Black Rail Occupancy in the CBBEP Boundary

Final Report

Publication CBBEP – 191
Project Number – 2320
May 2025

Prepared by:

Wei Xu
Department of Life Science
Texas A&M University - Corpus Christi
6300 Ocean Drive Corpus Christi, Texas 78412

Submitted to:
Coastal Bend Bays & Estuaries Program
1305 N Shoreline Blvd, Suite 205
Corpus Christi, TX 78401

PREPARED IN COOPERATION WITH THE TEXAS COMMISSION ON ENVIRONMENTAL QUALITY
The preparation of this report was financed through funding from the Texas Commission on Environmental Quality.

Black Rail Occupancy in the CBBEP Boundary

Prepared by

Wei Xu
Department of Life Science
Texas A&M University - Corpus Christi
6300 Ocean Drive Corpus Christi, Texas 78412

Beau Hardegree
U.S. Fish & Wildlife Service
4444 Corona Drive. Suite 215. Corpus Christi. TX 78411

Ashley Reed
U.S. Fish & Wildlife Service
4444 Corona Drive. Suite 215. Corpus Christi. TX 78411

Jaclyn Tolchin
Conservation Metrics, Inc.
145 McAllister Way. Santa Cruz, CA 95060

Abram B. Fleishman
Conservation Metrics, Inc.
145 McAllister Way. Santa Cruz, CA 95060

Submitted to:
Coastal Bend Bays & Estuaries Program
1305 N Shoreline Blvd, Suite 205 Corpus Christi, Texas 78401

TABLE OF CONTENTS

..... 1

Introduction 1

Figure 1. Site map of ARUs. Includes site locations as well as the locations of all 19 sensors. 3

Methods 4

Acoustic Sensors and Survey Design 4

Table 1. Deployment table showing each site, recording unit, site location, and the first and last recording from each site. 4

Analysis Environment 4

Automated Call Detection 5

BirdNET 2.4 5

Custom Classification Model 5

Model Review 6

Figure 2. Spectrogram of a black rail “kee-kee-jeer” song. Note that the energy band at 5 kHz is cricket signals, not part of the rail vocalization. 6

Figure 3. Spectrogram of a black rail “chatter” call. This is likely a pair giving a duet; pairs often alternate notes so rapidly it is difficult to distinguish that there are two individuals calling. 7

Acoustic Activity 7

Water sample collections 7

Table 2. Number of water samples collected from each ARU spot. 8

Water sample process and DNA extraction 8

qPCR with eDNA samples 8

Nondestructive vegetation sampling 9

Results 10

Acoustic Device Deployment 10

Table 3. Total number of days and hours of recordings that were included in the analysis for each site. 10

Figure 4. Total hours of recordings collected at each site per day that were included in the analysis. 11

Black Rail Vocal Activity 12

Table 4. Table summarizing black rail detections at high-activity sites. 12

Table 5. Table summarizing total black rail bouts detected at low-activity sites. 12

Figure 5. Map of survey locations in southern Texas. 13

High-activity Sites 13

Figure 6. Black rail vocalization rate (bouts per minute) per site at high-activity sites. 14

Figure 7. Black rail vocal activity throughout the season at high-activity sites. 15

Figure 8. Raster showing black rail vocal activity patterns throughout the day and season at each high-activity site. 16

Figure 9. Daily black rail activity patterns throughout the day at each high-activity site relative to sunrise. 17

Low-activity Sites 18

Figure 10. Number of black rail bouts detected per site at low-activity sites. 18

Table 6. Number of black rail bouts per date for each of the low-activity sites. 18

Table 7. Number of potential black rail bouts per date for low-activity sites CBBEP01, CBBEP04, and KlebergTract_02. 19

Detection of Black Rail eDNA 19

Figure 11. Identification of BLRA presence with the qPCR assay developed by Neice *et al*. 20

Vegetation Characterization of Sites 20

Discussion 24

Figure 12. Identification of BLRA presence with qPCR developed by Feist *et al*. 26

Literature Cited 28

Appendix 31

Introduction

Black rail (BLRA, *Laterallus jamaicensis*) is a secretive marsh bird with a sporadic breeding distribution throughout the Americas and requires tidal marshes, freshwater wetlands, or flooded grassy vegetation for nesting and foraging, thus, the species is currently restricted to small, fragmented areas where sufficient marshy habitat exists. Throughout the United States, there has been significant salt marsh and wetland removal (for land conversion) and degradation (due to pollution, water management) (Eddleman et al., 2020). This habitat loss has resulted in a disproportionately high number of threatened species that rely on wetland habitat, including the black rail. Most of the Black rail species were listed as endangered by the International Union for the Conservation of Nature (IUCN, 2021). Laws and framework that protect salt marsh habitat are likely the only way to prevent further population declines for black rail.

Males and females are similar in size, and adults are generally pale to blackish gray, with a small blackish bill and bright red eyes. BLRA forages on a variety of small (< 1 cm) aquatic and terrestrial invertebrates, especially insects, and seeds (e.g., *Typha*, *Scirpus*, *Spartina* spp.). Its habitat can be tidally or non-tidally influenced, and range in salinity from saltwater to brackish to freshwater. It requires dense overhead perennial herbaceous cover with underlying soils that are moist to saturated (occasionally dry) interspersed with or adjacent to very shallow water (typically $\leq$ 3 cm). Plant structure is considered more important than plant species composition in predicting habitat suitability since this species requires dense vegetative cover that allows movement underneath the canopy and is found in a variety of marsh habitats with a large salinity range (Flores & Eddleman, 1995). BLRA depends on this dense cover throughout their life cycle and is their primary strategy to avoid predation. Researchers working long periods (months to years) at locations where the species is present rarely see birds.

As a subspecies of BLRA, the eastern BLRA (*L. jamaicensis jamaicensis*) was listed as threatened in 2020 by the U.S. Fish and Wildlife under the Endangered Species Act. The wetlands in southern Texas were known to be the habitats of the eastern BLRA. The Coastal Bend Bays and Estuaries Program (CBBEP) has been dedicated to the restoration and protection of wetlands in these areas and the CBBEP boundary is just south of the known breeding range of eastern black rail and within the range of historical detections (Watts, 2016). However, little is known about the eastern BLRA's current distribution within the program boundary. The adult eastern BLRA are small (6in, 30g) and rarely seen among the concealing saltmarsh vegetation, making population monitoring particularly challenging for this species.

Acoustic cues have long been an important part of bird monitoring projects (Sauer et al., 1994). Technological innovations now make it possible to deploy weatherproof acoustic sensors that can reliably sample the acoustic environment for months at a time without maintenance. Hundreds of hours of field recordings can then be processed with pattern recognition software using deep learning and artificial neural network techniques to derive measures of acoustic activity rates for species of interest. This combination of passive acoustic sensors and automated call detection is especially powerful for monitoring rare/elusive species (Acevedo & Villanueva-Rivera,

2006; Scott Brandes, 2008).

In this study, the eastern BLRA surveys were performed within the CBBEP boundary using automated recording units (ARUs) in order to detect BLRA presence based on their vocalizations. This survey method takes advantage of the social behavior that occurs at and around breeding sites, including bird vocalizations for mate attraction and territory defense. Automated acoustic sensors and automated acoustic classification techniques now make it possible to efficiently detect and quantify vocalizations in large datasets. This technology enables researchers to greatly increase the spatial and temporal scale of acoustic surveys - improving detection probabilities for rare and elusive species. The increased survey effort enabled by passive acoustic monitoring is particularly helpful for identifying previously unknown breeding sites, as well as improving the statistical power of long-term monitoring projects when compared to less intensive monitoring methods (MacKenzie et al., 2002; MacKenzie et al., 2005)

In addition to the acoustic cue survey, several studies have attempted to detect the DNAs of birds mixed with the environmental DNAs (eDNA), which refer to the DNAs shed by the organisms. For example, by collecting the water samples from the drinking water sources of the Gouldian finch (*Erythrura gouldiae*), an endangered species from Australia, researchers identified the habitat of this bird (Day et al., 2019). Another study used the eDNA isolated from the saliva left on food remains to detect the presence of scarlet macaw (*Ara macao*) in Costa Rica (Monge et al., 2020). Most of the eDNA studies applied to the birds were based on two techniques. Metabarcoding DNA detections were done by the next-generation sequencing combined with universal primers designed for a phylogeny of organisms. For example, universal bird primers targeting the 12S subunit of RNA were used to enrich the avian DNA fragments from the eDNA. The enriched avian DNA fragments were then sequenced using the MiSeq platform to recognize the different species of birds (Ushio et al., 2018).

Another eDNA identification technique is based on the quantitative PCR (qPCR), which targets only one species. For this species-specific assay, mitochondrial genes were usually selected to be the target due to the advantage that individuals carry only one haplotype inherited through the maternal line. Additionally, the mitochondrial DNA is highly variable among species compared to the nuclear genomes of the organisms (Neiman & Taylor, 2009). Among those mitochondrial genes, cytochrome c oxidase subunit I (COI) was often chosen as the target because of its conserved sequences within species and high sequence variability between species (Hebert et al., 2003). A previous study suggested that a fragment in COI DNA with just 648 bases can distinguish over 260 bird species (Hebert et al., 2004).

Because of the secretive nature of this bird, very little is known about the occurrence of the eastern BLRA within the CBBEP boundary. Supporting inventory and monitoring efforts to improve our understanding of the distribution and abundance of existing BLRA populations is listed as a need for the species in the Recovery Outline. To help support this need, we conducted surveys of BLRA within the CBBEP boundary, including within the Nueces River Delta, Mission River Delta, and on Padre Island. (Figure 1). Surveys were conducted using ARUs and environmental DNA (eDNA).

Vegetation and wetland characteristics (species, cover, height, and soil moisture) were also collected at each site in an effort to understand the habitat use of black rails in the study area.

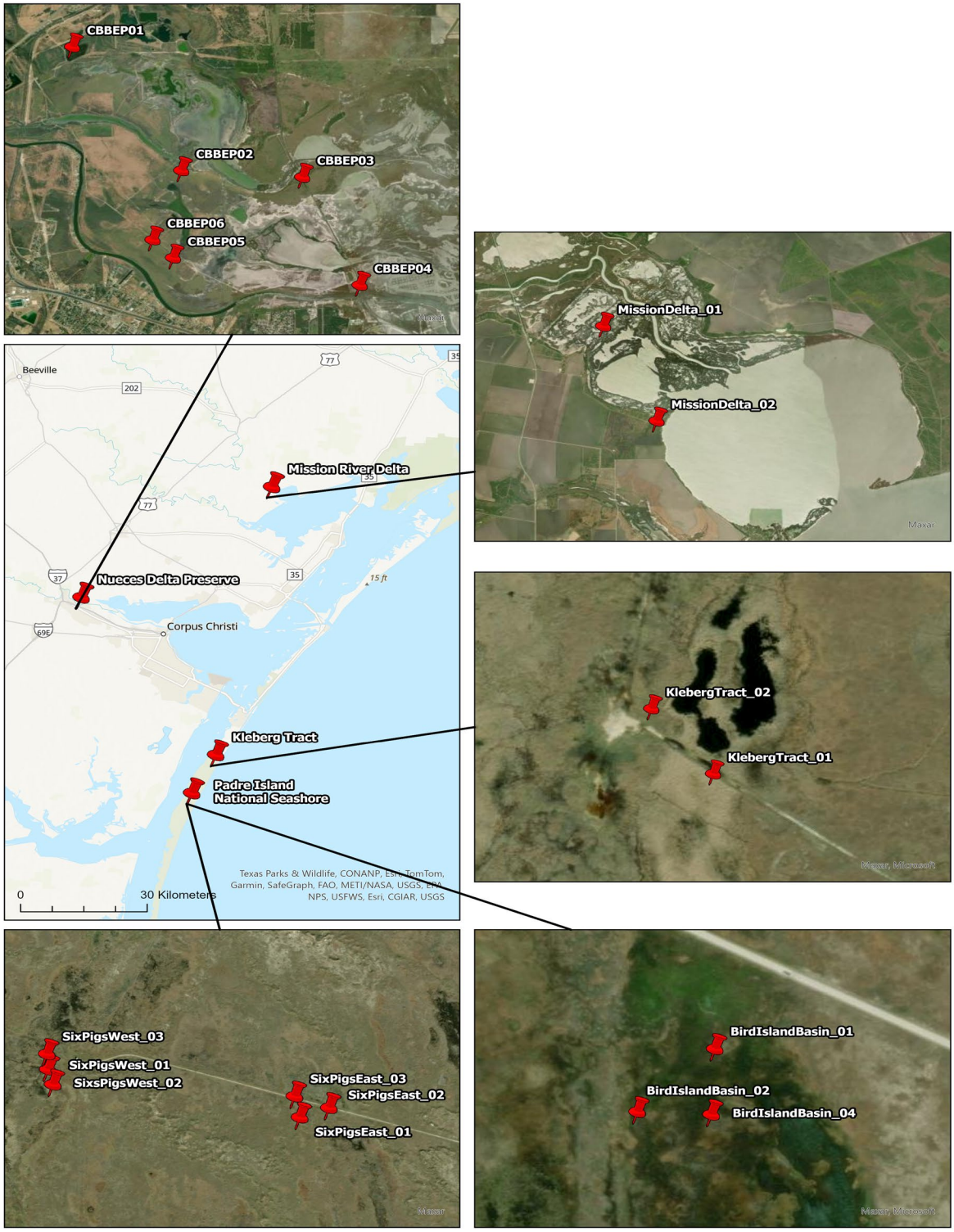

Figure 1. Site map of ARUs. Includes site locations as well as the locations of all 19 sensors.

Methods

Acoustic Sensors and Survey Design

Song Meter 4 (SM4) sensors, manufactured by Wildlife Acoustics, were used to collect acoustic recordings. Recordings were collected in stereo with two built-in SM4 microphones. The same settings were used for both channels: 24,000 Hz sample rate, 16dB gain, 26dB preamp, and no high-pass filter.

Sensors were positioned on T-posts approximately one meter above the ground in suitable wetland habitat within the CBBEP boundary at Padre Island National Seashore, the Nueces River Delta, and the Mission River Delta (Table 1). Sensors (Figure 1) were deployed from April to July 2024 and programmed to record continuously for three hours surrounding sunrise and sunset and record one of every five minutes throughout the rest of the day. A subset of the continuous three-hour recording blocks of each recording was analyzed.

Table 1. Deployment table showing each site, recording unit, site location, and the first and last recording from each site.

Survey Site	Recording Unit	Latitude	Longitude	First Recording	Last Recording
BirdIslandBasin_01	PAIS8	27.46532	-97.29695	2024-04-25 11:20:00	2024-06-22 12:55:00
BirdIslandBasin_02	PAIS9	27.46473	-97.29758	2024-05-20 11:05:00	2024-06-11 12:55:00
BirdIslandBasin_04	PAIS10	27.46471	-97.29698	2024-04-25 10:45:00	2024-06-26 15:30:00
CBBEP01	TAMU1	27.91353	-97.61055	2024-04-03 09:58:00	2024-07-01 13:10:00
CBBEP02	TAMU2	27.88724	-97.59023	2024-04-03 10:40:00	2024-07-01 13:40:00
CBBEP03	TAMU3	27.88585	-97.56764	2024-04-03 11:05:00	2024-07-01 13:50:00
CBBEP04	TAMU4	27.86284	-97.55696	2024-04-03 11:56:00	2024-06-25 02:25:00
CBBEP05	TAMU5	27.86864	-97.59176	2024-04-03 12:37:00	2024-07-01 14:30:00
CBBEP06	TAMU6	27.87251	-97.59567	2024-04-03 12:52:00	2024-07-01 14:20:00
KlebergTract_01	TAMU10	27.51303	-97.27017	2024-04-25 15:55:00	2024-07-03 11:40:00
KlebergTract_02	TAMU7	27.51364	-97.27068	2024-04-25 15:55:00	2024-06-06 04:30:00
MissionDelta_01	TAMU8	28.17673	-97.19594	2024-05-03 10:00:00	2024-07-01 16:40:00
MissionDelta_02	TAMU9	28.15658	-97.18599	2024-05-03 11:19:00	2024-07-01 16:00:00
SixPigsEast_01	PAIS7	27.37265	-97.32981	2024-04-25 06:40:00	2024-07-03 12:50:00
SixPigsEast_02	PAIS3	27.37299	-97.32890	2024-04-25 14:55:00	2024-07-03 12:55:00
SixPigsEast_03	PAIS6	27.37340	-97.32997	2024-04-25 13:50:00	2024-07-03 12:50:00
SixPigsWest_01	PAIS1	27.37436	-97.33769	2024-04-25 14:25:00	2024-07-03 12:45:00
SixPigsWest_02	PAIS2	27.37380	-97.33552	2024-04-25 14:35:00	2024-07-03 12:40:00
SixPigsWest_03	PAIS5	27.37491	-97.33772	2024-04-25 14:40:00	2024-07-03 12:45:00

Analysis Environment

A custom cloud-based workflow was used to process and review data from this project. Acoustic data were renamed and reorganized locally, then sent to Microsoft Azure Blob Storage, where they were processed on a scalable computer service called Azure Batch. Processing included four components: metadata extraction, acoustic metric generation, application of Convolutional Neural Network (CNN) detection models, and databasing the results. High probability detections were reviewed in the

Conservation Metrics web app (Auditor 2) which allows us to view spectrograms and listen to sound clips, facilitating data review and labeling.

Automated Call Detection

All acoustic recordings were analyzed using two CNN detection models: 1) BirdNET 2.4, an open-source bird detection and classification model (Kahl et al., 2021), and 2) a custom bird call detection and classification model trained specifically to detect and classify black rail “kee-kee-jeer” and “chatter” calls. Both models identify acoustic events with a high probability of containing focal signals.

BirdNET 2.4

BirdNET 2.4 is an open-source bird-sound detection and classification model (Kahl et al., 2021). It was trained on thousands of example recordings from North America and Europe and includes over 2,300 species. BirdNET is a CNN that relies on learned acoustic features to detect and classify bird sounds. Returned predictions consist of a predicted species and a confidence score that ranges between 0 and 1. Predictions can be filtered using records from eBird for the time of year and location where the recording was made. This works well when there are extensive eBird records for the study area, however, in remote locations or for projects that rely on site-to-site comparisons, spotty eBird records can confound results by providing an incomplete list of species in each week of the year or changing the list of species that are returned for each site. BirdNET was run without supplying a geographic location and week of year on all project recordings. This left the species list unrestricted so that predictions for all species were returned.

Custom Classification Model

A custom-built avian detection and classification model designed to detect black rail vocalizations was developed by Conservation Metrics (Santa Cruz, CA). The approach was based on a machine learning technique known as Convolutional Neural Network (CNN) models, where an algorithm is trained to detect a unique combination of spectro-temporal features found in target sounds (i.e., vocalizations from the species of interest). These models can then be used to search field recordings for sounds with the same combination of features. CNNs are a powerful classification tool used in many fields to perform speech recognition, image recognition, and computer vision tasks (Cichy et al., 2016; Deng et al., 2013; Schmidhuber, 2015; Yousef & Allmer, 2023).

The field recordings were first split into two-second clips and Mel spectrograms were used as raw data inputs. A multi-label CNN classification model was then trained using training and cross-validation datasets containing examples of positive sounds for each class (vocalizations from target species) and a representative example of “negative” sound clips (sound clips from all survey sites that do not contain the species of interest). The CNN learned what features from the Mel spectrograms best differentiate each target species from other sounds in the environment (and from other target species). The trained CNN was then applied to acoustic data from survey sites, returning a confidence score that a given two-second window of field recordings contained a sound produced by each target species.

A multi-class detection model ("Padre_Island_v01") was built for this project in 2023 using data from the 2022 season recorded at Padre Island National Seashore. Data from the nearby Texas Mid-Coast National Wildlife Refuge Complex, provided to us by the U.S. Fish and Wildlife Service, was also used, which included more examples of black rail vocalizations since rails were rare in the project data that year. The analysis targeted the black rail stereotyped "kee-kee-jeer" song that is given repeatedly during the breeding season, mostly at night (Figure 1) (Pieplow, 2019). The analysis also targeted the "chatter" call, which is often given by pairs in a duet during the breeding season (Figure 2) (Pieplow, 2019).

Model Review

A subset of the full dataset was analyzed to reflect the recording schedule in the contract: one of every five minutes for 24 hours per day. All the windows in the subset, which had a high BirdNET confidence score for "Black Rail" (confidence ≥ 0.1), were manually reviewed by Conservation Metrics. After this initial review of the data subset, the rest of the data at any site that had fewer than 100 black rail detections (low-activity sites) was reviewed. In other words, the entirety of the continuous recording blocks for low-activity sites was analyzed instead of subsetting to increase our chances of finding rails. Conservation Metrics also manually reviewed all windows from low-activity sites detected by the custom model that had a high probability score for the "kee-kee-jeer" (probability ≥ 0.65, Figure 2) and "chatter" calls (probability ≥ 0.03, Figure 3). In total, 37,717 two-second windows were reviewed.

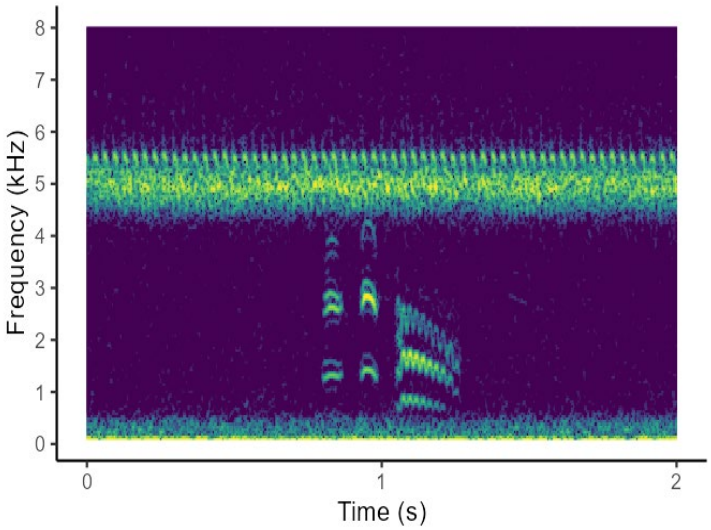

Figure 2. Spectrogram of a black rail "kee-kee-jeer" song. Note that the energy band at 5 kHz is cricket signals, not part of the rail vocalization.

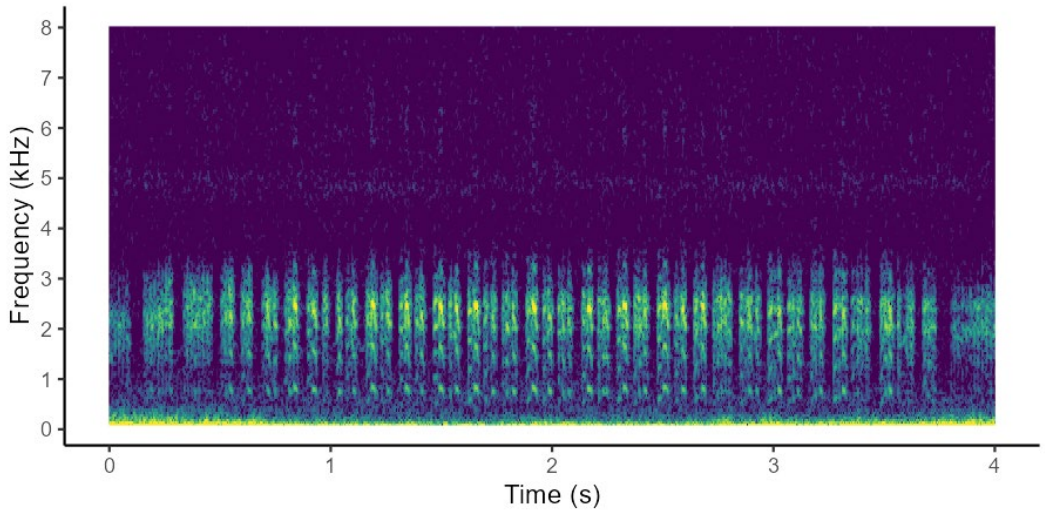

Figure 3. Spectrogram of a black rail “chatter” call. This is likely a pair giving a duet; pairs often alternate notes so rapidly it is difficult to distinguish that there are two individuals calling.

This year, the review protocol was changed slightly. In previous years, all windows detected were first reviewed by the custom model that had a high probability score for either call type, and then reviewed the BirdNET detections with high confidence scores. This year, the addition of new sites (CBBEP sites) included northern mockingbird (*Mimus polyglottos*) in the soundscape. Mockingbirds at these sites had a song element that mimicked black rail, and both the custom model and BirdNET struggled to differentiate between the mockingbird mimicry and the true black rail calls. BirdNET had less false positives than the custom model and thus BirdNET was utilized first in the analysis this year.

Acoustic Activity

Individual black rail vocalizations were aggregated into “bouts”, where a bout is defined as a sequence of calls with no more than six seconds between each call. For sites with greater than 100 rail detections (high-activity sites), daily and seasonal call rates (bouts per minute) were calculated. For sites with fewer than 100 rail detections (low-activity sites), the total number of bouts per site is presented.

Water sample collections

Depending on the availability of water volume in each ARU spot, we collected 1-2 liters of water samples using clean plastic bottles from different directions within 75 m from the ARU. The numbers of water samples collected from different ARU locations were listed in Table 2. The collected water samples were immediately kept on ice. All water samples were transported back to the lab and processed within 24 hours of collection.

Table 2. Number of water samples collected from each ARU spot.

Survey Site	Recording Unit	Latitude	Longitude	Number of Collected Water Samples
BirdIslandBasin_01	PAIS8	27.46532	-97.29695	0
BirdIslandBasin_02	PAIS9	27.46473	-97.29758	13
BirdIslandBasin_04	PAIS10	27.46471	-97.29698	9
CBBEP01	TAMU1	27.91353	-97.61055	26
CBBEP02	TAMU2	27.88724	-97.59023	10
CBBEP03	TAMU3	27.88585	-97.56764	5
CBBEP04	TAMU4	27.86284	-97.55696	11
CBBEP05	TAMU5	27.86864	-97.59176	15
CBBEP06	TAMU6	27.87251	-97.59567	10
KlebergTract_01	TAMU10	27.51303	-97.27017	30
KlebergTract_02	TAMU7	27.51364	-97.27068	26
MissionDelta_01	TAMU8	28.17673	-97.19594	36
MissionDelta_02	TAMU9	28.15658	-97.18599	27
SixPigsEast_01	PAIS7	27.37265	-97.32981	1
SixPigsEast_02	PAIS3	27.37299	-97.32890	5
SixPigsEast_03	PAIS6	27.37340	-97.32997	1
SixPigsWest_01	PAIS1	27.37436	-97.33769	10
SixPigsWest_02	PAIS2	27.37380	-97.33552	10
SixPigsWest_03	PAIS5	27.37491	-97.33772	5
Total number of samples				**251**

Water sample process and DNA extraction

The water samples were first filtered with filtered using wet-strengthened qualitative filter papers (>25 µm pore size) to remove the sand, mud, and other big particles. The flowthrough was further filtered by 0.45 µm filters in the lab. Total DNA isolation from the filter paper was performed with the PowerSoil® DNA Isolation Kit (Qiagen) following the manufacturer's instructions. Briefly, the filter papers were mixed with 2 ml cell lysis buffer from the kit and 3 mm diameter glass beads. The mixture was homogenized with a vortex at the maximum speed for 10 min. Then the homogenized samples were briefly centrifuged to remove the debris from the solution. The supernatant was then used for DNA extraction with the kit.

qPCR with eDNA samples

Primers and TaqMan probe that were used for qPCR were designed and reported by Neice and McRae (2021) based on the COI encoding gene. The primer/probe set included a forward primer (5'- CTT CCT CCC TCT TTC CTG CT -3'), a reverse primer (5'- GGA TAG TGC GGG TGG TTT TA -3'), and a TaqMan probe (6-FAM-CTA+C+TA+GCTT+C+A+TCA-IABkFQ). Thermocycling conditions were 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. To optimize the concentrations of eDNA for PCR reaction, we used 3 concentrations for each sample: original concentration (without dilution), 50 ng/µL, and 10 ng/µL. For every 10 µL reaction, 2 µL of DNA solution was used. The DNA samples isolated from eastern

BLRA (gift from Dr. Susan McRae at East Carolina University) were used as a positive control.

Nondestructive vegetation sampling

Nondestructive vegetation sampling followed methods adapted from the NOAA RESTORE Science Act Fire Bird Project (https://noaafirebird.home.blog/project-details/fieldsops/). At each ARU location, the dominant habitat type (>50% of the circle) was visually estimated within an 18m radius circle. Habitat types at site locations included low marsh and high marsh. At each ARU location, the percent cover of vegetation species within the 18m radius circle was visually estimated for all species making up > 5% of the circle. Then, located 10 m from the ARU, a 50 x 50 cm quadrat was used in the 4 cardinal directions to quantify percent vegetation cover. The percent cover within the quadrat was defined as the fraction of the total quadrat area that was obscured by a particular species when viewed from directly above. All species present in the quadrat were listed and percent cover noted. Soil moisture was characterized within each quadrant as either dry, moist, or standing water (depth). Vegetation height/visual obstruction was measured at each quadrat location using a Robel pole viewed from a distance of 4 m and 1 m above ground level. All data was recorded for each location on datasheets as described in the QAPP.

Results

Acoustic Device Deployment

Nineteen sensors were deployed between 02 April and 03 July 2024 and recorded a total of 11,007.45 hours of audio across 1,208 sensor-days (Table 3, Figure 4). All data gathered at each site were analyzed, with the exception of CBBEP02, CBBEP03, CBBEP05, and CBBEP06, where the data were subset to reflect the intended recording schedule in the contract (one of every five minutes for 24 hours). These sites had high black rail activity, so activity patterns could still be discerned using a subset of the data. In total, 9,480.99 hours of acoustic recordings were analyzed (Table 3). The recording quality was evaluated for each minute of acoustic data collected and deemed recording quality to be excellent, so no data was removed due to poor quality.

Table 3. Total number of days and hours of recordings that were included in the analysis for each site.

Survey Site	Total Days	Total Hours
BirdIslandBasin_01	53	465.92
BirdIslandBasin_02	24	211.30
BirdIslandBasin_04	54	480.73
CBBEP01	91	854.83
CBBEP02	89	410.83
CBBEP03	89	410.97
CBBEP04	74	680.80
CBBEP05	76	356.92
CBBEP06	76	356.07
KlebergTract_01	69	661.42
KlebergTract_02	42	396.90
MissionDelta_01	61	559.13
MissionDelta_02	61	559.10
SixPigsEast_01	57	509.28
SixPigsEast_02	63	479.60
SixPigsEast_03	56	509.37
SixPigsWest_01	63	585.17
SixPigsWest_02	61	556.20
SixPigsWest_03	49	436.45
Total	**1,208**	**9,480.99**

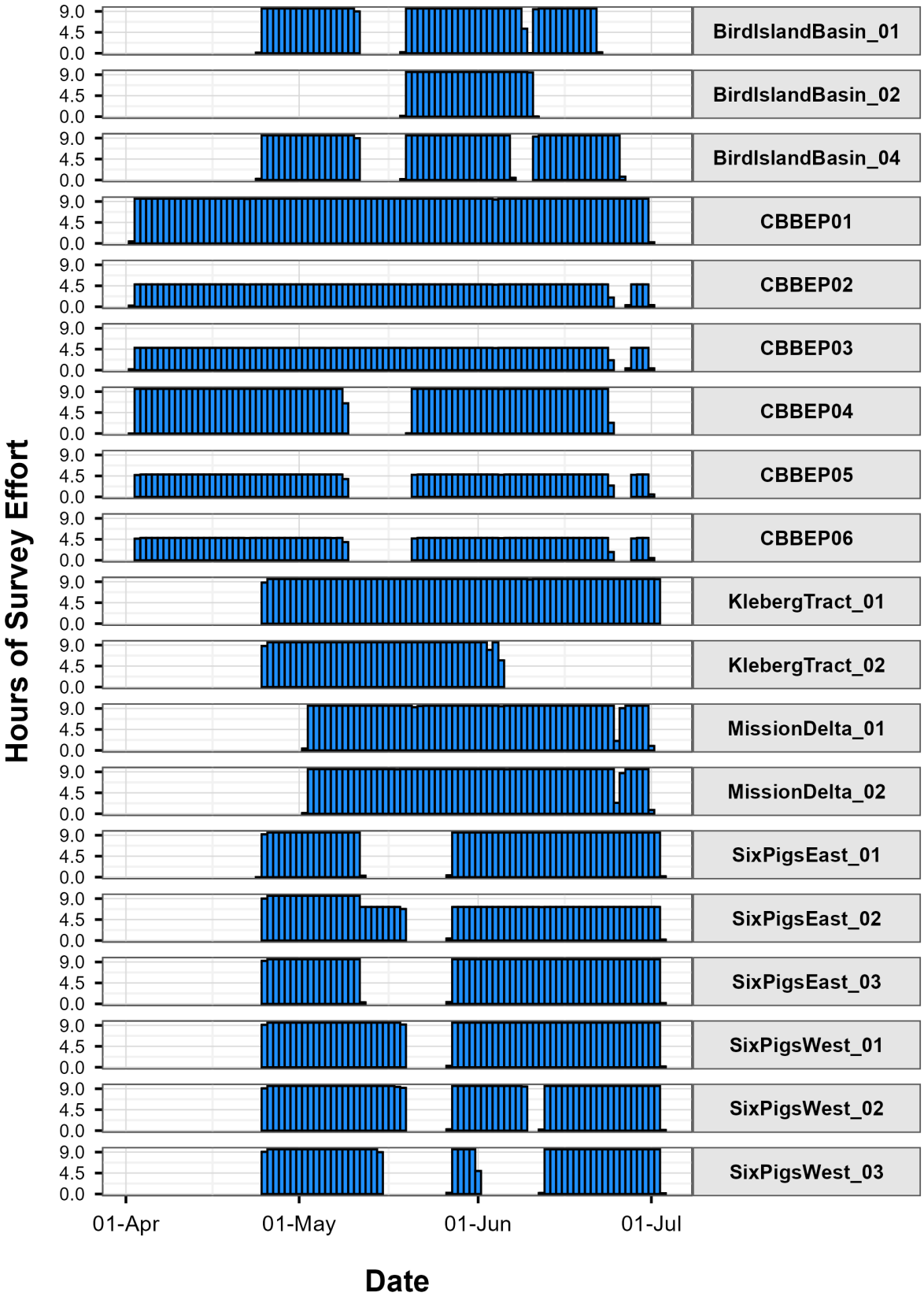

Figure 4. Total hours of recordings collected at each site per day that were included in the analysis. Note that a subset of the continuous recording block was analyzed around sunrise and sunset at CBBEP02, 03, 05, and 06, resulting in fewer hours of survey per day compared to other sites.

Black Rail Vocal Activity

Over 100 black rail bouts were detected at each of CBBEP02, CBBEP03, CBBEP05 and CBBEP06 and these sites were deemed high-activity sites (Figure 5 and Table 4). Between 1 and 29 bouts were detected at CBBEP01, CBBEP04, BirdIslandBasin_01, KlebergTract_02, and MissionDelta_01, and all other sites had zero black rail detections (low-activity sites; Figure 5 and Table 5).

Table 4. Table summarizing black rail detections at high-activity sites. Calls were aggregated into calling bouts, and call rates were calculated as bouts per minute.

Survey Site	Bouts/Min	SD	SE	Total Bouts	Nights Surveyed	% Nights Detected
CBBEP02	0.012	0.0412	0.0044	310	89	38.20
CBBEP03	0.006	0.0434	0.0046	144	89	22.47
CBBEP05	0.043	0.0994	0.0114	870	76	43.42
CBBEP06	0.056	0.1199	0.0138	1199	76	82.89

Table 5. Table summarizing total black rail bouts detected at low-activity sites.

Survey Site	Total Bouts	Nights	% Nights Detected
BirdIslandBasin_01	2	53	1.89
BirdIslandBasin_02	0	24	0
BirdIslandBasin_04	0	54	0
CBBEP01	29	91	9.89
CBBEP04	2	74	2.7
KlebergTract_01	0	69	0
KlebergTract_02	2	42	2.38
MissionDelta_01	1	61	1.64
MissionDelta_02	0	61	0
SixPigsEast_01	0	57	0
SixPigsEast_02	0	63	0
SixPigsEast_03	0	56	0
SixPigsWest_01	0	63	0
SixPigsWest_02	0	61	0
SixPigsWest_03	0	49	0

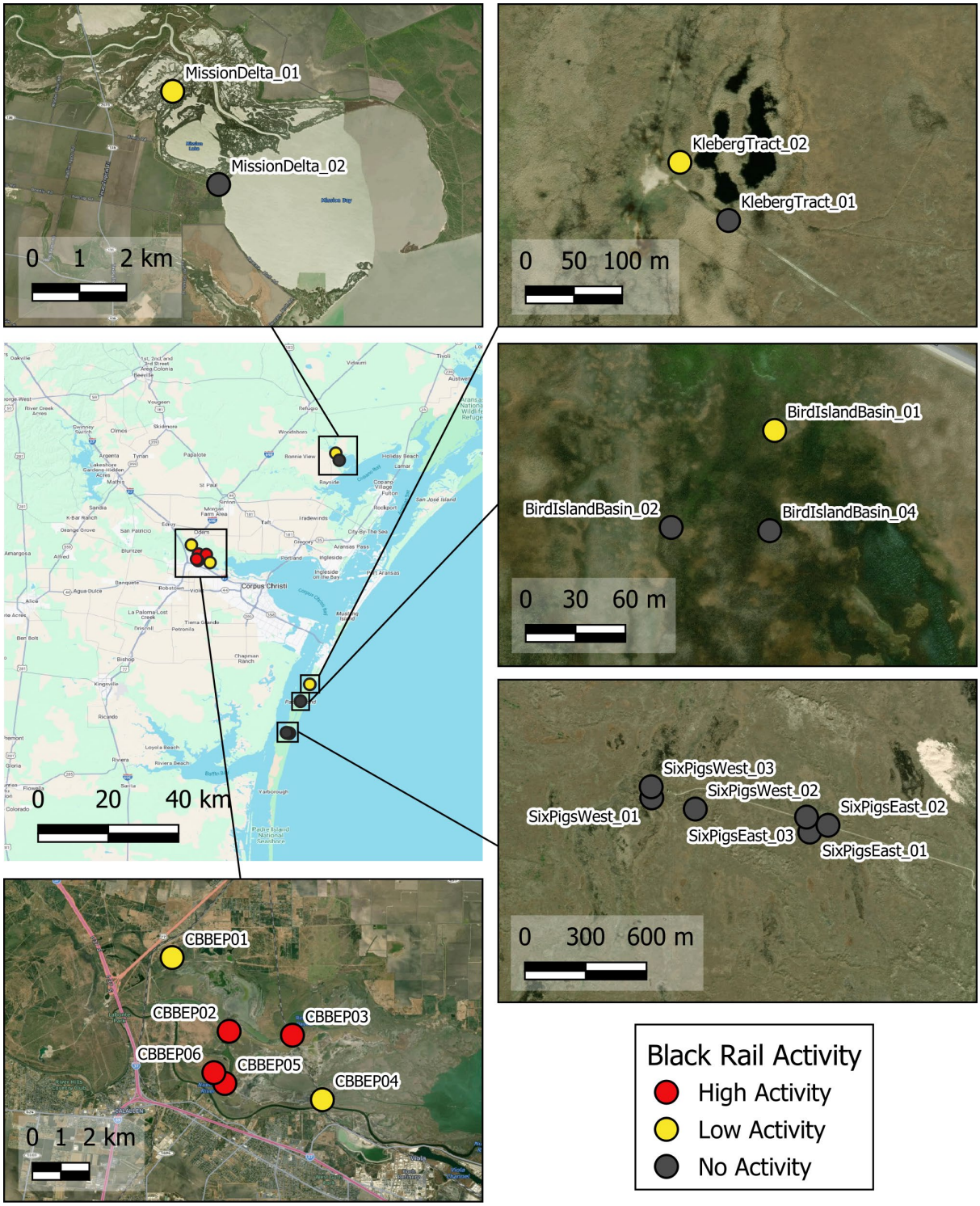

Figure 5. Map of survey locations in southern Texas. Points are color-coded to symbolize sites with high rail activity(100+ bouts), low rail activity (<100 bouts), and no rail activity.

High-activity Sites

CBBEP06 had the highest activity rate of 0.056 ± 0.1199 bouts per minute, followed by CBBEP05 (0.043 ±0.0994), CBBEP02 (0.012 ±0.0412), and CBBEP03 (0.006

±0.0434) (Table 4, Figure 6). The majority of the vocalizations at each site were detected throughout June, with the exception of CBBEP03, where 117 of the 144 bouts detected were on April 11 (Figure 7). Rail vocalizations peaked between sunset and sunrise, with another smaller peak within the two hours after sunrise (Figure 8 and Figure 9).

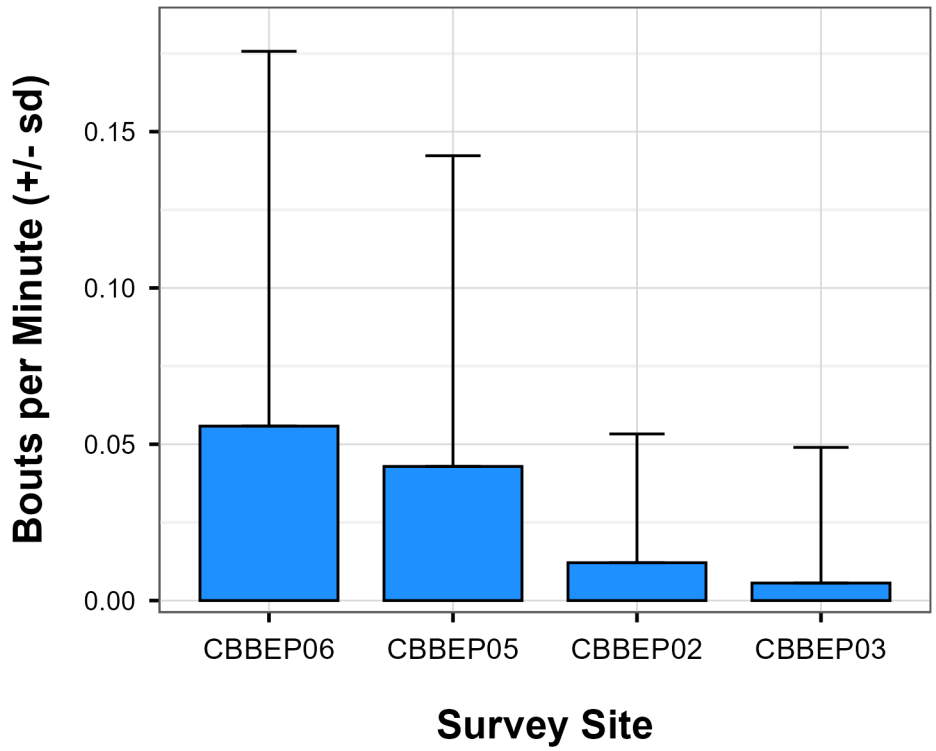

Figure 6. Black rail vocalization rate (bouts per minute) per site at high-activity sites.

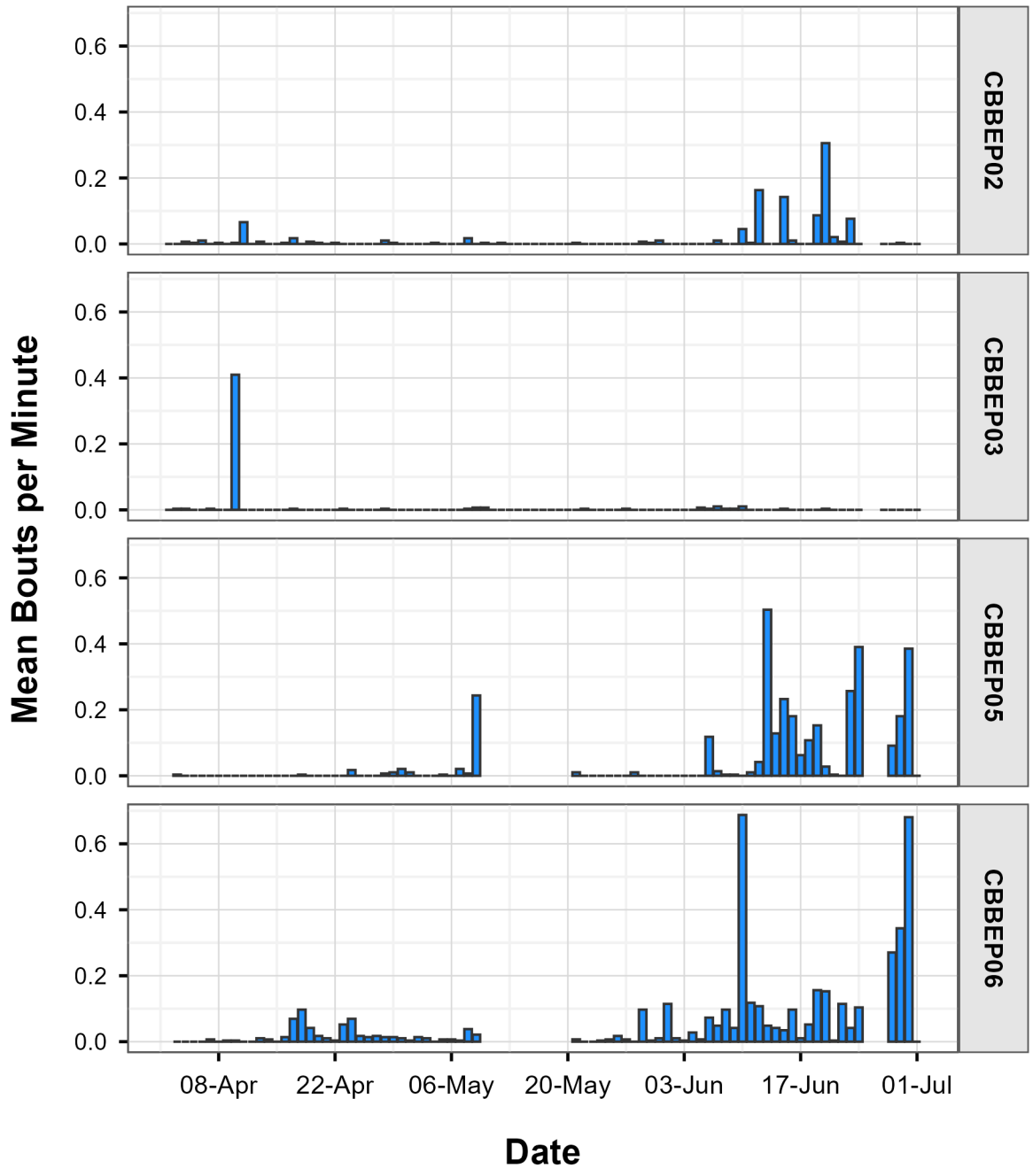

Figure 7. Black rail vocal activity throughout the season at high-activity sites.

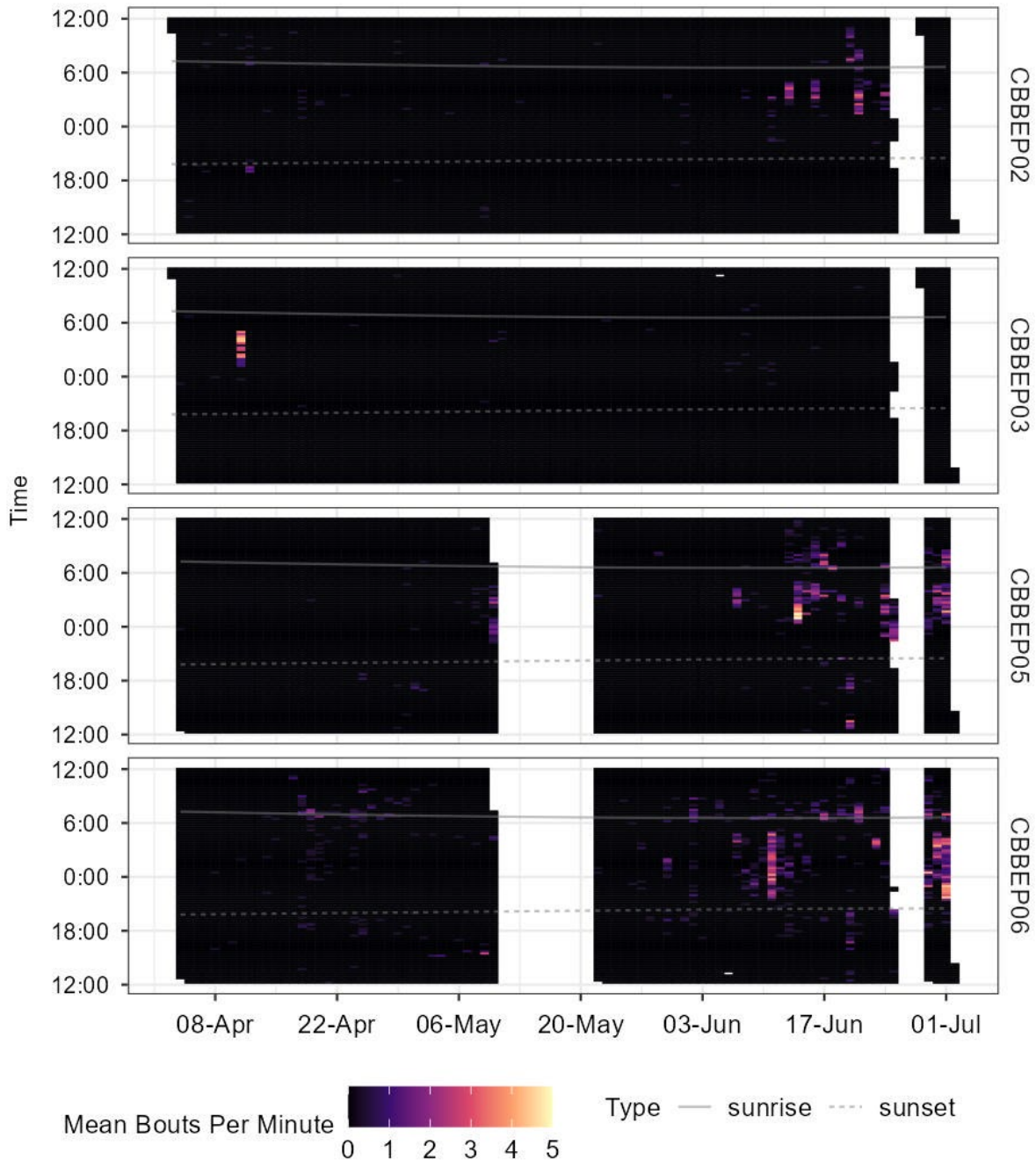

Figure 8. Raster showing black rail vocal activity patterns throughout the day and season at each high-activity site. Each panel is centered at midnight. The dashed gray line represents sunset, and the solid gray line represents sunrise. Brighter colors indicate higher vocal activity rates. Note that most vocal activity takes place between sunset and sunrise, as well as right after sunrise.

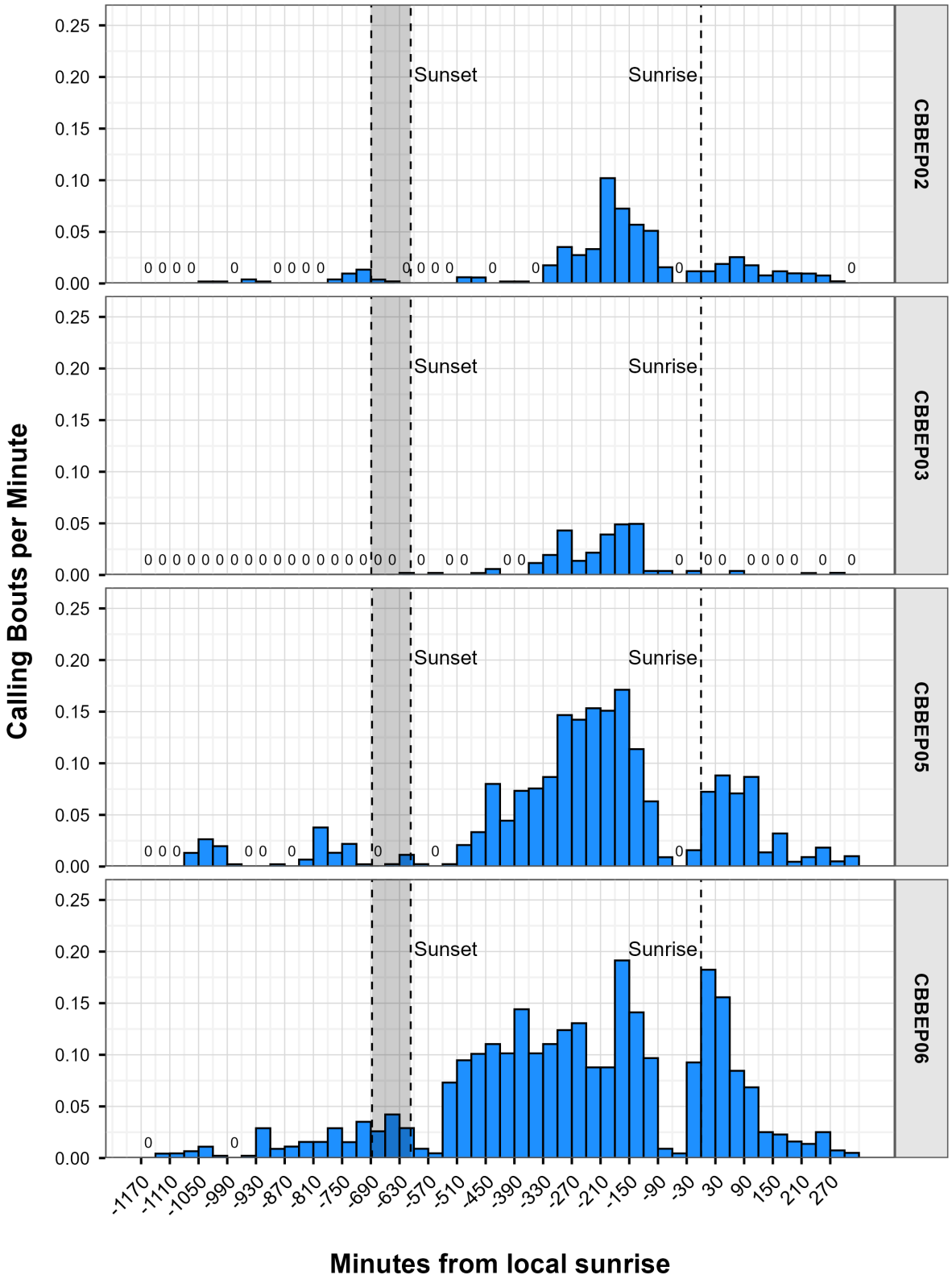

Figure 9. Daily black rail activity patterns throughout the day at each high-activity site relative to sunrise. Note a peak in vocal activity between sunset and sunrise, as well as right after sunrise.

Low-activity Sites

At the low-activity sites, CBBEP01 had the most black rail bouts (29), followed by BirdIslandBasin_01, CBBEP04, KlebergTract_02 (all 2), and MissionDelta_01 (Table 6 and Figure 10). CBBEP01, CBBEP04, and MissionDelta_01 had vocalizations during June, like the high-activity sites, while BirdIslandBasin_01 and KlebergTract_02 had detections on May 9 and May 8, respectively (Table 7). Some potential black rail detections were also identified at CBBEP01 (32), CBBEP04 (1), and KlebergTract_02 (1). These were signals that resembled the black rail "kee-kee-jeer" call but were too faint or masked by other signals to be confidently identified.

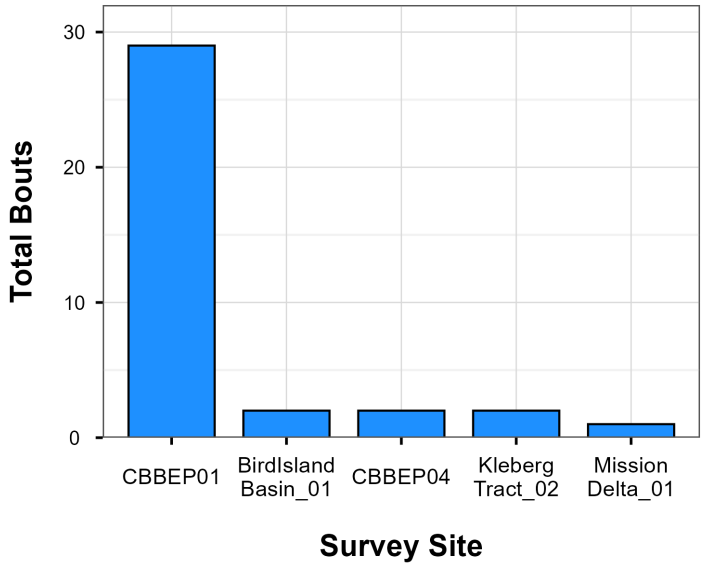

Figure 10. Number of black rail bouts detected per site at low-activity sites. Sites with zero detections are not shown.

Table 6. Number of black rail bouts per date for each of the low-activity sites.

Survey Site	Date	Total Bouts
BirdIslandBasin_01	2024-05-09	2
CBBEP01	2024-04-13	2
CBBEP01	2024-05-22	2
CBBEP01	2024-06-06	2
CBBEP01	2024-06-07	5
CBBEP01	2024-06-23	1
CBBEP01	2024-06-24	4
CBBEP01	2024-06-26	7
CBBEP01	2024-06-27	4
CBBEP01	2024-06-28	1
CBBEP01	2024-06-29	1
CBBEP04	2024-05-10	1
CBBEP04	2024-06-07	1
KlebergTract_02	2024-05-08	2
MissionDelta_01	2024-06-01	1

Table 7. Number of potential black rail bouts per date for low-activity sites CBBEP01, CBBEP04, and KlebergTract_02.

Survey Site	Date	Total Possible Bouts
CBBEP01	2024-04-05	1
CBBEP01	2024-04-07	3
CBBEP01	2024-04-08	1
CBBEP01	2024-04-13	2
CBBEP01	2024-04-14	2
CBBEP01	2024-04-15	1
CBBEP01	2024-04-17	2
CBBEP01	2024-04-30	1
CBBEP01	2024-05-01	2
CBBEP01	2024-05-19	2
CBBEP01	2024-05-20	1
CBBEP01	2024-05-22	2
CBBEP01	2024-06-05	1
CBBEP01	2024-06-07	1
CBBEP01	2024-06-10	4
CBBEP01	2024-06-13	1
CBBEP01	2024-06-16	1
CBBEP01	2024-06-25	1
CBBEP01	2024-06-26	2
CBBEP01	2024-06-29	1
CBBEP04	2024-04-29	1
KlebergTract_02	2024-05-07	1

Detection of Black Rail eDNA

Based on the concentrations of the eDNAs extracted from the water samples, 251 were confirmed with valid readings from the spectrophotometer. For each sample, we used two concentrations of DNA, 5 ng/μL (Figure 11a) and 0.5 ng/μL (Figure 11b), in the qPCR reactions. The BLRA genome DNA was used as a positive control in each run. Based on the amplification curves of all 251 samples, we were only able to identify the presence of BLRA in the positive control samples. The positive control was diluted to 50 pg/μL (exponential curves in Figure 11a) and 5 pg/μL (exponential curves in Figure 11b).

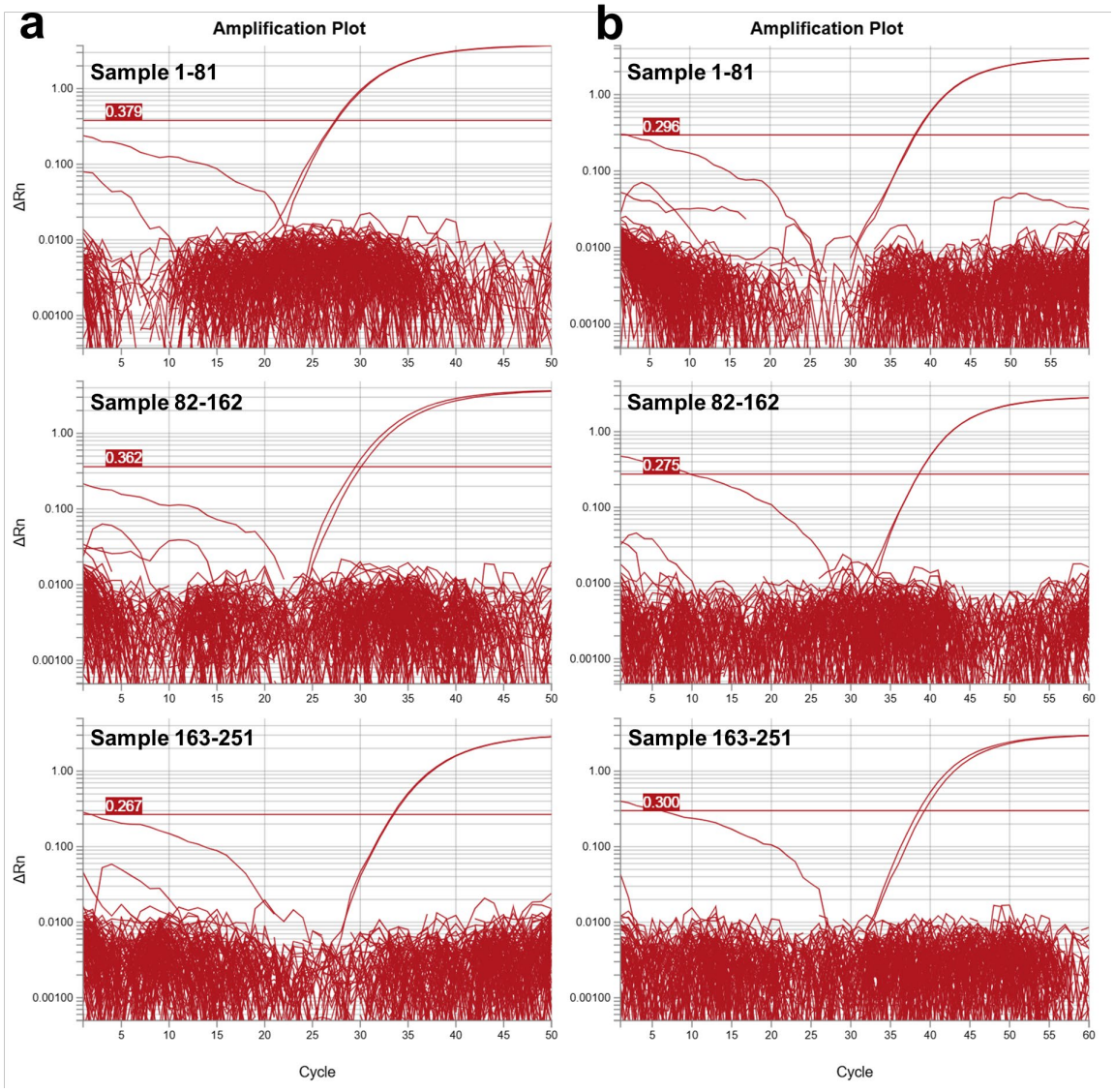

Figure 11. Identification of BLRA presence with the qPCR assay developed by Neice *et al*. Each panel in this figure represents a plate (384-well plate) of reaction. On each plate, BLRA genome DNA was used as a positive control and molecular water was used as a negative control. Each eDNA sample was diluted to 5 ng/μL (a) and 0.5 ng/μL (b) in the final reaction. ΔRn represents the magnitude of the normalized fluorescence signal generated by the reporter dye. The red horizontal line in each panel shows the threshold of the analysis, which determines the Ct values of all amplifications.

Vegetation Characterization of Sites

A total of 19 plots were established, with six that were located on the Nueces Delta Preserve (NDP), two in the Mission River Delta, and 11 on Padre Island. Two of the sites on Padre Island were located on the Kleberg Tract owned by Nueces County, while the others were all located within Padre Island National Seashore (see Figure 1). Vegetation sampling at each plot was carried out once during the study in June to limit potential disturbance to black rails if present. Plots were established in March, April, and May prior to installing ARUs. At the time of establishment, each site was

chosen based on dense vegetation and the presence of shallow water in the entire area or in certain parts of the plot. Data for each site is in Appendix 1 and vegetation data for each site is summarized below.

Sites within the NDP are characterized as high marsh and were dominated by *Spartina spartinae* (84%) with a lesser component of *Borrichia frutescens* (9 %). Only one site (CBBE01) had less than 90% *Spartina spartinae*. CBB01 had the most plant diversity of the sites at the NDP. This site was composed of approximately 45% *Borrichia frutescens*, 15% *Scirpus robustus*, and 10% *Distichlis spicata*. This was likely due to its location adjacent to a shallow moist soil unit with an active solar well that keeps the area wet even in drought. Vegetation height, as measured on the Robel pole, ranged from 68 to 49 cm, with the tallest vegetation at site CBBEP01. The other 5 sites dominated by *Spartina spartinae* averaged 53 cm tall.

Although all sites on the NDP, when established in March 2024, were adjacent to standing shallow water, by the time the vegetation samples were collected on June 5, 2024, after prolonged drought, all sites with the exception of CBBEP01 were dry. Because of the well at CBBEP01, quadrats on the South, East, and West continued to have moist soil conditions.

Two plots were established on property owned by CBBEP in the Mission River Delta on May 5, 2024. These two sites are tidally influenced and were characterized as high marsh. Vegetation data were collected at these sites on June 6, 2024. Site CBBEP08 is approximately 65% *Spartina spartinae*, 15% *Borrichia frutescens*,10% *Monanthochloe littoralis*, and 10% bare. This site is typical of salt marsh zonation on the mid-Texas coast, where elevation above the tide causes zonation. *Spartina spartinae* and *Borrichia frutescens* occupy the higher elevations of the site. Shallow tidal water was typically found approximately 12 m south of the ARU location at the center of the plot. The tallest vegetation was in the northern half of the plot circle dominated by *Spartina spartinae* with an average height of 47 cm. While the southern half of the plot was bare and dominated by very low-growing *Monanthocloe littorailis* (20 cm high). Site CBBEP09 had more abrupt zonation, forming a small (approx.30-50 cm) bluff with the high part of the plot dominated by *Spartina spartinae* and the lower elevation dominated by *Batis maritima*. The 18m plot was approximately 20% *Spartina spartinae* and 70% *Batis maritima*.

Eleven sites were established on Padre Island. These sites are typical of vegetated barrier island flats described by Britton and Morton (Britton & Morton, 1989). The sites were established in palustrine swales (depressions in the vegetated barrier island flat), and the plant communities of the sites on Padre Island were more species-rich (18 species) than those found at the mainland sites (NDP and Mission River Delta) combined (7 species). All 11 sites were established on April 25, 2024 and were selected for their dense tall vegetation and wetland characteristics (moist soil and shallow standing water) and are typical of barrier island palustrine wetlands. The sites were all characterized as low marsh. Prior to vegetation sampling in June, sites located within Padre Island National Seashore were burned between May 12, 2024, and May 15, 2024, as part of efforts to control a spreading wildfire (Sassine, Personal Communication).

The Kleberg Tract 01 plot was sampled on June 11, 2024, and was comprised of 45% *Schoenoplectus pungens*, 20% *Typha latifolia,* and 20 % *Spartina patens.* The

vegetation was tall and completely obscured the Robel pole to an average height of 66 cm. The soil in all 4 quadrats was characterized as damp with no standing water.

Kleberg Tract 02 plot was sampled on June 11, 2024. The plot circle was approximately 95% *Typha latifolia* and 5% Spartina patens. The average vegetation height was 89 cm, largely due to the dominance of the site by dense *Typha latifolia.* The soil moisture ranged from dry to water 14 cm deep.

Bird Island Basin 01 located in Padre Island National Seashore was sampled on June 18, 2024. The plot circle was approximately 40% *Schoenoplectus pungens*, 25% *Dichanthelium aciculare,* 15% *Centella erecta,* 10% *Samolus ebracteatus*, 5% *Phyla nodiflora*, and 5% *Rhynchospora caduca.* The soil was moist and due to the presence of some shallow standing water, not all of the vegetation burned during the fire in May; however it did top burn much of the wetland. The average height of vegetation obscuring the Robel pole was 21 cm. Although a large portion of the wetland was moist at the time of sampling, there was no shallow standing water in the area.

Bird Island Basin 02, located in Padre Island National Seashore, was sampled on June 18, 2024. The plant composition of the plot was similar to Bird Island Basin 02 with *Typha latifolia* accounting for 50% of the area. The average height of vegetation obscuring the Robel pole was 39 cm. The site substrate was moist with no standing water.

Bird Island Basin 04, located in Padre Island National Seashore, was sampled on June 18, 2024. This plot was made up of 50% *Typha latifolia,* 20% *Centella erecta,* 10% each of *Phyla nodiflora*, *Eleocharis montana, and Bacopa monnieri.* The average vegetation height was 25 cm and the soil was moist with no standing shallow water.

Six Pigs East 01, located in Padre Island National Seashore, was south of a road and was also dominated by *Typha latifolia.* The site was sampled on June 27, 2024. The plot circle was made up of 60% *Typha latifolia*, 20% *Sesbania herbacea*, 15% *Paspalum monostachyum* and 5% *Schizachyrium littorale*. The average height of the vegetation was 50 cm. At the time of sampling, two of the quadrats had dry substrate and two had moist substrate. There was no standing water in the wetland.

Six Pigs East 02 was in the same wetland as Six Pigs 03, both located north of the road in Padre Island National Seashore. This plot had a composition that included 30% each of *Paspalum monostachyum* and *Sesbania herbacea*, 20% *Schoenoplectus pungens,* and 15% bare road. The average height of the vegetation was 31 cm. The soil substrate at all four quadrat locations was dry.

Six Pigs East 03, located in Padre Island National Seashore, was sampled on June 27, 2024. The plot circle was made up of 70% *Typha latifolia*, 10% each of *Paspalum monostachyum* and *Sesbania herbacea*. The site circle was also comprised of 5% each of *Phyla nodiflora,* and bare road. The average height of the vegetation was 65 cm. At the time of sampling the site substrate was moist, but with no standing water.

Six Pigs West 01, located in Padre Isaland National Seashore, had a plot composition of 70% *Schoenoplectus pungens*, 20% *Eleocharis montana*, 5% *Paspalum monostachyum* and 5% *Andropogon glomeratus*. This site had an average vegetation height of 65 cm. The site ranged from dry to shallow standing water 4.5 cm deep.

Six Pigs West 02, located in Padre Isaland National Seashore, had a plot composition of 52% *Typha latifolia*, 35% *Schoenoplectus pungens*, 8% *Eleocharis montana*, and 5% *Paspalum monostachyum*. This site had an average vegetation height of 101 cm. The site ranged from moist to shallow standing water 4 cm deep.

Six Pigs West 03, located in Padre Isaland National Seashore, had a plot composition of 55% *Schoenoplectus pungens*, 30% *Paspalum monostachyum,* 10% *Phyla nodiflora*, and 5% *Typha latifolia*. This site had an average vegetation height of 50 cm. The site ranged from moist to shallow standing water 2 cm deep.

The fires that took place on Padre Island National Seashore between May 12 and May 15, 2024, largely impacted the uplands surrounding the vegetated wetlands. The sites at Bird Island Basin were visually more impacted than those at Six Pigs East and West. The wetlands at Six Pigs continued to support dense, tall wetland vegetation even though the surrounding uplands were burned to ground level.

Discussion

Overall, black rail seasonal and diel vocal patterns at high-activity sites were identified. In addition, rail presence at some of the low-activity sites was confirmed.

There was rail vocal activity at all six Nueces River Delta sites, with the four more centrally located sites having the greatest activity. Only one of the two Mission River Delta sites had a rail presence. On Padre Island, only one of the Kleberg Tract and Bird Island Basin sites had rail presence, similar to 2022, where only a few rail vocalizations were found at a few sites on the island: BigPond_01, BigPond_02, and NorthSite_03.

It is possible that rails are breeding at CBBEP02, CBBEP05, and CBBEP06, given the amount of vocal activity observed during June, while the vocalizations detected at CBBEP03 in April may indicate wintering or stopover use of that site. It may be of interest to extend future surveys into July, considering the relatively high vocal activity rates found at the end of June.

The threshold of 100 detections separating high- and low-activity sites was arbitrary and does not indicate anything specific about rail behavior, as daily and seasonal vocalization rate is known to vary widely between individuals (Eddleman et al., 2020). Although CBBEP01 had fewer than 100 detections, most of the detections were in June, which indicates the presence of rails during the breeding season.

Presence during May (BirdIslandBasin_01 and KlebergTract_02) may be important, even if activity was low. The Texas coast can be used by wintering, migrating, and breeding rails, so the presence of even a few rails suggests that sites may have the potential to support rails and thus should continue to be preserved and monitored further.

Northern mockingbird (*Mimus polyglottos*) was abundant at all CBBEP sites, and many instances of black rail mimicry were found, even at sites with few rails (CBBEP01 and 04). This mimicry was often identified by BirdNET as black rail, so careful manual review was required to separate true black rail vocalizations from mockingbird mimicry. Future surveys at these sites will likely continue to result in numerous false-positive detections that will require meticulous manual review, as even an expert analyst had trouble correctly removing false detections from BirdNET without inspecting long segments of recordings.

Although black rails were not detected at 10 of the sites, confirming the absence of a species is difficult to accomplish with any survey method, including acoustic monitoring, especially when vocalizations are rare or can be masked by other noises in the soundscape. Black rails are extremely secretive and notoriously difficult to survey for (Eddleman et al., 2020), so their absence in the data at some sites does not necessarily mean rails are not present. Even at the high-activity sites, rail vocalizations were relatively scarce across the survey period on average; a rate of 0.056 bouts per minute (at CBBEP06) equates to 3.36 bouts per hour, which could easily be missed with a recording schedule that is duty cycled.

Continuing acoustic surveys is recommended to further investigate the presence of

rails at low-activity sites, and potentially extending surveys into July since rails at the CBBEP sites had high vocal activity at the end of June. Since most of the rail detections in this data were during the night and early morning, survey effort could be increased at those times and decreased during midday. Usually, placing sensors at least 150 meters apart is recommended for samples to be independent. Most of the Padre Island sites were closer together than this, but interestingly, the rail detections at BirdIslandBasin_01 and KlebergTract_02 were not picked up by adjacent sensors.

The primer set and probes used in the present study to detect the presence of BLRA did not show any positive results in the eDNA samples, which is inconsistent with the results from the ARU analysis. There are several explanations for this inconsistency between the two assays. First, one of the advantages of the ARU is that the device can continuously record the sounds from the surrounding environment. Given the rarity of BLRA, continuous monitoring is critical to capture the sounds of BLRA at the right locations. Compared to the ARU monitoring, the eDNA analysis can only be done with the samples collected from certain time spots. Water sample collections in this study were only performed once for each ARU location. In order to successfully identify the presence of BLRA from a water sample, we expect a recent visit of BLRAs to the water location, and the DNA shed by the BLRA to the water would last long enough until the water collection. However, with the dramatic environmental changes during the sample collecting season, such as drought and storms, the DNAs shed by the BLRA were difficult to keep in certain spots for a relatively long period of time. This is considered the biggest hurdle for the eDNA technique. To solve this problem, repeated sampling for each location is likely to increase the success rate of eDNA detection.

Secondly, the sensitivity of Taqman qPCR for the eDNA technique largely relies on the primer sets and probes used in the study. The primers and probes used in this project were from a previous study (Neice & McRae, 2021) and the efficiency was confirmed by the study. However, most of the samples collected in their study were near the footprints of birds in the mud and the locations with recent video evidence for BLRA presence. The sample locations were more specific and the chance for BLRA DNA detection was expected to be high. In comparison, our sample locations were much broader. They were all close to ARU locations, which were selected by previous observations and estimation. To confirm the results of our study, we also used another set of primers and the probe (Feist et al., 2022), which were reported to have higher sensitivity compared to the ones used by Neice *et al*. The results were consistent with our original findings (Figure 12). Only valid amplification signals were seen from the positive control samples (both 50 and 5 pg/μL). BLRA was not identified from any of the eDNA samples from the environment, regardless of the DNA concentrations in the reaction (Figure 12a: 5 ng/μL; Figure 12b: 0.5 ng/μL).

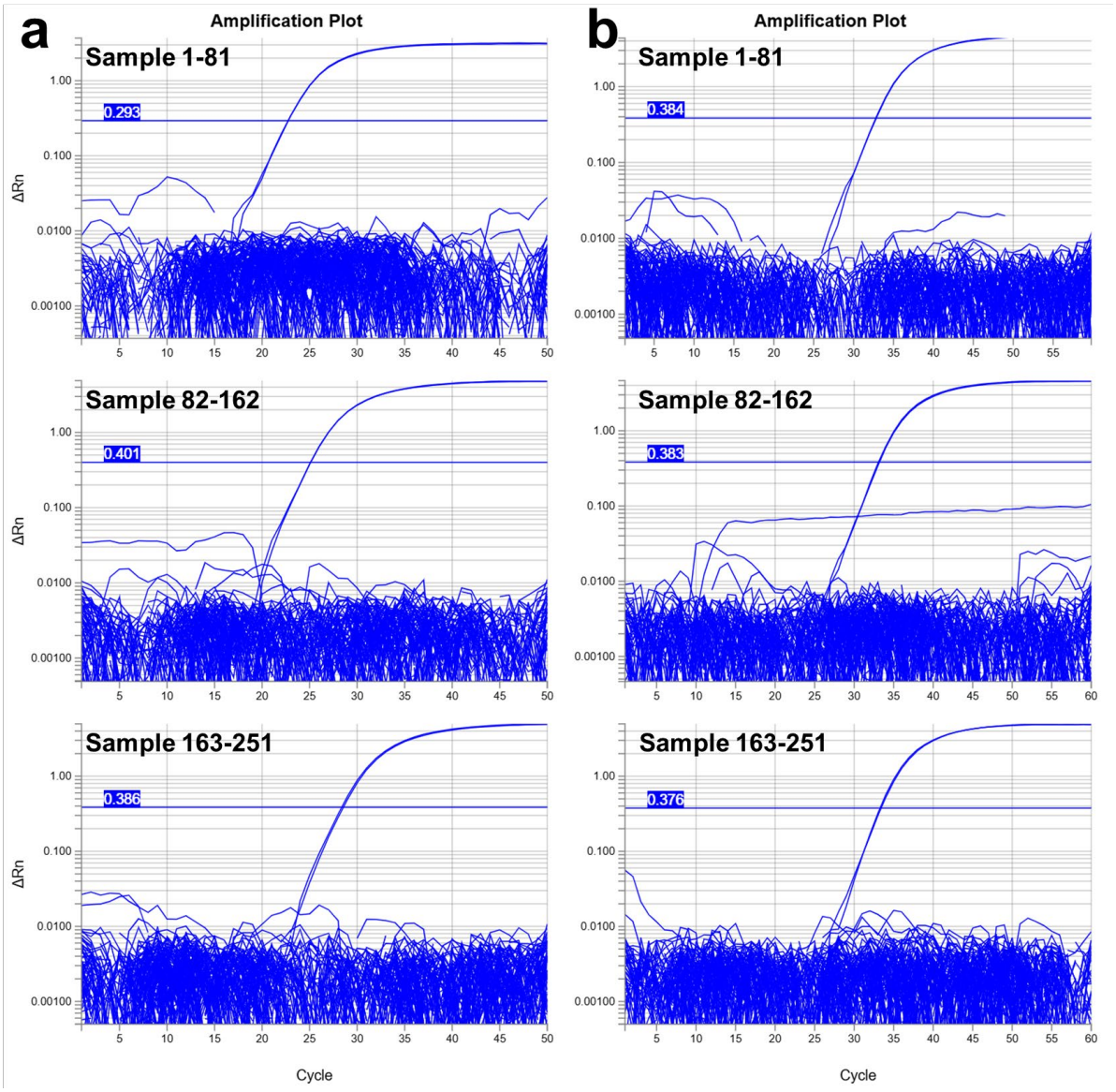

Figure 12. Identification of BLRA presence with qPCR developed by Feist *et al*. Each eDNA sample was diluted to 5 ng/μL (a) and 0.5 ng/μL (b) in the final reaction.

Finally, it was also widely reported that some inhibitors in the environmental water samples could stay with the DNA and increase the risk of false negatives of the eDNA technique (Burian et al., 2021; Buxton et al., 2021; Hunter et al., 2019). To rule out the possibility of PCR inhibition from the DNA samples, we designed a simple testing experiment. We randomly picked several eDNA samples, which were all negative for BLRA with qPCR analyses, and added very low concentrations of BLRA genome DNA (as low as 1 pg/μL). If the inhibition exists in the eDNA solution, it was expected to also inhibit the amplification of the BLRA DNA. However, this inhibition was not observed since the signals of BLRA DNA amplification from the eDNA samples were strong. Although the inhibition of PCR was not found in the eDNA solution, we were not able to identify the detection limit of the BLRA DNA from the environment.

Black rails were detected at all sites within the NDP with the highest activity at sites CBBEP 02, 03, 05, and 06 based on ARU data. These high activity sites were non-

tidal and dominated by dense near monotypic stands of *Sparatina spartinae* averaging 96% SD 2.5% of plot coverage across these sites. *Spartina spartinae*, is a robust bunch grass that forms dense stands. Because of its growth habit, there is overhead cover for the rail and often open trails beneath the dense canopy allowing for concealed movement. Tolliver *et al* (Tolliver et al., 2019) in a study of the mid- and upper-Texas coast, found that black rail occupancy was highest at locations with > 90% Spartina cover.

Although rails were detected at the high activity sites throughout the study on the NDP, it appears ARU detections peaked in June. It is interesting to note that these sites were completely dry with no noticeable soil moisture at the time of the vegetation sampling on June 5, 2024 even though they had moist soil and shallow standing water when established. National Weather Service records indicate that nearly 6 inches fell from June 19-21, 2024 at Corpus Christi International Airport and may have rehydrated soil conditions at the NDP.

CBBEP 01 and 04 are characterized as low activity sites based on ARU data. Both these sites had somewhat lower cover of *Spartina spartinae* 30% and 90% respectively than the high activity sites. CBBEP 01 had detections throughout the study but at low levels. This site had the highest plant diversity of any on the NDP likely due to site topography and the site being on the bank of a moist soil impoundment supplied with water from a solar well. CBBEP 04 had more sparse vegetation compared to other NDP sites, the plot included 10% bare area. The lower percent cover of *Spartina* and bare area of the plot may have contributed to the low activity.

Mission Delta 01 had 1 bout detected by ARU on June 1, 2024. This site was tidally influenced and is approximately 65% *Spartina spartinae*, 15% *Borrichia frutescens*, and 10% *Monanthochloe littoralis*, and 10% bare. Like the low activity sites on the NDP this site was also less dominated by *Spartina*.

Two of the Padre Island sites also had low activity detections of black rails based on ARU data. Kleberg Tract 02 and Bird Island Basin 01. The Kleberg Tract 02 had 1 bout on May 7th and 2 bouts on May 8th, while Bird Island Basin 01 had 2 bouts on May 9th. Both these sites are typical of barrier island swale wetlands and although they have no *Spartina spartinae* they do have very dense tall vegetation and moist soil or shallow standing water. The Kleberg Tract 02 plot was 95% *Typha latifolia* and 5% *Spartina patens*. While the Bird Island Basin 01 site was approximately 40% *Schoenoplectus pungens*, 25% *Dichanthelium aciculare,* 15% *Centella erecta,* 10% *Samolus ebracteatus*, 5% *Phyla nodiflora*, and 5% *Rhynchospora caduca.* The Bird Island Basin 01 site was burned in May during the study. The low activity found on Padre Island could be related to lower habitat suitability for the species or could be an indicator that Padre Island has fewer black rails compared to areas on the mainland with suitable habitat. Additional studies that included mainland palustrine marshes could help address these uncertainties.

Overall, sites with >95% cover of *Spartina* spartinae had more activity than other sites, and wetlands dominated by cattails and rushes on Padre Island had few detections.

Literature Cited

Acevedo, M. A., & Villanueva-Rivera, L. J. (2006). From the Field: Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians. *Wildlife Society Bulletin*, *34*(1), 211-214. https://doi.org/https://doi.org/10.2193/0091-7648(2006)34[211:UADRSA]2.0.CO;2

Britton, J. C., & Morton, B. (1989). *Shore Ecology of the Gulf of Mexico*. University of Texas Press.

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., & Sweet, M. (2021). Improving the reliability of eDNA data interpretation. *Mol Ecol Resour*, *21*(5), 1422-1433. https://doi.org/10.1111/1755-0998.13367

Buxton, A., Matechou, E., Griffin, J., Diana, A., & Griffiths, R. A. (2021). Optimising sampling and analysis protocols in environmental DNA studies. *Sci Rep*, *11*(1), 11637. https://doi.org/10.1038/s41598-021-91166-7

Cichy, R. M., Khosla, A., Pantazis, D., Torralba, A., & Oliva, A. (2016). Comparison of deep neural networks to spatio-temporal cortical dynamics of human visual object recognition reveals hierarchical correspondence. *Sci Rep*, *6*, 27755. https://doi.org/10.1038/srep27755

Day, K., Campbell, H., Fisher, A., Gibb, K., Hill, B., Rose, A., & Jarman, S. N. (2019). Development and validation of an environmental DNA test for the endangered Gouldian finch. *Endangered Species Research*, *40*, 171-182. https://www.int-res.com/abstracts/esr/v40/p171-182/

Deng, L., Hinton, G., & Kingsbury, B. (2013, 26-31 May 2013). New types of deep neural network learning for speech recognition and related applications: an overview. 2013 IEEE International Conference on Acoustics, Speech and Signal Processing,

Eddleman, W. R., Flores, R. E., & Legare, M. (2020). *Black Rail (Laterallus jamaicensis)* (1 ed.). Cornell Lab of Ornithology. https://doi.org/https://doi.org/10.2173/bow.blkrai.01

Feist, S. M., Guan, X., Malmfeldt, M. P., & Lance, R. F. (2022). Two novel qPCR assays to enhance black rail (Laterallus jamaicensis) eDNA surveys in the United States. *Conservation Genetics Resources*, *14*(3), 321-329. https://doi.org/10.1007/s12686-022-01279-y

Flores, R. E., & Eddleman, W. R. (1995). California Black Rail Use of Habitat in Southwestern Arizona. *The Journal of Wildlife Management*, *59*(2), 357-363. https://doi.org/10.2307/3808949

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proc Biol Sci*, *270*(1512), 313-321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P. D., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of Birds through DNA Barcodes. *PLoS Biol*, *2*(10), e312. https://doi.org/10.1371/journal.pbio.0020312

Hunter, M. E., Ferrante, J. A., Meigs-Friend, G., & Ulmer, A. (2019). Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. *Sci Rep*, *9*(1), 5259. https://doi.org/10.1038/s41598-019-40977-w

IUCN. (2021). *Laterallus jamaicensis* (https://doi.org/https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T22692353A178666347.en

Kahl, S., Wood, C. M., Eibl, M., & Klinck, H. (2021). BirdNET: A deep learning solution for avian diversity monitoring. *Ecological Informatics*, *61*, 101236. https://doi.org/https://doi.org/10.1016/j.ecoinf.2021.101236

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Andrew Royle, J., & Langtimm, C. A. (2002). ESTIMATING SITE OCCUPANCY RATES WHEN DETECTION PROBABILITIES ARE LESS THAN ONE. *Ecology*, *83*(8), 2248-2255. https://doi.org/https://doi.org/10.1890/0012-9658(2002)083[2248:ESORWD]2.0.CO;2

MacKenzie, D. I., Nichols, J. D., Sutton, N., Kawanishi, K., & Bailey, L. L. (2005). IMPROVING INFERENCES IN POPULATION STUDIES OF RARE SPECIES THAT ARE DETECTED IMPERFECTLY. *Ecology*, *86*(5), 1101-1113. https://doi.org/https://doi.org/10.1890/04-1060

Monge, O., Dumas, D., & Baus, I. (2020). Environmental DNA from avian residual saliva in fruits and its potential uses in population genetics. *Conservation Genetics Resources*, *12*(1), 131-139. https://doi.org/10.1007/s12686-018-1074-4

Neice, A. A., & McRae, S. B. (2021). An eDNA diagnostic test to detect a rare, secretive marsh bird. *Global Ecology and Conservation*, *27*, e01529. https://doi.org/https://doi.org/10.1016/j.gecco.2021.e01529

Neiman, M., & Taylor, D. R. (2009). The causes of mutation accumulation in mitochondrial genomes. *Proc Biol Sci*, *276*(1660), 1201-1209. https://doi.org/10.1098/rspb.2008.1758

Pieplow, N. (2019). *Peterson field guide to birds of North Americ*. Houghton Mifflin Co.

Sauer, J. R., Peterjohn, B. G., & Link, W. A. (1994). Observer Differences in the North American Breeding Bird Survey. *The Auk*, *111*(1), 50-62. https://doi.org/10.2307/4088504

Schmidhuber, J. (2015). Deep learning in neural networks: An overview. *Neural Networks*, *61*, 85-117. https://doi.org/https://doi.org/10.1016/j.neunet.2014.09.003

Scott Brandes, T. (2008). Automated sound recording and analysis techniques for bird surveys and conservation. *Bird Conservation International*, *18*(S1), S163-S173. https://doi.org/10.1017/S0959270908000415

Tolliver, J. D. M., Moore, A. A., Green, M. C., & Weckerly, F. W. (2019). Coastal Texas black rail population states and survey effort. *The Journal of Wildlife Management*, *83*(2), 312-324. https://doi.org/https://doi.org/10.1002/jwmg.21589

Ushio, M., Murata, K., Sado, T., Nishiumi, I., Takeshita, M., Iwasaki, W., & Miya, M. (2018). Demonstration of the potential of environmental DNA as a tool for the detection of avian species. *Sci Rep*, *8*(1), 4493. https://doi.org/10.1038/s41598-018-22817-5

Watts, B. D. (2016). *Status and distribution of the eastern black rail along the Atlantic and Gulf Coasts of North America* (The Center for Conservation Biology Technical Report Series: CCBTR-16-09, Issue.

Yousef, M., & Allmer, J. (2023). Deep learning in bioinformatics. *Turk J Biol*, *47*(6), 366-382. https://doi.org/10.55730/1300-0152.2671

Appendix

Vegetation Data

Vegetation Species List

BOFR - *Borrichia frutescens*
SPSP – *Spartina spartinae*
SCRO – *Scirpus robustus*
DISP - *Distichlis spicata*
BARE – bare ground
MOLI - *Monanthochloe littoralis*
BAMA5 - *Batis maritima*
SAVI- *Salicornia virginica (depressa)* Perennial glasswort
TYLA - *Typha latifolia*
SPPA - *Spartina patens*
PHNO2 - *Phyla nodiflora*
SCPUL4 - *Schoenoplectus pungens* var. *longispicatus*
CEER2 - *Centella erecta*
ELMO - *Eleocharis montana*
BAMO - *Bacopa monnieri*
SEHE8 - *Sesbania herbacea*
DIAC - *Dichanthelium aciculare*
SAEB2 - *Samolus ebracteatus*
PAMO4 - *Paspalum monostachyum*
RHCA9 - *Rhynchospora caduca*
SPVI3 - *Sporobolus virginicus*
DEAD – dead vegetation
SCLI11 - *Schizachyrium littorale*
EUEX5 - *Eustoma exaltatum*
ANGL2 - *Andropogon glomeratus*
EPLA3 – *Epilobium lactiflorum*
DIAN - *Dichanthium annulatum*
ROAD – caliche road

Nueces Delta Preserve Vegetation Plot Data

					Plot % Cover (18 m circle)					
Date	**Time Start**	**Time End**	**Plot #**	**Dominant e Habitat**	**BOFR**	**SPSP**	**SCRO**	**DISP**	**BARE**	**ELPA3**
6/5/2024	0929	1001	CBBEP01	High Marsh	45	30	15	10	-	-
6/5/2024	1036	1047	CBBEP02	High Marsh	5	95	-	-	-	-
6/5/2024	1117	1131	CBBEP03	High Marsh	-	100	-	-	-	-
6/5/2024	1212	1226	CBBEP04	High Marsh	5	90	-	-	10	-
6/5/2024	1254	1304	CBBEP05	High Marsh	-	95	-	-	-	5
6/5/2024	1316	1328	CBBEP06	High Marsh	-	95	-	-	-	5

CBBEP01 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
BOFR	45	North	75	Dry
SPSP	30	South	50	Moist
SCRO	15	East	75	Moist
DISP	10	West	70	Moist
Average	25		67.5	
Standard Dev	15.8113883		11.90238071	

CBBEP02 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
BOFR	5	North	40	Dry
SPSP	95	South	60	Dry
		East	50	Dry
		West	40	Dry
Average	50	**Average**	47.5	
Standard Dev	63.63961031	**Standard Dev**	9.574271078	

CBBEP03 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SPSP	100	North	65	Dry
		South	40	Dry
		East	65	Dry
		West	75	Dry
Average	100	**Average**	61.25	
Standard Dev	0	**Standard Dev**	14.93039406	

CBBEP04 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
BOFR	5	North	40	Dry
SPSP	90	South	45	Dry
BARE	10	East	92	Dry
		West	45	Dry
Average	35	**Average**	55.5	
Standard Dev	47.69696007	**Standard Dev**	24.44722206	

CBBEP05 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SPSP	95	North	45	Dry
ELPA3	5	South	52	Dry
		East	50	Dry
		West	60	Dry
Average	50	**Average**	51.75	
Standard Dev	63.63961031	**Standard Dev**	6.238322424	

CBBEP06 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SPSP	95	North	36	Dry
ELPA3	5	South	60	Dry
		East	45	Dry
		West	55	Dry
Average	50	**Average**	49	
Standard Dev	63.63961031	**Standard Dev**	10.67707825	

Mission River Delta Vegetation Plot Data

					Plot % Cover (18 m circle)				
Date	**Time Start**	**Time End**	**Plot #**	**Dominate Habitat**	**SPSP**	**BOFR**	**MOLI**	**BAMA5**	**BARE**
6/6/2024	1039	1107	Mission Delta 01	High Marsh	65	15	10	-	10
6/6/2024	1216	1237	Mission Delta 02	High Marsh	20	-	-	70	10

Mission Delta 01 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SPSP	65	North	42	Dry
BOFR	15	South	0	Moist
MOLI	10	East	20	Dry
BARE	10	West	52	Dry
Average	25	**Average**	28.5	
Standard Dev	26.77063067	**Standard Dev**	23.23072678	

Mission Delta 02 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SPSP	20	North	45	Moist
BAMA5	70	South	20	3.5 cm
BARE	10	East	30	5.5
		West	45	Dry
Average	33.33333333	**Average**	35	
Standard Dev	32.14550254	**Standard Dev**	12.24744871	

Padre Island Kleberg Tract Vegetation Plot Data

					Plot % Cover (18 m circle)			
Date	**Time Start**	**Time End**	**Plot #**	**Dominate Habitat**	**TYLA**	**SPPA**	**SCPUL4**	**PHNO2**
6/11/2024	1055	1118	Kleberg Tract 01	Low Marsh	20	15	45	10
6/11/2024	1011	1040	Kleberg Tract 02	Low Marsh	95	5	-	-

Kleberg Tract 01 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
TYLA	20	North	80	Moist
SPPA	15	South	36	Moist
SCPUL4	45	East	55	Moist
PHNO2	10	West	94	Moist
Average	22.5	**Average**	66.25	
Standard Dev	15.54563176	**Standard Dev**	25.82472975	

Kleberg Tract 02 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
TYLA	95	North	35	Moist
SPPA	5	South	0	14.5
		East	80	Moist
		West	152	Dry
Average	50	**Average**	66.75	
Standard Dev	63.63961031	**Standard Dev**	65.59153909	

Padre Island National Seashore Bird Island Basin (BIB) Vegetation Plot Data

					Plot % Cover (18 m circle)													
Date	**Time Start**	**Time End**	**Plot #**	**Dominate Habitat**	**SCPUL4**	**CEER2**	**PHNO2**	**TYLA**	**ELMO**	**BAMO**	**SEHE8**	**DIAC**	**SAEB2**	**PAMO4**	**RHCA9**	**SPVI3**	**DEAD**	**BARE**
6/18/2024	8:30	9:12	BIB01	Low Marsh	40	20	10	-	-	5	5	25	20	5	10	5	-	-
6/18/2024	9:17	9:53	BIB04	Low Marsh	-	25	10	50	10	10	-	-	-	-	-	-	-	-
6/18/2024	10:00	10:28	BIB02	Low Marsh	-	20	5	50	-	-	5	10	-	10	-	-	-	-

Bird Island Basin 01 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SCPUL4	40	North	33	Dry
CEER2	15	South	37	Moist
PHNO2	5	East	0	Moist
BAMO		West	15	Moist
SEHE8		**Average**	21.25	
DIAC	25	**Standard Dev**	17.095321	
SAEB2	10			
PAMO4	5			
RHCA9	5			
SPVI3				
Average	16.6			
Standard Dev	13.66			

Bird Island Basin 02 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
CEER2	20	North	20	Moist
PHNO2	5	South	115	Moist
TYLA	50	East	10	Moist
SEHE8	5	West	10	Moist
DIAC	10	**Average**	38.75	
PAMO4	10	**Standard Dev**	51.05144464	
Average	16.6666667			
Standard Dev	17.2240142			

Bird Island Basin 04 Quadrat Data				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
CEER2	20	North	12	Moist
PHNO2	10	South	25	Moist
TYLA	50	East	55	Moist
ELMO	10	West	10	Moist
BAMO	10	**Average**	25.5	
Average	20	**Standard Dev**	20.76053949	
Standard Dev	17.32			

Padre Island National Seashore Six Pigs East Vegetation Plot Data

					Plot % Cover (18 m circle)							
Date	**Time Start**	**Time End**	**Plot #**	**Dominate Habitat**	**PAMO4**	**SEHE8**	**TYLA**	**SCPUL4**	**SCLI11**	**EUEX5**	**PHNO2**	**ROAD**
6/27/2024	10:37	10:59	Six Pig 03	Low Marsh	10	10	70	-	-	-	5	5
6/27/2024	13:37	13:49	Six Pig 01	Low Marsh	15	20	60	-	5	-	-	-
6/27/2024	14:06	14:16	Six Pig 02	Low Marsh	30	30	-	20	-	10	-	15

Six Pigs East 01				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
PAMO4	15	North	82	Moist
SEHE8	20	South	38	Dry
TYLA	60	East	64	Moist
SCLI11	5	West	17	Dry
Average	25	**Average**	50.25	
Standard Dev	24.1522946	**Standard Dev**	28.59341416	

Six Pigs East 02				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
PAMO4	30	North	27	Moist
SEHE8	30	South	20	Dry
SCPUL4	20	East	25	Moist
EUEX5	10	West	54	Moist
ROAD	15	**Average**	31.5	
Average	21	**Standard Dev**	15.28615932	
Standard Dev	8.94427191			

Six Pigs East 03				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
PAMO4	10	North	100	Moist
SEHE8	10	South	5	Dry
TYLA	70	East	110	Moist
PHNO2	5	West	45	Moist
ROAD	5	**Average**	65	
Average	20	**Standard Dev**	49.15960401	
Standard Dev	28.0624304			

Padre Island National Seashore Six Pigs West Vegetation Plot Data

					Plot % Cover (18 m circle)					
Date	**Time Start**	**Time End**	**Plot #**	**Dominate Habitat**	**EPLA3**	**SCPUL4**	**TYLA**	**PAMO4**	**ANGL2**	**PHNO2**
6/27/2024	**11:27**	**11:42**	**Six Pigs West 02**	**Low Marsh**	**8**	**35**	**52**	**5**	**-**	**-**
6/27/2024	**12:10**	**12:35**	**Six Pigs**	**Low Marsh**	**20**	**70**	**-**	**5**	**5**	**-**

			West 01							
6/27/2024	**13:00**	**13:20**	**Six Pigs West 03**	**Low Marsh**	**-**	**55**	**5**	**30**	**-**	**10**

Six Pigs West 01				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
EPLA3	20	North	100	Moist
SCPUL4	70	South	70	Moist
PAMO4	5	East	67	Dry
ANGL2	5	West	25	4.5
Average	25	**Average**	65.5	
Standard Dev	30.82207	**Standard Dev**	30.83828789	

Six Pigs West 02				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
EPLA3	8	North	95	Moist
SCPUL4	35	South	140	Moist
TYLA	52	East	60	Moist
PAMO4	5	West	110	4
Average	25	**Average**	101.25	
Standard Dev	22.4944438	**Standard Dev**	33.26033674	

Six Pigs West 03				
Species	**Plot % Cover**	**Q Direction**	**Veg Height (cm)**	**Soil Moisture**
SCPUL4	55	North	100	M
TYLA	5	South	28	M
PAMO4	30	East	20	M
PHNO2	10	West	53	2
Average	25	**Average**	50.25	
Standard Dev	22.7303028	**Standard Dev**	36.02198403	