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*Article*

# Singing behaviour of ruby-crowned kinglets *Regulus calendula* in relation to time-of-day, time-of-year and social context

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Observational field studies provide insight on the multifunctional nature of birdsong. For example, if song production were limited to pre-fertilization, then that would suggest a mate attraction function. If it were used throughout the breeding season and in response to intruding males, then that would suggest a territorial defence function. In the present study, we determined the daily and seasonal singing patterns of male ruby-crowned kinglets *Regulus calendula* in Labrador, Canada, using microphone arrays in two breeding seasons. Using a playback experiment, we simulated a territorial intrusion to compare the structure of songs produced while defending a territory to the structure of songs produced during solo and contest singing. Singing peaked in the early part of the breeding season and then declined continuously for the remainder of the season, which suggests that the songs function in mate attraction. Singing peaked 2–3 h after dawn, and then declined steadily until it stopped at 10 pm. Some nocturnal singing was observed, but no dawn singing was observed. A high probability of signal overlap by heterospecific songs at dawn would hinder signal recognition and explain the observed delay in peak singing activity. Vocal responses to playback suggested a function in territory defence. However, there were no significant differences in the duty cycle, frequency modulation and bandwidth of songs in relation to the context of song production, though songs were shorter in the intrusion context than during solo singing. Overall, the study provides the first quantitative description of the effects of time of day, time of year and social context on singing behaviour in this understudied species.

Keywords: birdsong, countersinging, passerine, social behaviour, song contest, territory defence

## Introduction

Birdsong influences reproductive success in many species through its effects on territory defence and mate choice (Searcy and Andersson 1986, Andersson and Iwasa 1996, Collins 2004, Catchpole and Slater 2008, Mennill and Vehrencamp 2008). With respect to mate choice, song can convey to prospective mate information about the signaler's quality or ability to provide direct benefits, such as food availability (Berg et al. 2005,

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Ritschard and Brumm 2012). Several lines of evidence support the mate choice function of birdsong. First, females approach male taxidermic mounts and/or nest boxes coupled with a loudspeaker broadcasting male song more often than they approach silent controls (e.g. pied and collared flycatchers *Ficedula hypoleuca* and *F. albicollis*, Eriksson and Wallin 1986; European starling *Sturnus vulgaris*, Mountjoy and Lemon 1991), and some species respond with copulation solicitation displays following their approach (e.g. white-throated sparrow *Zonotrichia albicollis*, Wasserman and Cigliano 1991). Second, unpaired males and males that have lost their mates in the context of mate removal experiments sing more frequently than paired individuals in many species (sedge and reed warblers *Acrocephalus schoenobaenus* and *A. scirpaceus*, Catchpole 1973; plain titmouse *Baeolophus inornatus*, Johnson 1983; European starling, Cuthill and Hindmarsh 1985; black-capped chickadee *Poecile atricapillus*, Otter and Ratcliffe 1993). Third, song production in several temperate species diminishes or stops altogether for the remainder of the breeding season after a male has paired with a mate (e.g. sedge and reed warblers, Catchpole 1973; bluethroat *Luscinia svecica*, Merilä and Sorjonen 1994). A similar pattern has been documented in neotropical rufous-and-white wrens *Throthorus rufalbus*, where both males and females sing and breeding partners remain paired throughout the year. In this species, female song production is higher during the early part of the breeding season and immediately after nest failures than it is during incubation or the nestling period, suggesting that female song in this tropical species also functions to stimulate or coordinate mating activities (Topp and Mennill 2008).

Birdsong can also contribute to reproductive success by facilitating territory defence. Aggressive territorial encounters often involve singing interactions between rivals, since songs can reveal to the receiver the signaller's identity and aggressive intent (Stoddard 1996, Searcy et al. 2006, Searcy and Beecher 2009, Baker et al. 2012). Broadcasting a conspecific's song inside a bird's territory will, in many species, cause the resident bird to engage in territorial behaviour that usually involves the production of song (Mennill and Vehrencamp 2008) and, in some species, physical attacks on the speaker (Kroodsma and Byers 1991, Ręk and Osiejuk 2011, Baker et al. 2012). 'Speaker replacement' experiments further demonstrate the deterrent effect of song by removing a territory holder from its territory, replacing it with loudspeakers that broadcast song or an irrelevant control sound, and monitoring the rate at which neighbours intrude (Collins 2004). In red-winged blackbirds *Agelaius phoeniceus* and white-throated sparrows, neighbouring males hearing simulated song intruded into the removed males' territories less often and less quickly, as compared to silent controls (Yasukawa 1981, Falls 1988).

Describing patterns of song production provides insight into song function and a foundation for developing and testing functional hypotheses. For example, consistent song production throughout the breeding season suggests that song functions in territory defence, whereas a decline in song production after mate acquisition suggests a mate attraction function (Catchpole 1973, Merilä and Sorjonen 1994). Song production in relation to time of day can reveal specific periods of intense vocal output. Songbirds are well known for heightened vocal activity at dawn (i.e. dawn chorus), which has been attributed to intrinsic traits of the signaller, and social and environmental selective pressures (reviewed by Staicer et al. 1996). However, descriptions of diel singing activity are often overlooked (but see Sosa-López and Mennill 2014, Moran et al. 2019). Taken together, descriptions of diel and seasonal patterns of singing behaviour provide a basis for developing hypotheses about the selective forces that have shaped temporal patterns in birdsong.

In some species, different song types, or different components of a single song, serve different functions. For example, in chestnut-sided warblers *Setophaga pensylvanica*, songs with accented endings were produced more by unpaired males for mate attraction, whereas songs with unaccented endings were produced more by paired males for territory defence (Kroodsma et al. 1989). The use of different song types in particular social contexts has been studied in other North American wood warblers (Parulidae; reviewed by Spector 1992), Cuban grassquits *Tiaris canorus* (Baptista 1978), willow warblers *Phylloscopus trochilus* (Järvi et al. 1980), field sparrows (*Spizella pusilla*, Nelson and Croner 1991) and rufous-and-white wrens (Mennill and Vehrencamp 2008, Topp and Mennill 2008). If different song types, or different components within songs, serve different functions, then identifying the context in which they are used can provide insight into their function.

There are several methods for characterizing the singing behaviour of birds, but most have disadvantages. Direct observation is challenging because birds can be difficult to follow over long periods of time or through dense habitat. Furthermore, the presence of observers can alter a bird's behaviour (Magyar et al. 1978, Brandes 2008, Mennill et al. 2012). Autonomous recorders used in place of human observers can overcome these challenges, since multiple recorders can be deployed and programmed to record at relevant times of the day over long periods of time. A disadvantage, however, is that the range over which vocalizations are detected by a recorder varies substantially with habitat (Dabelsteen et al. 1993), weather (Lengagne and Slater 2002), species, and the location, orientation and motivation of the individuals being recorded (Witkin 1977). Unmeasured variation in the effective range of different recorders obscures estimates of song production and potentially confounds analyses of singing behaviour. Microphone arrays resolve this final challenge (Mennill et al. 2012). A microphone array consists of three or more time-synchronized audio recorders distributed throughout an animal's environment. The subtle differences in when an animal's vocalization arrives at each microphone can be measured and used to trilateralize the position of the vocalizing animal in three-dimensional space (Wilson et al. 2014). Locations can then be used to calculate spatially explicit measures of song density (i.e. number of songs per unit area per unit time) that can be compared directly among habitats and recording conditions (Marques et al. 2013, Stevenson et al. 2015).

The ruby-crowned kinglet *Regulus calendula* is a small, olive-green, migratory songbird that breeds in black spruce

*Picea mariana* forests and tamarack *Larix laricina* muskegs from northwestern Alaska to eastern Canada and New England (Swanson et al. 2008). Males sing a loud and complex song that comprises three parts, including a series of high-pitched notes at the beginning, a series of chatter notes that are an octave lower in the middle, and a series of loud, 4-note phrases at the end (Bent 1964; Fig. 1). Song production in ruby-crowned kinglets has been described qualitatively as 'spread and continuous from early spring to late fall, but with an interruption during the peak nesting period' (Bent 1964). Yet, in contrast to many other temperate species, little else is known about the singing behaviour of this species. In particular, the temporal patterns of song production, the structure of individual songs, and the social contexts in which songs are produced have yet to be quantified.

In this study, we used microphone arrays to record and quantify the singing behaviour of ruby-crowned kinglets. Our objectives were to: 1) quantify diel and seasonal patterns of song production during the breeding season; and 2) compare song structure among three social contexts, including during solo singing, during counter-singing exchanges with neighbours, and while responding to simulated territorial intrusions.

## Material and methods

The study was conducted within a 40-km radius of Happy Valley-Goose Bay, Labrador, Canada (centred at 53°250′010″N, 60°300′070″W) during the 2016 and 2017 avian breeding seasons. The area is part of the Boreal Shield Ecozone; it is dominated by black spruce *Picea mariana* and balsam fir *Abies balsamea*, but also includes deciduous trees, including paper birch *Betula papyrifera*, trembling aspen *Populus tremuloides*, balsam poplar *Populus balsamifera*, American mountain ash *Sorbus americana* and speckled alder *Alnus incana*.

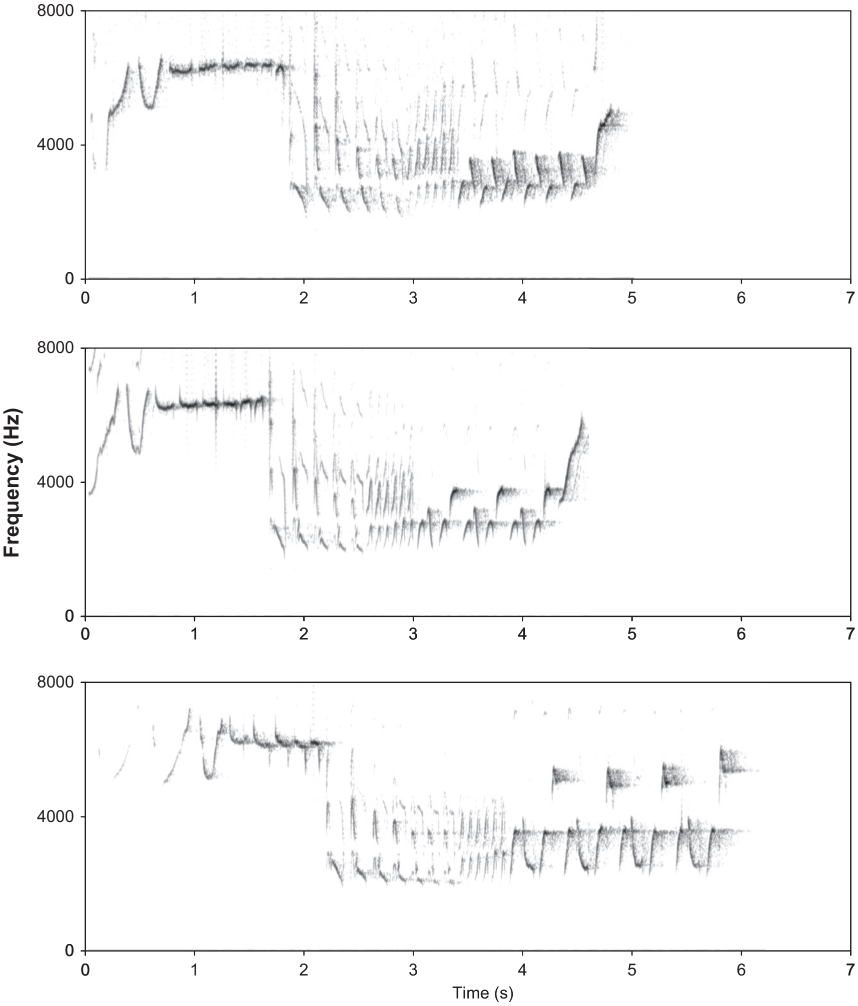

Figure 1. Songs from three different ruby-crowned kinglets. Songs were recorded during the 2016 breeding season in Labrador, Canada, using digital audio recorders (Song Meter SM3; Wildlife Acoustics Inc., Concord, Ma, USA; WAVE format, 24-kHz sampling rate, 16-bit amplitude encoding). Spectrograms were generated using a 1024-point FFT, and a Hamming window. The greyscale represents an amplitude range of 42 dB.

### Microphone arrays

We assessed diel and seasonal patterns of song production by setting up 112 microphone arrays for a minimum of 24 h each from 16 May to 14 July 2016 (n = 70), and from 16 May to 28 June 2017 (n = 42). Their locations were selected at random, but with the constraints that they were within 1 km of road or trail access, at least 500 m apart from each other, and not in a swamp, bog or body of water. The microphone arrays were also used in other projects investigating the relationships among habitat characteristics, anthropogenic disturbance, avian community composition and avian behaviour (Ethier and Wilson 2019, Hennigar et al. 2019). One of those studies involved broadcasting experimental noise or light, but those manipulations were only conducted at a given location after the data for the current study had been collected (Hennigar et al. 2019).

Each microphone array comprised three (n = 13) or four (n = 99) autonomous digital audio recorders (Song Meter SM3; Wildlife Acoustics Inc., Concord, MA, USA) arranged in either an equilateral triangle or square with sides approximately 40 m in length. After mounting the first recorder at the southwest corner of an array, the remaining recorder locations were determined by pacing approximate distances along compass bearings, or by using a handheld GPS unit for guidance (model: GPSmap 78s or eTrex 10; Garmin, Olathe, KS, USA). The recorders were attached to tree trunks approximately 1.5 m (range: 1.4–1.8 m) above the ground using two drywall screws. Each audio recorder was positioned so that the built-in microphone (frequency range: 50–20 000 Hz ± 10 dB) on the left channel pointed towards the center of the microphone array. A second external omnidirectional microphone (model AMM-A2; frequency range: 50–20 000 Hz ± 10 dB) connected to the right audio channel with a 3 m cable was elevated with a painter's pole and a wire hook and attached to a tree branch approximately 2.5 m above the other microphone. The elevated microphone also pointed towards the center of the array. The locations of the microphones were determined with a survey-grade GNSS with 10-cm accuracy (Trimble Geo 7X; Trimble, Sunnyvale, CA, USA). Each audio recorder had a GPS unit connected to it (Garmin SM3 GPS; Wildlife Acoustics Inc.), which synchronized its clock to within 1 ms of GPS time. Accurate microphone positioning and clock synchronization among recorders is critical for localizing sounds recorded with a microphone array (Mennill et al. 2012).

Recorders were programmed to record continuously until stopped, and to create a new stereo audio file every 2 h (WAVE format, 24 kHz sampling rate, 16-bit amplitude encoding). Each array was set up in the morning or early afternoon (8 am to 2 pm), and then left to record in the absence of human observers until at least 5 pm the following day; therefore, recordings sampled the evening chorus on the day of set-up plus the dawn chorus the following day. Arrays were left recording for an additional day as part of another experiment (Hennigar et al. 2019). If there was heavy precipitation (> 5 mm) during the first or second day, the array was left recording for an additional day.

Temperature affects the speed of sound and thus the localization process, and wind affects recording quality and signal detection. We accounted for these effects by placing a portable weather station (Kestrel 5500 Weather Meter; KestrelMeteres.com, Boothwyn, PA, USA) inside each array approximately 1 m above the ground. It measured and logged temperature (± 0.1°C) and wind speed (± 0.1 $\mathrm{km\,h^{-1}}$) every 20 min throughout the array deployment. Maximum windspeed at our sites was always low (mean ± SD = 0.75 ± 1.65 $\mathrm{km\,h^{-1}}$), so we did not consider its effects in subsequent analyses.

### Simulated territorial intrusions

We simulated territorial intrusions by broadcasting pre-recorded songs of ruby-crowned kinglets inside 24 arbitrarily selected microphone arrays from 28 May to 25 June 2016. Playback trials were always conducted after the 24-h period used to assess diel and seasonal patterns of song production. The playback apparatus comprised a digital audio player (Apple iPod) connected to a loudspeaker (PigNose Legendary 7-100 field speaker, PigNose) that was placed facing upwards approximately 30 cm above the ground. We had not mapped any territories, but ruby-crowned kinglets were common throughout our study area, so we assumed that most playbacks would have been inside or close to a ruby-crowned kinglet territory.

Each trial began by broadcasting a playback stimulus, which was a pre-recorded ruby-crowned kinglet song that was repeated every 10 s for 5 min (see stimulus preparation details below). Songs were broadcast at a peak amplitude of 80 dB sound pressure level, as measured with a digital sound level meter (Radioshack, model 33-2055; C-weighting, fast response) held 1 m from the speaker. During the playback, we sat on the ground 12 m away from the speaker. If a male was heard and seen within 30 m of the speaker during the playback, it became the focal subject. The trial ended when the subject was estimated to be 30 m away from the speaker, which only occurred after the playback had stopped. If more than one male responded, or if no male was detected, the trial was aborted and excluded from analysis. The final sample size was 20 playback trials.

We recorded the subject's vocalizations throughout the trial using a shotgun microphone (Sennheiser ME-67; frequency response 20–20 000 Hz ± 2 dB) and a solid-state digital recorder (Marantz PMD-661; Marantz America, LLC., NJ, USA; WAVE format, 44.1 kHz sampling rate, 16-bit encoding). We also estimated and dictated the distance between the subject and speaker whenever he changed positions so that we had a record of when the subject was within 30 m of the speaker.

Each playback stimulus was created from a single song of a different male, and each stimulus was used to test a maximum of 2 subjects (12 stimuli to test 24 subjects). Songs used to create playback stimuli were derived from the recordings of previous playback trials, except for the stimulus used in the first trial, which was derived from the Macaulay Library at the Cornell Lab of Ornithology. The stimulus male and the playback subject were separated by at least 500 m (the minimum distance between two arrays) to reduce the probability that they were familiar with each other.

When selecting a song for stimulus construction, we first reviewed the recording from which it was derived as a spectrogram (512-point fast Fourier transform, Hamming window, 87.5% overlap) in Audacity software (Audacity® software is copyright © 1999–2019 Audacity Team. The name Audacity® is a registered trademark of Dominic Mazzoni). We chose a song that had all three units (Fig. 1), high signal-to-noise ratio, and no distortion or overlapping sounds. We extracted the song, applied a high-pass filter (frequency = 1500 Hz, rolloff = 48 dB per octave) to remove background noise, and then normalized the song to a peak amplitude of −1 dB. The song was repeated every 10 s for 5 min, and the final sequence exported as a standalone audio file (WAVE format, 44.1 kHz sampling rate, 16-bit encoding) that was transferred to the playback device.

### Diel and seasonal patterns of song production

For each set of array recordings, we examined the first 5 min of every 20-min period over a 24-h period that began at 5 pm on the day the array was set up. Recordings were viewed as multichannel (6 channels for arrays with 3 recorders; 8 channels for arrays with 4 recorders) spectrograms (512-point FFT; Hamming window; gain = 25 dB; range = 78 dB) in Audacity, and songs were annotated when they were visible on one or both channels of at least 3 of the audio recorders composing the array. Songs that were visible on fewer recorders were considered too far away from the array to be localized (Wilson et al. 2014). Annotations were made by drawing a box around the song on the spectrogram. This technique automatically records the start and end times of each song, which are required for localization. While annotating songs, we discovered that two arrays had failed to record, and that 18 did not contain any ruby-crowned kinglet songs. Given the high rate of singing in this species (Results), it is likely that kinglets were absent from those 18 arrays, as opposed to being present and silent. A total of 20 407 songs from 92 arrays were annotated.

Annotated songs were localized automatically using a customized program in MATLAB (MATLAB 6.1). Prior to localization, the program bandpass filtered (2400–3000 Hz) songs to minimize background noise created by wind, traffic or other species. The selected frequency range excluded the faint introductory notes of the song, but included the song's louder third unit, which was always present (Fig. 1). The localization process estimates the GPS coordinates of the origin of a sound and provides a unitless measure of localization error that reflects the confidence of the estimate. The procedure included two steps. First, it identified the channel in which the song was the loudest (i.e. the reference channel), and then used waveform cross-correlation to measure the song's time-of-arrival differences between the reference channel and the other seven channels in the array. This step produced a vector containing seven observed time-of-arrival differences. Second, the program superimposed a three-dimensional lattice, with 2-m resolution, over the entire study site, which was defined as the area bounded by the microphone array, plus a 100-m buffer around the array and a 10-m buffer above and below the array. At each vertex in the lattice, the program used the temperature at the time of recording to calculate the speed of sound and the time it would take for a sound to travel from the vertex to each microphone. Those times were then used to produce a vector containing the seven theoretical time-of-arrival differences between the reference channel and the other seven channels. The vertex that minimized the sum of absolute differences between the observed and theoretical time-of-arrival vectors (i.e. localization error) was selected by the program as the most likely origin of the song. This second step was repeated 100 times, with each iteration using a finer-resolution lattice and a smaller study area (equal to the spatial resolution of the previous iteration) centred on the estimated origin of the song from the previous iteration. (Wilson et al. 2014, Ethier and Wilson 2019, Hennigar et al. 2019).

Only those vocalizations that met two inclusion criteria were retained in subsequent analyses. First, we used the 'sp' and 'rgeos' packages (Bivand et al. 2013, Bivand and Rundel 2017) in RStudio (ver. 1.0.136) to retain songs that had been localized to the area bounded by the microphones, or to a 10 m buffer around that. Previous research shows that localization accuracy declines for sounds produced more than 10 m beyond the array (Mennill et al. 2012, Wilson et al. 2014). Second, we retained only those songs with a localization error of 0.1 or less. We chose 0.1 because previous research showed that 95% of sounds with a localization error of 0.1 or less are localized to within 10 m of their true locations (Ethier 2018). That research involved broadcasting frequency upsweeps (200 ms in length, logarithmic increase from 0.5 to 2.5 kHz, broadcast at 90 dB sound pressure level, as measured 1 m from the speaker) from in and around eight of our arrays (but after our trials were complete), localizing them with our localization software, and comparing the location estimates to the true locations of the speaker, as determined with our GNSS. Of the 20 407 songs that we annotated in the current study, 3596 songs from 81 arrays met the inclusion criteria and were used for further analysis.

All remaining songs were categorized according to the hour of the day (1–24) when they were produced. Because only 5 min of every 20-min period were annotated, the number of songs was multiplied by four to estimate the total number of songs produced per hour. The frequency of songs produced in a given array during each hour was then divided by the area of the study plot (in hectares), including the 10-m buffer, to yield a spatially explicit measure of song density that could be compared among arrays (i.e. number of songs per hour per hectare). We also calculated the mean number of songs produced per hectare during each week of each breeding season to generate a plot depicting seasonal variation in song production. Spatially explicit measures of song production were necessary because there were significant differences in the sizes of arrays. The average size of an array, including the area bounded by the microphones and the 10-m buffer around that, was 2787 $m^2$, but they ranged from 1495 to 4434 $m^2$. Since larger arrays would likely enclose a greater proportion of the resident male's territory and be more likely to include sections of neighbouring males' territories, we would expect the number of songs detected by an array to correlate positively with array size. Using spatially explicit measures of singing activity based on localizations that met the inclusion criteria was therefore the only way to produce standardized estimates of song production that could be compared meaningfully among arrays of different size.

### Song structure in relation to social context

We tested whether song structure varied among three social contexts using recordings from 2016. Songs from the solo singing and counter-singing contexts were derived from the annotated songs from microphone array recordings. A song was considered 'solo' if it was not preceded or followed by the song of another male for 10 s (as determined by the time stamps of all annotated songs, not just those meeting the inclusion criteria), and 'countersinging' if it was. We chose 10 s for two reasons. First, studies of song-type matching, which is a particularly clear example of counter-singing, show that birds typically match their opponents' songs within 10 s (Foote et al. 2010, Price and Yuan 2011). Second, we reviewed our array recordings and noticed that consecutive songs sung by different males were typically separated by less than 10 s and were usually associated with two males singing in alternation. Those that were separated by more than 10 s were usually associated with the gap between the end of one male's singing bout and the start of another male's singing bout. Songs from different males could be distinguished because they were part of singing bouts that originated from different locations. Moreover, songs from different males differed in the fine acoustic structure of their 4-note phrases comprising the last section of the song (Fig. 1). Songs for the territorial intrusion context were derived from the recordings of our simulated territorial intrusion trials. A song was considered 'intrusion' if it was produced by a focal male during the 5-min playback period or within one minute after the end of the playback period. We retained songs that were not masked by the playback for the 'intrusion' context. For 'contest' and 'solo' contexts, we retained only high-quality songs for our structural analysis, which included songs with no distortion or overlapping sounds, and that were produced within 15 m from an array microphone (as determined by the localization process). We did not consider songs produced beyond 15 m because their softer elements might have attenuated before reaching the microphone. In total, we included 839 songs in our structure analysis (solo = 403 songs from 32 arrays, contest = 181 songs from 23 arrays, intrusion = 255 songs from 20 males).

We measured the acoustic structure of each song using Luscinia software (<http://rflachlan.github.io/Luscinia/>). Each song was viewed as a spectrogram (512-point fast Fourier transform, Hamming window, 88% overlap, 28 dB dynamic range, 100% dynamic compression), and the automatic signal detection algorithm was used to identify the elements composing the song and the silent intervals between them. We measured the following four structural features from each song:

1. Song duration: the length of time between the beginning of the first element of the song and the end of the last, including the silent intervals between each element. The end of a song was determined when there were no elements produced within 4 s after the last element.
2. Duty cycle: the sum of the durations of each element divided by song duration.
3. Frequency modulation: the frequency of maximum amplitude (i.e. peak frequency) was determined for each spectrum within each element of the song. The cumulative absolute change in peak frequency from one spectrum to the next was then divided by the cumulative duration of all signal elements within the song to yield a rate of peak frequency change ($Hz\,s^{-1}$).
4. Bandwidth: the difference between the 95th percentile and 5th percentile of all peak frequency values in a song.

We used a quantitative structural analysis, rather than one in which elements and songs were subjectively assigned to 'types,' because preliminary inspection of songs showed considerable structural variation within putative types and considerable overlap among types.

We compared song structure among the three social contexts using linear mixed models implemented in 'lme4' (Bates et al. 2015) in R (<www.r-project.org>). Array number was specified as a crossed random effect in the model to control for repeated sampling of songs within an array. Each response variable was tested separately in relation to social context, which was specified as a factor with fixed effects. If a model revealed a significant overall relationship between social context and a particular variable, as determined by an ANOVA (using 'anova' function in the R base package), we conducted pairwise comparisons between the three contexts using the 'multcomp' package and the Holm–Bonferroni method for controlling Type I error (Hothorn et al. 2008).

## Results

In 2016, song density peaked in week 24, which began on 7 June (mean ± SE = 1147 ± 434 songs per hectare per day; n = 9 arrays; Fig. 2), and then declined steadily until it all but ceased in the final week of sampling that year (i.e. week 28; Fig. 2). In 2017, song density was highest in the first week of sampling, which began on 19 May 2017 (mean ± SE = 724 ± 517 songs per hectare per day; n = 5 arrays; Fig. 2), and then again declined throughout the remainder of the sampling period (Fig. 2).

At the time and location of the study, civil twilight began between 2.40 am and 3.17 am and sunrise occurred between 3.33 am and 4.01 am. Sunset occurred between 7.56 pm and 8.33 pm and civil twilight ended between 8.40 pm and 9.26 pm. Ruby-crowned kinglets began singing after sunrise, between 4 am and 5 am, and did not reach peak song density until 2–3 h later (i.e. between 6 am and 7 am; Fig. 3). Song

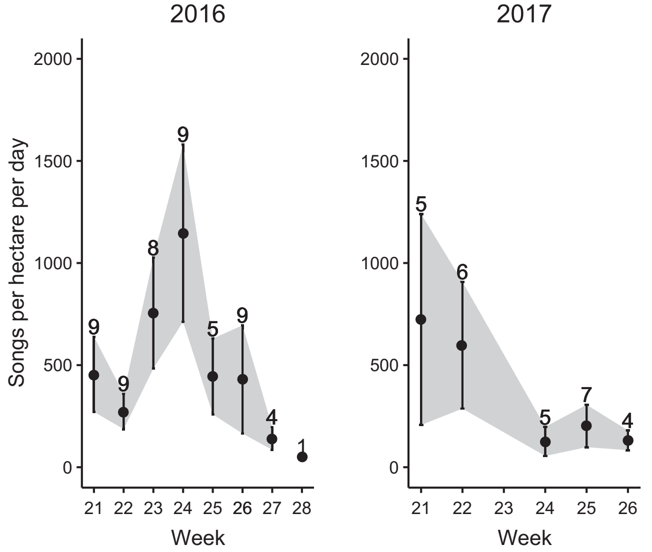

Figure 2. Seasonal singing activity of ruby-crowned kinglets shown for each week of the study in 2016 and 2017. The points represent the mean ± SE number songs produced per hectare per day. Sample sizes (i.e. the number of arrays) for each week are shown above each point.

density then declined steadily and approximately linearly between 08:00 and 22:00 h (Fig. 3). Some nocturnal singing (i.e. after civil twilight ended) occurred shortly after the end of civil twilight (mean ± SE = 7.84 ± 4.55 songs $ha^{-1}$; Fig. 3).

There was no significant relationship between social context and duty cycle (linear mixed effects model: $F_{2,777} = 0.26$, $p = 0.77$), frequency modulation ($F_{2,794} = 0.16$, $p = 0.85$) and bandwidth ($F_{2,776} = 2.82$, $p = 0.06$; Fig. 4). In contrast, there was a significant relationship between social context and song duration ($F_{2,734} = 4.85$, $p = 0.008$). Pairwise comparisons showed that songs produced in the solo context were significantly longer than songs produced in response to simulated intrusions ($p = 0.007$) and that contest songs did not differ significantly from songs produced in either the solo ($p = 0.44$) or intrusion context ($p = 0.44$; Fig. 4).

## Discussion

Describing the seasonal singing activity of birds provides important insight into the function of birdsong (sedge and reed warblers, Catchpole 1973; stonechat *Saxicota torquata*, Greig-Smith 1982; bluethroat, Merilä and Sorjonen 1994). Songs that function primarily in territory defence are expected to be heard consistently throughout the breeding season, since the resources being defended are important to the male and his mate and offspring throughout the entire season (Merilä and Sorjonen 1994). In contrast, diminished song production after pairing suggests that song functions more so in mate attraction (Catchpole 1973, Merilä and Sorjonen 1994). In this study, we provide the first quantitative description of seasonal singing pattern in the family Regulidae. Song production in ruby-crowned kinglets was not consistent throughout the sampling period (Fig. 2). In 2016, we observed a single pronounced peak in song production, followed by a sharp decline. This pattern suggests that song in ruby-crowned kinglets functions, in part, in mate attraction (Fig. 2). The pattern in 2017 also showed a single peak followed by a decline, but, compared to 2016,

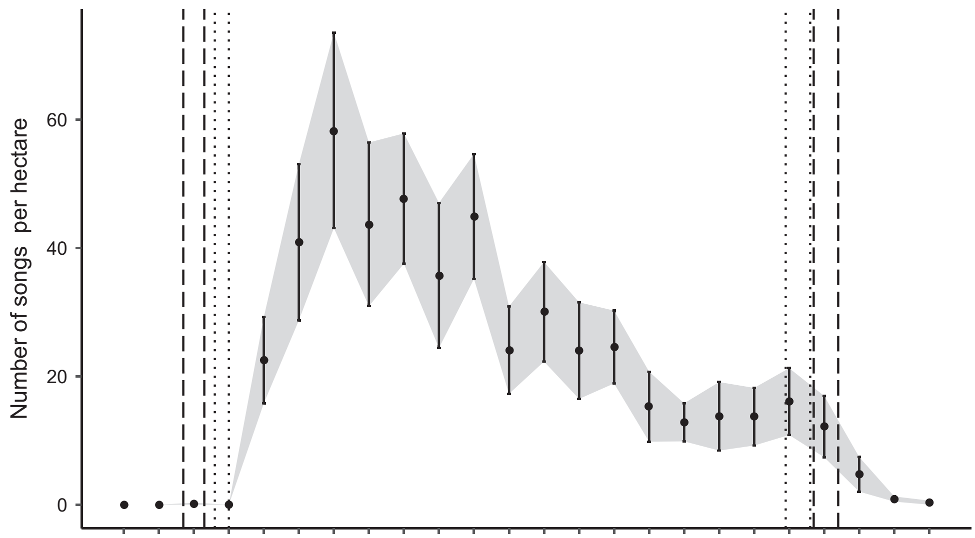

Figure 3. Diurnal singing activity of ruby-crowned kinglets. Shown for each hour of the day ('1' denotes the time from midnight until 1 am) are the mean ± SE number of songs produced per hectare from 81 arrays (n = 54 in 2016 and n = 27 in 2017) deployed during the breeding season. Vertical dotted lines indicate sunrise and sunset times and dashed lines indicate the start and end times of civil twilight at the study site during the sampling period, as determined by National Research Council Canada; <http://www.nrc-cnrc.gc.ca/eng/services/sunrise/>.

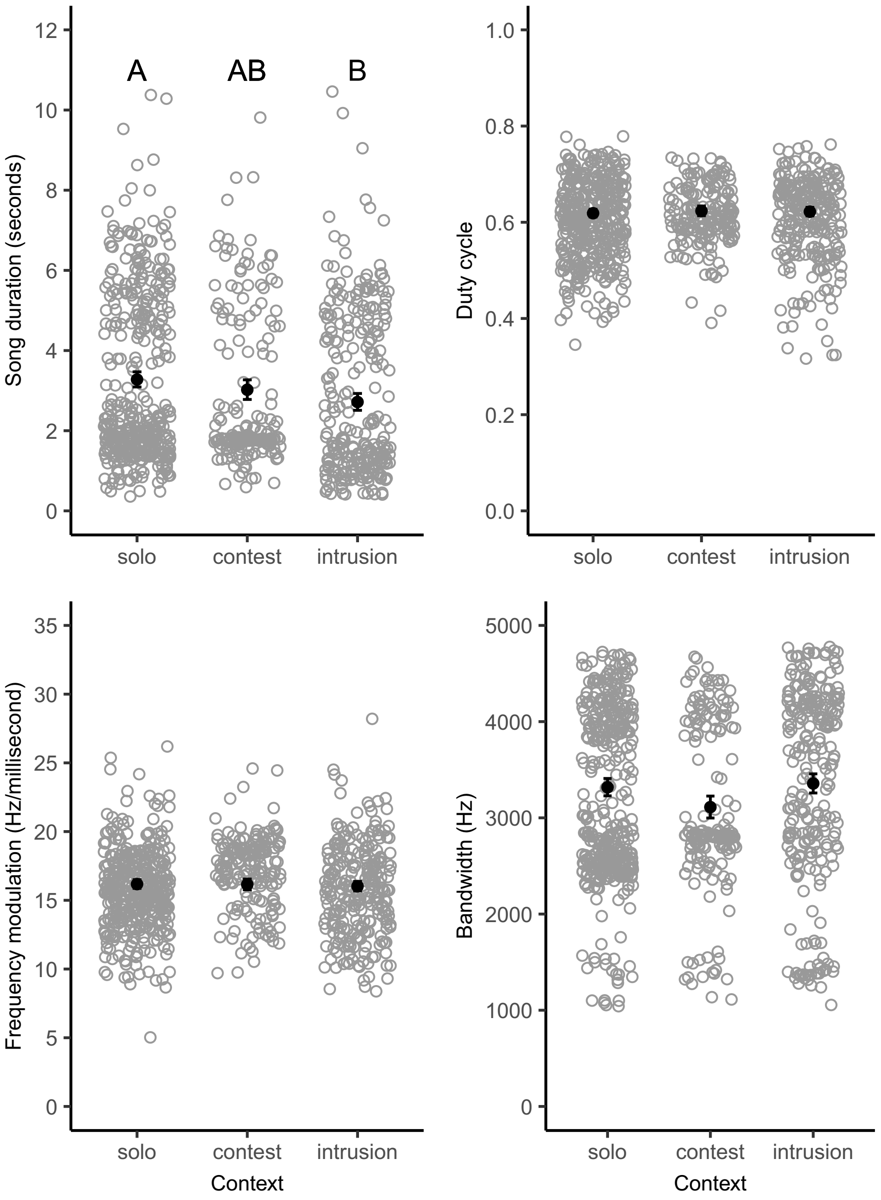

Figure 4. The relationships between social context and song structure in ruby-crowned kinglets. Song structure variables include (a) song duration, (b) duty cycle, (c) frequency modulation and (d) bandwidth. Songs were recorded during solo singing, song contests and territorial intrusions. Black dots with whiskers show mean ± SD and open grey circles are raw data points. For song duration, treatments with the same letters are not significantly different from each other.

the peak was less pronounced and the maximum rate of singing was lower. We suggest that cooler temperatures observed in 2017 account for this difference between years. The daily temperature (average of all 20-min samples obtained with our portable weather station) between 17 May and 30 June was 3.5 ± 4.8°C (mean ± SD) cooler in 2017 than in 2016. The cooler temperatures in 2017 were associated with delayed snowmelt (personal observation), as well as later (4 days) arrival of ruby-crowned kinglets, as determined by e-bird checklists (<ebird.org>) for our study site.

The dawn chorus in birds is well-known and widely observed in different environments (Henwood and Fabrick 1979, Brown and Hanford 2003). However, the peak singing activity of ruby-crowned kinglets occurred 2–3 h after dawn (Fig. 3). Several hypotheses to explain the dawn chorus revolve around the intrinsic characteristics of the singer (e.g. hormone levels), the social function of dawn singing (Poesel et al 2006), and/or the environmental pressures for dawn singing (Staicer et al. 1996). However, the occurrence of daytime singing can be under similar selective forces as well, attributed to long distance communication to attract females (chipping sparrow *Spizella passerina*, Liu et al. 2007). With respect to environmental pressures, an underlying assumption is that maximizing signal transmission distance would be beneficial because it can allow receivers to accurately retrieve information about the signaller's quality and location (Slabbekoorn 2004, Catchpole and Slater 2008). At dawn, low atmospheric turbulence and background noise reduce sound disruption and favour acoustic communication (Brown and Hanford 2003), which is not exploited by ruby-crowned kinglets and which therefore suggests other selective forces.

Temporal overlap between heterospecific songs can arise coincidentally due to a mutual preference for vocal display at certain times of the day, particularly at dawn (Slabbekoorn 2004). If signals also overlap in the frequency domain, then spectral overlap can create biotic interference for vocalizing animals (Cody and Brown 1969). Whether or not spectral overlap occurs depends on the species assemblage in an avian community and the corresponding range of frequencies occupied by their signals (Planqué and Slabbekoorn 2008). Due to its broad frequency range, the song of the ruby-crowned kinglet (Fig. 1) is more vulnerable than the songs of other species to spectral overlap by heterospecific songs. Shifting the peak diurnal singing activity away from dawn could reduce the probability of temporal and spectral overlap by songs of other species and ensure proper signal transmission. Whether the delayed peak in singing activity in ruby-crowned kinglets is innate or a result of active avoidance of heterospecific signal overlap remains unknown, but has been studied in other songbirds (e.g. nightingale *Luscinia megarhynchos*, Brumm 2006; red-eyed vireo *Vireo olivaceus* and least flycatcher *Empidonax minimus*, Ficken and Ficken 1974). If signal overlap avoidance is a learned behavioural response, then the number of species singing in a certain hour of the day should influence the singing activity of ruby-crowned kinglets.

Ruby-crowned kinglets sang in response to simulated intruders, which suggests that their song has the dual functions of mate attraction and territory defence that have been documented in many temperate and some tropical songbirds (Collins 2004, Mennill and Vehrencamp 2008, Topp and Mennill 2008). Interestingly, most song structure variables (duty cycle, bandwidth and frequency modulation) did not differ significantly among the three social contexts (Fig. 4), which suggests that the different units of the ruby-crowned kinglet's song do not serve separate functions. For example, if the heavily frequency modulated notes of the third unit (Fig. 1) were used for territory defence, then we should have observed songs with greater overall frequency modulation in the counter-singing and intrusion contexts than in the solo context. Therefore, our findings do not support the hypothesis that different units in the song of ruby-crowned kinglets serve different functions, as song types do in other temperate species (e.g. black-throated green warbler *Setophega virens* and Blackburnian warbler *Setophega fusca*, Morse 1967; chestnut-sided warbler *Setophega pensylvanica*, Kroodsma et al. 1989; hooded warbler *Setophega citrina*, Wiley et al. 1994). However, songs produced in response to simulated territorial intrusions were shorter than solo songs. Varying song length could encode information about a male's aggressive intent towards potential intruders, as has been observed in other species (e.g. yellowhammer *Embiriza citronella*, Rutkowska-Guz and Osiejuk 2004; banded wren *Thryothorus pleurostictis*, Hall et al. 2006; corn bunting *Miliaria calandra*, Osiejuk et al. 2007). Singing shorter songs could also be a mechanism for avoiding overlapping a rival's song. For example, when a simulated intruder overlaps the songs of black-capped chickadees, the chickadees shorten their songs by dropping the 'bee' note of the 'fee bee' song (Mennill and Ratcliffe 2004). A third possibility is that shorter songs in response to intrusions are a result of the rapid movements of ruby-crowned kinglets amidst the foliage while performing agonistic postural displays from different perches (e.g. leaning forward and erecting crown feathers accompanied by wing flicking; Swanson et al. 2008), which could interfere with vocal activity. Finally, reducing song length could be an artifact of increasing song rate. Increased song rate is energetically costly and can thus serve as an honest signal of an individual's ability or willingness to defend a territory (Searcy et al. 2014), as has been shown in black-capped chickadees (Baker et al. 2012). It is therefore possible that increases in singing rate are facilitated by reductions in song length that conserve energy or provide time to breathe. Baker et al. (2012) did not test whether increased song rate was associated with a concomitant reduction in the length of individual songs, and the current study did not test whether decreases in song length were associated with a concomitant increase in song rate. Similar to Searcy et al. (2006) and Baker et al. (2012), coupling a taxidermic mount with song playback would offer insights into the aggressive intent of ruby-crowned kinglets and allow the examination of potential trade-offs between song rate and song length during aggressive encounters.

Using microphone arrays, we quantified daily, seasonal and contextual singing patterns of breeding ruby-crowned kinglets to provide insight into their behaviour and ecology. Identifying when and in what context a bird sings allows one to further investigate the causes behind the patterns observed, and we suggest that our approach could be useful for studying other species. The current study provides a quantitative basis for understanding the acoustic communication of ruby-crowned kinglets in relation to territorial defence and mate attraction.

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*Permits* – All research complied with the 'Guidelines for the treatment of animals in behavioural research and teaching' set forth by the Animal Behavior Society and the Association for the Study of Animal Behavior and was approved under Memorial Univ. of Newfoundland animal use protocol number 15-02-DW.

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## Data availability statement

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.jm63xsj75> and <https://doi.org/10.5061/dryad.g1jwstqnh> (Fahmy and Wilson 2020).

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