VOCAL COMMUNICATION AND ACOUSTIC MONITORING OF THE KING RAIL (*Rallus elegans*)

by

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Rare and elusive species present a challenge for researchers, and are consequently often overlooked in evolutionary and behavioral studies. The King Rail, *Rallus elegans*, is a secretive marsh bird considered globally 'Near Threatened' by Birdlife International. Their simple, unlearned calls provide opportunity to decipher how information is aurally transferred during social interactions while excluding variation from learning and structural complexity. Furthermore, the dense, visually concealing habitat in which the species lives poses pressures on these calls for efficient sound transmission and reveals this system as a potential model of evolution. This thesis combines evolutionary ecology, behavioral observation, field experiments, and applied management approaches to examine behaviors associated with vocal communication in the King Rail and how these can be utilized to monitor populations more efficiently.

In order to use calls to research and monitor the King Rail, it is necessary to characterize their full vocal repertoire. Previous accounts for this species are incomplete and inconsistent. In Chapter 1, I review published findings and integrate these with my own field observations, recordings, and analyses to provide a comprehensive account of structure and function of the vocalizations produced by King Rails. Most calls are variations of a series of repetitive, pulsed notes created by altering peak frequency, bandwidth, amplitude, pulse rate, and note length.

In Chapter 2, I focus on the two most commonly used calls, the *grunt* (contact and disturbance context) and *kek* (mating and territorial context), to determine what information, such as individuality, might be encoded in these signals. While mean peak frequency of *grunts* increased in stressful contexts and mean peak frequency of *kek* notes increased over the course of the breeding season, I did not find sufficient evidence to suggest that these calls are individually variable. However, a playback experiment revealed that King Rails were more likely to vocalize and did so sooner in response to a neighbor's *grunt* rather than a stranger's *grunt*. This suggests that classifying information is perceived from this call, although it is unclear if King Rails can only distinguish between familiar and unfamiliar individuals or if they can tell caller identity.

In Chapter 3, I explored how vocal behaviors, such as temporal call rate patterns and likelihood of response to callback under certain conditions, could be used for auditory detection of breeding King Rails. I found that weather, number of human observers, and time of day and season did not affect the number of callback survey detections. Using computer-learning software, I created a signal recognizer to find King Rail calls in large recording files and automate the analysis of data from autonomous recording units (ARUs). These analyses revealed a significant seasonal decline and a clear crepuscular diel pattern in King Rail call rate. I also found that *grunt* call rate, but not *kek* call rate, was significantly positively correlated to King Rail density.

Overall, this thesis provides insight into the behavioral and evolutionary processes shaping the vocal communication system in a species with simple, unlearned calls. It has also generated tools for surveyors and managers to utilize while monitoring and implementing conservation plans for the King Rail.

VOCAL COMMUNICATION AND ACOUSTIC MONITORING OF THE KING RAIL (*Rallus elegans*)

A Thesis

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Master of Science in Biology

by

Katie M. Schroeder

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CHAPTER 1: VOCAL REPERTOIRE OF THE KING RAIL (*Rallus elegans*)

Abstract

Vocalizations are used to locate and monitor elusive species that are more likely to be heard than seen. Auditory callback is the standard protocol for censusing rails and other secretive marsh birds. Due to recent population declines, monitoring the King Rail, *Rallus elegans,* has become a conservation priority in many regions. However, previous accounts of the King Rail repertoire have been inconsistent and incomplete. Here, we combine prior knowledge of call context with interpretation from our own observations of a well studied King Rail population. Despite the lack of complex syringeal structure, King Rails produce a wide array of sounds that they combine in different contexts. We provide a comprehensive characterization of the structure and function of the most commonly heard King Rail calls, based on recordings taken with digital equipment and state-of-the-art sound analysis software. This synthesis is intended to serve as a tool for researchers, surveyors, and managers to help them interpret and report their observations in future efforts to conserve King Rail populations across their range.

Introduction

Species that are secretive by nature present a challenge for researchers and managers (Kolts and McRae 2016). Most bird populations can be surveyed using simple visual confirmation of presence or density. However, for species living in concealing environments, alternative methods must be used. Non-invasive methods include detection of vocalizations (Weir et al. 2005; Conway 2011), scat collection (Janečka et al. 2008), and camera trapping (Znidersic 2017). Each of these methods relies on prior species-specific information about the sampled tissue or behavior. Confusion arises when such accounts are incomplete or conflicting, as is often the case for rarely encountered species.

Owing to their densely vegetated habitats and tendency to stay hidden, marsh birds are widely surveyed using vocalizations (Conway 2011). In five regional waterbird conservation plans the King Rail, *Rallus elegans*, garnered ‘high concern’ or ‘immediate action’ status (Cooper 2008). Due to steep population declines over the past several decades, BirdLife International (2015) recently uplisted the King Rail to the designation globally ‘Near Threatened’. While auditory population monitoring remains crucial to future conservation efforts, the lack of a comprehensive repertoire description poses a barrier to this objective.

The most complete repertoire account for King Rail to date is found in Meanley’s (1992) species monograph. Tomlinson and Todd (1973) and Massey and Zembal (1987) provide additional descriptions of calls for two subspecies of the congeneric Ridgeway’s Rail, *Rallus obsoletus* (formerly *R. longirostris yumanensis* and *R. l. levipes*). The most recent repertoire accounts for the King-Clapper species assemblage include a useful, but brief overview of each call (Pieplow 2017). Detailed descriptions of species-specific repertoires are important because *Rallus* spp. have similar calls, resulting in confusion in the field between the King Rail, its salt marsh specialist sister species the Clapper Rail, *R. crepitans* (Maley and Blumfield 2013), and the smaller Virginia Rail, *R. limicola,* that is also sympatric with King Rails across parts of its range (Potter 1926; Graves 2001; Conway 2011). King Rails can be distinguished visually from Clapper Rails based on their brighter, more contrasted plumage and slightly larger average body size. Auditory distinction must be based on computer classification of spectral parameters of *kek* (mating) call recordings (Stiffler et al., in prep). In areas where their ranges overlap, species differentiation is complicated without analysis of recordings, and hybridization can occur (Maley and Brumfield 2013).

The problem of confusing calls of each species in the field is compounded by a lack of consistency in naming each call among commonly consulted field guides and research papers. Conway (2011) attempted to address this by providing standardized call names, but recent publications have not used his suggestions. For example, at least one field guide refers to the *kek* call as a 'clappering series*'* (Sibley 2016); which confounds two separate calls, highlighting the need for a review of the nomenclature of the repertoire of the King Rail and related species.

The purpose of this note is to provide a comprehensive overview of the structure and function of the King Rail vocal repertoire. We review past and recent findings, and update our understanding of call contexts using our own observations and recordings from a well-studied King Rail population analyzed using modern methods. We recommend standardized call names and relate King Rail calls to their functional equivalents in other North American rail species for more effective sharing of knowledge among researchers, surveyors, and managers.

Methods

Study site and strategy for studying breeding secretive rails

Breeding King Rails were studied from March through July 2015-2017 at Mackay Island National Wildlife Refuge (36°31′N, 75°58′W) in coastal North Carolina. Mackay Island includes 3,300 ha of freshwater and brackish marsh. The King Rail is the only long-billed rail species breeding on the refuge based on study of this population over the last seven years (Brackett et al. 2013; Clauser and McRae 2016; Kolts and McRae 2016; Clauser and McRae 2017). King Rail nests were found by walking transects through suitable habitat between April and July each year in addition to other systematic and opportunistic search methods (Clauser and McRae 2016). Active nests were monitored through hatching to collect vital rates for the long-term study. Nests served as focal points for behavioral observations.

Recording King Rail vocalizations in the field

King Rail calls were recorded during both breeding seasons using three methods. First, we opportunistically recorded King Rails found calling within an estimated distance of 10-60 m from points along refuge roads between 0600-1300 h and 1700-2000 h. Using a Sony linear pulse-code modulation (PCM) recorder and a hand-held Sennheiser ME 66 shotgun microphone with a windscreen (44.1 kHz sampling rate, 16-bit encoding), we made recordings of 5 min to 1 hr, depending on the continuity of calling. Second, we collected remote recordings using two autonomous recording units (ARU, Song Meter SM4, Wildlife Acoustics) (44.1 kHz sampling rate, 16-bit encoding). These were rotated among 10 sites on the refuge, and were deployed at each site for up to 72 hours once every 2-3 weeks. Each site was selected based on auditory and visual confirmation of King Rail presence in 2016. Last, calls were edited from the audio component (48 kHz sampling rate, 24-bit encoding) of video recordings taken with a camera (Sony Handycam) placed 1-3 m from selected nests for behavioral observation.

Responses to common call types using playback

To quantify the probability of response to each principal call type, morning and evening playback trials were performed opportunistically at irregular intervals during the breeding season. Subjects were selected from among King Rails found calling. In each case, we waited for the subject to stop calling before conducting the playback. Out of six possible recordings (two of each call type: *kek*, *grunt*, and *grunt duet*), one was chosen randomly for each trial. We directed the hand held speaker (Goal Zero, Rock Out 2 with Boostaroo audio amplifier) toward the bird(s) to play the call at maximum volume, then waited up to 1 min for a response. Vocal responses typically occurred within the first 30 seconds. In most cases, a single trial was performed at a given location, but on four occasions, two trials were performed within 8 minutes of one another at the same location.

Call characterization and sound analysis

Recordings were visualized and analyzed using Raven Pro 1.4 (Cornell Lab of Ornithology 2011) software, constructing spectrograms with a 1024 fast Fourier transform length and a Hann window. Frequency and time resolutions were 43.1 Hz and 11.6 ms, respectively (46.9 Hz and 10.7 ms for 20 *grunts* from camera audio). Peak frequency, the frequency most emphasized in a note, and note duration were measured for all calls from the power spectrum (Zollinger et al. 2012). In addition, call duration, number of notes, and pulse rate were measured for calls consisting of multiple notes. For all note measurements, the frequency range was truncated between 500-9000 Hz to capture only the full bandwidth of the notes. For *keks* and *alarms*, the ranges were further truncated to between 1500-7500 Hz, and *booms* were truncated between 50-500 Hz because these calls had narrower bandwidths. Means were calculated by first averaging all notes for an individual, and then averaging all individuals in the sample. This is with the exception of *grunts*, where peak frequency and note duration values only reflect average measurements from the first note in each individual's call because these parameters change from the beginning to the end of the call. All statistical analyses were performed using R software (Version 3.2.3).

Results

Six call types were given frequently by King Rails. Five additional calls were heard, but recorded fewer than five times each over the course of three breeding seasons. Mean values of key parameters are reported in Table 1.1 for the six frequently heard calls and the rare *kek-burr* call.

Grunt and grunt duet

Used year-round, this is the most commonly heard King Rail call. Although *grunt* is the most frequently used term for this pulsed call (Peterson and Peterson 2002; Kaufman 2005; Crossley 2011; Sibley 2016; Pieplow 2017), each note sounds phonetically more like a *jupe* or *cheup*, as first described by Meanley (1969). *Grunts* have also been referred to as the *clapper* (Sibley 2016) for which the Clapper Rail got its name, *clatter* (Conway 2011), or *chuff* by one contributor on the online song collection website Xeno-canto.org (Lane 2017). Another call, the *kek-hurrah*, sounds similar to the *grunt*, but has been listed separately (Conway 2011) without information distinguishing context. Virginia Rails also make a *grunt* call (Pieplow 2017).

The call is comprised of a series of pulsed notes (Figure 1.1A) that progressively accelerate and decrease in frequency (Figure 1.2A), note duration (Figure 1.2B), and amplitude. The notes particularly accelerate into a rapid trill at the end, similar to the sound of a dropped ball as its bounce descends in height and increases in speed. Sometimes the trill is absent or inaudible, due to the sound's low frequency and amplitude. On average, *grunts* consist of 17.6 notes, but they range from 4-39 notes. With each note in the *grunt* call, the entire body of a King Rail oscillates. The neck remains extended while the slightly open beak widens for each pulse (KMS, personal observation).

Grunts may be heard at any time of day. They are commonly heard during the breeding season, and less frequently at other times of year. Both sexes *grunt* in response to a disturbance such as the sudden loud noise made by a truck engine ignition, gunshots, or a door slamming. Human speech, and on one occasion, a sneeze, was also found to elicit *grunts*. Another context in which the *grunt* is used is between mates and family members. When uttered by an incubating bird, a possible interpretation is that it wishes its mate to relieve it at the nest or as a proclamation that 'all-is-well' (Meanley 1969) in the context of a 'roll call' (see below). We have additionally heard *grunts* in a territorial context. *Grunts* have been uttered during auditory simulations of territory intrusion by another bird (N =13) and by birds near a nest while a researcher was also within 20 m of the nest (N = 7).

Grunts can be heard singly or as a *duet* by a mated pair. Other studies have identified the functions of duetting in birds as enhancing territory defense and mate communication (Hall 2009; Kovach 2013), which is presumably also the case in King Rails. In the *duet*, *grunts* from two individuals overlap with staggered notes, creating the perception of a louder, faster-pulsed call (Figure 1.1B). They are asynchronous rather than precisely coordinated as in the duets of some tropical bird species (Thorpe 1963). It is unclear whether one sex is more likely to initiate a duet, but a male initiated and the female followed on at least one occasion. There does not appear to be consistency regarding whether the initiator or joiner has the longer call. Occasionally, the joiner only gives 2-4 notes. It is possible that these are accidental overlaps rather than intentional *duets*, explaining why the second bird quickly stops. Pieplow (2017) reported that Clapper Rail *duets* were longer and less accelerating than solo *grunts*. Student's t-tests indicated no difference in the number of notes, pulse rate, and acceleration between five *duets* and our sample of individual *grunts*. *Duets* lasted slightly longer on average, but this was not statistically significant (*grunt* length = 4.523 ± 0.168 s; *duet* length = 5.867 ± 0.711 s; $P = 0.10$).

The roll call is an interesting phenomenon observed in breeding rails. One individual initiates with a *grunt*, and this is followed immediately by *grunt* responses from one or more birds in the vicinity. Video observation has shown that incubating birds are among the responders. In order to quantify *grunts* given in the roll call context, we analysed 794 hrs of recordings from ARUs split into 5 min segments. For convenience of analysis, roll calls were defined as instances when three or more *grunts* were heard within a 5 min period. This may inflate the number of *grunts* in each roll call because responses are generally immediate and an entire roll call does not often exceed 30 s. Some *grunts* in a 5 min recording may not be part of a roll call. Equally, some *grunts* may have been missed if the roll call was split between two recordings. Thus, the following roll call rates are conservative because each 5 min period contained at least one roll call, but may have more. Up to 30 *grunts* were counted in one segment, although the majority of roll calls have six or fewer *grunts* ($N = 170$ out of 202; Figure 1.3A). The number of *grunts* in a roll call could be used as a measure of King Rail density, but we have observed cases where an individual responded more than once during a roll call, as well as cases where individuals did not respond at all. Use of *grunts* in the roll call context has been noted in other breeding populations of King Rails (Meanley 1969; 1992), and particularly in the evening for Clapper Rails (Simmons 1914; Johnson 1973; Mangold 1974; Massey and Zembal 1987) and Ridgeway's Rails (Dawson 1923). King Rails at Mackay Island NWR did not engage in roll calls evenly throughout the day ($\chi^2 = 99.5$, $df = 23$, $P < 0.001$). Most roll calls occurred in the morning between 0500-0800 h and in the evening from 1700-2000 h, whereas roll calls were infrequent or absent during the night (Figure 1.3B).

A couple of pairs of the confamilial Common Gallinule, *Gallinula galeata cachinnans* (Bangs; formerly *Gallinula chloropus cachinnans*), bred sympatrically at this site. Interestingly, individuals occasionally joined or initiated roll calls with King Rails. In the roll call context, Common Gallinules always used the *cackle*, also known as *wipeout* (Conway 2011) or *whinny* (Pieplow 2017). The *cackle* is a series of high-pitched, nasal-sounding notes that similarly begin high and fast then become longer and lower.

Table 1.1 Means (± SE) of 6 key parameters for common King Rail call types. Structural descriptions of calls are based on the sample sizes listed.

	Peak Frequency (Hz)	Note Duration (s)	Number of Notes	Call Duration (s)	Pulse Rate (notes/s)	Sample Size	
						Calls/Notes	Individuals
Grunt	2491 ± 124	0.114 ± 0.003	17.6 ± 0.6	4.523 ± 0.168	3.682 ± 0.071	61 calls	37
Kek series	2633 ± 30	0.042 ± 0.0006	31.7 ± 2.1	11.944 ± 0.719	2.610 ± 0.080	208 calls	46
Alarm	3814 ± 143	0.073 ± 0.003	NA	NA	NA	165 notes	7
Boom	184 ± 5	0.153 ± 0.006	6.6 ± 0.4	4.106 ± 0.222	1.342 ± 0.029	5 calls	5
Screech	2861 ± 176	0.338 ± 0.010	NA	NA	NA	47 calls	7
Churr	2226 ± 96	0.630 ± 0.019	NA	NA	NA	43 calls	10
Kek-burr (*kek*)	3304 ± 241	0.052 ± 0.004	NA	NA	NA	9 notes	3
Kek-burr (*burr*)	2584 ± 57	0.585 ± 0.028	NA	NA	NA	4 notes	3

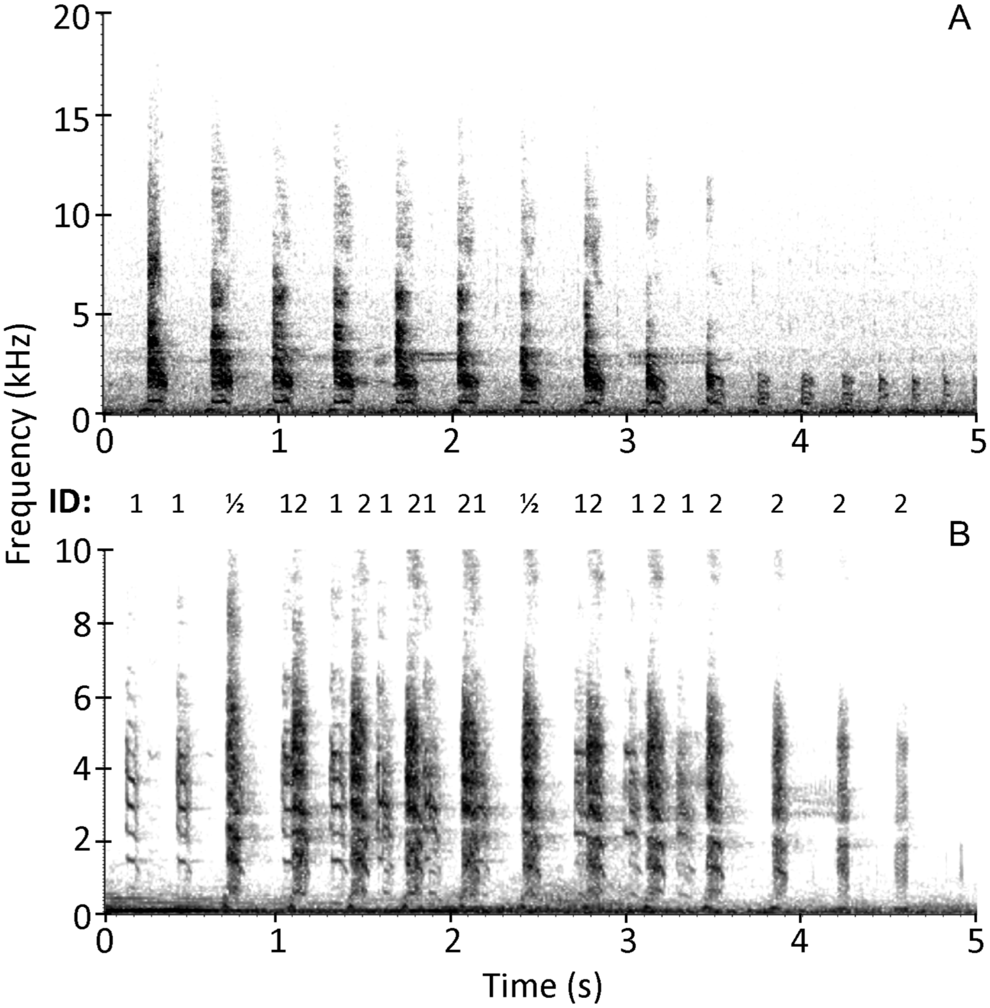

Figure 1.1 King Rail (A) *grunt* and (B) *grunt duet* spectrograms. Numbers above each syllable in the *grunt duet* indicate whether it is part of the first or second individual's *grunt*.

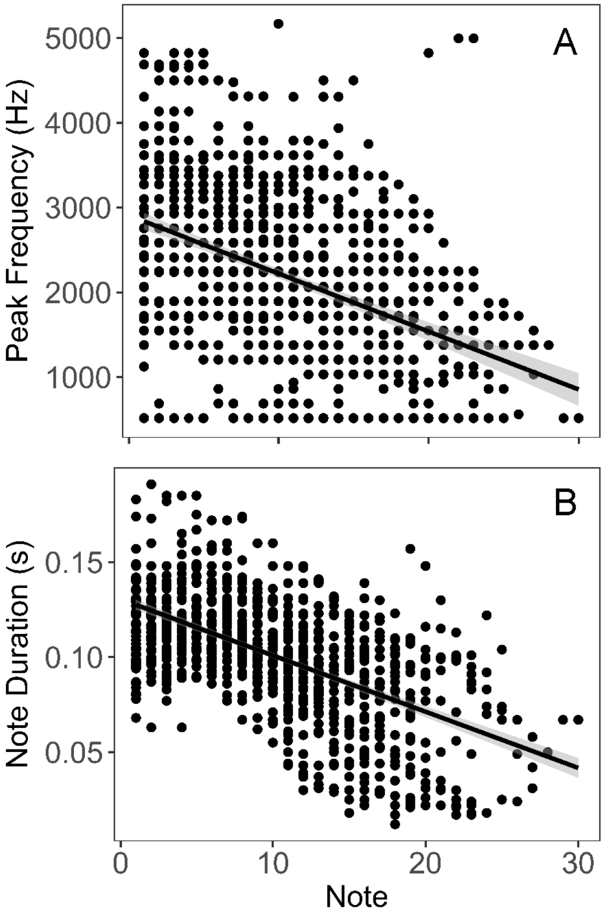

Figure 1.2 With each successive note in the *grunt* call, (A) peak frequency decreases by an average ± SE of 67.9 ± 4.2 Hz and (B) duration decreases by an average ± SE of 0.0028 ± 0.0001 s (N = 61 calls, both P < 0.001).

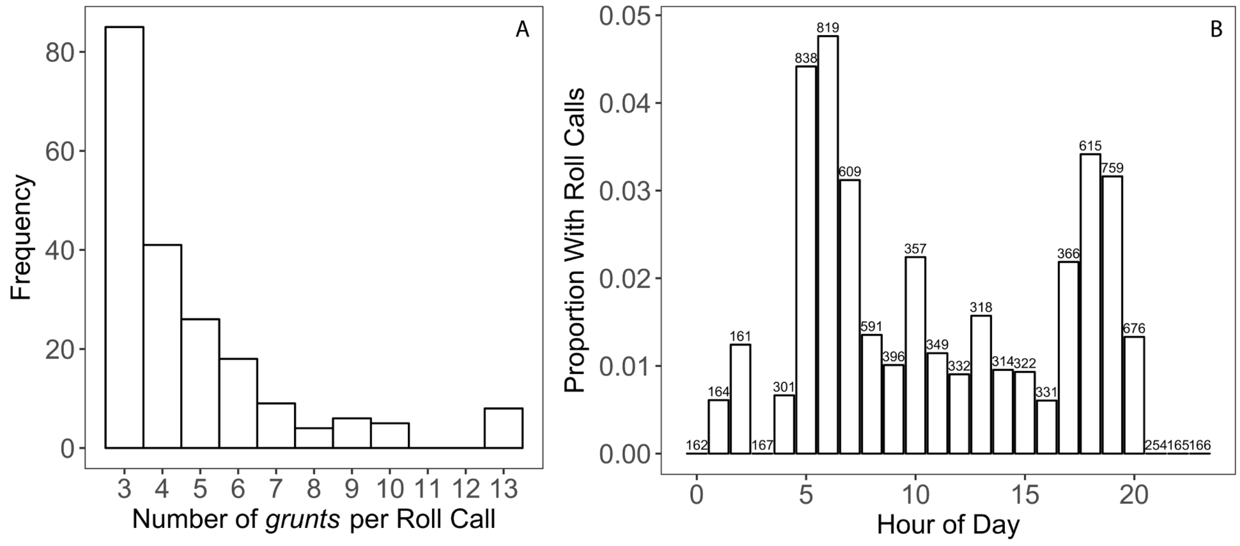

Figure 1.3 (A) Frequency distribution of *grunt* calls per roll call. Roll calls were considered instances when three or more *grunts* were heard within a 5 min period (N = 202 time periods with roll calls out of 9,532 time periods screened). Eight roll calls in the 13+ category included 13, 13, 13, 14, 15, 16, 23, and 30 *grunts*, respectively. (B) Roll calls per hour of day at Mackay Island NWR. Roll calls were considered instances when three or more *grunts* were heard within a 5 min period. During each hour of the day, the proportion of 5 min recordings that contained a roll call is shown. The number of 5 min recordings taken during each hour of the day is indicated above the bar, based on a total of 794.33 hrs of recordings from ARUs. Chi-squared analysis revealed a significant association between hour of day and the number of roll calls ($\chi^2 = 99.5$, df = 23, $P < 0.001$).

Kek

The *kek,* also *kik* (Meanley 1969) or *kek series* (Pieplow 2017), is heard commonly during the breeding season, and consists of a series of nearly identical, repeated notes (Figure 1.4A). It is similar in structure and function to the Virginia Rail *kiddik*, although lacks the characteristic paired syllables of the Virginia's call. This simple King Rail call sounds like two stones being struck together at a relatively constant rate, and is loud enough to be heard from 300 m away.

The dominant frequency of an individual's *kek* notes generally remained constant. The pulse rate was more consistent than in the *grunt*, but can change sporadically. Bursts of *kek* notes were typically 12.4 ± 0.4 s in duration and 2.61 ± 0.08 notes/s (N = 206), but individuals were observed to *kek* continuously for up to 2 hours, speeding up and slowing down intermittently with occasional breaks of silence. Fast bursts of *kek* notes were on average 2.6 notes/s. These bursts often started slowly, then accelerated to a consistent rate, and decelerate again before the end. Thus, the intervals between the first three notes decreased in duration in each burst series (all $P < 0.001$; Figure 1.5).

King Rails at Mackay Island NWR responded to *kek* playbacks with *keks* consistently by the second week of March, but were observed to spontaneously begin to *kek* only when the weather became mild. *Keks* continued throughout the breeding season, becoming less frequent during nesting. They could be heard throughout the day, but most often between sunrise and mid-morning and during the two hours before sunset. Early in the breeding season, in the hour before dusk, multiple King Rails have been heard *kekking* simultaneously within a 300 m radius, in an evening chorus. Anecdotally, if a *grunt* was heard during an ongoing *kek* call*,* the *kekker* was observed to either speed up or stop, seemingly to listen, and then resume. *Kek* choruses ended with last light, although a single male may continue *kekking* throughout the night as determined from passive recordings (N = 15). Night *kekking* was only observed between mid-April and mid-May in this population. *Kekking* has also been heard at night in King Rail populations in Maryland and Florida (Meanley 1969).

Kekking birds often pace back and forth within a small area. Early in the season, males *kek* while foraying outside of their territory for a mate (Kolts and McRae 2016). King Rails typically *kekked* from a hidden position, but did so occasionally out in the open or from an elevated clump of grass. For each note, the bill reopens widely and rapidly. It is possible that this sound is a clicking made within the throat or mouth rather than the syrinx.

Similar to song in songbirds, the *kek* serves both as a mating and a territorial call. Frequent changes in *kek* pulse rate may function to attract attention from females. Similar variation in display rate is seen in Fiddler Crabs, *Uca mjoebergi*, and experimental evidence reveals that females crabs prefer males with escalating sexual display rates (Mowles et al. 2018). *Keks* also illicit aggressive responses from males, as exhibited by our whoosh net capture efforts using a taxidermic mount and *kek* or *kek-burr* playback as a lure. Only males have been captured using this method (N = 9) (Clauser and McRae 2017; Kolts and McRae 2017), and we recorded video of one of them *kekking* while approaching the mount. *Keks* were also given as responses to *grunts* from nearby birds. Likewise, *grunts* were often heard while a King Rail has been *kekking* for a while, but it was not clear if this was directed at the *kekker* or if uttered for an unrelated purpose. The *kek* has been noted in an alarm context as well. On more than one occasion, adults have given the *kek* call when researchers were close by, when flushed from a nest, when directly defending a nest from researchers during nest checks, or in conjunction with *alarm* notes and/or *screeches*.

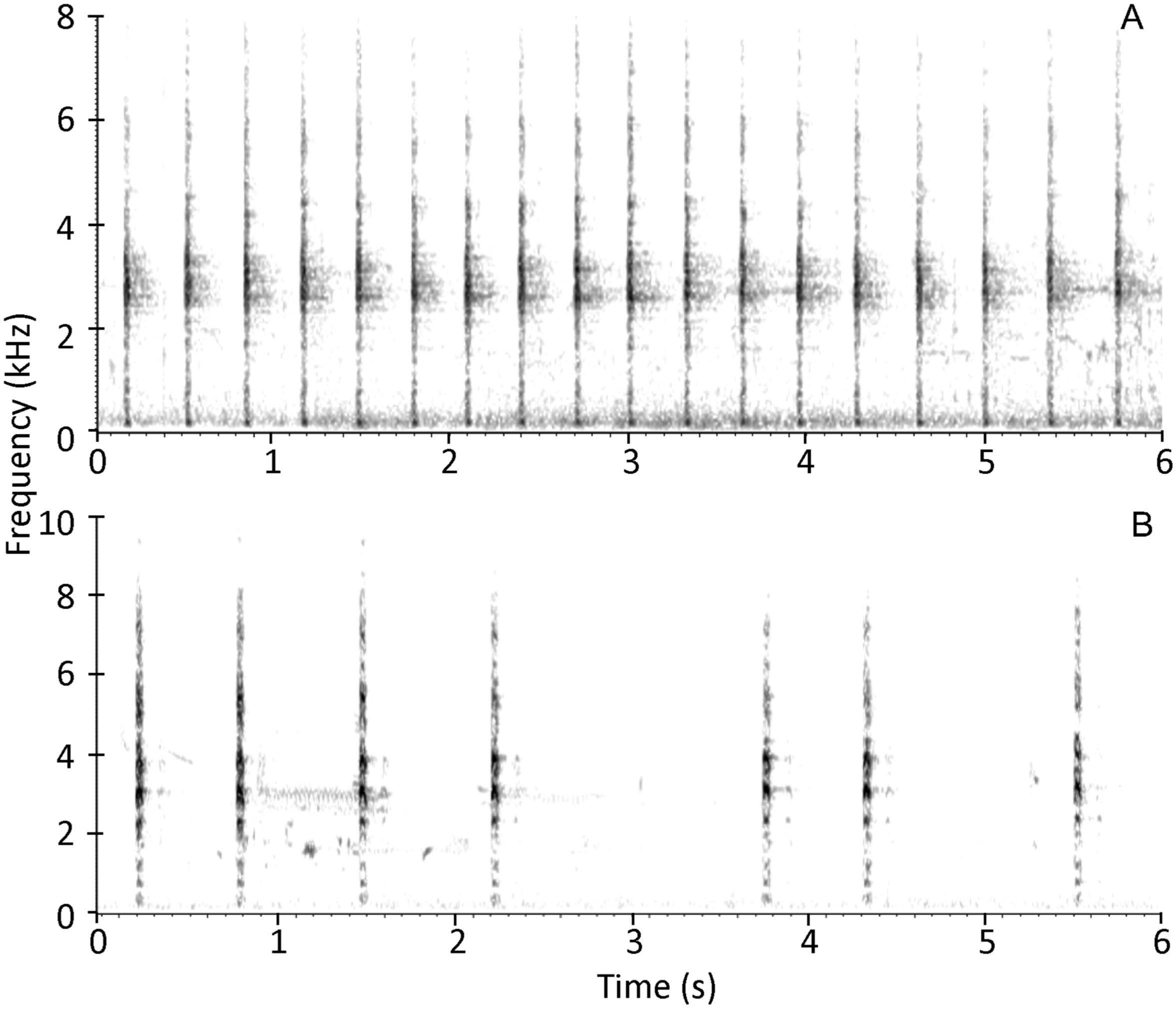

Figure 1.4 King Rail (A) *kek* and (B) *alarm* spectrograms. Note the higher maximum frequency and slower, more sporadic pulse rates in *alarm* calls as compared to *kek* calls.

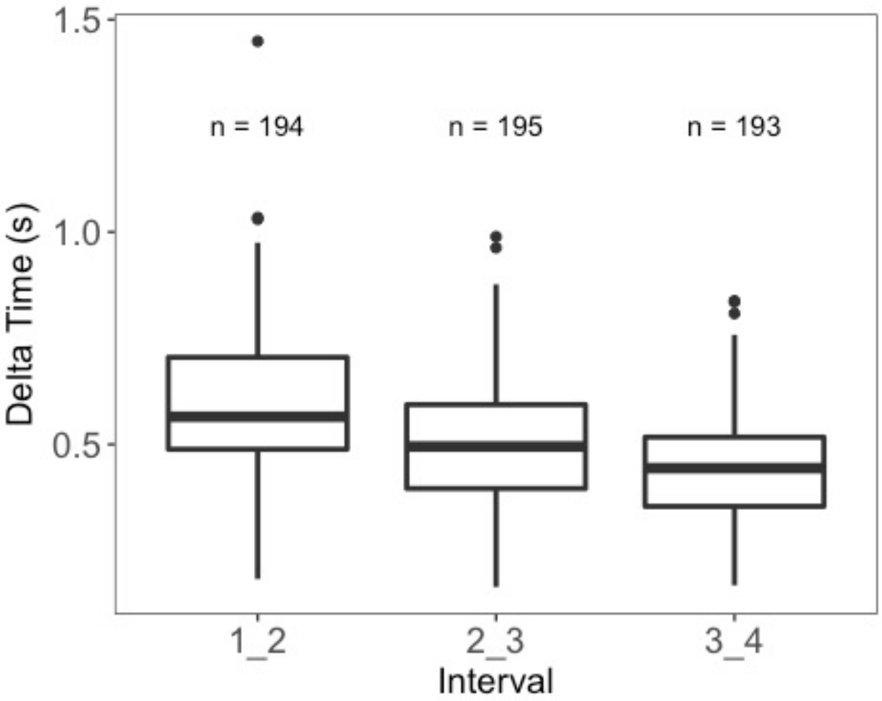

Figure 1.5 Duration of intervals between first four notes in King Rail *kek* calls measured between the end of one note and the beginning of the next note. One-way ANOVA ($F_{2,579}$ = 44.33, P <0.0001) and *posthoc* Tukey Tests (all Ps < 0.01) indicated that lengths of the first three intervals in a *kek* call are statistically different from each other.

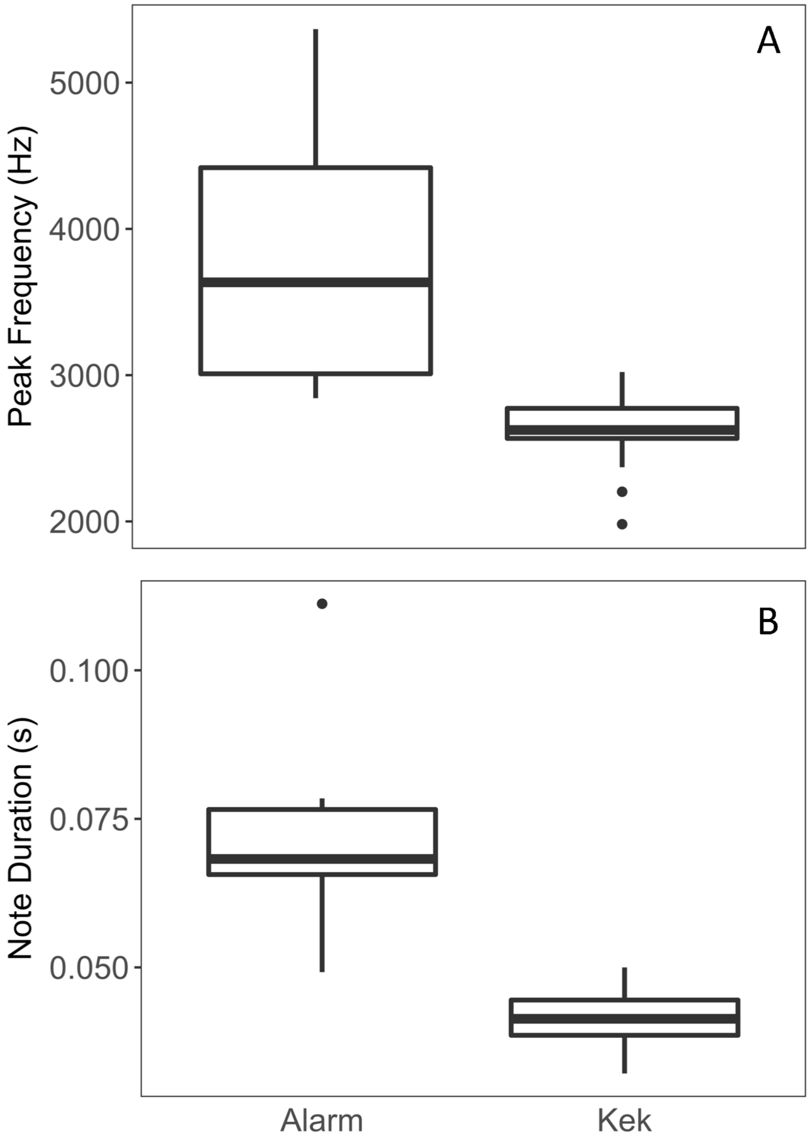

Figure 1.6 There were significant differences between *kek* and *alarm* calls in (A) Peak frequency (*kek* mean ± SE = 2633 ± 30 Hz, *alarm* mean ± SE = 3817 ± 143 Hz, $t_{6.07} = 3.11$, $P = 0.02$) and (B) note duration (*kek* mean ± SE = 0.042 ± 0.0006 s, *alarm* mean ± SE = 0.073 ± 0.003 s, $t_{6.10} = 4.37$, $P < 0.01$), based on 5,681 *kek* notes from 46 individuals and 168 *alarm* notes from 7 individuals.

Alarm

The *alarm* has previously been called the *agitated kek* (Tomlinson and Todd 1973) or *gip* (Meanley 1992). It is similar in structure to the *kek* (Figure 1.4B), but can be distinguished based on the sporadic nature of the pulses and higher frequency notes with a more wheezy or nasal timbre. The notes of a King Rail *alarm* do not come in a consistent burst, as in the *kek*. Rather, they are more spaced out, and often come in slow groups of 2-5 with a few seconds of silence before the next set of notes. Occasionally, one or two *alarm* notes in a sequence will be more shrill than the others, sounding like a squeaky toy. *Alarm* note peak frequency is significantly higher ($t_{6.07} = 3.11$, $P = 0.02$) and *alarm* note duration is significantly longer ($t_{6.10} = 4.37$, $P < 0.01$) than *kek* notes (Figure 1.6).

The *alarm* call resembles the pulsed alarm calls of other bird species in having short note duration and long pauses between notes. However, it differs structurally in having broad bandwidth. Most other North American rails have a similar, short pulsed and high frequency *alarm* call. Meanley (1992) briefly mentioned this call in context with birds that are flushed from the nest or an alarmed parent with a brood. *Alarm* calls were given in various contexts, for example, by a parent when researchers caught one of its 2-week-old chicks for sampling purposes. In another instance, one of at least three King Rails present made an *alarm* call during a whoosh net capture attempt using a taxidermic mount and audio lure. This bird was most responsive when playback consisted of the *kek-burr*. Just before dusk in mid-March, we recorded a King Rail *alarm* within 25 m of the road, and although the bird was not seen during the 30 min recording period, it gradually transitioned to higher frequency notes, possibly getting more agitated due to our presence.

Boom

The *boom* most likely is the same call referred to as a *hoo* or *hoot* in some sources (Tomlinson and Todd 1973; Pieplow 2017), although this call has not been well documented with respect to structure or context. Meanley (1969) was the first to mention a low, booming *oom-oom-oom* call given by males during the pre-nesting period. Only one previous recording is publically available, a seemingly incomplete *boom* from a Clapper Rail (University of Arizona). Here, we characterize for the first time, to our knowledge, the structure and function of the King Rail *boom*.

Booms consist of 5 to 10 very throaty, low frequency, low amplitude notes (Figure 1.7A). The spectrogram shows a fundamental and a strong first harmonic. To make the *boom* call, the bill remains closed or only slightly open. The neck contracts, but extends a few centimeters lengthwise during each note.

King Rails most likely use the *boom* as a warning or territorial call. *Booms* have been given by an incubating bird during simulated territory intrusions by another King Rail (N = 3) and on at least 35 occasions by birds near a field team in the marsh. The low frequency of the *boom* likely makes it difficult for many vertebrates to hear (Dooling et al. 2000) and difficult to locate outside of a short radius. Furthermore, the structure of these low frequency, low amplitude calls reduces attenuation, a cue that allows receivers to estimate distance from the sound source (Rek 2013). Thus, the *boom* is effective as a warning to close intruders without disclosing the position of the caller, such as when on a nest. Meanley (1969) observed that *booms* occurred during the pre-nesting period and that only males gave the *boom* call. In contrast, we have observed King Rails giving this call during the incubation period (N = 7). We observed four known males using the *boom* call, but also at least 40 King Rails of unknown sex, therefore we cannot refute the possibility that *booms* are also given by females.

Screech

The loud King Rail *screech*, also *squawk* (Conway 2011), can be quite squeaky and nasal. It has been described as being ‘highly plastic’ (Pieplow 2017), with a single bird often changing pitch and tonality significantly for consecutive *screeches*. The call consists of a single note, much longer than the short, staccato notes of the *kek* call. The note often decreases in frequency slightly from beginning to end and appears with several overtones on the spectrogram. However, harsher notes with more noise might lack semblance of pure tones (Figure 1.7B). *Screeches* are given repeatedly at non-uniform intervals, rarely for as long as an hour. The *screech* of a Virginia Rail is fundamentally indistinguishable from that of King and Clapper Rails (Potter 1926).

The *screech* is a distress call given in situations where there is immediate danger. Other researchers have described this call in Clapper Rails exhibiting behavior between aggressiveness and flight (Massey and Zembal 1987), suggestive of function both to deter and distract a predator. *Screeches* can be given by a lone King Rail during attempted predation, but are occasionally given concurrently by a mated pair defending their nest. Specific pairs have been found regularly to stand their ground during nest visits and respond vocally with *screeches*, or more commonly *churrs* (intensifying at later nest stages), while other parents more often leave the nest quietly.

Screeches were associated with predation events on both adults and nests. On one occasion, we observed a predation attempt on a *kekking* adult King Rail by a northern harrier, *Circus cyaneus.* The rail *screeched* many times and was able to dodge into dense vegetation to avoid the harrier's dive. On another occasion, we found a partially depredated nest where two birds were heard giving the *screech* call. The pair also gave intermittent *grunts* and one *kek-burr.* One punctured and two intact eggs remained in the nest, and we suspect that the predator had been chased away by the experimenter's approach. Likewise, a rail was heard giving the *screech* call by itself one morning. Subsequently, a second King Rail crossed a channel and entered into the patch of needlerush where the first bird had been calling. They both proceeded to *screech* for the next 15 minutes. Immediately after, our crew found three old nests in the same area as this pair, one of which possibly could have been depredated that day. No eggshells were found, consistent with a predator that swallows the eggs whole, such as a large bird or a black rat snake, *Pantherophis obsoletus*. A similar anecdote from Massey and Zembal (1987) described a pair of Clapper Rails *screeching* intensively at a murder of American crows, *Corvus brachyrynchos*, raiding their nest.

In all aforementioned instances where *screeches* were heard, danger of predation or intrusion was apparent and immediate. Of note, however, an ARU recorded *screeches* at the same location on three consecutive mornings in mid-June, mostly between 0600-0700. There was no known nest at this location during that time. It is unknown whether a predator or intruder may have returned each day at the same time to provoke this behavior.

Churr

Churrs have been alternatively referred to as a *rak-k-k* (Meanley 1992) or a *purr* (Massey and Zembal 1987; Conway 2011). Black Rails, *Laterallus jamaicensis*, and Virginia Rails also have their own version of this call (Pieplow 2017). *Churrs* consist of a prolonged, wide bandwidth trill, much like a growl, given repeatedly for the duration of a disturbance. The trill is similar to the sound of a rolled ‘r’. Each repetition typically has a constant dominant frequency, but it sometimes rises quickly and falls slightly towards the end. As with the *screech*, multiple overtones or harmonics can be distinguished in the *churr* spectrogram (Figure 1.7C).

The *churr* is most certainly a defensive or alarm call given when an adult or its nest is threatened. The call is given ubiquitously by bold parents that remain nearby while researchers are monitoring their nest, although *screeches*, *alarms*, *booms* and *grunts* have been heard in this situation as well. While giving a *churr* call, the bird will usually tilt its wings forward and raise them above their head, effectively making it look larger and more intimidating. *Churr* calls and displays have been observed during all daylight hours. This suggests that it is used by both sexes, because females tend to incubate in the morning and during the day, while males are more likely to be on the nest after 1700 h (Clauser and McRae 2017). Meanley (1969) has also noted a *churr* or *purr* given by the female King Rail during courtship.

Kek-burr

This call was heard fewer than 5 times over 3 breeding seasons. The *kek-burr*, also *kik-kurr* (Meanley 1969), consists of 1-5 slow *kek* notes followed by a trill, the *burr*, with little or no space between the last *kek* and the trill (Figure 1.8). The *burr* portion resembles the *churr* call, but can be slightly different in tone. The *kek* note is higher pitched, but similar in duration to the traditional notes in a *kek* call (Table 1.1).

Over 30 years ago, it was proposed that the *kek-burr* was the female advertising call (Zembal and Massey 1985). An ARU deployed at our field site recorded two *kek-burrs* an hour and a half before dawn in late April immediately followed by fast *kek* bursts from two other birds. One interpretation of this is that two males were excited by a female ready to mate. However, we propose that an alternative, or additional, function of the *kek-burr* in nest defense, potentially given by both sexes. The first account of this call suggested that it may be agonistic, only occurring when two males were in the same territory (Meanley 1969). On two separate occasions during morning nest checks, different parents attempting to defend their nests from the researcher used this call. It was also heard in conjunction with *screeches* and *grunts* during a nest predation event, as described above. Other accounts describe this call in conjunction with *churrs* (Massey and Zembal 1987; Simmons 2015), also suggesting a nest defense context. Tomlinson and Todd (1973) suggest that the *kek-burr* is probably given by both sexes. Taken together, evidence suggests that the interpretation that this call is for mate solicitation is inaccurate or incomplete.

Other calls

A number of soft communications have been observed through video footage of a banded female incubating her clutch one hot morning in mid-July. These started ~20 min after she had returned to the nest, beginning with short, single- or double-syllable calls similar to a whispered *grunt*. The call was given every few minutes for the next 45 minutes, at which time her calls began to get slightly louder and more *grunt*-like with more syllables. However, these calls were still quiet compared to the normal volume of a *grunt*. After about 2 hours on the nest, she was now in full sun and had begun to shade her eggs. At this point she began occasional, soft *churring*. Shortly after, she gave a high, whiny *squeal* and displayed bouts of panting. About ten minutes after the *squeals*, she left her nest to take a recess, and *grunted* softly. We inferred that these calls were likely to communicate to her mate that she wanted to be relieved of nest attendance duty due to becoming overheated.

A soft *tuk* call, noted in King Rails (Meanley 1969) and in Common Moorhens, *Gallinula chloropus chloropus* (Linnaeus) in the U.K. is given to communicate softly between mates (*puck-puck*; McRae 1996), or between parents and chicks (SBM, personal observation). The *tuk* is a quiet, throatier note given in a short series. For example, an adult King Rail was observed foraging on an open mudflat one morning when it gave 4 short *tuk* syllables and walked out of sight behind a patch of Typha sp. Shortly thereafter, it returned into the open followed by 4 chicks and its mate. Young chicks call to their parents with a high-pitched *chee-up* (Meanley 1969) note that falls in pitch.

Responses to Playback

We conducted playback experiments where we played one of three different King Rail calls and recorded the call type of any responses. *Grunt* and *duet* calls were pooled for both playback recordings and responses. A Fisher's Exact test using the Freeman-Halton extension was used to compare probability of *grunt*/*duet*, *kek*, *distress*, or no response to both *grunt*/*duet* and *kek* playback. Playback call type had a marginal influence on response call type (P = 0.08). King Rails tended to respond to playback with the same call type (Figure 1.9). *Duets* were more likely to illicit responses than single *grunts*. Call type of the individual when initially found had no effect on response call type (P = 0.15), but birds found *kekking* and *screeching* always responded with *keks* and *screeches*, respectively.

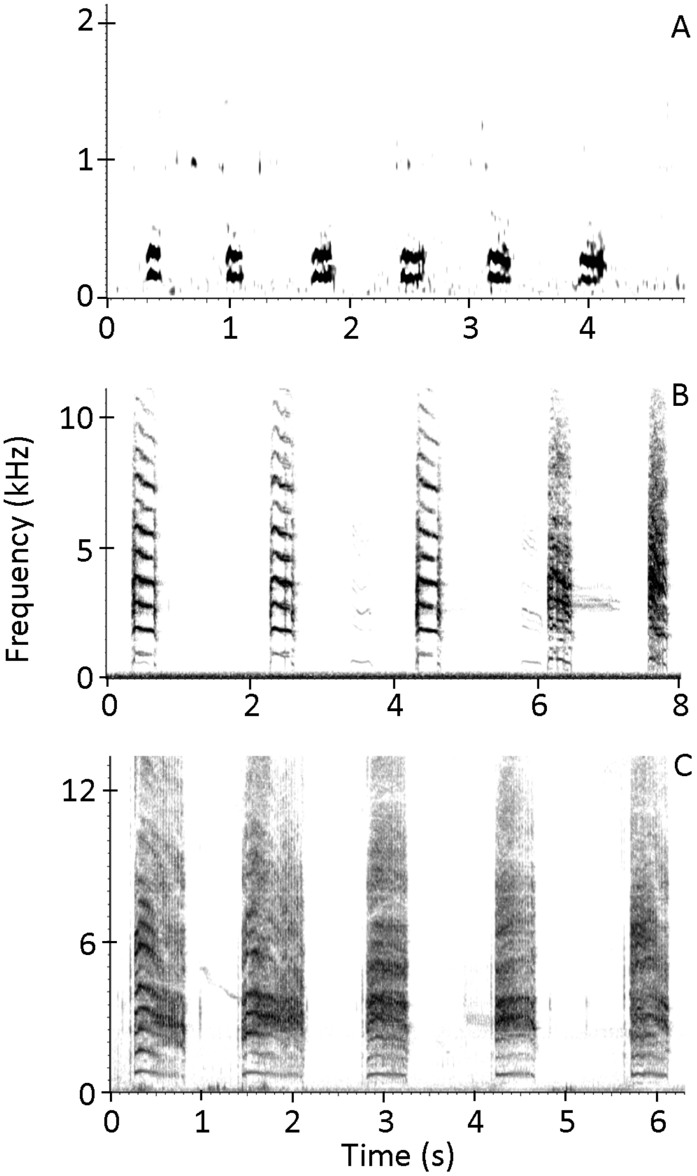

Figure 1.7 King Rail (A) *boom*, (B) *screech*, and (C) *churr* spectrograms.

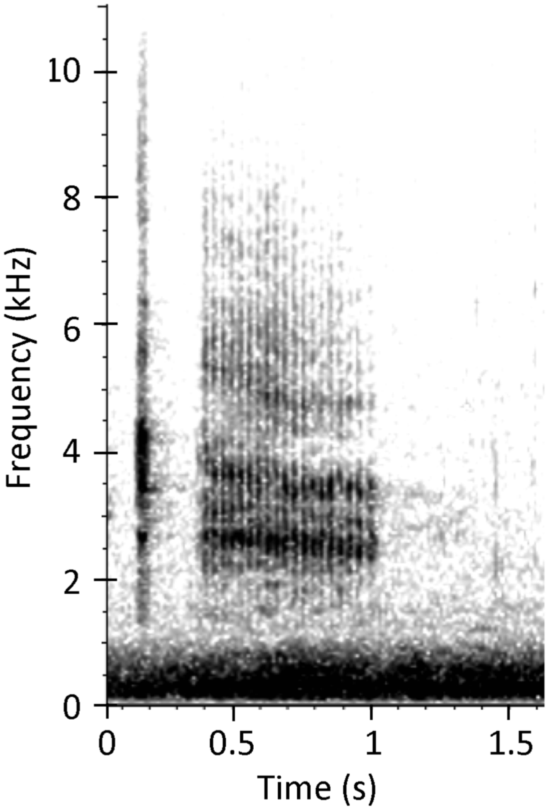

Figure 1.8 King Rail *kek-burr* spectrogram. Note similarities in frequency, note length, and structure between this call and *kek* and *churr* calls. The high amplitude signal < 1 kHz is traffic noise from a nearby causeway.

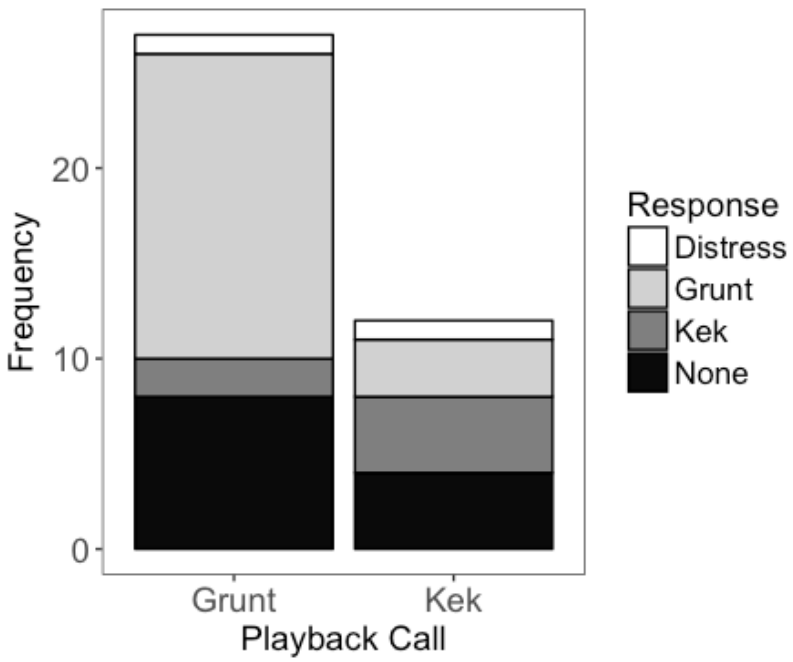

Figure 1.9 Number of responses of each call type to playback of either a *grunt* or a *kek*. *Grunt duet* playback and responses were combined with *grunt* playback and responses. A Fisher's Exact test showed that response call type was marginally more likely to match playback call type (N = 27 *grunt* or *grunt duet* and 12 *kek* playbacks, P = 0.08).

Discussion

In spite of their lack of complex syringeal morphology, King Rails can produce an array of subtly different sounds that have been shaped by natural and sexual selection to produce a reasonably diverse repertoire. King Rail calls are variations on a theme, most consisting of a repetitive series of pulsed notes. The full repertoire is produced by altering frequency, bandwidth, number of harmonics, note length, call length, pulse rate, and amplitude. With the exception of the kek-burr, there is little change in structure within the same call.

King Rails use multiple call types in similar contexts, complicating the interpretation of call function. Stressed or agitated birds may make an assortment of sounds regardless of their intended usage. It has also been suggested that only male King Rails *kek* (Meanley 1969), though we have anecdotal evidence that females may also kek in a territorial context. Therefore, we urge caution in interpreting calls and call combinations without visual evidence of context.

The vocal repertoires of King and Clapper Rails overlap completely (Pieplow 2017), and contextual usage is expected to be equivalent in both species. Although Clapper Rail *keks* and *grunts* have higher average frequencies (Stiffler et al., in prep) and faster pulse rates (Pieplow 2017), differences are small enough to present a significant challenge in the field. Our hope is that this characterization and parameterization of the complete repertoire will provide a useful tool for surveyors, and facilitate standardization of terms for consistency in future communications among researchers and managers.

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CHAPTER 2: VOCALIZATIONS IN A NON-PASSERINE: FACTORS AFFECTING CALL STRUCTURE AND PLAYBACK REPSONSES IN KING RAILS

Abstract

The signals that animals use to communicate with conspecifics are vital for survival and reproduction. Often these signals appear simple. Yet, they can encode detailed information about the caller such as its identity, motivation or condition. To determine how information is encoded in simple, unlearned calls, I analyzed variability in the structure and context of the King Rail's *kek* mating and territorial call, and the *grunt* disturbance/contact call. While neither call was found to be individually distinctive, a playback experiment showed that King Rails were more likely to vocalize and did so sooner when presented with the call of a neighbor rather than a stranger. These results suggest that King Rails are capable of class-level, and possibly individual-level, recognition. Distinct characteristics of *grunt* calls, particularly in a roll call context, offers the possibility that passive call recording could be used to track King Rails through space and time for the purpose of censusing breeding populations, determining breeding densities, and potentially return rates.

Introduction

Social behavior, often involving complex interactions between multiple individuals, requires the ability to discriminate among of conspecifics (Sherman et al. 1997). Signaling systems have evolved to convey information about the signaler to others (Zahavi 1975) and to facilitate exchange of information during mating, territorial defense, parental care, and predatorprey interactions. Effective signaling, and the ability to learn unique features of others' signals, is therefore central to survival and reproduction.

Discrimination can occur at the species, class, or individual level. Organisms exhibit class-level recognition when they are able to categorize conspecifics into groups based on information such as dominance, kin or non-kin, quality, and familiar or non-familiar (Sherman et al. 1997). When discrimination is so specific that individual conspecifics can be differentiated, it is considered true individual recognition (Dale et al. 2001).

Recognition capability, even as specific as individual recognition, is widespread across taxa. Multiple sensory systems are also utilized for recognition. For example, scent signals discriminate individuals in Eurasian Otters, *Lutra lutra* (Kean et al. 2015). Blue-footed Boobies, *Sula nebouxii*, know the unique call of their mates (Dentressangle et al. 2012). Conspecifics can identify individually variable Electric Organ Discharges in the elephantfish species, *Pollimyrus adspersus* (Paintner and Kramer 2003). *Polistes* wasps recognize individual nest mates based on facial pattern (Injaian and Tibbetts 2014). These systems are all markedly different, yet recognition behavior shares a few key similarities.

According to Sherman et al. (1997), and revisited by Tibbetts et al. (2008), a species must meet three requirements in order to exhibit recognition. These requirements include (i) production of a signal or cue from a sender, (ii) matching of the signal to a template, or internal representation, by a receiver, and (iii) a unique behavioral response by the receiver. In other words, in individual recognition an individual sends a unique signal, which a receiver perceives and mentally matches to a representation of the sender, causing the receiver to act in a certain way that shows recognition of the sender. Thus, the signal, template, and response must all be individually specific (Tibbetts et al. 2008).

In some of the above examples of recognition, the discriminating signal is seemingly simple, yet still able to encode complex information. This raises the question of what other species might show recognition capabilities. The King Rail, *Rallus elegans*, provides an excellent example because they are seasonally territorial and socially monogamous. Thus, the need to maintain long-term relationships should promote the ability to recognize 'dear enemies' (Temeles 1994) and mates (Tibbetts and Dale 2007). King Rails live in densely vegetated, visually concealing habitats (Meanley 1969), largely limiting their communication to vocalizations. King Rails have relatively small, unlearned vocal repertoires consisting of six frequently used calls, most of which consist of variations of a series of repetitive, pulsed notes (see Chapter 1). The confamilial Corncrake, *Crex crex*, has similar, simple calls, that have been shown to be individually unique (Budka et al. 2015). This supports the possibility of the highest level of recognition in rails through calls, but subsequent unique behavioral responses in receivers were not evaluated in order to confirm that Corncrakes use those individually specific cues.

At what level are King Rails able to discriminate conspecifics based on their simple calls? What call parameters encode distinguishing information? Answering these questions, regardless of the answers, would allow for comparative analysis of the King Rail to other species and shed light on the common circumstances that favor evolution of various levels of recognition in communication systems. Furthermore, King Rails are declining across their global range, and are listed as endangered in 12 states in the United States (Cooper 2008). The ability for researchers and managers to learn about breeding populations of King Rails based solely on call recordings would be an effective and non-invasive method of obtaining census data.

Here, I evaluate the evidence for individual recognition in King Rails. Following the requirements outlined by Sherman et al. (1997), I tested the hypotheses that 1. King Rails should produce vocalizations that are individually variable. and 2. King Rails should have unique responses to calls of different individuals. This led to the following specific predictions: First, King Rail *kek* and *grunt* calls should be highly variable in one or many parameters. Second, discriminant function analysis should allow statistical separation of calls by individual King Rails based on specific call parameters. Last, King Rails should respond differently to calls of familiar and unfamiliar conspecifics when played from a location associated with the known individual. If King Rails exhibit all three of these qualities, then I can confirm that they are capable of true individual recognition. I additionally assessed factors affecting call structure and responses to calls to determine what information other than identity King Rails may encode in their vocalizations.

Methods

Refer to Chapter 1 for details regarding the study site and recording methods.

Sound analysis

Recordings were digitized using Raven Pro 1.4 (Cornell Lab of Ornithology 2011) software. The general spectrogram and power spectrum window settings were: Hann window, DFT = 1,024 samples, overlap = 50%, hop size = 512 samples, 3 dB bandwidth = 61.9 Hz, frequency resolution = 43.1 Hz. Due to different sampling rates, frequency resolution of 20 *grunts* edited from the audio component of video recordings was instead 46.9 Hz. Spectrograms were used for visualization and temporal measurements. Frequency parameters were measured from the power spectrum.

Sample sizes of *grunt* and *kek* calls are shown in Table 2.1. Recordings were chosen based on both quality and confidence of caller identity. For analyses testing identity signatures in call structure, I eliminated recordings taken at the same location on different days within the same year since I could not be certain if these were from the same or different individuals. This removed the possibility of incorrectly classifying calls from the same individual as independent. To select for high quality, recordings with low signal to noise ratios were rejected. I excluded entire calls where the beginning and ending were unclear on the spectrogram due to masking of notes by background noise including conspecific or heterospecific calls such as those from red-winged blackbirds (*Agelaius phoeniceus*) and green tree frogs (*Hyla cinerea*) or significant noise from wind, rain, airplanes, or car traffic. Individual call notes were also excluded if overlapped by background noise. Some notes appeared to include an echo or tail on the sonogram, which was excluded from the measurements.

Because King Rails sometimes produce *kek* notes continuously over the course of minutes or hours, a *kek* series, or one call, was defined as a series of at least 10 notes where the interval between notes was no greater than two times the mean gap between notes. Thus, a *kek* series was considered to be a separate call if it occurred after a pause of more than twice the average interval between notes. *Kek* note bandwidth was truncated to 1,500-7,500 Hz to avoid low frequency background noise. King Rail notes were rarely visible above this range. I measured 8 frequency and temporal parameters for individual *kek* notes in addition to the number of notes, pulse rate, and the intervals between the first three notes for each full call (Table 2.2).

Unlike *kek* calls, each *grunt* call has an obvious beginning and end. I measured the same 8 parameters as before for individual notes as well as call duration, number of notes, pulse rate, pulse rate of the first 10 notes, and pulse acceleration for each call (Table 2.2). Trills usually occurring at the end of *grunt* calls could not consistently be visualized due to their low frequency and amplitude. This contributed to variation in measurement of the full pulse rate, so the pulse rate of the first 10 notes was included as an additional parameter. *Grunt* notes were truncated to 500-9,000 Hz because these recordings generally included less low frequency noise than *kek* recordings and *grunt* notes reached higher frequencies.

Table 2.1 Origin of samples for both call types.

Call Type	Individuals	Mean Calls per Individual	Calls per Individual Range	Total Number of Calls
Grunt	37	1.6	1-11	61
Kek	46	4.5	1-15	208

Table 2.2 Descriptions of parameters measured for King Rail calls (first 8 parameter descriptions taken from Charif et al. 2010). A = *alarm*, B = *boom*, D = *distress squawk*, Gl = *growl*, Gt = *grunt*, K = *kek*, and Kb = *kek-brr* indicate which parameters were measured for each call.

Parameter	**Call(s)**	**Units**	**Definition**
Peak Frequency	A, B, D, Gl, Gt, K, Kb	Hz	The frequency at which Peak Power occurs within the note.
1st Quartile Frequency	Gt, K	Hz	The frequency that divides the note into two frequency intervals containing 25% and 75% of the energy in the note.
3rd Quartile Frequency	Gt, K	Hz	The frequency that divides the note into two frequency intervals containing 75% and 25% of the energy in the note.
Inter-quartile Range Bandwidth	Gt, K	Hz	The difference between the 1st and 3rd Quartile Frequencies.
Frequency 5%	Gt, K	Hz	The frequency that divides the note into two frequency intervals containing 5% and 95% of the energy in the note.
Frequency 95%	Gt, K	Hz	The frequency that divides the note into two frequency intervals containing 95% and 5% of the energy in the note.
Bandwidth 90%	Gt, K	Hz	The difference between the 5% and 95% frequencies.
Note Duration	A, B, D, Gl, Gt, K, Kb	s	The time from the beginning to the end of a note.
Call Duration	A, B, D, Gl, Gt, K, Kb	s	The time from the beginning of the first note to the beginning of the last note in a call.
Number of Notes	B, Gt, K		The total number of notes in a call.
Interval	K	s	The time from the end of the first note to the beginning of the second note in a sequence.
Pulse Rate	B, Gt, K	Notes/s	Total number of notes in a call minus 1, divided by the time from the beginning of the first note to the beginning of the last note.
Pulse Rate 10	Gt	Notes/s	Pulse rate of the first 10 notes. 10 divided by the total time from the beginning of first note to the beginning of the 11th note.
Acceleration	Gt	Notes/s^2	Increase in pulse rate over time.

Playback experiment

Using calls I recorded at the site, I conducted a playback experiment loosely following the design of Falls and Brooks (1975) to test whether King Rails can distinguish between a familiar and an unfamiliar conspecific call. Test males were observed on their nests in a series of trials where I either played the *grunt* call of a neighboring bird recorded 100-370 m from the test male's nest or that of a stranger from a distant territory 4-5 km from the test male's nest. To explore the effect of directionality, calls were played either from an area between the test bird and the neighbor's territory ("right" location), or from the opposite side of the test male's territory relative to the neighbor ("wrong" location). This resulted in four different treatment types: "neighbor right", "neighbor wrong", "stranger right," and "stranger wrong."

Each custom experimental playback recording consisted of a *grunt* followed by 5 minutes of silence and the same *grunt* again. In similar experiments with passerines, songs can be played continuously. However, given the ephemeral nature of the King Rail *grunt* call, the two *grunts* were included and temporally separated to ensure that the test subject heard the playback while still reasonably simulating the natural use of the call. *Grunt* recordings were acquired using the methods described in Chapter 1. They were band-pass filtered between 500 and 20,000 Hz and edited in Raven to remove all other conspecific and heterospecific calls. To avoid pseudoreplication (Kroodsma 1989), each *grunt* call was used with a single test male as either a neighbor or stranger, with the exception of one call that was used twice, once as a neighbor and once as a stranger for two separate test males. The order of treatment type was randomized for each test male to account for habituation.

Trials were conducted at least 24 hours apart during the test male's incubation period. Trials took place before dark, but after 17:00 h when the male was most likely to be attending the nest (Clauser and McRae 2016). Before each trial, a video camera (GoPro HERO5 and/or Sony Handycam) was placed at the nest to confirm adult presence and provide a visual and auditory record of responses to playback. Speakers were operated by a field assistant hidden 30 m from the nest. Trials began at least 10 min after the subject returned to the nest, and observations continued 10 min after playback or until the bird returned to his previous activity. If the subject did not return to the nest within 1 hour of camera placement, the trial was canceled for that evening. For each assay, I recorded whether the test male vocalized, its latency to vocalize, vocalization type, approach distance to the speakers, and time off the nest until the subject returned or resumed non-territorial activities (e.g. foraging). Peak frequency, pulse rate 10, and number of notes of both experimental *grunts* and response *grunts* by test males were also measured to determine if parameter variance of the stimulus had an effect on playback responses.

Statistical analysis

All analyses were performed using R statistical software (Version 3.2.3). Further detail on statistical analyses is included in the Results section.

Results

Variability of kek calls

Means of *kek* parameters are shown in Table 2.3. Spearman's Rank Correlation, using Holm's method to correct for multiple inferences, revealed some frequency parameters significantly correlated with temporal parameters. Peak frequency correlated positively with note duration ($r = 0.23$, $P = 0.04$). Also, 1st quartile frequency correlated negatively with pulse rate ($r = -0.28$, $P < 0.01$). Taken together, these correlations show a tradeoff between frequency and temporal factors, indicating that it might be difficult to produce high frequency notes that are short and rapidly pulsed.

Principal components analysis (PCA) was used to determine which combinations of parameters explained the most variation among *keks* within the sample. PCA using all parameters of *kek* calls yielded four PCs with eigenvalue >1 explaining a cumulative 85.4% of the variance (Table 2.4). The first two PCs loaded most heavily on frequency parameters, while temporal parameters were more important in PC3 and PC4. PC1 was positively correlated with frequencies at the top end of the bandwidth, while PC2 was positively correlated with frequencies at the bottom end of the bandwidth. Thus, taken together, bandwidth explains the majority of the variation among King Rail *kek* calls. PC3 was associated with shorter calls and PC4 was associated with shorter notes and slower-pulsed calls.

Plotting PC2 against PC1 did not clearly separate individuals for which I recorded multiple calls (Figure 2.1). Discriminant Function Analysis (DFA), used to determine how well the model can distinguish individuals while still accounting for highly correlated parameters, was able to correctly classify 34.2% of 197 calls from 38 individuals. When only including calls from the five King Rails with >10 calls, 66.7% of these 57 calls were correctly assigned to an individual.

I used linear mixed models accounting for individual ID to test for factors affecting *kek* parameter values other than identity. PC1 increased by 0.05 ± 0.02 ($P < 0.01$) with each day increase in Julian date (Figure 2.2). This suggests that *kek* calls increase in frequency as the breeding season proceeds.

Table 2.3 Mean (± 1 SE) parameter values for *kek*s based on 5,681 notes (above dotted line) and N = 208 calls (below dotted line).

Parameter (units)	Mean ± SE
Peak Frequency (Hz)	2633 ± 30
1st Quartile Frequency (Hz)	2387 ± 28
3rd Quartile Frequency (Hz)	2984 ± 39
Bandwidth 90% (Hz)	1874 ± 83
Frequency 5% (Hz)	1924 ± 29
Frequency 95% (Hz)	3798 ± 84
Interquartile Bandwidth (Hz)	597 ± 26
Note Duration (s)	0.042 ± 0.001
Call Duration (s)	11.944 ± 0.719
Number of Notes	31.7 ± 2.1
Pulse Rate (notes/s)	2.610 ± 0.080

Table 2.4 Principal component (PC) loadings and proportion of variance explained for all parameters of *kek* calls. Only PCs with eigenvalue >1 are shown. Highlighted values indicate loading > ±0.4.

	PC1	PC2	PC3	PC4
Proportion of Variance	0.382	0.210	0.169	0.092
Peak Frequency (Hz)	0.392	0.252	-0.132	-0.103
1st Quartile Frequency (Hz)	0.332	**0.428**	-0.176	-0.061
3rd Quartile Frequency (Hz)	**0.476**	-0.016	-0.003	0.003
Bandwidth 90% (Hz)	0.384	-0.348	0.138	0.037
Frequency 5% (Hz)	0.137	**0.515**	-0.210	0.005
Frequency 95% (Hz)	**0.442**	-0.177	0.068	0.040
Interquartile Bandwidth (Hz)	0.350	-0.380	0.143	0.055
Note Duration (s)	0.114	0.037	-0.174	**-0.624**
Call Duration (s)	0.001	-0.214	**-0.650**	0.300
Number of Notes	-0.038	-0.297	**-0.640**	-0.033
Pulse Rate (notes/s)	-0.108	-0.238	-0.037	**-0.707**

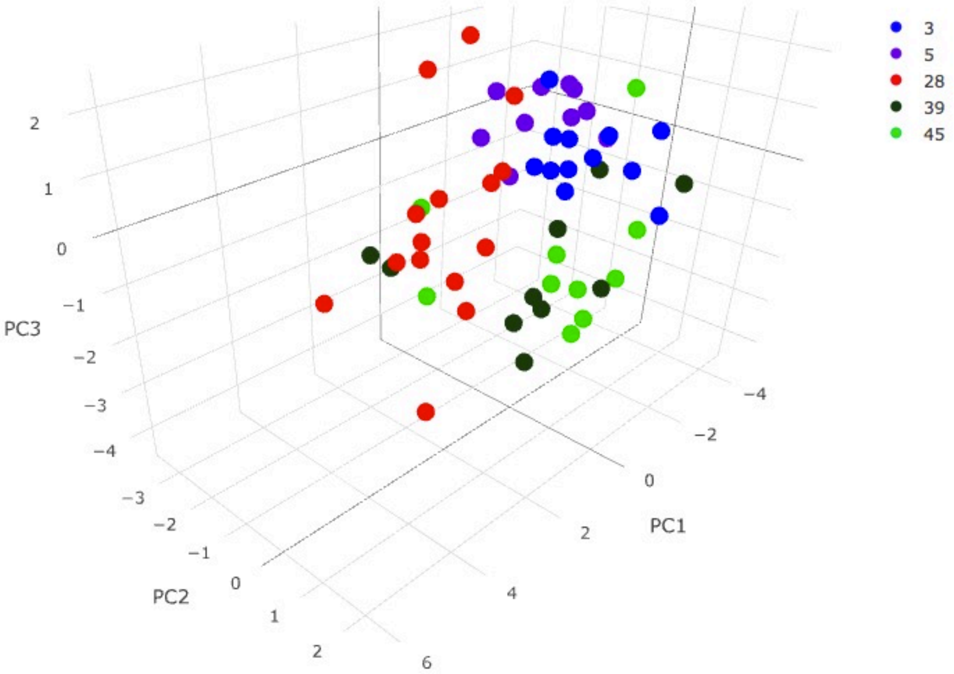

Figure 2.1 PCA scores from analysis using *kek* parameters for 38 individuals. The 5 individuals with ≥ 10 calls are depicted here, each represented with different colors. Points represent one call from an individual.

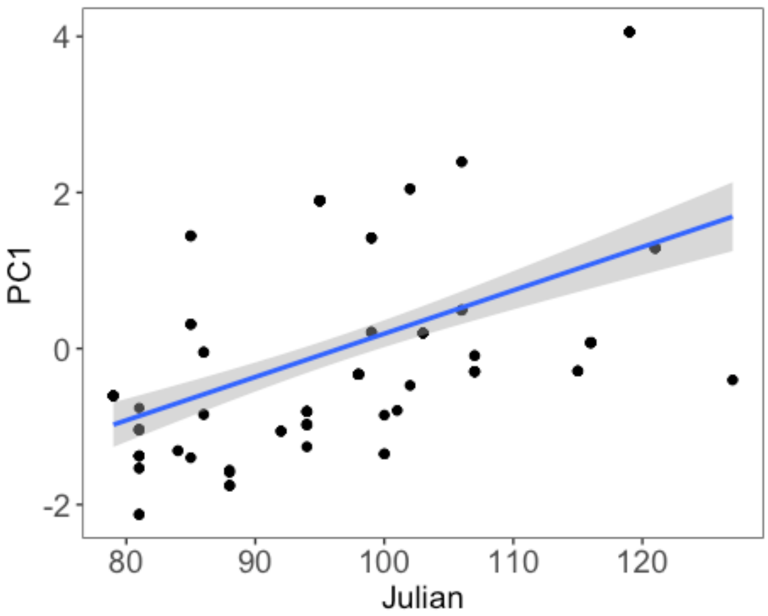

Figure 2.2 Linear mixed model predictions show a significant positive correlation between PC1 and Julian date (P < 0.01). Each point represents an individual *kek* call (N = 197 calls from 38 individuals).

Variability of grunt calls

Grunt calls showed a wide range of variability among individuals. Mean peak frequency ranged from 517 to 4223 Hz (Figure 2.3), pulse rate 10 from 2.7-4.0 notes/s (Figure 2.4), and the number of notes from 8-30 (Figure 2.5). The individuals with more than one call and of known sex are representative of the ranges of the full sample for all parameters. Mean parameter values are reported in Table 2.5.

I performed Principal Components Analyses (PCA) to determine which parameters described most of the variation among *grunt* calls. This resulted in five principal components (PCs) with eigenvalue >1, together explaining 88.7% of the total variance in the population (Table 2.6). The first PC, explaining about 30% of the variation, was associated with frequencies at the high end of the bandwidth. The second PC was associated with energy distributions of *grunt* notes beginning at higher frequencies.

Principal components analysis (PCA) on *grunt* parameters was not able to separate individuals (Figure 2.6). Using the first five PCs, Discriminant Function Analysis (DFA) was able to correctly classify only 37.5% of 32 calls to one of eight individuals for whom I had > 1 call. The majority of calls correctly classified were from the individual with the most calls, indicating that small sample size per individual may have caused this low classification percentage. Caller identity was thus not generally distinguishable based on the parameters measured here.

To test for variance in *grunt* calls based on context, I performed one-way ANOVAs using the first five PCs for the 48 *grunts* where the context was known: Callback survey, roll call, spontaneous while incubating, video camera at nest, or in response to playback with video camera at nest (see Figure 2.7 for descriptions). *Posthoc* Tukey tests on significant variables showed that *grunts* directed at a camera placed at the individual's nest had lower PC2 scores than *grunts* given during playback experiments (P = 0.03) (Figure 2.7). This suggests that birds that may have been more agitated or stressed called at higher frequencies than birds in more natural contexts.

Sex of the recorded bird could be deduced in only a small number of cases. Male King Rails are more likely to incubate in the evening (Clauser and McRae 2016), so adults observed on the nest after 17:00 h Eastern Standard Time were assumed to be male. One known male was captured and banded. Three *grunts* were recorded from two birds known to be female: one was captured and banded and the other was observed laying an egg on video. The three *grunt* calls from two females were shorter and faster accelerating than 30 *grunt* calls by 8 males. The female *grunts* also had higher peak frequencies, but energy in each note was spread over a wider bandwidth. However, student's t-tests, and Wilcoxon signed rank tests for non-parametric call parameters, showed that calls of the two sexes were not significantly different.

Based on the smaller body size of females relative to males, the trends for peak frequency and acceleration are what we would expect. In fact, comparing body size to peak frequencies of one banded male and one banded female did not eliminate the possibility that smaller birds have higher, faster calls. Using tarsus length as a proxy for size, the banded female was in the 60th percentile of 36 females banded at the site, and in the 19th percentile by *grunt* peak frequency of all 37 individuals sampled (lower of the two known females). The male was the smallest male out of 33 banded at the site, smaller than the banded female. He was in the 76th percentile overall by *grunt* peak frequency (2nd highest of the eight known males), in the 65th percentile overall by pulse rate 10 (4th highest of eight males), and in the 35th percentile overall for note duration (4th shortest of eight males).

One-way ANOVA revealed no statistical differences between *grunt* calls recorded by different methods except that Sony Handycam *grunts* had higher PC2 scores than calls recorded using the PCM ($P < 0.01$), indicating that they were higher and faster-pulsed but slower-accelerating calls. This was likely due to confound of context in these recordings. All *grunts* given in a ‘Nest Camera’ context were recorded with the Handycam, and as previously discussed, King Rail *grunts* were higher and faster in this potentially stressful situation.

Table 2.5 Mean parameter values for first notes of the full sample of *grunt* calls (population), the subsample of calls assigned to males, and the subsample of known females (± 1 SE).

Parameter (units)	Full Sample (N = 61 calls from 37 individuals)	Males (N = 30 calls from 8 individuals)	Females (N = 3 calls from 2 individuals)
Peak Frequency (Hz)	2491 ± 124	2475 ± 148	2858 ± 1074
1st Quartile Frequency (Hz)	2033 ± 86	2103 ± 104	2043 ± 482
3rd Quartile Frequency (Hz)	3274 ± 109	3313 ± 154	3415 ± 958
Bandwidth 90% (Hz)	3063 ± 133	2851 ± 156	3348 ± 1118
Frequency 5% (Hz)	1325 ± 78	1455 ± 99	1444 ± 352
Frequency 95% (Hz)	4389 ± 131	4306 ± 149	4792 ± 1462
Interquartile Bandwidth (Hz)	1241 ± 92	1210 ± 139	1372 ± 485
Note Duration (s)	0.114 ± 0.003	0.113 ± 0.004	0.105 ± 0.019
Call Duration (s)	4.523 ± 0.168	4.519 ± 0.266	3.265 ± 0.683
Number of Notes	17.6 ± 0.6	17.3 ± 1.0	13.0 ± 2.9
Pulse Rate (notes/s)	3.682 ± 0.071	3.616 ± 0.060	3.623 ± 0.127
Pulse Rate 10 (notes/s)	3.385 ± 0.042	3.469 ± 0.056	3.284 ± 0.198
Acceleration (notes/s^2)	0.303 ± 0.004	0.295 ± 0.005	0.316 ± 0.023

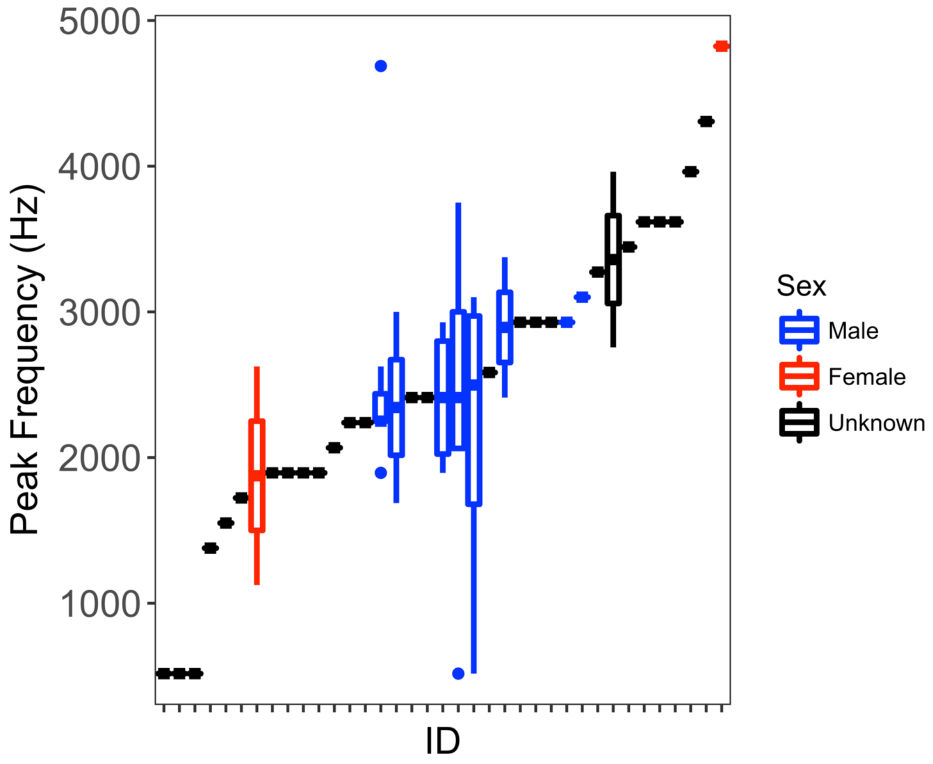

Figure 2.3 Peak frequency of first notes of *grunts* from 37 individuals reordered by median to show range. Boxplots illustrate minimum, first quartile, median, third quartile, maximum, and outliers (values > 1.5 times the inter-quartile range) for the eight individuals with more than one call. Ten individuals where sex is known are color-coded.

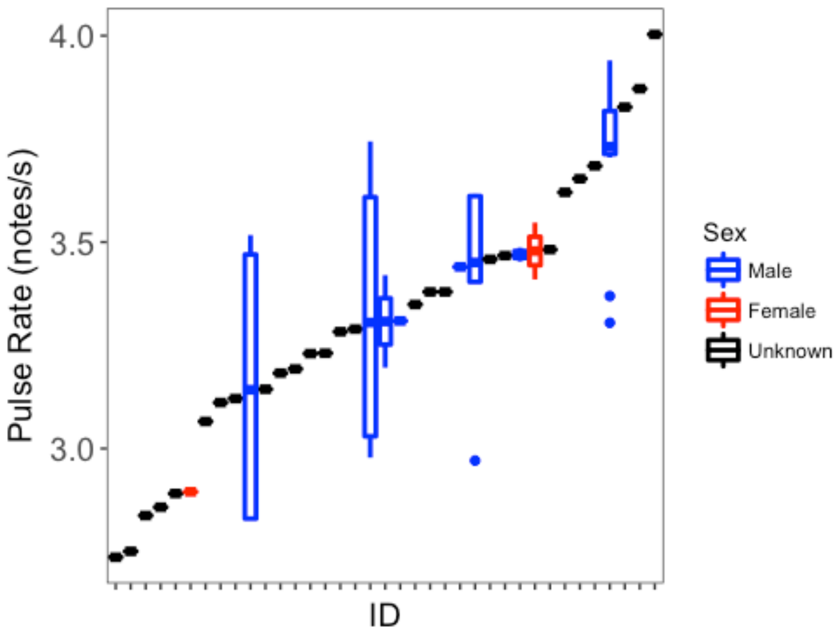

Figure 2.4 Pulse rate 10 of *grunts* from 37 individuals reordered by median to show the range. Boxplots illustrate minimum, first quartile, median, third quartile, maximum, and outliers (values > 1.5 times the inter-quartile range) for the eight individuals with more than one call. Ten individuals where sex is known are color-coded.

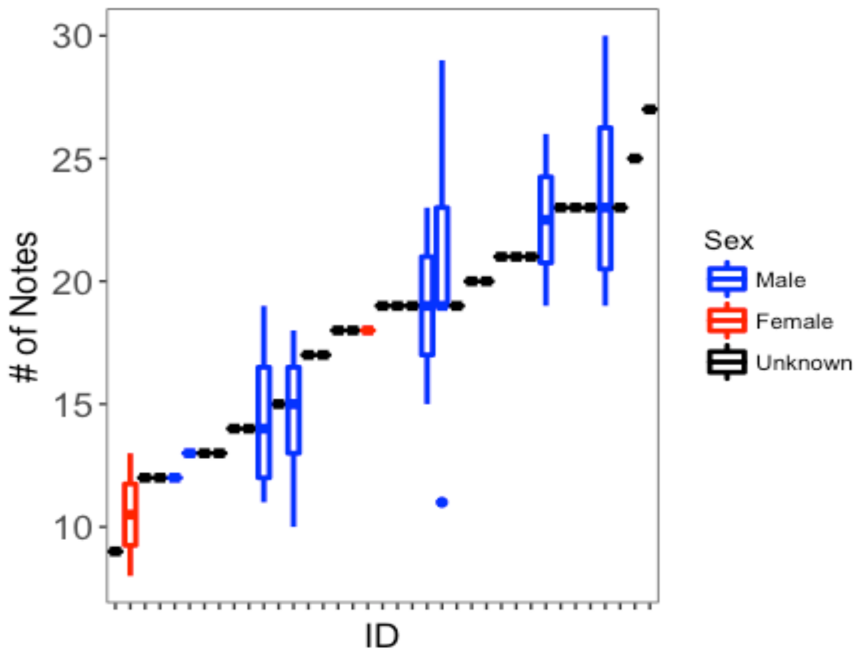

Figure 2.5 Number of notes in *grunts* from 37 individuals reordered by median to show range. Boxplots illustrate minimum, first quartile, median, third quartile, maximum, and outliers (values > 1.5 times the inter-quartile range) for the eight individuals with more than one call. Ten individuals where sex is known are color-coded.

Table 2.6 Principal component (PC) loadings and proportion of variance explained for all parameters of *grunt* calls. Only PCs with eigenvalue >1 are shown. Bolded values indicate loading > ± 0.4.

	PC1	PC2	PC3	PC4	PC5
Proportion of Variance	0.299	0.204	0.158	0.142	0.084
Peak Frequency (Hz)	0.350	0.290	-0.164	-0.009	-0.057
1st Quartile Frequency (Hz)	0.314	**0.436**	-0.164	-0.049	0.015
3rd Quartile Frequency (Hz)	**0.429**	0.105	0.185	0.185	-0.161
Bandwidth 90% (Hz)	0.314	-0.363	0.228	0.131	0.030
Frequency 5% (Hz)	0.206	**0.507**	-0.051	-0.026	-0.123
Frequency 95% (Hz)	**0.440**	-0.064	0.200	0.116	-0.042
Interquartile Bandwidth (Hz)	0.216	-0.285	0.374	0.267	-0.206
Note Duration (s)	0.284	-0.111	-0.217	-0.011	0.464
Call Duration (s)	-0.129	0.011	-0.334	**0.553**	-0.329
Number of Notes	-0.124	0.055	-0.234	**0.663**	0.055
Pulse Rate (notes/s)	-0.019	0.140	0.156	0.311	**0.762**
Pulse Rate 10 (notes/s)	-0.219	0.300	**0.494**	0.105	0.008
Acceleration (notes/s^2)	0.225	-0.339	**-0.443**	-0.088	0.054

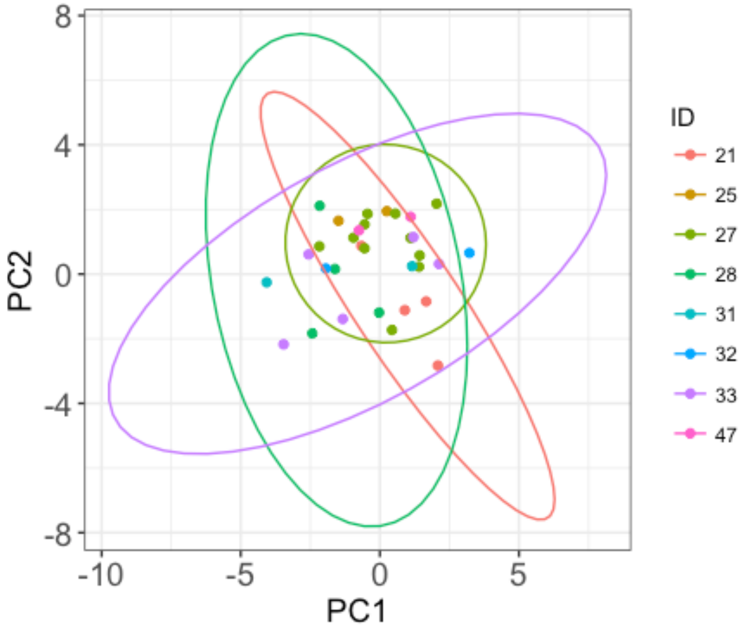

Figure 2.6 PCA scores from analysis of *grunts* for 8 individuals with more than one call. Points represent one call from an individual. Ellipses represent 95% confidence intervals, which could not be calculated for 4 individuals due to small sample size.

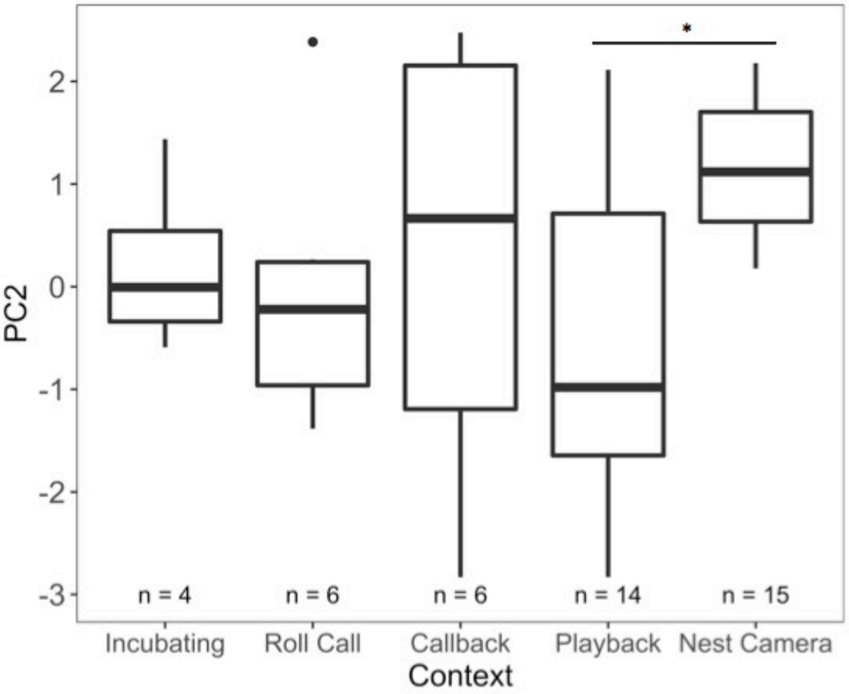

Figure 2.7 Average PC2 score for *grunt* calls recorded in various contexts. Boxplot hinges illustrate first quartile, median, third quartile; whiskers are minimum and maximum values < 1.5 times the inter-quartile range; points are outliers. The ‘incubation’ context includes spontaneous *grunts* uttered while sitting on a nest, representing the least stressful context. ‘Roll Call’ *grunts* were given as part of a chain of calls among neighbors, and is thus an induced call, but may begin as a spontaneous call similar to those in the ‘incubation’ context. ‘Callback’ *grunts* were elicited by *kek/grunt/kek-burr* playback during standardized callback surveys, thus simulating a nearby King Rail. The playback experiment, as described above, simulates a territory intrusion by another King Rail using the *grunt* call. ‘Playback’ *grunts* are responses to this intrusion. ‘Nest Camera’ *grunts* were directed at a video camera within 20 min of placement at the bird’s nest, simulating a novel object and the most stressful context of the five categories. Contexts are ordered left to right in the order of least to most stressful. *Grunts* directed at a recently placed nest camera had significantly higher PC2 scores than playback experiment *grunts* (P = 0.03), indicating higher low-end frequencies. No other significant differences were observed.

Response variability and playback experiment

I analyzed responses to the playback experiment using generalized mixed models based on AD Model Builder in R with a negative binomial distribution for each continuous response variable and a logistical regression for the categorical response variable of vocal response (yes or no). I assumed that the data followed these distributions, but did not have enough resolution with my small sample size to properly assess this assumption. Subject identity and number of days after onset of incubation were used as random effects. Trial number was initially added as a random effect to account for habituation to playback. However, variance of the factor was consistently estimated as zero, indicating singularity, so trial number was consequently removed from the model.

Seven males were sampled to test their responses to *grunt* calls of neighbors and unfamiliar conspecifics in different locations. Each received the 'neighbor right,' 'neighbor wrong,' and 'stranger right' treatment once. Two of the seven subjects were additionally treated once with the 'stranger wrong' treatment. Treatments are described above.

Test males were almost 2 times more likely to vocalize when the playback was of a neighbor's call regardless of location (Figure 2.8). Movement towards the speakers and time spent away from the nest were not different when the playback was a neighbor or a stranger. However, given that the test bird responded vocally to playback, test males responded to neighbors on average 3:20 min:sec sooner after the first playback call than they did to strangers ($P < 0.05$) (Figure 2.9). The peak frequency, pulse rate 10, and number of notes in *grunt* responses from test males did not differ significantly based on treatment. There were no significant effects of location on responses by the test male, and I did not test for any interactions between call type and location due to lack of power to find such effects.

Test males also responded more strongly with respect to certain parameters of the playback *grunt*. They moved closer to the speakers when the playback *grunt* had a higher peak frequency ($P < 0.001$, Figure 2.10A) and faster pulse rate 10 ($P < 0.01$, Figure 2.10B). In addition, linear mixed models, accounting for subject identity and number of days after onset of incubation, showed that *grunt* stimuli that elicited a vocal response from test males had an average pulse rate 10 that was 0.3 notes/sec slower ($P = 0.01$, Figure 2.11A) and mean peak frequencies 387 Hz higher ($P < 0.01$, Figure 2.11B) than *grunts* that did not elicit a vocal response.

Paired student's t-tests, and Wilcoxon signed rank tests for non-parametric call parameters, showed no significant parameter differences between neighbor and stranger *grunts* selected for playback to the same test male. On the other hand, non-paired tests showed that *grunts* used for playback had on average significantly faster pulse rates ($W = 174$, $P = 0.03$), but not pulse rate 10 ($W = 357$, $P = 0.26$), and significantly longer note length ($W = 151$, $P < 0.01$) than *grunts* from the rest of the population. This occurred in part due to constraint on acquiring neighbor *grunt* recordings within a maximum radius of the test male's nest. However, experimental calls were also chosen for quality, therefore these recordings included less noise, thus allowing for measurement of the end trills and more accurate visualization of note/call beginnings and endings. This explains discrepancies in both pulse rate and note length.

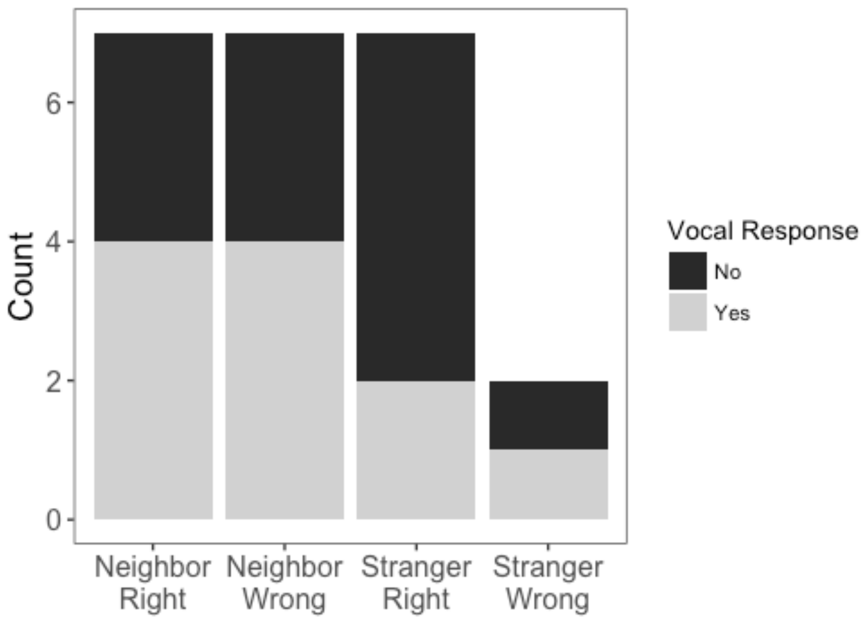

Figure 2.8 Number of responses including and not including vocalization in response to experimental *grunt* playbacks in each treatment. Seven test subjects received each of the first three treatments, and two of those seven subjects received all four treatments.

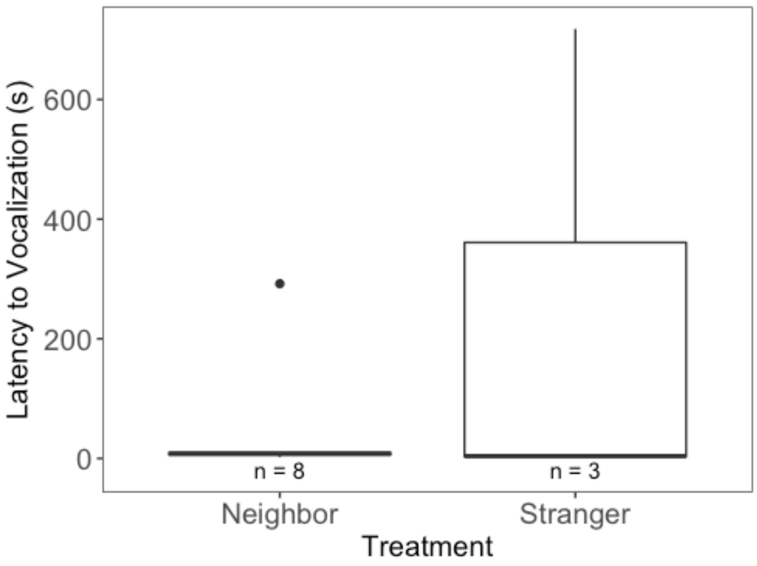

Figure 2.9 Test male latency to vocalization in response to playback of a neighbor's or stranger's *grunt*. Boxplot hinges illustrate first quartile, median, third quartile; whiskers are minimum and maximum values < 1.5 times the inter-quartile range; points are outliers. Latency was measured as seconds after the first *grunt* on the recording. Test males responded sooner to neighbors than strangers ($P < 0.05$).

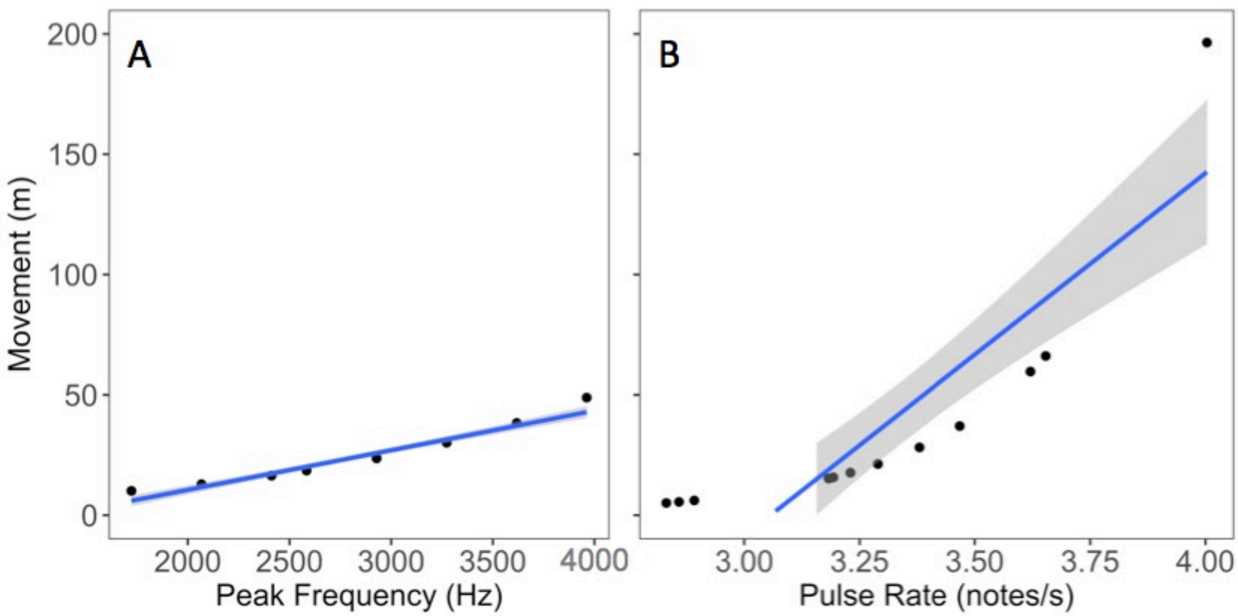

Figure 2.10 Model predictions of test male movement as a function of playback *grunt* peak frequency (A) and pulse rate 10 (B). Test males moved closer to the speakers when the playback *grunt* had a higher peak frequency ($P < 0.001$) and a faster pulse rate 10 ($P < 0.01$).

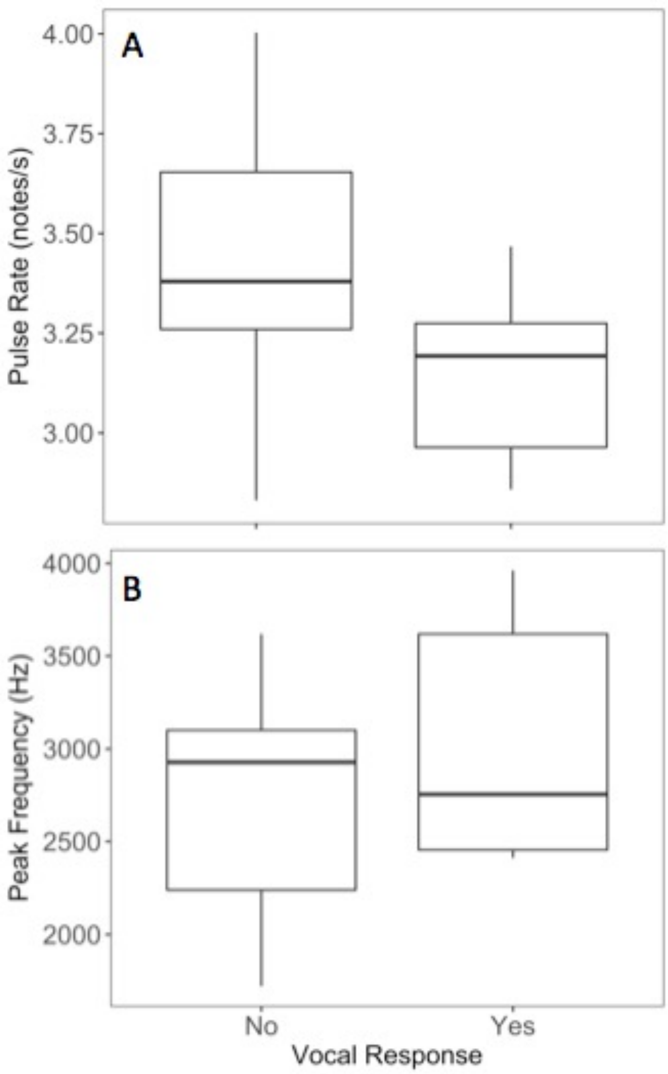

Figure 2.11 Pulse rates (A) and peak frequencies (B) of *grunts* that did (N = 11) and did not (N = 12) elicit vocal responses from test males. Boxplot hinges illustrate first quartile, median, third quartile; whiskers are minimum and maximum values < 1.5 times the inter-quartile range; points are outliers. *Grunt* stimuli that elicited a vocal response from test males had an average pulse rate 0.3 notes/s slower (P = 0.01) and mean peak frequencies 387 Hz higher (P < 0.01) than *grunts* that did not elicit a vocal response (P = 0.01).

Discussion

Individually variable calls in the King Rail

King Rail *kek* and *grunt* calls showed a high degree of variation in multiple parameters, yet appeared to have more variation among than within individuals. DFA was only able to classify 34% of calls to the correct individual based on the PCs calculated from those parameters. Based on these results, I am compelled to reject the hypothesis that King Rail calls are individually variable, at least given the parameters measured in this study.

Kek calls are given primarily for male mating advertisement (Meanley 1969). Therefore, this call may signal genetic quality or condition to potential mates, rather than identity. Vocal consistency has been shown to correlate with reproductive success in several species of oscines (reviewed in Sakata and Vehrencamp 2012). Some King Rails' calls clustered together tightly on the PCA plot, while others were highly dispersed (Figure 2.1). This difference in degree of variation within individuals is consistent with the hypothesis that *kek* calls function as a signal of mate quality, since high quality males may achieve greater consistency. Given that *keks* are also used in a territorial context (see Chapter 1), it is still possible that this call might carry an individual signature. When I considered data from only King Rails for which I recorded >10 *kek* calls (N=5), DFA was able to correctly assign 66.7% of calls. This reanalysis suggests that sample size per individual has a significant effect on classification ability, indicating either that classification could be improved with more data, or that correct classifications arose only through random chance.

I predicted that *grunts*, given their use in the roll call and as a contact call between mates, would be more likely to encode identity. Sample to parameter ratio for *grunt* PCA was only 5:1. Sample limitations aside, weak classification ability may also be an artifact of my decision to compare just the first notes of *grunt* calls. I did this because both frequency and temporal parameters change from the beginning to the end of the call, but this first note may not always be representative of the individual's voice. This could perhaps be the case, for example, if they need to warm up following a long period of silence. Additionally, it is possible that the individually unique parameters of male and female *grunts* are different and should be tested separately. This is the case, for example, in Blue-footed Boobies, *Sula nebouxii*, where the female identity is encoded by frequency and male identity by temporal cues (Dentressangle et al. 2012). Therefore, the prospect that King Rails have individually unique elements to their most common calls cannot be fully dismissed. Nevertheless, King Rails had higher calls in the 'Nest Camera' context versus the 'Playback' context, indicating that their calls likely do encode information about the caller's motivation or situation.

Individually specific responses in King Rails

Test males were more likely to vocalize, and did so significantly sooner, in response to playback of *grunts* from familiar versus unfamiliar King Rails. These findings provide some evidence in support of the hypothesis that King Rails recognize familiar individuals and respond in a consistent manner toward them. Latency to first song in White-throated sparrows, *Zonotrichia albicollis*, was also different between neighbors and strangers, although they responded sooner to strangers (Falls and Brooks 1975). In fact, Falls' and Brooks' results align more closely with the expected results based on the dear enemy hypothesis, which states that territory holders can reduce energy expenditure through focus on defense against potential usurpers, such as non-territorial strangers, as opposed to their territory-holding neighbors (Temeles 1994). Others studies in birds also report stronger responses to strangers than neighbors

(Wiley and Wiley 1977; Stoddard et al. 1991; Moser-Purdy and Mennill 2016; Stermin et al. 2017).

There are a couple of possible explanations for discrepancies in response between these previous studies and my observations in King Rails. The first is that new stimuli may cause a temporary suspension of normal behaviors. In zebra finches, for example, the “response latency” is much longer after hearing a novel song compared to a song they heard the day before (Stripling et al. 2003).

The second explanation arises because King Rails, and other rails, exhibit roll call behavior (see Chapter 1) where one bird *grunts* and several others in the area respond. Thus subjects in my experiment may have responded to a neighbor’s roll call from the vicinity of his territory more readily. Neighbors are already likely to know the location and stage of the territory holder’s nest. By contrast, responding vocally to a stranger would mean giving away your position.

An alternative explanation is that neighbors may be perceived as a greater threat than strangers because of their more intimate experience of the territory and nest. Higher aggression to neighbors relative to strangers has been shown, for example, in Northern Harriers, *Circus cyaneus* (Temeles 1990), Red-winged Blackbirds, *Agelaius phoeniceus* (Olendorf et al. 2004), and Meadow Voles, *Microtus pennsylvanicus* (Ferkin 1988). An estimated 25% of nests in this King Rail population had offspring sired by extra-pair males, and one extra-pair male that was identified was a territory neighbor (Brackett 2013). Neighboring males may pose the greatest threat of cuckoldry. Test males may have had recent experience with neighbors, and been focused on guarding their mates from them during this experiment. Similarly, Leiser (2003) found evidence that the presence of a female increases aggression between neighboring male pupfish. This could explain why King Rails responded more strongly to neighbors.

What remains to be explained, is how test males distinguished between neighbors and strangers, whether through class-level or individual recognition. It can be difficult to distinguish between the two. The simplest explanation is that *grunt* recognition, specifically during roll calls, may rely more on where the signal is coming from rather than the signal itself. This idea can be ruled out because in this study, playback did not occur from neighbor territories. Alternatively, King Rails may simply remember that they heard a particular *grunt* before, but not associate it with a specific individual, classifying calls only as familiar and unfamiliar. Otherwise, King Rails recognize the calls of individual conspecifics. To distinguish between these last two possibilities, Falls and Brooks (1975) suggest that simple discrimination between familiar or unfamiliar individuals only indicates class-level recognition, and that differences in location are an additional factor needed to show individual recognition. There must be a stronger response to a stranger than the neighbor at the test male's territory boundary with the neighbor, but equal strength of response to the neighbor and stranger from the opposite side of the test male's territory. This would show both that the test male recognizes the neighbor's call and can associate it with an expected location. Their study, as well as others with similar experimental designs (e.g. Wiley and Wiley 1977; Stoddard et al. 1991), placed speakers at or just inside territory boundaries in addition to the territory center. In my study, playback location may not have caused a significant change in response to neighbors because we were not able to determine King Rail territory boundaries, and therefore, all trials simulated intrusion within a territory in close proximity to the test male's nest. The direction of the speakers from the nest may not matter to the test male in such a situation.

It is unclear at what level King Rails recognize conspecifics. There is likely some information about the caller encoded in the *grunt*. Although there was insufficient evidence from discriminant function analysis to support individuality of *grunt* calls, King Rails responded differently to neighbors and strangers. To remember a specific *grunt*, it must contain elements that are consistent and individually unique. Individually specific cues may be present, but not used to distinguish conspecifics at that level. King Rails moved closer to the speakers in response to higher and faster calls, and were more likely to vocalize after higher and slower calls. These differential responses to varying parameters also suggest that class-related information such as size or aggression might be encoded in the *grunt*. Regardless of the subject's motivation in this experiment, the ability to discern which calls were from neighbors suggests that King Rails remember unique elements of the *grunts* of their neighbors and can classify conspecifics at either the class or individual level.

Contextual variation in grunt structure

The structure of King Rail *grunts* was only significantly different when comparing the two most stressful contexts represented in this study (Figure 2.7): during a simulated territory intrusion by another rail in close proximity to a nest ('Playback') and after a video camera (novel object) was placed within 1 m of a nest ('Nest Camera'). The latter is potentially the situation with the most direct and immediate threat, and elicited *grunts* with higher low-end frequencies. Many parameters of Black-capped Chickadee alarm calls, including bandwidth and interval length between notes, also change in relation threat level (Templeton et al. 2005). Still, in this study, the fact that only the two most stressful situations were different in pairwise comparisons implies that anxiety may not have a clear directional affect on *grunt* parameters. Rather, stress may alter calls in a more random fashion, causing them to deviate in both directions from the natural state. Alternatively, intended receiver, e.g. a response to an intruder (“Playback”) versus a warning to mate of a novel object (“Nest Camera”), may have caused the difference.

Temporal variation in kek structure

One interesting finding of this study was an increase in the frequency of *kek* calls over the course of the breeding season. Possible explanations for this phenomenon include physiological factors such as fatigue, stress, improvement from practice, or environmental factors such as temperature and humidity. Previous studies have shown similar temporal changes in call structure in various songbirds. For example, note consistency and rate increased over the course of the breeding season in Banded Wrens, *Thryophilus pleurostictus*, either due to additional singing practice or increased aggression (Vehrencamp et al. 2013). Song motor performance, as measured by frequency excursion, of Adelaide’s Warblers, *Setophaga adelaidae*, increased with the cumulative number of songs and not the time of day, indicating that this change was related to a ‘warming up’ effect rather than temperature, time since waking, or social interactions (Schraft et al. 2017). Higher frequencies and longer calls are likely to be physically demanding for birds, and it might take time to reach maximum performance of these parameters. Although this was not seen over shorter time scales in the King Rail, this could explain the increase in *kek* frequency throughout the season. On the other hand, birds experience higher stress as the breeding season progresses, often causing negative effects such as decrease in mass and immunocompetence (e.g. Hanssen et al. 2003). For example, Sedge Warblers, *Acrocephalus schoenobaenus,* with parasites spent less time conducting song flight displays and had smaller song repertoires than individuals without parasites (Buchanan et al. 1999). Such physiological changes may alter *kek* structure in King Rails over time.

It is unlikely that environmental factors play a significant role in changes of King Rail calls over time. Recordings were only taken from birds within 60 m, which is not far enough for attenuation to cause this significant change. Additionally, higher temperature and lower humidity each result in an increase in sound absorption and attenuation, and higher frequencies attenuate faster than lower frequencies (Wiley and Richards 1982). Therefore, neither behavioral changes by the calling King Rail to increase sound transmission, nor alterations in signal perception by the receiver resulting from these climate changes would likely cause higher call frequencies at the end of the day and season when temperatures tend to be highest.

Implications for spatial and behavioral monitoring of King Rails

Acoustic monitoring is an important tool in research and wildlife management. It is a non-invasive means of observation, especially useful for species that live in dense habitats or are nocturnal. Simple signals, such as the pulsed calls of the Corncrake that can be classified to the correct individual >98% of the time based on pulse-to-pulse duration (Budka et al. 2015), can be used for discrimination. Given their conservation status and generally secretive nature, passive recording of calls could prove important for the future of King Rail conservation.

In order to track individuals through space and time, the species must be loudly vocal to facilitate easy recording, have individually distinct vocalizations, and the individually distinct features must persist over time (Mennill 2011). King Rails are vocal throughout the breeding season. In this study, I have also shown that *grunt* calls could convey unique information about the caller based on differential responses to neighbors and strangers in my playback experiment.

Further research is required to pinpoint individually-specific parameters and confirm that they are stable over time. Nevertheless, King Rail *grunts* may be a useful call type to use for passive acoustic monitoring, especially given the roll call behavior where many individuals in an area call in short succession. This method can provide information on the timing and density of territorial occupancy, site fidelity, population dynamics, and habitat selection of King Rails. The next chapter will explore this application further.

Conclusion

Although I did not find evidence that King Rail *kek* and *grunt* calls vary among individuals, King Rails are able to distinguish between *grunts* of neighbors and strangers. These findings suggest that *grunt* calls have individually specific characteristics that King Rails use for at least class-level, if not individual-level, recognition. Thus, the simple, unlearned calls of these species are still able to convey complex information.

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CHAPTER 3: USE OF AUTONOMOUS RECORDING UNITS AND CALLBACK SURVEYS TO MONITOR A THREATENED, SECRETIVE MARSH BIRD, THE KING RAIL (*Rallus elegans*)

Abstract

Conducting auditory surveys of the King Rail, a rare and secretive species, is complicated by low detection rates. This study aimed to improve detections using two auditory methods. I report the relationship between call rates detected by autonomous recording units (ARUs) and density of breeding King Rails as determined based on callback surveys. I also assessed the effect of weather and temporal factors on callback survey detections and used ARUs to determine temporal variation in calling rates. Wind, temperature, cloud cover, number of observers, time of day, and time of season did not have significant effects on the number of callback survey detections. However, ARU data revealed a seasonal decline in King Rail call rates as well as a crepuscular diel pattern. Thus, breeders may respond to callback over a broader period of seasonal daylight hours, but when using a passive acoustic monitoring method, there is a significantly higher probability of detections early in the season and around dawn and dusk. *Grunt* call rate remained constant throughout the breeding season and is associated with a ‘roll call’ behavior, in which an individual gives a spontaneous *grunt* call and others in the vicinity respond with the same call in quick succession. *Grunts*, but not *keks*, were significantly positively correlated with breeder density and are a valuable tool for abundance estimation. My findings regarding the range and sensitivity of passive detection and field callback surveys will help inform decisions about best practices for auditory surveys to improve detection efficiency and achieve more effective monitoring of this imperiled species.

Introduction

Adaptive management strategies and conservation plans for threatened species rely foremost on the availability and quality of occupancy and abundance estimates. Rare species that are additionally elusive or secretive present a formidable challenge for surveyors and managers. Modern wildlife monitoring programs are being assisted by research into improved survey methods (Warton et al. 2017) and technological innovation such as GPS tracking and camera traps (e.g. Marvin et al. 2016).

Marsh birds are difficult to monitor due to their habit of remaining hidden in dense reeds. For this reason, Conway (2011) established a standardized callback survey protocol to utilize their loud vocalizations for censusing. Conway's protocol relies on multiple visits to each site at specified times of day, within specific date intervals, requiring many man-hours in the field. As an alternative, many researchers have begun using autonomous recording units (ARUs) to study marsh birds (Duke and Ripper 2013; Butler et al. 2015; Gibson 2017; Stiffler et al. 2018). ARUs provide a minimally invasive method of collecting large amounts of data, but these data must either be reviewed manually, still requiring many man-hours, or by computer-based signal recognizers that perform automated analysis but do not always have high accuracy (Lemen et al. 2015; Knight et al. 2017).

In this study, I present results from both callback surveys and ARU deployments for detecting a declining marsh bird, the King Rail, *Rallus elegans* (Cooper 2008). King Rails are rare and elusive. They are listed as 'Near Threatened' (BirdLife International 2015), and low detections have been reported for King Rails using the standardized protocol across studies (Pickens and King 2012; Harms and Dinsmore 2014). They are also difficult to distinguish vocally from their sister species, the Clapper Rail, *Rallus crepitans*, which can live sympatrically with King Rails in brackish marshes, and hybridization is known to occur (Maley and Brumfield 2013). This compels many auditory surveyors to lump the two species (Nadeau et al. 2008; Conway 2011; Stiffler et al. 2017). There is little information regarding the best conditions and times of day and season for surveys to increase detection probability of King Rails. Likewise, for researchers and managers wishing to use ARUs, availability of call recognizers and vocalization rates for this species would greatly enhance efficiency and inferences.

I used a well-studied King Rail population at Mackay Island National Wildlife Refuge in coastal North Carolina near the Virginia border to test the effectiveness of survey methods. This relatively dense breeding population of King Rails has been extensively monitored over the last nine years, and represents the only long-billed rails breeding at the site (Brackett et al. 2013; Rogers et al. 2013; Clauser and McRae 2016; Kolts and McRae 2016; Clauser and McRae 2017). My goals were 1. to assess the influence of temporal and environmental factors on the number of King Rail detections during callback surveys, 2. to use ARUs to assess temporal variation in King Rail call rate, 3. to determine which call type(s) best represent density, and 4. to report a model relating King Rail call rate to density to be used for abundance estimates at other sites.

Methods

Study site and survey locations

The study was conducted at Mackay Island National Wildlife Refuge (36° 31′ N, 75° 58′ W) on the Atlantic flyway. The refuge includes over 2000 ha of freshwater to moderately brackish marsh with a high probability of King Rail occurrence (Rogers et al. 2013). Breeding King Rails have been studied intensively there over the past seven years (Brackett et al. 2013; Clauser and McRae 2016; Kolts and McRae 2016; Clauser and McRae 2017). Ten locations throughout the refuge were chosen as survey sites based on auditory and/or visual confirmation of King Rail presence in March 2016. Locations were at least 370 m apart, and all were in proximity to access roads.

Auditory King Rail surveys

Two Song Meter SM4 units, hereafter Autonomous Recording Units (ARUs), from Wildlife Acoustics were rotated independently among the 10 survey locations during two breeding seasons. Each location was sampled four times in 2016 (April 16-July 4) and five times in 2015 (March 31-July 1), with two to three weeks between visits within a season. Extended periods of rain and high winds were avoided whenever possible. ARUs were mounted approximately 5 ft above the ground on a tree or pole. Recordings were taken at 44.1 kHz with 24 dB preamplifier, 12 dB gain, and 16-bit files split into 5 min blocks.

Initially in 2016, ARUs were deployed to record continuously for 72-hour periods. In order to reduce the amount of data produced, I used early-season recordings to compare the number of King Rail detections using recording schedules of 10 min on/10 min off, 20 min on/20 min off, and 30 min on/30 min. None was statistically superior, so subsequent deployments were scheduled to record continuously from 1 hr before and 3 hrs after sunrise, continuously from 2 hrs before and 1 hr after sunset, and 10 min on/10 min off during all other times. Deployments were reduced to 48 hrs but used the same daily schedule during the 2017 season.

At the end of each ARU deployment, a callback survey including only King Rail calls was performed based on the Standardized Marsh Bird Monitoring Protocol (Conway 2011). ARUs were retrieved and callback surveys occurred within the 3 hr after sunset or between 2 hr before and 1 hr after sunset. Morning and evening surveys were performed at all locations and were equally spaced among the early and late parts of the season. Callback surveys included 5 min of passive listening followed by 1 min of King Rail playback consisting of a *grunt duet*, *keks*, and a *kek-burr* for 10 s each separated by 15 s of silence. King Rails heard outside of the 6-minute survey period were not included as callback detections in survey statistical analysis, but were used for density estimation at that location (see below). In 2017, two additional callback surveys, one the in the morning and one in the evening, were conducted at each location late in the season, and did not follow deployment of an ARU. All auditory responses and sightings were recorded, including call type and estimated distance and direction of the rail from the surveyor.

Analysis of field recordings

I used Kaleidoscope Pro 4.5.5 Beta2 from Wildlife Acoustics to analyze ARU sound files. This software uses computer learning to create a recognizer based on negative and positive training examples given by the user to find calls of interest in large batches of sound files. I created a separate cluster template for *grunts, keks,* and a negative training example of all sounds other than King Rails. ARU recordings were run through the recognizer, and additionally filtered by selecting for vocalizations 0.1-6s in length, between 1500-7000 Hz, and with a 0.3 s maximum inter-syllable gap.

Due to low detection accuracy, I used Kaleidoscope to narrow down the number of vocalizations requiring manual review. A researcher screened 162,793 vocalizations flagged as either a King Rail *kek* or *grunt*, and marked them as *grunt*, *kek*, other King Rail call, or not a King Rail. The average length of true King Rail vocalizations found by Kaleidoscope was 1.7 s, thus some vocalizations represented segments from the same call. *Grunt* segments within 10 s were visually reviewed and removed for analysis if determined to be part of the same call. *Keks* within 11.9 s, the average actual length of *kek* calls, were automatically removed because the beginning and ending of this call are more arbitrary than in *grunt* calls (Chapter 1). The results below inherently underrepresent the number of King Rail vocalizations in my ARU recordings because a vocalization does not appear in the results file for review if it does not closely match the learned King Rail call template.

King Rail density estimation

King Rail density was estimated for comparison to detections during auditory surveys. A variety of information was used, including detections during callback surveys, observation of King Rails outside the survey periods, and nest density. King Rails move around during the course of the breeding season, especially early in the breeding season (Kolts and McRae 2016). Density was therefore estimated separately for each survey period at each survey location.

I used my callback survey observations to estimate breeder densities within a 100 m radius of the ARU location. The callback phase of the protocol was recorded by the ARUs. Scrutiny of ARU detections revealed that true positives tended to be calls of high amplitude. Comparison of ARU detections during the callback phase of the deployment with ground-truthed callback survey observations revealed that King Rail calls detected by ear and estimated to be beyond ~100 m away were not reliably flagged by the software.

A minimum number of King Rails within 100 m of each recording location was determined based on detection numbers during callback surveys as well as opportunistic auditory and visual detections of rails outside of survey periods. Individuals were assumed to be in the area for two days before and after the observation, and were not considered present during auditory surveys that took place outside of this time period.

Intensive nest searches were performed at least twice throughout the breeding season near each survey location by walking transects through suitable nesting habitat. Nests within 100 m of survey locations were considered within range of survey locations. Nests were monitored at least every three days through hatching or failure. First egg dates were back-dated from the hatch date, in the case of successful nests, or estimated using developmental stage of the eggs when found, as determined by floating (Rush et al. 2007). Two adults for each nest were assumed to be present starting one week before the first egg date, or one week before the date the nest was found if unable to estimate first egg date, through either the predation or hatch date. For nests found depredated, I used the condition of the nest and eggshells to estimate predation date where possible, and I only included breeders that were likely to have had active nests during a survey at that location.

Density estimates were highly conservative. Detections using the methods described above were not considered additive. Rather, they were combined into a single density estimate reflecting the minimum possible number of King Rails present during each individual auditory survey. For example, I did not count two birds with an active nest at the location if I had already heard at least two birds there during the callback survey.

Statistical analysis

All statistical analyses were performed using R statistical software (Version 3.2.3). Note that ARU statistical analysis was performed using raw counts, but graphs show call rates taking into account the amount of time sampled for ease of interpretation.

Results

Factors affecting the number of detections during callback surveys

I used model comparison to assess the relative roles of environmental factors on the number of detections during each callback survey. The full model was a generalized mixed model based on AD Model Builder with zero inflation, a negative binomial distribution, and location as a random effect. Fixed effects included Julian date, cloud cover, temperature, wind speed measured on the Beaufort scale, the number of human observers present, and time of day (morning or evening). An automated model selection process was then used to evaluate all possible combinations of fixed effects, and models within 2 ΔAIC_C of the top model were averaged (Table 3.1). Conditional averages were used to calculate estimates and the relative importance of each fixed effect.

None of the fixed effects had a significant influence on the number of detections in callback surveys. Cloud cover was the only fixed effect not included in any of the top models. Temperature and whether the survey took place during the morning or evening were each included in 7 of the 11 top models, and were the most important environmental factors (Table 3.2).

Although the number of detections did not correlate with Julian date (Figure 3.1), many of the surveys with the highest number of detections occurred within the three survey windows suggested by Conway (2011). More initial detections of individuals occurred during the passive listening period (0.91 ± 0.12 detections) than during the call broadcast period (0.64 ± 0.10 detections), but this difference was not significant according to a paired Wilcoxon signed rank test ($P = 0.11$).

Table 3.1 Top 11 models based on Akaike Information Criterion adjusted for small sample size explaining effects of environmental factors on the number of detections during callback surveys. Only these models were averaged to produce the relative effect size of each predictor. Cloud cover was the only fixed effect not included in any of the top models.

Fixed effects	df	AIC_C	ΔAIC_C	w_i
Temperature, Time	6	371.0	0.00	0.069
Temperature, Wind, Time	7	371.3	0.35	0.058
Null	4	371.4	0.38	0.057
Temperature, Time, Julian	7	371.5	0.47	0.055
Wind	5	371.6	0.59	0.051
Temperature	5	372.0	1.00	0.042
Time	5	372.2	1.19	0.038
Temperature, Wind	6	372.3	1.28	0.036
Wind, Time	6	372.4	1.41	0.034
Temperature, Wind, Time, Julian	8	372.9	1.87	0.027
Temperature, Time, Observers	7	372.9	1.95	0.026

Table 3.2 Influence of fixed effects on the number of detections during callback surveys. Estimates are derived from the averaging of the top 11 models < 2 ΔAIC_C from the top model. No fixed effects were significant.

Variable	Estimate	SE (Estimate)	Relative importance
Intercept	1.55	1.16	-
Temperature	-0.02	0.01	0.63
Time	-0.35	0.22	0.62
Wind	-0.16	0.12	0.42
Julian	0.01	0.01	0.17
Observers	0.07	0.12	0.05

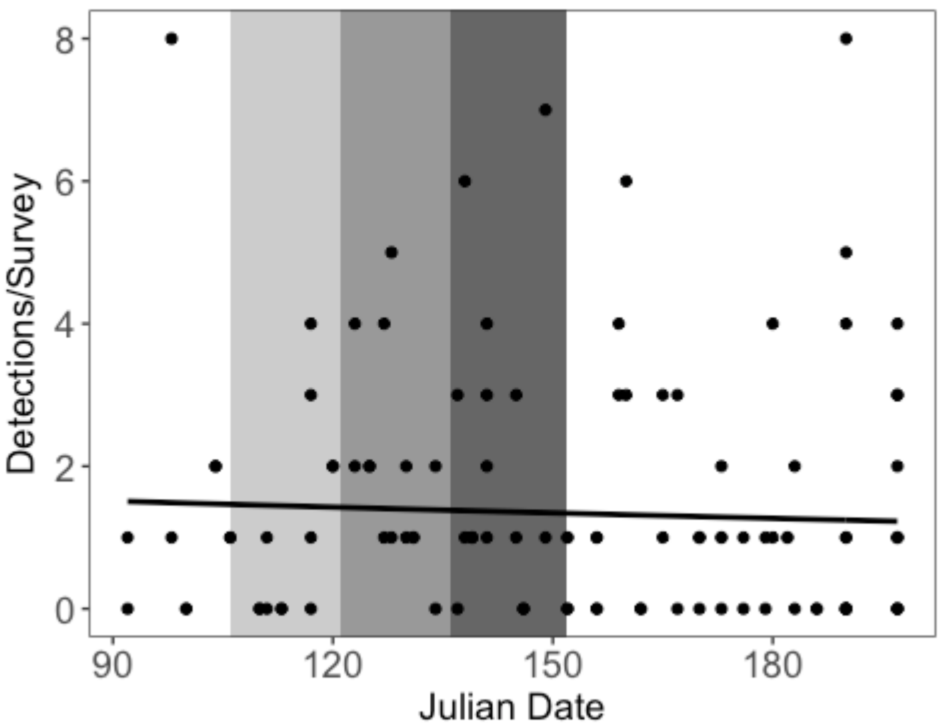

Figure 3.1 Number of detections in callback surveys as a function of Julian date (April 2-July 16). The black line shows model predictions, with detections decreasing slightly over the course of the season. Shaded rectangles represent the three survey windows suggested by Conway (2011) for our study site latitude. There was no significant correlation, but many of the highest single-survey detections occurred within these windows.

Performance of signal recognizer

Kaleidoscope Pro acoustics cluster analysis software was used to find King Rail calls in 3,331 h 5 min of passive recordings taken from the 10 survey locations at Mackay Island NWR. The program found 772 *grunts*, 2147 *keks*, and 1180 other King Rail calls including *alarms, churrs, screeches,* and *kek-burrs*.

Due to the relative indistinctiveness of the King Rail's pulsed calls among other marsh species with overlapping bandwidth, only 3.6% of vocalizations flagged as King Rails were true positives. Red-winged Blackbird, *Agelaius phoeniceus*, *kon-ka-reee* calls and frog mating calls, especially Green Tree Frogs, *Hyla cinerea*, and Southern Leopard Frogs, *Lithobates sphenocephalus*, accounted for 24.5% and 25.0% of false positives, respectively.

King Rail call types were frequently confused. Out of the vocalizations that were actually King Rails, 51% of those labeled as *grunts* were actually *keks* and 16% of those labeled as *keks* were actually *grunts*. Also, other King Rail calls made up 4% of vocalizations labeled as *grunts* and 26% of those labeled as *keks*. Thus, more vocalizations were matched to the *kek* template than the *grunt* template, indicating a more permissive representation of the *kek* call within the recognizer.

The recognizer also had a high rate of false negatives. Out of the total 3,331 h 5 min of data scanned, I manually scanned a 41.33 hr subset of files and found 122 *grunts* and 226 *kek* bouts. In the same set of files, Kaleidoscope only found 14 *grunts* and 50 *kek* calls. I address this discrepancy in the discussion section. Overall, inability to discern King Rail calls with low signal strength or overlapping heterospecific calls contributed to the low performance metrics shown in Table 3.3.

Table 3.3 Kaleidoscope recognizer evaluation metrics based on a 41.33 hr subset of recordings. Detections found during manual review in Raven Pro 1.3 were used as ground truth. Equations are adapted from Knight et al. (2017). tp = true positives, *fp* = false positives, *fn* = false negatives. I used β = 1 for F-score calculation.

Metric	Equation	*Grunts*	*Keks*
Precision	$tp/(tp + fp)$	0.07	0.04
Recall	$tp/(tp + fn)$	0.11	0.22
F-score	$\frac{(\beta^2 + 1) * precision * recall}{\beta^2 * precision + recall}$	0.04	0.03

King Rail call rates over time and density using ARUs

King Rails called at all hours (Figure 3.2). However, chi-squared analysis revealed a significant association between hour of day and call rate ($\chi 2 = 39.086$, df = 23, $P = 0.02$). Most calls were detected around sunrise and within the 2 hours before sunset. Call rates were higher during the morning peak. Lowest call rates occurred at night. The quietest period began after sunset and extended until about 0300 h when some males would begin to *kek,* especially early in the season.

I used a generalized linear mixed model with a negative binomial distribution and an offset term for the log of the number of 5-minute time blocks sampled each day to determine the association between date and the number of all call types detected. Total calls decreased by 0.98 ± 1.00 each day over the course of the breeding season ($P < 0.001$). *Keks* followed the same trend as all calls combined, although *grunts* remained relatively constant through the breeding season (Figure 3.3).

If ARUs are to be used to assess breeding populations, the calling rate per unit density must be calibrated. To determine whether breeding rail density could be estimated by the number of ARU call detections, I again used a generalized linear mixed model with a negative binomial distribution and an offset term incorporating the log of the number of 5-minutes time blocks sampled during each ARU deployment. *Keks* were not significantly linearly related to density. The model using all call types combined was marginally significant, showing an increase of 1.15 ± 1.08 calls with each unit increase in density ($P = 0.08$). However, a model using the number of *grunts* as a predictor and location as a random factor showed a significant correlation with breeder density. The number of *grunts* increased by 1.17 ± 1.07 calls per individual ($P = 0.03$, Figure 3.4). Thus, the number of King Rails within a 100 m radius (~3.14 ha) can be approximated by the equation below. I solved the negative binomial model equation for the x variable, density, inserted the slope (0.15425) and intercept (-4.5258), and added the multiplier 12 to convert hours to the number of 5 min blocks, the offset term used in my model.

$$density\ (d) = \frac{\#\ KIRA}{3.14\ ha} = \frac{\log\left(\frac{\#\ grunts}{12(\#\ hrs\ sampled)}\right) + 4.52584}{0.15425}$$

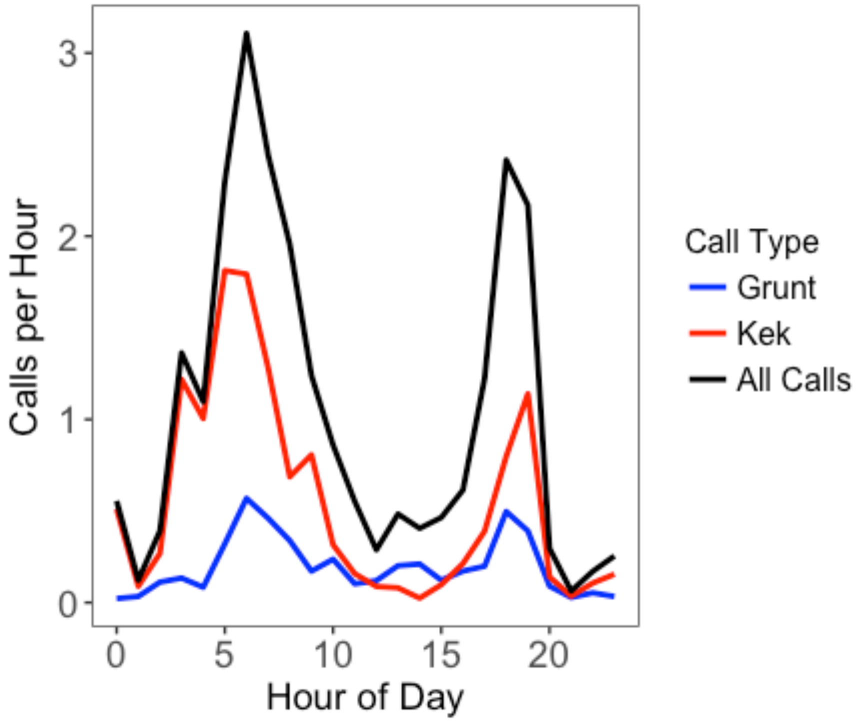

Figure 3.2 King Rail call rate during each hour of the day for *grunts*, *keks*, and all call types combined. Chi-squared analysis revealed a significant association between hour of day and call rate (χ2 = 39.806, df = 23, P =0.02). Raw data is shown.

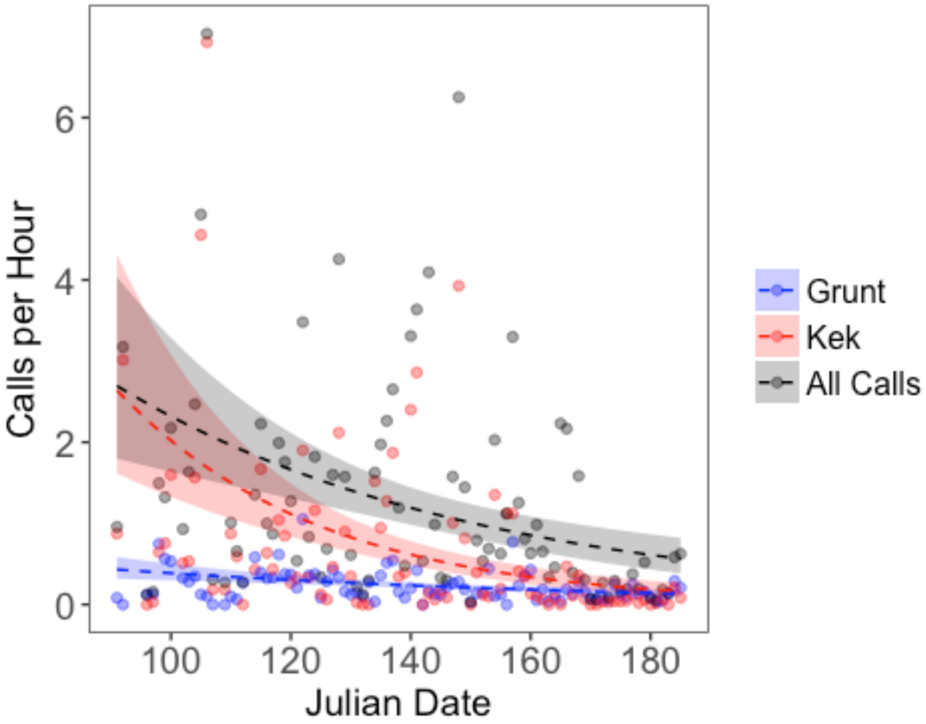

Figure 3.3 Model predictions of King Rail call rate over the course of the season (March 31-July 4) for *grunts*, *keks*, and all call types combined. Points represent raw data. Dashed lines show regression model predictions with 95% confidence intervals.

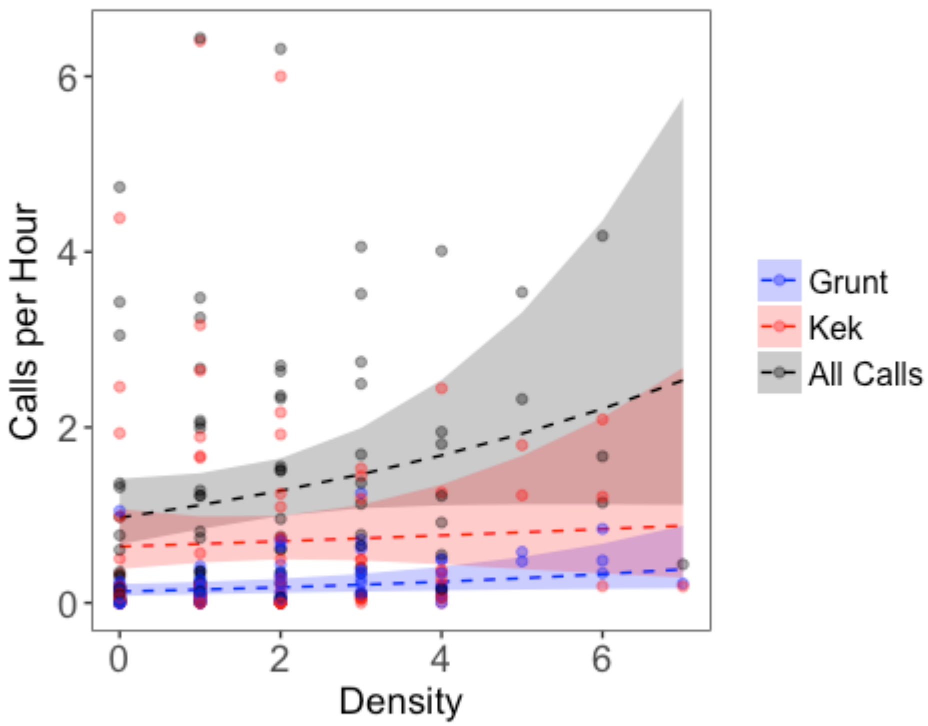

Figure 3.4 Model predictions of King Rail call rate for *grunts*, *keks*, and all call types combined as a function of density (number of rails per 3.14 ha). Points represent raw data. Dashed lines show regression model predictions with 95% confidence intervals.

Discussion

These results allow me to recommend best practices for auditory surveys of King Rails. Callback survey detections were not affected significantly by weather conditions, time of day, time of season, or the number of human observers present. Despite lack of evidence showing a detrimental effect of wind, calmer days facilitated call identification and accuracy of distance estimates by human observers.

ARU data reveal a crepuscular pattern of call rate in King Rails, with a higher call rate peak in the morning than the evening. By contrast, there were no significant differences in detection rate between callback surveys conducted in the morning or evening. I recommend that both callback surveys and ARU sampling periods occur within the time windows suggested by Conway (2011): 30 minutes before sunrise to three hours after or two hours before sunset to 30 minutes after. Programming ARUs to limit sampling to only these periods will preserve memory and battery life and reduce the amount of data requiring review while still giving an accurate representation of call rate. We easily detected even distant rails *kekking* during the quiet, calm early morning periods, therefore recording early in the season between 0300 h and dawn is a strategic method of sampling breeding males.

King Rail detections during callback surveys did not vary significantly with day of the breeding season between April 2 and July 16, but some of the highest single-survey detections occurred during Conway's (2011) latitude-based three recommended survey windows suggesting their appropriateness for this species. Alternatively, I found a seasonal decline in detection rate based on ARU survey data, similar to other studies on *Rallus* spp. (Conway et al. 1993; Stiffler et al. 2017). These data suggest that callback surveys for King Rails can occur throughout the breeding season, but early season surveys best reduce variation in occupancy and abundance estimates (Wiest and Shriver 2016).

King Rail density was significantly correlated with *grunt* call rate but not *kek* call rate or overall call rate. This is not surprising based on the behaviors associated with each call's usage. *Keks* are used by males for advertisement, and only one or a few males are likely to call at any given time within the area sampled, but they may do so intermittently for up to 2 hours (Chapter 1). During the breeding season, *grunts* are often given in a 'roll call' format where one bird's *grunt* is followed by *grunt* responses from other individuals nearby (Chapter 1). Therefore, *grunt* call rate is likely to give a more representative sample of the breeders at a given location. *Grunt* call rate also remained more constant than *kek* rate over the course of the season. Moreover, Kaleidoscope software had more difficulty discriminating *kek* calls than *grunts*. Thus, for researchers and surveyors using ARUs to monitor King Rails, focusing only on *grunts* could save time.

While this study did not directly compare callback survey and ARU detections, it nevertheless revealed some important considerations when choosing a survey method. First, when conducting callback surveys, the number of individuals detected during the initial passive listening period was greater than the number first detected during the minute of call broadcast. Thus, broadcast does not always significantly increase detections, contrary to findings in a number of other King Rail studies (Conway and Gibbs 2005; Pierluissi and King 2008; Soehren et al. 2009; Conway and Nadeau 2010). Call broadcast is likely not a novel stimulus for rails at my study site because they breed at relatively high densities and show conspecific attraction (unpublished data). ARUs still have promise to be an efficient method of detecting King Rail occupancy, particularly in areas with low population densities, possibly in combination with automated periodic call broadcasts. Signal recognizer accuracy and/or time investment to conduct manual analysis of sound files would be the limiting factors.

Second, estimated effective area sampled varied among survey methods. Field observers were able to detect calls within ~300 m radius (28 ha), manual review of ARU data within ~200 m (13 ha), and the automated signal recognizer in Kaleidoscope within ~100 m radius (3 ha). Given that mean home range size and territory size in this population are estimated to be 19.8 ± 2.5 ha and 2.7 ± 1.0 ha, respectively (Kolts and McRae 2016), survey and data analysis method can have a large impact on the number of King Rails sampled. This could explain, in part, the discrepancy in detections between the Kaleidoscope recognizer and manual review. Previous studies have likewise found counts from field observers to be more accurate and sensitive for marsh birds (e.g. Digby et al. 2013; Vold et al. 2017). Only one study so far has found that the number of individuals estimated through manual review of ARU data was higher than through field observation (Lambert and Mcdonald 2014).

Third, caution should be taken when making inferences from signal recognition software. The signal recognizer in Kaleidoscope had a high rate of false positives and false negatives. Accurate detection of calls of interest are made difficult with low signal strength, high background noise, and complex signals (Charif et al. 2010), which were all issues encountered within this dataset. Trial and error should be implemented when choosing training files. Including only examples of good quality and high signal to noise ratio resulted in a large number of false negatives, while including too many examples with overlapping heterospecifics increased the number of false positives. Recognizer accuracy was reduced by convergence of other marsh species on calls within the same peak frequency range of the King Rail, presumably due to similar selective pressures on sound transmission properties (Morton 1975). To increase discrimination of the recognizer, frequency range should be minimized. However, for my data set, minimizing frequency range did not eliminate many overlapping heterospecifics due to this call frequency convergence. Discrimination was further complicated because King Rail call rate is highest at dawn and dusk when call rate is also high for other marsh species. Kaleidoscope's user interface only offers the ability to change signal length, maximum inter-syllable gap, and frequency range in designing a signal recognizer. It does not currently support user-entry of parameters such as minimum inter-syllable gap, syllable length, or minimum number of pulses, which would potentially increase accuracy in detection of the King Rail's pulsed calls. Despite the limitations and tribulations of this software, the resulting data still captured a representative snapshot of King Rail calls and was able to reveal significant patterns.

Future studies on rails should consider the R program MonitoR (Katz et al. 2016) or convolutional neural networks (Knight et al. 2017), which have shown success over other recognition software methods. The development of new analysis methods and algorithms is ongoing, and will continue to be a fertile area for research. Currently, densities estimated by different signal recognition methods might not be comparable due to low agreement across methods (Lemen et al. 2015). My Kaleidoscope signal recognizer will be available upon request.

This study was intended to determine the best conditions and times to survey for King Rails, a threatened species that is declining across much of its range (Cooper 2008). Although the Kaleidoscope signal recognizer had low precision and recall, it still produced reliable data showing clear patterns. My model relating *grunt* call rate with King Rail breeding density can be used to generate an estimate of breeding density at other sites with ARUs. Moreover, the considerations of analysis performance reported here will help inform decisions by researchers and managers considering callback versus passive auditory surveys.

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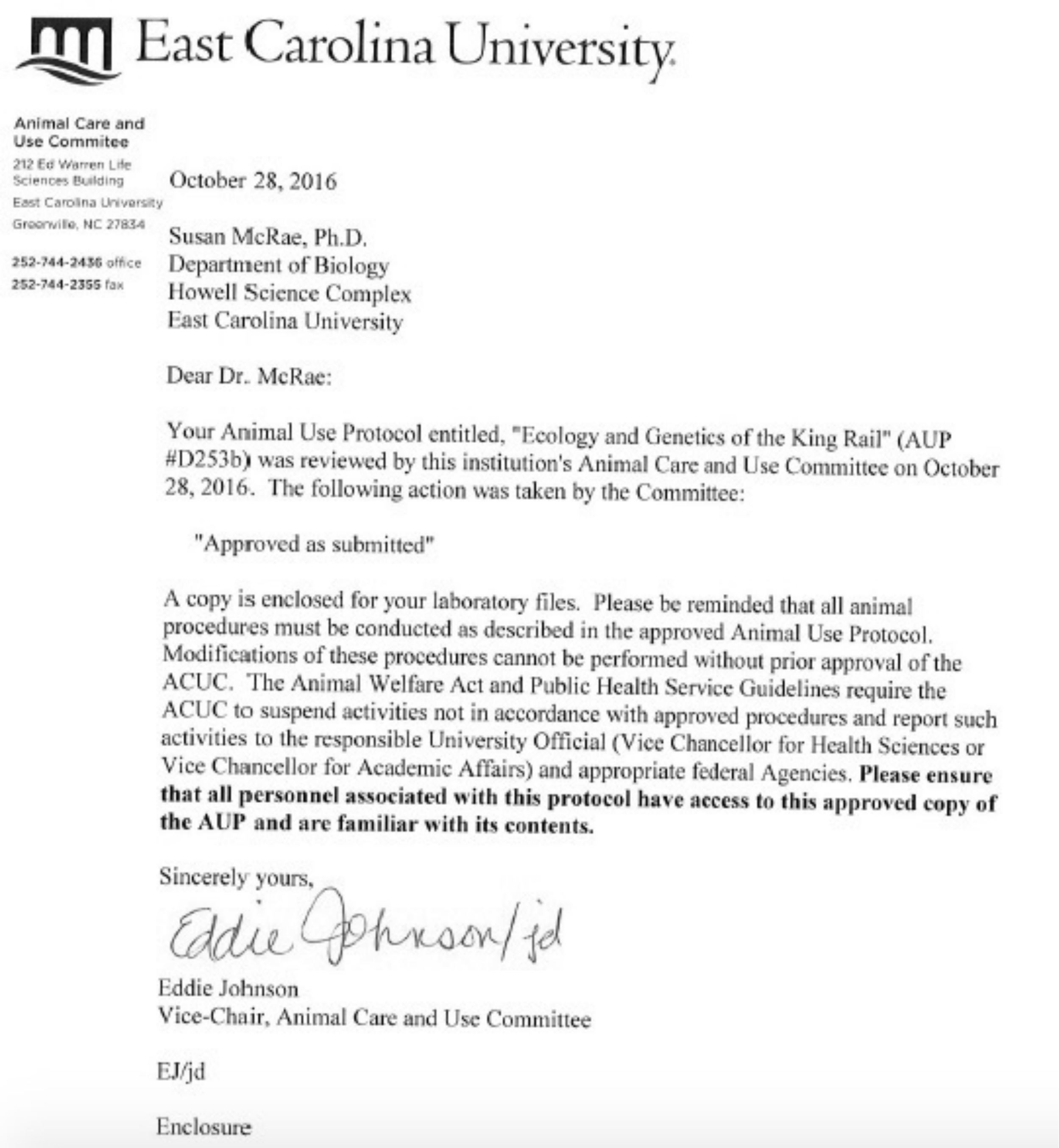

East Carolina University.

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

October 28, 2016

Susan McRae, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. McRae:

Your Animal Use Protocol entitled, "Ecology and Genetics of the King Rail" (AUP #D253b) was reviewed by this institution's Animal Care and Use Committee on October 28, 2016. The following action was taken by the Committee:

"Approved as submitted"

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

Sincerely yours,

Eddie Johnson/jd

Eddie Johnson
Vice-Chair, Animal Care and Use Committee

EJ/jd

Enclosure