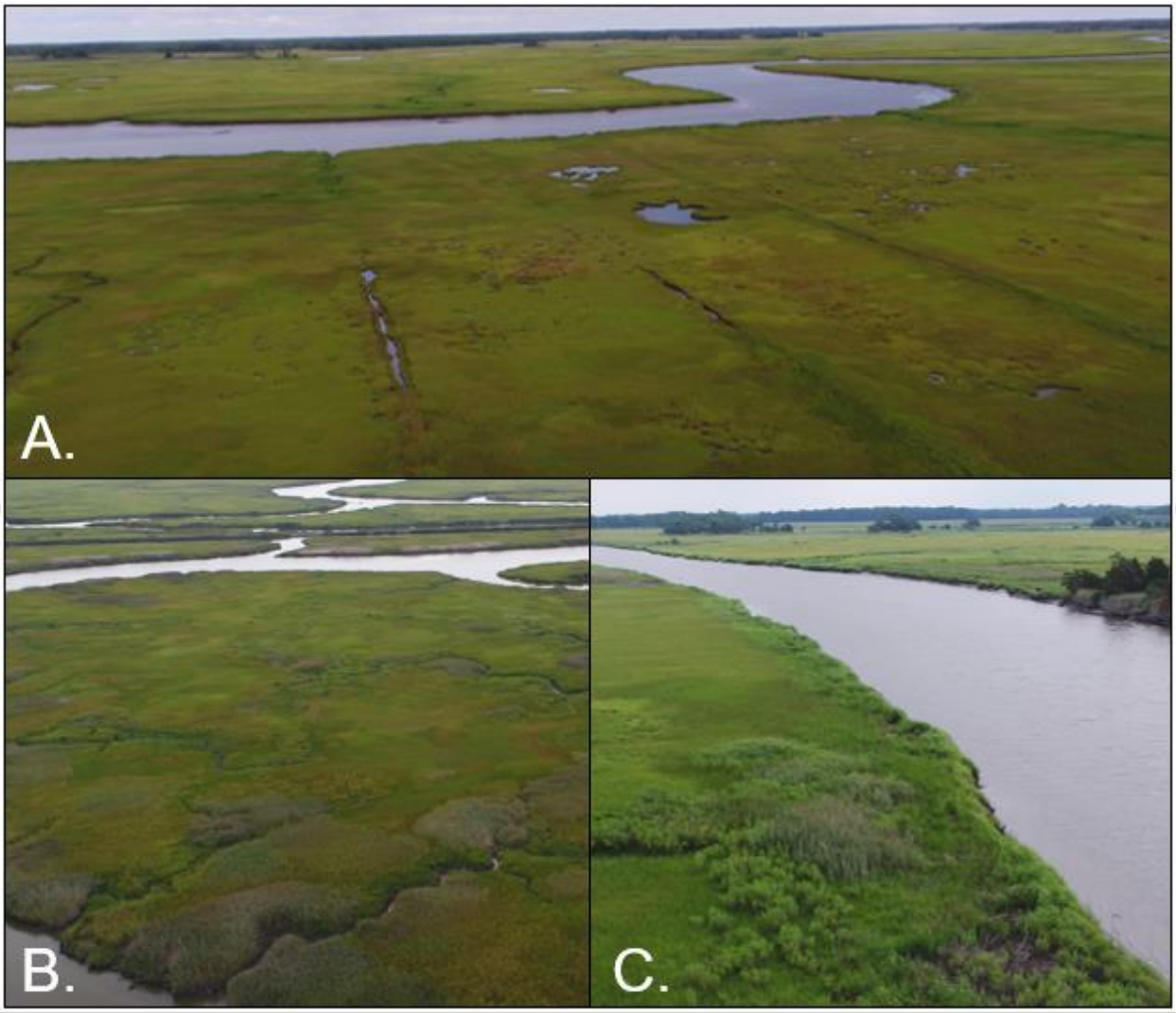

Figure 1.2. UAV aerial imagery of the Mispillion River (A), Woodland Beach (B), and St. Jones (C) sites in Delaware taken in 2021 and 2022.

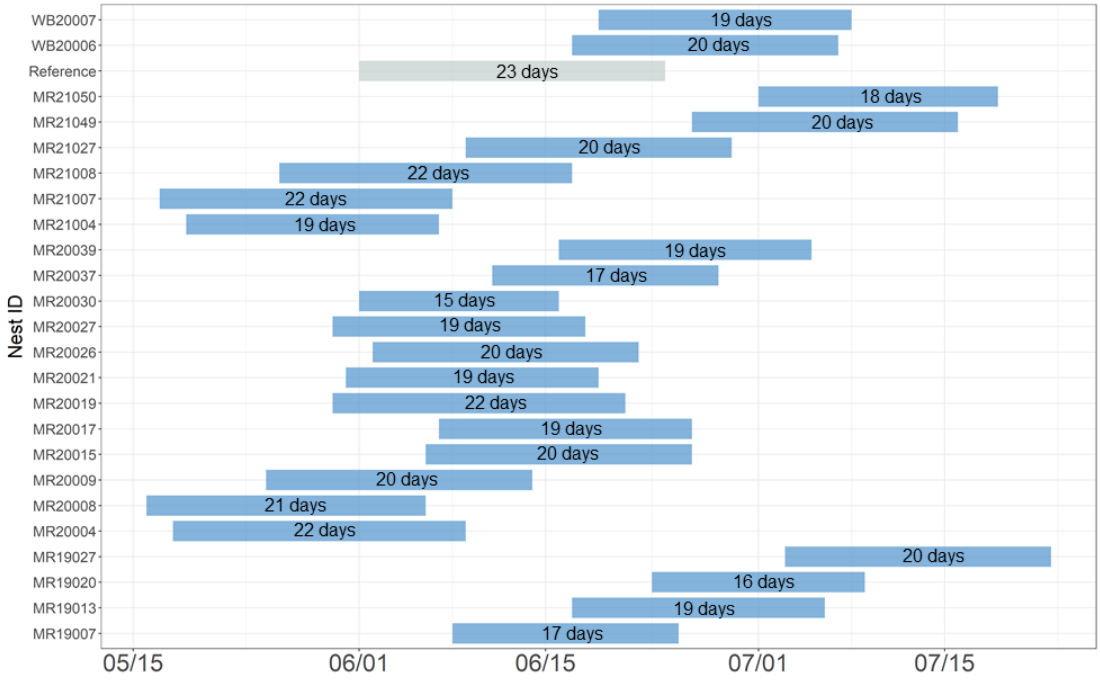

Figure 1.3. Clapper Rail incubation time (days) of nests discovered in Delaware from 2019-2021 during the laying period and monitored until hatch (n = 24). Mean incubation period for Delaware nests was 19.3 days.

Figure 1.4. VHF tag attachment to a Clapper Rail chick using eyelash adhesive (top) and the area of tag attachment (bottom). No feathers were removed during processing.

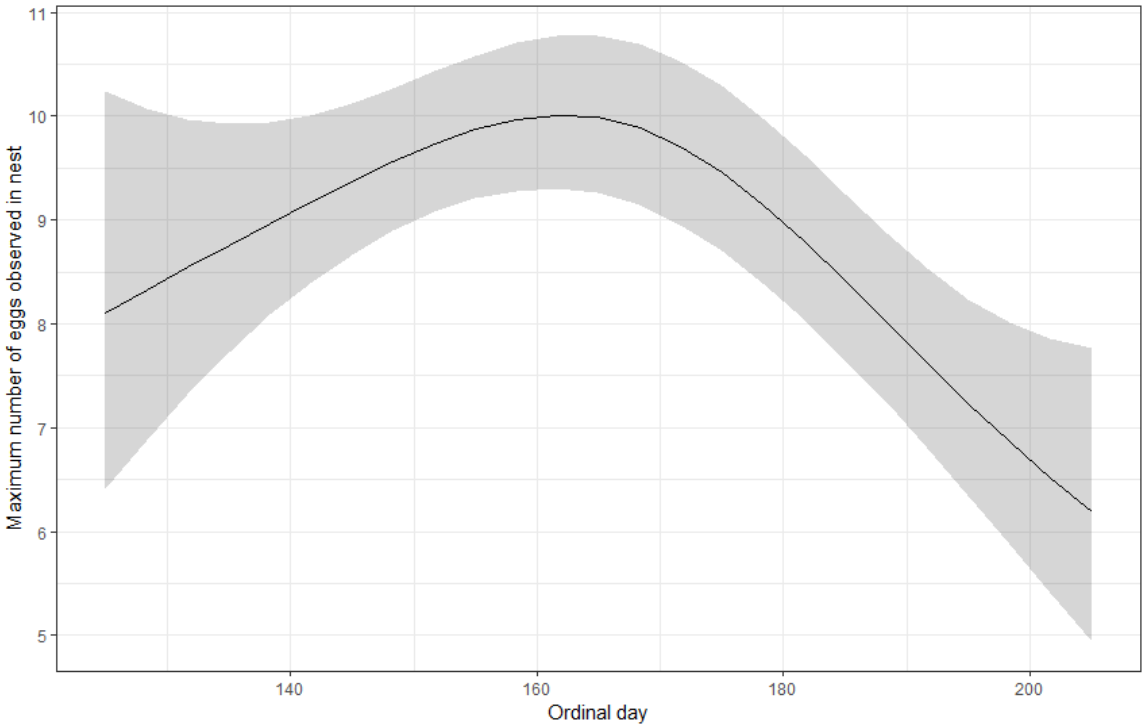

Figure 1.5. The predicted general additive model curve for maximum number of eggs (our proxy for clutch size) found in the Clapper Rail nests in Delaware from 2019-2021 decreased across the breeding season, suggesting that clutch size follows a pattern similar to nest survival. The effect of ordinal day presented here was highly significant ($p < 0.001$).

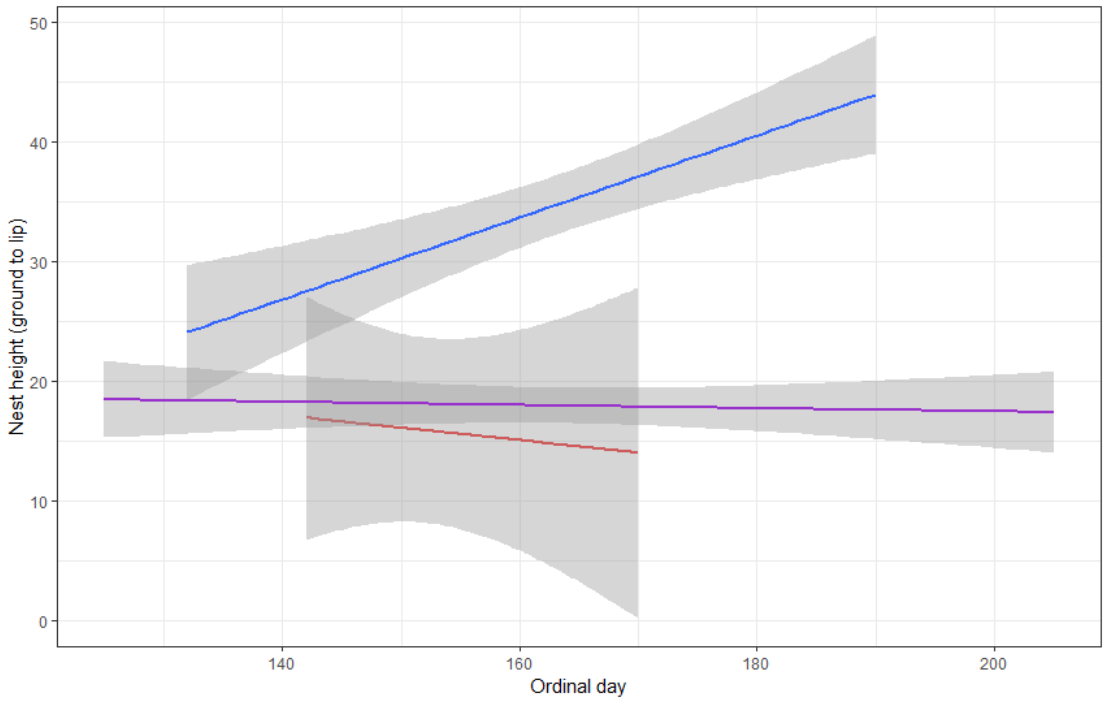

Figure 1.6. Nest height (cm) over time at Woodland Beach (blue), St. Jones (red), and Mispillion River (purple). Nest height increased with ordinal day at Woodland Beach ($\beta = 0.342$, se = 0.079, $p < 0.001$).

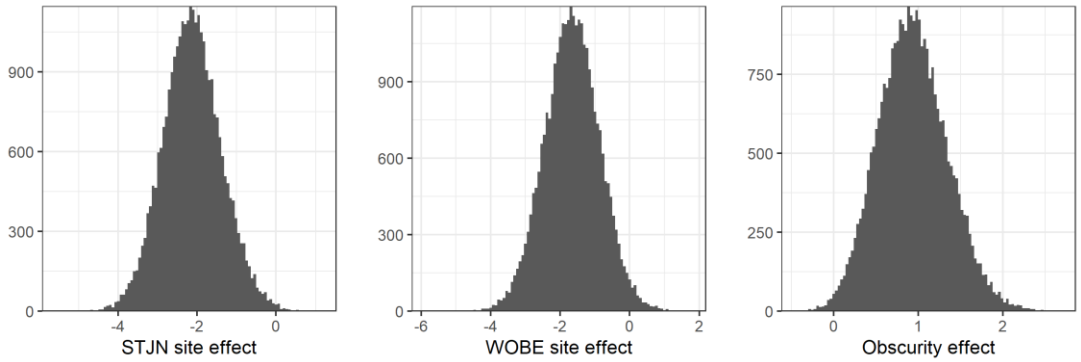

Figure 1.7. The three significant linear effects in Clapper Rail nest survival from the top competing model. STJN is the St. Jones site effect and WOBE is the Woodland Beach site effect (Mispillion River was modeled as the intercept). Obscurity is visual obscurity as measured with a Robel pole.

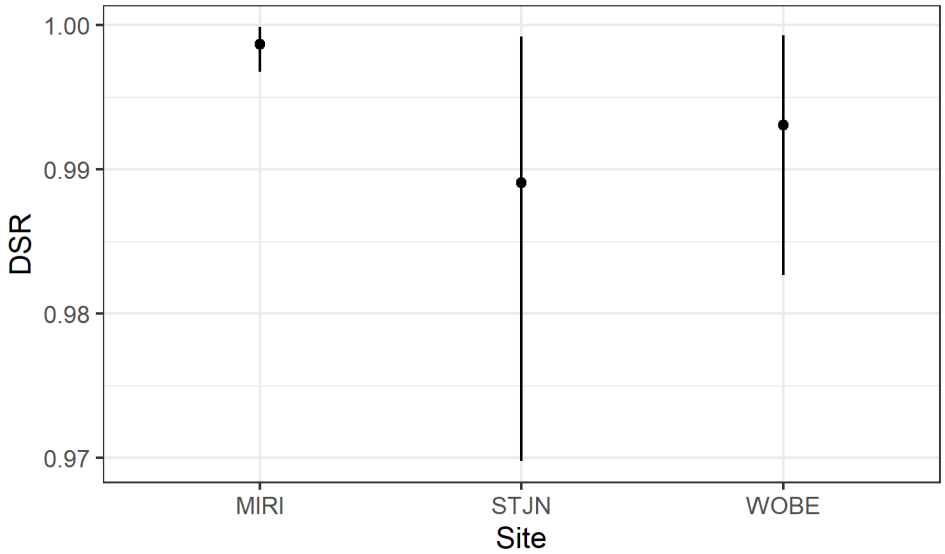

Figure 1.8. The predicted Clapper Rail nest daily survival rates (DSR) and 90% credible intervals for each site (MIRI = Mispillion River, STJN = St. Jones, and WOBE = Woodland Beach).

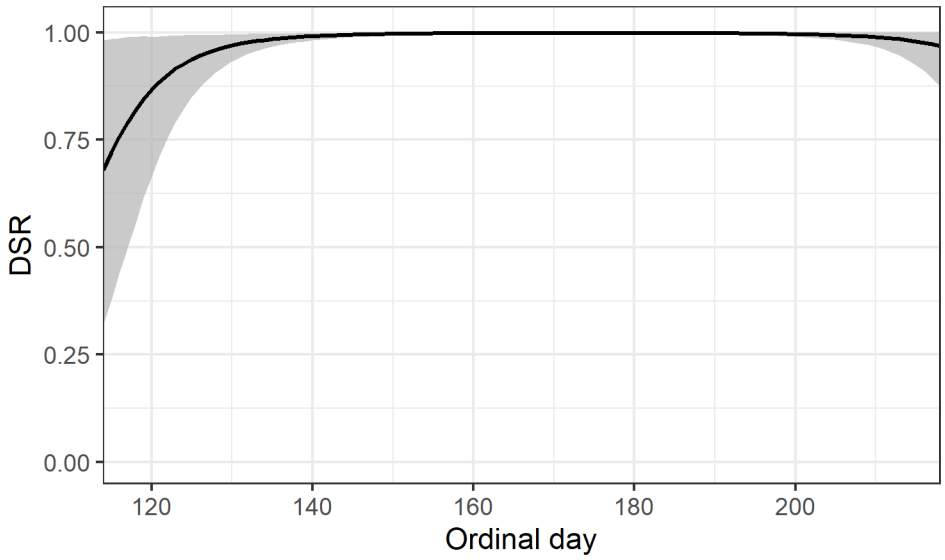

Figure 1.9. The predicted Clapper Rail nest daily survival rate (DSR) across the nesting season at the Mispillion River site.

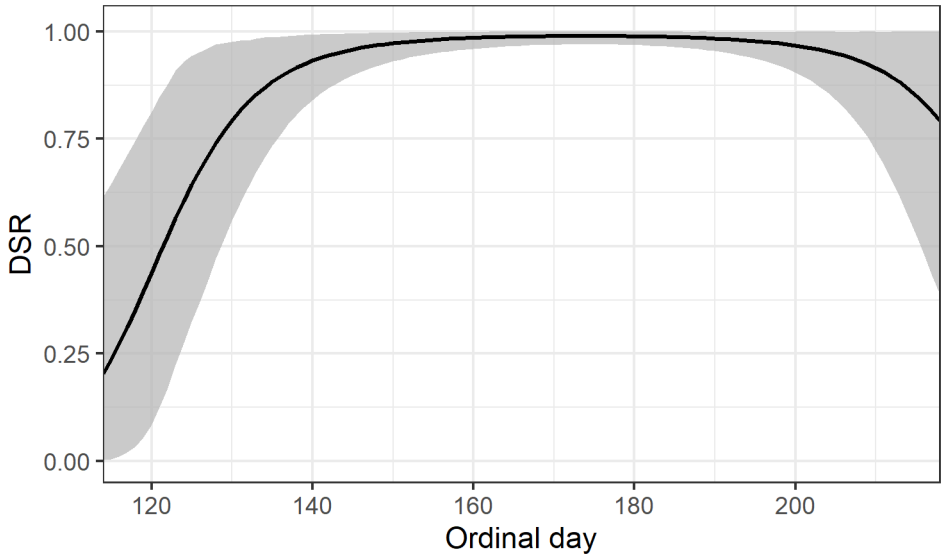

Figure 1.10. The predicted Clapper Rail nest daily survival rate (DSR) across the nesting season at the St. Jones site.

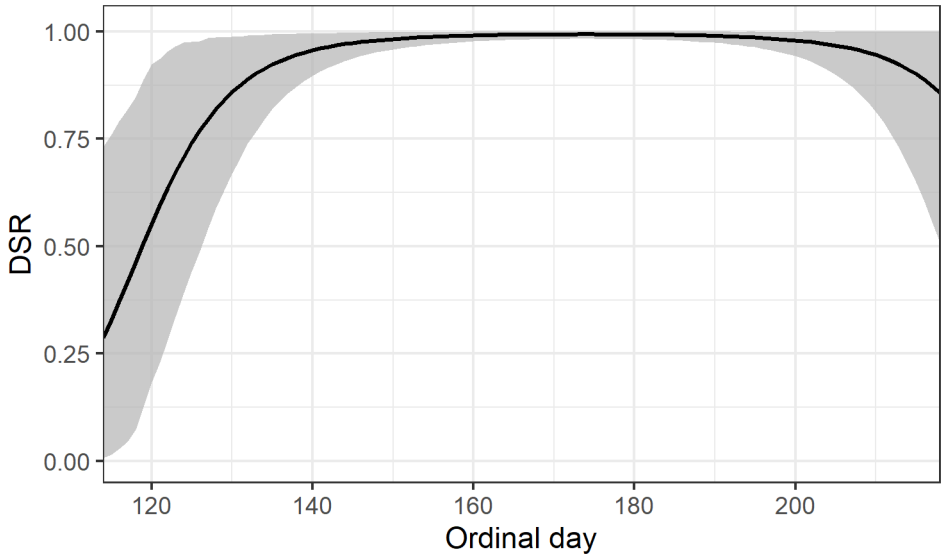

Figure 1.11. The predicted Clapper Rail nest daily survival rate (DSR) across the nesting season at the Woodland Beach site.

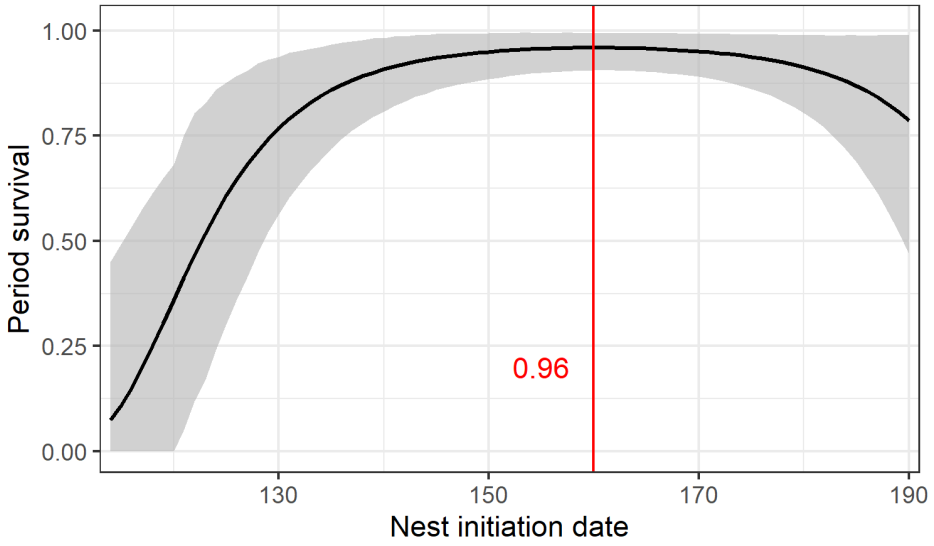

Figure 1.12. The predicted Clapper Rail nest period survival based on nest initiation date across the season at the Mispillion River site. The peak period survival estimate is on day 160 and is denoted with a red line. The red value is the peak survival probability.

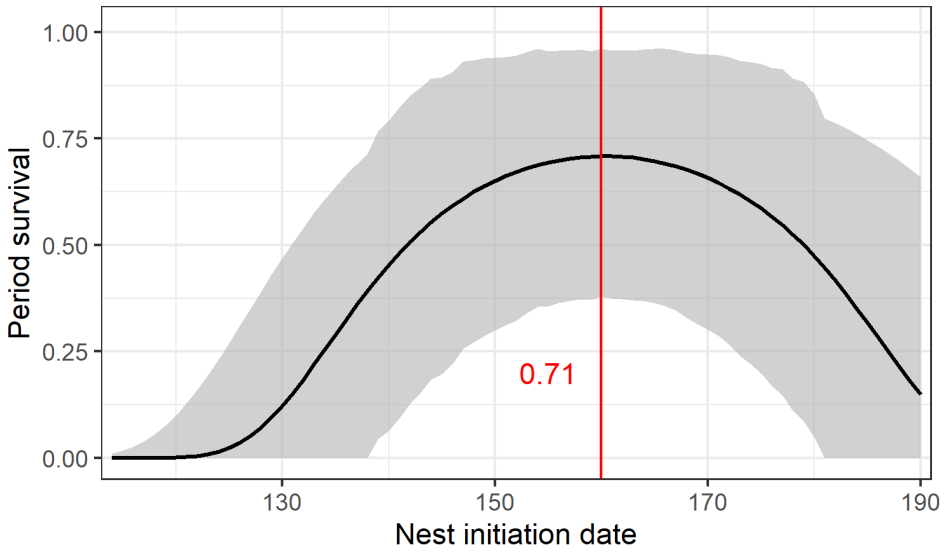

Figure 1.13. The predicted Clapper Rail nest period survival based on nest initiation date across the season at the St. Jones site. The peak period survival estimate is on day 160 and is denoted with a red line. The red value is the peak survival probability.

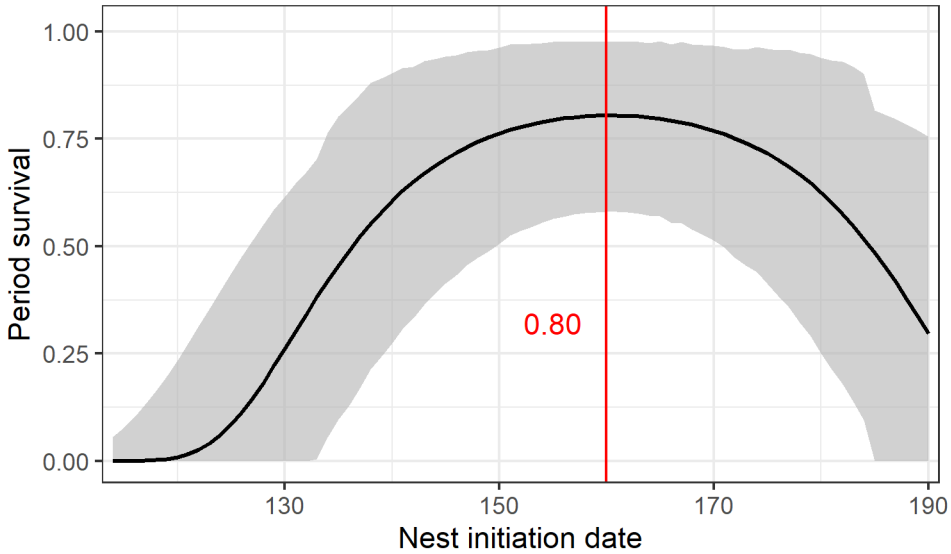

Figure 1.14. The predicted Clapper Rail nest period survival based on nest initiation date across the season at the Woodland Beach site. The peak period survival estimate is on day 160 and is denoted with a red line. The red value is the peak survival probability.

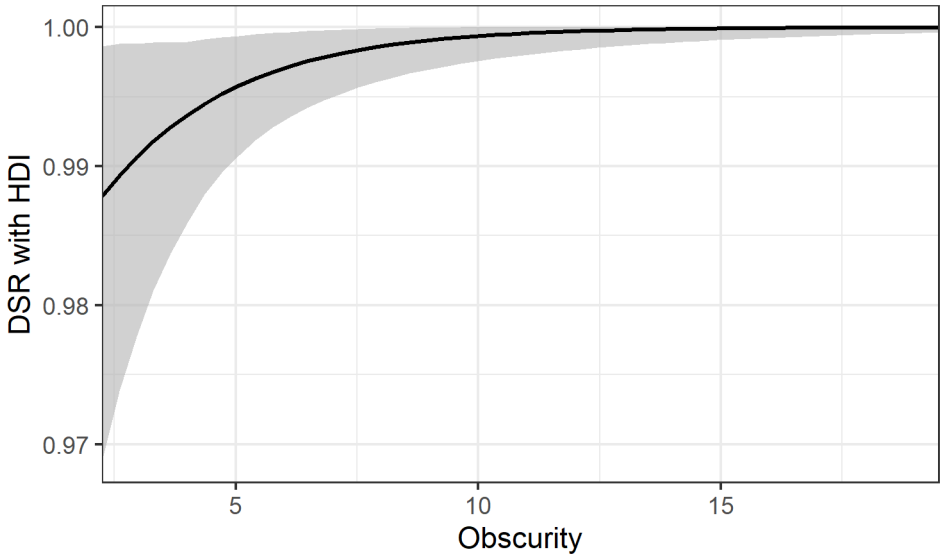

Figure 1.15. The predicted Clapper Rail nest daily survival rate (DSR) across the range of observed visual obscurity values.

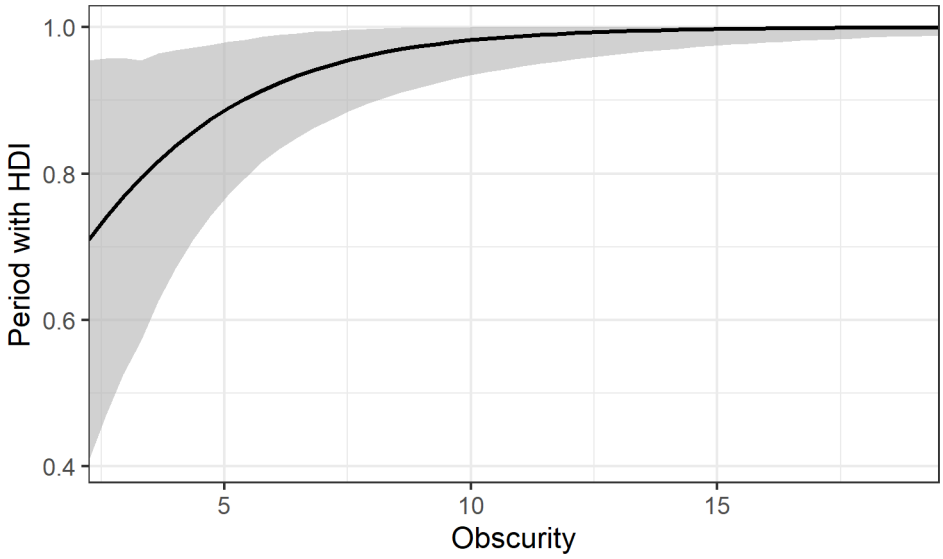

Figure 1.16. The predicted Clapper Rail nest period survival in Delaware across the range of observed visual obscurity values. Estimates based on data collected from 2019-2021.

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Chapter 2

CLAPPER RAIL MOVEMENT ECOLOGY

Introduction

To best manage wildlife population, it is imperative to gather data from across their full annual life cycle (Marra et al. 2015). Estimates of North American avifauna show a decline in breeding bird populations of an estimated 2.9 billion breeding birds since 1970, amounting to a nearly 30% reduction (Rosenberg et al. 2019). 2.5 billion of these individuals were from migratory species, with the highest proportional loss being seen in species overwintering in coastal regions (Rosenberg et al. 2019). Migratory species are particularly difficult to monitor across their full annual cycle as they can travel long distances (Skagen and Knopf 1993) and exhibit varying degrees of site fidelity to their wintering, stop-over, and breeding grounds. Understanding vital rates and space use across all seasons is necessary to effectively understand and manage these populations. Recent improvements in tag technology allow us to directly tag and track migratory birds in ways that were previously impossible, increasing the potential to understand migratory species connectivity and its implications for conservation (Cohen et al. 2018, Knight et al. 2021). These advances have opened to door to better understanding avian space use and population dynamics by allowing for finer resolution data to be collected and for even small-bodied avian taxa to be fitted with tags (Cohen et al. 2018, Holton et al. 2021).

The Clapper Rail (*Rallus crepitans*) is one avian species of conservation concern that can benefit from the use of advanced tag technology. The Clapper Rail is

a highly vocal but otherwise cryptic bird species endemic to North American salt marshes (Rush et al. 2020). Clapper Rail are among the few vertebrate species that have colonized tidal salt marshes, a system characterized by regular influxes of tidal waters that create varying water and salinity levels and support small numbers of low-growing halophytic plants (Greenberg et al. 2006). Tidal salt marshes can exert intense selective pressures as the limited availability to fresh water and cover can lead to thermoregulatory stressors, the lack of tall cover can lead to increased predation, and the varying flooding levels can cause direct mortality and nest loss. Although Clapper Rail are adapted to this dynamic habitat, anthropogenic impacts from development and climate change are altering these salt marshes at a pace that may prove impossible to adapt to without substantial conservation actions. There is limited information related to Clapper Rail movements and/or fine scale space use patterns, precluding management agencies from estimating the extent of habitat necessary to support Clapper Rail populations.

To plan and enact conservation action, it is necessary to have monitoring data, information on vital rates (such as nest, juvenile, and adult survival), and knowledge of the habitat and spatial extent of that habitat that is necessary to support target populations. Current efforts to monitor salt marsh bird populations at the state, federal, private, and academic levels provide critical information on population trends, but fail to address the underlying drivers of those trends. Efforts led by the Saltmarsh Habitat Avian Research Program show that the Northern Clapper Rail subspecies (*R. c. crepitans*) is declining at a rate of ~4.6% annually (Correll et al. 2017). To effectively

manage Clapper Rail populations, however, both vital rate data (as is partly addressed in my previous chapter) and information about their habitat use are needed.

Presently, there is no information related to the Northern Clapper Rail (*R. c. crepitans*) within breeding season space use or movements from the breeding season to the non-breeding season. Understanding basic space use and movement patterns is a first step in determining the extent of habitat necessary to support viable populations and the connectivity among different stages during the annual cycle. During the breeding season, both the male and female Northern Clapper Rail incubate the nest and defend the territory, but territory sizes, nest locations within the territory, and sex-specific patterns of habitat use are largely unknown (Rush et al. 2020). Space-use shifts after broods hatch or how the broods move across the landscape is also unknown and increasing our understanding of these basic life history parameters can aid in developing appropriate and targeted management actions. Tracking individual Clapper Rail chicks is particularly important to determine if brood ranges overlap as brood amalgamation can have large impacts on survival estimation (Flint et al. 1995, Orange et al. 2016). Many of these questions are best addressed by directly monitoring individual birds using radio telemetry.

The Northern Clapper Rail is the only Clapper Rail subspecies considered to be completely migratory (Rush et al. 2020). As a migratory species, they likely experience additional sources of mortality that commonly impact migrants such as additional exposure to open hunting seasons, encountering storms during migrations (Newton 2007), colliding with human-made structures (Loss et al. 2015, Hager et al.

2017), or generally lower survival due to the energetic costs and risks associated with migration (Sergio et al. 2019, Buechley et al. 2021). However, winter records of Northern Clapper Rail regularly occur in the southern portions of its range (including Delaware), suggesting that some individuals may overwinter near their breeding grounds. Currently, it is unknown what proportion, if any, of Northern Clapper Rail winter near their breeding grounds and for those that do migrate, what routes they take and when they depart.

The majority of the Clapper Rail range is in the U.S. (Figure 4.1), making it possible for wildlife management implementation to be conducted across the full annual cycle of this species by the United States (Marra et al. 2015, Knight et al. 2021). The first steps to developing conservation strategies for this species is understanding its spatial use across the full annual cycle and whether these patterns vary by sex. To that end, my objectives were to use tag technologies to address fundamental space-use and movement questions about three important life stages in the Clapper Rail annual cycle: (1) breeding adults, (2) young chicks, and (3) migrating adults and juveniles.

Methods

Rail capture and tagging

I captured Clapper Rail at three demographic sites along Delaware Bay; Woodland Beach State Wildlife Area, St. Jones National Estuarine Research Reserve, and along the Mispillion River (Milford Neck Wildlife Area and The Nature

Conservancy property) (Figure 1.1; see Chapter 1 for study area descriptions). I captured adult and juvenile rails using 60 mm mist netting, noose carpets (Harrity and Conway 2020), and/or modified walk-in funnel traps (Figure 2.1; Low 1935, Stewart 1951, Kearns et al. 1998) between April and September of 2019- 2021. Playback was actively used in response to rail behavior during mist net and noose carpet capture attempts while walk-in traps played a pre-recorded audio loop. Chicks were captured at nests on their hatch day (see Chapter 2 for additional details).

Upon capture, I banded, tagged, and drew blood from adult and juvenile Clapper Rails (USGS Bird Banding Lab Permit #23475, AUP #1157). Breeding adults were fitted with Lotek pinpoint transmitters (Lotek Wireless, Inc., Wareham, UK) that did not exceed 3% of their body weight using a modified leg-loop harness (Rappole and Tipton 1991) that included a neck-loop. I deployed 4.5 g downloadable GPS/VHF pinpoint tags in 2019 and 2020. I deployed solar powered, Argos-enabled pinpoint transmitters in 2019-2021. In 2019 and 2020, the solar Argos tags weighed 6 g and were 11 mm tall while in 2021 the tags weighed 6.5 g and were 16 mm tall. Harnesses were constructed using natural tubular Teflon tape (Bally Ribbon Mills, Bally PA) and the weight of tag harnesses varied by individual, with larger individuals needed more material, but did not exceed 2 g. The GPS tags were programmed to take points every 3-7 hours and send data to the Argos satellite every three fixes. Lotek proprietary software (PinPoint Host version 2.12.3.4) identified any low quality fixes and I removed all “bad” or “ok” fixes and any fixes that were not designated “3D”.

To capture juvenile (hatch year) rails, I deployed walk-in traps constructed with chicken wire and hardware cloth in 2019-2021 in August and September (Fig. 2.1). Although it is also possible to capture adults through this method, this approach more often results in the capture of juvenile rails (Stewart 1951). When possible, I also deployed speakers with a 4-hour playback loop at one of the trap cells. I used speakers similar to Kearns (1998) powered by a 12 Volt, 15 Amp battery and FoxPro Fusion speakers. Juveniles that were > 5 weeks of age were fitted with 0.9 g nanotags (Lotek Wireless, Inc., Wareham, UK) that weighed <1% of their body weight to track juvenile dispersal. In 2020, I secured nanotags using JB weld marine epoxy. In 2021, I secured nanotags using a leg loop harness (Rappole and Tipton 1991). Nanotags are coded radio tags that are detected when they come within range of the collaborative Motus Wildlife Tracking System. Thus, these tags can only provide location data from areas that have Motus coverage. Despite this drawback, they are an effective method to study animal movement with reduced tag weights (Paxton et al. 2022).

Home range estimation

I estimated home range size using the adehabitathr package in R (Calenge 2006, version 0.4.20). Rails are physically capable of traveling long distances in short time frames, therefore I considered points taken >3 hours apart to be independent and used these data to generate kernel density estimates. I generated 50%, 75%, and 95% kernels for each individual's overall home range, nesting range, and 3-week brooding range using the getverticeshr function with the default h smoothing parameter. I

classified points as being from the nesting period if the individual was regularly visiting a nest within their home range that I was able to locate and monitor. I included only data from the active incubation period of the nest, not from the laying period. If the nest subsequently hatched during the monitoring efforts, the day following the hatch-date began the brooding period and continued for three weeks. Although rail chicks continue to receive parental care for up to 6 weeks post-hatch, after 3 weeks post-hatch they are able to forage independently (Meanley 1985). For each category of home range, I included only individuals with a minimum of 30 points over at least 14 unique days per bird (i.e. bird-days).

When tag data showed highly clustered points in the same area for at least a 48-hour period or when tag data showed a dramatic departure from the previous area, I checked on the status of the tag on foot to determine if the tag was still on a live bird. In instances where the tag was no longer on a living bird, I documented the tag location, condition, and details about the carcass if it was present.

Chick movements

Chick locations were recorded in the field during tracking with Garmin GPS units (Oregon or Map64) and chick status was assessed at every tracking encounter. I included all points of chicks that were determined to be alive during the tracking occasion, either by a visual resight or by clear movement of the tag location during the tracking occasion. I then calculated the distance of each point from the nest at which it was captured using the “distVincentyEllipsoid” function in the R geosphere package

(version 1.5-1.4, Hijmans et al. 2021) to determine how far the chick moved from the nest on a given day.

Migratory movements

I processed the nanotag data using the motus package in R following recommendations by Motus. Briefly, I removed nanotag detections with a run length of less than three, detections that were flagged by the "motusFilter" function, and detections that were outside of the probable tag life span. Additionally, I restricted detections to receivers between 39.6 and 28 degrees Latitude and between -71 and -85 in Longitude. I considered both nanotagged and GPS tagged rails to have initiated migration if they traveled >40 km from their capture location after the month of July.

Genetic sexing

I determined the sex of the rails with a genomic approach to differentiate space use by male and female adult rails during the breeding season. In short, DNA was extracted using a Zymo mini-prep plus DNA extraction kit (Zymo, Irvine, CA, USA) following the manufacturer's protocol. ddRAD libraries (Peterson et al. 2012) were prepared and sequenced with a 15% PhiX Spike-in on either an Illumina Hiseq Platform PE150 or a NovaSeq-6000 (Novogene Co). I sexed the rails by aligning our ddRAD sequences to our Clapper Rail reference genome that includes the Z chromosome (see Chapter 3).

Results

Breeding season adult home ranges

I captured 51 adult rails and deployed 40 GPS transmitters (Table 2.1). 12 of these individuals were mortalities based on the presence of a carcass or damage to the tag (3 F, 9 M). Seven of the moralities showed evidence of raptor depredation, such as marks from the bill on the tag or directly proximity to a raptor roost with rail feathers present. Two mortalities showed evidence of mammal depredation, such as fresh mammal scat or tooth marks on the carcass or tag. Two mortalities showed evidence of depredation, but lacked sufficient evidence to attribute to a specific group of predators. One mortality was due to unknown causes as no signs of perimortem trauma on the carcass were evident at the time of its discovery. On average, depredated individuals were monitored for 22.67 bird-days (± 6.22) prior to the mortality event and the lowest proportion of mortalities occurred at the Mispillion River site (Table 2.2). Seven of the mortalities occurred in 2021, five of which were confirmed as raptor predation. In one instance, a female was apparently depredated during the incubation stage by a barn owl. The male continued to tend the nest and the nest successfully hatched 10 days later.

Twenty-three birds met the criteria for inclusion in the general, overall home range estimations. On average, each bird generated 207.78 points of data across 41.35 bird-days (Table 2.3). Overall home range estimates (n=23) had overlap in their confidence interval across sites and sexes (Figures 2.2 and 2.3). The average 50%, 75%, and 95% home ranges were 0.53, 1.16, and 2.94 ha respectively (Table 2.4).

50% and 75% nesting home ranges (n = 7) were approximately one-third the size of the brooding home ranges (n = 9) and one-fifth the size of the overall home ranges (Table 2.4). In one instance, an individual's 95% nesting range included 5 nests that were concurrently active (Figure 2.4). Only two of the nests fell within the 50% range and they were ~5 m apart. GPS points and manual tracking of the GPS/VHF tag on the bird confirmed one of those two was the primary nest the bird was associated with and the data from that nest was used to define the breeding status of that individual for home range estimation. Although brood home range estimates were larger, the core home ranges were generally closely centered around/contained the core nesting home range (Figure 2.5). However, as the broods presumably aged the adults sometimes ventured further from the nests (Figures 2.5 and 2.6).

Only one individual was documented to shift their home range (i.e. moved to a new home range with no overlap with their previous range) while the remaining birds had stable core ranges across the breeding season, thereby supporting the assumption that >14 bird/days of data would yield samples representative of their overall territory. In the case of the individual that shifted their range, the shift occurred in late July after a nest depredation event and thus likely represented the end of breeding activity for that individual. I documented the nest failure of another tagged individual earlier in the season that renested ~30 m from the original nest (Figure 2.7) which, coupled with the fact that no other large home range shifts occurred, suggests that nest failure does not necessarily cause a large deviation in home range.

Chick movement

I tagged 82 chicks from 33 unique nests (Table 2.5). When chicks were captured, an average of 3.25 (SE = 0.20, range = 1-8) chicks were tagged per nest. Tag retention varied and many of the tags were recovered from the nest or at nearby brood platforms within the first few days of tagging, suggesting adult intervention in tag removal (see Chapter 1 for further detail). 18 chicks from 15 unique nests were tracked for at least a week (Figure 2.8)

The distance chicks traveled in the first day after hatching was on average 11.91 m (n = 39, ± 2.19). By the fourth day, the mean distance traveled from the nest was 30.37 m (n = 16, ± 5.11) and remained relatively stable for the first two weeks. The average distance from the nest did not increase significantly after day three post-hatch, although there was a large amount of individual variation (Figure 2.9). In two instances, I was able to tag chicks from adjacent territories for two or more weeks. In the first of these instances, one chick from MR20059 (hatched 7/28) was tracked for 14 days and a neighboring chick at nest MR20050 (hatched 7/19) was tracked for 16 days (Figure 2.10). Although the nests were only ~43 m apart, there appeared to be little overlap in brood movements. The chick at MR20059 did appear to cross the border into the neighboring territory on two occasions, but not until it was at least 12 days old. In the second of these instances, two chicks at MR21026 (hatched 6/23) were both tracked for 22 days and a chick at MR21027 (hatched 29 June) was tracked for 18 days (Figure 2.11). These two nests were situated approximately ~60 m apart and again showed little apparent overlap in brood movements. The chick from

MR21027 moved into the territory of the other brood twice, once at 8 days of age and the other at 11 days of age. For the remaining week of tracking, however, that individual did not enter the adjacent territory again.

Migration

I captured 54 rails (49 HY, 5 AHY) in total using walk-in traps in 2020 and 2021 and deployed 28 nanotags on juvenile rails (Table 2.5). An additional nanotag was deployed on an adult captured in the walk-in trap. I detected migratory movements for four Argos GPS-tagged birds and eight nanotagged birds between 2019 and 2021 (Figure 2.12). The earliest detected departure date was a juvenile rail on 10 September and the latest detected departure was an adult rail that successfully reared chicks on 18 October. One nanotagged juvenile traveled north to the New Jersey side of Delaware Bay and was not detected further. The remaining birds traveled south with most birds traveling to South Carolina. One probable mortality event was detected in North Carolina when the GPS location data began to be transmitted from a forested area for an extended period of time rather than suitable rail habitat.

One Argos-tagged individual took an inland path through North Carolina that seemed to follow interstate 95. The bird traveled from Delaware to South Carolina over two days and with a stopover in forested habitat. It ultimately traveled to marshes in coastal Georgia where it remained until the transmitter ceased transmissions. Although several nanotagged birds were detected in South Carolina, none were

detected between South Carolina and Mackay Island near the border of Virginia and North Carolina.

Discussion

Adults

Adult home ranges did not differ in size between sexes. Although my sample size was low, this is consistent with work conducted in South Carolina (Ricketts et al. 2020). Our mean home range size across all sampled individuals was larger than work conducted elsewhere in the Clapper Rail range; overall (95%) home ranges were reported to be 1.37 ha in Mississippi (Rush et al. 2010), 1.06 ha in South Carolina (Ricketts et al. 2020) and 1.2 ha in Georgia (Cumbee et al. 2008) compared to our 2.94 ha estimate. However, these cannot be considered one-to-one comparisons as our analytical methods, temporal scale, and sample size differed considerably. Notably, only Rush (et al. 2010) employed a kernel density estimate method while Ricketts utilized the Local Convex Hull method and Cumbee et al. (2008) employed the Minimum Convex Polygon method. Even in the instance where a kernel approach was taken, a direct comparison is difficult to make as they used likelihood cross-validation to select their smoothing parameter, while I used an *ad hoc* approach that has been shown to produce good estimates in cases where the utilization distribution density is unimodal (Worton 1995).

A key assumption of many home range estimators is that the points used in the analysis are independent. In previous studies, this was accomplished by trying to take

only one point per tidal cycle. However, further stratification to sample across varying times at varying tides may be important to understanding Clapper Rail movements. The effect of tides on the marsh surface can be both difficult to account for and difficult to quantify as it varies between sites and even within the same marshes depending on hydrology and marsh topography. Our study sites experience particularly large shifts in tidal height relative to other portions of the Clapper Rail range, with Woodland Beach experiencing as much as 7 feet of difference between high and low tide. Throughout the tidal cycle, portions of the rail's territory may be submerged and thus "unavailable" to the bird at that time. However, these tidal mudflat areas are important foraging habitat and are used disproportionately when they are available (Rush et al. 2010, Ricketts et al. 2020). By leveraging GPS tags, I collected higher resolution data across the full daily cycle, therefore my home range estimates may by larger as they represent a more complete estimate of rail home ranges.

Despite the utility of GPS tags, this technology can negatively influence bird survival in some instances (Severson et al. 2019). Although I did not concurrently monitor rails with VHF only to directly compare survival, a spike in mortalities in 2021 coincided with our deployment of larger GPS tags. It is unclear if the tags contributed to these mortality events and future work focusing on these impacts is necessary.

In addition to the overall home ranges, I subset the nesting and brooding location for known breeding status birds and employed the same minimum cutoffs.

This resulted in a small but comparable sample size of 7 nesting and 9 brooding adults. The average number of points and unique bird days used in estimates for both periods were very similar; on average, each range was produced using data from ~20 days, which amounts to a full incubation period (Table 2.3). The nesting ranges were approximately one-third of the brooding ranges. Despite the expansion during the brood period, the center of the core range around the nest remained relatively stable in the first several weeks after the nest hatched, consistent with the brooding movement data collected (discussed further below).

Chick movements

These data provide the first direct glimpse into Clapper Rail brood movements. The chicks remained very close to the nest in the first few days of hatching. Although the chicks are ambulatory during this time frame, their legs are not yet developed enough to move quickly through the marsh in the first 24-48 hours post hatch. Even as the chicks grew, however, they remained close to the nest and therefore likely well within the territory established by the adults. The fidelity to the nesting site suggests that Clapper Rail do not exhibit brood amalgamation, at least during the days immediately post-hatch. One possible explanation of this behavior is that adult rails are avoiding investing parental care in unrelated chicks. Rail chicks are unable to break apart larger and tougher food items and require extensive parental care immediately after hatching (Meanley 1985). Food availability may also be a factor influencing the movement of broods. Although I did not quantify food availability,

fiddler crabs were observed in high densities across the site and make up the largest component of the Clapper Rail diet (Meanley 1985, Rush et al. 2020). The broods may not be food limited during this early developmental period and therefore may not have any need to stray far from their nest and natal territory. The tagged broods I tracked that were in the closest proximity were 43 m apart and they had very limited overlap in movements, however, I did document several pairs of nests without tagged chicks that were in closer proximity to one another. Nests in such close proximity may have had higher degrees of overlap in brood ranges.

Brood amalgamation behavior has important implications for survival modeling and is a strategy employed by other precocial bird species (Flint et al. 1995, Orange et al. 2016, Morandini et al. 2021). Brood amalgamation or crèching behavior can reduce the chance that any individual chick in the original brood is depredated, but species known to utilize this strategy demonstrate less parental care than Clapper Rail. Although I did not capture whole broods, my tracking data suggest that the chicks remain very close to their nests even up to three weeks of age. In instances where they strayed from their early natal range, the chicks returned to the area near their nest (and thus the core of the adult/brood home range). In contrast, other precocial species living in areas dominated by grass species exhibit brood amalgamation early in the brooding period. Up to 10% of Northern Bobwhite broods exhibit brood amalgamation within the first 3-4 days post-hatch and by day 12 of age, the rate of amalgamation can be as high as 67% (Faircloth et al. 2005).

Migration

I documented a previously unknown migration route using GPS tag technology. Although the juvenile nanotagging could not provide points along this route due to a limited number of Motus towers, the lack of detections between Virginia and South Carolina suggest that some juveniles may be taking this route as well. Additionally, historical band records report few recoveries of mid-Atlantic banded birds in North Carolina, which may have been in part due to Northern Clapper Rail undertaking migration through inland North Carolina rather than the coast.

Summary and conclusions

Quantifying avian movements across their full annual cycle provides important data for the designation of conservation and management areas(Hostetler et al. 2015, Buechley et al. 2021). Without space-use information, it is difficult to construct conservation plans at the state, federal, or non-governmental organization levels as it is impossible to estimate the number of territories a given property might support or the degree to which populations may be exposed to anthropogenic structures (Powell 2000, Watson et al. 2014). Our breeding season movements provide a baseline for strategic management and for future modeling efforts (Hostetler et al. 2015, Dunn et al. 2022).

My chick results reveal that Clapper Rail broods stay within their parental territory, at least during the first two weeks post-hatch. Therefore, it is not necessary to set aside separate brooding habitat, an approach used in other species that select different habitats for nesting vs brooding (Brennan et al. 2020). I also did not detect

any apparent instances of brood amalgamation, suggesting that future brood survival studies need not include modeling components to incorporate uncertainty due to brood amalgamation.

The discovery of a novel migratory pathway for Clapper Rail has large implications for the management of this species. Managing for coastal wetland stop-overs may be insufficient to support migrating rails if they are utilizing inland routes. Inland records of Clapper Rail may be written off as vagrants, however, the GPS data in this case show a clear, deliberate, and expeditious flight across North Carolina. I also did not detect any movements into the Gulf Coast or western Florida despite a lack of genetic population structure between these populations (see Chapter 4).

My research has expanded our knowledge of the full annual cycle movements of Clapper Rail considerably. I have provided robust home range estimates in this region using GPS tags to support future modeling and study designs, directly monitored chick movements to make inferences about brood amalgamation and produced the first GPS data of Clapper Rail migratory routes.

TABLES

Table 2.1. The number of breeding season adult Clapper Rail captured and tags deployed by year in Delaware.

	2018	2019	2020	2021
Captured	5	13	14	24
GPS/VHF tag	--	8	5	--
GPS Argos	--	5	9	13

Table 2.2. The number of tagged male and female Clapper Rail and the number of mortalities by site.

Site	Male	Female	Mortalities	Percent mortalities
Mispillion River	14	2	3	18.75%
St. Jones	8	2	4	40.00%
Woodland Beach	9	5	4	28.57%

Table 2.3. The sample sizes, number of points, and number of bird days used to generate kernel density home range estimates for Clapper Rail breeding in Delaware from 2019-2021.

		n	mean points	points range (min, max)	mean bird-days	bird-day range (min, max)
General	Overall	23	207.78 (±31.56)	53, 582	41.35 (±5.29)	15, 117
	Female	6	147.33 (±17.57)	93, 203	25.17 (±4.12)	15, 41
	Male	17	229.12 (±41.35)	53, 582	47.06 (±6.52)	21, 117
Nesting	Overall	7	105.14 (±18.33)	34, 170	19.86 (±0.91)	15, 23
	Female	1	87	87	20	20
	Male	6	108.17 (±21.39)	34, 170	19.83 (±1.08)	15, 23
Brooding	Overall	9	99.56 (±13.98)	25, 176	20.22 (±0.68)	17, 22
	Female	4	110.5 (±10.44)	97, 141	19.75 (±1.11)	17, 22
	Male	5	90.80 (±24.40)	25, 176	20.6 (±0.93)	17, 22

Table 2.4. The 50%, 75%, and 95% home range estimates for male and female Clapper Rail across the three Delaware demographic sites, 2018 - 2021. The male and female home range estimates had overlapping confidence intervals. General home ranges were larger than nesting and brooding ranges, but covered a larger period.

		n	50% (ha)	75% (ha)	95% (ha)
General	Overall	23	0.53 (±0.18)	1.16 (±0.36)	2.94 (±0.85)
	Female	6	0.60 (±0.13)	1.35 (±0.28)	3.29 (±0.75)
	Male	17	0.50 (±0.24)	1.09 (±0.48)	2.82 (±1.14)
Nesting	Overall	7	0.10 (±0.02)	0.25 (±0.04)	0.69 (±0.11)
	Female	1	0.15	0.33	0.90
	Male	6	0.09 (±0.02)	0.24 (±0.05)	0.65 (±0.13)
Brooding	Overall	9	0.32 (±0.07)	0.68 (±0.16)	1.77 (±0.47)
	Female	4	0.42 (±0.12)	0.90 (±0.26)	2.15 (±0.56)
	Male	5	0.24 (±0.08)	0.51 (±0.18)	1.47 (±0.74)

Table 2.5. The number of Clapper Rail chicks and juveniles captured and tagged by year. Note that all chicks were tagged with VHF tags and all juveniles were tagged with nanotags.

	2020		2021	
	Captured	Tagged	Captured	Tagged
Chicks	104	35	177	47
Juveniles	28	15	21	14

Table 2.6. The locations of band recoveries from Clapper Rails banded in New Jersey, New York, and Virginia from 1933-1978 by state in which they were encountered.

	FL	GA	NJ	NY	NC	SC	VA
New Jersey	6	42	488	0	4	35	12
New York	1	2	0	11	0	1	0
Virginia	6	8	0	0	5	14	58
Total:	13	52	488	11	9	50	70

FIGURES

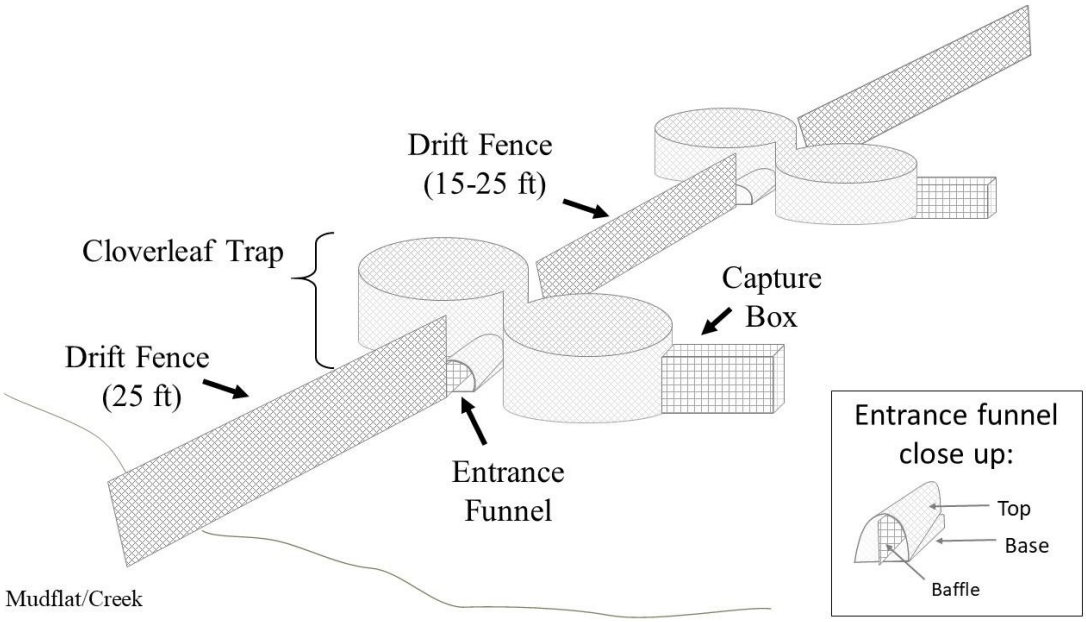

Figure 2.1. The trap design for walk-in traps deployed in 2019-2021 to capture juvenile Clapper Rail in Delaware.

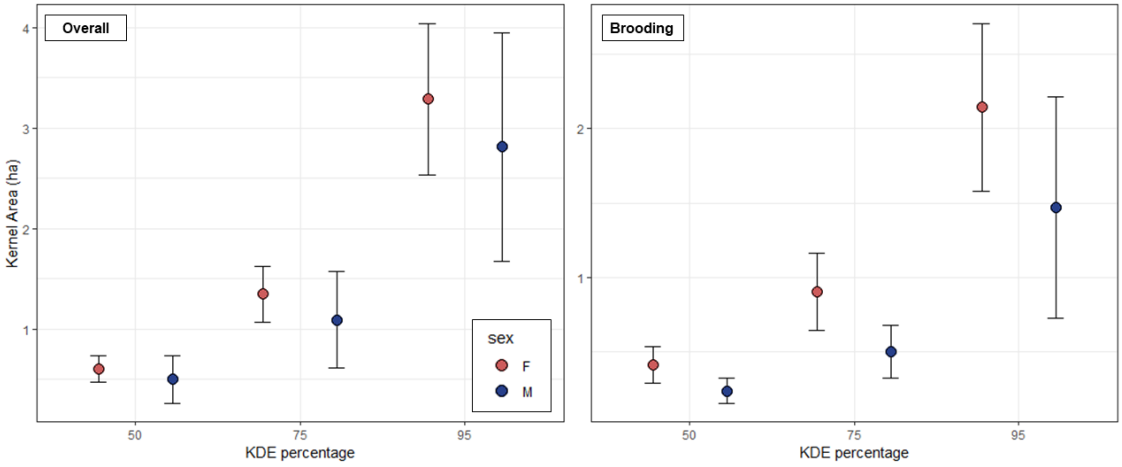

Figure 2.2. The overall and brooding home ranges of Clapper Rail in Delaware by sex from 2019-2021. The confidence intervals at all kernel levels overlapped.

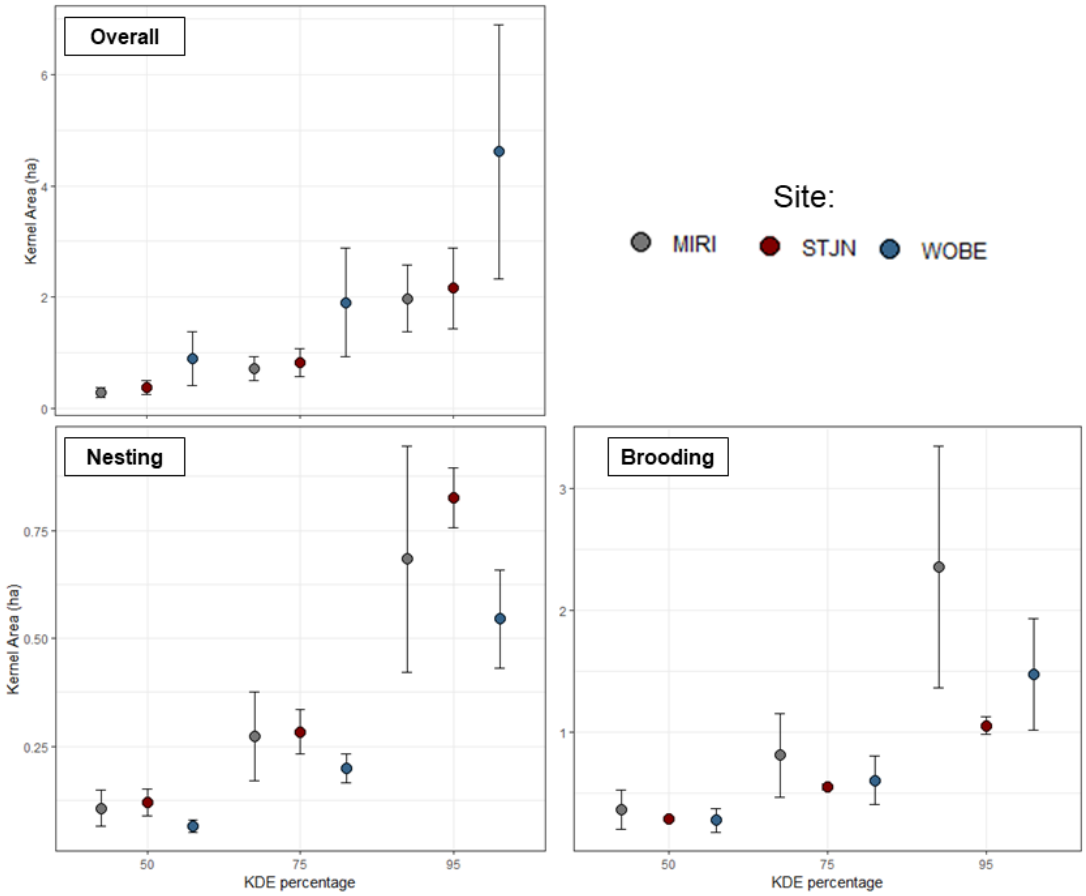

Figure 2.3. The overall, nesting, and brooding Clapper Rail home ranges by site in Delaware. The home range estimate confidence intervals overlapped in all but one case, the 95% brooding interval. The Mispillion River estimate was higher than the St. Jones estimate, but only at the 95% kernel level.

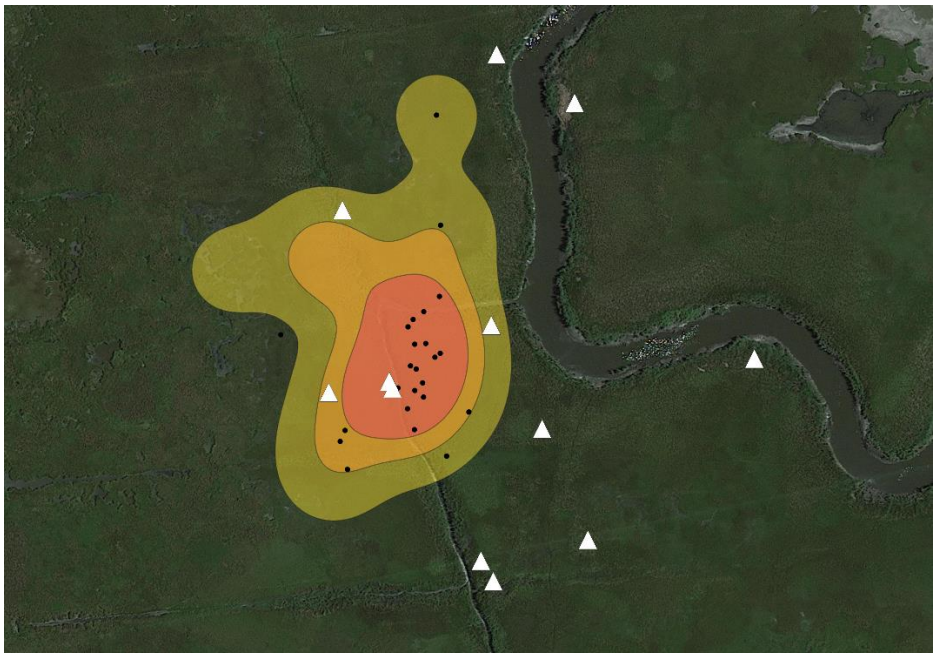

Figure 2.4. The nesting home range of an individual Clapper Rail in Delaware tracked in 2019 with nests denoted by white triangles. The core range included two nests that were 5 m apart and an additional three nests fell within the 95% home range.

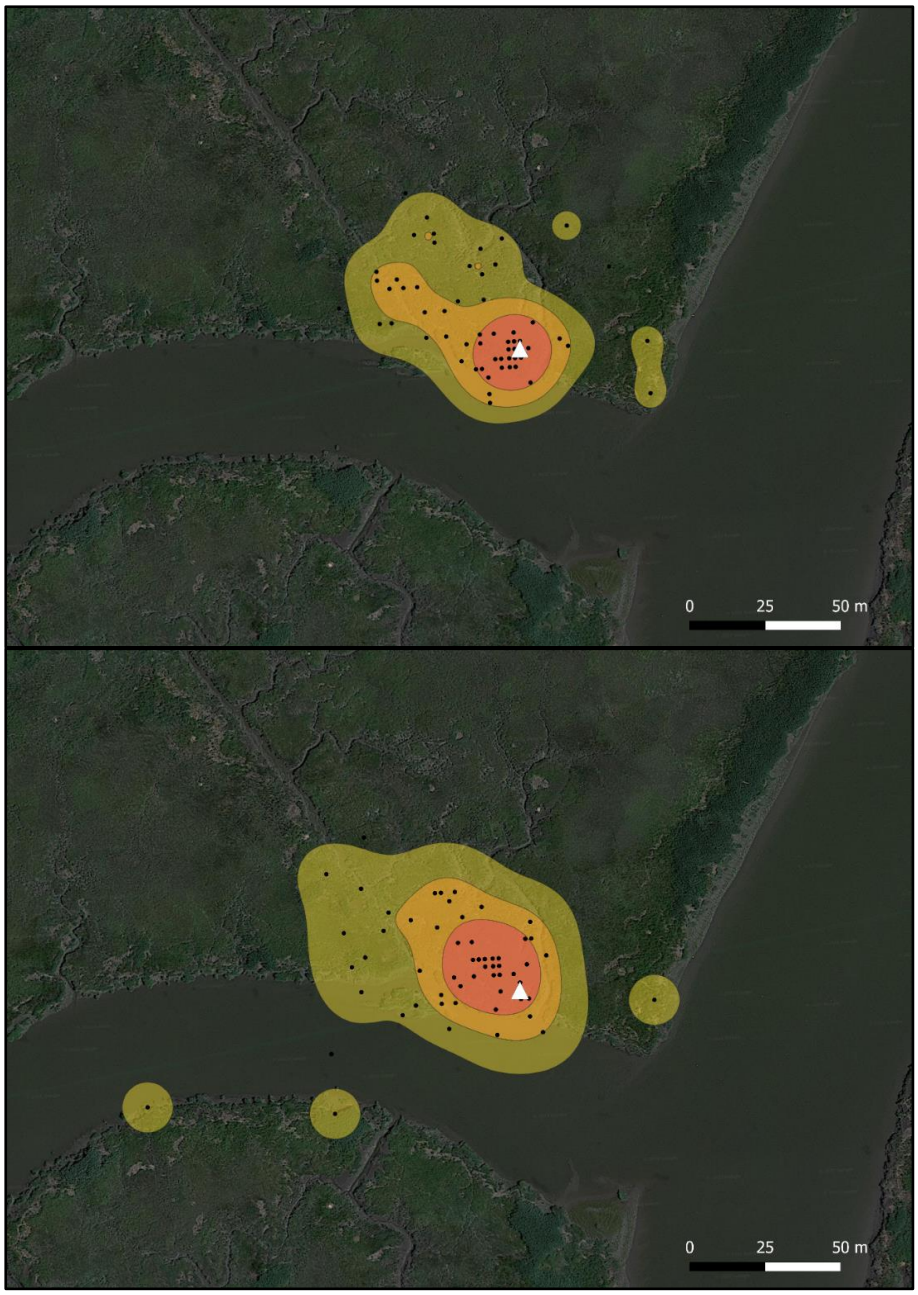

Figure 2.5. An example of the nesting (above) and brood (below) home ranges for an individual Clapper Rail tracked in 2021 at Woodland Beach in Delaware. The individual's nest is denoted by a white triangle. Although the range expanded during the brooding period, the core range included the nest. The 95% brooding range reflects foraging movements across creeks.

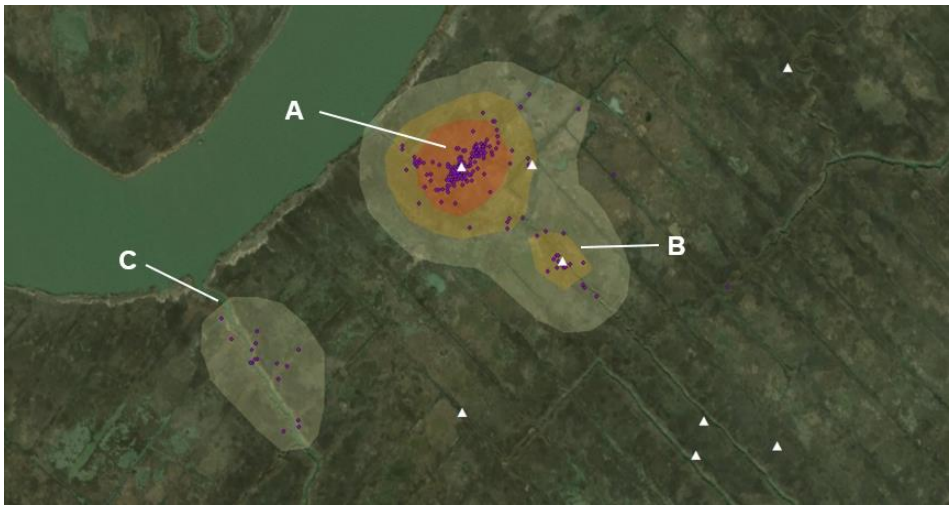

Figure 2.6. The brooding range of an individual Clapper Rail at Mispillion River, Delaware in 2020 with nests denoted by white triangles. The individual remained near their nest (A) in the early days of brooding. On one occasion, she used a nearby inactive nest (B) overnight. She then moved to a close-by creek (C) before going missing.

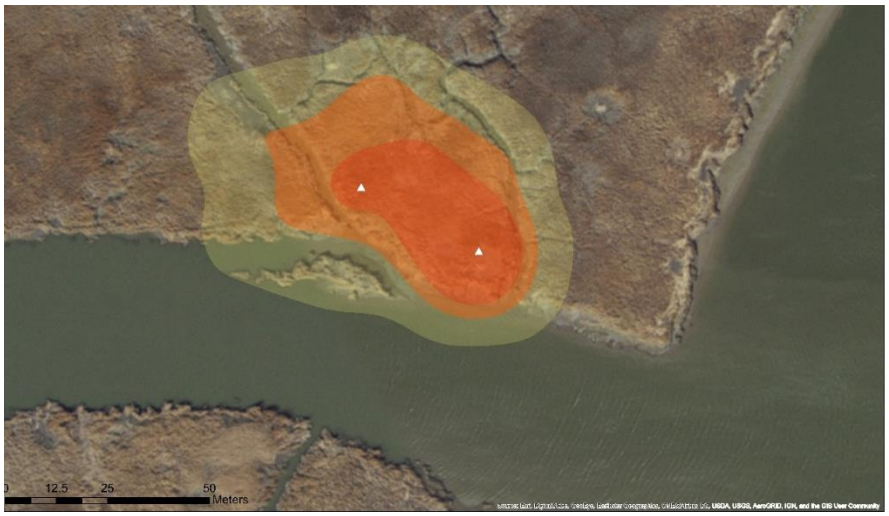

Figure 2.7. The overall range of an individual Clapper Rail at Woodland Beach, Delaware in 2021. The bird's two nests are denoted by white triangles. After an initial nest failure (left), a new nest site was selected closeby (right).

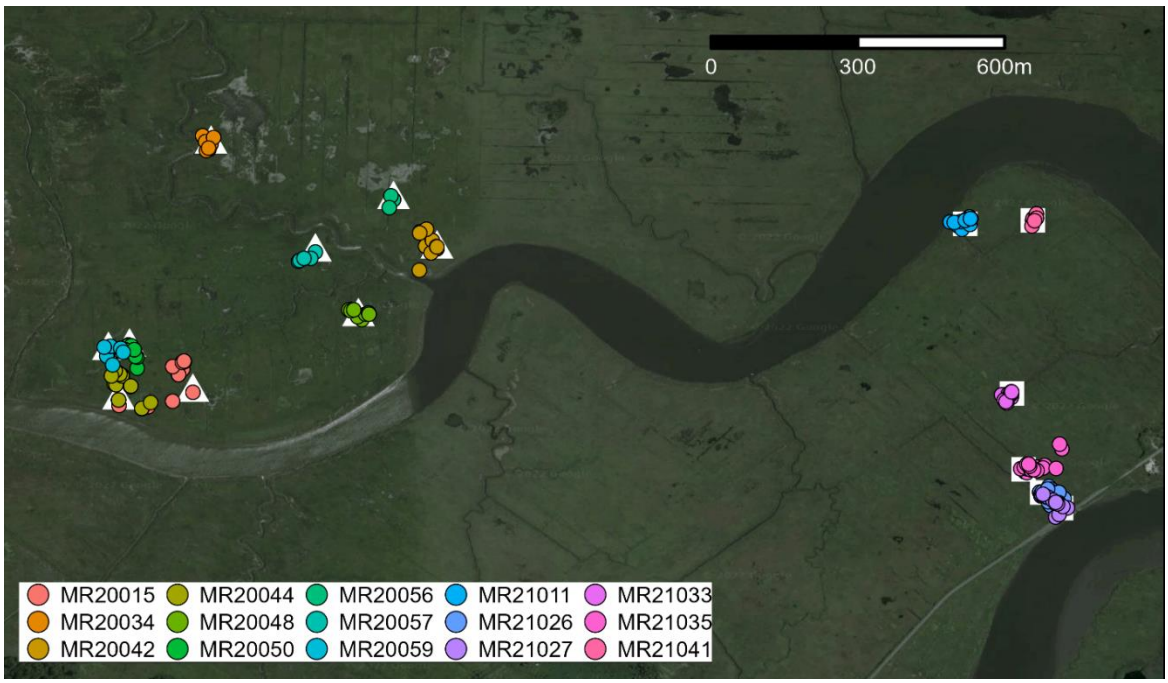

Figure 2.8. The Clapper Rail nests the chick were tagged at in Delaware from 2020-2021. All chick locations associated with a given nest (regardless of individual chick) are shown.

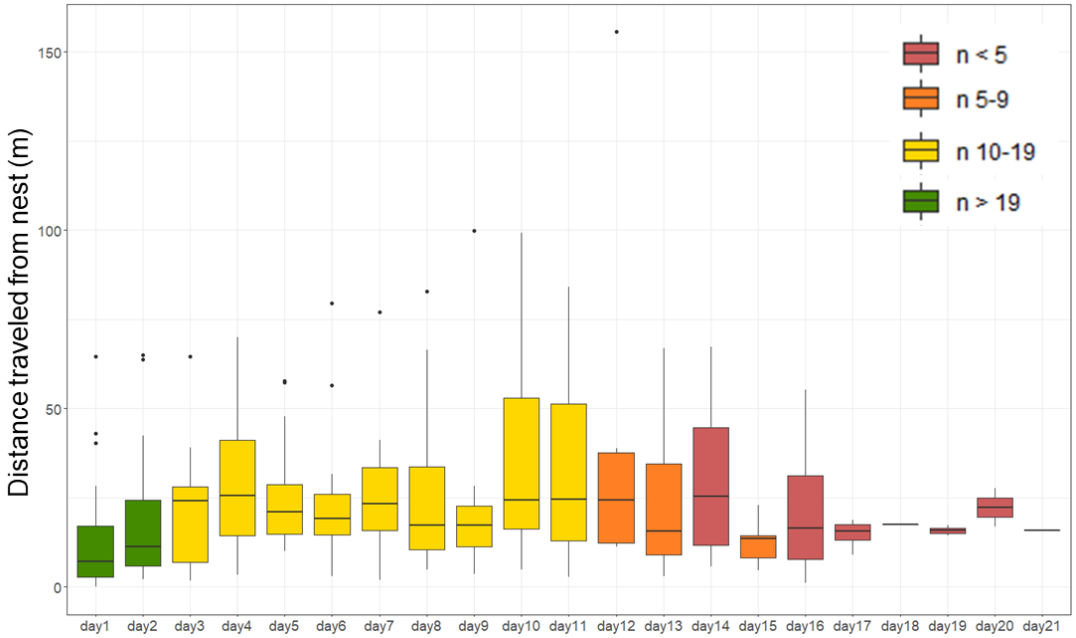

Figure 2.9. The distance of Clapper Rail chick locations from their nest of origin in Delaware from 2020-2021. Each day is color coded by number of chick locations presented (>19 in green, 10-19 in yellow, 5-9 in orange, and <5 in red). The averages remained constant among the first several weeks, although there was considerable individual variation.

Figure 2.10. An example of the behavior of Clapper Rail broods tagged near each other along the Mispillion River, Delaware in 2020. White triangles denote nests with tagged chicks and diamonds denote other nearby nests. A tagged chick appeared to cross the border into the neighboring territory on two occasions around two weeks of age.

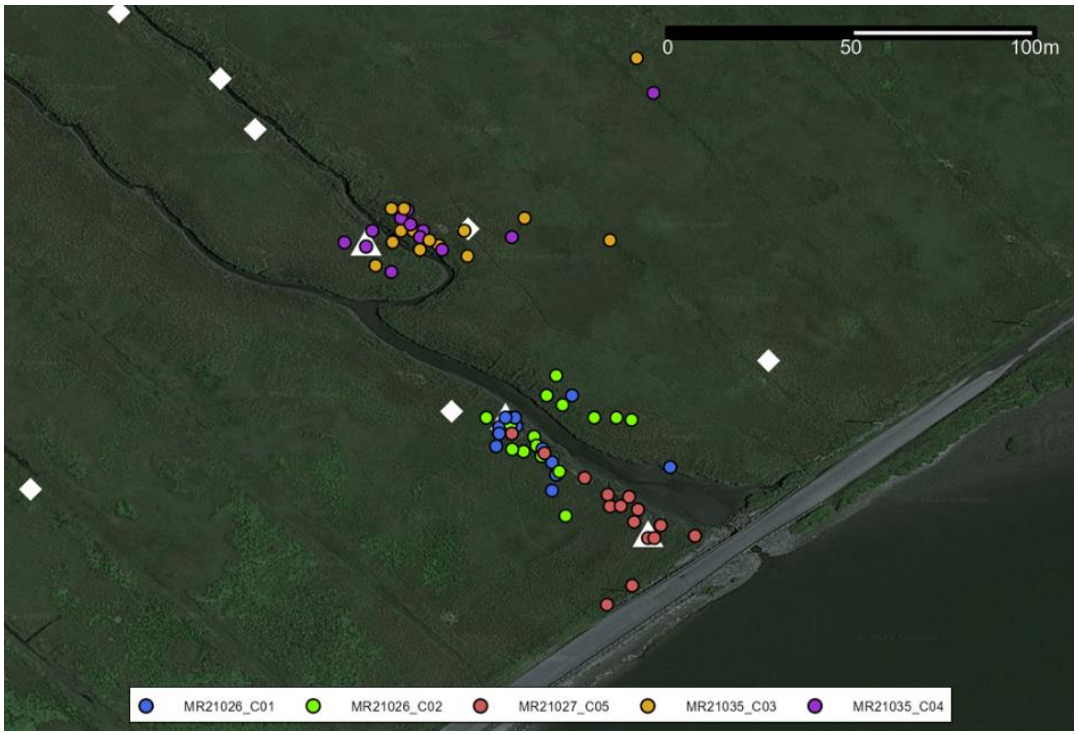

Figure 2.11. An example of the behavior of Clapper Rail broods tagged near each along the Mispillion River, Delaware in 2021. White triangles denote nests with tagged chicks and diamonds denote other nearby nests. One chick moved into the presumed territory of the other brood twice at 8 and 11 days of age, but subsequently returned to the immediate vicinity of its nest.

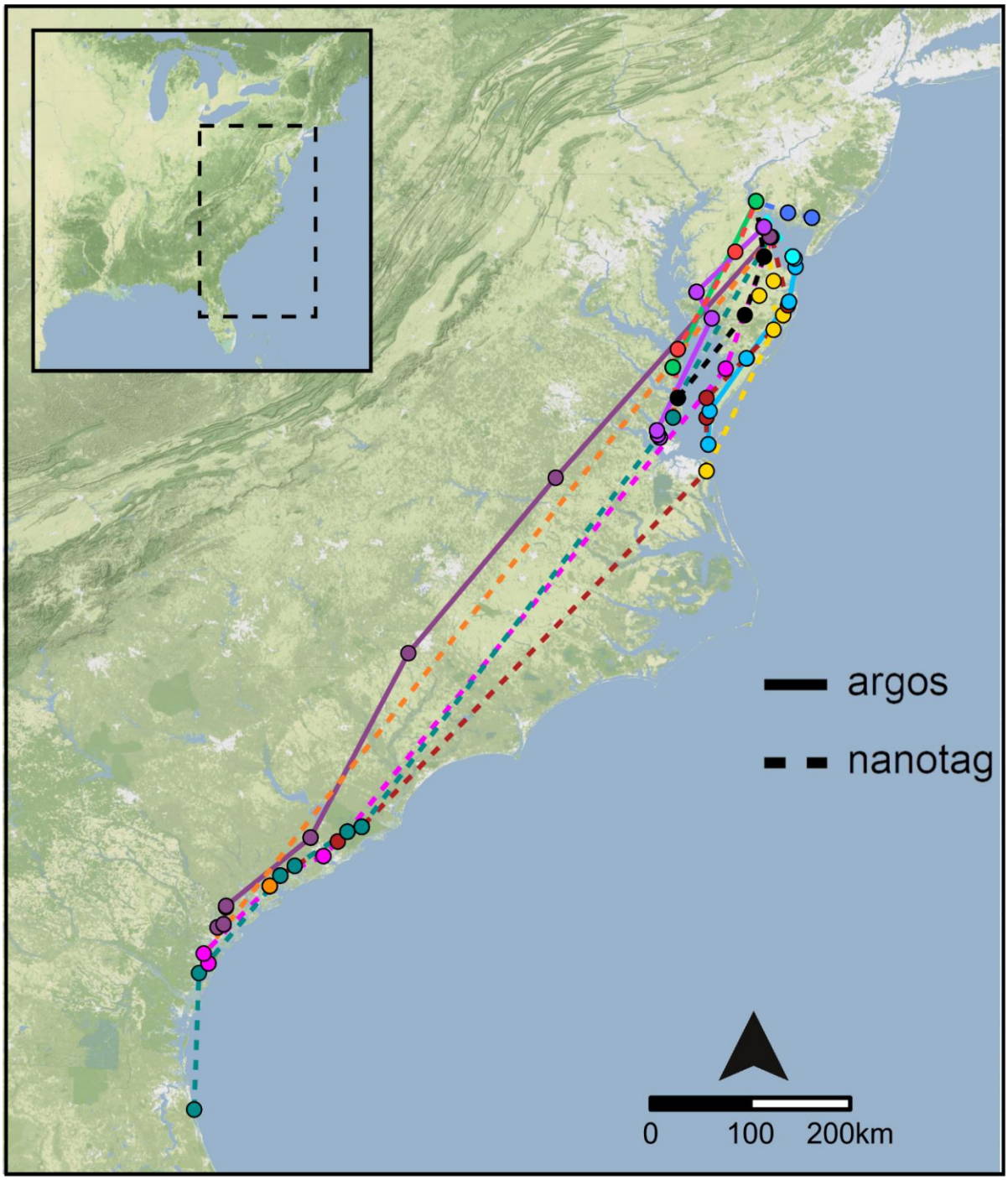

Figure 2.12. The migratory detections of birds tagged in Delaware in 2019-2021. The nanotag detections were recorded when tagged individuals traveled within the range of a Motus network tower while the Argos tags were GPS locations transmitted remotely via the Argos satellite.

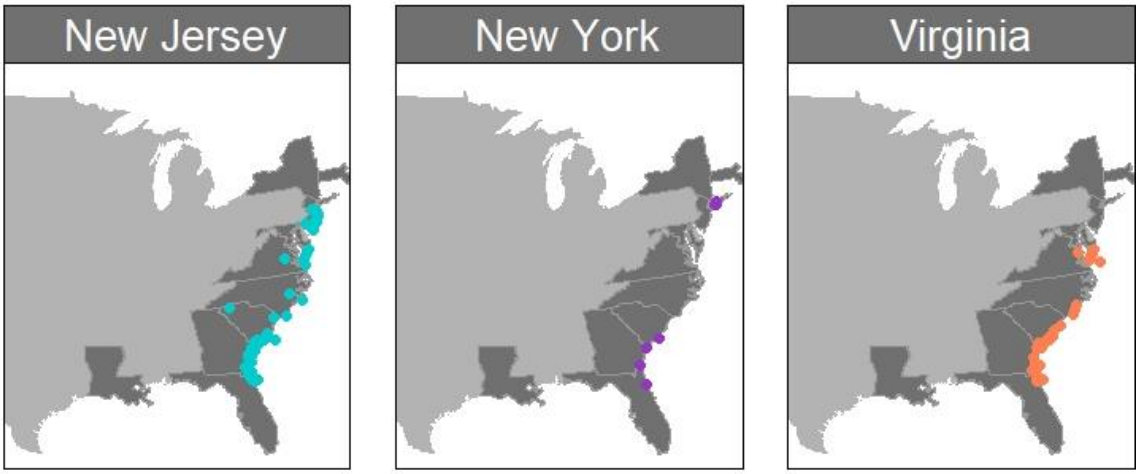

Figure 2.13. Clapper Rail band recovery data from the USGS Bird Banding Lab (banded from 1933-1978) by state in which they were banded. The majority of the birds were banded in New Jersey and subsequently recovered in New Jersey, however, among the recoveries in other states Georgia and South Carolina were the most prevalent.

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Chapter 3

A HIGH QUALITY DE NOVO CLAPPER RAIL REFERENCE GENOME

Introduction

Rallids (Aves: Rallidae) include 37 genera and 159 globally distributed species that occur primarily in wetlands, jungle lowlands, and montane forests (Garcia–R et al. 2019, Winkler et al. 2020). Despite their global distribution, most rallid species remain poorly understood because of their secretive nature. The type genus Rallus includes thirteen species of slim bodied, long-billed rails that occur in the Americas, Eurasia, Africa, and Madagascar (Winkler et al. 2020). Clapper Rail (*Rallus crepitans*) and King Rail (*Rallus elegans*) are two closely related species that occur along the eastern coast of North America, south to the Caribbean (del Hoyo et al. 2016, Rush et al. 2020). Clapper and King rail are similar in plumage, vocalization, and morphology (Maley and Brumfield 2013), but they exhibit different habitat preferences for saltwater (clapper rail) and freshwater (King Rail) wetlands. The internal nasal salt glands of clapper rails are larger than those of King Rail, and this adaptation is believed to contribute to the salinity tolerance (Conway et al. 1988) of clapper rails, although salt gland size is known to be a plastic trait that varies based on the water salinity to which the birds are exposed (Conway et al. 1988, Olson 1997). Osteologically, a narrower interorbital bridge in clapper rails accommodates its larger salt gland, and this species difference does not appear to be plastic, at least to the same extent as the salt gland (Olson 1997). Clapper and King rail populations hybridize where they co-occur in brackish marsh (Olson 1997, Maley 2012).

Avian hybridization along salinity gradients in North American marshes occurs not only between Clapper and King rails, but also between Nelson's sparrows

(*Ammospiza nelsoni*) and saltmarsh sparrows (*Ammospiza caudacuta*) (Shriver et al. 2005, Walsh et al. 2016b, 2019). Similar to King Rail, Nelson's Sparrows are more closely associated with fresh and brackish wetlands while Saltmarsh Sparrows, like Clapper Rails, are considered salt marsh obligates (Greenberg et al. 2006, Greenlaw et al. 2018). In the Nelson's/Saltmarsh sparrow hybrid zone, genes associated with osmoregulation and salinity tolerance exhibit increased introgression, leading to improved fitness when hybrids are compared to Nelson's Sparrows nesting in brackish and salt marshes (Walsh et al. 2016a). This observation suggests that for some organisms, hybridization may facilitate expansion into increasingly saline environments and additional work is warranted to explore these dynamics in other taxa. As climate change and sea level rise alter tidal marsh salinity gradients, it is increasingly important to understand how organisms can adapt to these changes in salinity.

To facilitate molecular investigations of the underlying mechanisms of saltwater tolerance and adaptive divergence between clapper and king rail populations, we completed the first genome assembly for Clapper Rail using DNA from a vouchered, wild female bird collected in Louisiana. To produce a chromosome-level assembly, we scaffolded contigs assembled using Meraculous (Chapman et al. 2011) and Spades (Bankevich et al. 2012) using Chicago and Hi-C libraries (Dovetail Genomics LLC). The resulting reference genome will be foundational to future studies investigating adaptation to high salinity environments, species limits in actively hybridizing populations, the conservation of Rallus species, and the genetic effects of sea level rise on marsh taxa.

Methods

Specimen collection and DNA extraction

We flash froze pectoral muscle, cardiac muscle, liver, and other tissues in liquid nitrogen from a wild female clapper rail collected in Louisiana. A round skin of the voucher specimen (LSUMZ 199649) is archived in the Collection of Birds of the Louisiana State University Museum of Natural Science (LSUMNS), and tissues associated with the specimen are preserved in the LSUMNS Collection of Genetic Resources (LSUMZ B-95207). We shipped liver tissue to Dovetail Genomics, LLC (Scotts Valley, CA) where Dovetail staff performed high molecular weight (HMW) DNA extraction using the Blood and Cell Culture Midi Kit (Qiagen, Gmbh).

Library preparation, sequencing, and assembly

Following HMW DNA extraction, Dovetail staff fragmented the DNA, prepared short insert sequencing libraries using an Illumina TruSeq DNA PCR-free kit, and sequenced the DNA using paired-end (PE) 150 base pair (BP) sequencing on an Illumina Hiseq X. The resulting data were trimmed to remove bases with quality scores lower than 20 using Trimmomatic (Bolger et al. 2014). Dovetail staff used in-house software to profile the trimmed reads at a variety of k-mer values (19, 31, 49, 79, 109) in order to estimate genome size and to fit negative binomial models to the data to determine the best k-mer value for assembly. The constrained heterozygous model with 49-mers and a homozygous peak depth of 42.0 was selected as optimal for the assembly. Dovetail staff then assembled contigs using Meraculous (Chapman et al. 2011) with a k-mer value of 49, a minimum k-mer frequency of seven, and the diploid nonredundant haplotigs mode.

Following contig assembly, Dovetail staff used remaining tissue to prepare a single, proprietary “Chicago” library following the methods described in Putnam et al. 2016 and summarized in Salter et al. 2019. They sequenced the resulting Chicago library on an Illumina HiSeq X using PE, 150 BP reads to a depth of approximately 70X. Similarly, Dovetail staff prepared one HiC library from remaining tissue following the methods described in Lieberman-Aiden et al. (2009) and summarized in Salter et al. 2019. Dovetail staff sequenced the resulting HiC library to a depth of approximately 45X using PE, 150 bp reads on an Illumina HiSeq X. After preparing and sequencing Chicago and HiC libraries, Dovetail staff used HiRise (Putnam et al, 2016) to conduct two rounds of scaffolding: (1) using the Chicago reads to scaffold the Meraculous contigs, and (2) using the HiC reads to scaffold the Chicago scaffolds. We refer to the resulting assembly as the “Dovetail HiC Assembly”.

After receiving the Dovetail HiC Assembly, we computed contiguity statistics using assembly-stats (https://github.com/sanger-pathogens/assembly-stats) and estimated assembly completeness using BUSCO v4.0.6 (Manni et al. 2021). While evaluating this version of the assembly, we noticed that the Z chromosome appeared to be missing. Specifically, after aligning scaffolds and contigs from the Dovetail HiC Assembly to the chicken genome assembly (UCSC galGal6; NCBI GCF_000002315.5) using ragtag v1.0.1 (Alonge et al. 2019), we did not recover any contigs or scaffolds that aligned to the Z chromosome, suggesting Z chromosome contigs and scaffolds were not present. This problem has been observed in other Dovetail assemblies of birds (Del-Rio et al. 2021, Recuerda et al. 2021, Shakya et al. 2021) and may have resulted from the coverage parameters used by Dovetail during

the Meraculous assembly process inadvertently excluding contigs representing sex chromosomes.

We addressed this problem by maintaining the macrochromosomes (scaffolds > 20 Mbp) from the Dovetail HiC Assembly while re-assembling and re-scaffolding contigs representing the microchromosomes. To start the microchromosome reassembly process , we trimmed the short-insert sequencing reads with trimmomatic v0.39 and corrected the trimmed reads using Musket v1.1 (Liu et al. 2013) and a kmer value of 61. We then performed a second de novo assembly using spades v3.14.0 (Andrey et al. 2020) with error correction turned off (--only-assembler`) on a high-memory (1.5 TB) compute node, and we filtered the resulting assembly using faFilter (Kent et al. 2002) to remove contigs < 1 Kbp. We extracted macrochromosomes (scaffolds > 20 Mbp) from the Dovetail HiC Assembly using faSize (Kent et al. 2002) and custom Python code (Supplemental File), concatenated each into a single file, and used ragtag to align the contigs output by spades to this file macrochromosomes. Because of the way that ragtag formats output files, we were able to separate the contigs that aligned to macrochromosomes from those that did not, and we used custom Python code to create a file of contigs that did not align to the macrochromosomes. We provided this file of contigs to Dovetail staff, who re-ran the Chicago and HiC scaffolding processes using their proprietary HiRise pipeline.

After rescaffolding, we merged the resulting scaffolds (many representing microchromosomes) into the file of macrochromosomes to produce an assembly representing the entire genome, and we sorted the file by descending scaffold length using sortbyname in BBMap 38.78 (Bushnell 2014). We used custom Python code to rename all scaffolds, and we used faFilter to remove contigs/scaffolds shorter than

1000 bp in length. To ensure that the updated assembly contained scaffolds representing the Z chromosome, we performed a second alignment of the updated assembly to the chicken genome assembly (galGal6).

After validating that the updated assembly contained a large scaffold representing the Z chromosome, we used bwa v0.7.17 (Li 2013) to align reads from the short-insert libraries to the assembly, SAMtools v1.1.0 (Li et al. 2009) to sort and index the resulting BAM file, and Pilon 1.23 (L. et al. 2022) to polish the assembly by fixing `--all` of the issues identified. We used custom Python code to clean the polished scaffold names, we computed contiguity statistics using assembly-stats, and we estimated assembly completeness using BUSCO v4.0.6. Finally, we identified repeats in the polished assembly using RepeatModeler v2.0.1 (Smith and Hubley 2008) and RepeatMasker v 4.1.0 (Smith et al. 2013), and we used bedtools (Quinlan and Hall 2010) to soft-mask the polished assembly.

Data availability

Data from all sequencing runs will be available on NCBI. Short-insert, Chicago, and HiC reads and the final assembly with the Z chromosome will also be available on NCBI Genome.

Results and Discussion

The sequencing of short-insert libraries produced 325 million read pairs with an approximate insert side of 382 bp. Analysis of k-mer histograms suggested the Rallus genome size was 1.3 Gbp. Meraculous assembly using a k-mer value of 49 output 55,528 contigs with a total length of 990.8 Mbp, a N50 of 50 kbp (L50 = 5,380), and a maximum contig length of 606.9 Kbp (Table 3.1). Meraculous estimated

that the assembled contigs comprised 97% of the estimated, non-repetitive genome size and 78% of the entire genome.

Chicago library sequencing produced 254 million read pairs, and HiRise made 27,838 joins and 24 breaks to the Meraculous assembly, producing an intermediate Chicago assembly including 19,218 scaffolds and having a total length of 994.3 Mbp, a N50 of 1.8 Mbp (L50 = 128), a N90 of 0.06 Mbp (L90 = 1384), and a maximum scaffold length of 13.8 Mb. HiC library sequencing produced 170 million read pairs, and HiRise made 5,992 joins and zero breaks to the Chicago assembly. Fifty-seven gaps in the resulting assembly were closed using short-insert reads to produce the Dovetail HiC Assembly that included 13,226 scaffolds having a total length of 994.9 Mb, a N50 of 82.7 Mb (L50 = 4) scaffolds, a N90 of 10.8 Mbp (L90 = 18), and a maximum scaffold length of 204 Mbp. BUSCO completeness estimates for the Dovetail HiC Assembly are provided in Table 3.2.

Contig re-assembly using spades output 55,026 contigs having a total lenth of 1.1 Gbp, a N50 of 58.0 kbp (L50 = 4,904), a N90 of 9.5 kbp (L90 = 22,795), and a maximum contig length of 907 kbp. We identified 24,773 contigs that did not align to macrochromosomes in the Dovetail HiC assembly that we submitted to Dovetail for re-scaffolding, which output a set of 12,193 scaffolds having an N50 of 15.3 Mbp (L50 = 5) and an N90 of 8 Kbp (L90 = 673). The longest scaffold in the re-assembly was 76.1 Mbp in length and primarily aligned to the chicken Z chromosome. After merging the macrochromosomes with these scaffolds, polishing the assembly, and cleaning all scaffold names, the assembly included 12,167 scaffolds having a total length of 1.1 Gbp, a N50 of 82.9 Mbp (L50 = 4), a N90 of 12.2 Mbp (L90 = 20), and a maximum scaffold length of 204.5 Mbp. We refer to this assembly as Rc_LA_1.0, and

BUSCO completeness estimates for the Rc_LA_1.0 improved on the results from the Dovetail HiC Assembly (Table 3.2), although several BUSCOs remained fragmented (n=17; 7%) or were not detected (n=13; 5%).

The Rc_LA_1.0 assembly we produced is the first for a species in the genus Rallus and one of six assemblies representing taxa within the Rallidae (Table 3.3). Our assembly is among the most contiguous for the taxonomic family, and the availability of a genome assembly representing this genus will facilitate investigations of salinity tolerance, interspecific hybridization, and mechanisms of speciation in Clapper and King rails.

TABLES

Table 3.1. Contiguity statistics for *Rallus crepitans* assemblies comparing the Dovetail HiC Assembly and the Rc_LA_1.0 assembly.

	Dovetail HiC Assembly	Rc_LA_1.0
Scaffolds	13,226	12,167
Total length (Mb)	994.8	1,108.0
N50 (Mb)	82.7	82.9
N90 (Mb)	10.8	12.2
L50	4	4
L90	18	20
Longest Scaffold (Mb)	204.0	204.6
# N's	4,085,069	3,899,784
# Gaps	42,269	41,488

Table 3.2 Estimates of assembly completeness using the BUSCO aves_odb10 database (n = 8338 BUSCOs) showing the improvements in completeness between the Dovetail HiC Assembly and the Rc_LA_1.0 assembly, which includes the Z chromosome.

	Dovetail HiC Assembly		Rc_LA_1.0	
	Count	Percentage	Count	Percentage
Complete BUSCOs	6993	83.9	7673	92.0
Complete and single-copy BUSCOs	6979	83.7	7621	91.4
Complete and duplicated BUSCOs	14	0.2	52	0.6
Fragmented BUSCOs	384	4.6	216	2.6
Missing BUSCOs	961	11.5	449	5.4

Table 3.3. Reference genome metrics for other publicly available Rallidae assemblies (as provided by GenBank). BUSCO completeness run with aves_odb10 (n = 8338).

	Rc_LA_1.0	*Atlantisia rogersi*	*Fulica atra*	*Gallirallus okinawae*	*Porphyrio hochstetteri*	*Zapornis atra*
GenBank accession	(pending)	GCA_013401215.1	GCA_013372525.1	GCA_013400835.1	GCA_020800305.1	GCA_013400835.1
Scaffolds	12,167	277,915	17,827	768,680	174	120,944
Total length (Mb)	1,108.0	1,202.6	1,167.6	1,114.5	1,270.3	1,127.6
N50 (Mb)	82.9	0.04	6.39	0.01	71.56	0.13
L50	4	8,560	46	48,754	5	2,081
Complete BUSCOs	92.0%	--	94.5%	42.9%	96.9%	--

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Chapter 4

CLAPPER RAIL POPULATION GENOMICS AND HYBRIDIZATION WITH KING RAIL

Introduction

Tidal marshes are dynamic and productive ecosystems at the interface of terrestrial and marine biomes characterized by daily tidal inundation. Tidal marshes provide substantial ecosystem services as they buffer storm events, sequester carbon, and mediate nutrient cycling (Costanza et al. 2008, Mcleod et al. 2011, Huxham et al. 2018, Gilby et al. 2021*a*). Tidal marshes also support wildlife populations, including specialist species endemic to tidal marshes (Greenberg et al. 2006, Whitfield 2017, Crotty et al. 2018). Tidal marshes have been lost and degraded by anthropogenic activities which has directly impacted the extent and condition of salt marshes through land conversion, altering hydrological regimes, pollution, and the introduction of invasive species (Kirwan and Megonigal 2013, Gilby et al. 2021*b*). In addition to these direct effects, anthropogenically accelerated climate change will continue to reduce and degrade salt marsh habitat through the increased rate of sea level rise and increased storm intensity and frequency. Sea level rise also reduces marsh stability, alters flooding regimes, and changes marsh distribution across the landscape (Morris et al. 2002, Schuerch et al. 2018*a*). Although inland marsh migration may follow increases in sea levels, in many areas human development restricts marsh migration limiting compensation for marsh loss (Schuerch et al. 2018*b*). Understanding how endemic tidal marsh wildlife will respond to marsh loss and altered hydrology caused

by increasing sea levels and storms is a conservation priority. Planning for how tidal marsh dependent wildlife will respond to rapidly changing habitat quality and extent requires an understanding of population dynamics, adaptive potential, and resiliency of coastal marsh species.

Genetic data can provide key insights into population dynamics, resiliency, and adaptative potential. The application of genetic data in the management and conservation of wildlife species is becoming increasingly feasible with advances in sequencing technology and bioinformatic approaches (Hohenlohe et al. 2021). Next Generation Sequencing (NGS) technology has increased our scope of inference for delineating species and populations (Toews et al. 2016, Feng et al. 2020), estimating effective population sizes (Waples et al. 2016), identifying selection pressures (Catchen et al. 2017, Ryan et al. 2018), and understanding adaptive capacities of different populations and species (Andrews et al. 2016, Benham and Cheviron 2020*a*, Albecker et al. 2021, Hohenlohe et al. 2021, Johnson et al. 2022). Reduced-representation NGS approaches such as Restriction site-associated DNA sequencing (RAD-seq) yield thousands of single nucleotide polymorphism (SNP) markers and, consequently, often reveal finer-scale population structure than microsatellite and other molecular markers (Narum et al. 2013, Bohling et al. 2019, Sunde et al. 2020). High resolution genetic data can be particularly useful in migratory bird population management, as birds are difficult to monitor across the full annual cycle and capable of long-distance dispersal. Many avian taxa are easily sampled on the breeding grounds due to their displays, facilitating the collection of genetic samples.

Additionally, bird genomes are relatively small (Gregory et al. 2007) and thus more cost effective to sequence than other vertebrate taxa.

Evolutionarily, tidal marshes provide a selection gradient along which closely related avian taxa have been shown to vary in their adaptations to the stressors of salinity and frequent flooding. Passerelidae sparrows, for example, have repeatedly evolved various degrees of salinity tolerance that vary both within and between species (Walsh et al. 2019, Benham and Cheviron 2020*b*). For example, the closely related, congeneric Saltmarsh (*Ammospiza caudacutus*) and Nelson's (*Ammospiza nelsoni*) sparrows vary in their adaptation to salt marshes and exhibit bidirectional introgression where they are sympatric (Walsh et al. 2016*c*, 2018). Saltmarsh Sparrow are highly adapted to salt marsh systems while only one sub-species of Nelson's Sparrow (*A. n. subvirgatus*) has evolved salinity tolerance allowing it to colonize brackish and salt marshes. *A. n. subvirgatus* appears to have fewer adaptations to coastal salt marsh conditions relative to Seaside and Saltmarsh sparrows (Walsh et al. 2021). This system of passerines with varying degrees of adaptation to tidal marshes has yielded valuable insights into patterns of population structure (Walsh et al. 2016*a*), hybridization (Shriver et al. 2005, Walsh et al. 2016*b*), and adaptive introgression (Walsh et al. 2018).

A similar and less studied system of hybridizing freshwater and salt marsh avian species exists between the King (*Rallus elegans*) and Clapper rail (*Rallus crepitans*). The King Rail occurs primarily in freshwater marshes while the Clapper Rail occurs in tidal salt marshes (Meanley 1969, 1985). The two species differ in

morphology and plumage coloration (Maley 2012), vary in salinity tolerance (Olson 1997), and are known to hybridize in brackish marshes (Meanley and Wetherbee 1962), an intermediate habitat that will shift its distribution as sea level rise progresses. Clapper Rail are coastally distributed along eastern North America and comprise eight subspecies (Figure 4.1) , of which at least one (*R. c. crepitans*) is declining (Correll et al. 2017). The proximity of their populations to oceans makes them highly subject to direct impacts of two aspects of climate change: sea level rise and increased storm frequency and intensity. Although Clapper Rail may fare better than other saltmarsh obligate taxa in the near-term response to rising sea levels (Hunter et al. 2017, Klingbeil et al. 2018, Field et al. 2019), their persistence on the landscape is likely to be driven by marsh migration that may be prevented by anthropogenic structures and/or coastal forests (Field et al. 2016, Schuerch et al. 2018*b*). The coastal distribution of Clapper Rail not only subjects them to increasing sea levels, but also places them in the path of increasing coastal storm frequency and intensity. Coastal storms cause direct mortality (especially in young birds), nest loss, habitat loss, and displacement into unsuitable habitat (Carlson et al. 2002).

King Rail are primarily a freshwater species, although the coast adjacent freshwater and brackish marshes serve as important habitat (Cooper 2008, Rogers et al. 2013, Glisson et al. 2015) and much of their current distribution follows coast lines (Figure 4.2). This proximity to coastlines may lead to salinity changes in the freshwater marshes King Rail inhabit, resulting in resource competition and potential for hybridization with Clapper Rail. The patterns of introgression between King and

Clapper rail are poorly understood, but this hybridization can be cryptic and may favor Clapper Rail phenotypes (Coster et al. 2018*b*). King Rail are listed as threatened or vulnerable in Canada and in multiple U.S. states (Cooper 2008). This climate induced habitat shift may result in Clapper Rail the genetic swamping of King Rail in coastal wetlands where they co-occur, similar to the genetic swamping effect Mallard populations have on American Black Duck populations (Ankney et al. 1987, Lawson et al. 2021).

To date, little information exists regarding the range-wide population connectivity of Clapper rail. Previous work suggested that Clapper Rail populations along the East coast of the U.S. were not genetically distinct (Coster et al. 2018*a*), but this may have been a limitation of the resolution of a small number of genetic markers; recent advances in genetic techniques provide the opportunity for more fine-scale patterns of population structure to be identified with large SNP datasets. Both King and Clapper rails have resident and migratory populations that winter sympatrically, but gene flow between these breeding populations has not been quantified. Range-wide characterization of patterns of introgression has also not been conducted. Better understanding this system will lead to opportunities to study the underlying genomics of resiliency and adaptation in the face of osmoregulatory stress, as well as provide an opportunity to study the role of hybridization in adaptation and its response to climate change. Our objectives were therefore to: (1) to assess population structure of breeding Clapper rail populations along the North American Atlantic and Gulf coasts, and (2) to

determine the extent of introgression between King and Clapper Rail on both the Atlantic and Gulf coasts.

Methods

Sample Collection

We collected 50 King Rail, 162 Clapper Rail, and 15 putative hybrid samples using various methods including blood draws on captured birds, lethal specimen collection, hunter harvest, and opportunistic collection of genetic material. King Rail in North Carolina were captured and banded by East Carolina University (ECU) at Mackay Island, NC and blood samples were stored in ethanol. We collected Clapper Rail samples from adult birds captured in Delaware during the breeding season (April-August) from 2018-2020 at Woodland Beach Wildlife Area, the St. Jones National Estuarine Research Reserve, and Milford Neck Wildlife Area, and we stored blood samples in Queens lysis buffer (Seutin et al. 1991) immediately after collection.

King Rail, Clapper Rail, and putative hybrids specimens were collected in Louisiana by the Louisiana State University Museum of Natural Science from 2008-2014 and tissue samples were shipped in ethanol. In 2020, we lethally collected samples from North and South Carolina prior to their fall migration using a twenty-gauge shotgun and playback from a FoxPro (Lewiston PA) game caller (federal collections permit MB61020D-0, AUP 1157). North Carolina samples were collected from Roanoke Island and from south of Wilmington, NC. South Carolina samples

were collected from the Nemour's Foundation property near Yemassee, SC and from South Carolina state property near Fripp Island, SC.

Samples were also obtained from hunters who harvested early season Clapper Rail individuals between 5 and 19 September 2020 at East River Wildlife Management Area (WMA), Great Harbor WMA, and Nell's Island WMA in Connecticut prior to their fall migration. The carcasses were immediately frozen then shipped whole on ice to the University of Delaware. Samples from harvested rails in Georgia were collected on Nobuto blood filter strips on 27 October 2019 and thus may have had migrant birds present in the sample.

Salvaged samples from Connecticut nest monitoring efforts from 2017-2019 were provided by the University of Connecticut. These consisted of abandoned eggs or dead chicks from failed or partially failed nests (typically due to predation or flooding). Additional salvaged samples from North Carolina were contributed by the North Carolina Museum of Natural Sciences, which provided whole carcasses frozen immediately after death at wildlife rehabilitation centers.

Sequencing & Bioinformatic Processing

We extracted DNA from blood and tissue samples for all but the North Carolina King Rail samples, which were extracted at ECU. Blood and muscle tissue samples were extracted using a Zymo mini-prep plus DNA extraction kit (Zymo, Irvine, CA, USA) following the manufacturer's protocol. Blood samples stored on Nobuto blood filter strips were extracted using an Omega Bio-tek E.Z.N.A. Blood

DNA Mini Kit (Omega Bio-tek Inc., Norcross, GA, USA). We quantified the DNA concentration in each sample using a Qubit 3.0 fluorometer Broad Range double-stranded DNA assay kit (Life Technologies, NY, USA). We normalized DNA concentrations to 25 ng/µl and prepared four double digest restriction site-associated DNA sequencing (Peterson et al. 2012) libraries using 500 ng of input DNA per sample, when available for 50 King Rail, 162 Clapper Rail, and 15 putative hybrid samples (see Figure 4.3 for break down by geographic location). Ten samples had <500 ng of input DNA; input DNA for these ranged from 50-390 ng. We grouped the low quantity samples together in one index group to maintain across-index group molarity consistency. DNA was digested using the enzymes *SbfI* and *MspI* and ligated to P1 and P2 adapters using t4 DNA ligase (30 min at 37° C, 60 min at 20° C, held at 10° C). We subsequently pooled samples into index groups by their unique P1 adapter sequence and cleaned using 1.5 x Agencourt AMPure XP magnetic beads (Beckman Coulter, IN, USA). We conducted size selection of fragments between 400 and 700 bp using BluePippin (Sage Science, MA, USA). Illumina TruSeq primer sequences were incorporated using low cycle Polymerase Chain Reactions (PCR) and a final clean up using AMPure XP beads was completed. We visualized libraries on a fragment Bioanalyzer to verify library fragment size/distribution. Resulting libraries were sequenced with a 15% PhiX Spike-in on one lane of an Illumina Hiseq PE150 and three partial lanes on a NovaSeq-6000 (Novogene Co).

DNA sequence data were demultiplexed by index group by Novogene Co. then we demultiplexed to the individual level using the *Stacks* version 2.55

process_radtags pipeline with reference alignment (Catchen et al. 2011, 2013). We aligned the demultiplexed reads to our reference genome (see Chapter 3) using the Burrows-Wheeler Aligner (BWA) package with the BWA-MEM algorithm (Li and Durbin 2009). To identify SNPs, we ran the *ref_map.pl* pipeline in *Stacks* with the minimum percentage of individuals across populations required to process a locus (-r) set to 70% and the minor allele count (-min-mac) set to three to remove singletons and doubletons, but maximize rare alleles, which provide high resolution for discriminating population structure (O'Leary et al. 2018, Díaz-Arce and Rodríguez-Ezpeleta 2019). We restricted the data export to one random SNP per locus (-write-first-snp). The VCF file was subsequently filtered further using vcftools (Danecek et al. 2011). Any SNPs with more than two alleles or a mean read depth (-min-meanDP) of less than 10 were removed. SNPs within 500 bp of one another were omitted to reduce linkage. Individuals missing data for more than 50% of the remaining SNPs were removed from the analysis. A second VCF file with one parameter difference was produced at this stage with a more stringent individual missing data filter of 90% to explore the effects of missing data on population inference

Genetic Sexing

We used our aligned ddRAD sequencing data to determine sex by comparing autosomal chromosome sequencing depth to Z chromosome sequencing depth. We calculated the mean sequencing depth across 30 autosomal chromosome scaffolds and compared it to the sequencing depth of the Z chromosome scaffold. As females have

only one copy of the Z chromosome, their ratio of autosomal chromosome depth to sex chromosome depth should be greater than the ratio for males. We determined the cutoff values for sexing using 78 known sex King and Clapper rails. We considered a ratio of >1.2 autosomal depth/sex chromosome depth to be female and <1.05 to be male.

Population structure

To evaluate genetic differentiation among the sampling groups, we calculated pairwise F_{ST} (Figure 4.4, Weir and Cockerham 1984) using the R package *hierstat* (version 0.5-10, Goudet 2005). We also calculated observed and expected heterozygosity and the inbreeding coefficient among these groups to assess population genetic diversity and potential signatures of inbreeding.

To characterize population structure across all sampling populations, we ran ParallelStructure (version 2.3.4, Pritchard et al. 2000, Besnier and Glover 2013) using the Cyberinfrastructure for Phylogenetic Research (CIPRES) science gateway (Miller et al. 2010) and modeled 1 to 5 populations with 100,000 iterations, following a 200,000 burn-in. We used STRUCTURE HARVESTER (Earl and VolHoldt 2012) to visualize the plateau in LnPd as well as to implement the delta K method (Evanno et al. 2005) and identify the most likely number of populations (K).

We also used a multivariate clustering approach to visualize population structure. We created subsets of the filtered data by species and conducted Principle Component Analyses (PCA) using the glPca function in R package *adegenet* (version

2.1.5, Jombart and Ahmed 2011) for Clapper Rail samples only, King Rail samples only, and all rail samples together. We repeated the PCAs for the stringently filtered data set (allowing only 10% missing data) to ensure that missing data did not alter the pattern of our results.

The two species can be difficult to differentiate in-hand, particularly if they are introgressed, and we discovered inconsistencies in the genetic data and the field IDs. We therefore used the genetic data to correct species identification when both the PCA and program STRUCTURE results indicated that an individual had been misclassified at the species level. Using this corrected dataset, we conducted a Discriminant Analysis of Principal Components (DAPC) to look for evidence of fine scale population differentiation. *A priori* groups were formed by sampling location and species (for example, NC Clapper Rail were in a separate group than NC King Rail). Using the R package *adegenet* (version 2.1.5, Jombart and Ahmed 2011), we performed stratified cross-validation with varying numbers of PCs to select the appropriate number of PCs to retain. After performing cross-validation with 30 replications across the full array of PCs, we isolated the PC range that had the greatest proportion of successful outcome prediction (40-70 PCs). To select a single PC number for the DAPC, we ran cross validation with only these 30 PCs for 1000 replications and used the number of PCs with the lowest root mean squared error, 51 (Jombart 2008).

Hybrid Identification

To characterize patterns of introgression in our dataset (or across our sampling locations), we classified the level of introgression of individuals. We first selected reference individuals for each species, using phenotypically pure King Rail from North Carolina and Louisiana. Due to apparent introgression in some of the sampled populations, we implemented a 90% cut off from the Program STRUCTURE population assignments to remove putative hybrids from our selection of reference individuals. We selected Clapper Rail reference individuals from areas where they were least likely to be sympatric with King Rail in highly saline marshes in Connecticut, South Carolina, and Louisiana and selected individuals that were phenotypically pure. We removed birds with <99% probability of assignment to the apparent Clapper Rail population and <90% assignment to the King Rail population. Our subsequent hybrid analysis was based upon 28 reference King Rail and 39 reference Clapper Rail. To maximize the number of SNPs assessed for fixation, we used a less filtered dataset than that used for the analyses described above. The SNPs were run through populations with the same parameters, including missing data of 0.7 and a MAC of 3, but were not filtered in VCFtools, resulting in a dataset consisting of 27,706 SNPs.

We identified 142 fixed SNPs between our two reference groups (parental species). Using R package *introgress* (version 1.2.3, Gompert and Buerkle 2010), we used these fixed SNPs to calculate hybrid index and interspecific heterozygosity values for each individual sampled. Pure individuals were defined by a hybrid index

value of either <0.05 (King Rail) or >0.95 (Clapper Rail). Individuals with intermediate hybrid index values (0.25-0.75) and high heterozygosity (>0.3) were considered to be recent generation hybrids (F1/F2). Individuals with low heterozygosity (<0.3) and hybrid index values of 0.05-0.25 or 0.75-0.95 were considered backcrossed (Milne and Abbott 2008, Walsh et al. 2015).

Results

Data description and summary statistics

Following demultiplexing and filtering, the dataset contained 8,834 SNPs. We removed one individual from Connecticut due to excess missing data. The pairwise F_{ST} values among Connecticut, Delaware, North Carolina, and South Carolina Clapper Rail sampling groups all fell below 0.01. The F_{ST} values between Louisiana and other Clapper Rail sampling groups ranged from 0.010 to 0.012. The F_{ST} value between the Louisiana Clapper Rail and hybrid samples fell within this range at 0.011. South Carolina had the largest F_{ST} value of any of the Clapper Rail groups paired with the Louisiana hybrids at 0.026 (Figure 4.4). The Louisiana and North Carolina King Rail groups had a paired F_{ST} of 0.010. When compared against the Clapper Rail sampling groups, the North Carolina King Rails had higher F_{ST} values ranging from 0.169 (South Carolina Clapper) and 0.140 (Louisiana Clapper). The Louisiana King Rail sampling group had lower F_{ST} values when compared to Clapper populations than did the North Carolina King Rail sampling group (Figure 4.4).

Observed heterozygosity (H_O) was lower than the expected heterozygosity (H_S) and consistent across all sampling groups, ranging from 0.10 to 0.11. The inbreeding coefficient (F_{IS}) was low across sampling locations, ranging from 0.04 to 0.010 (Table 4.2).

Population structure

STRUCTURE results supported a K = 2 model, as evidenced by both the plateau in estimated log likelihood values and the Delta K (Table 3). The two populations appeared to cluster along species delineations and nearly all of the King Rail samples had some percentage of assignment to the presumed Clapper Rail population (Figure 4.5).

The two species showed distinct groupings along PC1, which accounted for nearly 9% of the variation (Figure 4.6). Some individuals were intermediate between the two species groups clustering in the center of PC1. These samples included individuals identified in the field as hybrids and had partial STRUCTURE assignment to both groups indicating potential introgression. The PCA with the more stringent missing data filter showed similar patterns (Figure 4.7).

The PCA including only Clapper Rail samples showed weak differentiation of the Gulf Coast Louisiana Clapper Rail from a large cluster containing individuals of all of the Atlantic Coast sampling populations along PC1, which explained 2% of the variation in the data (Figure 4.8). Several Delaware Clapper rails, all of which had 30-50% assignment to the King Rail group in STRUCTURE, clustered with the Louisiana

Clapper rails along PC1. Three individuals separated along PC2, which accounted for 1.2% of the variation in the dataset. The PCA with the more stringent missing data filter showed similar patterns (Figure 4.9).

The PCA including only King Rail samples seperated three Louisiana individuals along PC1, (Figure 4.10), all of which had > 99% assignment to the Clapper Rail STRUCTURE cluster. Two individuals from the North Carolina population were seperated on PC2, which explained less than half of the variation that PC1 did (3.3%). The PCA run with the more stringent missing data filter showed similar patterns (Figure 4.11).

The DAPC, defined with groups based on sampling locality and STRUCTURE corrected species assignment, produced a similar pattern to the PCA with individuals separating by species along the first discriminant axis, with structure-identified Louisiana and Delaware hybrids in the middle. The second axis parsed out the North Carolina King Rail group and the Louisiana King Rail group (Figure 4.12). These two axes accounted for 89% of the variation in the dataset.

We found 142 fixed SNPs between our reference King and Clapper rail individuals. Using these markers, we identified 78.4% of the individuals (n = 178) as pure King or Clapper rail, 16.7% of the individuals (n = 38) as backcrossed, and 4.8% of the individuals as recent generation hybrids (n = 11; Figure 4.13).

Discussion

Here we provide the first analysis using genome-wide SNP markers to assess the breeding population structure and patterns of introgression for Clapper and King Rail. Our findings confirm previous research conducted during the migration period (Coster et al. 2018a), which indicated that the Clapper Rail populations along the Atlantic Coast have a high degree of genetic connectivity, despite geographic distance and differences in migratory behaviors. The northernmost subspecies of Clapper Rail (*R.c.crepitans*) is migratory, but they winter sympatrically with residents birds along the southern Atlantic coast (Elizondo unpublished data, Stewart 1954). Our results also reveal a high degree of gene flow and population connectivity between the Gulf and Atlantic coast Clapper Rail populations, with evidence of only weak population structure based on our DAPC results. Clapper Rail are capable of long-distance dispersal (Hon et al. 1976) and can travel several hundred miles over inland habitats during migration (see Chapter 2), which may explain this lack of population structure. Among the two sampled King Rail populations, the Atlantic Coast and Gulf Coast populations demonstrated weak population structure, which could be driven by a number of factors including isolation by distance. The disjunct nature of King Rail's freshwater habitat may act as a barrier to gene flow that is not present in Clapper Rail populations, which occupy the relatively more continuously distributed salt marsh habitat (Maley 2012).

We detected two distinct clusters in our samples that corresponded to King and Clapper rail species assignment. Individuals with intermediate clustering occurred in

all sampled breeding populations, supporting evidence of fairly widespread introgression. Based on fixed markers between the two species and interspecific heterozygosity, roughly one-fifth of the rails sampled were introgressed. Less than 5% of the introgressed individuals were classified as recent generation (F1/F2) hybrids and the remainder were back-crossed. Individuals classified as recent hybrids were often 'cryptic hybrids' not discernible in-hand to even experienced observers, in concurrence with previous observations (Maley 2012, Coster et al. 2018*b*). Our Clapper Rail sampling was focused on saltmarshes (as defined by vegetation communities), yet even our most saline sampling locations had evidence of King Rail geneflow. Our King Rail samples from freshwater marshes showed more extensive backcrossing than the Clapper Rail from highly saline areas based on our hybrid index and heterozygosity analyses. Although both King and Clapper rail have nasal salt glands which plastically increase in size when exposed to salinity, Clapper Rail consistently outperform King Rail at expelling salt water (Conway et al. 1988) and have structural differences in their skull to accommodate larger salt glands (Olson 1997). We postulate that salt marsh is a greater barrier to King Rail than freshwater marsh is to Clapper Rail as freshwater does not require physiological adaptations, saltmarsh requires both physiological adaptations to tolerate salinity and behavioral adaptations to tolerate tidal cycles. Due to this differential adaptation, it is likely that sea level rise will force further secondary contact as salt marsh habitat diminishes, possibly pushing Clapper Rail into fresher marshes.

The North Carolina King Rail samples were collected from locations in close proximity to saltwater, potentially fostering rail dispersal between fresh and saline marshes. In Louisiana, however, it was noted that despite close proximity of freshwater impoundments to saltwater, King rail phenotypes were still the predominant phenotypes in the impoundments (Maley 2012). Given the importance of brackish and coast adjacent marshes to King Rail populations, it is likely that all but the most inland populations have some degree of introgression.

Tidal systems are characterized by varying salinity and water levels, which may create additional barriers to King Rail colonization of salt marsh systems. Clapper Rail exhibit a wide array of behavioral adaptations to living in salt marsh tied to their nesting and foraging habits. For example, Clapper Rail eggs are capable of withstanding extensive submersion in salt water and adults may spend considerable time preventing eggs from flooding out (Apgar pers. comm.) and retrieving those that do (Pettingill 1938*b*).

Previous work in the Gulf Coast suggests that this boundary is maintained by ecological speciation along a salinity gradient zone (Maley 2012), but human alterations of landscapes, including the creation of freshwater impoundments, might produce a mosaic landscape that precludes straightforward delineation of a hybrid zone. Our results support the conclusion that the King and Clapper rail species boundary is maintained despite frequent secondary contact and may exhibit a mosaic hybrid zone similar to that found in tidal marsh sparrows (Maley 2012, Walsh et al. 2016*b*).

TABLES

Table 4.1. The number of variant loci retained as additional filtering parameters were added.

Software	Filter	# variant loci
Stacks: gstacks	NA	423,631
Stacks: populations	Min. percentage of individuals with the loci (-r) set to 0.7 AND Min. MAC set to 3	27,706
VCFTools	Minimum mean depth of 10	9,760
VCFTools	Thin by 500 bp	8,834

Table 4.2. Expected heterozygosity (H_S), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) for Clapper and King rail sampling groups. Observed heterozygosity was consistently slightly lower than expected heterozygosity.

		H_S	H_O	F_{IS}
Clapper Rail	Connecticut	0.110	0.106	0.038
	Delaware	0.112	0.098	0.095
	North Carolina	0.114	0.104	0.073
	South Carolina	0.111	0.106	0.049
	Georgia	0.103	0.093	0.066
	Louisiana	0.115	0.104	0.079
Hybrid	Louisiana	0.123	0.109	0.077
King Rail	Louisiana	0.123	0.111	0.090
	North Carolina	0.114	0.108	0.095

Table 4.3. Average LnP(K) and Delta K values for each K value modeled in program STRUCTURE.

K	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	-740514.74	9.64	—	—	—
2	-700283.82	87.51	40230.92	44558.56	509.18
3	-704611.46	518.05	-4327.64	7881.30	15.21
4	-701057.80	214.68	3553.66	2265.60	10.55
5	-699769.74	808.03	1288.06	—	—

FIGURES

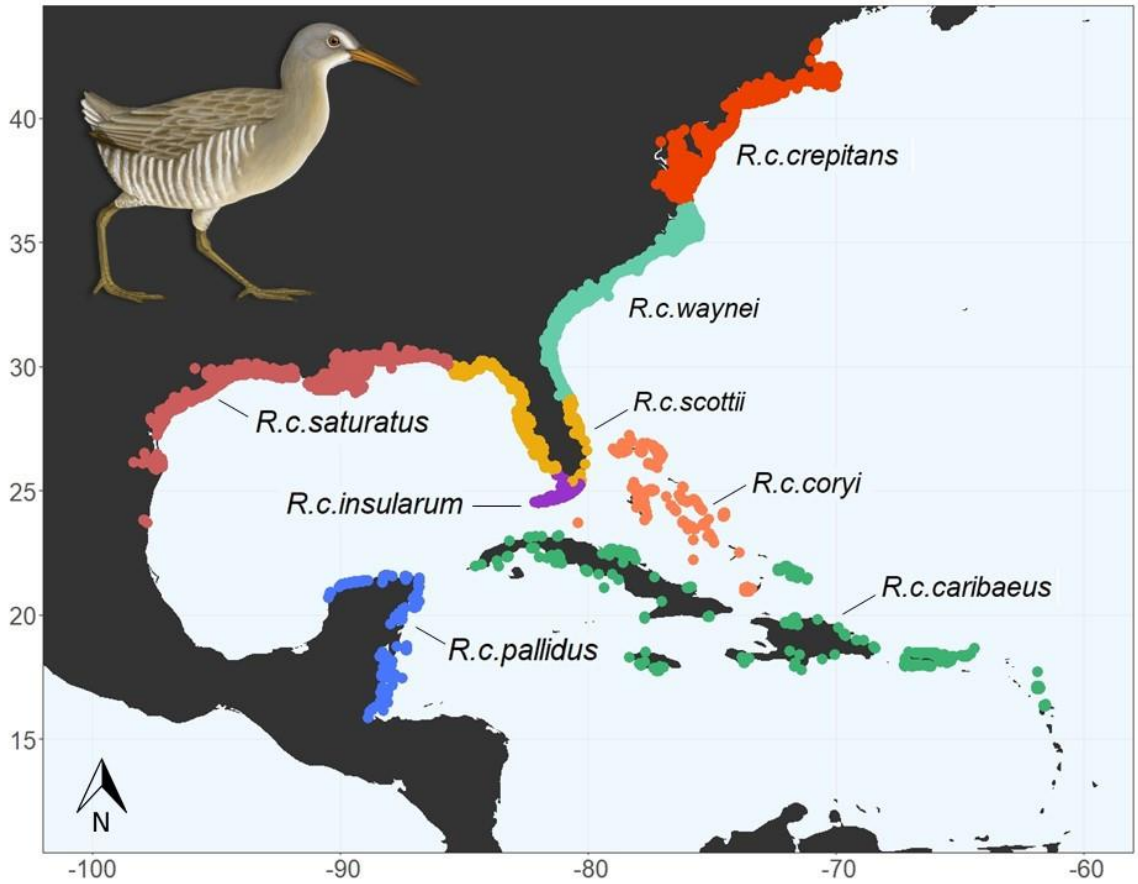

Figure 4.1. The distribution of the eight Clapper Rail subspecies generated using eBird data.

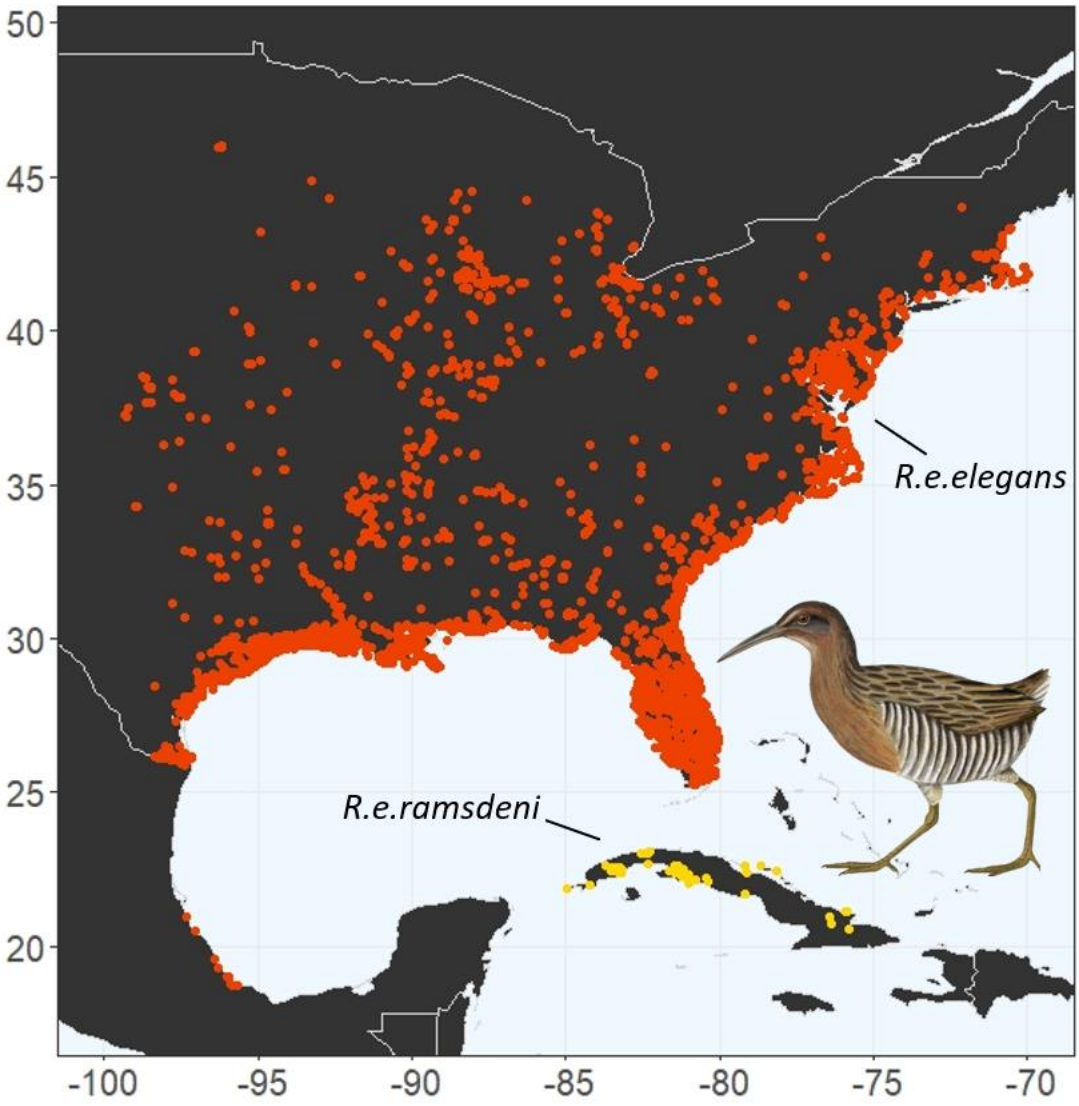

Figure 4.2. Reports of King Rail based on eBird data (excluding Canada due to data restrictions on endangered species).

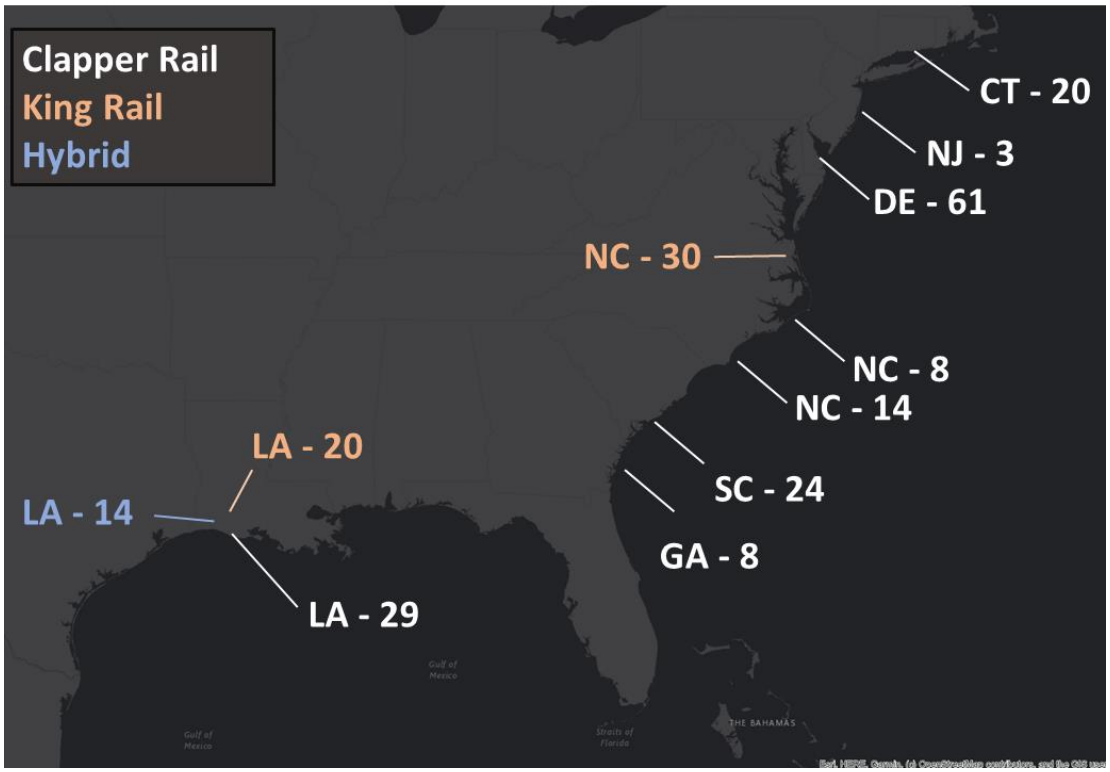

Figure 4.3 The geographic locations of the King, Clapper, and hybrid rail samples used in the genetic analyses.

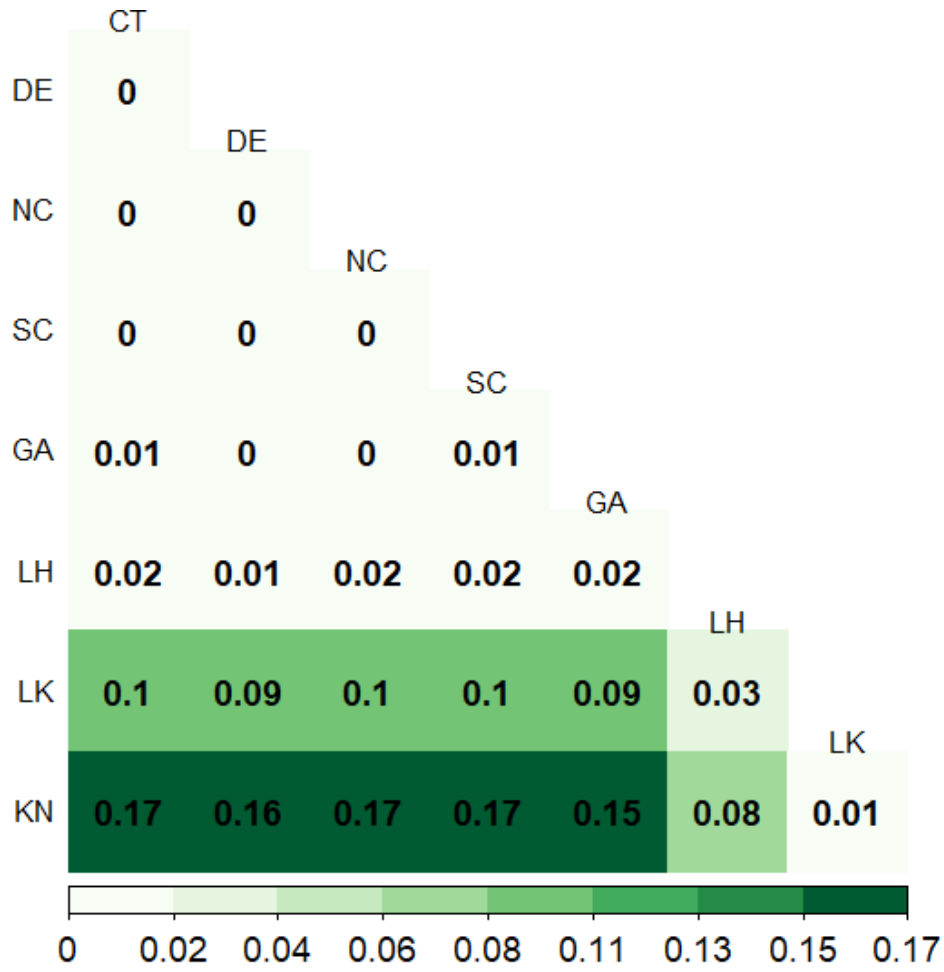

Figure 4.4. Pairwise F_{ST} values between the Clapper and King rail sampling groups (CT = Connecticut Clapper Rail, DE = Delaware Clapper Rail, NC = North Carolina Clapper Rail, SC = South Carolina Clapper Rail, GA = Georgia Clapper Rail, LH = Louisiana hybrid rail, LK = Louisiana King Rail, KN = North Carolina King Rail).

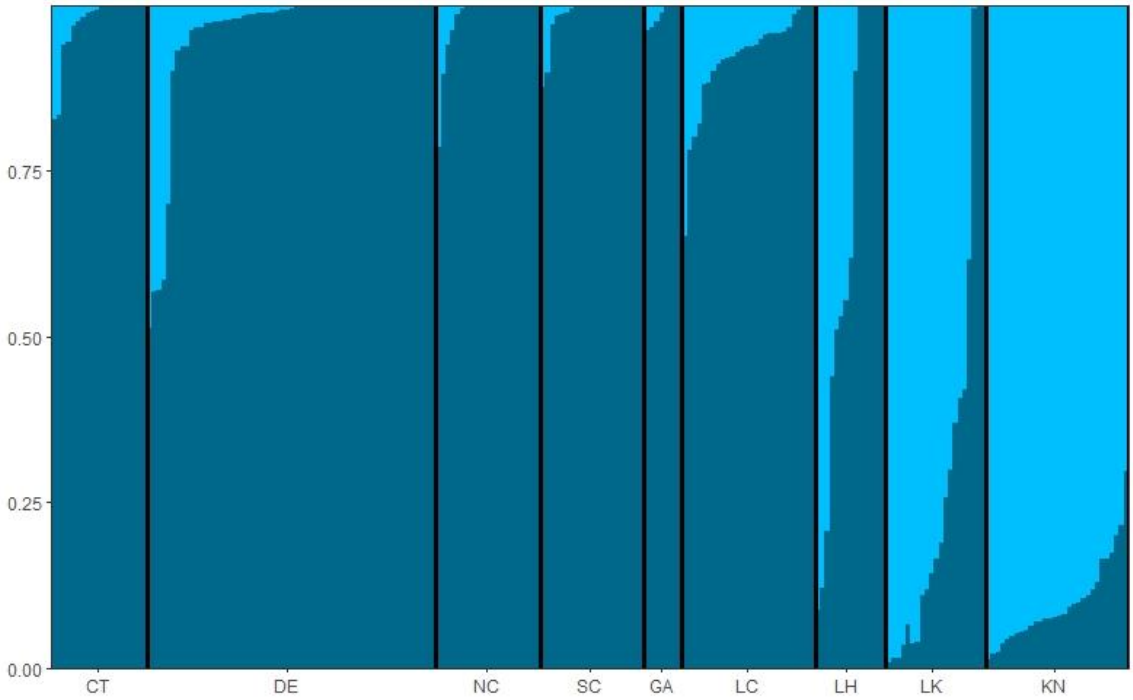

Figure 4.5. Program STRUCTURE population assignments for all Clapper and King individuals sampled from 8 sampling locations plus putative hybrids. K1 (dark blue) corresponds to Clapper Rail and K2 (light blue) corresponds to King Rail. The Clapper Rail samples (CT, DE, NC, SC, GA, LC) suggest low levels of introgression across the populations. There were putative hybrids from Louisiana (LH) with high assignment to the Clapper Rail group. Most of the Louisiana King Rails (LK) had at least some assignment to the Clapper Rail group.

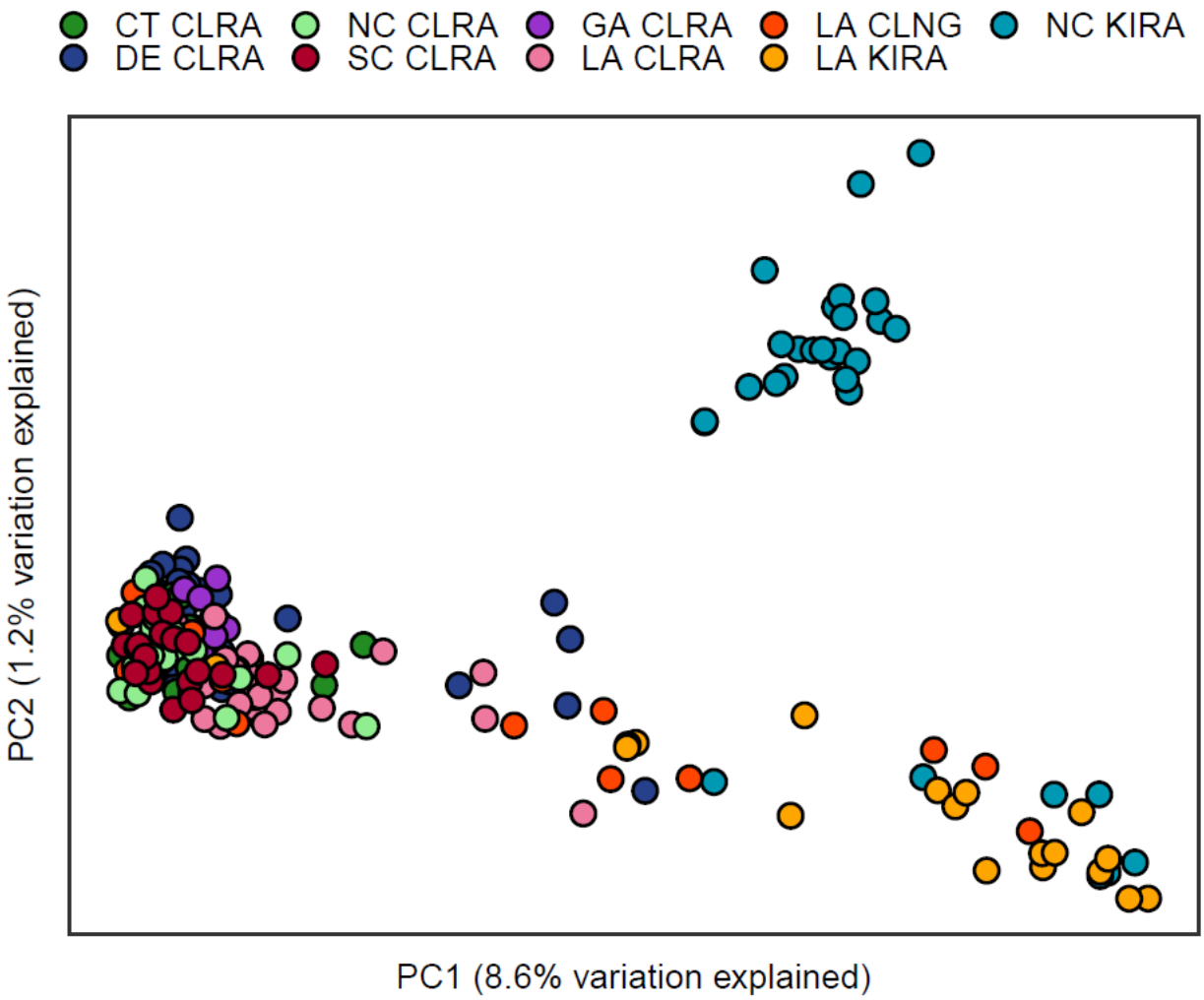

Figure 4.6. PCA results plotted by PC1 (x axis) and PC2 (y axis) across all samples collected from nine sampling locations of Clapper Rail (CLRA) and King Rail (KIRA). Each point is colored according to their original species identification made at the time of sample collection. Individuals grouped toward the left correspond with pure Clapper Rail and individuals on the far right correspond with pure King Rail, with potential hybrids in the middle.

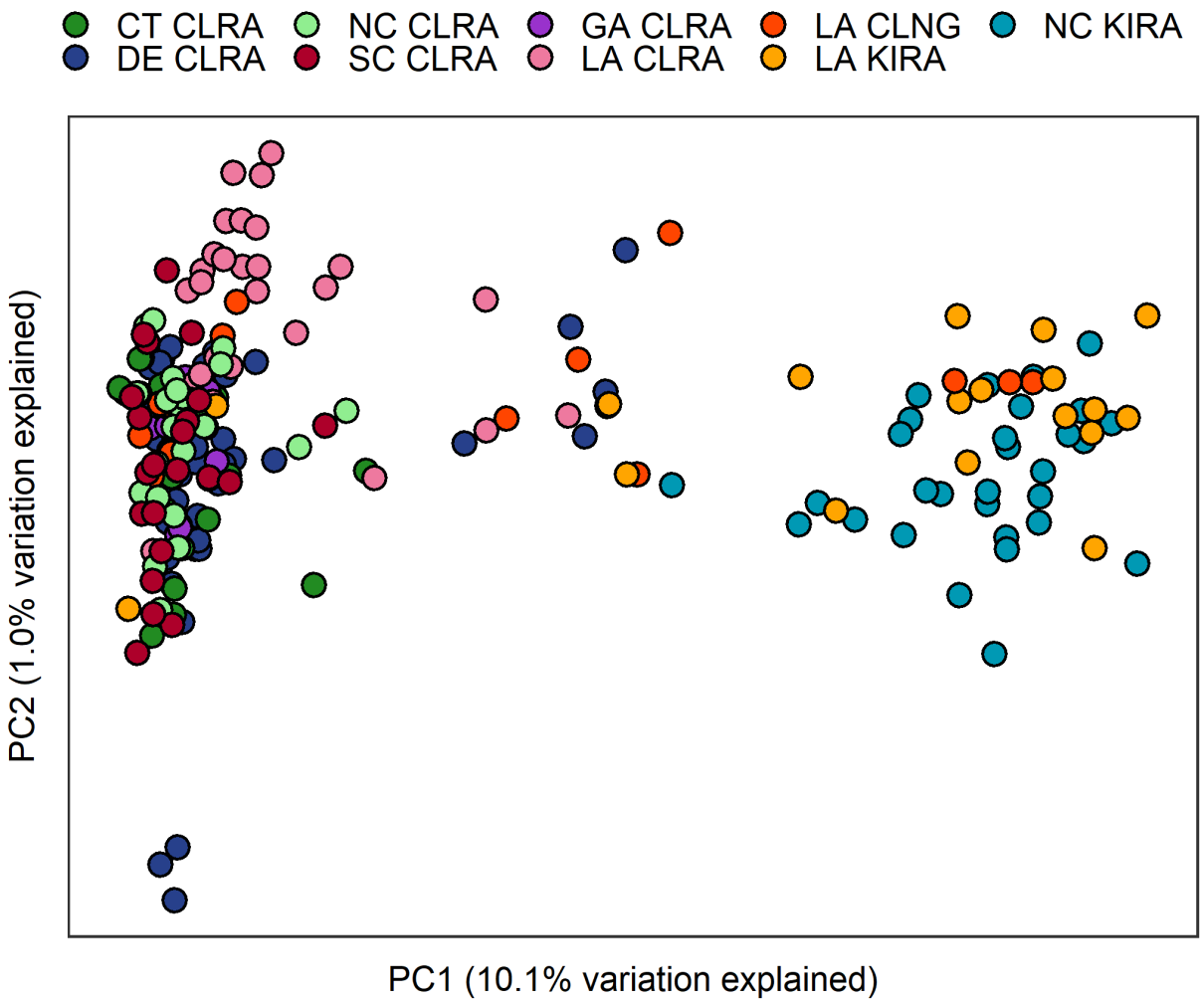

Figure 4.7. PCA results of the dataset derived with the more stringent missing data filter of 90% plotted by PC1 (x axis) and PC2 (y axis) across all samples collected from nine sampling locations of Clapper Rail (CLRA) and King Rail (KIRA). Each point is colored according to their original species identification made at the time of sample collection. The PCA is largely consistent with the full dataset (see Figure 4.5).

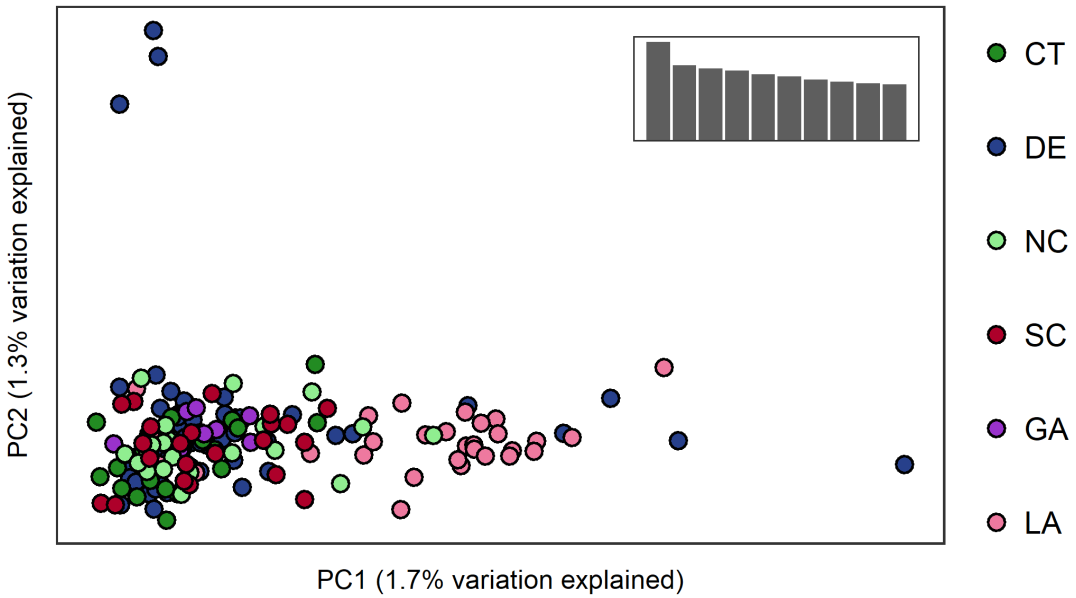

Figure 4.8. PCA results plotted by PC1 (x axis) and PC2 (y axis) across all Clapper Rail samples collected from 6 sampling locations. PCs one through ten (left to right) are plotted in an inset scree plot. Each point is colored according to their original species identification made at the time of sample collection. The five rightmost Delaware samples had greater than 0.4 assignment to the presumptive King Rail group in Program STRUCTURE.

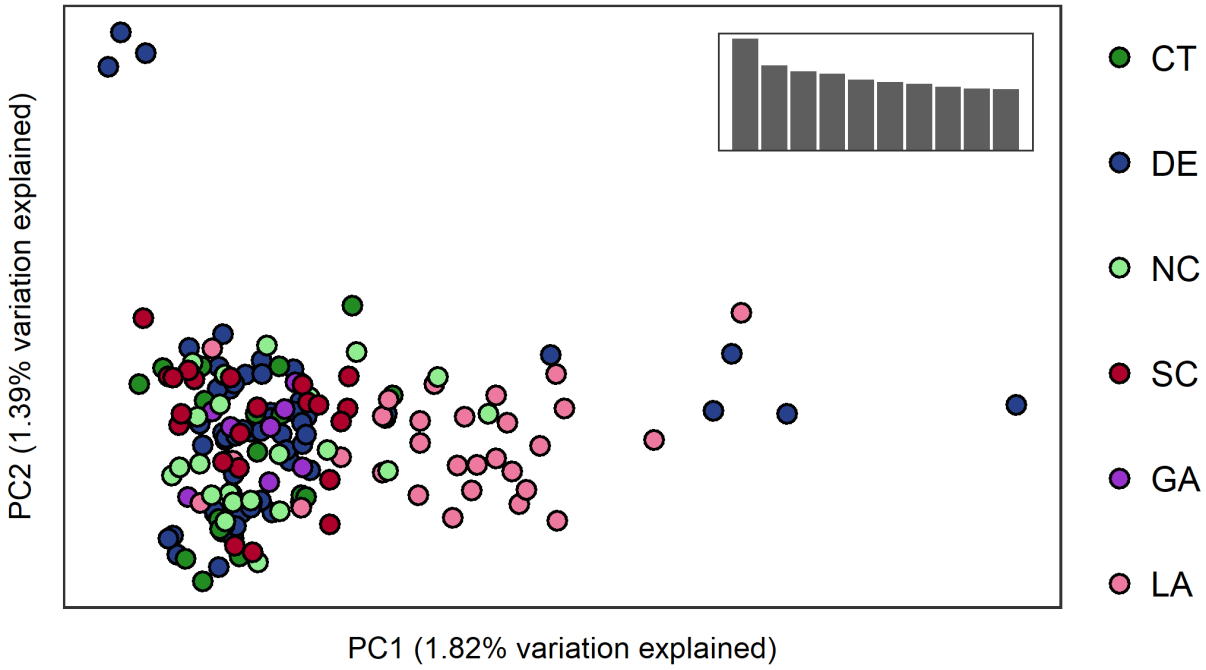

Figure 4.9. PCA results of the dataset derived with the more stringent missing data filter of 90%. plotted by PC1 (x axis) and PC2 (y axis) across all Clapper Rail samples collected from six sampling locations. PCs one through ten (left to right) are plotted in an inset scree plot. Each point is colored according to their original species identification made at the time of sample collection. The five rightmost Delaware samples had greater than 0.4 assignment to the presumptive King Rail group in Program STRUCTURE.

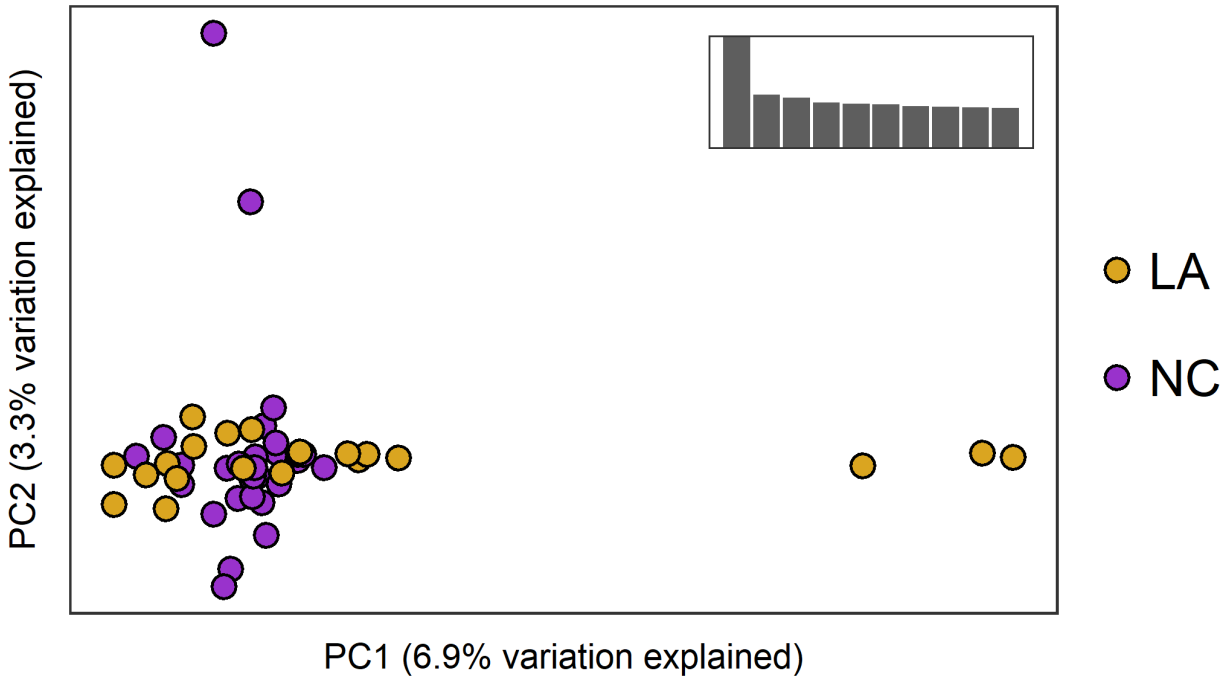

Figure 4.10. PCA results plotted by PC1 (x axis) and PC2 (y axis) across all King Rail samples collected from the two sampling populations. PCs one through ten (left to right) are plotted in an inset scree plot. Each point color corresponds to the individual's original species identification made at the time of sample collection. The three rightmost individuals had greater than 0.99 assignment to the presumptive Clapper Rail group in Program STRUCTURE.

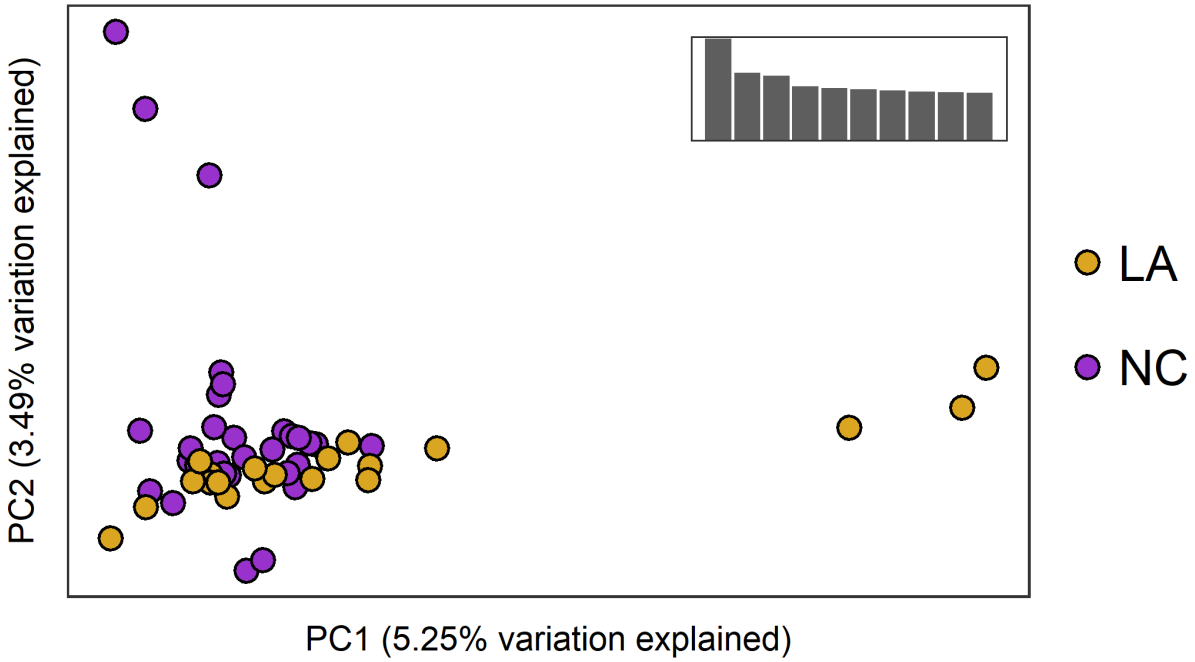

Figure 4.11. PCA results for a more stringently filtered King Rail only dataset with a missing data filter by site of 90%. PCs one through ten (left to right) are plotting in an inset scree plot. The patterns reflected in Figure 10 remain the same; several individuals cluster out along PC1 and those individuals all had greater than 0.99 assignment to the presumptive Clapper Rail group in Program STRUCTURE.

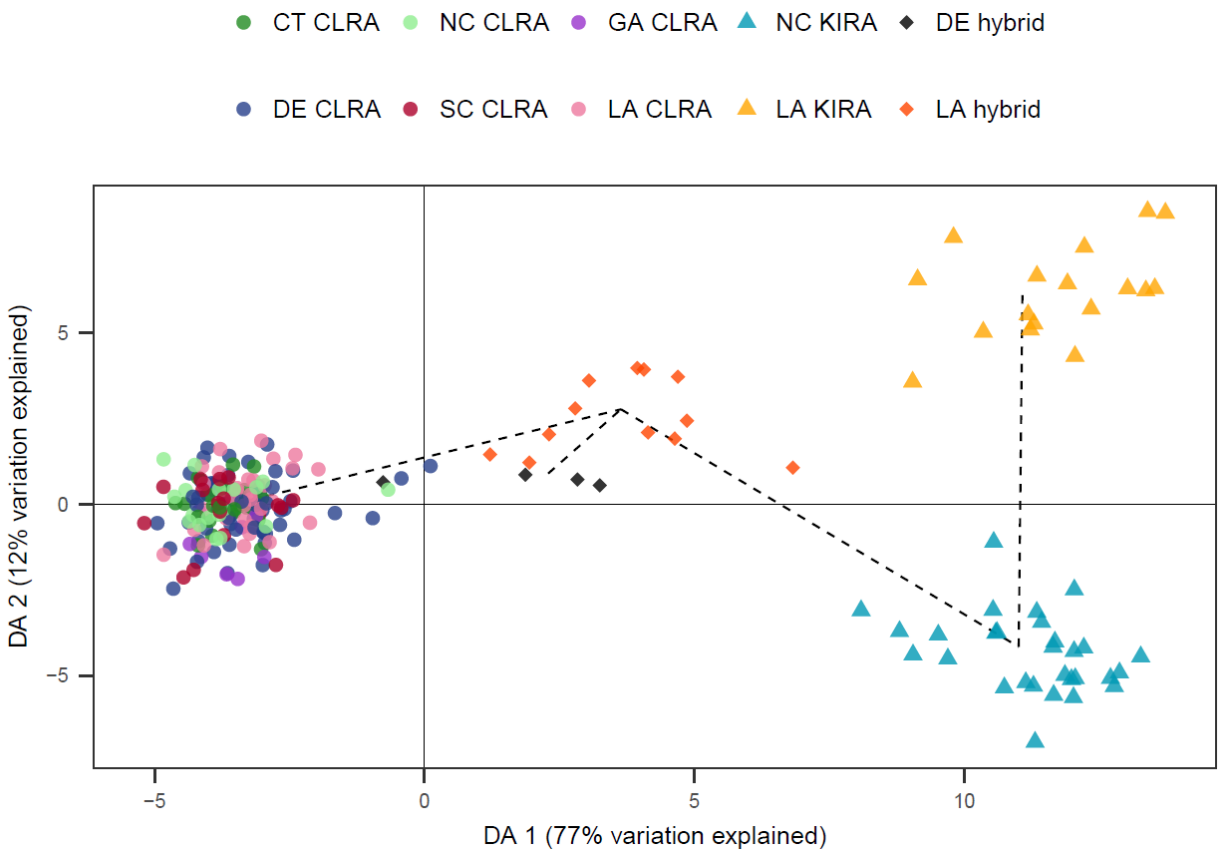

Figure 4.12. DAPC results plotted along the first two discriminant axes. Each individual is colored by its corrected species assignment for each sampling group: Connecticut Clapper Rail (CT CLRA), Delaware Clapper Rail (DE CLRA), North Carolina Clapper Rail (NC CLRA), South Carolina Clapper Rail (SC CLRA), Louisiana Clapper Rail (LA CLRA), North Carolina King Rail (NC KIRA), Louisiana King Rail (LA KIRA), and hybrids from Delaware and Louisiana (DE hybrid and LA hybrid respectively). Clapper Rail are denoted with circles, King Rail with triangles, and hybrids with diamonds.

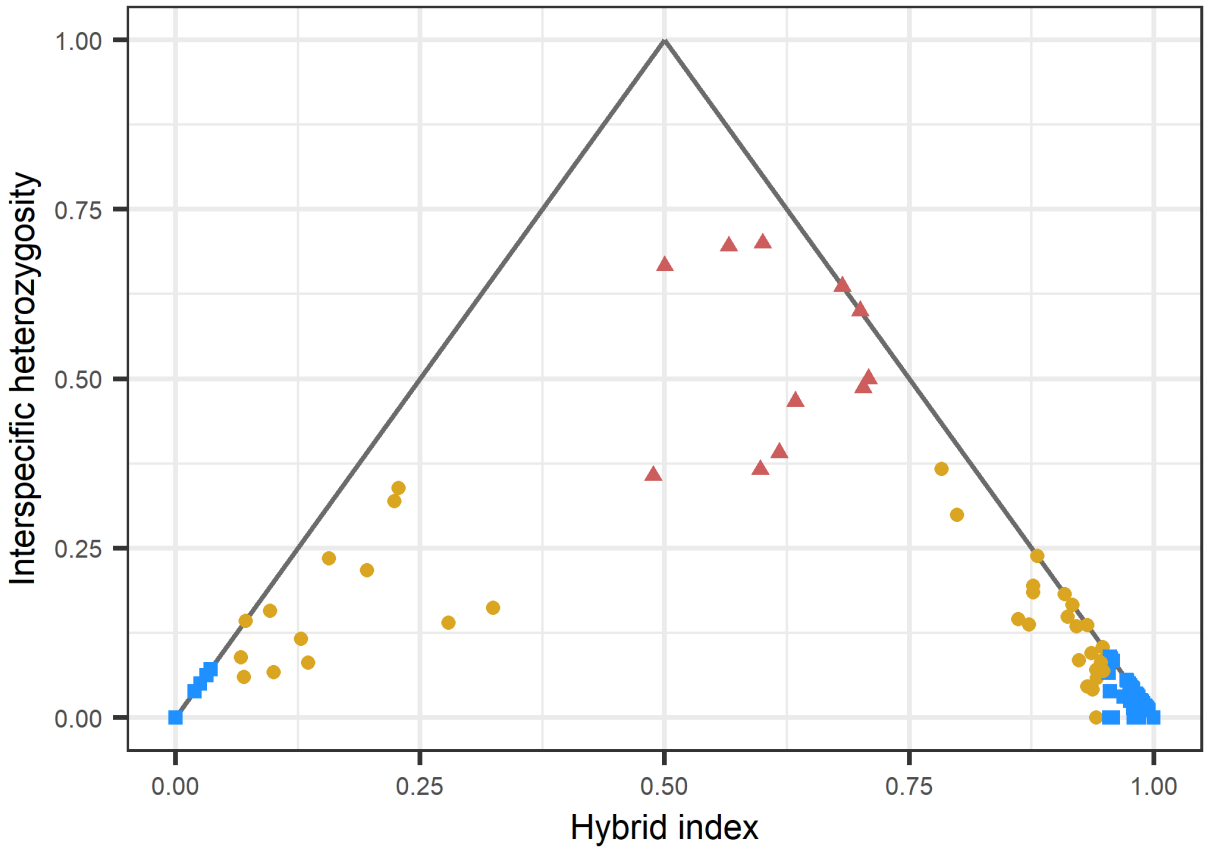

Figure 4.13. Hybrid index and interspecific heterozygosity results for all King and Clapper rail samples. Recent generation hybrids (F1/F2) are denoted by triangles, backcrossed individuals by circles, and pure individuals by squares.

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Appendix A

FRONT PAGE OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) APPLICATION

University of Delaware
Institutional Animal Care and Use Committee

Application to Use Animals in Research

(New and 3-Yr submission)

Title of Protocol: Research on avian populations in the mid-Atlantic	
AUP Number: 1157-2021-0	**← (4 digits only — if new, leave blank)**
Principal Investigator: W Gregory Shriver	
Common Name (Strain/Breed if Appropriate): Seaside Sparrow, Saltmarsh Sparrow, Nelson's Sparrow, Carolina Wren, Grey Catbird, Wood Thrush, Clapper Rail, and King Rail **Genus Species:** *Aimospiza maritimus, Aimospiza caudacutus, Aimospiza nelsoni, Thryothorus ludovicianus, Dumetella carolinensis, Hylochicla mustelina, Rallus crepitans*	
Date of Submission: 4/20/2021	

Official Use Only

IACUC Approval Signature: [illegible], DVM

Date of Approval: 7.1.2021

Revised: 05/2020

Page **1** of **22**

Appendix B

NEST FATE ASSIGNMENT DICHOTOMOUS KEY

Categorical fates (i.e. ultimate fates)
1 = Fledged
2 = Failed - flooded
3 = Failed - depredated
4 = Failed - unknown
5 = Unknown (if failed or fledged)
6 = Never had eggs

If there is evidence of even 1 chick hatching and successfully leaving the nest bowl, fate =Fledged, regardless of if the rest of the nest fails

Categorical fate key
1 - At final visit*, chicks seen (**fledged = 1**)
1' - At final visit, nest structure intact but eggs are cold/not attended for two consecutive visits (**failed - unknown = 4**).
1'' - Eggs were never present at previous checks (**never had eggs = 6**)
1''' - At final visit, no eggs present in nest bowl (2)
1'''' - At final visit, some eggs disturbed or missing (2)

2 - Eggs were pipping** (at any level) at previous visit (3).
2' - Eggs were within 4 days of hatching or float at (≥G) but no pipping on previous visit (4)
2'' - Eggs were not within 4 days of hatching based on known or estimated clutch completion data from float data (5)
2''' - No float data are available (**unknown = 5**)

3 - Indicators of hatch present and no signs of disturbance (**fledged = 1**)
- Pipping fragments present
- Food fragments at nest
- Small fecals in or directly around nest bowl
- Active eggs still present in the nest when the first egg went missing. If nest was still attended/developing after first egg went missing, assume that egg hatched.
- Eggshell "Tops or Bottoms" with detached membranes found nearby
- Injury display by parents
- See nest fate assignment SOP for additional details

3' - Signs of disturbance, such as damaged nest bowl/eggs, signs of flooding, or dead chicks (**unknown = 5**)

3’’ – No indications of hatch or nest disturbance (**unknown = 5**)

4 - Indicators of hatch present and no signs of disturbance (**fledged = 1**)

- Pipping fragments present
- Food fragments at nest
- Small fecals in or directly around nest bowl
- Active eggs still present in the nest when the first egg went missing. If nest was still attended/developing after first egg went missing, assume that egg hatched.
- Eggshell “Tops or Bottoms” with detached membranes found nearby
- See nest fate assignment SOP for additional details

4’ - Signs of disturbance, such as damaged nest bowl/eggs, signs of flooding, or dead chicks, or signs of abandonment such as cold eggs (**unknown = 5**)
4’’ – No indications of hatch or nest disturbance (**unknown = 5**)

5 - Clear signs of depredation (see nest fate assignment SOP for predator appendix; **failed - depredated = 3**)

- the nest is found with its structure pulled apart
- the nest is found with obvious depredation remains
- egg shells that remain are primarily large (>5mm) pieces (sometimes with attached membranes with blood or yolk) (2011-2014: this will be the most likely instance in which ‘depredation’ will be assigned)

5’ - Clear signs of flooding (see nest fate assignment SOP for flooding evidence criteria; **failed - flooded = 2**)

- the nest is observed underwater during a high tide and a subsequent nest check confirms that the nest is missing contents
- the nest is found with intact eggs outside the nest
- the nest is found with intact cold or dirty eggs in the nest, and eggs do not subsequently hatch
- the nest is found with intact dead chicks in, or close to, the nest
- the nest is found with barely-alive nestlings
- the nest is found to be empty and soaking wet immediately (next day) after a high tide, and was known to have been active immediately prior to the high tide

5’’ - Insufficient information to ascertain flooding or depredation (**failed - unknown = 4**).

* “Final visit” is generally the last visit to the nest, however, this also includes the first visit at which hatchlings were observed (regardless of any further nest checks that occur).
**For our purposes, pipping includes any cracks in the egg that are attributable to the eggs beginning to hatch. This includes small cracks, starring, and true pipping (i.e. hole in egg)