HABITAT SELECTION, PRESCRIBED BURN EFFECTS, AND PASSIVE MONITORING OF EASTERN BLACK RAILS (*Laterallus jamaicensis jamaicensis*) IN NORTH CAROLINA

by

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THESIS ABSTRACT

Eastern black rail (*Laterallus jamaicensis jamaicensis*) populations have experienced rapid declines from habitat loss exacerbated by sea level rise and human development. The suitable habitat for this species is broadly described as marsh containing dense, herbaceous vegetation at high elevations with little to no standing water (~ 0-5 cm). However, habitat characteristics, such as vegetation species composition and hydrology, vary by location where black rails are found. In North Carolina, birds are concentrated in only a few areas of coastal marsh that are subject to erosion and likely to be lost within the century. Additionally, this species' behavior and phenology is poorly understood. As the black rail faces threats of extinction, it has become increasingly vital to better understand the species' activity patterns and identify viable management strategies to facilitate marsh migration away from the shoreline and preserve existing populations. Prescribed fire may be a useful tool for maintaining patches at an early-successional state and promoting quality habitat, but its effectiveness has not yet been assessed for the mid-Atlantic coastal plain.

In Chapter 1, I identify and discuss characteristics of suitable habitat as they relate to burn management and the probability of black rail occupancy. I conducted callback surveys

during the breeding season at sites that were either burned in mid-winter or left unburned in 2022. I created occupancy models to identify predictors of suitable habitat. In separate analyses, I directly compared these variables among sites left unburned, within one year of burn, and one-year post-burn. These results were then integrated to assess the effects of prescribed fire management on black rail habitat and the effect of burn status on habitat use.

In Chapter 2, I uncover temporal patterns of vocal activity and propose a phenological timeline for black rails in coastal North Carolina. I used passive monitoring techniques to document aspects of phenology and behavior during the breeding season. During 2023, I deployed recording units and monitored water levels at Cedar Island NWR. Lunar illumination values were extracted for each hour that was recorded. Counts of verified detections (3-second segments of recordings of black rail vocalizations) were then incorporated into models that identified temporal predictors of 1) vocalization probability and 2) counts of call segments during active periods. Predictors of vocalization probability were assessed separately from estimated counts.

Over the 2-year period, I deployed motion-activated trail cameras at locations where black rails were detected to uncover evidence of phenology and breeding activity. I captured evidence of a presumed immature black rail after the completion of its juvenile molt. This 2022 observation is the first record of confirmed breeding by black rails in North Carolina in over 120 years. Results of this project provide insights into habitat preferences, vocalization patterns, and breeding phenology of Eastern black rails in North Carolina.

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CHAPTER 1: EFFECTS OF PRESCRIBED BURNS ON EASTERN BLACK RAIL OCCUPANCY AND HABITAT USE IN NORTH CAROLINA

Abstract

Marsh habitat in the mid-Atlantic is rapidly being lost. Consequently, the Eastern black rail is experiencing severe population declines and is now listed as federally Threatened. Where black rails are found, they are scarce and are notoriously difficult to detect. Suitable habitat for the species and appropriate management tools are in urgent need of assessment. Prescribed fire is used to maintain or restore habitat for black rails, but its effectiveness has not yet been verified. In coastal North Carolina during the breeding season, I conducted callback surveys, measured vegetation and water levels, and generated occupancy models that identified predictors of black rail presence. In separate analyses, I directly compared these variables by burn status of each site, then by occupancy to assess the relationship between black rail habitat use and prescribed fire management. Given that black rails are sensitive to inundation, I additionally monitored water levels throughout the breeding season to qualitatively assess habitat suitability within each study area.

Survey detection was negatively influenced by wind. Timing of burn as a covariate did not predict black rail occupancy, but there was an increase in detections within the fire-treated extent the second year, indicating that black rail recruitment increased one year post-burn. Occupancy was negatively affected by the presence of open water. When compared to untreated sites, prescribed fire reduced vegetation density within the year of burn. Results presented here will inform habitat management strategies by providing insights into the effects of prescribed fire, hydrology, and habitat characteristics on occupancy.

Introduction

Wetlands are degrading rapidly under stressors brought on by climate change and increased urbanization (Lee et al. 2006; Chen et al. 2024). More than 50% of wetlands have been lost in the United States since the European settlement, subjecting many obligate marsh species to severe habitat loss (Dahl 1990). Absent land management, accelerated sea level rise is expected to negatively affect the quality of existing habitats for marsh birds (Mitchell et al. 2006). Marsh birds are increasingly threatened by habitat loss, yet their detection remains challenging (Conway 2011). Many marsh bird species have adopted secretive strategies that make them difficult to observe (Eddleman et al. 1988). Consequently, conservation and management decisions for these species must account for both the loss of habitat and the inherent difficulties in detecting elusive species within their remaining environments (Steidl et al. 2013).

Perhaps the species of rail most impacted by the loss of wetlands is the black rail (*Laterallus jamaicensis*). The black rail is a rare and secretive marsh bird in dramatic decline across its range in North America (Watts 2016; Conway & Sulzman 2007). The species is considered globally Endangered (Birdlife International 2021) and was listed as one of 70 North American ‘tipping point’ species likely to face extinction in the next 50 years absent conservation action (North American Bird Conservation Initiative 2022).

Out of five recognized black rail subspecies, two occur in North America: the California black rail (*L. j. coturniculus*) and the Eastern black rail (*L. j. jamaicensis*). The California black rail, found primarily in California and Arizona wetlands west of the Rocky Mountains (Evens et al. 1991), has declined by 22.5% annually since 1973 (Conway & Sulzman 2007), and is currently listed among taxa in Category 1 candidates for federal

uplisting (U.S. Fish and Wildlife Service 1989). Within the past century, the Eastern black rail has suffered a range reduction greater than 90% where it occurs in the American mid-west region, parts of Colorado, and across the Atlantic and Gulf Coasts of North America (Eddleman et al. 1994; Watts 2016). Severe wetland degradation caused by human activity and sea level rise has exacerbated the decline of the eastern subspecies (Watts 2016; Stevens & Conway 2021), leading to its recent uplisting to federally Threatened (U.S. Fish and Wildlife Service 2020).

Black rails occupy high marsh habitats with dense, herbaceous vegetation cover and consistently low (~ 0-5 cm) water level (Eddleman et al. 1994). Populations have been found in freshwater, brackish, and salt marshes with moist to saturated soils (Flores & Eddleman 1995; Legare & Eddleman 2001). A higher proportion of marsh area is associated with higher occupancy in California, and both the Atlantic and Gulf coasts (Richmond et al. 2012; Roach & Barrett 2015; Tolliver et al. 2019). Preferred vegetation cover varies by location (Watts 2016). For example, multiple studies have reported a positive relationship between black rail occupancy and habitats with emergent grass dominance (Repking & Ohmart 1977; Eddleman et al. 1994; Legare & Eddleman 2001). Black rail habitat has also been associated with larger proportions of *Spartina spp*. in the Texas Gulf Coast (Tolliver et al. 2019), gulf cordgrass (*S. spartinae*) in coastal Louisiana (Johnson & Lehman 2021), and sawgrass (*Cladium jamaicense*) in the Albemarle-Pamlico Peninsula of North Carolina (Taillie & Moorman 2019) as well as the saltmarsh basins of eastern Florida (Legare & Eddleman 2001). High marsh often serves as a buffer between coastal waters and upland habitats, explaining associations with proximity to water (Tolliver et al. 2019; Neice & McRae 2021b) and to the forest line (Roach & Barrett 2015; Taillie & Moorman 2019). However, more

research is needed to determine how to appropriately manage for these habitat requirements while maximizing conservation effort for existing populations.

Black rails are notoriously difficult to detect, as birds may call irregularly and infrequently depending on site or survey conditions (Legare et al. 1999). Without sufficient monitoring effort of secretive species, there is an increased risk of not detecting present birds which may result in unsuitable management (Robinson et al. 2018). Callback surveys are the standard method for detecting secretive rails (Conway 2011). These involve broadcasting a standardized recording of species-specific calls into a given area and recording responses of the focal species (Repking & Ohmart 1977). Black rail callback responses are highest within crepuscular and nocturnal hours, so callback surveys are usually conducted during these times (Repking & Ohmart 1977; Spear et al. 1999; Wilson et al. 2016; Tolliver et al. 2019; Neice & McRae 2021a; Butler et al. 2023). The standardized protocol for marsh bird callback surveys recommends at least three visits per year, typically during the breeding season (Conway 2011). However, because of the difficulty in detecting black rails, some models predict up to fifteen surveys being necessary to obtain detection probabilities greater than 90% (Conway et al. 2004). While the appropriate survey effort may vary by location, conducting more than three surveys is recommended for marsh birds with low detection probability (Butler et al. 2023). However, it is important to balance survey effort with the potential disturbance to the birds caused by too many visits (Bibby et al. 1992).

Occupancy models estimate detection probability and the proportion of sites occupied by species within a given extent (MacKenzie et al. 2002). The model can additionally incorporate covariates that may explain variation in detection probability or occupancy state, such as habitat characteristics specific to the survey site or observational conditions of each survey conducted (Fiske & Chandler 2011). Implementing repeated measures with multiple

survey visits into the model accommodates the imperfect detection rate typically observed with elusive species (MacKenzie et al. 2006) This type of modelling is therefore useful for monitoring black rails and identifying habitat requirements that could guide future management plans.

Most inland marshes near the Atlantic Coast were extensively drained and altered by agricultural practices and human development (Eddleman et al. 1988). Concurrently, tidal marshes are actively experiencing a 'coastal squeeze' (Tono & Chmura 2013), which occurs when the marsh vegetation retreating from high water is eventually obstructed by man-made structures and steep elevational gradients (Doody 2004). Across the coastal regions between Maine and Texas, the projected 100-year impact of sea level rise is greatest in the upper to mid-Atlantic (Marsooli et al. 2019). As sea level has increased on the Atlantic Coast, black rails have retreated closer to the forest edge (Roach & Barrett 2015; Taillie & Moorman 2019).

In North Carolina, occupied habitat is in tidal marshes under threat of inundation from sea level rise and increasing storm surge (Johnston et al. 2021). In the survey studies conducted within the state recently, the numbers of black rail detections were limited (Taillie & Moorman 2019; Paxton & Hines 2024). A state-wide assessment found sparse black rail hotspots concentrated to ~ 25% of habitat, much of which is threatened by rising sea level (Stevens & Conway 2021). Management decisions in the state must therefore be enacted quickly to preserve remaining populations (Neice and McRae 2021b). Where black rails are found in eastern North Carolina, recommended management strategies include the enactment of buffer zones that can facilitate marsh migration and promote suitable habitat conditions (Watts 2022).

Prescribed fire is used by land managers to keep marsh habitat in an early successional state (Krebs et al. 2010). This method is also used to control woody encroachment and prevent invasive plants like common reed (*Phragmites australis*) from dominating the landscape (Nyman & Chabreck 1995; Kimura & Tsuyuzaki 2011). Furthermore, thinning of emergent vegetation removes thick overgrowth from previous years (Hackney 1981). Fire regimes have been shown to improve wetland quality as well as promote suitable habitat characteristics for other North American rails (Hackney & de la Cruz 1981; Conway et al. 2010). However, fire can pose danger to resident birds under certain conditions. Burn application during sensitive life stages (e.g. incubation, brood rearing, or flightless molt) may lead to mortality of black rails within the burned extent (Grace et al. 2005; Hand et al. 2021). In the Texas Gulf Coast, burns with patchy mosaics applied on rotational schemes have been more successful in minimizing black rail mortality compared to complete and frequent burns (Grace et al. 2005). Federal and state land managers use prescribed fire as their primary management strategy on historically occupied coastal black rail sites along the East Coast including in North Carolina (Freske 2006; Coastal Land Trust 2019). These burn treatments provide an opportunity to assess the effects of prescribed fire on black rail occupancy. In this study, I evaluated the effectiveness of prescribed fire for black rail management, as it has not yet been formally validated as an appropriate strategy for this species in the Atlantic coastal plain.

Prescribed burns are also predicted to have effects on the hydroperiod in wetland habitats. By reducing vegetation density and altering the structure of plant communities, burns can affect water infiltration rates, soil moisture retention, and surface runoff patterns (Moody & Martin 2001). The removal of leaf litter and organic matter can enhance soil permeability, leading to increased infiltration and reduced surface runoff, thereby potentially

decreasing the risk of flooding (Certini 2005). Over time, the cumulative effects of prescribed burns can contribute to lower water table depths (Clay et al. 2009; Holden et al. 2015).

My goals in this chapter were to 1) compare survey detections across sites, 2) compare habitat characteristics in burned and unburned plots, 3) determine which habitat characteristics are related to black rail occupancy, and 4) identify which survey conditions increase the probability of black rail detections. During the 2022 and 2023 breeding seasons, I conducted standardized marsh bird callback surveys for black rails at sites in two non-adjacent coastal counties in North Carolina, with the following treatments: burned within-year, one year post-burn, and unburned. I performed vegetation surveys to document vegetation structure and community composition at each site. Water levels were monitored using water pressure data loggers deployed continuously during the breeding seasons at representative sites for each treatment. I applied an occupancy model to understand which covariates have the greatest influence on occupancy as well as detectability. These findings will help inform later survey efforts for black rails in coastal environments in addition to refining prescribed burn management for black rail habitat.

Methods

Study areas

This study was conducted on properties adjacent to the Pamlico Sound, a large and shallow estuarine system in eastern North Carolina. Monitored sites experienced both semidiurnal and wind-driven tidal influences and featured a mixture of forest, impoundments, and tidal marsh habitat. Coastal marsh landscapes were dominated by stands of black needlerush (*Juncus roemerianus*), interspersed with patches of mixed grasses (*Distichlis spicata* and *Sporobolus pumilus*). Managed properties were overseen by either state or federal agencies.

Goose Creek Game Lands

Goose Creek Game Lands (GL) is a 7,308-acre (~ 2957-hectare) complex in Pamlico County, North Carolina, with disjunct units comprised of wetlands, pine forest, and impoundments. There is brackish marsh on all land tracts (Mast et al. 2015). This land was acquired by the state for habitat management in 1944 after the area was previously used for timber harvesting, tar production and local farming practices (Mast et al. 2015). Semidiurnal tide influences are present, but the primary driver of hydrology here is wind-driven circulation of water from the Pamlico Sound. Impoundments, a habitat type likely beneficial to black rails (Roach & Barrett 2015), make up approximately 17% of the current land extent. There are six impoundments in the complex: Pamlico Point, Campell's Creek, Hunting Creek, Spring Creek, Smith Creek, and Hobucken (Figure 1.1).

The complex has historically been managed by the North Carolina Wildlife Resources Commission (NCWRC) using prescribed burns on a 3- to 5-year rotational scheme (Mast et al. 2015). Several plots of land were burned in 2022, both before and after the first field

season. In 2023, a small wildfire occurred on the Hobucken impoundment (Figure 1.1) in early May. This wildfire was followed by a prescribed burn of pine-dominated forest and surrounding marsh in early August. Here, 4 survey points were selected that were burned in 2022, 15 points where no burns occurred during the study, and 2 points burned only in 2023.

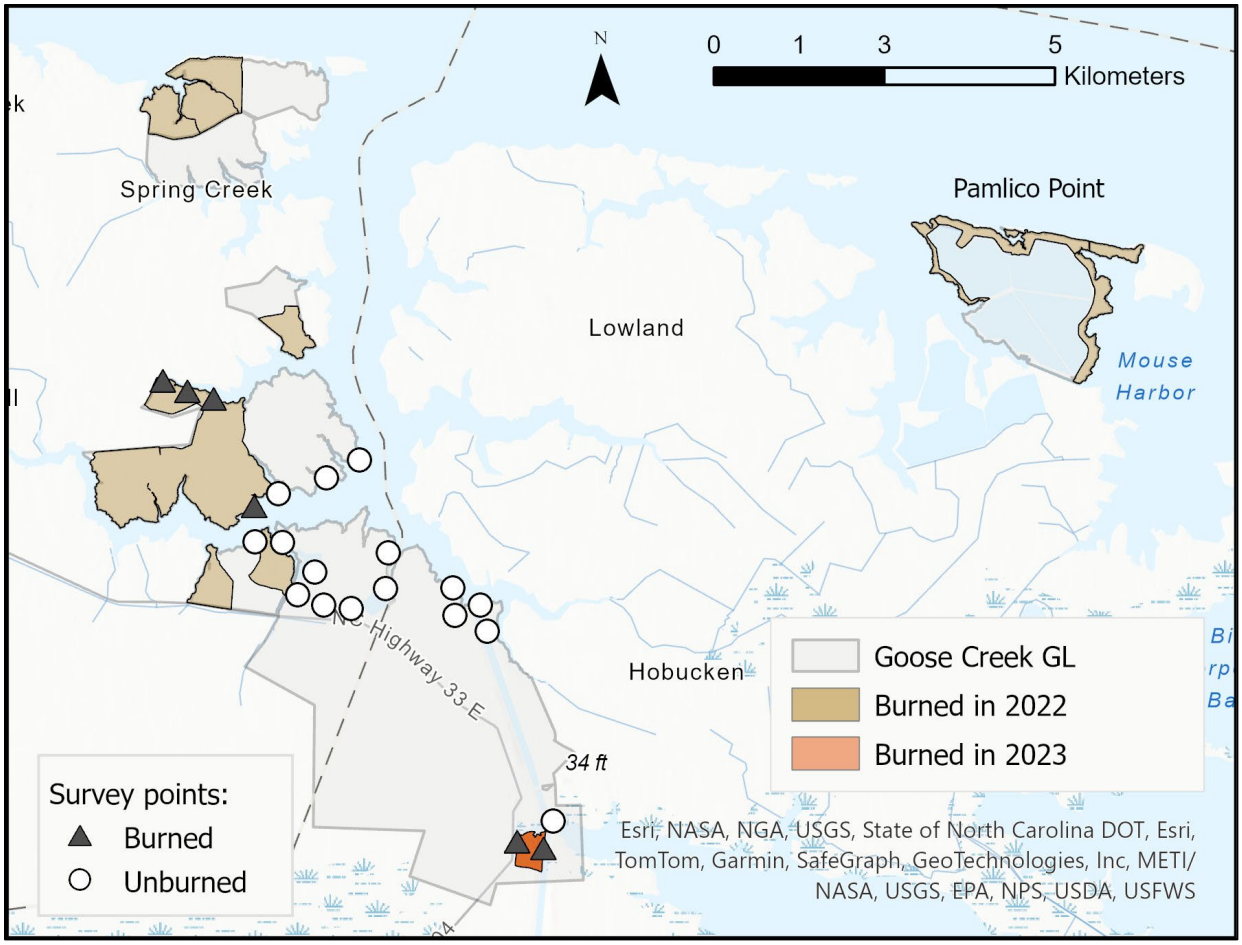

Figure 1.1 Goose Creek Game Lands in Pamlico County, NC (shaded areas), with callback survey point positions indicated and the timing of burn of specified parcels indicated by color. Only areas shaded in light brown were burned prior to the 2022 breeding season. The small area in orange indicates the extent of a wildfire that occurred mid-May 2023.

Salter's Creek Game Lands

Salter's Creek GL (also known as Carteret County GL) consists of 6,000 acres (2,428 hectares) acquired by the North Carolina Coastal Land Trust in 2019. Most of the property (~ 5,170 acres/2,092 hectares) was transferred to NCWRC for habitat management in the same year (Coastal Land Trust 2019). State-managed land includes 1,600 acres (~ 647 hectares) of estuarine marsh and approximately 17 miles (~ 27 km) of shoreline on the sound. Historically, black rail detections have been reported north and south of the land extent (Wilson et al. 2016; Neice & McRae 2021b). Salter's Creek GL is adjacent to a historical source population for black rails in the state (Cedar Island NWR) and is therefore likely to be colonized if managed appropriately (Watts 2016; Figure 1.2).

The first prescribed burns for this property occurred in March 2022. In March 2023, only a small (~ 30-hectare) portion was burned after a controlled fire failed to catch properly and the application could not be completed across the planned extent. Selected survey points in this area were distributed across 12 unburned sites, 11 sites burned in 2022, and 1 site burned in 2023.

Cedar Island National Wildlife Refuge

Cedar Island NWR has approximately 14,500 acres (~ 5,868 hectares) of salt marsh. The U.S. Fish and Wildlife Service first purchased the land for conservation management in 1964. The current acreage was acquired by 1992 (Freske 2006). Once host to the largest number of black rails in the state, an estimated 80 or more breeding pairs were reported here in the 1970s (Watts 2016).

A wildfire covered ~ 1,400 acres (~ 567 hectares) in the northeastern part of the refuge in June 2021 (Kendall Smith, refuge manager, pers. comm.), resulting in a patchy mosaic of black needlerush (*Juncus roemerianus*) and mixed salt grasses. The land managers conducted a prescribed burn on the same side in March 2022. However, the fire did not catch well where the wildfire extent overlapped (Figure 1.2). No further prescribed burns were completed at the refuge until March 2024, after the study period concluded. At Cedar Island NWR, I selected 17 survey points in unburned sites and 13 in sites burned in March 2022.

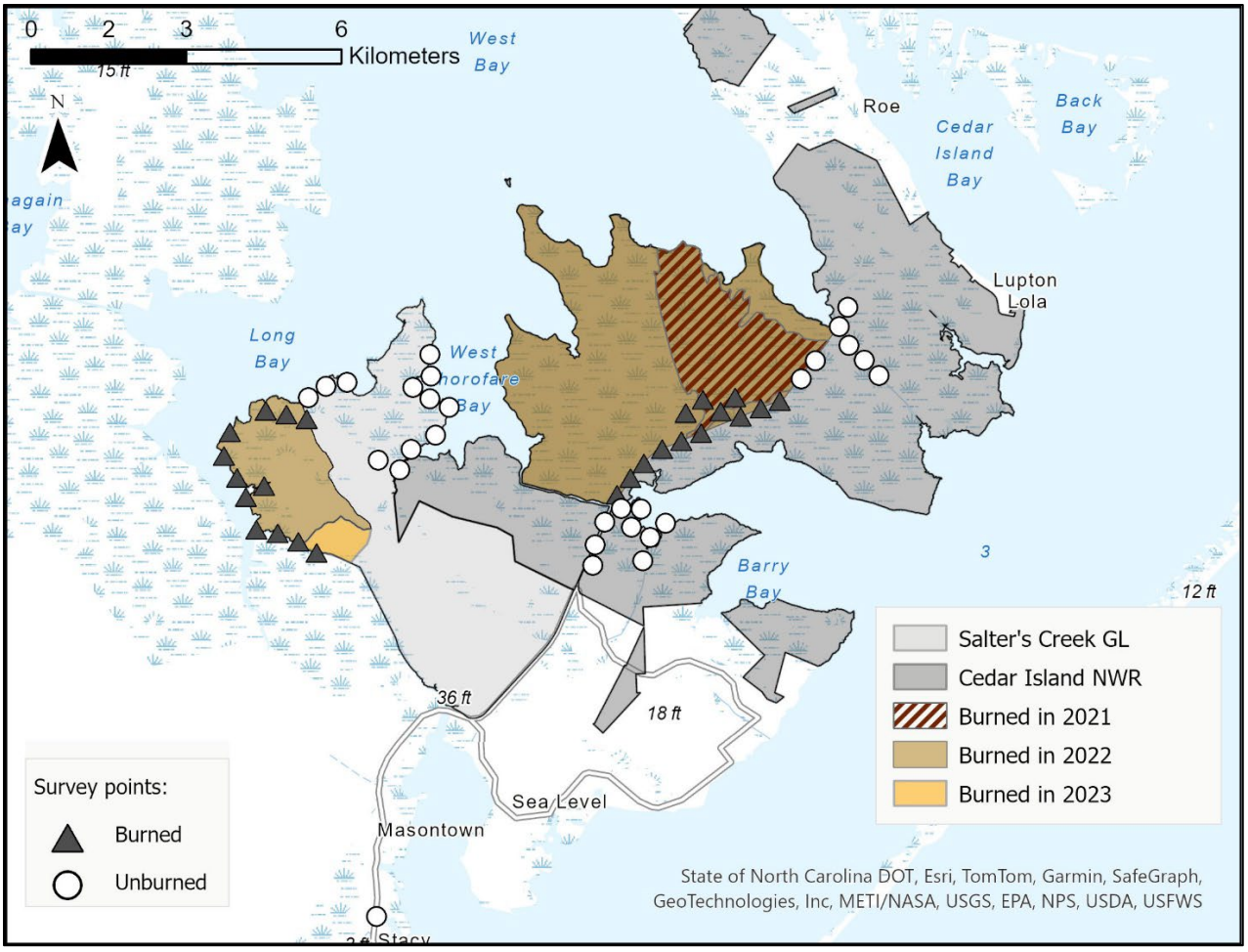

Figure 1.2 Study area of Salter's Creek Game Lands and Cedar Island National Wildlife Refuge located in Carteret County, NC, with each callback survey point position indicated. The unmanaged portion is indicated in grey while the boundary of the first prescribed burn (conducted in March 2022) is in light brown. The dark brown, crosshatched patch represents the extent of a wildfire that occurred in 2021.

North River site

A small parcel of saltmarsh along the North River near Beaufort, NC, of approximately 40 acres (~ 16 hectares) was historically occupied by black rails despite no land management in recent years (Neice & McRae 2021a). The habitat consists of high marsh vegetation with a pine forest edge. Due to previous detections, this area (referred to as 'North River site') was monitored for comparison to occupied sites with active management. Two survey points categorized as 'unburned' were selected here.

Standardized callback surveys

Callback surveys involve broadcasting species-specific calls intended to elicit a response from focal species, if present. In this study, I conducted callback surveys based on the South Carolina Department of Natural Resources (SCDNR) survey protocol for black rails, which was derived from the Conway (2011) Standardized Marsh Bird Survey Protocol. I completed a series of callback surveys at 4 to 9 points daily from early May to late August, omitting days with unsuitable survey conditions (high winds, thunderstorms, heavy rain, or thick fog). All surveys were conducted in the morning (between 30 minutes before and 3 hours after sunrise) or evening (2 hours before and 1 hour after sunset). Each point had at least one visit in both the morning and evening survey windows. Each survey point was visited at least 3 times in 2022 and 5 times in 2023. There was a minimum of 10 days between visits to minimize disturbance (Conway 2011) and to ensure that surveys are conducted at different times in the breeding cycle. The number of visits was increased in 2023 to increase detection probability (Spear et al. 1999; Butler et al. 2023).

Each survey consisted of a 10-minute recording containing a sequence of black rail vocalizations and passive listening periods. This was broadcast between 90 -100 dB from selected points with a FoxPro game caller (Table 1.1). Surveyors were either on foot in the marsh, or in a kayak with the broadcast directed toward the marsh. All detections (auditory or visual) of black rails within each minute of the broadcast period were recorded. If the response was auditory, the type of call elicited was also noted. Rails commonly respond to calls of confamilial species (Schroeder & McRae 2019), so I documented any responses by Virginia rails (*Rallus limicola*) and clapper rails (*Rallus crepitans*). During the surveys, I recorded visual detections of least bitterns (*Ixobrychus exilis*) that are less apt to respond vocally. These additional species were documented to determine if the presence or absence of co-occurring wading birds affected black rail occupancy or detection probability. Survey conditions such as time of day, cloud cover, wind speed, temperature, moon phase, tide level, and noise interference were also documented, as well as number of surveyors present (Table 1.2).

In the mid-Atlantic region, black rails are confined to a limited number of suitable areas, while many other habitats that appear suitable remain unoccupied (Roach & Barrett 2015; Stevens & Conway 2021; Paxton & Hines 2024). To locate habitats with high black rail abundance (i.e., ‘hotspots’), survey points were selected based on visual determination of suitable habitat from previous studies (Flores & Eddleman 1995; Neice & McRae 2021a). In this environment, it is estimated that call playback at 100 decibels cannot be heard beyond about 200 m. Therefore, to avoid multiple detections of the same bird, survey points were spaced at a minimum distance of 400 meters apart, as per the recommendations of the standardized marsh bird callback protocol (Conway 2011).

Table 1.1 Broadcast sequence used in callback surveys. The call playback tape was developed and provided by the SCDNR (2022).

Minute	Playback sound
1	Silence (passive listening)
2	Silence (passive listening)
3	*Kick-kee-doo* calls
4	*Kick-kee-doo* and *churt* vocalizations
5	*Kick-kee-doo* and *grr* vocalizations
6	Silence (passive listening)
7	Silence (passive listening)
8	*Kick-kee-doo*, *grr* and *churt* vocalizations
9	Silence (passive listening)
10	Silence (passive listening)

Table 1.2 Survey-specific measurements along with their descriptions and corresponding units or scales. These were incorporated into occupancy models to fit probability of detection.

Measurement	Description
Time	The start time of the survey (24-hour time format).
Cloud cover	Cloud cover scale (0-5): 0 = clear skies, 1 = sunny with a few clouds, 2 = partly cloudy, 3 = cloudy, 4 = overcast, and 5 = overcast with rain during at least 30 seconds of the survey.
Wind	The wind speed at the beginning of each survey (in mph, measured with a Kestrel 2500).
Temperature	Recorded at the start of each survey, (in ºC, The Weather Channel).
Lunar illumination	Amount (in lux) of moonlight on the ground relative to site location and elevation (Śmielak 2023). Extracted with 'moonlit' R package (version 0.1.0).
Tide level	The tide level (in meters) at the start of each survey (data obtained from the nearest NOAA buoy station).
Noise	Background noise on a scale of 0-4, where 0 = no noise, 1 = faint noise, 2 = moderate noise (probably cannot hear black rails beyond 100 m for > 30 seconds), 3 = loud noise (probably cannot hear black rails beyond 50 m for > 30 seconds), 4 = intense noise (probably cannot hear black rails beyond 25 m for > 30 seconds).
Surveyors	The number of trained personnel participating in a survey (1-3 individuals).

Coarse-scale elevation

To determine if elevation affected occupancy probability, I measured reference elevation across the sites surveyed using the Real-Time Kinematic (RTK) method with an Emlid Reach RS2 Global Navigation Satellite System (GNSS) receiver. A standard GNSS receiver measures distance and elevation by calculating the time it takes for a signal to pass between a satellite and the receiver; however, these measurements can be less accurate when weather conditions change, such as cloud cover (Schaefer & Pearson 2021). The RTK method accounts for this by incorporating a stationary device (i.e. base station) that sends corrections to the receiver while ellipsoidal height data is collected. This allows for centimeter-level accuracy in each measurement (Emlid 2023). Although measurements can vary by position within the landscape, relatively little above-water topographic relief is found in marsh habitat (Neice & McRae 2021b).

I measured elevation at the center points of all coarse-scale vegetation surveys conducted in 2023. When measuring elevation, the receiver was placed on a pole that was precisely 1.8 m above ground level. The base station was set to stand at ~ 4 m above ground level within detectable range (~ 2 miles) of the receiver. Elevation was measured by calculated ellipsoidal height based on the GEOID18 geoid model and the NAVD 88 vertical control datum as defined by the National Oceanic and Atmospheric Administration (NOAA). All measurements were collected in July 2023, near the end of the period of field data collection.

Vegetation sampling

Vegetation sampling was conducted at select survey and detection sites to evaluate both coarse-scale and fine-scale aspects of habitat. Representative sites were chosen to balance the number of points by management category (burned and unburned) where possible. Coarse-scale measurements involved visually estimating the percentage cover of different vegetation species present. Fine-scale measurements focused on structural characteristics of the vegetation, indicative of density. These dual protocols were designed to capture variations in species composition and vegetation structure, providing a comprehensive understanding of the habitat.

Coarse-scale measurements

Coarse-scale vegetation sampling based on the Florida Fish and Wildlife Commission (FWC) Coarse Vegetation protocol (2022) was conducted at all game land sites and at sites where black rails were detected. At each point, a 50-meter radius circle was designated around the survey center point and then divided into four 90° quarters. Within each quarter, I visually estimated the percent coverage of each land cover category so that all categories added up to 100% (Table 1.3). Call broadcasts were unidirectional. Therefore, the center point of the associated vegetation survey was set to 9 meters in the direction of the broadcast, either perpendicular to the habitat edge (e.g. if the survey was from a kayak), or 9 meters northward if the point was inland. This was to ensure that the edge vegetation considered irrelevant to black rail habitat did not bias samples on points near the marsh edge. Only cover categories that made up at least 5% of each quarter were recorded. All vegetation surveys were completed near the end of the growing season from July 1 - August 9 during both years.

Fine-scale measurements

In addition to coarse-scale samples, I conducted fine-scale vegetation sampling based on the 2021 NOAA Firebird Vegetation Sampling Protocol. This protocol includes measurements of visual obstruction height and disc settling height, where higher values are indicative of higher vegetation density (Robel et al. 1970). At each point surveyed, I measured the average visual obstruction height and disc settling height across five 50x50 subsample quadrats. Subsamples were chosen based on standardized positions from the survey center point: 4 plots were sampled 10 m out in each cardinal direction, and the fifth plot 5 m to the northwest. The survey point center was established 18 meters inland on edge points and 18 meters northward on inland points to minimize the bias of edge vegetation. I measured visual obstruction height within each subsample by placing a Robel pole marked with 10-cm increments in the center of the 50x50 quadrat, then having an observer step 4 meters to the east and report the height at which the pole was just visible. To measure disc settling height, I placed a 1” PVC pipe in the quadrat center and a perforated disc 30 cm in diameter and exactly 1 kg in weight over the pipe. The disc was dropped from precisely 1.5 m off the ground, and the height at which the disc naturally settled on the vegetation was recorded to the nearest centimeter.

Table 1.3 Cover types measured in coarse vegetation sampling. The percent coverage of each land cover type was estimated in each plot.

Cover type	Description
Mixed grasses (*Distichlis spicata* and *Sporobolus pumilus)*	*Distichlis spicata* and *Sporobolus pumilus* (also known as *Spartina patens*) are fine, salt-tolerant grasses. These were largely intermixed, exhibiting similar levels of dominance in patches where present.
Black needlerush (*Juncus roemerianus*)	Patches of black needlerush, a salt-tolerant rush, often co-occurred with patches of mixed salt grasses. This rush often dominates salt and brackish marsh in coastal North Carolina.
Forest cover	Forest dominated by pines *Pinus spp*. that intersected the 50-m radius of the land cover sampling area, representing the upland edge of the marsh habitat.
Water cover	Open water that intersected the 50-m radius of the vegetation sampling area, representing the shoreline edge of fringe marsh.
Marsh elder (*Iva frutescens*)	Perennial, saline-tolerant shrub known to grow in high marsh.
Common reed (*Phragmites australis*)	A tall, perennial grass species that grows along the edge of water bodies. Clonal and invasive, it has spread rapidly across North American wetlands.
Low marsh cordgrasses (*Spartina alterniflora* and *Sporobolus cynosuriodes*)	Smooth cordgrass (*Spartina alterniflora*) and big cordgrass (*Sporobolus cynosuriodes*) are tall, perennial grass species with rigid, hollow stems. Native to the Atlantic and Gulf Coasts. These thrive in the lower zones of tidal marsh, particularly in areas with brackish water.
Common cattail (*Typha latifolia*)	A tall, perennial plant native to North America often found in aquatic habitats and low marshes. Can form dense patches that outcompete native plants where present.

Schoenoplectus, Bolboschoenus, and *Cladium* spp.	*Schoenoplectus cynosuriodes, Bolboschoenus maritimus,* and *Cladium mariscus* are all perennial sedge species found in brackish and saltwater wetland habitats along the Atlantic and Gulf Coasts. These were grouped into one cover type based on similarity in structure and co-occurrence.
Switchgrass (*Panicum virgatum*)	A grass species primarily found in prairies, woodlands, along roadsides, and in a variety of wetland environments. Characteristic of elevated areas where water drainage is better.
Sea oxeye daisy (*Borrichia frutescens*)	A perennial shrub species that forms dense, rounded mound of foliage and flowers. Native to the Atlantic and Gulf Coasts.
Dead/bare ground	Patch of dead vegetation or unvegetated mudflat.

Hydrological monitoring

I deployed HOBO U20L water data loggers (pressure sensors; MicroDAQ, LLC) in select sites to better understand how hydrological patterns affect black rail habitat use and how these patterns could be influenced by prescribed burns. Loggers were programmed to take atmospheric pressure measurements (atm) every 30 minutes. Each logger was tethered within a mounted 1-2 m length of 1.75" PVC pipe with holes drilled every ~ 10 cm to prevent water buildup. The logger was anchored with a piece of paracord such that the device was suspended at ground level within the PVC tube. I measured the height of each PVC tube from the ground and manually recorded a water level reference for all deployments every ~ 2-3 weeks. This was later used to correct logger-recorded water levels. I inserted a tent peg through the perforations in the PVC just above the suspended logger to ensure each device was sufficiently stable (i.e. not rising with the tide).

In 2022, I deployed 3 HOBO U20L water data loggers between May 10 - July 28 in Salter's Creek GL and 2 loggers between June 1 - July 27 at Goose Creek GL to record water level variation during the breeding season in areas representative of recently burned and unburned sites. Three were deployed at sites burned within the year and 2 at unburned sites. On July 5th, a Salter's Creek GL logger was transferred from a recently burned site to an occupied, unburned site to obtain hydrologic data in areas used by black rails. I checked each logger at least every 2 weeks to record a reference water level and to download the data using a portable HOBO Optic USB Base-U-4 Station (MicroDAQ, LLC), for storage on a portable hard drive.

Because I had access to more loggers, I was able to monitor hydrology at each of 4 main study sites during the second field season. In 2023, I deployed 4 loggers at Salter's Creek GL between April 9 - August 25, 3 at Goose Creek GL between April 16 - August 1, 2

at Cedar Island NWR between April 9 - August 15, and 1 logger at the North River site between May 3 - August 10. Seven of these sites were occupied and 3 had no detections. In terms of management, there were 5 unburned, 4 one year post-burn, and 1 burned site monitored. Each year, an additional logger was deployed above ground (suspended from a tree) at central locations in Salters Creek GL, Goose Creek GL, and North River to adjust for local barometric pressure. Cedar Island NWR is close to Salters Creek GL and was adjusted according to the control logger deployed there.

Statistical analyses

Habitat variables related to black rail occupancy and burn treatment

To determine how habitat use relates to burn management, I tabulated sites where vegetation was sampled by occupancy, then by burn. All sites with at least one black rail detection were considered occupied. Sites in the 'burned' category were burned between February – April 2022, and those that had not undergone prescribed fire in ≥ 2 years were considered 'unburned'. This criterion for the 'unburned' category was used so that study areas with different burn schedules could be compared.

Mixed grass, marsh elder (*I. frutescens*), common reed (*P. australis*), black needlerush (*J. roemerianus*), forest, and open water cover were expected to differ by burn status and occupancy and were prioritized over the other cover types for analysis. I standardized vegetation species cover estimates (%) with an arcsine square root transformation and averaged the measurements per site of visual obstruction and mean disc settling height (cm). I then checked each habitat variable for normality with a Shapiro-Wilk test.

I performed paired nonparametric (Mann-Whitney U) tests to compare percentage cover type estimates, mean disc settling, and mean visual obstruction between burned and

unburned sites as well as occupied and unoccupied sites. The Mann-Whitney test was chosen due to its robustness in handling non-normal data distributions and its suitability for comparing two independent groups with relatively small sample sizes (Corder & Foreman 2009).

Occupancy modelling

To estimate occupancy and detection probability across sites from surveys conducted in 2022 and 2023, I used the package 'unmarked' (v. 1.4.1) in the R statistical software package (version 4.4.0) to fit a single-season, single-species occupancy model to the data from callback surveys and vegetation samples (MacKenzie 2002). Occupancy (ψ) refers to the probability that a species is present at a site, whereas detection (p) reflects the probability that the species will be detected at a site (i) during a survey (t), given the species is present (MacKenzie et al. 2002). The models for occupancy and detection probability can be expressed with the following equations:

$$\psi_i = \text{logit}^{-1}(X_i\beta)$$

$$p_{it} = \text{logit}^{-1}(W_{it}\alpha)$$

Covariates corresponding to each site, hereafter 'site covariates', are represented as (X) with an effect size of (β). Covariates specific to each survey, hereafter 'survey covariates', are represented as (W) with an effect size of (α). Covariates considered as survey covariates included wind (mph), semidiurnal tide (m), lunar illumination (lx), start time and day of survey, cloud cover, and noise level (Table 1.2). Site covariates included point-level elevation (m) as well as estimated % cover of mixed grasses, open water, forest, marsh elder (*Iva frutescens*), smooth cordgrass (*Spartina alterniflora*), and common reed (*Phragmites australis).*

An occupancy model accounts for imperfect detection by incorporating detection probability into the estimation process, treating each 0 as a 'non-detection' rather than absence, thereby adjusting for the likelihood that the species may be present but not observed during surveys (Fiske & Chandler 2011). Assumptions are that 1) occupancy does not change during the sampling period or occupancy variation is specified, 2) surveys at each site are independent or variation is specified, and 3) there were no false-positive detections. To satisfy the first assumption, callback surveys were conducted during the breeding season when black rails stay close to their nesting sites (Tsao et al. 2009). To account for among-year variation, ‘year’ was incorporated as a fixed covariate in all fit models. This approach better captures variability in space and time within a short-term study without the risk of over-parameterization that comes with using dynamic occupancy models designed for longer sampling periods (Linden & Roloff 2013; Fuller et al. 2016). Survey points were at least 400 m apart to ensure that all survey detections were independent. To prevent false-positive detections, surveyors received comprehensive training on recognizing all potential black rail call types.

Detections were formatted as a binary variable, where a “1” indicated a black rail detection during a survey visit and a “0” indicated no detection. The detection history for each site was recorded in a dataframe where each row represented a survey point in a given year and each column represented a visit. There were up to 5 visits per point in 2023, but only 3 visits per point in 2022. For site-year combinations with fewer than 5 visits, the detection history matrix included missing values that represent the absence of survey effort rather than a non-detection. Missing values did not contribute to the likelihood calculation for detection, and detection probability was only applied to actual survey visits (Fiske & Chandler 2011).

Each model covariate was standardized with a z-score transformation using the scale function in R (version 4.4.2). To generate a global model, covariates were modeled separately for occupancy and detection. Each single-covariate model was evaluated using Akaike's Information Criterion (AICc), which adjusts for models with low sample sizes and multiple parameters by penalizing model complexity more heavily as the sample size decreases, thereby reducing the risk of overfitting (Burnham et al. 2011). Covariates were retained in the global model if they performed better than the null occupancy model.

I used the 'dredge' function in the R package 'MuMIn' (version 1.48.4) to generate all combinations of models with retained covariates estimating (p) and (ψ), then ranked them by AICc value using R package 'AICcmodavg' (version 2.3-3). To account for variation in the two years of surveys, 'year' was incorporated into all models during the dredge exercise. Effect sizes from top models with ΔAICc values <2 were averaged using the model averaging function ('modavg') in 'AICcmodavg' due to top model weights <0.1 (Symonds & Moussalli 2011). I used a Mackenzie-Bailey goodness-of-fit test to assess the fit of the model with the fewest parameters and lowest AICc as well as the 'global model' that included all site and observation covariates selected (MacKenzie & Bailey 2004).

Results

Only 3 existing sites were burned during 2023, the second year of study. Two sites were within the extent of a wildfire that burned ~ 0.06 km^2 at Goose Creek GL in June 2023. In March, a prescribed burn at Salter's Creek GL covered the area surrounding one site. Though this site was completely burned, the treatment was limited to ~ 0.02 km^2 due to poor weather conditions. Unless otherwise specified, sites in the 'burned' category represent habitats that were subject to fire treatment between February - April of 2022 (that is, "burned" = burned within-year if sampled in 2022, one year post-burn if sampled in 2023).

Black rail detections: spatial and temporal distribution

A total of 675 surveys were completed during the study period: 255 from May 15 - August 18 during 2022 and 420 from May 4 - August 25 during 2023. I performed callback surveys at 32 burned and 53 unburned points in 2022. In 2023, surveys included 39 burned points (3 burned within-year, 36 one year post-burn), and 47 unburned points. The mean time (± SD) between surveys was 27 ±13 days (range = 11 - 66) in 2022 and 20 ±10 days (range = 11 - 57) in 2023. Due to the increase in visits during the second year, more surveys were conducted in the mornings in 2023 than in 2022.

Overall, there were 26 individual survey detections of at least one black rail at 19 different points. Nine opportunistic detections occurred outside of traditional callback surveys, bringing the total number of in-person detections to 35. At least one black rail was detected at a total of 24 points. In 2023, new detections occurred at two points, while only one point with a detection in 2022 had no detections in 2023. In each year, black rails were detected during surveys from May through July, but not in the last 3-4 weeks of the season during August.

Black rails were rarely detected within 50 meters of the waterway. Out of 35 opportunistic and survey detections, only 3 occurred when surveying from the water's edge from a kayak.

Characteristics of occupied habitat

To meet assumptions of the model, we could include only detections made during callback surveys in occupancy modeling. Black rails were detected during at least one callback survey at 5 unburned points, 5 points burned within-year, and 6 points one-year post-burn. Nine of the points with vegetation data had at least one black rail detection and 43 points had no detections.

Prescribed fire management

There was some evidence of black rails using habitat one year post-burn. Within Salter's Creek GL, I detected only one black rail on a unit burned within year in 2022, but this increased to 4 black rails detected in units one year post-burn in 2023. Taking account of all sites in 2023, 8 (23%) of 35 points surveyed in areas one year post-burn had at least one detection, compared to 5 (16%) out of 32 points surveyed in the burned area in 2022 (Fisher's exact test $p = 0.54$). In unburned areas, the proportion of points with at least one detection was consistent between years at ~ 10% (5 of 52 points surveyed in 2022, and 5 of 47 points in 2023).

Coarse-scale land cover

In 2022, occupied sites had significantly more mixed grass cover ($U = 333.5$, $p = 0.035$), less open water ($U = 27$, $p = <0.001$), and less low marsh cordgrasses ($U = 119.5$, $p = 0.009$; Figure 1.3). In 2023, occupied sites had significantly more *J. roemerianus* ($U = 308.5$, $p = 0.045$), less *P. australis* ($U = 138$, $p = 0.015$), less low marsh cordgrasses ($U = 138$, $p = 0.015$), and less open water ($U = 67$, $p <0.001$). In 2023, there was also a trend of

more mixed grass cover at occupied sites, but this difference was not significant (U = 297.5, p = 0.078; Appendix Table A1).

Vegetation structure

There was no significant difference in visual obstruction between occupied and unoccupied sites (Mann-Whitney U = 163, p = 0.195). There was a trend toward lower disc settling heights in occupied sites, although not significant at the 95% confidence level (Mann-Whitney U = 145, p = 0.088; Appendix Table A2).

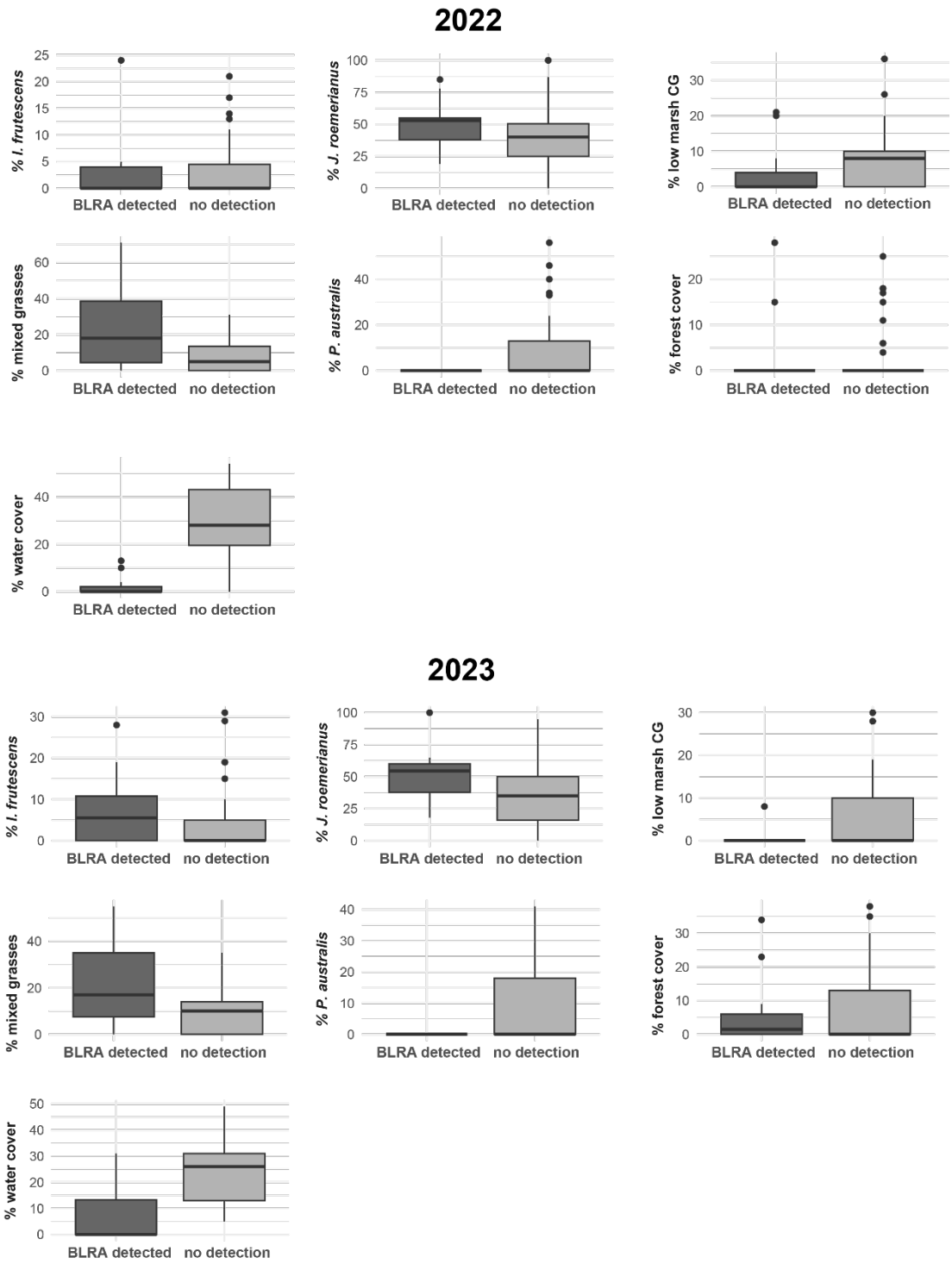

Figure 1.3 Land cover percentages in relation to black rail occupancy status for callback survey points. Eleven occupied sites were sampled for land cover in 2022 and 12 in 2023. Sites without detections were assumed to be unoccupied (43 in 2022 and 37 in 2023). Box plots indicate the 25th and 75th percentiles with the median indicated by a bar, whiskers spanning 1.5x the interquartile range, and outliers as points. There was significantly less open water cover in occupied sites in both years. In 2022, occupied sites had more mixed grasses (*D. spicata* and *S. patens mix*) than unoccupied sites.

Occupancy model results

To meet assumptions of the model, we could include only points with detections made during callback surveys and corresponding habitat measurements in occupancy modeling. Survey date, survey time, lunar illumination, cloud cover, and noise as observation covariates and low marsh cordgrasses, marsh elder (*I. frutescens*), black needlerush (*J. roemerianus*), forest cover, years since burn, and point-level elevation as site covariates did not meet the criteria for the global model (Table 1.4-1.5). The global model included tide and wind as detection (p) covariates and mixed grasses, open water, and common reed (*P. australis*) as occupancy (ψ) covariates (Table 1.7). 'Year' was also included as a fixed effect on occupancy (ψ) to account for potential variability between years. A MacKenzie-Bailey goodness-of-fit test indicated that this model fit the data adequately with little overdispersion (χ^2 = 42.165, p = 0.209, $\hat{C}$= 1.17).

The global model was then 'dredged' for all model combinations using the package 'MuMin', with 'year' forced as a factor in each. This generated 32 candidate models, where 3 had Δ AICc values ≤ 2 from the top-ranked model (Table 1.7). After averaging the top 3 models, the effect sizes for tide, wind, open water, and *P. australis* cover remained consistent in the full-averaged model (Table 1.8). With mixed grass cover, the effect size was lower in the full averaged model compared to the conditional average (β = 0.44, SE = 0.586, z = 0.270, p = 0.787; Table 1.8). This suggests that including all model terms in the full averaged model dilutes the specific impact of mixed grass cover on occupancy. Year was included as a site covariate in all averaged models because most survey points were visited in both years, but there was no significant effect of year (β = 0.27, SE = 0.525, z = 0.516, p = 0.606).

Black rail detection was negatively affected by wind speed (β = -0.70, SE = 0.307, z = 2.289, p = 0.022; Figure 1.4). One unit increase in wind resulted in a decrease in the log-odds of detection probability by 0.702. The positive effect size estimate for tide level was not significant in the full-averaged model (β = 0.24, SE = 0.267 z = 0.909, p = 0.363).

Black rail occupancy was negatively affected by open water cover (β = -1.74, SE = 0.609, z = 2.864, p = 0.004; Figure 1.5). One unit decrease in open water cover decreased the log-odds of occupancy by 1.74. Additionally, the percent cover of *P. australis* showed a negative trend with black rail occupancy, decreasing the log-odds of occupancy by 0.271 per unit increase. However, the full average model estimate for *P. australis* had a high standard error, likely resulting from non-normal data distribution and a high frequency of zeros, and was not significant (β = -9.94, SE = 277.79, z = 0.036, p = 0.971).

In the full-averaged global model, covariates that retained significant influence were wind speed (mph) on detection and % open water on occupancy (Figure 1.6). Under average survey (0.60 m tide level, 3.17 mph wind speed) and site conditions (6% *P. australis,* 14% mixed grasses, and 16% open water cover), mean (± SE) species occupancy probability % was 0.27 (± 12)% and detection probability was 16.6 (± 7)%. Across study sites, occupancy and detection averaged to 27 (±7)% and 36 (± 6)%, respectively. Occupancy estimates varied by location. The highest occupancy was observed at North River (ψ= 85 ± 7%), followed by Cedar Island (ψ= 29 ± 2%) and Salter's Creek GL (ψ= 29 ± 2%), then the lowest at Goose Creek GL (ψ= 7 ± 2%).

Table 1.4 Models for selecting **survey-specific *(p)*** covariates in the global model. These covariates were date (days since the beginning of the survey season), wind (speed in mph), tide (level in meters as recorded by the nearest NOAA data buoy station), lunar illumination (relative moonlight at the ground level in lux), time (survey start time), cloud cover (on a 3-point scale), and noise level (on a 3-point scale). Every model contained 'year' as a fixed effect (ψ). Covariates were incorporated into the global model if ΔAICc < 2 compared to the null model, which included only 'year'. Covariates that met the criteria for the global model are bolded.

Model parameter	AICc	ΔAICc (null)	K
$\hat{p}$ (.) ψ(.)	173.72	0.00	3
$\hat{p}$ (date) ψ(.)	173.74	0.02	5
$\hat{p}$ (lunar illum) ψ(.)	174.06	0.34	5
$\hat{p}$ (cloud) ψ(.)	175.36	1.64	5
$\hat{p}$ (time) ψ(.)	175.45	1.73	5
$\hat{p}$ (lunar illum) ψ(.)	175.81	2.09	5
$\hat{p}$ (noise) ψ(.)	175.89	2.17	5
$\hat{p}$ (wind) ψ(.)	**170.86**	**2.86**	**5**
$\hat{p}$ (tide) ψ(.)	**166.25**	**7.47**	**5**

Table 1.5 Models for selecting **site-specific *(ψ)*** covariates in the global model. These covariates included percentages of *I. frutescens* (Iva), mixed *D. spicata* and *S. pumilus* (mixed_grass), open water cover (open_water), forest cover (forest), *P. australis* (Phragmites), *J. roemerianus* (Juncus), low marsh cordgrass patches of either *S. alterniflora* or *S. cynosuriodes* (low_marsh_CG), and the number of years since prescribed fire (years_since_burn). Covariates were incorporated into the global model if the ΔAICc < 2 compared to the null model, which included 'year' as a fixed effect. Each covariate model also contained 'year' as a fixed effect for (ψ). Covariates that met the criteria for the global model are bolded.

Model parameter	AICc	ΔAICc (null)	K
$\hat{p}$ (.) ψ(.)	173.72	0.00	3
$\hat{p}$ (.) ψ(low_marsh_CG)	172.44	1.28	5
$\hat{p}$ (.) ψ(Iva)	173.18	0.54	5
$\hat{p}$ (.) ψ(years_since_burn)	174.95	1.23	5
$\hat{p}$ (.) ψ(forest)	175.46	1.74	5
$\hat{p}$ (.) ψ(Juncus)	175.59	1.87	5
$\hat{p}$ (.) ψ(elevation)	175.89	2.17	5
$\hat{p}$ (.) ψ(mixed_grass)	**167.54**	**6.18**	**5**
$\hat{p}$ (.) ψ(Phragmites)	**164.00**	**9.72**	**5**
$\hat{p}$ (.) ψ(open_water)	**158.54**	**15.18**	**5**

Table 1.6 Model selection results for black rail occupancy with year as a fixed effect in all combinations, ranked by AICc value. This table shows the results after all possible combinations of variables from the global model were evaluated. Survey-specific covariates retained in the global model included wind speed (mph) and tide level (m) according to the nearest NOAA data buoy station. Site-specific covariates included percentages of *Phragmites* (*P. australis*), mixed grasses (*D. spicata* and *S. pumilus*), and open water. Effect sizes were evaluated with model averaging.

Model	AICc	ΔAICc	log-likelihood	weight
$\hat{p}$ (tide + wind), ψ (*Phragmites* + open water)	143.76	0.00	-64.26	0.31
$\hat{p}$ (wind), ψ (*Phragmites* + open water)	144.04	0.28	-65.56	0.27
$\hat{p}$ (tide + wind), ψ (*Phragmites* + mixed grass + open water)	150.51	1.66	-69.93	0.13

Table 1.7 Results of averaging covariate effect sizes from the top models. “Full (β)” refers to the average estimate across all models, and “conditional (β)” is where only the covariate is present. Comparing the discrepancies between the ‘full’ and ‘conditional’ effect sizes provides a way to assess if the predictor’s influence is true or model-dependent. Survey-specific covariates included wind speed (mph) and tide level (m) according to the nearest NOAA data buoy station. Site-specific covariates included percentages of *Phragmites* (*P. australis*), mixed grasses (*D. spicata* and *S. pumilus*), and open water. *P. australis* had a large negative effect size but was considered non-significant due to the large standard error.

	$\hat{p}$ *(tide)*	$\hat{p}$ *(wind)*	ψ *(open_water)*	ψ *(mixed_grass)*	ψ *(Phragmites)*
Full (β)	0.24	-0.70	-1.71	0.08	-12.97
Conditional (β)	0.38	-0.70	-1.70	0.45	-12.97

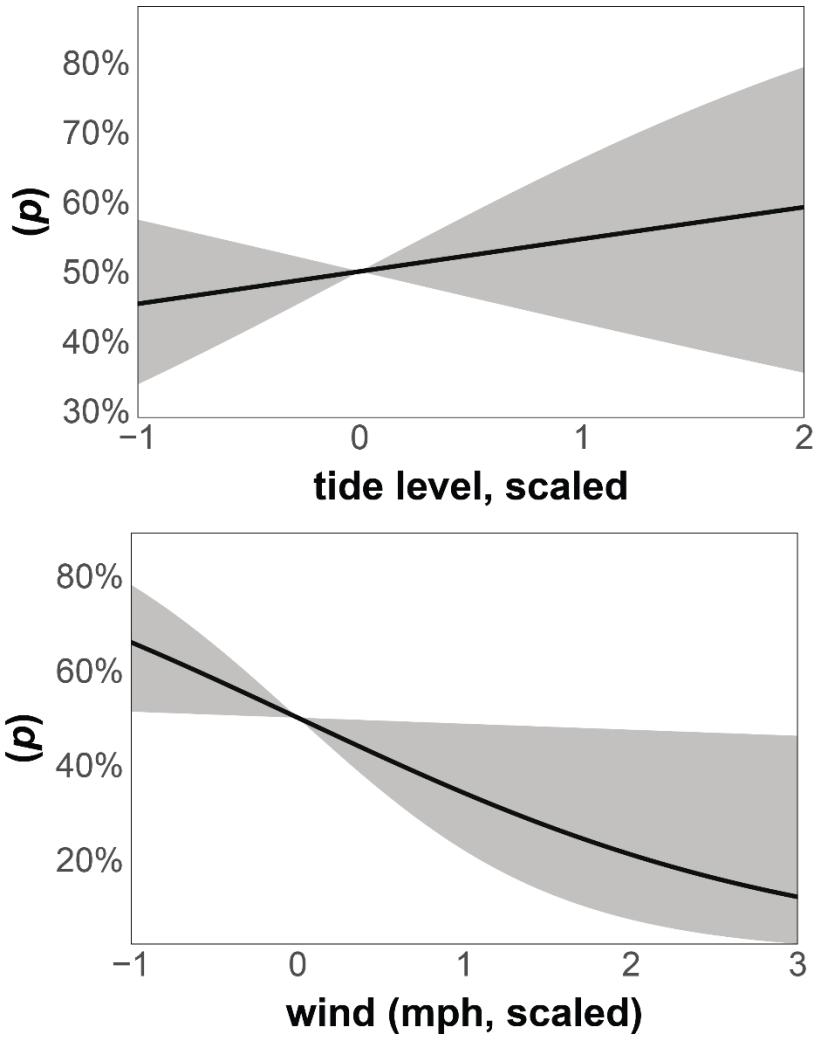

Figure 1.4 Estimated survey-specific covariate relationships with black rail detection probability (*p)*. The black lines represent the estimated (*p)* from the full-averaged occupancy model. The shaded areas represent the 95% confidence intervals. The trend of tide level was positive on detection probability, though not statistically significant. The confidence intervals increased at higher tide levels, suggesting greater uncertainty as tide level increased. Detection probability decreased as wind speed increased.

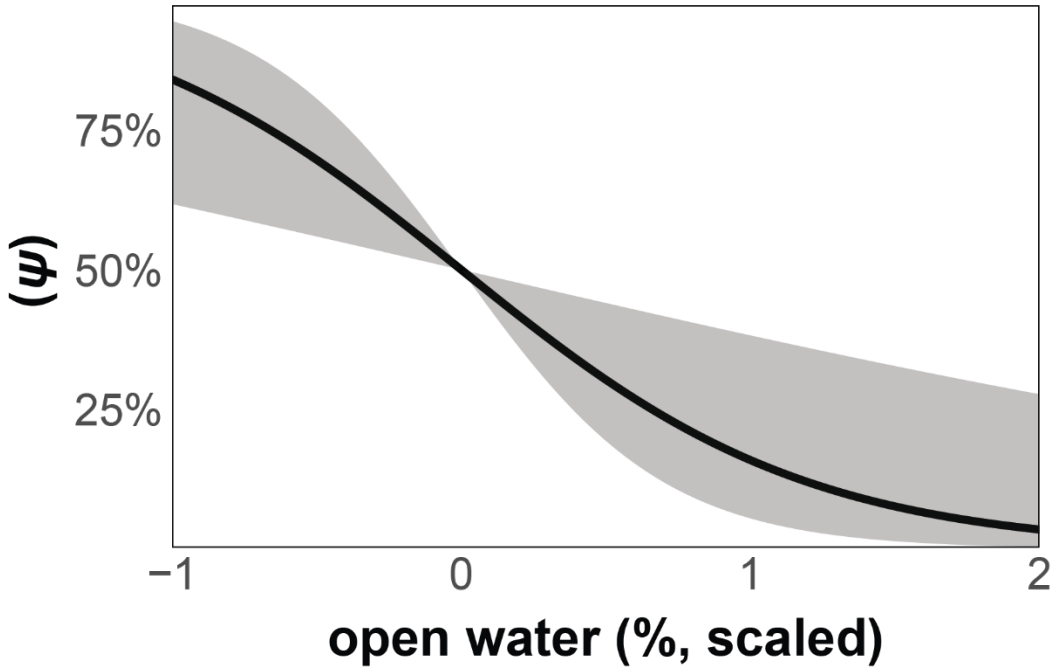

Figure 1.5 Estimated relationship between percent open water cover and black rail occupancy probability (*ψ*). The black lines represent the estimated (*ψ)* from the full-averaged occupancy model. The shaded areas represent the 95% confidence intervals. Occupancy probability decreased as percent open water cover increased.

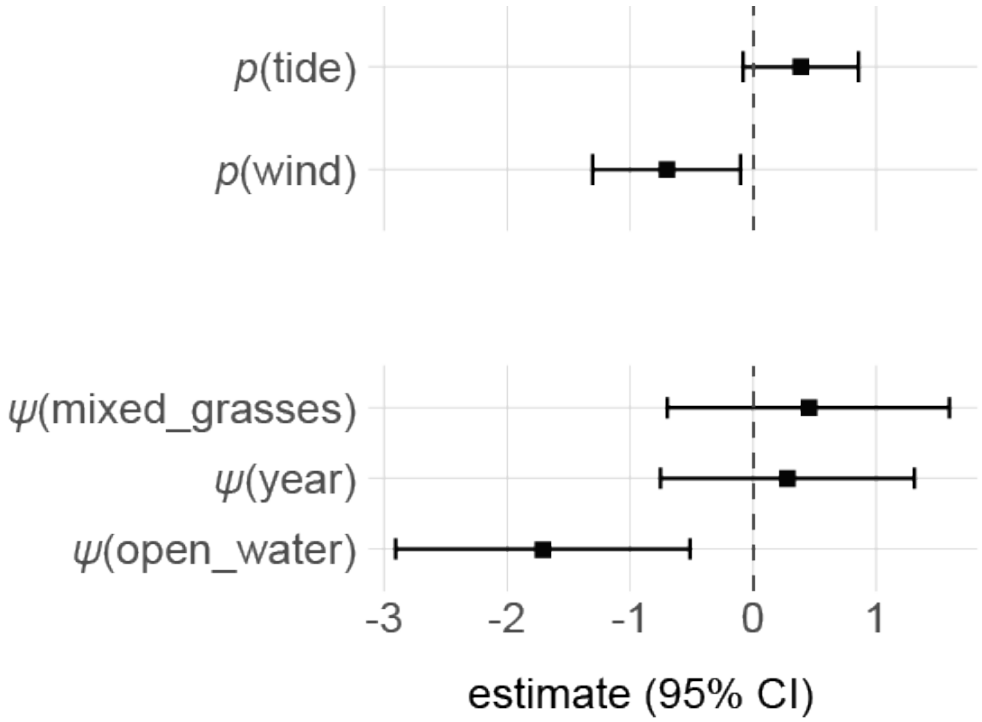

Figure 1.6 Estimated covariate relationships with black rail detection probability (*p*) and occupancy (*ψ)*. Points and whiskers represent the estimated effect size and 95% confidence intervals from the full-averaged occupancy model. The effect of tide level trended positively on detection probability but was not significant. Detection probability decreased as wind speed increased. Greater abundance of mixed grasses trended upward on occupancy probability but was not statistically significant. Open water was the factor with the strongest negative influence on black rail occupancy.

Prescribed burn effects on vegetation

Land cover type percentages and mean vegetation heights were estimated at a total of 35 unburned, 20 burned within-year (2022), and at 18 points one-year post-burn (2023; Figure 1.7). Black rails were detected during at least one survey at 5 unburned points, 5 burned within-year, and 6 points one year post-burn. Not all detections were at points where vegetation was surveyed due to the prioritization of game land sites to meet federal grant obligations. Due to constraints on time and personnel, I was only able to sample 17 burned points in both years.

Coarse-scale land cover

Land cover type data were not distributed normally even after transformation to z-scores. Therefore, I compared average cover type percentages by burn status using non-parametric tests.

Burned and unburned plots– The only cover type that differed significantly between burn states was black needlerush (*J. roemerianus*), which occurred at higher percentages in the burned sites in both years (2022: Mann-Whitney $U = 450$, $p = 0.05$, 2023: $U = 385$, $p = 0.029$; Appendix Table A3).

Burned plots between years– Tests revealed no significant change in forest cover ($W = 6$, $p = 0.402$), *P. australis* ($W = 9$, $p = 0.108$), low marsh cordgrasses ($W = 24$, $p = 0.721$), *I. frutescens* ($W = 7$, $p = 0.141$) one year post-burn (Appendix Table A4).

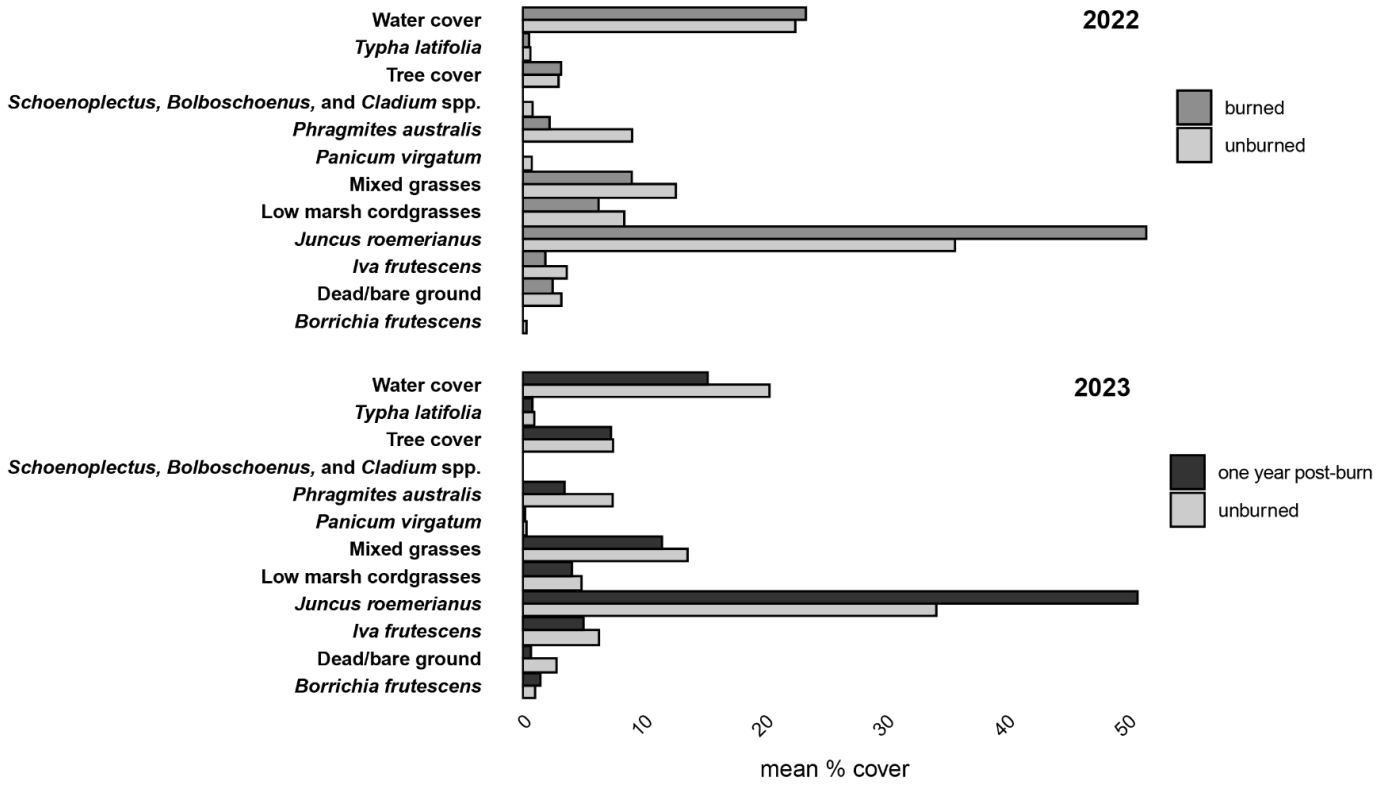

Figure 1.7 Mean percent land cover estimates by burn status, separated by year. Here, all sites in the 'burned' (*N* = 20) and 'one year post burn' (*N* = 18) categories represent habitats that were subject to fire treatment between February-April of 2022. Sites in the 'unburned' category in 2022 (*N* = 34) and 2023 (*N* = 31) were untreated for $\geq$ 2 years. Only *J. roemerianus* differed between burned and unburned sites both years and was significantly more abundant in burned sites. This is due to land managers selecting burn sites with large *J. roemerianus* patches.

Vegetation structure

In 2022, I conducted fine-scale vegetation sampling on 9 unburned points and 7 points burned within the year. In 2023, fine-scale samples were completed on 30 unburned, 17 one-year post-burn, and 3 freshly burned points.

Burned and unburned plots– Fine-scale vegetation measurements confirmed that burning reduced vegetation density. There was a significant difference between burned and unburned plots in mean visual obstruction (obtained using the Robel pole) within the year of burn (Figure 1.8). Mean visual obstruction was significantly lower in plots burned the same year than in unburned areas (i.e. not burned for ≥ 2 years; Mann Whitney $U = 3.0$, $p = 0.02$). Mean disc settling height also trended lower in burned plots than in unburned plots but was not significant (Mann-Whitney $U = 6.0$, $p = 0.07$; Appendix Table A2).

In 2023, there was a marginally significant increase in disc settling height measurements between sites unburned and one year post-burn (Mann-Whitney $U = 207.5$, $p = 0.049$; Figure 1.9). However, no significant differences were found in visual obstruction measurements between burned and unburned sites (Mann-Whitney $U = 271.5$, $p = 0.462$).

Burned plots between years– Average disc settling height had a mean (± SE) difference of 0.9 (± 4.4) cm from time of burn to one year post-burn, but did not significantly change between years ($t_4 = 0.20$, $p = 0.85$). However, visual obstruction measurements significantly increased by 22.1 ± 4.7 cm on average between the burn year and one year post-burn ($t_4 = 4.29$, $p = 0.013$).

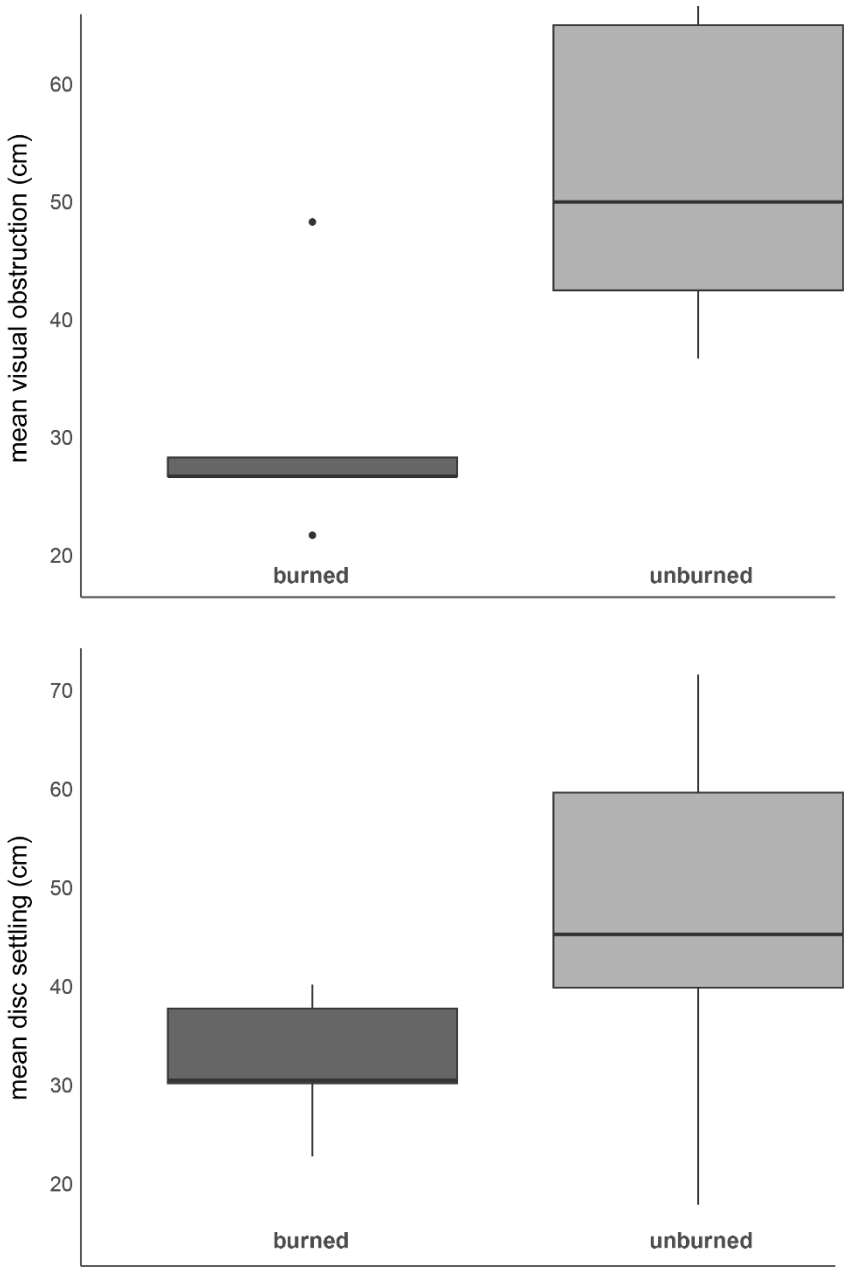

Figure 1.8 Box plots of mean visual obstruction (top) and disc settling height (bottom) at burned (N = 5) and unburned (N = 7) plots sampled in 2022. Mean visual obstruction was higher in unburned sites (i.e. sites untreated for $\geq$ 2 years). Mean disc settling heights were not significantly different between treatment groups at the 95% confidence level.

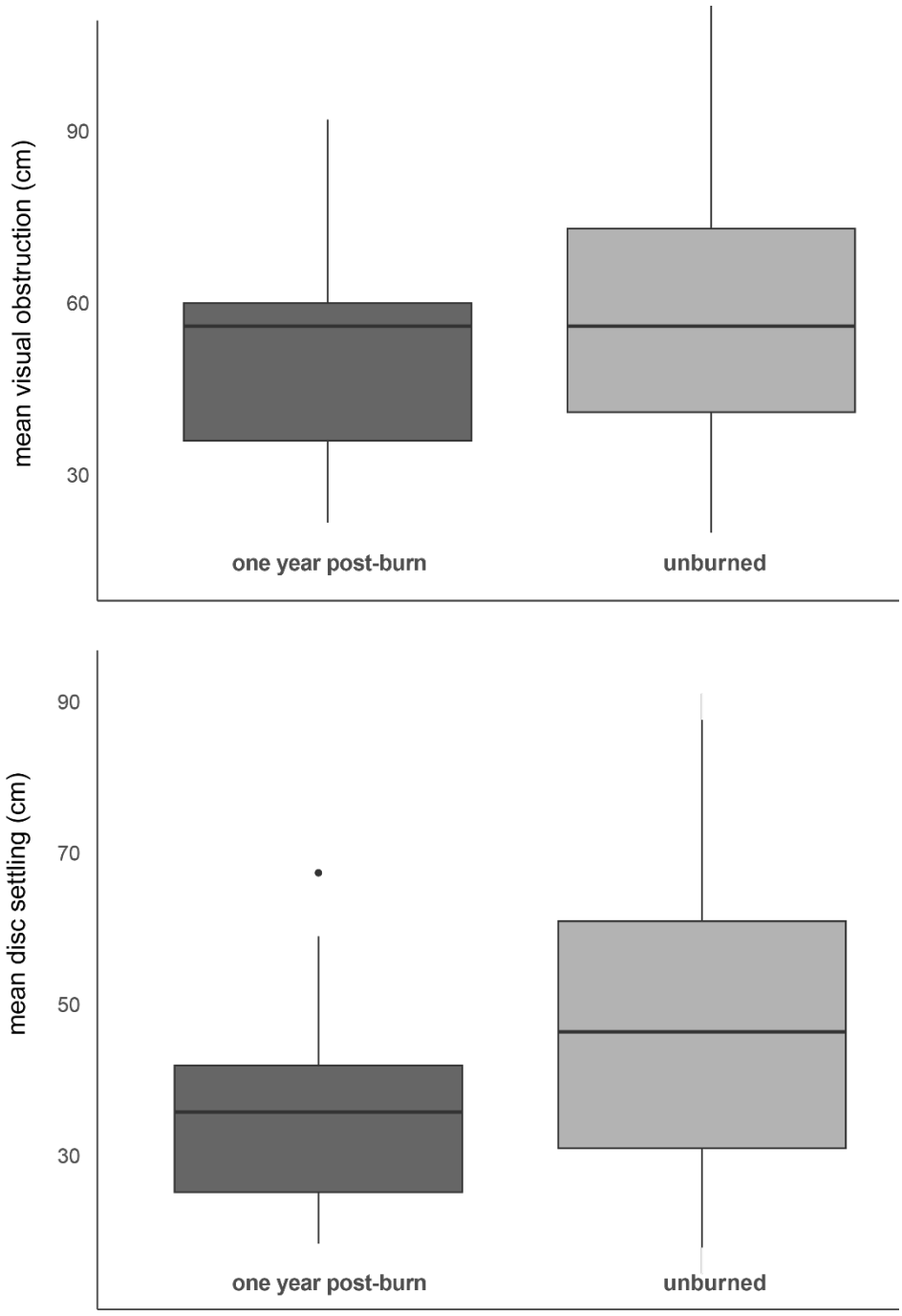

Figure 1.9 Box plots of mean visual obstruction (top) and disc settling height (bottom) at plots sampled one year post-burn (*N* = 20) and unburned (*N* = 31) in 2023. Sites one year post-burn were subject to fire treatment in February-April 2022. Visual obstruction measurements did not differ between treatments. Disc settling heights were marginally higher in unburned sites.

Hydrologic conditions

Black rails are thought to prefer habitats with damp soil and standing water depths ranging between ~ 0-5 cm (Eddleman et al. 1994). Sites where water levels frequently surpass this range are not considered viable for supporting black rail populations long-term (Stevens & Conway 2021). I therefore evaluated hydrologic patterns based on degree of water level stability, and how frequently depths exceeded the 5-cm threshold (Figure 1.10-1.13).

Comparison by burn status

Water level fluctuations consistent with a modest lunar tide were detected by each of the water loggers at Goose Creek GL (Figure 1.10). The percentage of days with water levels below 5 cm increased from 8.4% to 15.6% and from 10.2% to 15.6% in the unburned extent between years (Tables 1.8-1.9). The unburned site experienced a period of inundation between May 21 and August 1, 2023, where water levels fluctuated between 7-50 cm. The average range of water level fluctuation per day decreased from 14.1 ± 0.8 in 2022 to 11.9 ± 0.8 cm in 2023 in the burned extent and from 10.2 ± 0.9 to 8.4 ± 0.6 cm in the unburned area.

At Salter's Creek GL, high water levels occurred in early May 2022 due to the confluence of a king tide with an unusually strong wind tide caused by a 'noreaster' (Figure 1.11). This produced higher water levels in the burned area than in the unburned area. A similar tendency for the unburned area to absorb the excess water was seen on days with high rainfall, though this may have been influenced by regional differences. In 2023, flooding occurred in the burned extent on the week of April 9 due to a storm surge, but quickly receded below 5 cm by April 11. On the burned side, the percentage of days where water levels remained below 5 cm increased from 24.1% to 67.9%, and the average water level

fluctuation per day decreased from 9.5 ± 0.8 in 2022 to 4.4 ± 0.5 cm in 2023 (Tables 1.8-1.9). The percentage of days below 5 cm on the unburned side went from 58.8% in 2022 to 48.2% in 2023, driven mainly by daily fluctuations of ~ 20 cm observed between June 17 and August 22. On the unburned side, mean water level fluctuation per day also marginally increased from 5.9 ± 0.6 in 2022 to 6.8 ± 0.6 cm in 2023.

The Cedar Island NWR and North River areas were only able to be hydrologically monitored in 2023. At Cedar Island NWR, the unburned site experienced inundation from April 9-June 6 (with occasional dips below 5 cm) where water level recorded at the one year post-burn site remained above 10 cm between May 21 - June 19 (Figure 1.12). Here, water levels remained under 5 cm for 59.7% of days in unburned and 60.1% in the one year post-burn extent. The average daily fluctuation of water level was only 3.2 ± 0.2 in the area one year post-burn and 2.9 ± 0.5 cm in the unburned area.

Water levels at the North River site fluctuated between 0-10 cm with some peaks exceeding 10 cm between May 25 - June 8, June 15-23, July 1-11, and August 1-5 during 2023 (Figure 1.13). The percentage of days observed where the water level remained under 5 cm was 72.3%. The average daily fluctuation of water level was ~ 5 cm.

Comparison by black rail presence

By the end of the 2022 season, 2 of the 5 HOBO U20L logger sites were occupied by at least 1 bird and 3 had no black rail detections. Four detections occurred near logger sites in 2022 when water levels ranged between ~ 0-5 cm. In 2023, 17 detections occurred near logger sites when water level was low or receding.

At Goose Creek GL, only one bird was detected in 2022 near an impoundment not burned for ≥2 years (Figure 1.11). At the time of detection, the recorded water level was ~ 5 cm but water levels ≥20 cm were recorded the next day. The presence of tides that regularly

exceeded ~ 20 cm suggests that the natural marshes in this area are poorly suited to black rails (Tables 1.8-1.9).

It is noteworthy that black rails were only detected at Salter's Creek in 2022 after the flooding subsided. Black rails were detected at this site more frequently in 2023, and at additional points that mostly remained within their preferred range of 0-5 cm. In 2022, two birds were detected when the water level was at 0.1, then 1.7 cm. In 2023, excursions from the range of tolerance for black rails (0-5 cm water depth) occurred throughout the summer of 2023 during bouts of heavy rain (Figure 1.11).

As Cedar Island NWR contains over 5,000 hectares of marshland, accumulation of high water at one point may not reflect inundation of the entire landscape. The observation of multiple detections despite excursions from the range of tolerance for black rails optimistically suggests that birds are finding refuge from flooded areas here. However, after heavy rain, it took longer for the area burned within one year to drain compared with unburned areas (Figure 1.12).

At North River, water level rarely exceeded the ~ 5 cm threshold of tolerance (Figure 1.13). When this site was hydrologically monitored in 2023, only one bird was detected overall. More detections occurred at this site the previous year.

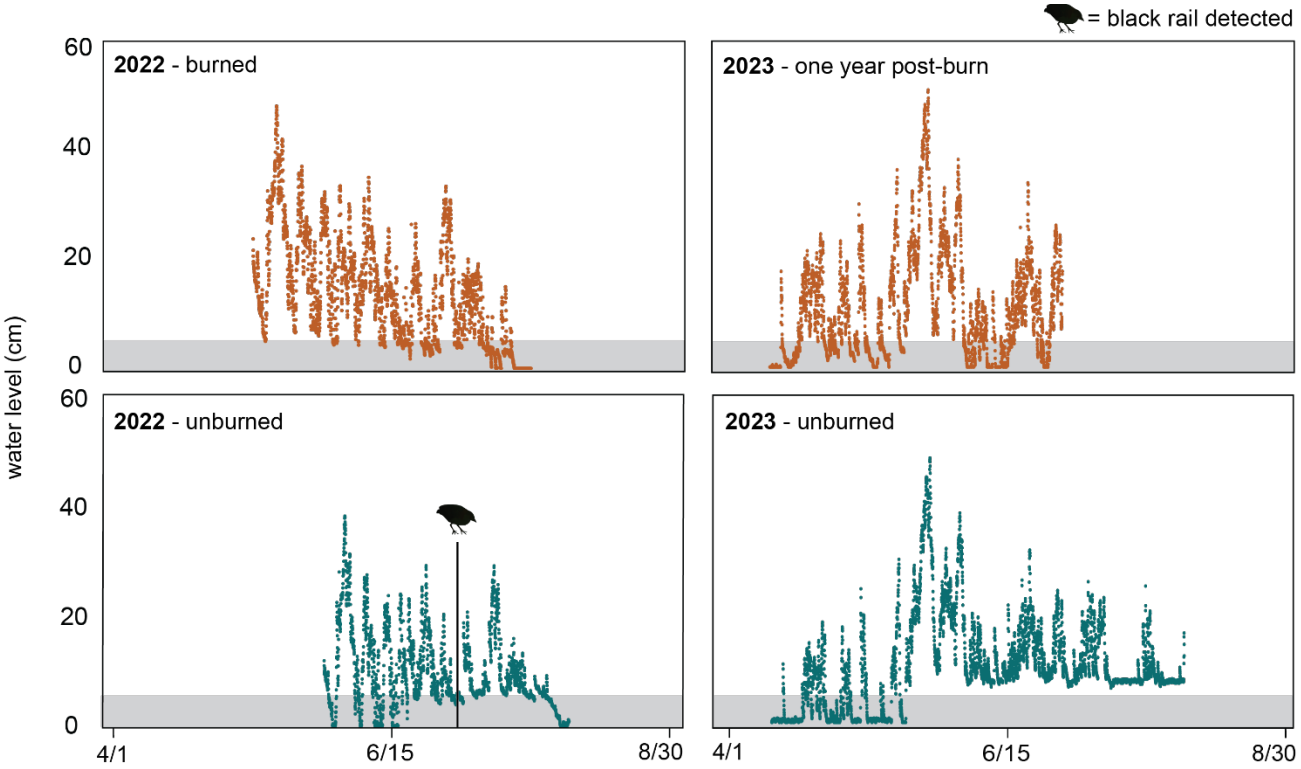

Figure 1.10 Water levels recorded at Goose Creek GL during the study period. The shading indicates the range of tolerance for black rails (between 0-5 cm; Eddleman et al. 1994). In 2022, there was only one detection in the unburned area. The detection occurred when the water was below 5 cm. Several days of heavy rainfall and thunderstorms caused a high-water surge between May 21-31, 2023. This site was characterized by combined wind-driven and lunar tide influences that caused larger daily water level fluctuations.

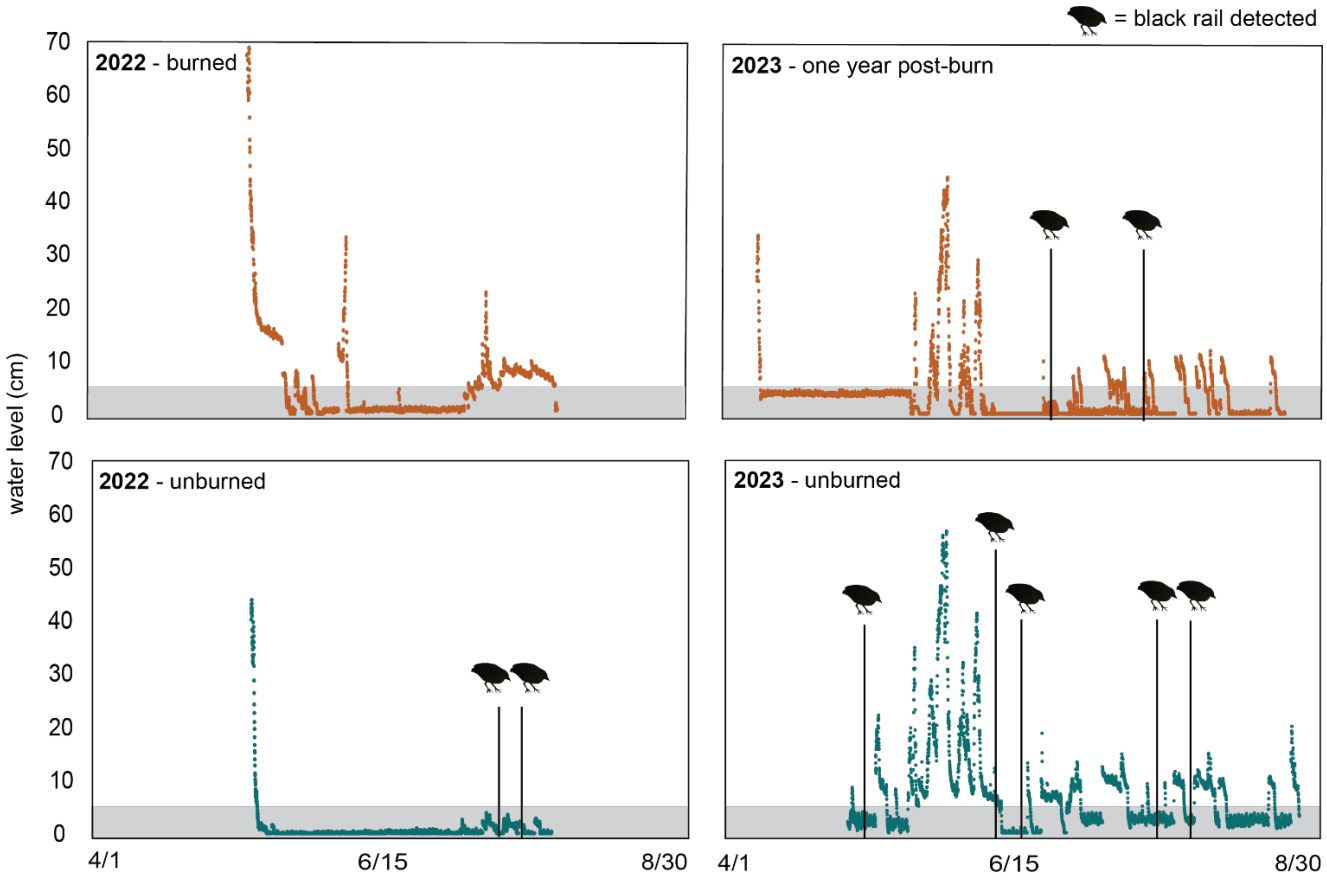

Figure 1.11 Water levels recorded at Salter's Creek GL during the study period. The shading indicates the range of tolerance for black rails (between 0-5 cm; Eddleman et al. 1994). In 2022, the effects of the 'noreaster' and king tide effects caused water levels to rise above 40 cm at both burned and unburned sites. A flood was observed early at the one year post-burn site in 2023 but receded in less than a day. A storm surge prompted flooding between May 21-31 at all Salter's Creek sites in 2023. In 2022, the water level remained below 5 cm when birds were detected at the unburned site. More detections occurred here in 2023 when water levels were low (near 5 cm or lower). No birds were found at the burned site in 2022, but 2 birds were detected here in 2023 when the water levels were between 0-5 cm.

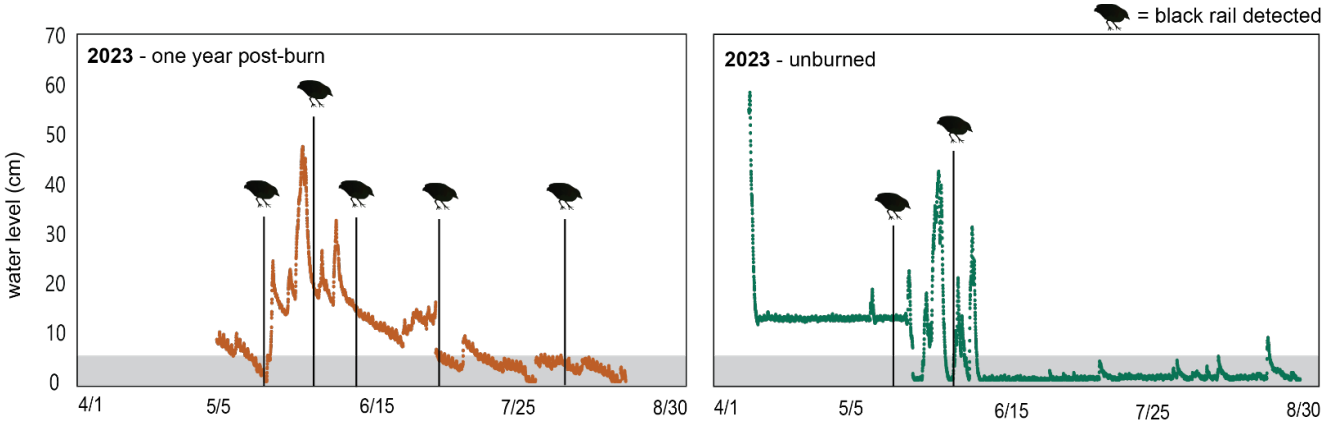

Figure 1.12 Water levels recorded at Cedar Island NWR during the study period in 2023. The shading indicates the range of tolerance for black rails (between 0-5 cm; Eddleman et al. 1994). Heavy rainfall prompted flooding between May 21-31 at both sites. High water over 50 cm was recorded at the unburned site on April 9. After 3 days, inundation above 10 cm then persisted until June 6. Recorded water levels were occasionally higher than 5 cm when detections occurred, the range being between 0.3-21.8 cm.

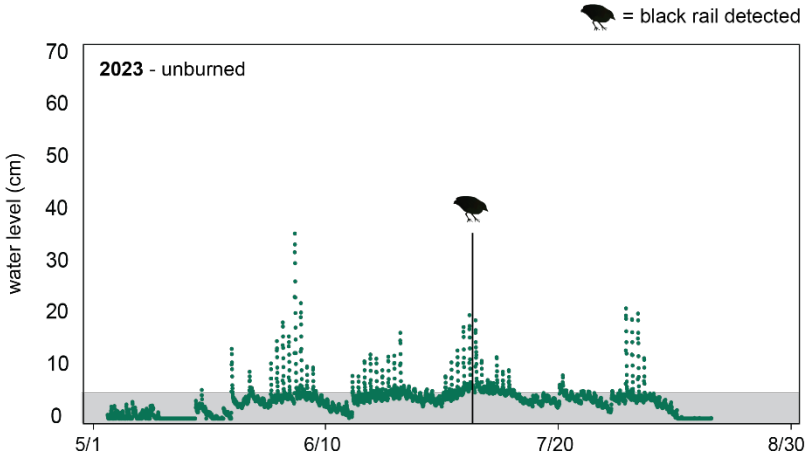

Figure 1.13 Water levels recorded at the North River site during the study period in 2023. The shading indicates the range of tolerance for black rails (between 0-5 cm). Some high water levels were recorded during heavy rainfall. However, inundation above 10 cm did not last longer than 24 hours during the observed period. Only one bird was detected here in 2023 when the water level was near the optimal 5-cm threshold (Eddleman et al. 1994).

Table 1.8 Summary of hydrology at each site in 2022, separated by burn status. Data loggers were deployed at Goose Creek GL from June 1 - July 27 and at Salter's Creek from May 10 - July 28.

		Total days observed	Days below 5 cm	Days below 10 cm	Mean daily fluctuation (cm)
Burned sites	Goose Creek GL	59	5 **(8.4 %)**	10 **(16.9 %)**	14.1 ± 0.8
	Salter's Creek GL	79	19 **(24.1 %)**	31 **(39.2 %)**	9.5 ± 0.8
Unburned sites	Goose Creek GL	59	6 **(10.2 %)**	17 **(28.8 %)**	10.2 ± 0.9
	Salter's Creek GL	80	47 **(58.8 %)**	67 **(83.8 %)**	5.9 ± 0.6

Table 1.9 Summary of hydrology at each site in 2023, separated by burn status. Data loggers were deployed at Goose Creek GL from April 16 - August 1, at Salter's Creek GL from April 9 - August 25, at Cedar Island NWR from April 9 - August 15, and at North River from May 3 - August 10.

		Total days observed	Days below 5 cm	Days below 10 cm	Mean daily fluctuation (cm)
Burned sites (One year post-burn)	Goose Creek GL	77	12 **(15.6 %)**	22 **(28.5 %)**	11.9 ± 0.8
	Salter's Creek GL	134	91 **(67.9 %)**	109 **(81.3 %)**	4.4 ± 0.5
	Cedar Island NWR	103	37 **(35.9 %)**	62 **(60.2 %)**	3.2 ± 0.2
Unburned sites	Goose Creek GL	108	14 **(13.0 %)**	35 **(32.4 %)**	8.4 ± 0.6
	Salter's Creek GL	139	67 **(48.2 %)**	92 **(66.2 %)**	6.8 ± 0.6
	Cedar Island NWR	139	83 **(59.7 %)**	85 **(61.2 %)**	2.9 ± 0.5
	North River	105	54 **(51.4 %)**	76 **(72.4 %)**	5.0 ± 0.6

Discussion

This study identified environmental factors related to black rail occupancy and investigated how prescribed burn management relates to black rail habitat use. Other studies have used occupancy models to identify predictors of suitable habitat (Richmond et al. 2012; Roach & Barrett 2015; Taillie & Moorman 2019), but few have done so across sites of varying burn regimes. As sea level rise continues to extirpate wetland loss, identifying management tools that facilitate buffer zones from threatened habitats is essential for black rail conservation (Watts 2020). These results provide additional guidance to wildlife managers on strategies to enhance black rail habitat.

Factors affecting black rail detection probability

In the global occupancy model, detection was influenced positively by tide level. Detection increasing with the tide contrasts what has been observed with California black rails in the Suisan Marsh of California, where detection was highest at low tide during surveys (Spear et al. 1999). The Suisan Marsh provides ≥ 88,000 acres (~ 35,000 hectares) of high marsh habitat, where some locations surveyed in this study (e.g. Goose Creek and Salter's Creek GL) contain less than 8,000 acres (~ 3,000 hectares). Therefore, the effect of tide on detection may differ by marsh size. Furthermore, black rails may become more easily detected as they retreat to high ground (Thorne et al. 2019). As black rails concentrate in less inundated areas during high tides, increasing survey effort in these refugia may increase detection probability in coastal habitats.

Wind speed was negatively correlated with detection probability, as seen in previous studies (Conway et al. 2004; Tolliver et al. 2019). The effect of wind speed has been shown to decrease when surveys are not performed while wind speeds exceed 25 km/h, or ~ 15

mph (Evens et al. 1991; Conway 2011). After further restricting maximum wind speeds for surveys, Butler et al (2023) did not detect an effect of wind on black rail detection probability. By contrast, surveys in this study were only performed at wind speeds <20 km/h (~ 12 mph) and a significant effect was still present. Maximum wind speed recommendations for black rail surveys may need to be re-evaluated to minimize the negative impact of wind on sound transmission and survey results. Coastal regions will almost always have some level of wind.

Black rail occupancy in relation to habitat characteristics

Mean visual obstruction and disc settling height values did not statistically differ between occupied and unoccupied sites. This lack of statistical significance could be attributed to the small number of detections or the small number of plots sampled. Alternatively, the fine-scale protocol derived from the NOAA Firebird Project protocol (2022) may not have appropriately captured the vegetation structure due to the degree of heterogeneity observed in sampled areas and the small number of points. The disc settling heights recorded at one North River site, for example, ranged from ~ 26-105 cm. The mean disc settling height (~ 74 cm) may not have accurately represented the structural range present at this site. Only five repeated measures were performed per plot, which also may have limited the accuracy of the averaged measurements. Trends in disc settling height and visual obstruction as measured in this study have been successfully linked to other marsh bird occupancy trends, but with a greater number of sites sampled (Van't Hul et al. 1997; Brewer et al. 2023). Future studies that measure fine-scale vegetation characteristics should consider increasing the number of repetitions per site, increasing the number of sites sampled, or accounting for the range of values recorded.

There was a strong negative effect of *P. australis* cover on black rail occupancy in the global and full-averaged occupancy models, but the standard error was notably high. This

invasive species was present at only 14 out of 49 survey points, but it was never found where black rails occurred. The negative trend between *P. australis* and occupancy is noteworthy, however, as it contradicts the positive association between *P. australis* and black rail occurrence found by Tsao et al. (2015) in California. Rather than representing a difference in preference between subspecies, it is likely related to species assemblage differences in each state and competition between emergent species in wetlands. Water limitation is so significant in the Sacramento-San Joaquin Delta and the foothills of the Sierra mountains in California, that dominant plant species in wetlands with suitable hydrology may be of less import. In any case, tolerance by black rails of this invasive species warrants further investigation. If *P. australis* is negatively associated with black rail habitat, prescribed fire could be used to significantly reduce its presence if implemented with hydrologic management (Hazelton et al. 2014).

Mixed grass cover (*Distichlis spicata* and *Spartina pumilus*) was more prevalent in areas where black rails were found in 2022. However, this variable did not strongly predict occupancy in the full-averaged model. This discrepancy is likely due to the sampling design, where survey points were selected based on visual assessments of habitat suitability informed by previous studies (Eddleman et al. 1995; Tolliver et al. 2019; Johnson & Lehman 2021). Although this cover type was present at all sites where black rails were detected, it was also found in areas without detections. This is corroborated by findings of concentrated hotspots occupying a small percentage of suitable habitat (Stevens & Conway 2021). Greater distance from waterways may be more crucial in habitat selection for coastal areas due to the increased risk of inundation from rising sea levels (Wilson et al. 2007; Taillie & Moorman 2019).

Occupancy was negatively influenced by the presence of open water. I had survey points along the shoreline edge of the marsh as well as further inland, and larger percentages of open water land cover were recorded mostly at points at the shoreline of the sound or at creek edges. Most black rail detections occurred when traversing into the marsh by foot. Surveyors would benefit from avoiding shoreline edges, as they may negatively impact detection (Flores & Eddleman 1995) and potentially bias occupancy estimates (Bart & Peterjohn 1995).

Occupancy modelling suggested that black rail detection increased at high tide, but hydrology measurements show that detections more often occurred when water level was low. The larger marsh area at Cedar Island NWR coincided with higher variation in water levels during detections, possibly reflecting small elevational differences, and offering more refugia for the rails from flooding. This agrees with previous findings that water management is essential to maximizing occupancy probability in smaller, fragmented marshes (Roach & Barrett 2015; Hand et al. 2021). Despite the presence of high marsh, Goose Creek GL was subject to boat wakes and wind tides that caused hydrological spikes well beyond what black rails can tolerate. The only instance of a detection at this site was within an impounded area with controlled water flow. Across all tidal areas, flooding and storm surge were observed during the breeding season in both years of the study, indicating extreme inundation risk. Water control is greatly needed in these areas if more habitat is to be restored.

Effects of prescribed fire on high marsh habitat

There was more black needlerush (*J. roemerianus*) at burned sites compared to unburned sites in both years, indicating that sites selected for burning initially had higher levels of black needlerush than untreated sites. Vegetation species composition did not differ significantly between treatment groups otherwise. Therefore, prescribed burns in high marsh

habitat can reduce vegetation density without significantly altering plant communities. Vegetation density, as measured by the disc settling height method, was reduced at sites burned within the year compared to unburned sites. Similar studies of prescribed burns in wetlands have observed that burn treatments can significantly impact vegetation density and biomass while having little to no effect on species composition (Austin et al. 2007; Ford & Grace 1998). Visual obstruction increased significantly at burned sites between the year of burn and one year post-burn, indicating that the habitat recovered after one year.

Importantly, the number of detections in the burned extent increased by 50% one year post-burn, but opportunistic detections contributed to this result. Fire has previously been reported to not affect black rail occupancy in Colorado (Conway et al. 2010) but has been anecdotally linked to sustained occupancy between years in coastal Louisiana (Johnson & Lehman 2021). More work is needed to fully understand the use of prescribed fire for black rail habitat management over multiple years. However, this study provides evidence that fire does not negatively impact black rail occupancy and suggests that recruitment may increase one year post-burn.

Fire frequency and severity has impacted multiple co-occurring marsh birds. In North Carolina, king rail (*Rallus elegans*) occupancy probability was higher in marshes burned within-year and one year post-burn (Rogers et al. 2013). Seaside sparrow densities increased between 0-4 years post-burn and were higher at sites exhibiting a patchy burn structure compared to those burned completely (Mitchell et al. 2006). The number of years post-burn was not influential enough to include in the global occupancy model, but the increase in black rail occupancy within burned sites over time indicates that habitat suitability may increase after the first year. Two multi-year studies in southern Texas found no significant relationship between time since burn and black rail occupancy (Haverland et al.

2019; Butler et al. 2023). While fire regime alone may be an insufficient predictor of black rail presence, the increase in occupancy observed one year post-burn suggests that annual burns may not be appropriate for black rail management.

Studies have confirmed interactions between prescribed burn and marsh vegetation (Nyman & Chabreck 1995; Conway et al. 2010; Bickford et al. 2012), but the direct relationship between hydrology and fire is not well understood. Fire may have an indirect relationship with hydrologic conditions, as it may affect soil conditions impacting evapotranspiration and water retention (Moody & Martin 2001). Mean daily rates of fluctuation in water level were higher at sites burned within the year than at the same sites one year post-burn. When hydrology patterns stabilized at burned sites one year post-burn, the number of black rail detections in these areas increased. More research involving pre- and post-fire treatments is needed to better understand how fire-induced hydrological changes impact black rail habitat use over time. This study, however, provides evidence of stabilized water levels in habitat one year post-burn.

Management implications

Overall, this study investigated the use of fire management and hydrologic monitoring to maintain optimal habitat for black rails. It also highlights the importance of accounting for tide and wind effects in planning callback surveys and suggests increasing survey point distance from waterways to enhance detection probability. Lastly, findings suggest that fire management would be better allocated by conducting burns on more marshes less frequently, rather than on fewer marshes annually. Additionally, prescribed fire between February-April does not appear to negatively impact black rail occupancy. Future research should focus on long-term habitat and population responses to fire under varying burn regimes to refine the most effective management strategies for this species.

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CHAPTER 2: ACTIVITY PATTERNS OF EASTERN BLACK RAILS IN NORTH CAROLINA REVEALED WITH PASSIVE MONITORING TECHNIQUES

Abstract

Black rails are notoriously difficult to observe, and their ecology and behavior are poorly understood. Passive monitoring equipment such as Autonomous Recording Units (ARUs) and on-the-ground camera traps offer a minimally invasive strategy for observing black rail activity and breeding phenology, yielding insights that can guide future monitoring and inform management strategies. In this study, I used ARUs to document circadian and seasonal patterns of black rail vocalizations and deployed motion-activated trail cameras to document aspects of phenology and behavior during the breeding season. I programmed recording units to record between 17:00 - 10:00 h at two sites within Cedar Island NWR. Audio recordings were analyzed by the BirdNET Analyzer GUI (version 1.0.2, Cornell Lab of Ornithology), then the automated detections were manually verified. Verified detections were modelled against temporal covariates to determine primary predictors of circadian vocalization patterns. Vocalization probability and frequency was highest during the night (23:00 - 4:00 h) and decreased as the hours since midnight increased. Higher vocalization frequency was associated with higher lunar illumination and low standing water depths. Birds were additionally more likely to call when the moon was brighter. Trail cameras captured evidence of juvenile black rails and confirmed breeding success in North Carolina. Findings are discussed in relation to future monitoring and management efforts for the species.

Introduction

Little is known about black rail behavior and life history strategies. Like other rallids, black rails move silently through dense vegetation and are adept at evading detection (Eddleman et al. 1988; Eddleman et al. 1994). This elusive escape strategy makes monitoring efforts challenging and impedes conservation efforts for the species (Watts 2016). Knowledge of breeding phenology is key to timing management treatments, such as prescribed fire (Nyman & Chabreck 1995).

Prescribed fire can be an effective tool for maintaining black rail habitat (Chapter 1). However, fire applications during vulnerable life stages may induce bird mortality, both from fire and smoke-attracted predators (Grace et al. 2005; Mitchell et al. 2006). Additionally, a poorly timed, high-intensity burn within the coastal range could alter black rail breeding cycles and reduce survival (Legare et al. 1998; Hand et al. 2021).

Seasonal and circadian patterns of black rail activity appear to vary between locations (Bobay et al. 2018). Therefore, local assessment of vocal activity patterns during the breeding season is vital to maximizing the outcome of monitoring strategies that rely on auditory detections, including eliciting vocal response to playback of species-specific calls.

Long-duration acoustic recordings have been useful as a supplemental monitoring strategy for secretive marsh birds that are vocal but call irregularly (Bobay et al. 2018; Znidersic et al. 2020; Znidersic et al. 2021). Autonomous Recording Units (ARUs) can be programmed to record continuously over extended periods (Wildlife Acoustics 2019), which can reduce the probability of false negatives for occupancy (Acevedo & Villanueva-Rivera 2006). Recorders can also reduce the impact of habitat disturbance from surveyors by decreasing the amount of time spent in the field (Swiston & Menhill 2009; Stiffler et al. 2018).

However, long ARU deployments result in weeks or months of audio recordings that require large storage capacities and hours of investment in analysis (Towsey et al. 2014).

The use of call-recognizing software has been developed to automate the analysis process, where a machine-learning model uses neural network algorithms to identify focal species in small audio segments (Kahl et al. 2021). While convenient, this methodology is also prone to high error rates and must be manually validated (Pérez-Granados 2023). With a combination of call-recognizing software and manual validation, long-duration recordings permit the capture of daily and seasonal fluctuations in black rail vocal activity throughout the breeding season (Butler et al. 2015).

Studies from South Carolina, and Florida have reported Eastern black rail populations nesting between May-September (Legare et al. 2001; Hand et al. 2021). As reported in South Carolina, independence occurs ~ 40 days post-hatch, and the flightless molt for young adults lasts ~ 21 days after the end of the breeding season (Hand et al. 2021). Multiple broods have been documented from black rail pairs within a single year, suggesting that they have high fecundity if brood disturbance is minimized (Legare & Eddleman 2001; Hand et al. 2019). Despite a repertoire of at least 5 vocalization types (Pipelow 2017), the only call for which the context is clearly understood is the *kick-kee-doo* mating call used by breeding males to attract females and defend territories (Eddleman et al. 1994). This distinctive call is primarily documented between March-June, though has been heard as late as August (Pipelow 2017; this study).

Other black rail vocalizations include the *grr* and the *churt*, both of which are likely alarm calls where the former is more often used during territorial defense (Eddleman et al. 1994). The least understood vocalization documented is the *ink-ink-ink*, otherwise referred to as the *tch* call. The context of this call has not been formally defined, but has been

documented when parents are nesting (Conway 2011; Hand et al. 2019). Although the lexicon of the black rail is poorly understood, monitoring these vocalizations may indicate the breeding status of observed individuals within the study area. Incubating and brood-rearing adults are often more secretive (Eddleman et al. 2020). Therefore, seasonal peaks in calling activity may delineate intervals prior to and between broods.

Neither seasonal nor circadian patterns of vocal activity have been documented for North Carolina populations (Watts 2016). When determining occupancy, the ideal survey window for black rails may vary across the range due to population differences in seasonal and diel activity (Bobay et al. 2018). For example, a decline in calling rate was reported for black rails after mid-June in a single-season Texas Gulf Coast study (Butler et al. 2015). However, California black rail callback detections were reported to increase between mid-June and early-July (Spear et al. 1999). Traditional callback survey and passive monitoring studies have shown periodicity in detectability of black rails coinciding with moon phase (Tolliver et al. 2019) and time of year (Butler et al. 2015). Nevertheless, seasonal vocalization patterns are not well-documented across the species range.

Motion-activated infrared trail cameras have been used successfully to document rail occupancy, breeding phenology, and temporal variation in activity (Znidersic 2017; Hand et al. 2021). In the impounded wetlands of South Carolina, breeding black rails with broods of 1-7 chicks were documented with custom-focused trail cameras (Hand et al. 2021). This study was remarkable for providing the first breeding records of black rails in South Carolina in 170 years. When deployed on breeding territories, trail cameras can also reveal the range of nest predators and co-occurring species present in the area (Kane et al. 2023).

My goals in this chapter were to characterize seasonal and circadian patterns of activity to inform monitoring efforts. Specific aims were to 1) determine how black rail

vocalization probability and frequency changed within the season and in relation to moon phase and semidiurnal tide cycle, and 2) document evidence of breeding and phenology. In this study, I deployed ARUs from May-August at two locations for one breeding season. Hourly counts of vocalization detections were modeled with covarying conditions such as hour of day, diel phase, hydrologic conditions, and lunar illumination. After obtaining auditory or visual detections of black rails, I deployed trail cameras in occupied sites during the 2022 and 2023 breeding seasons. To determine how camera detections related to breeding phenology, I noted the date and development stage of each black rail detected. These detections further revealed what times of day black rails are active.

Methods

Bioacoustic data

ARU deployments

I performed standardized callback surveys (Chapter 1) to verify black rail presence on historically occupied sites in Carteret, Pamlico, and Beaufort County, NC. Within Cedar Island NWR, I deployed Song Meter Mini ARUs (Wildlife Acoustics 2019) between May 29 - July 21, 2023, at locations ~ 200-400 meters apart with confirmed black rail occupancy and near data loggers monitoring water level. During the previous year, 24-hour ARU recordings revealed little to no black rail activity during the afternoon. Additionally, a study conducted in remote areas of the Albemarle peninsula (~ 100 km north of Cedar Island NWR) found that ARU detection probability for black rails was highest between midnight-04:00 h (Bobay et al. 2018). Units were therefore set to record overnight continuously between 17:00 - 10:00 h (EDT). Each ARU was mounted on a notched metal fence post that stood ~ 90 cm from the ground.

Water level measurements

At the two sites where ARUs were deployed, I additionally set out HOBO U20L data loggers (MicroDAQ, LLC) that were tethered within 1.75”-diameter PVC pipes (Chapter 1 Methods, pg. 30). Hourly pressure sensor readings were collected throughout the day at both locations from April 1 - August 15. Water level measurements corresponding to date and hour were used in later model analyses.

Audio data processing

After recordings were collected, recordings were parsed into a series of one-hour recordings in .wav file format then processed using the BirdNET GUI Analyzer (version 2.4) --

an automated call recognizer algorithm developed by the K. Lisa Yang Center for Conservation Bioacoustics at Cornell Lab of Ornithology and Chemnitz University of Technology (Kahl et al. 2021). This recognizer identified 3-second audio selections with potential black rail calls and tagged them with a confidence score, i.e. an index of probability for correctly identifying a species-specific vocalization (Wood & Kahl 2024).

We set a low confidence score threshold to reduce the chances of missing detections. For flagged items with a confidence score of 0.1 or greater, sonograms were scrutinized visually. If the selection resembled the structure of a black rail call, I also listened to it for confirmation. If the selection was not visually similar to known black rail vocalizations, the observation was not used in model analysis. To verify the chosen selection threshold, I calculated the minimum confidence score required to ensure the probability of a correctly flagged black rail vocalization <90% (Pérez-Granados 2023).

Analyses of vocal activity

On the Atlantic Coast, documented black rail home ranges average less than 1 hectare during the nesting season (Legare et al. 2001). Therefore, black rail detections greater than 200 meters apart (i.e. 2 hectares) were considered separate individuals. Since recording unit deployment locations were based on where detections occurred in the field, the estimated number of birds recorded was informed by detections from callback surveys, opportunistic in-person detections, and the presence of overlapping vocalizations during recordings.

Temporal variables

I summed verified flags from each site by recording hour and placed them into a matrix with detection date and time (24-hour, EDT). To investigate how vocalization

frequency varied on a circadian scale, I used two approaches to characterize the timing of vocalizations. Hours were first symmetrically scaled in their proximity to midnight (0:00 h), that is, hours nearest midnight had low values and hours further were high (e.g. 0:00 h = 0, 17:00 h = 7, 7:00 h = 7). Second, I categorized hourly intervals into diel phases ('evening'= 17:00 h - 22:59 h, 'night' = 23:00 h - 03:59 h, and 'morning' = 04:00 h - 10:00 h). To compare how activity differed between crepuscular and nighttime phases, I also created a category that separated hourly observations that occurred at night from those that did not ('night'= 23:00 h - 03:59 h and 'not night'= 17:00 h - 22:59 h, 04:00 h - 10:00 h). Hours were standardized using the scale function in R (version 4.4.2) and the diel phase categories were converted into factors.

I collected water level and lunar illumination data for each hour of recording to determine how environmental variables impacted vocalization. I extracted and scaled water level measurements (cm) from the nearest data logger deployed for each verified flag count/hour observation. I used the R package 'moonlit' (version 0.1.0) to obtain lunar illumination relative to the time, elevation, and location of the study area (Śmielak 2023). This package calculates lunar illumination by estimating the approximate amount of moonlight (in lux) reaching the ground after adjusting for site-specific atmospheric absorption, the distance between the moon and the Earth, and twilight illumination (Austin et al. 1976**;** Śmielak 2023). The resulting values for lunar illumination were then log-transformed and standardized.

Statistical analysis

Partially due to the elusive nature of black rails, many one-hour periods contained zero vocalizations. An excess of zeros can obscure the influences of model covariates on response variables, such as vocal activity (Mullahy 1986). To accommodate this characteristic of the data, I fit a two-stage hurdle model with zero-inflated and truncated

count components to assess the effect of diel phase, moonlight, and hydrology on black rail vocal activity. This distributional framework accommodates the high frequency of zero observations while addressing overdispersion in count data for verified flags of activity (Clink et al. 2021).

The first hurdle stage employed a zero-inflated logistic regression to fit the probability of observing zero vocal activity, as demonstrated by the following equation:

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right) = X_1\beta_1 + Z_1u_1$$

The probability of observing a zero, or true absence of activity, is represented with (p). Covariates are represented as (X_1) with an effect size of (β_1) while (Z_1u_1) represent the terms and matrix of included random effects. This model stage estimated how each specified covariate influences the probability of black rails vocalizing ($1-p$).

Once observations surpassed the 'hurdle' of 0, the second hurdle stage applied a truncated count model with a negative binomial distribution to predict non-zero counts and estimate the effects of the specified covariates. This equation has the following form:

$$\log(\lambda) = X_2\beta_2 + Z_2u_2$$

Here, (λ) is the expected count of verified flags, given it is non-zero, with predictor covariates (X_2), associated covariate coefficients (β_2), and random effects (Z_2u_2). The hurdle model combined these stages by first assessing the probability of silence (zero verified flags) and, if non-zero activity was predicted, estimating the count of vocalizations with the truncated negative binomial component (Welsh et al. 1996). This setup allows for predicting 1) the probability of a black rail vocalizing or not, then 2) the number of black rail vocalizations when non-zero.

‘Site’ was included as a random effect in all models to account for variation in vocalization between different population locations. To evaluate the independent contributions of temporal variables to vocal activity patterns, each covariate was assessed separately (Welsh et al. 1996). The model was implemented using the R package glmmTMB (version 1.1.10; Brooks et al. 2024).

Trail cameras

Hyperfire 2 motion-activated infrared trail cameras (Reconyx) custom-focused to ~ 1 m were deployed at occupied sites identified based on callback surveys and historical knowledge. These sites included habitat in Cedar Island NWR, Salter’s Creek GL, and sites along the North River near Beaufort, NC. Each camera was programmed to take a burst of 3 still photographs, 1 second apart when triggered. Cameras were mounted with gear ties on metal fence posts, positioned facing downward at a 45° angle, in small clearings in the dense vegetation. A garden pole marked at 3, 5, 10, and 20 cm above ground level was placed within the field of view to document water level when photographs were triggered.

Trail camera images of black rails were assumed to be of different individuals if they were detected at camera traps >200 meters apart, or at the same camera deployment if differentiated by sex or age based on plumage characteristics. I estimated age and sex using plumage characteristics, where possible using published phenology information (Taylor 1988; Pyle 2008; Hand et al. 2021; Olsen et al. 2024). All motion-triggered captures were archived and the number of photographs with detections were summarized. The level of standing water, temperature, day, and time were noted for each detection.

Results

ARU deployments and recordings

To inform ARU unit placements, I performed 215 callback surveys at 30 points within Cedar Island NWR ($\bar{x}$ = 4.73, 4–5 surveys per point) from May 5 - August 25, 2023. We detected birds at 7 of the callback survey points 400 meters apart and at 3 points at least 200 meters apart where opportunistic detections occurred. Recorders detected up to three black rails calling simultaneously at two locations, while a maximum of two black rails were recorded calling simultaneously at the other two locations. Based on this information, I conservatively estimated that ~ 10 individual birds were detected using auditory recordings during the study period.

In 2023, I collected 1,390 hours of recordings during the breeding season. ARUs were deployed in two different marsh units at Cedar Island NWR between May 5 - June 28 and May 29 - July 21, respectively, except for a period between June 28 - July 2 where no recording units were operating. Seasonally, ARUs collected 340 hours of recordings during the month of May, 802 in June, and 248 in July. For each scheduled hour between 17:00 and 10:00 EDT, 80-84 hours of recordings were collected. During each diel phase time block, 501 hours were recorded during the evening, 404 hours overnight, and 485 hours during the morning.

The BirdNET recognizer tagged 66,309 segments of audio containing potential black rail vocalizations. After manual validation of all selections with confidence scores > 0.1, 46,004 (69.4%) were identified as true positives. False positives flagged by the recognizer were primarily red-winged blackbird (*Agelaius phoeniceus*) calls. Selections with confidence scores $\geq$ 0.7 had a 90% chance of containing true black rail vocalizations. True positives with

lower scores either demonstrated low signal-to-noise ratio from birds vocalizing far away from the recorder or contained interference from passing cars, high winds, and other birds. There were more than 10,000 identified selections (~ 16.1% overall and ~ 23.1% of verified true positives) scored < 0.7 by BirdNET that contained black rail calls. Detections with confidence scores < 0.7 occurred during the morning, night, and evening.

Nearly all vocalizations detected were segments of *kick-kee-doo* calls. However, there were two *churt*, two *grr,* and 140 *ink-ink-ink* calling bouts correctly detected by the recognizer as well. The two *churt* and *grr* calls occurred during the morning phase between 6-9:00 h between June 11th and 13th, whereas all *ink-ink-ink* calls detected occurred during morning (7-9:00 h) or evening hours (17-20:00 h) between June 6th and 11th. Though *kick-kee-doo* calls were detected throughout the evening, night, and morning, this was the only call type observed at night. Additional vocalizations (*churt*, *grr* and *ink-ink-ink* calls) were detected during the evening and morning periods.

ARU detections on a seasonal and circadian scale

The calling rate, defined as the number of flags per recorded hour each month, was highest during June (42.7 flags/hour), whereas May (22.4 flags/hour) and July (23.2 flags/hour) had rates similar to one another. The highest level of vocal activity was recorded during a 4-day period (July 18 – 21), whereas little to no vocal activity was observed between July 3-14 (Figure 2.1).

Black rail vocalizations occurred throughout the daily 17:00 - 10:00 h EDT recording window, but the number of verified flags peaked at 7,703 selections between 0:00 h and 1:00 h (Figure 2.2). Vocal activity was highest between 23:00 h and 2:00 h EDT, where the number of verified flags each hour surpassed 7,000 selections. During the evening and

morning hours, small peaks occurred between 19:00 h and 20:00 h and between 6:00 h - 7:00 h EDT. Less than 500 verified flags were observed within each hour interval between 17: 00 h and 18:00 h and between 8:00 h and 10:00 h. It is important to note that the temporal patterns of calling activity reflect counts of 3-second recorded segments with vocalizations rather than individual calls or calling bouts.

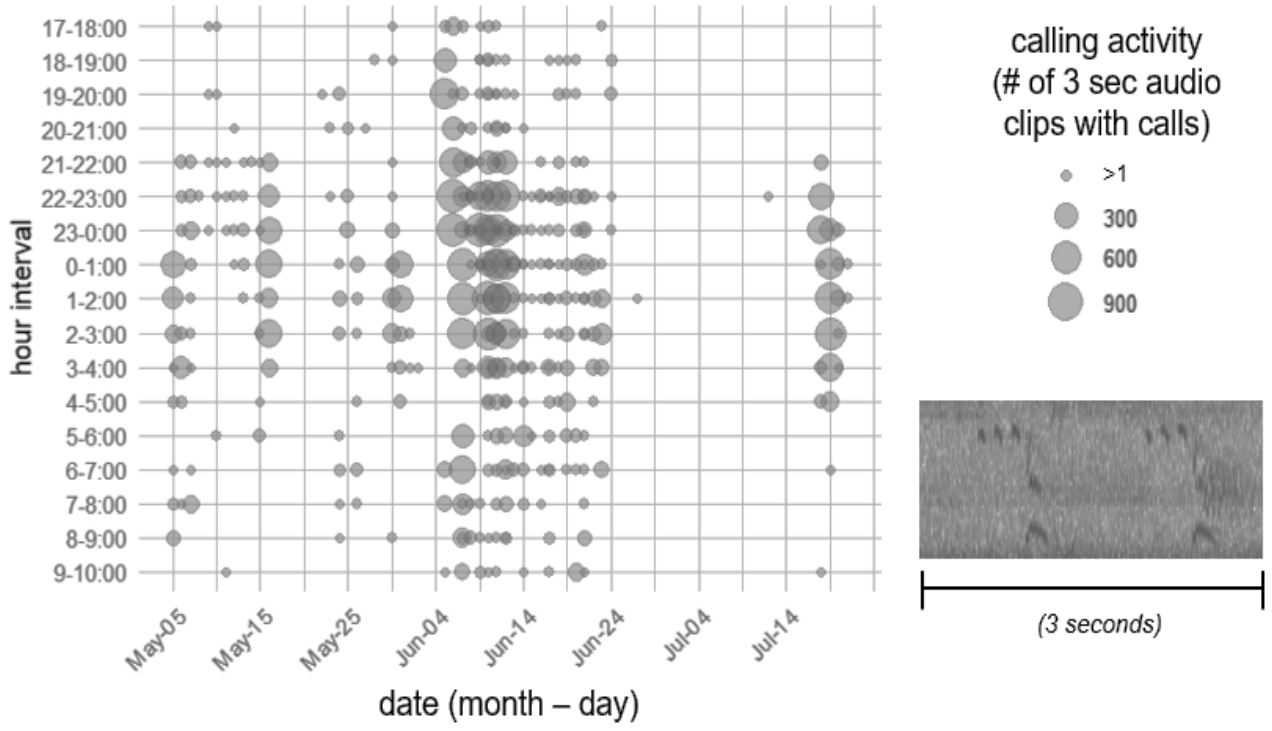

Figure 2.1 Plot showing the count of 3 second audio clips with verified black rail calls by hour (from daily recording schedule start to end) and date, as obtained from recordings of vocal activity from ~ 10 individuals occupying Cedar Island NWR. The spectrogram (bottom right) represents a typical selection with a confirmed black rail mating call, showing the spectral layout of two *kick-kee-doo* call segments. Vocalization counts were highest between 21:00 and 3:00 h and during the month of June. A lack of auditory detections was observed between June 25 and July 2, and no verified activity was present in recordings between July 3-14.

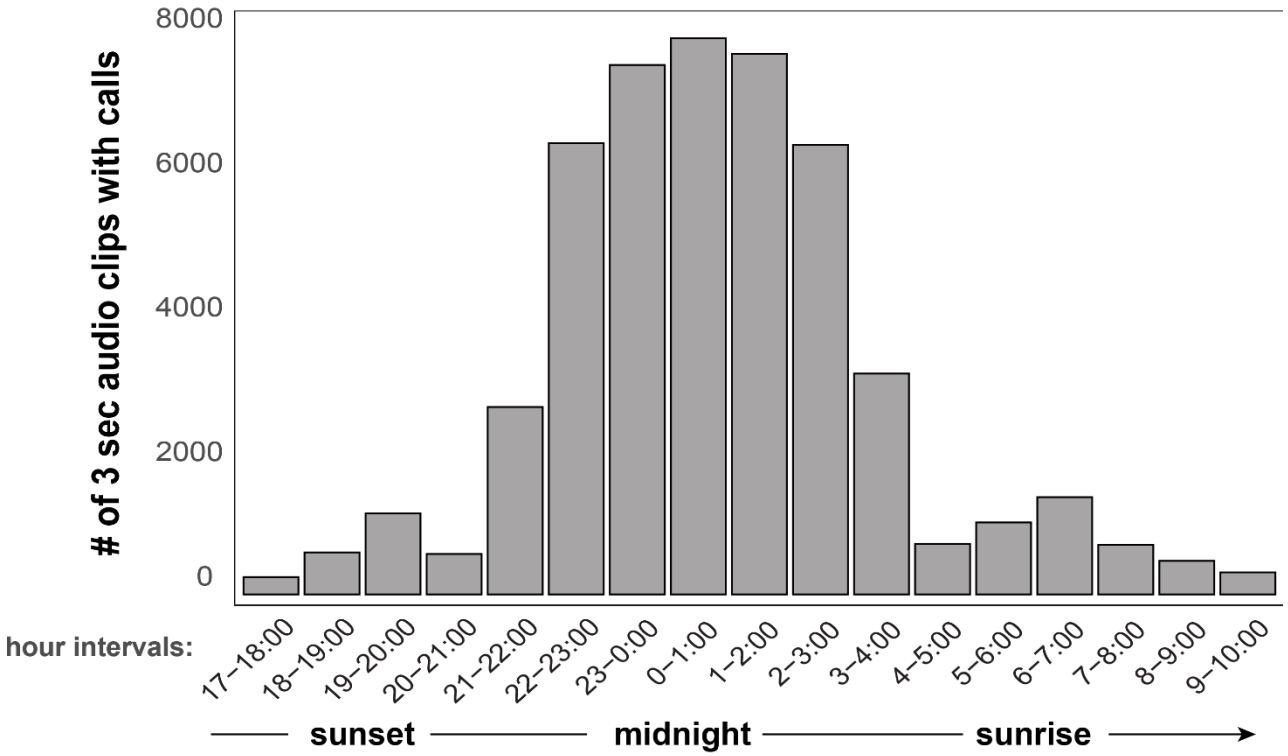

Figure 2.2 The number of 3 second audio clips with verified black rail calls by hour, from daily deployment schedule start to end. Recording cycles started at 17:00 h during the evening, recorded overnight, and ended at 10:00 h the next morning.

Models to identify temporal effects on black rail vocal activity patterns

I assessed the effects of 4 temporal covariates on hourly vocal activity patterns in separate two-stage hurdle models, all with the ARU deployment site included as a random effect. Timing covariates included diel phases as factors (evening, night, or morning) and the hours from midnight (0:00 h). Remaining temporal covariates included environmental conditions such as water level (in cm, measured once hourly) and lunar illumination (lux). For each covariate, results from the zero-inflated binary logistic regression stage (predicting the probability of observing zero) as well as the truncated count model stage (estimating the intensity of vocal activity when >0) are presented.

Black rail vocal activity over time

The probability of black rails calling was significantly affected by diel phase. Zero vocal activity was significantly more likely during the evening (β = 0.66, SE = 0.143, p < 0.001) and morning (β = 1.02, SE = 0.163, p < 0.001) compared to overnight (Figure 2.3). When compared to both morning and evening hours combined, zero activity was significantly less likely during the night (β = -0.81, SE = 0.127, p < 0.001). Considering the counts of 3-sec segments with call detections, black rails were calling significantly less during the morning and evening hours compared to night (β = -0.81, SE = 0.127, p < 0.001). In comparison to both morning and evening phases combined (i.e. night vs. not night), counts were also significantly higher at night (β = 1.2, SE = 0.313, p < 0.001).

As the number of hours away from midnight increased, so did the probability of zero vocal activity (β = 0.59, SE = 0.068, p < 0.001; Figure 2.3). When activity was present, hourly vocalization estimates decreased with increasing distance from midnight (β = -0.86, SE = 0.138, p < 0.001).

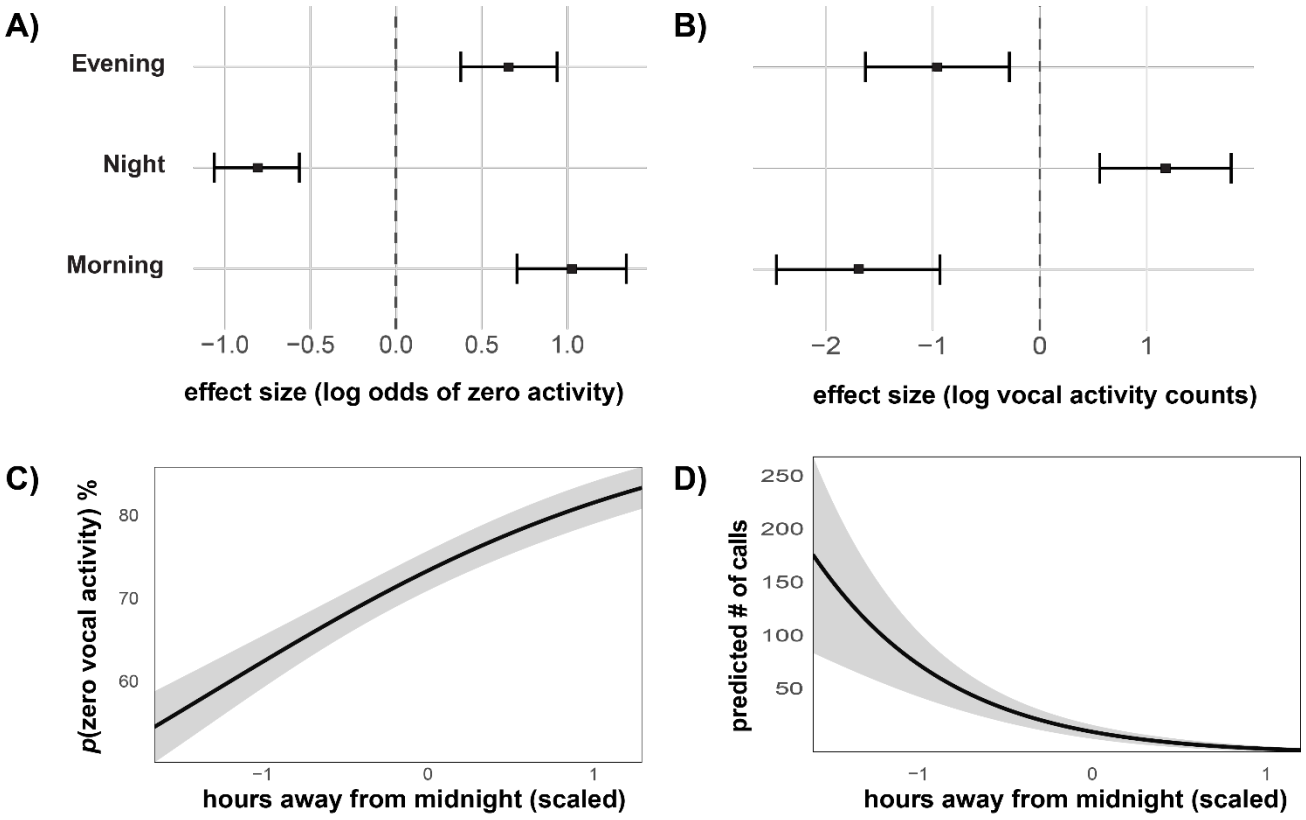

Figure 2.3 Effects of temporal covariates on (A & C) the probability of zero and (B & D) estimated counts of black rail vocal activity. A and B show effect sizes based on hurdle models with the nocturnal period differing significantly from morning and evening hour combined. C shows the increased probability of silence/no activity with the number of hours away from midnight and D illustrates the estimates for counts of 3-sec recordings in relation to hour as the number of hours away from midnight increase.

Effect of environmental conditions on vocal activity patterns

Water level did not strongly influence the probability of black rail vocal activity, but did influence calling rate when activity was >0. As the water level increased, the estimated number of vocalizations decreased significantly (β = -0.08, SE = 0.018, $p < 0.001$; Figure 2.4). There was a slight positive trend of predicted zero activity as water level increased, but this effect was not significant (β = 0.01, SE = 0.008, $p = 0.148$).

Moonlight had a significant effect on the probability and intensity of black rail vocalization. As lunar illumination increased, the probability of zero vocal activity significantly decreased (β = -0.35, SE = 0.070 $p < 0.001$). The estimated number of vocalizations also increased with illumination (β = 0.64, SE = 0.165, $p < 0.001$).

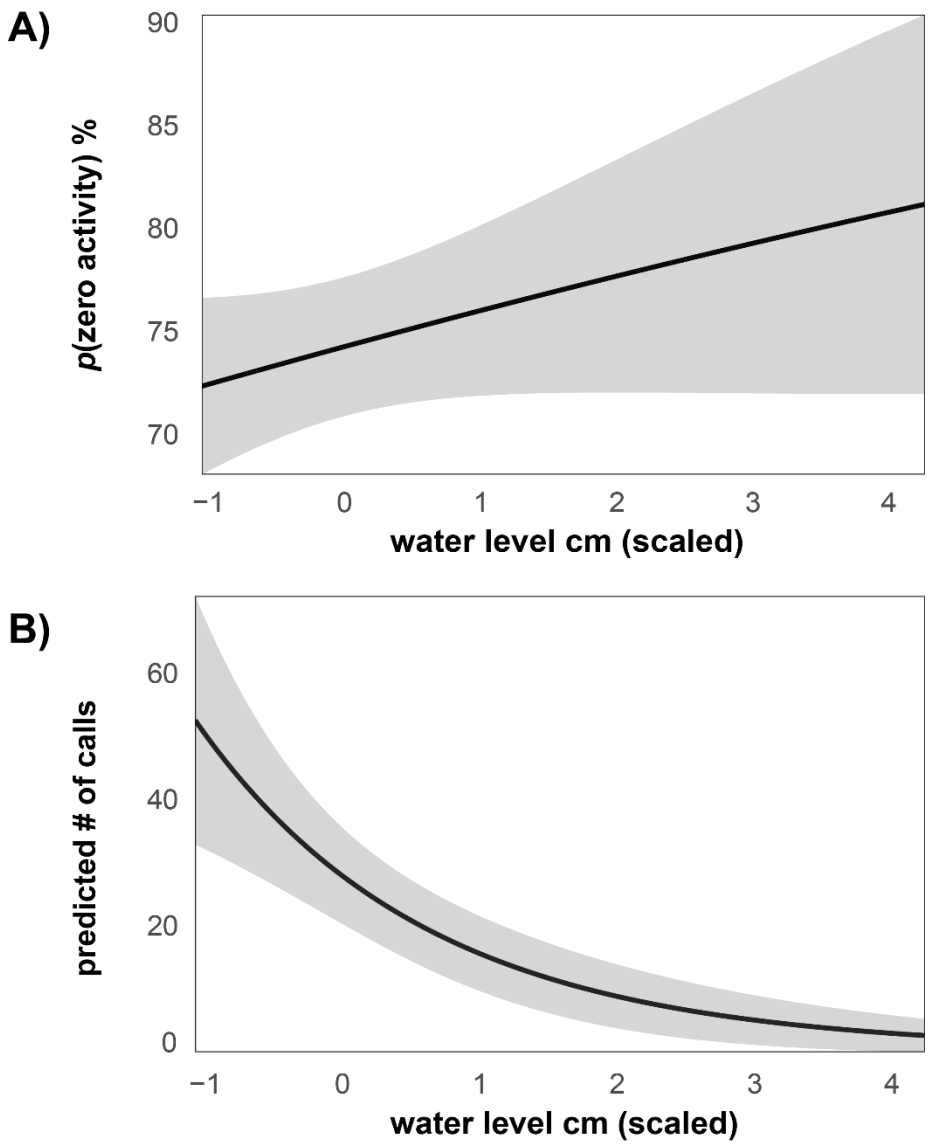

Figure 2.4 Effects of standing water level on the probability of zero and estimated counts of black rail vocal activity over time.

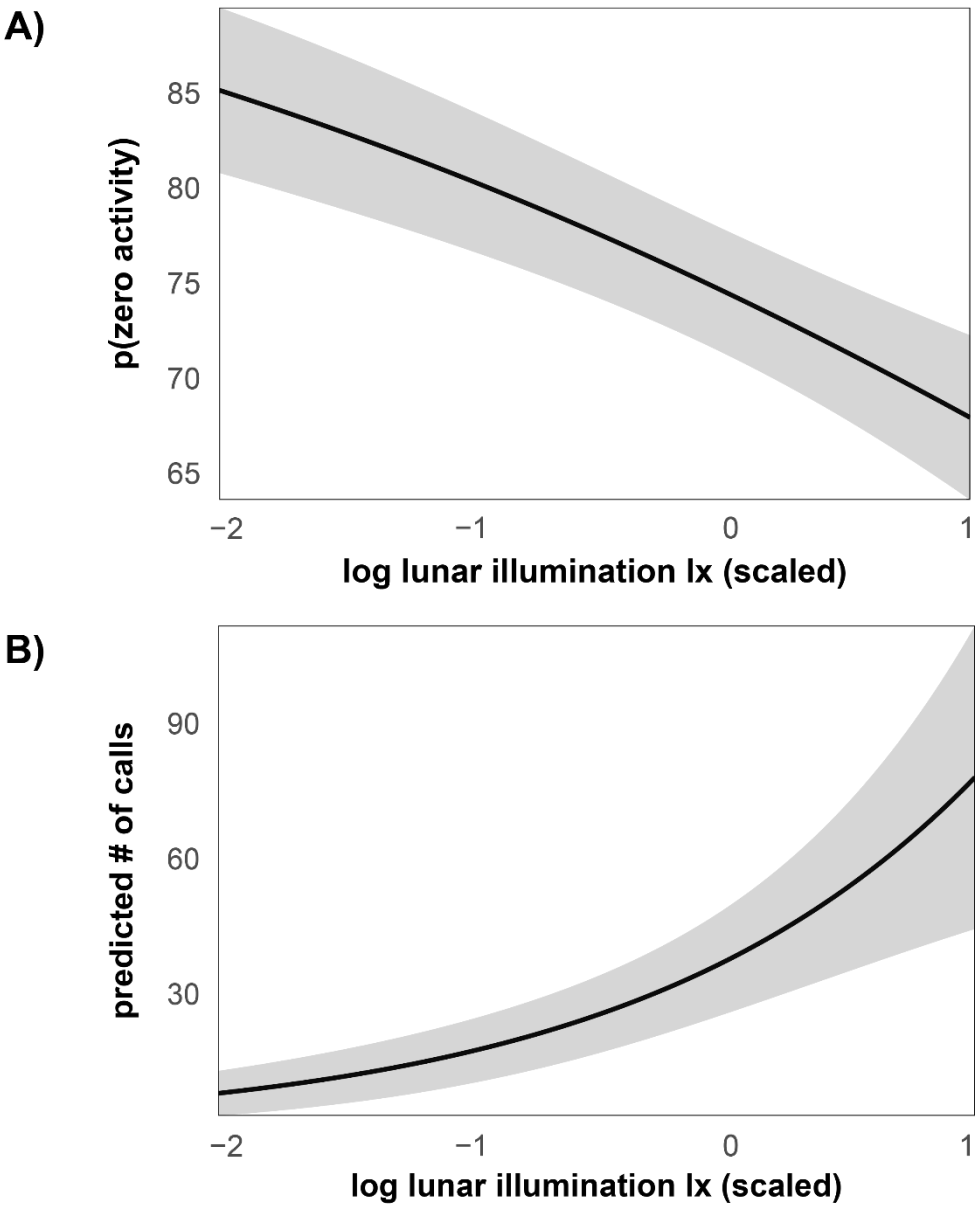

Figure 2.5 Effects of lunar illumination on the probability of zero and estimated counts of black rail vocal activity over time.

Black rail detections with trail cameras

Trail camera deployments and photographs

I deployed 12 trail cameras at Cedar Island NWR, North River, and Salter's Creek GL sites over the course of two breeding seasons. Out of 186,336 total motion-triggered photographs, 116 (0.06%) contained black rails. At North River, black rails were captured by trail cameras both years, while camera detections occurred only in 2022 at Cedar Island NWR and only in 2023 at Salter's Creek GL. Images of co-occurring rallids, such as Virginia rails (*Rallus limicola*), clapper rails (*Rallus crepitans*), and soras (*Porzana carolina*) were documented at all three sites. Many photographs were triggered by windblown vegetation. Other sources of camera triggers included raccoons, rodents, rabbits, and red-winged blackbirds.

The number of black rails and life stages documented

I captured images of 8 black rails: 5 in 2022 and 3 in 2023. One individual was an immature with complete juvenile plumage (≥40 days old) as defined by Hand et al. (2021). This observation confirmed breeding in North Carolina for the first time in the 21st century (Watts 2016). We also captured images of one adult male and three adult females in 2022, followed by two adult males and one adult female in 2023 (Figure 2.6).

Day, time, and habitat conditions at time of detection

Camera detections occurred between June 29 - August 9 in 2022 and between May 29 - July 9 in 2023 (Figure 2.7). The immature triggered the camera on July 14 and July 20, 2022. On August 2, the camera was triggered by an individual likely to be the same individual with more mature plumage and slightly darker eye color (Hand et al. 2021).

One adult male at Cedar Island NWR triggered a camera 6 times from July 13 - August 9 and three females (two at Cedar Island NWR, one at North River) triggered four times between June 29 - August 10 in 2022. In 2023, two males were photographed at Salter's Creek GL five times between May 29 and June 9 in adjacent territories and one female was photographed a single time at North River on July 9 in the same place the immature was detected in 2022.

In spite of the cameras having an infrared flash capable of taking nocturnal photographs, black rails were only captured on camera during the morning, afternoon, and evening between ~ 7:00 - 20:00 h, and never overnight (Figure 2.7). Five out of eight black rails (62.5%) revisited the same camera trap more than once, and one bird triggered the camera twice within a three-hour interval in the afternoon.

At the time of each black rail detection, the peat layer was either dry or saturated with water (well below <5 cm water depth). Temperatures recorded by the trail cameras at the times of black rail detections ranged from 16-40°C.

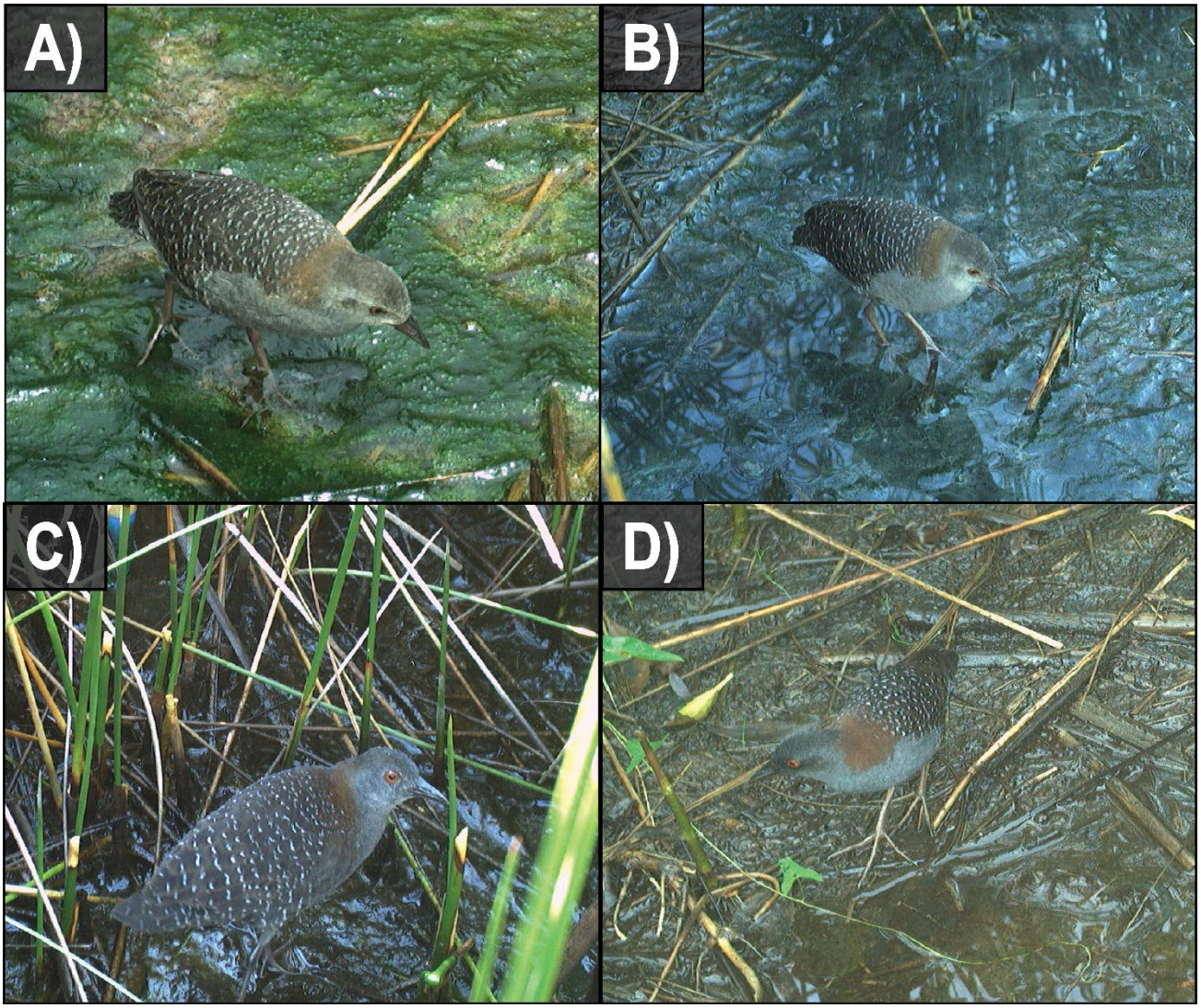

Figure 2.6 Different life stages of black rails captured by trail cameras during the study. The immature bird in complete juvenile plumage (A-B) was identified based on pale plumage over the entire face and nape, faintness or lack of spotting on primaries, and dark amber color of the eye. Plumage maturation and brighter eye color was observed on the immature on August 2, 2022 (B) compared to July 14, 2022 (A). Females (C) were distinguished by the fully red coloration of the eye, contrasting plumage color between the top and sides of the head, and pale chin and throat. Adult males (D) were identified based on the fully red coloration of the eye, dark facial plumage (especially near auricular), and walnut brown hue of the nape (Hand et al. 2021).

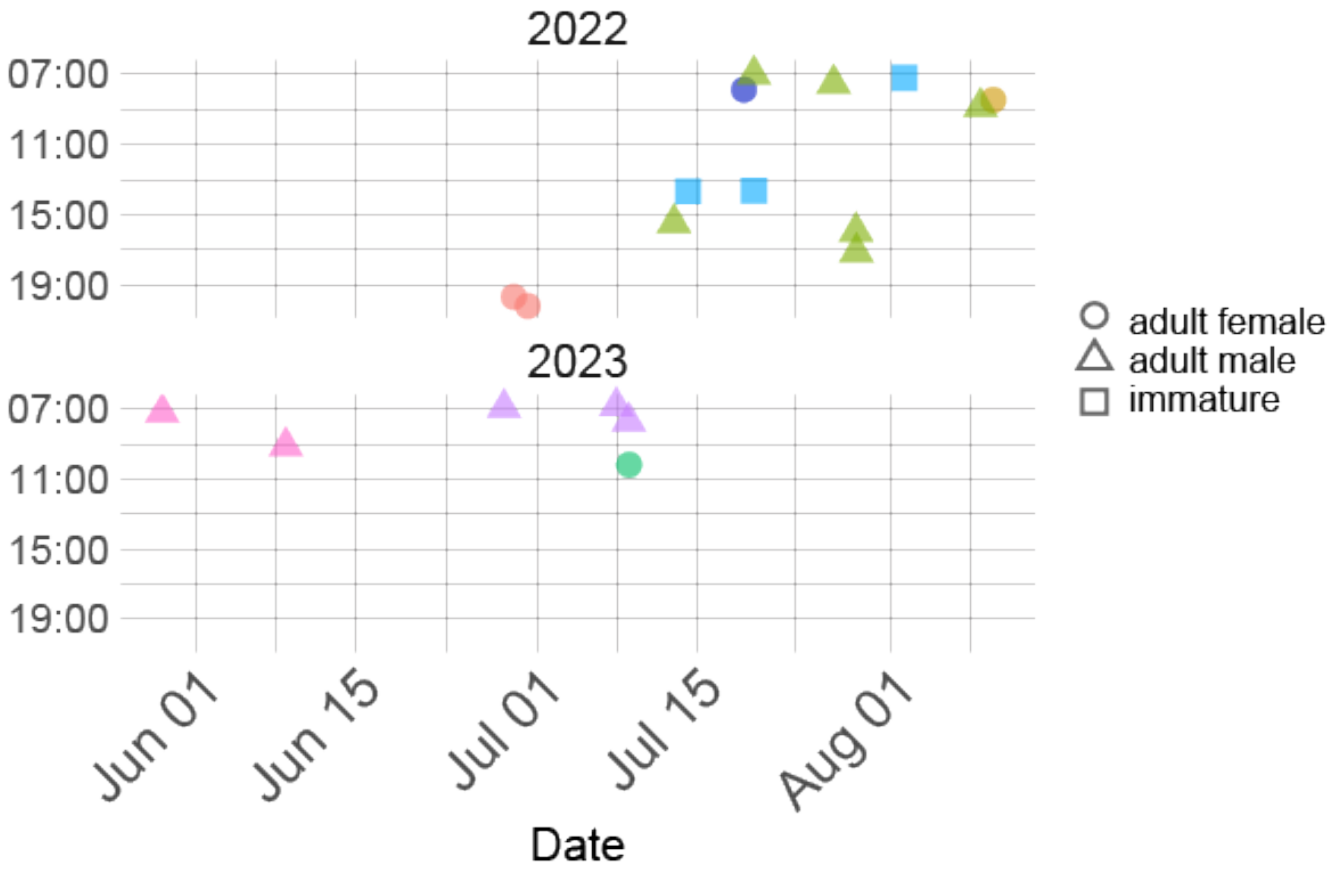

Figure 2.7 Timing of trail camera detections by season and time, with age and sex indicated by shape and individuals by color. All black rail camera detections occurred between May 29 and August 9, between the hours of 7:00 and 21:00 h. In 2022, two adult females were detected at Cedar Island NWR in the same territory (red and yellow circles) and one female was detected at North River (navy blue circle). One adult male (green triangles) was detected six times at Cedar Island NWR. The immature (light blue squares) was detected three times at North River. In 2023, two adult males (pink and purple triangles) were observed at Salter's Creek GL in adjacent territories. One female (green circle) was detected at North River in 2023 in the same location as the immature bird the previous year.

Discussion

I documented black rail activity patterns using passive acoustic monitoring techniques, which will inform knowledge gaps in phenology and behavior during the breeding season. Traditionally, black rails have been monitored with in-person callback surveys that demand considerable effort to attain sufficient detection probability (Flores & Eddleman 1995; Richmond et al. 2008; Johnson & Lehman 2021; Butler et al. 2023). The recommended survey window is during the hours of dawn and dusk for all marsh birds (Conway 2011) but does not consider site-specific variation of calling activity within species. Here, I report that spontaneous black rail vocal activity occurs cyclically outside of this window. Additionally, this study provides the first evidence of black rails breeding successfully in North Carolina in ~ 124 years (Watts 2016). I related the timing of vocal activity and observations of juvenile birds to identify trends in breeding phenology. These results will be used to enhance species monitoring efforts and provide insight regarding the timing of habitat management.

Recognizer performance and detections

I set a low threshold confidence score of $\geq$0.1 when defining criteria for flagged selections from the BirdNET GUI recognizer to review manually. This meant ~ 31% of selections were false positives that required time and labor to sift through. However, the recognizer also identified black rail vocalizations correctly in high and low-noise soundscapes. Out of the selections with true positives, ~ 35,000 (~ 76.9%) were scored $\geq$0.7, but there were more than 10,000 identified selections (~ 23.1%) that were scored lower by BirdNET yet contained black rail calls. True positives with lower scores either demonstrated low signal-to-noise ratio from birds vocalizing far away from the recorder or contained interference from passing cars, high winds, and other birds. While low confidence score thresholds tend to generate more false positives, thresholds set too high increase the

probability of missing detections (Katz et al. 2016; Brauer et al. 2016; Knight & Bayne 2019). Traditional threshold recommendations are generally meant for those using ARUs to assess occupancy of a focal species (Wood & Kahl 2024). Since there are not yet many studies presenting recognizer-detected black rail activity over time, this study provides important baseline information on black rail vocalizations that will be used to set recommendations for confidence thresholds in the future. Findings, which include numerous verified detections below the 90% confidence threshold, suggest that a lower threshold of ~ 70% would be more appropriate for the species instead of 90%.

Each BirdNET recognizer detection was a 3 second clip of audio containing one or more potential vocalizations (Kahl et al. 2021). These detections were manually verified before being included in later analyses. Most of these verified selections included ~ 1-3 *kick-kee-doo* syllables, as this is the primary vocalization type heard during the breeding season (Eddleman et al. 1994). However, the BirdNET model was trained on and was able to correctly identify *churt*, *grr* and *ink-ink-ink* vocalizations as well. It is important to note that the temporal influences on activity examined here reflect the probability and estimated counts of 3-second periods with vocalizations, rather than individual call types.

Seasonality of vocal activity

The calling rate of black rails reached its peak in June, with most vocalizations being the *kick-kee-doo* call. Breeding males use this call to attract mates and possibly broadcast territory ownership, but may be less likely used when actively nesting (Pipelow 2017). A similar seasonal trend in vocal activity (peaking around June) was reported by Butler et al. (2015). However, it remains unclear whether calling birds were between broods or not yet paired during this time.

There was a gap in the days recorded between sites that may have limited the ability to capture the full seasonality of vocal activity. Recording unit deployments were informed by in-person callback surveys, and survey detections did not occur in one of two locations being monitored until late May. Unit deployment was delayed at this location as a result. Due to an ARU malfunction, I was also unable to record here between June 25 - July 2. These circumstances may have led to an incomplete view of the seasonal calling behavior across sites.

Temporal variability in vocal activity

Black rail calls were detected during each hour of the recording cycle, but the probability and frequency of vocalization was highest during the middle of the night. This agrees with the circadian timing of black rail vocal activity reported along the Texas Gulf Coast (Butler et al. 2015). Probability of vocalization and the estimated counts of vocalizations were greater overnight compared with evening and morning, which corroborates the high probability of passive detection between midnight and 04:00 previously reported within the Albemarle-Pamlico Estuary System (Bobay et al. 2018). It was not determined whether callback surveys conducted during the night would yield higher detection probabilities. However, birds can be detected using callbacks during dawn and dusk in spite of less spontaneous vocal activity during the evening (19-20:00 h) and morning (6-7:00 h) periods (Chapter 1).

Only *kick-kee-doo* calls were observed during the night, whereas additional vocalizations (*churt*, *grr* and *ink-ink-ink* calls) were detected during crepuscular hours. Therefore, while vocalization intensity of breeding males is higher overnight, aspects of the broader repertoire used by black rails of both sexes occur on a crepuscular schedule. This coincides with the peaks of activity observed with trail cameras.

These findings are most useful for guiding appropriate hours for passive monitoring. When using acoustic recorders to assess occupancy, deployments scheduled to record between 23:00 h – 04:00 h are significantly more likely to yield detections where black rails are present. It is logical to assume surveying when birds are most active will increase probability of detection overall, but it is not yet known whether elicited calls via playback would yield better results overnight compared to other times. Further investigation is needed into the relationship between the probability of passive vocalizations and playback-initiated response.

Effect of environmental conditions on vocal activity

When birds were active, the frequency of vocalization appeared to be influenced by water level. Vocalization counts were significantly lower when the water level was high. This result illustrates the significant impact of rising water levels on black rail behavior. Black rails are intolerant of water depths greater than a few centimeters (Todd 1977; Flores & Eddleman 1995) and are also more susceptible to predation when habitat is flooded (Evens & Page 1986). The severe negative impact of flooding on black rail nesting success has been documented across states and subspecies (Flores & Eddleman 1993; Legare & Eddleman 2001). The low calling frequency observed during highwater suggests that birds are preoccupied with seeking higher ground, reducing their chances of being detected.

Vocalization probability did not increase significantly at lower water depths, likely because Cedar Island NWR is a larger refuge with extensive marsh and more habitat upslope that birds can retreat to during high water events (Freske 2006). Thus, birds may not have been driven out by excess water but were disrupted in calling frequency when active. The lack of significant effect from water level on vocalization probability points to the benefits of high marsh coverage in managed sites. Increased marsh cover, wetland size, and

proximity to large marsh have all been linked to greater black rail abundance (Spautz et al. 2005; Hargett 2024). Additionally, predation pressures have been shown to significantly increase for tidal marsh species in smaller, fragmented patches (Thorne et al. 2019). While negative impacts of increased flooding on black rail presence may be lessened to some extent in larger tidal habitats, high water could still disrupt breeding behavior.

Black rails were more likely to call when more moonlight was present. Similarly, calling frequency increased with lunar illumination. Moonlight avoidance, i.e., decreased activity during moonlit nights, has been documented in coastal birds such as petrels (*Procellariidae* spp.) due to increased predation pressure (Mougeot & Bretagnolle 2000). In other nocturnal species including the willie wagtail (*Rhipidura leucophrys*), call rate was positively influenced by lunar illumination (Dickerson et al. 2020). Although previous black rail survey study in Florida found higher detection probability at fuller moon phases (Legare & Eddleman 2001), this trend was not observed when modelling moon phase alone. A significant effect on vocal activity was detected, however, when lunar illumination was evaluated with increased temporal and site-specific resolution. Moon phase is often used as a proxy for night brightness when monitoring nocturnal species, but it does not fully capture the way lunar illumination on the ground varies by latitude, elevation, and moon altitude (Kyba et al. 2017; Śmielak 2023). Results here indicate that, when compared to moon phase, ground-level moonlight brightness is a better predictor for the fluctuations in calling behavior. Since only *kick-kee-doo* calls used by breeding males were recorded at night, the benefits of advertising to potential mates during nocturnal hours appear to outweigh the risk of potential predation (Schmidt & Belinksy 2013).

Limitations were still present in site-specific accuracy of moonlight brightness, as cloud cover and other weather variables are not accounted for. It is also possible that night

activity was affected by artificial light pollution, as there were streetlights and traffic along the highway intersecting the marsh, ~ 200 meters from each site where the ARUs were deployed.

Breeding phenology and activity documented

Photographs of an immature black rail were captured in Carteret County, NC. This marks the first photographic evidence of breeding success for the species in North Carolina. Breeding records for black rails in the state are scarce: only three known accounts exist (Watts 2016). In 1887, a single nest with six eggs was reported by natural historian John S. Cairns in Buncombe County (Simpson 1978). Between 1890-1898, ornithologist Witmer Stone received seven nests collected from Raleigh, NC (Stone 1900). Many of these records are of breeding attempts, as collected nests were found damaged or destroyed (Simpson 1978; Stone 1900). In 1942, T. Gilbert Pearson documented the only black rail juveniles within the state: two downy chicks captured and donated by a local farmer in 1893 (Pearson et al. 1919). This study provides confirmation of successful breeding and reports the only documented independent young.

Calling activity was greatest in June, and the immature black rail was captured on a trail camera in July and early August. Additionally, *ink* calls—vocalizations primarily associated with nest defense (Conway 2011)— were captured on ARUs in mid-June. During the 2024 breeding season, a ~2 week old chick was detected on a trail camera during the second week of July in the same area as the immature reported here in 2022 (S. McRae, pers. comm.). Though vocal activity was not documented during every date range, evidence from vocal activity and the camera observations of the immature bird (~6 weeks old in mid-July and ~8.5 weeks in early August) suggest that it hatched around the first week of June. Therefore, the clutch was likely laid in the second week of May, brood rearing likely occurred

in early June, and independence (i.e., no parental care) occurred in late June (~3 weeks of age). This estimated timeline is based on the breeding schedules described for a well-studied population in South Carolina (Hand et al. 2021) and is also consistent with the detection of a black rail chick captured on a trail camera during late June in Louisiana (Monopoli et al. 2024). There may be variation in the duration of the breeding season of each population, and more observations are needed to determine the breeding phenology of this population. More passive acoustic studies using recorders in conjunction with camera traps will be used in future to determine whether this pattern is typical.

Black rails were detected on cameras only during morning and evening hours, but most often in the morning. Individual black rails that triggered the camera traps on multiple days did so at nearly the same time and approached from the same direction, revealing habitual movement patterns. One adult male triggered the camera trap within the same hour interval about once per week over a period of 27 days between July 13- August 9. Generally, when multiple visits occurred, they were observed between 2 and 11 days apart.

Foraging behaviors were observed when the field of view had ~3 cm of standing water. When the substrate was dry, birds moved through the frame of view quickly. Black rail diet and feeding behavior is not well documented, but past observations have reported observing birds foraging for small aquatic invertebrates near edges of emergent vegetation stands (Eddleman et al. 2020). These behaviors suggest that shallow, saturated conditions may be important to maintain in areas selected for management.

Implications for monitoring and management

Passive monitoring revealed that black rails in this area vocalize at times spanning the evening, night, and morning, but mating calls are heard significantly more during the night. There is a clear circadian pattern of vocal activity when passively sampled. This information

could lead to more efficient survey efforts. Overnight point visits between 23:00 - 04:00 h on nights with high lunar illumination could improve detection rates, but safety and risk for personnel should be considered. While overnight hours may be the best time to assess occupancy via passive acoustic monitoring, whether call-response survey detection would improve in this time period remains to be investigated.

Trail cameras and ARUs reveal that black rail activity is significantly impacted by hydrology. At Cedar Island NWR, management actions that facilitate marsh migration have been identified as a priority so that birds may retreat from habitat affected by sea level rise (Watts 2020). These management actions are critical for maintaining suitable habitat in tidally influenced wetlands and ensuring black rails can persist in these newly confirmed breeding sites.

The detection of an immature black rail provides evidence of breeding activity spanning at least May through July. Management actions, such as prescribed burns, on occupied habitat during this time could negatively impact breeding success if caution is not taken. Early winter burns are recommended in areas with breeding rail populations (Haverland 2019). Further, burns conducted in early winter that were patchy and did not burn all emergent vegetation facilitated the maintenance of suitable breeding conditions (Hand et al. 2021), whereas more complete winter burns have resulted in direct mortality of black rails (Legare et al. 1998). Results here and in Chapter 1 support prescribed fire management for black rails with consideration of timing and burn intensity.

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Appendix A

Table A1. Comparison of coarse vegetation cover type estimates at sites where black rails were detected and not detected, separated by year. In 2022, this included 11 occupied sites and 43 unoccupied sites with 25 at Salter's Creek GL, 22 at Goose Creek GL, 5 at Cedar Island NWR, and 2 at North River. In 2023, this included 12 occupied sites and 37 unoccupied sites with 24 at Salter's Creek GL, 20 at Goose Creek GL, 4 at Cedar Island NWR, and 1 at North River. Significant results are bolded.

Parameter	**Mann-Whitney *U***	***p***
2022		
% marsh elder *(Iva frutescens)*	243.0	0.87
% black needlerush (*J. roemerianus*)	310.5	0.11
% low marsh cord grasses	152.5	0.06
% mixed grasses	**333.5**	**0.04**
% common reed (*P. australis*)	**154.0**	**0.03**
% forest cover	234.5	0.96
% water cover	**27.0**	**>0.0001**
2023		
% marsh elder *(Iva frutescens)*	276.0	0.19
% black needlerush (*J. roemerianus*)	**308.5**	**0.045**
% low marsh cord grasses	**135.0**	**0.02**
% mixed grasses	297.5	0.08
% common reed (*P. australis*)	**138.0**	**0.01**
% forest cover	224.5	0.96
% water cover	**67.0**	**0.0003**

Table A2 Mean values and standard errors for the visual obstruction and disc settling height measurements at points sampled between years (N= 12). During fine-scale vegetation samples, six measurements were taken for each variable per site. Mean visual obstruction increased significantly one year post-burn, but disc settling height did not.

	2022				2023			
	burned	unburned	occupied	unoccupied	**burned**	unburned	occupied	unoccupied
Visual obstruction (cm)	**30.3 ± 4.6**	52.6 ± 4.8	35.0 ± 5.0	49.3 ± 6.5	**52.4 ± 4.7**	56.9 ± 7.8	63.2 ± 9.1	49.1 ± 4.3
Disc settling height (cm)	**32.3 ± 3.1**	47.7 ± 6.9	30.2 ± 4.5	49.3 ± 5.9	**33.2 ± 3.6**	48.1 ± 6.9	47.5 ± 9.6	37.9 ± 4.5

Table A3. Comparisons of coarse vegetation cover type estimates at burned and unburned sites, separated by year. In 2022, vegetation was surveyed at 20 burned and 34 unburned sites. In 2023, vegetation was surveyed at 18 burned and 31 unburned sites. Significant results from Mann-Whitney *U* tests are bolded: only black needlerush was significantly more prevalent at burned sites.

Parameter	2022		2023	
	Mann-Whitney *U*	*p*	Mann-Whitney *U*	*p*
% marsh elder *(Iva frutescens)*	273.0	0.15	235.0	0.34
% black needlerush (*J. roemerianus*)	**450.0**	**0.05**	**385.0**	**0.03**
% low marsh CG	274.5	0.23	259.0	0.64
% mixed grasses	252.5	0.11	241.5	0.44
% common reed (*P. australis*)	264.5	0.09	243.0	0.36
% forest cover	339.0	0.99	256.5	0.62
% water cover	338.0	0.98	220.0	0.22

Table A4. No change in land cover types in burned areas between year of burn and one year post-burn. Land cover data were from coarse vegetation surveys conducted at the same 17 points in areas burned in March 2022. Paired comparisons using Wilcoxon's ranked sign tests revealed no significant differences.

Parameter	*W*	*p*
% marsh elder *(Iva frutescens)*	26.0	0.15
% black needlerush (*J. roemerianus*)	47.0	0.17
% low marsh cordgrasses	47.0	0.29
% mixed grasses	62.0	0.58
% common reed (*P. australis*)	41.0	0.67
% forest cover	40.0	0.63
% water cover	39.5	0.08

Appendix B

ECU

Animal Care and Use Committee
003 Ed Warren Life Sciences Building | East Carolina University | Greenville NC 27834 - 4354
252-744-2436 office | 252-744-2355 fax

February 24, 2022

Susan McRae, Ph.D.
Department of Biology, ECU

Dear Dr. McRae:

Your Animal Use Protocol entitled, "Monitoring secretive marshbirds in coastal refuges and associated wetlands" (AUP #D339b) was reviewed by this institution's Animal Care and Use Committee on 02/24/2022. The following action was taken by the Committee:

"Approved as submitted"

****Please contact Aaron Hinkle prior to any hazard use****

A copy of the protocols is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP/Amendment and are familiar with its contents.**

Sincerely yours,

Jamie DeWitt, Ph.D.
Vice-Chair Animal Care and Use Committee

JD/GD

enclosure