EMERGING TECHNOLOGY FOR THE STUDY OF ONE OF NORTH AMERICA'S MOST ELUSIVE BIRDS, THE BLACK RAIL (*LATERALLUS JAMAICENSIS*)

By

DAVID TILSON

(Under the Direction of Clinton Moore & Robert Cooper)

ABSTRACT

Black Rails (*Laterallus jamaicensis*) are small migratory marshbirds that occur in emergent, fresh- and saltwater wetlands. Little is known about even the basic ecology and life history of this species, and metrics of occupancy and abundance are difficult to gauge. However, emerging technologies present new tools that may shed light on previous knowledge gaps. In this thesis, I discuss the applications of Autonomous Recording Units (ARUs) and Automated Radio-Telemetry Systems (ARTS) as they relate to Black Rail research. I developed a vocalization-specific, audio recognizer using the Animal Sound Identifier (ASI) framework that allows the user to control false positive rates of vocalization detection. Additionally, I present a case study for the application of radio signal strength (RSS)-based ARTS to Black Rail research.

INDEX WORDS: Black Rail, *Laterallus jamaicensis*, Autonomous recording units, Animal sound identifier, Automated radio-telemetry systems.

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DEDICATION

This thesis is dedicated to LGBTQIA+ individuals working in STEM and elsewhere.

Your voices matter.

Never cease to be your authentic selves.

ACKNOWLEDGMENTS

This work would not have been possible without the help of many individuals with whom I am grateful to have worked alongside. I'd first like to thank my co-advisors Clint Moore and Bob Cooper for giving me the opportunity to work on this project and for providing guidance through unexpected challenges. Additionally, I'd like to thank Adam Smith who has served as a mentor to me since shortly after completing my undergraduate degree and who first sparked my passion for coastal saltmarshes and the birds that live there. He has been extremely supportive over the years, and I am grateful he was a part of my graduate experience as a committee member. I'd also like to thank Jeff Hepinstall-Cymerman for providing guidance and insight in his role as a committee member.

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CHAPTER 1

INTRODUCTION

BACKGROUND

Black Rail Species Summary

Black Rails (*Laterallus jamaicensis*) are small migratory marshbirds that occur in emergent, fresh- and saltwater wetlands (Eddleman et al. 2020). Black Rails require dense herbaceous cover and shallow (~2cm) water coverage (Flores and Eddleman 1995). These birds are rare and have a reputation for elusivity beyond that of even other rails, a group known for their secretive habits, making them a highly sought-after quarry by recreational birders (Eddleman et al. 2020).

Black Rail populations, like populations of many North American rail species, are currently in decline, primarily due to wetland degradation and loss (Eddleman et al. 1988, Conway et al. 1994). The Black Rail has experienced population declines, breeding range retractions, and reductions in the number of breeding locations within its core range (Davidson 1992, Watts 2016). The eastern subspecies (*L. j. jamaicensis*) is listed as Threatened under the Endangered Species Act (83 Fed. Reg. 195). The Southeast U.S. Regional Waterbird Plan (Hunter et al. 2006) identifies the Black Rail as a species of continental and regional concern in need of immediate management action. In the U.S., the eastern subspecies historically occurred along the Atlantic Coast from Massachusetts south to Florida and along the Gulf Coast from Florida to south Texas, with inland locations scattered from east of the Appalachian Mountains westward to Colorado and north to the Great Lakes (Eddleman et al. 2020). However, the distribution of recent observations indicates a substantial southward contraction in the subspecies' range by approximately 450km (Watts 2016). Coastal areas support most remaining breeding populations and almost all wintering populations (Hunter et al. 2006, Watts 2016).

Black Rails are rarely seen, and managers rely almost exclusively on vocalizations to assess site occupancy and abundance for this species. Peak vocalization times for Black Rails in Maryland were reported 1-2h after sunset and 1-2h before sunrise, with individuals rarely heard during the day (Weske 1969, Reynard 1974). In Florida, peak vocalizations were reported 1-2h before sunset and 1-2h after sunrise (Eddleman et al. 2020). Vocalization times varied between two populations in New Jersey, with one never vocalizing at night and another vocalizing primarily at night (Kerlinger and Wiedner 1991).

Legare and Eddleman (2001) reported a larger home range size for Eastern males (0.82-3.1ha) compared to females (0.51-0.86ha) during the nesting season. Similarly, Tsao et al. (2009) found that male California Black Rails (*L. j. coturniculus*) have home ranges 46% larger than females' during the breeding season. Additional home range reports include winter-spring home ranges of 0.52-0.91ha in Texas (Moore et al. 2018) and 0.22-1.59ha in Louisiana (Johnson and Lehman 2019) for the Eastern subspecies (both sexes). The largest reported territory was 3.34ha of a male in Maryland (Weske 1969). California Black Rails have reported home ranges of 0.26-0.43ha (Flores and Eddleman 1991, Tsao et al. 2009). The smaller home range of the California subspecies relative to Eastern Black Rails could be due to the larger size of the Eastern subspecies or differences in predation risk, food nutritive value, or habitat availability (Tsao et al. 2009). Other rails have smaller home ranges during the breeding season compared to the non-breeding season (Bookhout and Stenzel 1987, Conway et al. 1993); however, Kolts and McRae

(2017) reported larger home ranges for King Rail (*Rallus elegans*) during the brood-rearing period when parents are no longer constrained to the nest. Flores and Eddleman (1991) reported that home ranges did not differ between seasons for the California Black Rail, possibly due to stable year-round water levels.

Autonomous Recording Units

Conducting auditory point count surveys using intermittent broadcasts of conspecific vocalizations has been the primary method for surveying Black Rails (Conway et al. 2004). However, recent studies have started to investigate the use of Autonomous Recording Units (ARUs) for secretive marshbirds in general (Sidie-Slettedahl et al. 2015, Drake et al. 2016, Schroeder and McRae 2020, Znidersic et al. 2020) and Black Rails specifically (Butler et al. 2015, Bobay et al. 2018, Znidersic et al. 2021). ARUs are weatherproof devices that can record audio for long periods of time. ARUs can be set to record continuously or be programmed to record at specific times of interest. These units allow vocalization data to be collected over long periods of time relative to manual surveys (e.g., point counts) and/or at times when in-person surveying is logistically challenging (e.g., nighttime hours). ARUs may be a particularly useful technology for detecting elusive species that vocalize infrequently (Celis-Murillo et al. 2009, Hutto and Stutzman 2009, Drake et al. 2016, Bobay et al. 2018).

ARUs can help mitigate some of challenges related to surveying Black Rails such as their scarcity, infrequent vocalizations, difficult-to-navigate terrain, and high variability in peak vocalization times across populations. However, efficient and cost-effective identification of vocalizations from ARU-produced libraries is one of the greatest challenges for data collection via ARUs (Hutto and Stutzman 2009, Shonfield and Bayne 2017).

Automated recognizers reduce review time and can make data processing more manageable for large audio file libraries (Knight et al. 2017). However, automated analysis is prone to higher false positive and false negative rates compared to manual review (Sidie-Slettedahl et al. 2015, Bobay et al. 2018). Bobay et al. (2018) reported a high false positive rate (91 true positives out of 11,872 predictions of vocalization) and an unknown false negative rate using Kaleidoscope software (Wildlife Acoustics 2017) to detect Black Rail vocalizations. Butler et al. (2015) reported a 37% false positive and a 9% false negative rate when using Song Scope software (Wildlife Acoustics 2011) for Black Rail.

Automated Radio-Telemetry Systems

Much of the basic ecology and life history of Black Rails remains unknown, and telemetry studies may offer the best opportunity to study this cryptic species (Case and McCool 2009). Automated radio-telemetry systems (ARTS) use receivers that automatically record signals from radio transmitters. ARTS were introduced in one of the earliest wildlife telemetry studies (Cochran et al. 1965), but many studies favor manual radio-tracking due to the high cost associated with automated telemetry (Ward et al. 2013). However, ARTS are not as costly as they once were. Additionally, the advent of digitally coded tags that all transmit on the same frequency now allows ARTS to track multiple animals simultaneously. ARTS can achieve large sample sizes through higher sampling frequencies compared to manual radio-tracking (Paxton et al. 2022), and ARTS are particularly valuable for species that are too small to carry global positioning system (GPS) trackers or for species where recovery of position-logging trackers is unlikely due to low recapture rates.

A relatively recent form of ARTS is the use of a network or grid of omnidirectional receivers that use radio signal strength (RSS) to estimate an animal's location (Krull et al. 2018, Paxton et al. 2022, Wallace et al. 2022). RSS-based ARTS use a known relationship between RSS values and distance, established through in situ calibrations, to determine tag position via multilateration.

STUDY SIGNIFICANCE

Black Rails are difficult to study because of their scarcity and elusive habits. Much remains to be learned about the life history of this species, and mapping of populations and assessing patterns of occurrence and abundance are vital for conservation efforts (Hands et al. 1989). ARUs are an emerging tool for surveying Black Rail occupancy but are currently limited by the lack of accurate automated classifiers. RSS-based ARTS are a recently developed tool for wildlife monitoring that have never before been applied to Black Rails. ARTS have great potential in exploring breeding ecology, movement ecology, habitat selection, and patterns of activity in Black Rails. The overarching goal of this thesis is to further explore these two emerging technologies and their applications to Black Rails. Specifically, my objectives were to 1) investigate the potential of the Animal Sound Identifier framework (ASI; Ovaskainen et al. 2018) to build vocalization-specific recognizers for Black Rails and compare my recognizer with previous classifiers for the species, 2) present a case study using a RSS-based ARTS to track a transmittered Black Rail and discuss best practices and potential applications, and 3) summarize potential applications of both ARUs and ARTS and areas for future research as they apply to Black Rails.

THESIS STRUCTURE

This chapter provides a general overview and background information on Black Rails and introduces emerging tools, ARUs and ARTS, with potential applications to aid research of this difficult to study species.

In chapter two, I introduce the ASI framework (Ovaskainen et al. 2018) and adapt it to build Black Rail recognizers that are vocalization-type specific and validated against an independent audio library (i.e., a separate library than the one used to build the models). I then compare this method to prior recognizers for this species and discuss limitations and potential next steps for the application of ARUs to Black Rail research.

In chapter three, I present a case study of the first RSS-based ARTS to be applied to Black Rail research. This chapter discusses best practices and potential applications of this method.

The fourth chapter summarizes major findings and conclusions and highlights areas for future work based on the findings from each study and the relevant knowledge gaps for the species.

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CHAPTER 2

APPLICATION OF THE ANIMAL SOUND IDENTIFIER (ASI) FRAMEWORK TO DEVELOP VOCALIZATION-SPECIFIC BLACK RAIL CLASSIFIERS[1]

[1]Tilson, D.A. and A.D. Smith. To be submitted to *Avian Conservation & Ecology.*

ABSTRACT

Targeted surveys for Black Rails have commonly consisted of auditory point count surveys using intermittent broadcasts of conspecific vocalizations. However, these surveys are labor-intensive, and survey reliability can be affected by temporal calling behavior, surveyor experience, survey effort, and environmental factors. For these reasons, autonomous recording units (ARUs) are receiving increased popularity for passive acoustic monitoring. However, efficient and cost-effective identification of vocalizations from ARU-produced libraries is one of the greatest limitations of this method of data collection. In this paper, we apply the Animal Sound Identifier (ASI) framework to develop vocalization-specific classifiers that allow the user to control false positive rates. We then validated the most common vocalization type, the Black Rail's *ki-ki-doo* song, and found ASI predicted probability was strongly related to the presence of the vocalization ($z = 7.14$, $p < 0.0001$). Lastly, we discuss the potential of further development of vocalization-specific classifiers and their application to Black Rails.

INTRODUCTION

Black Rails (*Laterallus jamaicensis*) are small migratory marshbirds that occur in emergent, fresh- and saltwater wetlands (Eddleman et al. 2020). These birds have a reputation for elusivity beyond that of other rails, a group known for their secretive habits. The Black Rail has experienced population declines, breeding range retractions, and reductions in the number of breeding locations within its core range (Davidson 1992, Watts 2016). Additionally, the Eastern subspecies (*L. j. jamaicensis*) is listed as Threatened under the Endangered Species Act (83 Fed. Reg. 195).

Targeted surveys for this species have commonly consisted of auditory point count surveys using intermittent broadcasts of conspecific vocalizations (Conway et al. 2004). However, survey reliability of labor-intensive point count surveys for secretive marshbirds can be affected by temporally-varying calling behavior, surveyor experience, survey effort, and environmental factors (Conway and Gibbs 2011). Furthermore, wetlands can be logistically challenging to survey over multiple repeated visits due to their mucky terrain and often dense vegetation (Bobay et al. 2018, Znidersic et al. 2020). For these reasons, recent studies have started to investigate the use of autonomous recording units (ARUs) for secretive marshbirds in general (Sidie-Slettedahl et al. 2015, Drake et al. 2016, Schroeder and McRae 2020, Znidersic et al. 2020) and Black Rails specifically (Butler et al. 2015, Bobay et al. 2018, Znidersic et al. 2021).

Efficient and cost-effective identification of vocalizations from ARU-produced libraries is one of the greatest challenges for data collection via ARUs (Hutto and Stutzman 2009, Shonfield and Bayne 2017). Manual review of audio recordings or spectrograms to identify vocalizations is time intensive, often requiring more time per file than the length of the audio itself (Celis-Murillo et al. 2009, Hutto and Stutzman 2009). Manual inspection is often not feasible for large audio libraries that can include days or months of audio recordings. Automated recognizers can reduce review time and make data processing more manageable for large audio file libraries (Knight et al. 2017). However, automated analysis is prone to higher false positive and false negative rates compared to manual review (Sidie-Slettedahl et al. 2015, Bobay et al. 2018), and accuracy may be particularly low for less complex vocalizations (Swiston and Mennill 2009, Sidie-Slettedahl et al. 2015, Bobay et al. 2018, Schroeder and McRae 2020).

Recognizers are typically scored using human listening and/or manual inspection of spectrograms as a benchmark, and two common score metrics of recognizer performance are precision and recall (Knight et al. 2017). Precision is the proportion of recognizer-suggested positives that are true detections of the vocalization of interest, and recall is the proportion of true vocalizations that the recognizer correctly identifies (Knight et al. 2017). Users often select a score threshold according to their specific objectives to balance the tradeoff between precision, to minimize false positives, and recall, to minimize missed detections (Knight and Bayne 2019).

In this study, we adapted the Animal Sound Identifier framework (hereafter ASI; Ovaskainen et al. 2018) to build Black Rail recognizers that are vocalization-type specific. We then validated the model for the most common Black Rail vocalization against an independent audio library (i.e., a separate library than the one used to build the models) and compared our findings with previous recognizers for Black Rails. Finally, we discuss the current applicability of ARUs to Black Rail studies and highlight areas for continued development.

METHODS

Building the Audio Library

We built recognizers in ASI for four general Black Rail vocalizations – *ki-ki-doo*, *growl*, *churt*, and *ik-ik* (Eddleman et al. 2020). The most distinct and common Black Rail vocalization is their song, *ki-ki-doo*, primarily given by males (Reynard 1974, Flores and Eddleman 1991). While typically 3 notes, there is some variation with 1-3 *ki*'s and occasionally 2 *doo*'s. Thus, we also constructed a recognizer that incorporated partial components of the *ki-ki-doo* song. The second most common call type is an agitated "growl", a multi-note *grr-grr-grr-grr* that can continue in extended sequences. Other vocalizations include *churt* calls that can occur singly or in groups of twos or threes and *ik-ik* calls that occur in clusters of two to several notes.

Our reference library was collected or solicited from partners throughout the Eastern Black Rail's breeding range (CO, FL, NC, SC, TX) and was made using various Song Meter (Wildlife Acoustics, Inc., Maynard, MA) and iPhone (Apple Inc., Cupertino, CA) devices. We sought audio files of diverse vocalization strength and quality resulting from varied recording environments (e.g., distance from ARU, background noise and non-target species composition, type of recording device). Prior to use with ASI, audio files were divided into ≤ 1-minute segments using the open-source SoX – Sound eXchange audio editor (v. 14.4.2; https://sourceforge.net/projects/sox/files/sox/). Our full reference library contained the subset of segments known or suspected to include Black Rail vocalizations. We further subset from this library those segments known a priori to contain Black Rail vocalizations.

Setting ASI Parameters

The audio file conversion and cross-correlation calculation parameters used are available on GitHub (Script_0_defining_project_parameters.m; https://github.com/adamdsmith/BLRA_ASI_scripts). In general, we used ASI default parameters as identified in Ovaskainen et al. (2018), with the primary exceptions that we adjusted the frequency parameters with which to search for Black Rail vocalization letter candidates (parts of animal vocalizations potentially useful for identification). We searched for letter candidates with frequencies between 450 - 4500 Hz and allowed for letters to occupy 500 - 3500 Hz bandwidth. Other modifications were minor and are noted in the Script_0_defining_project_parameters.m as noted above.

Identifying Letter Candidates

The first steps of ASI (Ovaskainen et al. 2018) involve the selection, refinement, and annotation of parts of animal vocalizations that are useful for identification. These audio segments, termed "letters" by Ovaskainen et al. (2018), form the basis from which the vocalization-specific recognizer models are built. Although ASI can auto-select letter candidates, we found this feature inadequate for Black Rails given the relative infrequency of vocalizations in audio recordings, especially the rarer call types (i.e., *churt* and *ik-ik*). Instead, we manually identified letters representing the focal call types from our selection of one-minute reference segments known a priori to contain Black Rail vocalizations. More than one letter for a given vocalization type can be created per reference segment for those containing multiple vocalizations or constructed from vocalization fragments.

We considered two recognizers for the *ki-ki-doo* song – a general *ki-ki-doo* recognizer that included letters representing a complete 3-part song as well as letters comprising fragments of the song (i.e., the *ki-ki*, *ki-doo*, and *doo* components; hereafter the KKD recognizer) and a recognizer including only letters containing the complete 3-part *ki-ki-doo* vocalizations (hereafter the KKD0 recognizer). *Growl* calls can be long and variable, so we standardized our construction of letters for this vocalization by extracting the opening sequence of 3 notes, and alternating sequences of 4 and 3 notes for the duration of a particular growl vocalization. Letters from *ik-ik* calls were selected from their natural groupings of 2+ notes. Letters from *churt* calls were always selected using single notes, even if given in a series as doubles or triples.

Turning Letters into Vocalization-Specific Models

Each letter was then cross-correlated with our full reference library of one-minute segments known or suspected to include Black Rail vocalizations from which a letter-specific, probit regression model was constructed for each individual letter (described here, but see Ovaskainen et al. 2018 for more details). This step requires the user to classify potential letter matches as a positive/negative match while ASI adaptively refines the letter-specific models with each input. Models span the entire predictor space, but ASI prompts particular focus on areas of high vocalization uncertainty (i.e., potential matches occurring near the inflection point on the probit models). Model training continues until the mapping from correlation to classification probability converges and classifying additional potential matches no longer exerts a meaningful change on the fitted model.

Next, all letters of a particular vocalization are used collectively to generate predictors of probability that a given vocalization type occurs in a particular audio segment. Those predictors are then used to build vocalization-specific probit models, in a way similar to the letter-specific model construction described previously (see Figures 2.1 & 2.2 for examples of ASI-generated models). ASI presents model quality in terms of its discrimination power, measured by Tjur R^2 (Tjur 2009). Each model report includes an observed R^2 (R2T) from the training data and a predicted R^2 (R2A). Note, R2A often exceeds that of R2T because training data are specifically selected to involve cases that are especially difficult to classify when fitting the vocalization probit models (Ovaskainen et al. 2018). We build all vocalization-specific models, except the KKD, from 50 inspected and classified segments; we used 110 segments for the KKD model due to its more variable vocalization fragments.

When ASI uses a vocalization-specific model to estimate the probability of an audio segment containing a given vocalization, the first predictor is the highest probability/correlation of that audio segment with the letters constructed for that vocalization type (called *Lmax*, for maximum letter probability). For example, of the set of KKD0 letters used to build the KKD0 species model, the first predictor is the highest correlation among all those letters and the new, unknown audio segment. The second predictor is a modified first principal component capturing variation not explained by *Lmax*. It can represent various information, including the predictive combinations of several letters, their frequencies, or their autocorrelation structures (additional detail provided in Ovaskainen et al. 2018). The second predictor typically explains much less variation than *Lmax*, and we evaluated its usefulness on a vocalization-specific basis.

Validation

We validated one of our fitted models, the KKD0 classifier, against an independent set of data from South Carolina containing 1440 one-minute segments randomly sampled from nearly 720 hours of audio recordings from South Carolina. Of these, we selected 276 audio segments spanning the full range of output probabilities and manually inspected them for the presence of a full *ki-ki-doo* vocalization to evaluate our model's predictive ability. For each audio segment, we also noted whether incomplete forms of the vocalizations were present (e.g., *ki-doo* only) and if the vocalization was so faint as to be detectable audibly but visually absent (or nearly so) from the corresponding spectrogram (generated by Audacity v. 3.1.3; The Audacity Team 2021). We then constructed a logistic generalized linear model to estimate the relationship between predicted probability of presence of vocalization and true presence in R v. 4.2.1 (R Core Team 2022). This allowed us to explore false positive rates, model precision, and recall a function of predicted probability of the KKD0 model. Precision is defined as TP/(TP+FP), where TP represents true positives and FP represents false positives (Knight and Bayne 2019). Recall is defined as TP/(TP+FN), where FN represents false negatives (Knight and Bayne 2019). We did not validate the KKD model (i.e., containing partial call fragments), because we found it more prone to mistaking vocalizations from non-target species for Black Rail. We also lacked sufficient independent audio segments to validate *growl*, *churt*, or *ik-ik* calls, although this could be completed in the future once enough relevant audio is compiled.

All ASI-related code was run in MATLAB version 2020a (The MathWorks Inc. 2020) and is available at https://github.com/adamdsmith/BLRA_ASI_scripts.

RESULTS

Our full reference library was comprised of 6149 one-minute segments. Of these, 251 segments were known a priori to contain Black Rail vocalizations. We created 1564 letters representing the four Black Rail vocalization types from the reference segments of known vocalizations (Table 2.1). Most letters (61%) derived from recordings collected in North Carolina, and the remainder derived from 4 other states representative of the Eastern Black Rail's breeding range (Table 2.2). In 276 audio segments obtained from our validation data set, we determined that the full *ki-ki-doo* vocalization occurred in 74 of them and incomplete vocalizations occurred in 37 others.

The model for complete *ki-ki-doo* vocalizations (KKD0; R2T = 0.72, R2A = 0.90; Figure 2.1) far outperformed the *ki-ki-doo* model that included incomplete vocalization fragments (KKD; R2T = 0.48, R2A = 0.62; Figure 2.2). The vocalization-specific models for *ik-ik* and *growl* also performed well with high expected predictive ability for new files (i.e., R2A > 0.8), while the *churt* model appeared to lack predictive ability (R2A = 0.31; Table 2.2). For all models, we report predictive ability based on a model containing the first two predictors. In all cases, the first predictor (*Lmax*) was the most important, with the second predictor expected to explain less than 3% variation in independent audio segments in all models. Figures for *churt*, *ik-ik*, and *growl* models are available in the supplementary (S2) figures (Figures 2.5-2.7).

ASI predicted probability of KKD0 vocalization in an independent data set was strongly related to the presence of the vocalization from manual review ($z = 7.14$, $p < 0.0001$; Figure 2.3A). This model offers insight into expected false positive rates based on the predicted ASI probability. To put expected rates of false positives into perspective, for example, at an ASI probability 0.75, the logistic model estimated a 44% chance of containing KKD, or roughly equal parts true vocalizations and false positives. Many of the segments with ASI probabilities greater than 0.7 classified as not containing a full KKD0 did contain incomplete KKD vocalizations. When we relaxed our definition of a successful identification to include those audio segments with incomplete KKD, the predictive power of the logistic model improved substantially at higher ASI probabilities (Figure 2.3B), with an ASI probability of 0.75 corresponding to an estimated 67% chance of a segment containing a KKD0 or incomplete KKD vocalization (equivalent to a 2:1 true positive to false positive ratio, or 33% false positive rate).

We observed similar effects in the precision and recall of the vocalizations from the KKD0 model (Figure 2.4). Recall of *ki-ki-doo* vocalizations declines steadily with predicted probability but is relatively consistent whether we used a strict (Figure 2.4A) or relaxed (Figure 2.4B) definition of a successful identification. In other words, the relative proportion of true positives and false negatives was consistent between the two definitions of successful classification. Precision increases with the predicted probability used to identify vocalizations but is distinctly improved when using a relaxed definition of a successful Black Rail vocalization in manually-reviewed files, reflecting the relative decrease in false positives under this scenario.

Processing new audio files to generate estimates of the probability of containing a given vocalization related nearly 1:1 with audio file length. For example, the time to process 24 hours of 1-minute segments with the final model to generate the probability of files containing KKD0 vocalizations took approximately 24 hours of runtime (Intel® Core i7 11^{th} generation processor). However, we sped up the runtime considerably by performing the calculation in parallel (approximately 4 hours on 8 threads with the same processor).

DISCUSSION

Classification of Black Rail vocalizations directly from field data rather than from a reference library is difficult due to the relative infrequency of vocalizations. This is particularly true for rarer call types. However, we feel that it is important to use field recordings representative of the natural variation in call strength and non-target sounds found in field data. Training recognizers on only “clean” vocalizations where the desired call-type is dominant in its frequency band may lead to poorer performance for audio when these ideal conditions are not met (Znidersic et al. 2021). We found it necessary to curate a reference library that had a higher number of files containing Black Rail vocalizations than expected from a single field effort. Even so, Black Rail vocalizations were so infrequent relative to non-target species that we opted for manual letter selection over ASI’s auto-selection feature for letter candidates. We were also unable to find sufficient audio containing any target call-type for model validation except the most common Black Rail vocalization, the *ki-ki-doo* song.

Our validation model of the KKD0 classifier and evaluations of precision and recall may allow researchers to a priori set their threshold of model performance based on their preferences and ability to manually review audio. The selected ASI threshold theoretically quantifies the expected maximum proportion of false positives. For example, with a KKD0 ASI probability > 0.75, less than ½ of the files should be false positives since higher probability files should have fewer false positives according to the logistic model (under the relaxed definition of success). Our classifier is likely most practically used to identify files with a high probability of containing KKD0 calls while offering reasonable false positive rates. Researchers will likely want to balance precision and recall, but they may prioritize one over the other depending on their specific goals. Setting excessively strict false negative rates likely results in unacceptable false positive rates for most studies, and so precision may be the more important metric. However, if the main goal is to document the presence of Black Rail vocalizations in each audio file regardless of how many false positives must be reviewed, then recall becomes more important.

Under the relaxed definition of successful identification, our inferred false positive rate is 33% at a threshold of 0.75 ASI probability. This is a lower false positive rate than previous studies using Kaleidoscope (99.2%; Wildlife Acoustics 2017; Bobay et al. 2018) and Song Scope (37%; Wildlife Acoustics 2011; Butler et al. 2015). Black Rail KKD0 calls in audio segments with ASI probability < 0.25 were very faint and not easily discernible on the spectrogram. This was expected because letters are built from spectrogram characteristics of the audio (i.e., it is not possible to build letters from audio that is visually absent from the spectrogram). It is important to note that these faint vocalizations are prone to false negatives with any classifier and even with manual review. In fact, many of these faint calls were incorrectly annotated as negative in initial stages of validation and were only corrected after comparing notes between multiple reviewers and revisiting audio with discrepancies.

Our model for complete *ki-ki-doo* vocalizations (KKD0) far outperformed our KKD model including vocalization fragments. Vocalization fragments from non-target species, particularly Icterids, were often mistaken for KKD fragments, particularly the *doo-* and *ki-ki-* only letters. This can likely be attributed to the relative simplicity of these letters. Likewise, *churt* calls have little complexity, and identifiability may have been improved had we also included letters from the occasional double or triple *churt*. For example, the *ik-ik* model had the highest R^2 value and single notes are similarly noncomplex; but unlike *churt* calls, *ik-ik* calls almost always occur in clusters of 2+ notes and all our letters included multiple notes.

ASI and other recognizers require computational skills that may be beyond the training of some ecologists (Znidersic et al. 2021). BirdNET (Kahl et al. 2021), an analysis software that can be applied to extremely large collections of audio, is a more user-friendly alternative but costs money and is yet unevaluated for Black Rails. ASI is performed in MATLAB which requires a paid license unlike some other programming platforms (e.g., R). Additionally, ASI requires some modest pre-processing (e.g., splitting the files into 1-minute segments), and processing new audio files to generate probabilities of vocalization occurrence in MATLAB requires running in parallel on multicore processers or running on more powerful high-performance computing (HPC) clusters to reduce processing times.

While recognizers for Black Rails are still limited, our study demonstrates the potential of classifiers that allow the user to control false positive rates and successfully identify Black Rail vocalizations. We also highlight areas for continued investigation, specifically the development of vocalization-specific models for less frequent call-types. *Ki-ki-doo* songs are given primarily during the breeding season, but little is known about the temporal frequency of other vocalization types, which may be important for developing survey methods for occupancy during the non-breeding season. Another area of potential research is the development of chick and juvenile call-type recognizers. We briefly explored this, but it quickly became apparent that our library contained far too few of these vocalizations to make the models. However, if developed, these recognizers could be instrumental in surveys of breeding occupancy and success.

CONCLUSIONS

We expect to see an increased popularity of passive acoustic monitoring via ARUs as field equipment both improves and becomes less costly. Augmenting point-count surveys with ARU surveys may increase detection probability for Black Rails and thus increase the ability to make statistical inferences about occupancy (Bobay et al. 2018). ARUs have the capacity to greatly increase the temporal span of acoustic surveys while reducing visits to easily disturbed sites with hazardous access. Additionally, ARUs could be used to survey occupancy across larger tracts of land allowing for a more targeted approach for in-person abundance surveys.

ARUs may one day replace in-person surveys as the preferred method of surveying occupancy for Black Rails and similar species. However, a few barriers must be crossed before this becomes possible. First, classifiers must be made more accessible. This can be achieved by developing classifiers that are more user-friendly to users with limited computational skills or by making outsourcing classification less cost prohibitive. Second, the time between ARU deployment and the acquisition of actionable data for analysis and evaluation must be reduced. In-person surveys produce data that is actionable as soon as it is collected and entered. Conversely, audio files collected via ARUs may sit on hard drives indefinitely due to limited time for review. Developing automated classifiers that can process audio quickly and accurately is the key to unlocking the full potential of ARUs. Lastly, we encourage clear evaluation of false positive and false negative rates, precision, and recall for all classifiers. This will allow researchers to clearly define goals for their monitoring programs based on their ability to review output from automated classification.

ACKNOWLEDGMENTS

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TABLES

Table 2.1. Number of letters identified for each vocalization type from 251 one-minute reference segments.

Vocalization type	# letters identified
KKD	1274
KKD0	368
KK	257
KD	368
D	281
GRR	99
IKIK	100
CHURT	91

Table 2.2. Number of letters identified from each state throughout the Eastern Black Rail's breeding range.

State	# letters identified
NC	960
SC	228
CO	177
TX	169
FL	30

Table 2.3. Observed (R2T) and predicted (R2A) Tjur R^2 (Tjur 2009) values for fitted vocalization-specific models based on both predictors for each.

Model	R2T	R2A
KKD0	0.72	0.90
KKD	0.48	0.62
IKIK	0.88	0.90
GROWL	0.66	0.83
CHURT	0.55	0.31

FIGURES

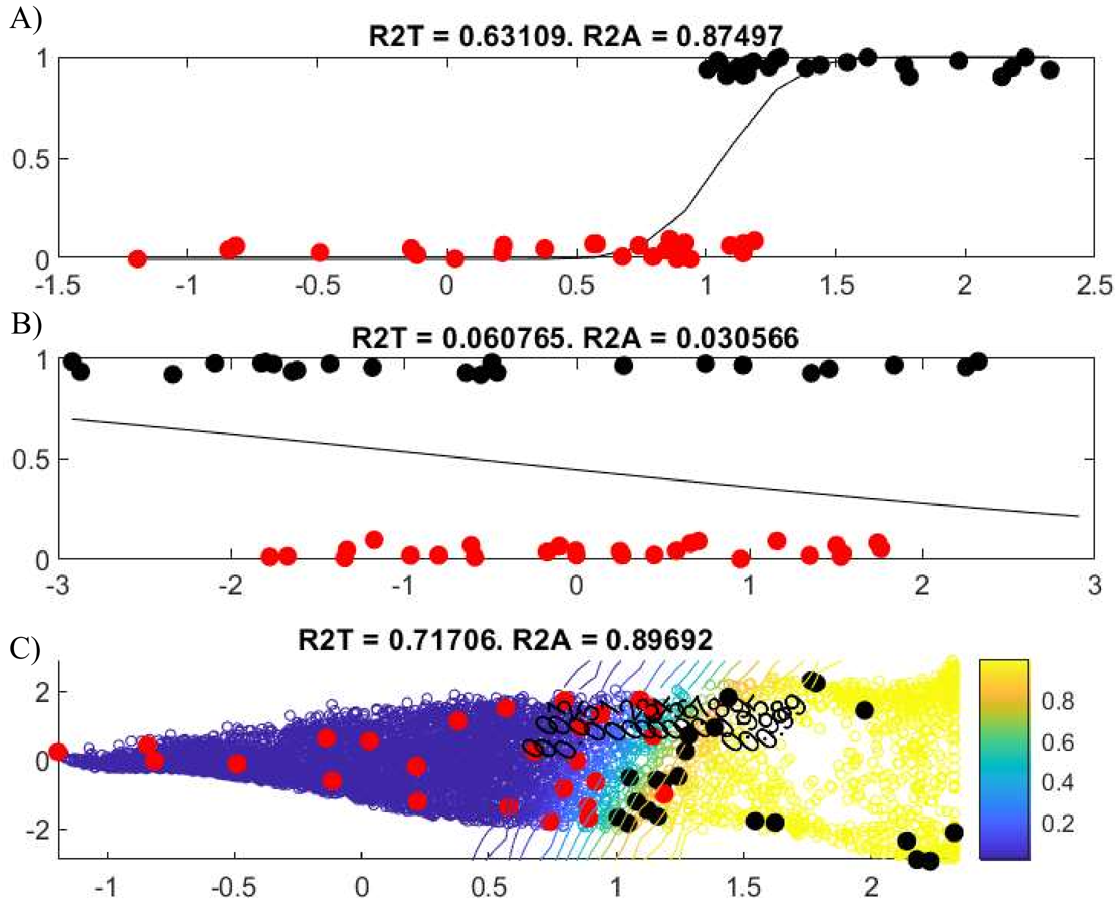

Figure 2.1. Vocalization-specific model built from letters containing only full *ki-ki-doo* vocalizations (KKD0), estimated from a sample of 50 manually classified segments. Panels illustrate the predictive ability using only predictor 1 (A; horizontal axis; the letter with maximum correlation, *Lmax*; see text for details), the predictive ability of a second predictor alone (B; horizontal axis), and the combined predictive ability of both predictors (C; predictor 1 along horizontal axis, predictor 2 along vertical axis). Red points indicate audio files not containing the vocalization and black points indicate files containing the vocalization; points are jittered to improve visibility. Predicted probability of a file containing the KKD0 vocalization is displayed as the vertical axis in panels A and B and is displayed as contour isoclines and a color ramp in panel C.

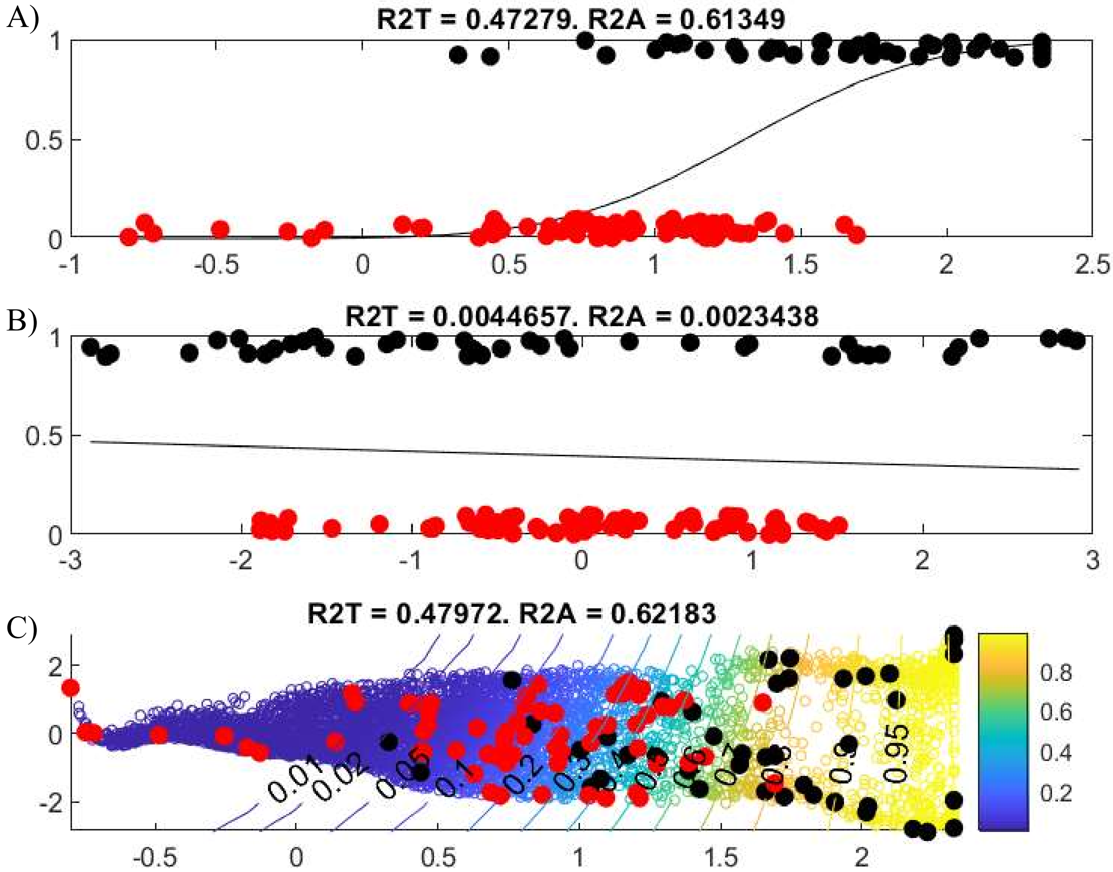

Figure 2.2. Vocalization-specific model built from letters containing full or fragmented *ki-ki-doo* vocalizations (KKD), estimated from a sample of 110 manually classified segments. Panels illustrate the predictive ability using only predictor 1 (A; horizontal axis; the letter with maximum correlation, *Lmax*; see text for details), the predictive ability of a second predictor alone (B; horizontal axis), and the combined predictive ability of both predictors (C; predictor 1 along horizontal axis, predictor 2 along vertical axis). Red points indicate audio files not containing the vocalization and black points indicate files containing the vocalization; points are jittered to improve visibility. Predicted probability of a file containing the KKD vocalization is displayed as the vertical axis in panels A and B and is displayed as contour isoclines and a color ramp in panel C.

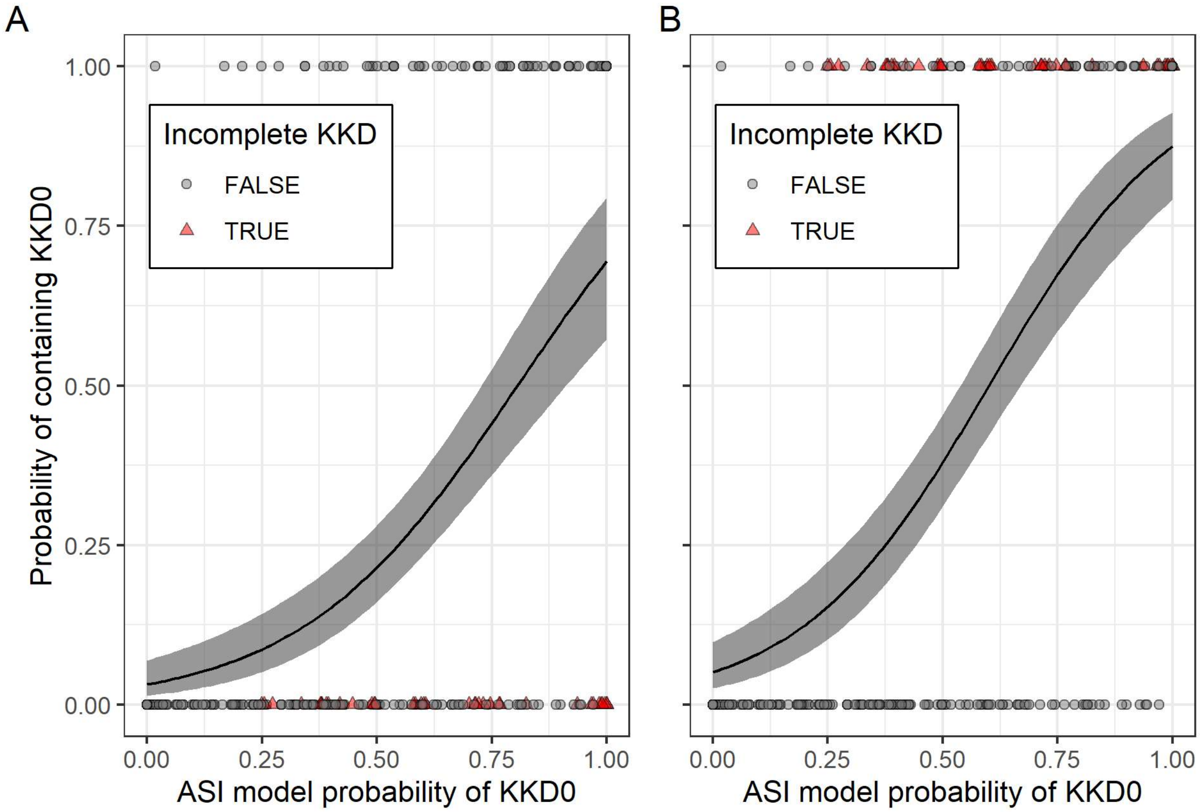

Figure 2.3. Logistic generalized linear model displaying the estimated relationship between KKD0 model-predicted probability and occurrence of a KKD0 vocalization. Audio containing incomplete KKD vocalizations only are classified as no-detection under a strict definition of success (A; i.e., requiring a complete vocalization to consider a vocalization present) and positive-detections under a relaxed definition of success (B; i.e., partial vocalizations accepted as a positive identification). The shaded areas represent 95% pointwise confidence intervals.

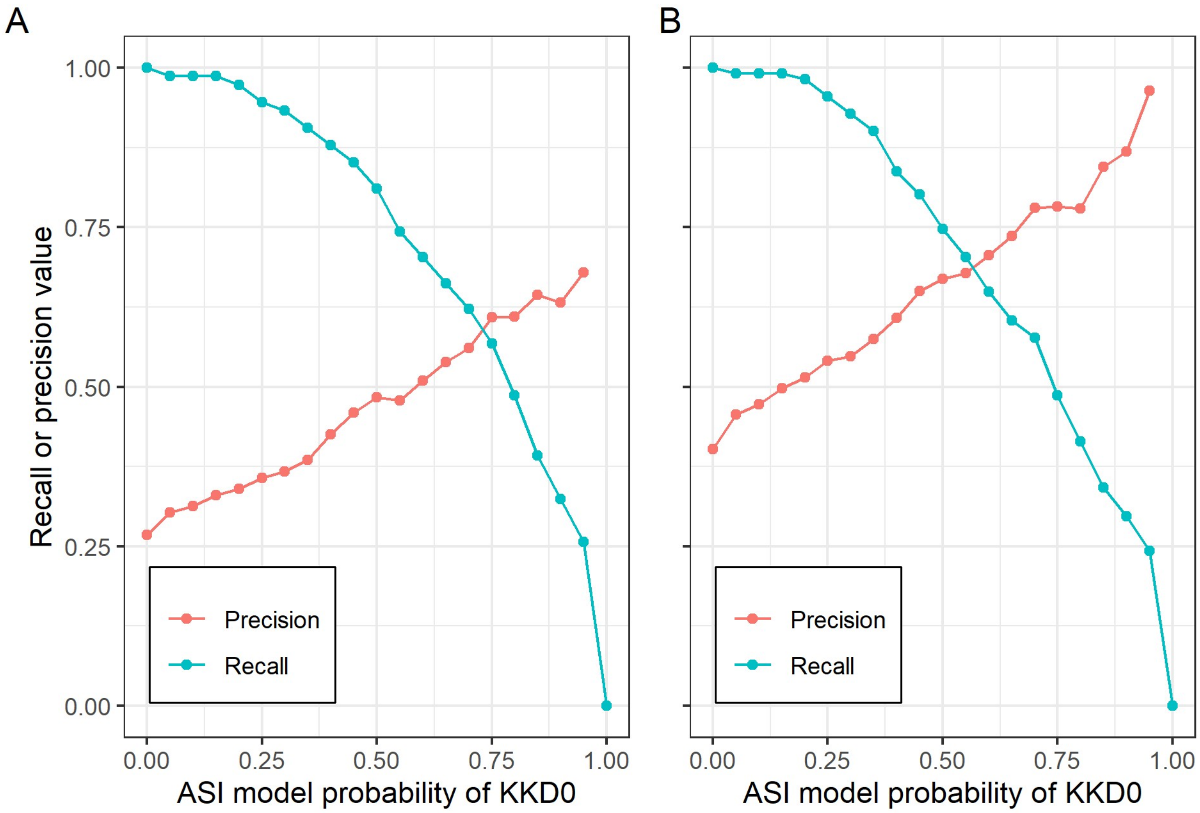

Figure 2.4. Recall and precision in the KKD0 model relative to the ASI model probability used to classify an audio file as containing Black Rail vocalization. Panel (A) reports recall and precision when requiring a complete vocalization to consider a vocalization present, whereas panel (B) reports recall and precision when accepting partial vocalizations as a positive identification.

SUPPLEMENTARY (S2) FIGURES

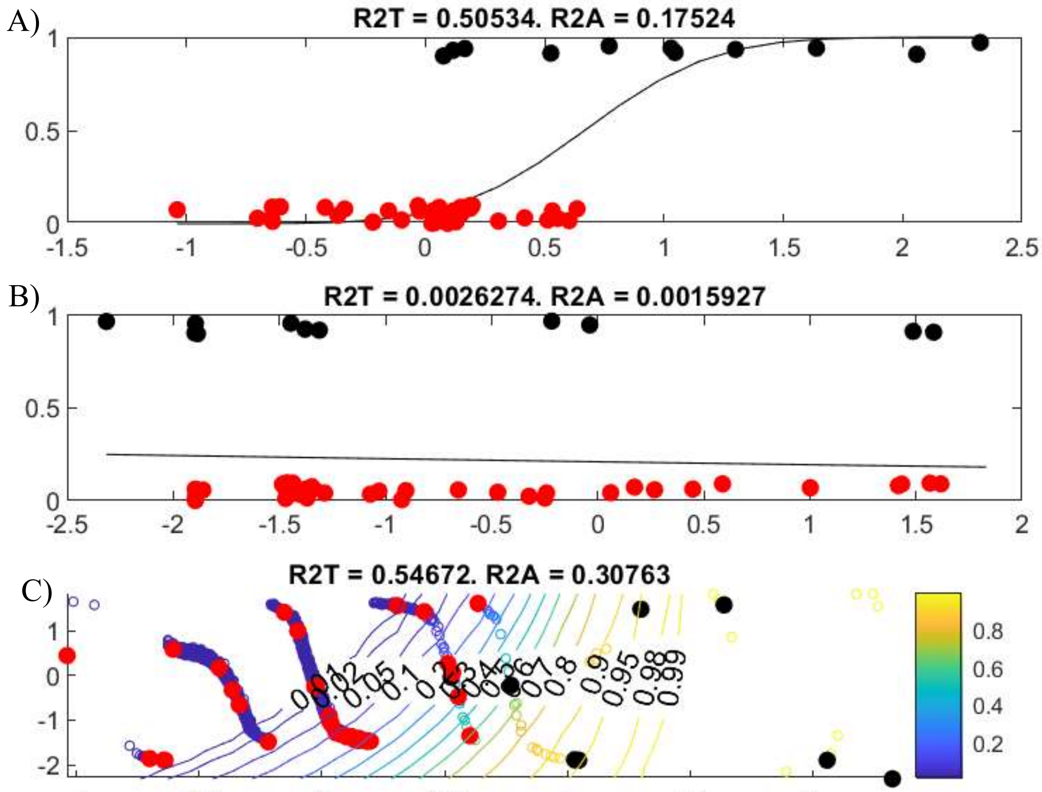

Figure 2.5. Vocalization-specific *churt* model, estimated from a sample of 50 manually classified segments. Panels illustrate the predictive ability using only predictor 1 (A; horizontal axis; the letter with maximum correlation, *Lmax*; see text for details), the predictive ability of a second predictor alone (B; horizontal axis), and the combined predictive ability of both predictors (C; predictor 1 along horizontal axis, predictor 2 along vertical axis). Red points indicate audio files not containing the vocalization and black points indicate files containing the vocalization; points are jittered to improve visibility. Predicted probability of a file containing the *churt* vocalization is displayed as the vertical axis in panels A and B and is displayed as contour isoclines and a color ramp in panel C.

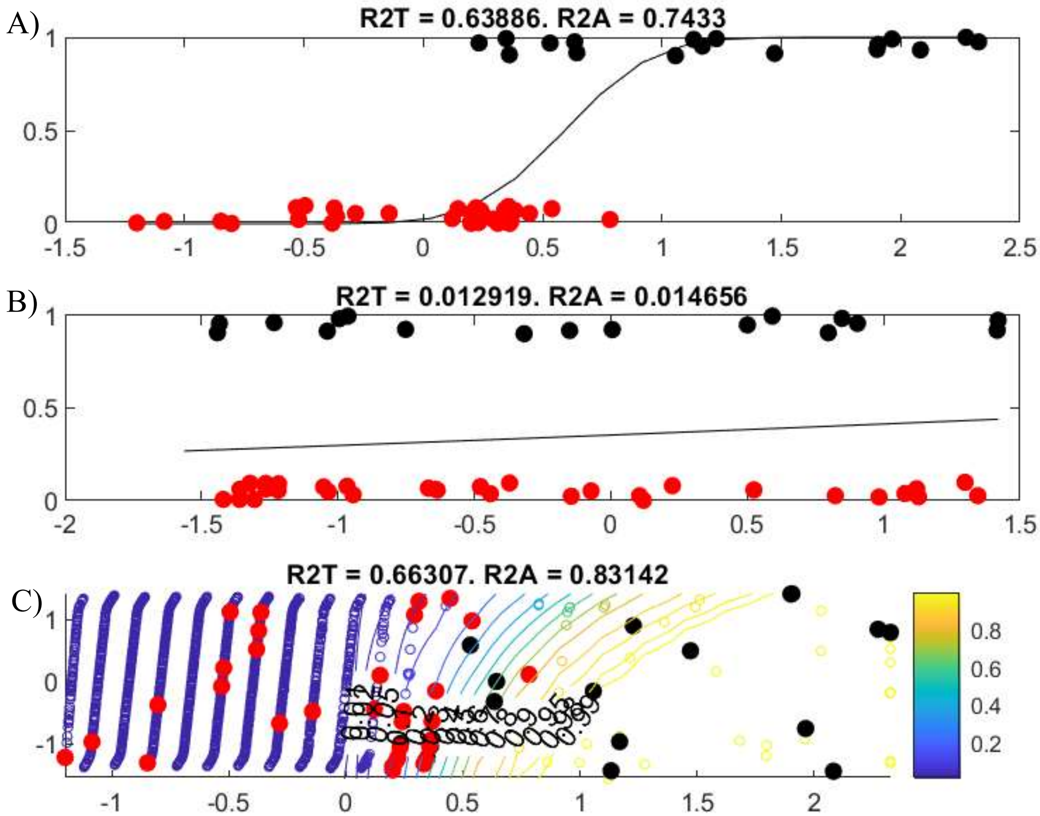

Figure 2.6. Vocalization-specific *growl* model, estimated from a sample of 50 manually classified segments. Panels illustrate the predictive ability using only predictor 1 (A; horizontal axis; the letter with maximum correlation, *Lmax*; see text for details), the predictive ability of a second predictor alone (B; horizontal axis), and the combined predictive ability of both predictors (C; predictor 1 along horizontal axis, predictor 2 along vertical axis). Red points indicate audio files not containing the vocalization and black points indicate files containing the vocalization; points are jittered to improve visibility. Predicted probability of a file containing the *growl* vocalization is displayed as the vertical axis in panels A and B and is displayed as contour isoclines and a color ramp in panel C.

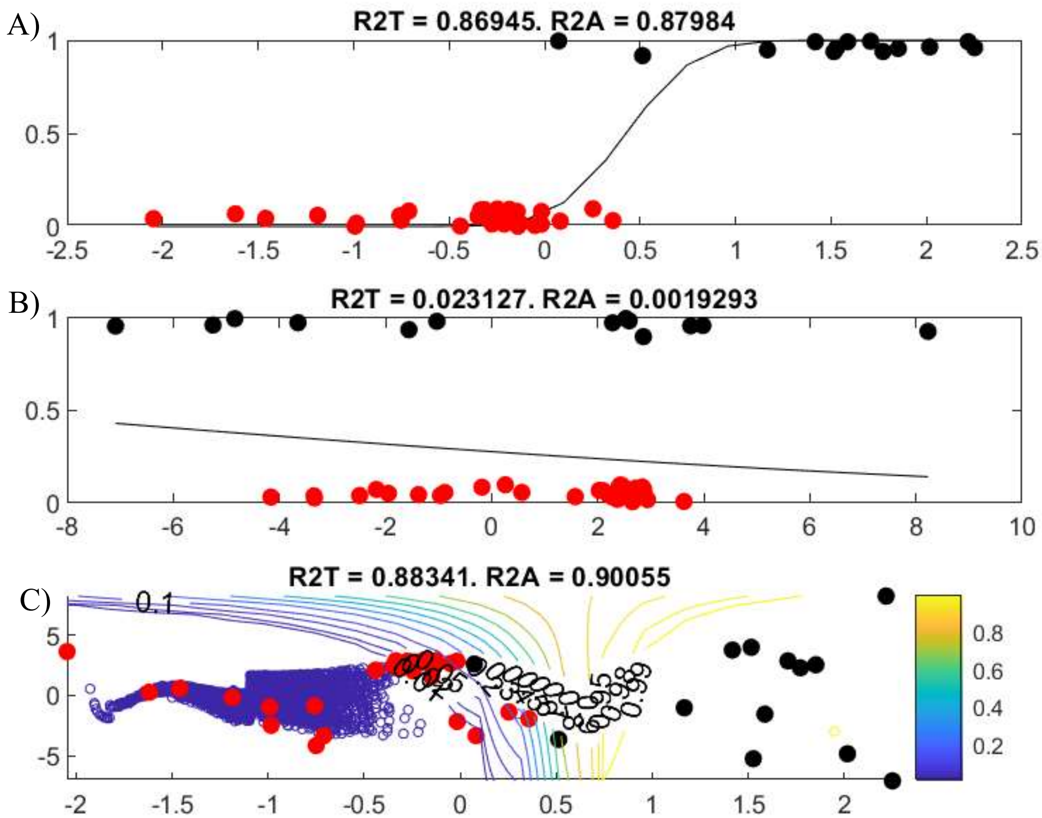

Figure 2.7. Vocalization-specific *ik-ik* model, estimated from a sample of 50 manually classified segments. Panels illustrate the predictive ability using only predictor 1 (A; horizontal axis; the letter with maximum correlation, *Lmax*; see text for details), the predictive ability of a second predictor alone (B; horizontal axis), and the combined predictive ability of both predictors (C; predictor 1 along horizontal axis, predictor 2 along vertical axis). Red points indicate audio files not containing the vocalization and black points indicate files containing the vocalization; points are jittered to improve visibility. Predicted probability of a file containing the *ik-ik* vocalization is displayed as the vertical axis in panels A and B and is displayed as contour isoclines and a color ramp in panel C.

CHAPTER 3

MULTILATERATION TRACKING OF A BLACK RAIL USING AN AUTOMATED RADIO-TELEMETRY SYSTEM[1]

[1]Tilson, D.A. and A.D. Smith. To be submitted to *Methods in Ecology & Evolution*.

ABSTRACT

Much of the basic ecology and life history of Black Rails (*Laterallus jamaicensis*) remains unknown, and telemetry studies may offer the best opportunity to study this cryptic species. Automated radio-telemetry systems (ARTS) use receivers that automatically record signals from radio transmitters and can achieve large sample sizes through higher sampling frequencies. ARTS may be particularly valuable for species where GPS tracking is not feasible. A relatively recent form of ARTS is the use of a network or grid of omnidirectional receivers that use radio signal strength (RSS) to estimate an animal's location through multilateration based on a known distance-RSS relationship. However, the RSS-based approach is poorly understood in different outdoor environments. We present a case study demonstrating the application of RSS-based ARTS for researching Black Rail movement, home range, and habitat uses. ARTS are well suited for researching life history patterns of rails and other marshbirds. The relative structural simplicity of marshes (i.e., lacking structural complexity and containing limited topographic variation) makes it easy to deploy node arrays with even spacing, and radio signals have a lower tendency to bounce and attenuate.

INTRODUCTION

Wildlife telemetry was introduced in the 1960s (Adams 1965, Cochran et al. 1965) and has proven a valuable method for studying animals in the wild. Automated radio-telemetry systems (ARTS) use receivers that automatically record signals from radio transmitters. ARTS were introduced in one of the earliest telemetry studies (Cochran et al. 1965), but most studies use manual radio-tracking due to the high equipment cost associated with automated telemetry (Ward et al. 2013). However, ARTS are not as costly as they once were. Additionally, the advent of digitally coded tags that all transmit on the same frequency now allows ARTS to track multiple animals simultaneously. ARTS are being applied to a wide range of taxa including reptiles (Ward et al. 2013, Tucker 2014), mammals (Wallace et al. 2022), insects (Fisher et al. 2020), and birds (Lenske 2018, Schofield et al. 2018, Bircher et al. 2020). Like global positioning system (GPS) datasets, ARTS can achieve high temporal resolution through higher sampling frequencies (Paxton et al. 2022), but ARTS are particularly valuable for species that are too small to carry GPS trackers or for species where recovery of position-logging trackers is unlikely due to low recapture rates.

A relatively recent form of ARTS uses a network or grid of omnidirectional receivers (nodes) to record radio signal strength (RSS) from which an animal's location can be estimated (Krull et al. 2018, Paxton et al. 2022, Wallace et al. 2022). RSS-based localization requires an established relationship between RSS values and distance in the focal environment. Factors such as background radio noise, weather, and structural environment can affect the accuracy of such localizations (Paxton et al. 2022), making in situ calibrations vital for studies using a RSS-based approach (Krull et al. 2018). In situ calibrations are also used to estimate the signal strength at which RSS values no longer convey useful information for localization. Inclusion of RSS information from all nodes in a network can produce inaccurate localizations; therefore, it is important to filter the nodes contributing to a given localization (Paxton et al. 2022).

RSS-based localization can produce large sample sizes of fine-scale movement data for species previously difficult to study due to their rare and elusive nature. However, the RSS-based approach is poorly understood in different outdoor environments. In this paper, we apply the approach to the Black Rail (*Laterallus jamaicensis*), North America's rarest and most elusive rail species. Black Rails are small migratory marshbirds that occur in emergent, fresh- and saltwater wetlands (Eddleman et al. 2020). Much of the basic ecology and life history of Black Rails remains unknown, and telemetry studies may offer the best opportunity to study this cryptic species (Case and McCool 2009). The objectives of this study were to present a case study using an RSS-based ARTS to track a transmittered Black Rail and discuss best practices and potential applications, as well as areas for future research.

MATERIALS AND METHODS

Study Area

We evaluated RSS-based localization for Black Rails at St. Johns National Wildlife Refuge (SJNWR) located 5-km west of Titusville, Brevard County, Florida. SJNWR is located in a relict saltwater basin bordering the St. Johns River. SJNWR is dominated by emergent marsh that is maintained in a structurally simple state through regular controlled burns to reduce woody vegetation. Dominant plant species are sand cordgrass (*Sporobolus bakeri*), Jamaican swamp sawgrass (*Cladium jamaicensis*), black needle rush (*Juncus roemerianus*), and wax myrtle (*Morrella cerifera*).

Trapping & Transmitter Attachment

We captured a Black Rail in March 2022 using double-door box traps placed along drift fences made from polyethylene netting, similar to the method used previously at the site (Legare and Eddleman 2001, Legare et al. 1999). The trapline was placed between an area of standing surface water and a salt pan in an area that had mud and dense vegetation where rails were expected to move. Once we heard a Black Rail vocalizing, we used an audio lure on the opposite side of the trapline. The captured rail was given a U.S. Geological Survey (USGS) aluminum leg band placed above the intertarsal joint on the right leg. The rail was fitted with a digitally-coded UHF transmitter (CTT PowerTag, Cellular Tracking Technologies, Rio Grande, New Jersey, USA) using a modified leg-loop harness (Haramis and Kearns 2000) that includes a waist loop due to the short caudal region of rails. The PowerTag transmitted a signal every 27s, weighed just more than 1g with the harness (less than 3% of bird's body weight), and had an expected battery life of ~90-200 days.

Tracking

We monitored rail movements using an array composed of a network of independent, solar-powered receiving nodes (CTT Node v. 2, Cellular Tracking Technologies, Rio Grande, New Jersey, USA) within and surrounding the bird's territory. Detection data from the nodes was transmitted and offloaded to a larger base station composed of a SensorStation (v.2, Cellular Tracking Technologies, Rio Grande, New Jersey, USA) and Yagi antennas fixed to the top of a 6-meter mast and tripod.

Prior to capture, we approximated the center of activity for the bird based on patterns of the presumed individual's vocalizations. We employed a soft deployment method for nodes by evaluating patterns of detection on a daily basis from the partial node array to hone in on the bird's center of activity, allowing us to actively refine the array as it was being constructed. Initial node deployment focused on surrounding the bird's area of use rather than increasing node density, so areas of interest were identified based on total node detection activity (i.e., strongest average signal strength on a daily time frame) rather than localization. The complete node array was deployed over the course of approximately 1.5 weeks. Nodes were installed on the top of 3-meter electric metallic tube (EMT) conduit inserted 0.6m into the ground, leaving the node 2.4m above ground level when fully installed. Nodes were organized at the centers of a hexagonal grid resulting in a distance of 25m between nodes within an east to west within a row and ~22m north to south between rows, centered on our expected activity center (Figure 3.1).

Tag and Node Calibration

ARTS should be calibrated to their particular application (e.g., species habits and the habitat or landscape composition and structure). Presumably, tags exhibit some degree of variation in the signal strength they emit. Likewise, nodes may vary in reception ability and measurement of signal strength for a given tag detection. We conducted tag and node calibrations to quantify internode and intertag variation and enable tag and node-specific adjustments to RSS prior to localization (see the Supplementary Information S3 for calibration details).

In addition to internode and intertag variability, a key relationship that must be estimated for RSS-based localization is the decrease in received signal strength as a function of tag distance from a node. The objective of RSS vs. distance calibrations is to quantify the relative change in received signal power of a tag in situ as a function of distance from a node. We conducted this calibration, in representative habitat (i.e., vegetation composition, structure, and density) on SJNWR known to have breeding Black Rails. The specifics of the calibration are provided in Supplementary Information (S3), but the resulting data set comprises an average of RSS measurement within two-minute intervals at specific and known distances from a node. The average RSS measurements at each distance were then used to fit a statistical model describing this relationship and its uncertainty (Supplementary Information S3). Paxton et al. (2022) used an exponential decay model to describe this relationship. We evaluated the exponential decay model, but also considered a model based on the inverse square law for electromagnetic radiation given the relatively simple environmental structure of Black Rail habitat at SJNWR (Supplementary Information S3).

We selected an RSS value of -90 as our filter threshold based on the inverse square law and exponential decay models nearing asymptotic behavior at this RSS, as well as the increasing uncertainty associated with the estimated distance (see Supplementary Information S3). We expected RSS values above this threshold to be useful for localization estimates. Due to the increasing uncertainty in predicted distance from node with weaker RSS values, we also weighted node detections by the inverse of their estimated distance from the transmitter, thus giving longer range detections less weight in the multilateration process. Like Paxton et al. (2022), we did not incorporate uncertainty in the estimate of RSS_0 or K into the estimate of distance from node, and thus this uncertainty was not propagated into the localization estimate. Doing so is possible (e.g., via bootstrapping), but would be computationally intensive and time-consuming to propagate into the multilateration estimates for such high temporal resolution data.

Localization Validation

Because distance estimation error scales with distance, grid design should affect localization (i.e., estimation of tag location) accuracy. To evaluate the localization accuracy provided by our grid, we generated a test set of tag detections at 37 random, spatially-balanced locations within and around the node array. At each location, we allowed the ARTS to measure RSS of a tag for two minutes (see Supplementary Information S3 for additional details). We then localized the tag at each test location using the filtering threshold and multilateration approach described previously. We also explored the localization accuracy using a reduced node array to simulate a sparser grid with increased node spacing. Specifically, we omitted data from nodes in every other row to produce an array with a node spacing of 25m east to west within row, and ~45m north to south between rows reducing the number of nodes by approximately 33% (Figure 3.1). For additional comparison, we calculated the location accuracy of a naïve approach where the tag was assigned the location of the node with the highest RSS value (i.e., the strongest average signal strength).

We evaluated the relationship between multilateration error (i.e., absolute distance between estimated location and known location) and the number of nodes detecting the tag (i.e., those meeting our RSS criterion) using a generalized linear model with the MASS package in R. We modeled multilateration error as Gamma, with a log link function, given the continuous but non-negative multilateration error. We only considered validation points located within the minimum bounding polygon of the node grid and meeting our RSS filter criteria.

Home Range & Diel Patterns of Activity

We compared home range sizes by generating autocorrelated Kernel Density Estimations (aKDE) as described in Signer and Fieberg (2021) for the multilateration and naïve approaches for full and reduced grids. Because ARTS typically produce data at much higher temporal resolution compared to manual telemetry, we also compared home range estimates of the multilateration full grid at four different temporal resolutions: localization occurring every 15 minutes, 1 hour, 4 hours, and 8 hours. We estimated all home ranges at two different home-range levels (50% and 95%).

We inferred Black Rail activity from fluctuations in RSS from one or more nodes. Received RSS will fluctuate more when rails are active, as the attached transmitter is moving and antenna orientation is changing. Receivers should record more consistent RSS values when rails are at rest and the transmitter remains consistently oriented. Our estimate of activity was a weighted average of the standard deviation of received RSS among detecting nodes; weights were based on the number of detections available to calculate the standard deviation. To summarize activity on a daily timeframe, we aggregated our estimate of activity into 30-minute time intervals on the 24-hour clock to explore diel activity patterns.

All analyses were conducted in the R Statistical Software Environment version 4.2.1 (R Core Team 2022).

RESULTS

Estimated parameters of the inverse square model for the distance-RSS relationship were K = 35.72 (SE = 1.08) and RSS_0 = -29.47 (SE = 0.61). At the filtering threshold value of RSS = -90, the model-predicted mean distance was 69.5m (95% parametric bootstrap confidence interval 61.4 – 70.5m).

We captured and transmittered an adult male Black Rail on March 18, 2022. Soft deployment of the node array started on March 20th and was refined over 10 days with the finalized grid completely deployed on March 30th. In the days following April 14, 2022, it became apparent from very consistent RSS values that the bird had either lost its transmitter or died. Investigation confirmed the latter with strong evidence pointing towards predation by a bird of prey. We thus report on continuous tracking period of 14 days under the complete grid, March 31 – April 13, 2022. From vocal and movement behavior, the bird was presumed unmated and no nesting activity was indicated for the duration of its monitoring period.

Our validation of the RSS-based localization approach using multilateration resulted in a median absolute localization error of 13.2m for the full grid and 23.0m for the reduced grid (Figure 3.2A). Under the naïve approach, median absolute errors were 13.8m and 19.8m for the full and reduced grids, respectively (Figure 3.2B). For our evaluation of multilateration error and number of detecting nodes, 44 validation locations met our criteria (24 with the full grid and 20 with the reduced grid). Multilateration error decreased with the number of nodes used in the estimation ($t_{41} = -4.22$, $p < 0.0001$; Figure 3.3) but was similar in the full node grid and the reduced node grid ($t_{41} = -0.9935$ $p = 0.33$). We considered whether the relationship between number of detecting nodes and multilateration error varied between grid configurations. There was little support for this interaction ($t_{40} = -1.35$ $p = 0.18$), so we did not include this interaction in the final model. Each additional detecting node reduced average multilateration error by approximately 12%.

Home range estimates for the tagged bird were larger under the naïve approach at either node grid density (Figure 3.4). Using a full grid, the home range estimated from the multilateration and naïve approaches overlapped by 90.5% and 84.6% at the 95% and 50% kernel density levels, respectively. The reduced grid resulted in a similar but slightly smaller home range estimation compared to the full grid for the multilateration approach. Conversely, grid reduction resulted in a slightly larger home range estimation for the naïve approach. Under the multilateration approach, home range estimated from the full and half grids overlapped by 81.0% and 84.6% at the 90.5% and 50% kernel density levels, respectively. Temporal resolution of localization had a relatively small effect on home range size and overlap for the full grid under the multilateration approach (Figure 3.5).

Over the two weeks of continuous tracking, the Black Rail exhibited very consistent activity patterns relative to civil sunrise and sunset, as inferred from RSS variation. Specifically, the rail was consistently diurnal, showing increased movement during daylight hours (Figure 3.6). On two occasions (April 10 and April 11), the bird left its typical territory, and both occurred during the day. Nightly activity was more variable, with most nights indicating little activity throughout, but occasional periods of activity throughout the nighttime period (Figure 3.6). Estimates of activity aggregated into 30-minute time intervals over the course of its tracking additionally highlights the largely diurnal nature of this individual (Figure 3.7).

DISCUSSION

In some ways, Black Rails are a model species to investigate monitoring via ARTS, both in terms of methodology and conservation. The relative simplicity of marshes (i.e., lacking structural complexity and containing limited topographic variation) makes it easy to deploy node arrays with even spacing and radio signals have a lower tendency to bounce and attenuate. Black Rails primarily move by walking and rarely fly except when migrating. Their profile so near to the ground simplifies methods by removing the need for vertical calibrations, but also greatly reduces the detection range to achieve RSS measurements adequate for localization. The Black Rail is one of North America’s least studied species and ARTS have great potential to inform knowledge gaps vital to conservation efforts.

The soft deployment of node arrays allows for the adaptive refinement of grid placement during construction, saving time and effort and reducing disturbance to sensitive habitats by mitigating the need to readjust the grid after deployment. We recommend node spacing at ~75% of the maximum detection range within the filtering parameters as a good starting point. Once the grid has been centered and encompasses the area of interest, then nodes can be added to reach the desired final grid density.

Median absolute localization errors for the full grids were relatively similar under the multilateration and naïve approaches. However, median absolute error for the reduced grid was lower for the naïve approach than for the multilateration approach. With the naïve approach, there is a more clearly defined upper threshold for error. Validation points within the grid are not expected to have an absolute error greater than approximately half the distance of the spacing between nodes when using the naïve approach. Points occurring outside the grid are capped at the maximum distance of detection meeting our filtering parameters (i.e., RSS > -90), which our calibrations suggest is likely to be just above 60 meters. Multilateration error decreases with more detecting nodes above the filter threshold. This suggests that the multilateration approach performs best when an array is large enough so that the target animal's home range falls within the grid, as locations near the grid edge will have fewer surrounding nodes. This is supported by the findings of Paxton et al. (2022), that reported a decrease in localization error inside the grid with increased distance from the array edge. Our system is structurally simple, but in more complex environments, radio signals may bounce and attenuate more readily (Paxton et al. 2022), likely increasing error for both approaches but disproportionately so for naïve approaches.

General biological inferences about home range cannot be made over 14 days of tracking of a single bird; however, the experience did provide preliminary insight about effects of spatial and temporal resolution of localization on home range estimation. Full and reduced grids had similar home range estimations in both size and overlap under the multilateration approach. This suggests a tighter spacing in the full grid than required for our tagged bird. In situ range tests are valuable to inform node spacing to balance both localization accuracy and area coverage. Range tests require only two nodes, a transmitter, and a base station and can be performed prior to larger equipment purchases as an economic way to a priori evaluate equipment needs and feasibility for a given ARTS application. We note that removing alternate rows of nodes (along the north-south gradient; Figure 3.1) to create the reduced grid could have introduced a spatial bias in home range estimation. In practice, at any node density, node spacing in orthogonal directions should be consistent.

Temporal resolution had a relatively low effect on home range estimations for the resolutions investigated for this individual. Researchers must weigh costs and benefits of temporal resolution with tag longevity based on their study objectives.

The tagged Black Rail was more active during the day than at night. This result matches the findings of Legare and Eddleman (2001) showing Black Rails were active throughout the daylight hours when not incubating. However, changes in Black Rail activity during the day are not well understood. Flores and Eddleman (1995) reported Black Rails in Arizona to be inactive at night but did not investigate changes in activity patterns during the day. Diel patterns of vocalization are highly variable among Black Rail populations (Eddleman et al. 2020). Two avenues of future research that ARTS would be well suited to investigate are 1) do diel patterns of movement activity likewise vary by population? and 2) what is the relationship between vocalization rates and movement activity?

A potential application of ARTS that we were unable to investigate with our unmated bird, is the theoretical ability to determine the start and cessation of incubation. Male and female Black Rails share incubation duties (Eddleman et al. 2020), so evidence of a bird’s inactivity, in a single location, for approximately half of its normally active time is a good indicator that the bird has started incubating. This could be particularly useful for studies investigating nest success and/or sources of nest mortality. Only a few nodes are needed to monitor nesting activity if localization is not required for a study's research objectives.

ARTS can provide very robust datasets in both location estimation and diel patterns of activity. These values can be summarized to explore activity differences at different times of day, in different seasons, among age and sex classes, and to inform the identification of behavioral states (e.g., onset and cessation of nest incubation). As the technology and methodology continues to develop, ARTS are likely to see increased prevalence in ecological research.

ACKNOWLEDGMENTS

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FIGURES

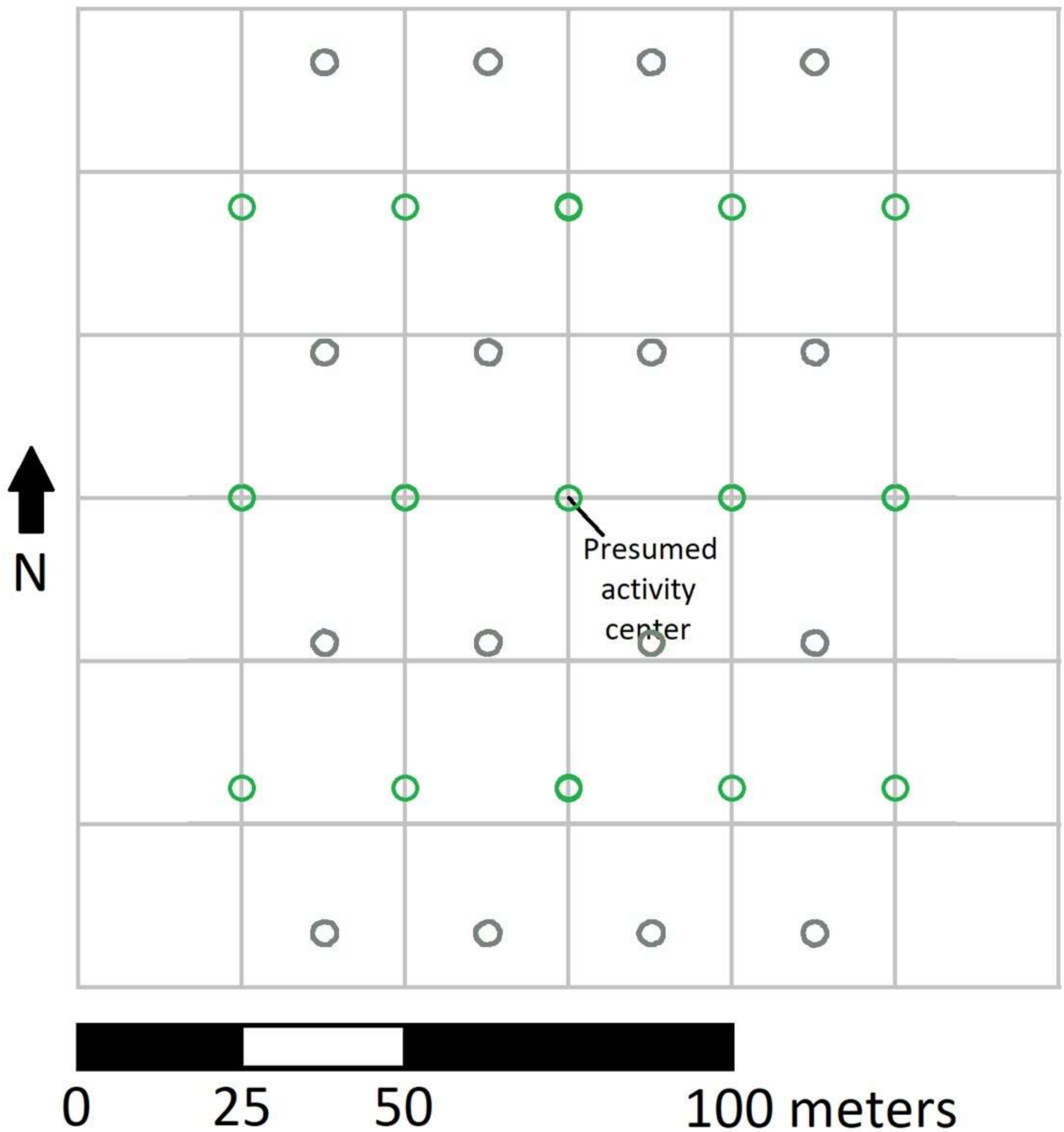

Figure 3.1. Initial placement of 31 receiving nodes (circles) around the presumed center of a transmittered Black Rail's activity. Spacing between nodes is 25 m east to west within a row, and ~ 22 m north to south between rows. Alternate rows are shifted so traps occur halfway (east to west) between nodes in adjacent rows. Gray circles represent nodes from which data was omitted for the reduced grid.

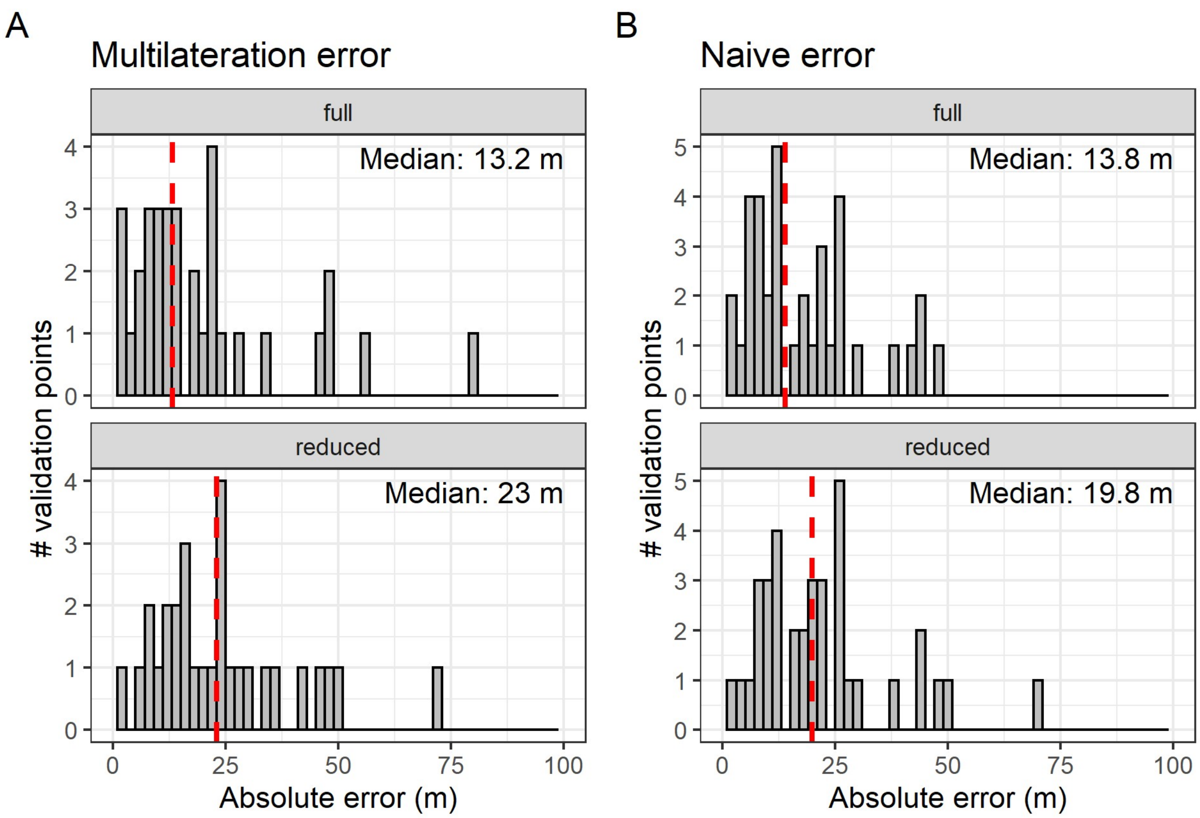

Figure 3.2. Absolute errors of validation points (n = 37) under full and reduced grids using multilateration and naïve approaches. Red dashed lines represent the median absolute error.

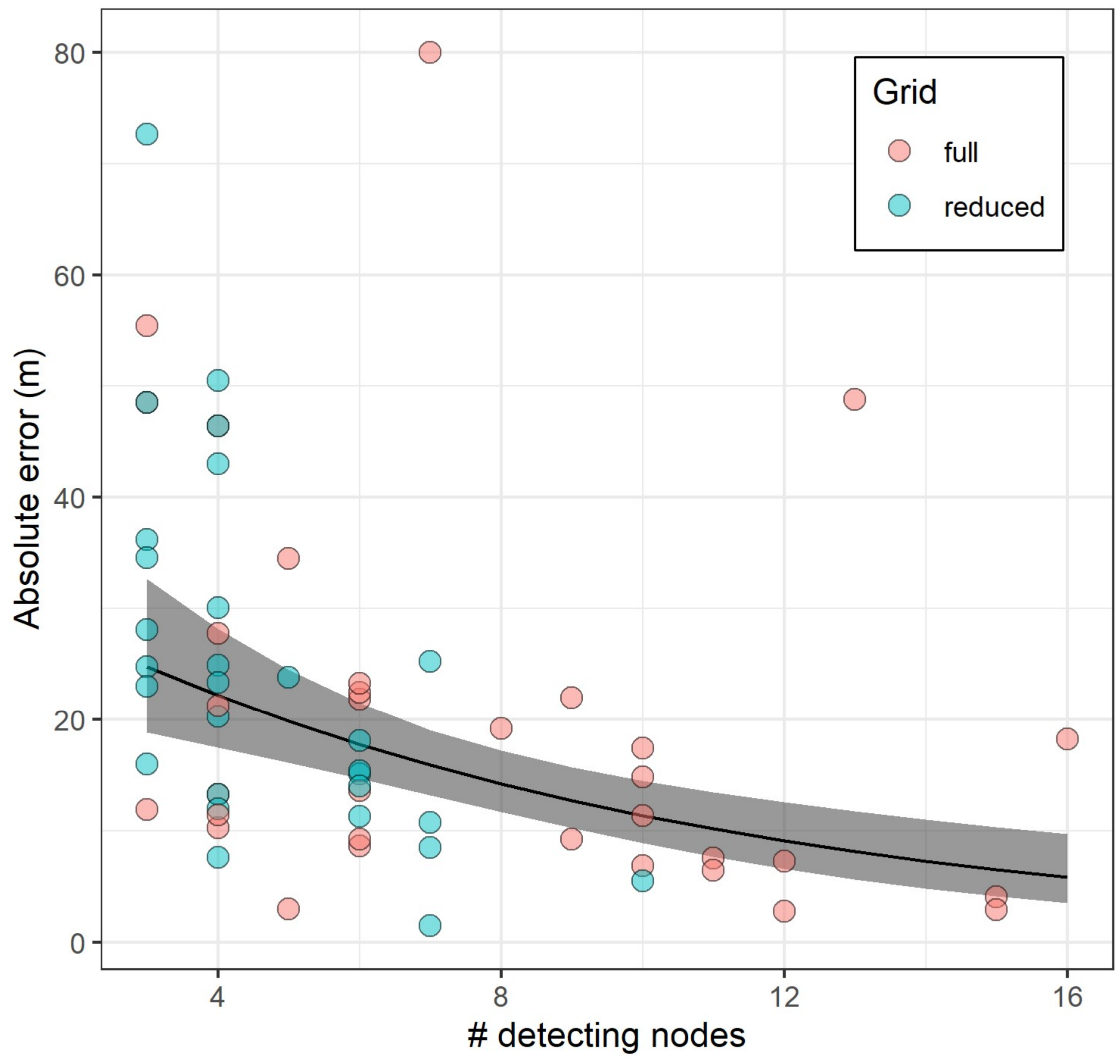

Figure 3.3. Gamma generalized linear model fit (line) between absolute errors of validation points over the number of detecting nodes. Shaded area represents 95% confidence interval.

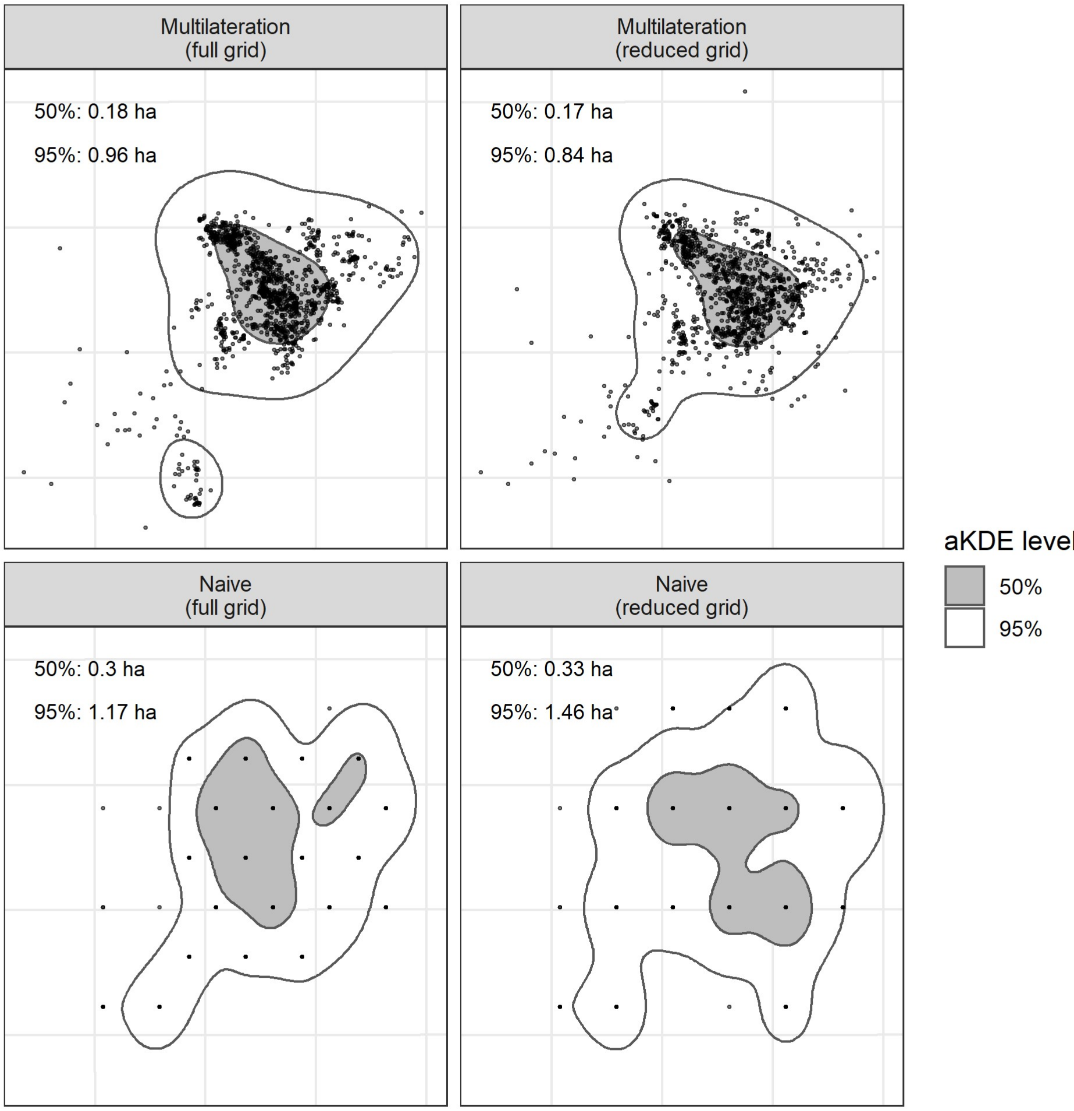

Figure 3.4. Autocorrelated kernel density home range estimates under full and reduced grids using multilateration and naïve approaches. Home ranges represent a single tagged Black Rail from March 31, 2022 - April 13, 2022.

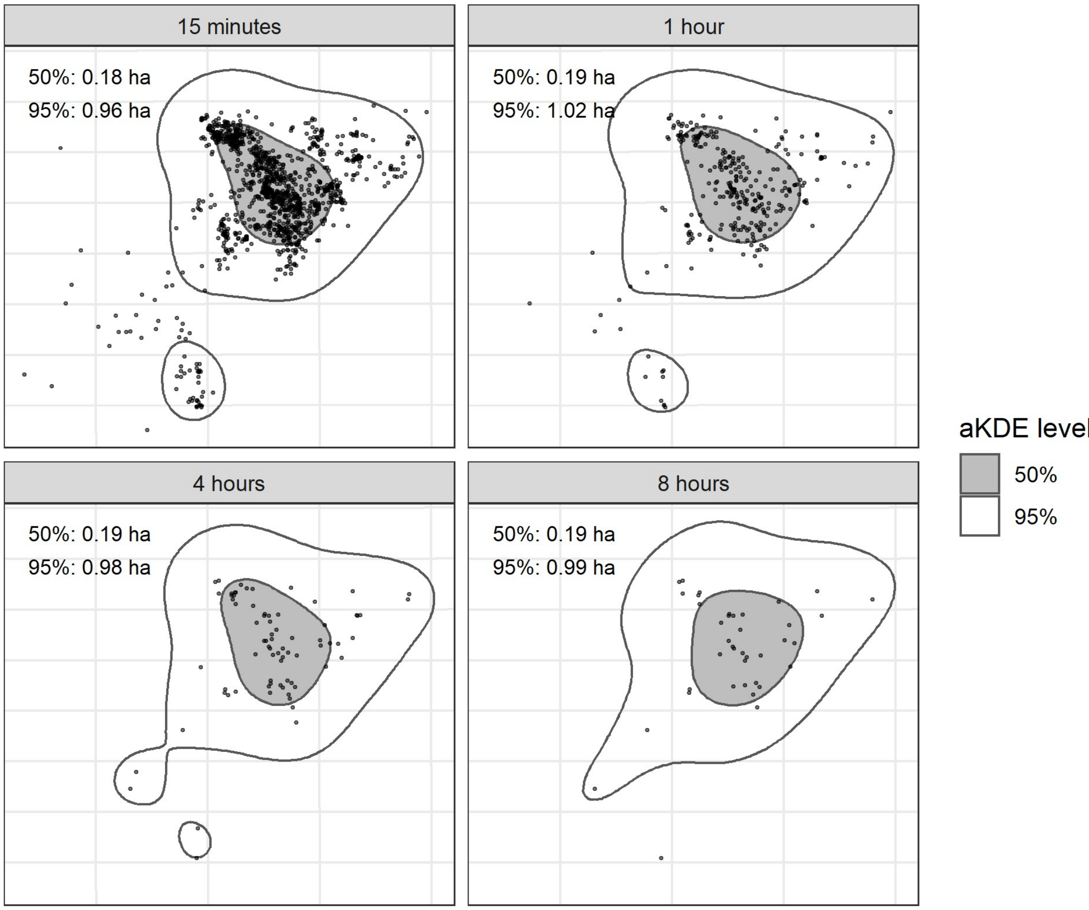

Figure 3.5. Autocorrelated kernel density home range estimates at four different temporal resolutions. Home ranges represent a single tagged Black Rail from March 31, 2022 - April 13, 2022.

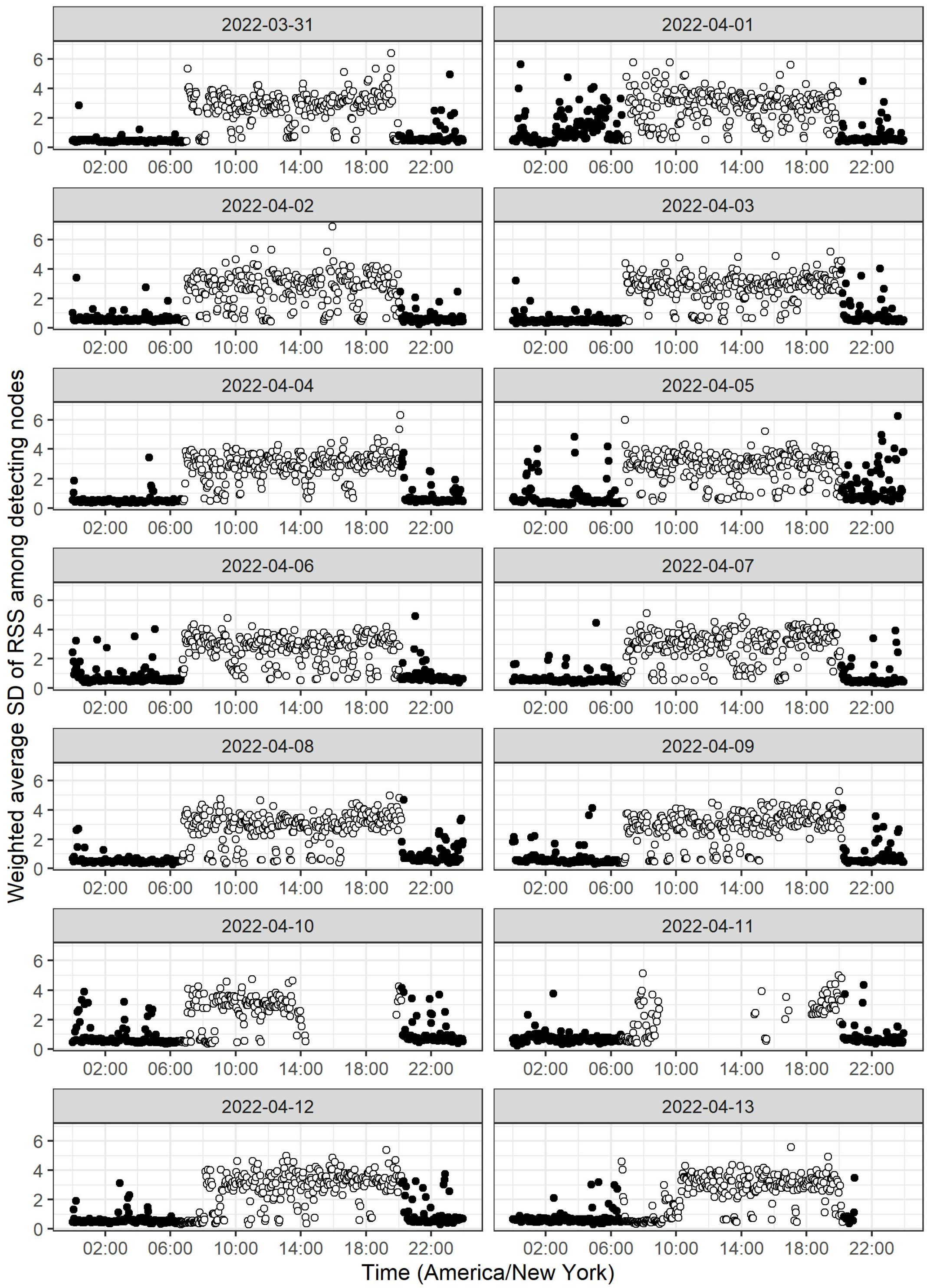

Figure 3.6. Patterns of activity of a tagged Black Rail from March 31, 2022 – April 13, 2022. A higher weighted average standard deviation indicates more activity in a given 3-minute interval. White and black circles indicate periods of activity during the day (dawn to dusk) and night (dusk to dawn), respectively. Periods without activity indicate periods of time where the individual left the monitored area and could not be adequately tracked.

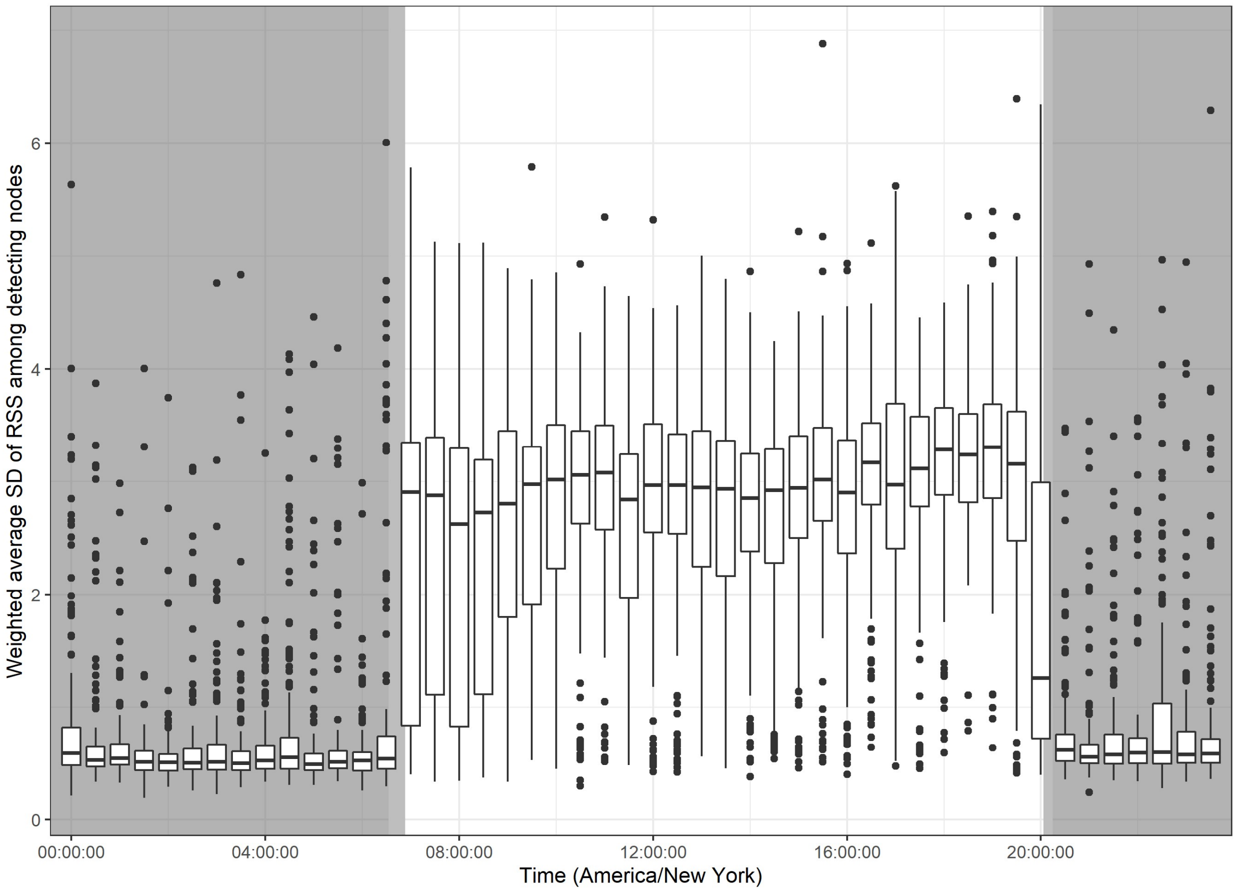

Figure 3.7. Boxplots summarizing diel patterns of activity of a tagged Black Rail from March 31, 2022 – April 13, 2022. A higher weighted average standard deviation indicates more activity in a given 30-minute window. Background shading indicates the extent of the nighttime period (dusk to dawn) during this date range.

SUPPLEMENTARY INFORMATION TEXT (S3)

Node Calibration

Nodes presumably will vary to some extent in the signal strength they record for a given tag detection. Quantifying that variation in a controlled environment is helpful to estimate node-specific RSS corrections to put all nodes on the same RSS scale.

In this calibration, a node was placed every 30° in a circle of 2m radius around a single tag, for a total of 12 nodes per trial. Nodes were installed at an equal height of 1.34m above the ground. The tag was secured vertically, with the antenna pointed up, on top of a non-conductive pole at a height of 1.05m above the ground. Each group of nodes was calibrated for a total of 3 minutes and the start and end times were noted according to the 0Official U.S. Time (time.gov) to facilitate matching with detection data. The same tag was used in all calibrations. We assumed that amplitude magnitude (signal strength) in the cyclical spline model (below) was not dependent on the specific tag used, but this is an assumption that could be further investigated by repeating the calibration with other tags. We estimated node-specific corrections for 191 nodes.

It was expected that the specific position a node occupied in the circle around the tag may influence its received RSS due to an imperfect antenna (i.e., the tag antenna may not emit signals in a perfectly omnidirectional manner). Thus, to allow for estimates of node-specific RSS corrections, we tested 24 nodes a second time in a different position from their initial test; these nodes had their positions shifted 30° clockwise and we selected nodes such that replicate measurements occurred at all 12 node positions. Received RSS data support the idea of an effect of node position (Figure 3.8).

To calculate node-specific adjustments to RSS, we estimated a generalized additive model (Wood 2006) that related measured RSS by each node's identity as well as a cyclical spline for node position around the tag using the `mgcv` package, v. 1.8.40 in R v. 4.2.1 (R Core Team 2022). RSS measurements were centered prior to fitting, and we suppressed the intercept from the model so that node-specific corrections were generated directly as model output. Node position was an important factor in node RSS (Figure 3.9). Node-specific RSS corrections ranged from -14.9 to 6.3 (mean ± SD: 0.15 ± 3.50; Figure 3.10). See GitHub (https://github.com/adamdsmith/blra_telemetry) for code.

PowerTag RSS Calibration

Likewise, tags vary to some extent in the strength of the signal they emit. Quantifying the variation in a controlled environment is helpful to estimate tag-specific RSS corrections necessary to put all tags on the same RSS scale.

This calibration used a similar, but importantly distinct, setup to the node calibration. For this calibration, a single tag was placed at a horizontal distance of 1m from each of four nodes placed in the four cardinal directions. The tag was mounted horizontally (antenna parallel to ground) at a height of 1.20m above the ground on a non-conductive pole. All nodes were installed on EMT conduit at a height of 1.38m above the ground.

To start each trial, the tag's antenna was oriented directly at the node located to its due North. Every four minutes, the non-conductive pole holding the tag was rotated 90° such that the tag's antenna pointed directly at the next node around the array. Thus, each tag spent 4 minutes with its antenna pointed directly at, directly away from and perpendicular (right and left) to each node. Tags were calibrated for a total of 16 minutes. Calibration timing was noted according to the Official U.S. Time (time.gov) to facilitate matching with detection data. All tags (n = 20) were calibrated using identical arrangements and node identities. We assumed calibration model results (below) were not dependent on these specific four nodes, but this is an assumption that could be further investigated by repeating the calibration with other nodes. Upon inspection of the data, there was consistent RSS information recorded when the tag antenna was oriented perpendicular to the receiving node, but considerable variation when the tag antenna was oriented towards or away from the receiving node. Thus, we used only detection data for detections with the tag oriented perpendicular to the receiving node, although we retained antenna orientation information (left vs. right).

To calculate tag-specific corrections to RSS, we first applied the node-specific RSS adjustment for each node used in the calibration as calculated in the `Node Calibration` section. We then estimated a generalized additive mixed model (Wood 2006) that related measured RSS by each tag's identity as well as a fixed effect for antenna orientation (left vs. right) using the `mgcv` package, v. 1.8.40 in R v. 4.2.1 (R Core Team 2022). As with node calibration, we suppressed the intercept from the model so that node-specific corrections were generated directly as model output. We included a random effect for node identification. Tag-specific RSS corrections were bimodal but ranged from -8.44 to 2.83 (mean ± SD: -4.05 ± 4.59; Figure 3.11). See GitHub (https://github.com/adamdsmith/blra_telemetry) for code.

RSS vs Distance Calibration

The objective of RSS vs. distance calibrations is to quantify the relative change in received signal power of a tag in situ as a function of distance from a node.

For this calibration, a node, installed atop conduit in the same fashion as in our arrays, was placed on each end of a 60-m transect (i.e., nodes were 60m apart). A test tag was secured 6-8cm above the bottom of a non-conductive 1-m pole with the tag near horizontal but with the antenna angled slightly downward. This tag arrangement was consistent with the height and orientation of tags when deployed on Black Rails. To start the calibration, the pole with the tag was placed directly under the starting node (distance = 0m) so that the bottom of the pole touched the ground. The pole was held at arm's length, with the user standing on either side of the tag relative to the transect such that the user did not obstruct the sight line between the tag and either node. The pole was rotated slowly such that the tag completed a full turn each minute for a total of two minutes. Calibration times were tracked according to the Official U.S. Time (time.gov). This procedure was conducted at 15 different distances along each transect: 0, 1, 2, 4, 8, 15, 25, 30, 35, 45, 52, 56, 58, 59, and 60 m. We repeated this procedure for a total of seven transects using the same tag. We assumed calibration model results (below) were not dependent on the specific tag and nodes used, but this is an assumption that could be further investigated by repeating the calibration with other tags and nodes.

Paxton et al. (2022) used an exponential decay model to describe this relationship. We evaluated the exponential decay model, but also considered a model based on the inverse square law for electromagnetic radiation given the relatively simple environmental structure of Black Rail habitat at SJNWR. In short, RSS is proportional to distance as described by the following linear equation:

$$RSS \propto -K * \log_{10}(\text{Distance}) + RSS_0$$

where K is proportional to a signal propagation constant or exponent describing the particular relationship between RSS and distance in our system, and RSS_0 is essentially the RSS measured at distance 0 (intercept). We estimated K and RSS0 using a linear mixed model relating RSS to log10(distance) using the lme4 package (v. 1.1.30; Bates et al. 2015) in R v. 4.2.1 (R Core Team 2022). We included a random effect for the transect (n = 7) of data collection. See GitHub (https://github.com/adamdsmith/blra_telemetry) for code.

In our particular application, the inverse square law and exponential decay models produced relatively similar relationships (Figures 3.12 & 3.13), although the inverse square law had better predictive ability as evaluated by RMSE over 500 parametric bootstrap iterations of the fitted model (Figure 3.14). Thus, we report results from the inverse square law model in the manuscript. With estimates of K and RSS_0 from the linear mixed model, we can estimate tag distance from a node using:

$$\text{Distance} = 10\text{^}((RSS_0 - RSS_{average})/K)$$

where $RSS_{average}$ is the average RSS over a given time interval (2 or 3 minutes in our application), corrected according to tag- and node-specific variations in RSS. A visualization of the uncertainty around estimated distances as a function of RSSI from the inverse square law model is illustrated in Figures 3.15 and Figure 3.16.

LITERATURE CITED (S3)

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48.

PAXTON, K. L., BAKER, K. M., CRYSTSER, Z. B., GUINTO, R. M. P., BRINCK, K. W., ROGERS, H. S. & PAXTON, E. H. 2022. Optimizing trilateration estimates for tracking fine-scale movement of wildlife using automated radio telemetry networks. Ecology and Evolution, 12(2), e8561.

R CORE TEAM 2022. R: a language and environment for statistical computting. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

WOOD, S. N. 2006. Generalized additive models: an introduction with R (2nd edition). Chapman and Hall/CRC. New York, USA.

SUPPLEMENTARY (S3) FIGURES

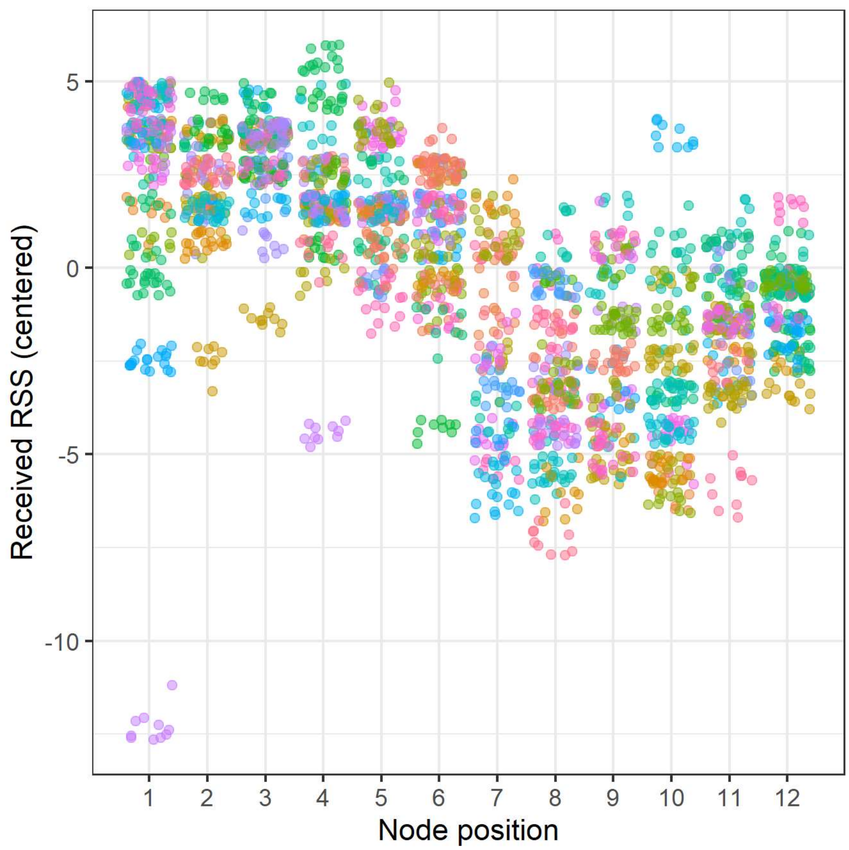

Figure 3.8. Received signal strength by Node position during calibration. Each node (n = 191) is colored distinctly. 24 nodes were calibrated at two positions. Points are jittered to improve clarity.

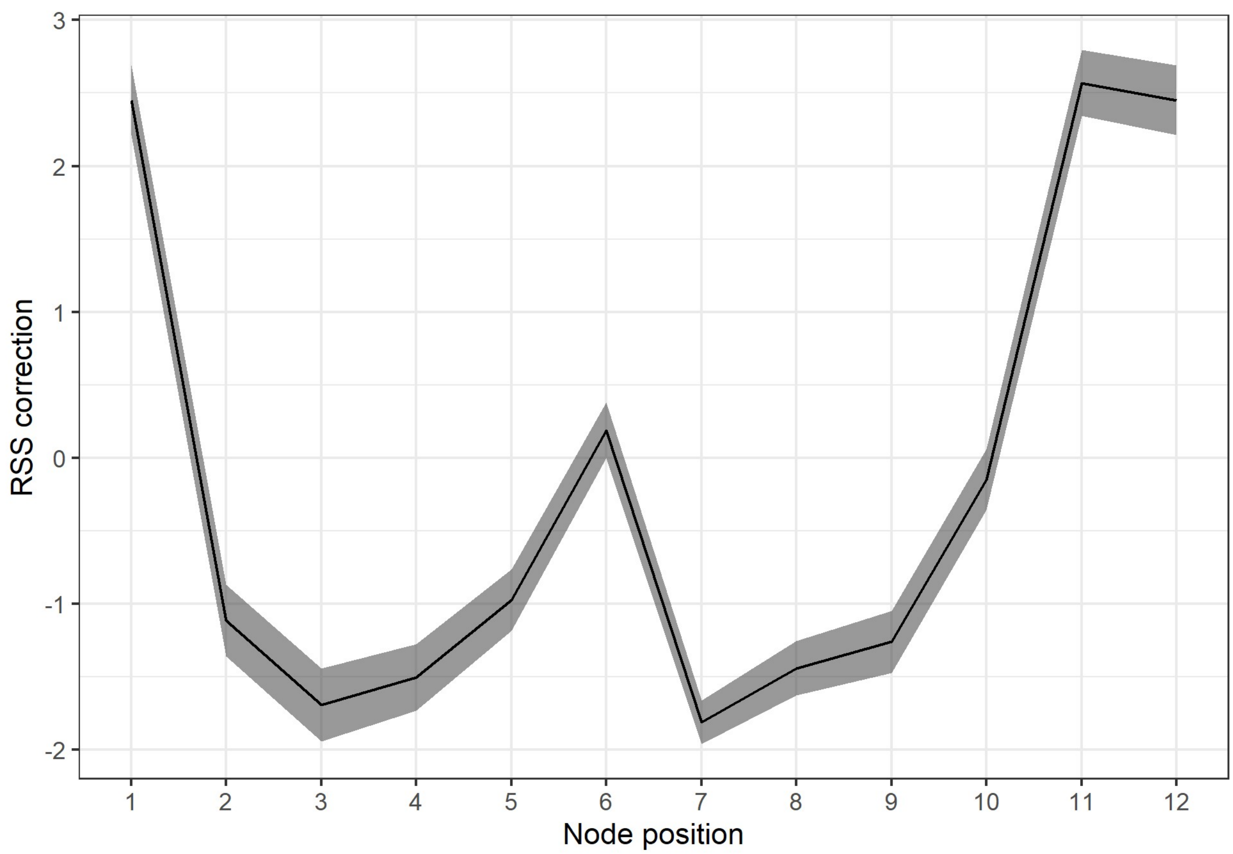

Figure 3.9. Estimated effect on node position during calibration on received signal strength. Band indicate pointwise standard error.

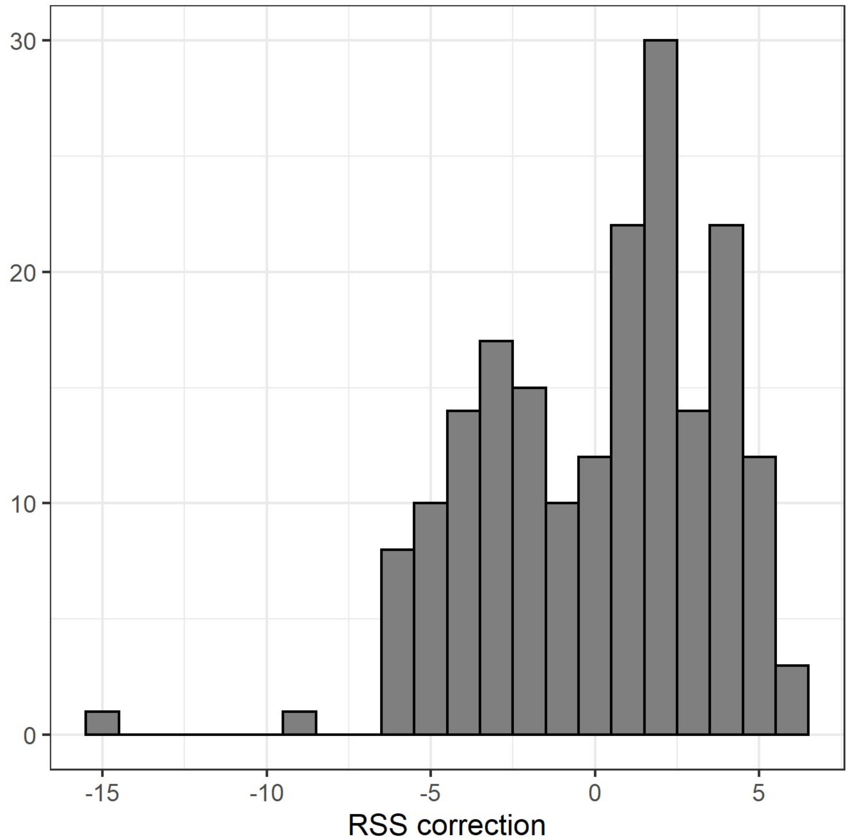

Figure 3.10. Histogram of node-specific corrections for received signal strength (RSS).

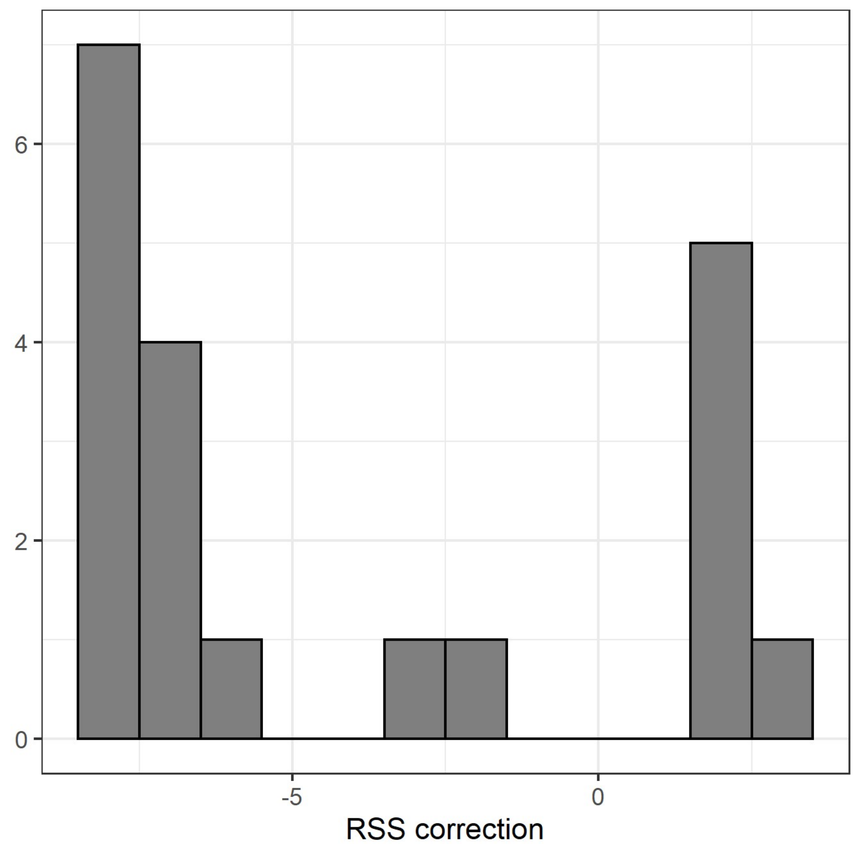

Figure 3.11. Histogram of tag-specific corrections for received signal strength (RSS).

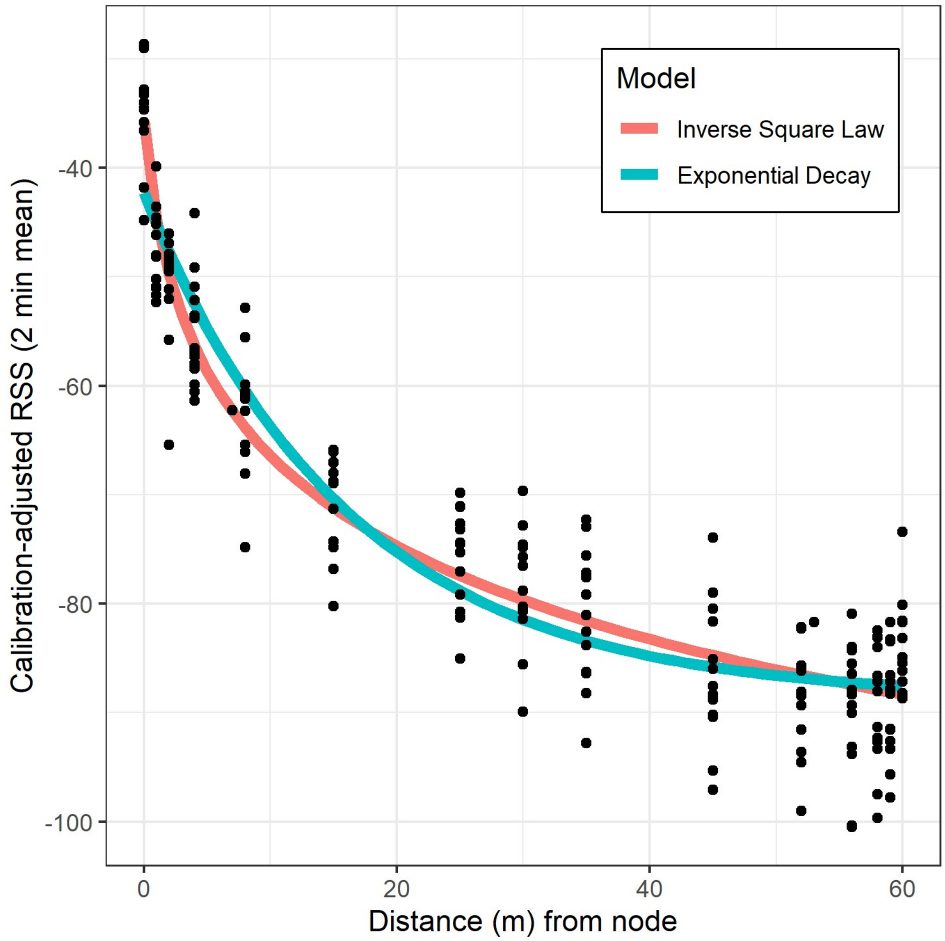

Figure 3.12. Fitted relationship between calibration-adjusted RSS values (averaged over two minutes) and distance from the detecting receiver node modelled as an exponential decay function and according to the inverse square law for electromagnetic radiation. Values represent a single test tag used on 4 transects with 2 nodes per transect.

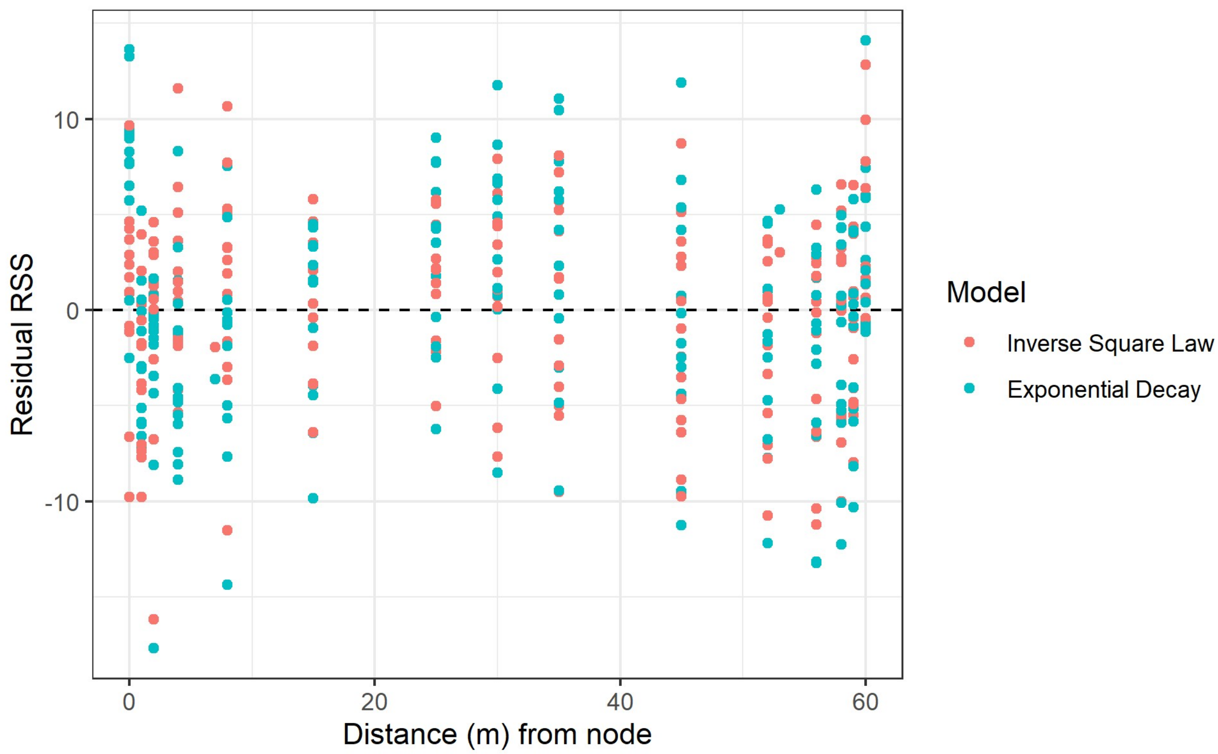

Figure 3.13. Residuals from fitted relationship between calibration-adjusted RSS values (averaged over two minutes) and distance from the detecting receiver node modelled as an exponential decay function and according to the inverse square law for electromagnetic radiation.

Figure 3.14. Violin plots illustrating density of root mean square error (RMSE) for 500 bootstrapped simulations from the modelled relationship between calibration-adjusted RSS values (averaged over two minutes) and distance from the detecting receiver node using an exponential decay function and according to the inverse square law for electromagnetic radiation.

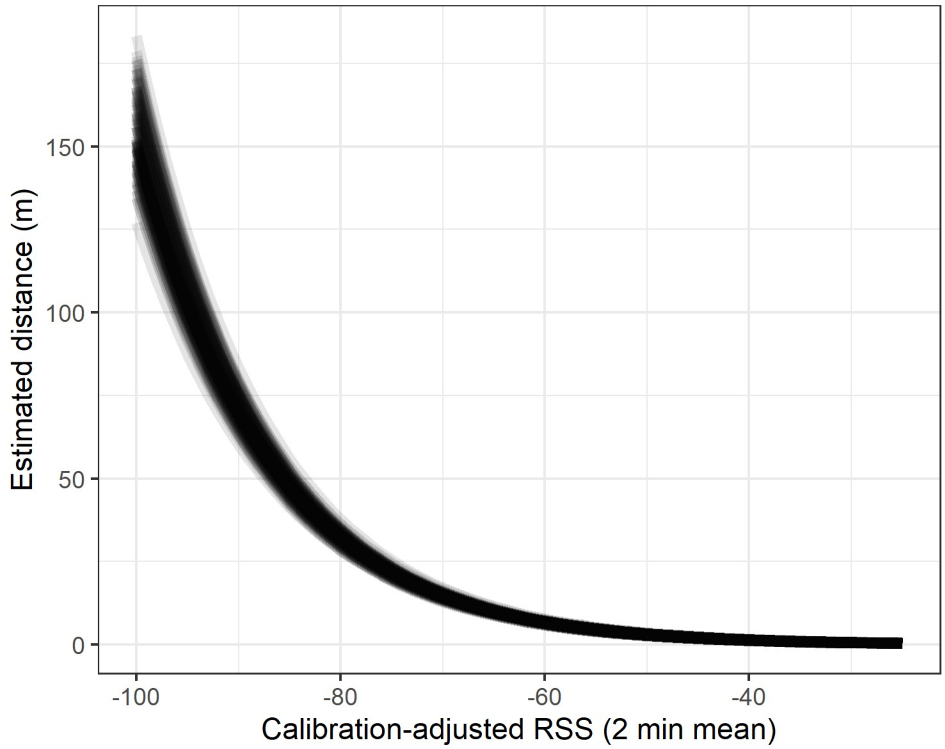

Figure 3.15. Bootstrapped simulations from the final modelled relationship between calibration-adjusted RSS values (averaged over two minutes) and distance from the detecting receiver node using the inverse square law for electromagnetic radiation. The variation in the fitted lines illustrate the relative uncertainty in estimated distance from node for a given RSS value.

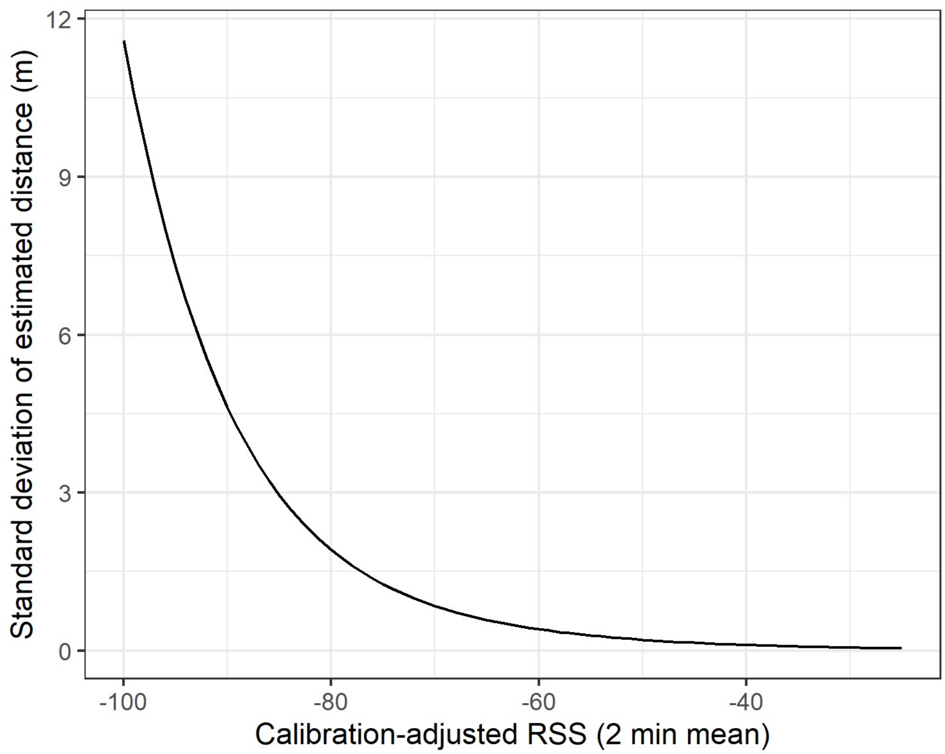

Figure 3.16. Uncertainty (standard deviation) in the predicted distance of a transmitter from the detecting node as a function of average received signal strength (RSS).

Chapter 4
CONCLUSIONS

Broadly, the goal of this thesis was to evaluate technological advances and their potential to shed light on an understudied species, the Black Rail (*Laterallus jamaicensis*). Specifically, our objectives were to 1) investigate Animal Sound Identifier (ASI) software (Ovaskainen et al., 2018) to see if it was suitable to develop vocalization-specific recognizers that aid users in classifying large audio libraries of field data collected by autonomous recording units (ARUs), 2) to demonstrate the use of radio signal strength (RSS)-based automated radio-telemetry systems (ARTS) for researching Black Rails, and 3) to summarize potential applications of both ARUs and ARTS and areas for future research as they apply to Black Rails.

Both ARUs and ARTS are likely to see increased use as the technologies and methodologies continue to advance. This may be especially true for species like the Black Rail that are difficult to research by conventional methods because of their relative rarity, cryptic behavior, and the difficult-to-access habitat. Black Rails are an ideal species to investigate monitoring via ARUs and ARTS, both in terms of methodology and conservation.

Population and occupancy surveys for this species are conducted almost exclusively by auditory surveys, and ARUs have great potential to increase the accuracy and cost-effectiveness of surveys and may provide higher quality estimates to inform management decisions. ARUs can be used to augment in-person surveys of occupancy, increasing the detection probability for Black Rails and thus increasing the quality of statistical inferences about occupancy. Additionally, ARUs could be used to survey occupancy across larger tracts of land allowing for a more targeted approach for in-person abundance surveys.

ARUs may one day replace in-person surveys as the preferred method of surveying occupancy for Black Rails and similar species; however, a few barriers must be crossed before this becomes possible. First, classifiers must be made more accessible to users with limited computational skills and/or to those with limited funds for outsourcing classification. Second, the time between ARU deployment and the acquisition of actionable data for analysis and evaluation must be reduced. In-person surveys produce data that is actionable as soon as it is collected and entered, while audio files collected via ARUs may sit on hard drives indefinitely due to limited time for review. Developing automated classifiers that can process audio quickly and accurately is the key to unlocking the full potential of ARUs. Lastly, we encourage clear evaluation of false positive and false negative rates, precision, and recall for all classifiers. This will allow researchers to clearly define goals for their monitoring programs based on their ability to review output from automated classification.

While vocalization recognizers for Black Rails are still limited, our study demonstrates the potential of classifiers to identify Black Rail vocalizations reasonably well with user control of false positive rates. Continued research applying ARUs to Black Rails is needed, specifically 1) improving existing recognizers for the species, 2) continued development and standardization of occupancy and abundance modeling methods specific to ARU data collection, and 3) building recognizers for all Black Rail vocalization types (including vocalizations by non-adult birds) and evaluating their potential to inform studies of vocalization behavior and occupancy.

RSS-based ARTS have great potential to inform knowledge gaps vital to the conservation efforts of Black Rails, such as studies of movement, activity patterns, habitat selection, and breeding ecology. It is our hope that this study can provide a reference baseline from which future work applying ARTS to Black Rails and similar species can stem. We recommend researchers interested in RSS-based ARTS perform in situ range tests prior to larger equipment purchases as an economical way to inform node spacing and study feasibility for the given application. For researchers interested in home range and location monitoring via node arrays, we recommend soft deployment of arrays to allow for the adaptive refinement of grid placement during construction, saving time and effort and reducing disturbance to sensitive habitats by mitigating the need to readjust the grid after deployment.

LITERATURE CITED

OVASKAINEN, O., MOLITERNO DE CAMARGO, U. & SOMERVUO, P. 2018. Animal Sound Identifier (ASI): software for automated identification of vocal animals. Ecology Letters, 21, 1244-1254.