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ORIGINAL ARTICLE

Singing in a fragmented landscape: wrens in a tropical dry forest show sex differences in the effects of neighbours, time of day, and time of year

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Abstract

Songbird vocalizations serve diverse functions including territory defence against neighbouring conspecific animals. In tropical and south-temperate regions, it is commonplace for both female and male songbirds to sing solo songs and coordinated vocal duets to defend their territories. The Área de Conservación Guanacaste in Costa Rica is the site of an ambitious conservation effort to protect and regrow tropical dry forests; it consists of a mosaic of regenerating and mature forest patches, presenting a special opportunity to study the effect of different numbers of neighbours on male and female wrens living in these fragmented forests. We analyzed recordings of Rufous-and-white Wrens (*Thryophilus rufalbus*) over a 17-year period, focusing on vocal behaviour and variation in the number of territorial neighbours within the fragmented landscape in the conservation area, and on diel and seasonal variation in female and male song. We hypothesized that the number of conspecific neighbours would influence vocal behaviours, including female and male solo song rate, duet responsiveness rate, repertoire use, and song-switching rate. For females, we found that wrens change song-types more often in areas with more neighbours, whereas the other aspects of female vocal behaviour did not vary with the number of neighbours. For males, we found no aspect of vocal behaviour that varied with the number of neighbours. For both sexes, we found variation in vocal behaviours with time of day and time of year, in keeping with previous research. Our results underscore the idea that we must explore female and male birds independently, because the sexes may respond differently to external factors. We did not find strong links between wren vocal behaviour and variation in the number of neighbours, yet we encourage further behavioural studies on vocal behaviour of birds with varying numbers of neighbours in fragmented landscapes.

Keywords Conspecific neighbours · Duetting · Female song · Forest fragmentation · Rufous-and-white Wren · *Thryophilus rufalbus* · *Thryothorus rufalbus* · Tropical dry forests

Zusammenfassung

Singen in einer fragmentierten Landschaft: Zaunkönige in einem tropischen Trockenwald zeigen Geschlechtsunterschiede hinsichtlich des Einflusses von Nachbarn, Tages- und Jahreszeit.

Die Lautäußerungen von Singvögeln erfüllen verschiedene Funktionen, einschließlich der Verteidigung des Reviers gegen benachbarte Artgenossen. In tropischen und südlich-gemäßigten Regionen setzen üblicherweise sowohl weibliche als auch männliche Singvögel Sologesänge sowie aufeinander abgestimmte Duette zur Revierverteidigung ein. In der Área de Conservación Guanacaste in Costa Rica bemüht sich ein ambitioniertes Projekt darum, die dortigen tropischen

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Trockenwälder zu schützen und nachwachsen zu lassen. Das Gebiet besteht aus einem Mosaik regenerierender und etablierter Waldstücke, was eine besondere Gelegenheit bietet, den Einfluss der Anzahl von Reviernachbarn auf männliche und weibliche Zaunkönige, die in diesen fragmentierten Wäldern leben, zu untersuchen. Über einen Zeitraum von 17 Jahren analysierten wir Gesangsaufnahmen von Rotrückenzaunkönigen (*Thryophilus rufalbus*) in der fragmentierten Landschaft des Schutzgebiets, wobei wir uns auf das Lautverhalten und die Variation in der Anzahl von Reviernachbarn sowie auf tägliche und saisonale Schwankungen im Gesang von Weibchen und Männchen konzentrierten. Wir nahmen an, dass die Anzahl der Artgenossen in Nachbarrevieren das Lautverhalten beeinflussen würde, einschließlich der Sologesangsraten von Weibchen und Männchen, der Reaktionsbereitschaft in Gesangsduetten, dem Repertoireeinsatz und der Rate, mit der zwischen verschiedenen Gesängen gewechselt wurde. Wir fanden, dass weibliche Zaunkönige in Gebieten mit mehr Nachbarn den Gesangstyp häufiger wechselten, während andere Aspekte des weiblichen Lautverhaltens nicht mit der Anzahl der Nachbarn zusammenhingen. Bei Männchen variierten keinerlei Aspekte des Lautverhaltens mit der Anzahl der Nachbarn. Für beide Geschlechter schwankte das Lautverhalten abhängig von der Tages- und Jahreszeit, was mit vorherigen Befunden übereinstimmt. Unsere Ergebnisse unterstreichen die Idee, dass weibliche und männliche Vögel getrennt untersucht werden sollten, da die Geschlechter auf externe Faktoren unterschiedlich reagieren könnten. Wir fanden keine starken Zusammenhänge zwischen dem Lautverhalten von Zaunkönigen und der Anzahl von Reviernachbarn, ermutigen jedoch die Durchführung weiterer Studien zum Lautverhalten von Vögeln mit unterschiedlich vielen Nachbarn in fragmentierten Landschaften.

Introduction

Birdsong research has traditionally focused on male song, but, more recently, a growing body of research has revealed that female song is widespread, especially in the tropics and south-temperate regions (Langmore 1998; Odom et al. 2014). The lack of understanding on the subject of female song has been the consequence of historical biases towards research in north-temperate ecosystems where female song is less common (Stutchbury and Morton 2001; Odom et al. 2014; Tobias et al. 2016). Several large-scale analyses have revealed that female song is associated with life history traits that include year-round territory defence, social monogamy, and sexual monochromatism (Najar and Benedict 2015; Odom et al. 2015; Tobias et al. 2016), and that female song serves similar functions to male song including territory defence, mate attraction, and mate guarding (Langmore 1998; Hall 2004; Cain and Langmore 2015; Najar and Benedict 2015). Following a call for increased focus on female song more than two decades ago (Langmore 1998), there has been growing documentation of female singing behaviour, and yet there is still much research to be done and many unanswered questions about female song (Odom and Benedict 2018). In particular, little is known about how variation in neighbouring territories due to habitat fragmentation, as well as patterns of diel and seasonal variation, influences female singing behaviour.

In species where both sexes sing, breeding partners may combine their vocalizations into duets, which occur when one member of a pair responds to the song of its mate, thus coordinating their behaviour in a jointly-produced vocalization (Hall 2004). Duets serve multiple functions that vary with context and species (Hall 2004; Mennill and Vehrencamp 2008). For example, in Rufous Horneros (*Furnarius rufus*), females and males use duets to cooperatively defend year-round territories, and to mutually guard mates (Diniz et al. 2018). In Venezuelan Troupials (*Icterus icterus*), duets are used to defend territory and maintain contact (Odom et al. 2017). In Barred Antshrikes (*Thamnophilus doliatus*), pairs produce duets to defend territories from rival pairs (Koloff and Mennill 2013). These examples indicate that duetting behaviour serves multiple functions, with joint territory defence being a common function across the diverse avian taxa in which duetting occurs (reviewed in Hall 2004). Whether duetting behaviour varies with the number of territorial neighbours has not been addressed previously.

In many taxa of birds, vocal activities are influenced by the number of nearby conspecific neighbours (Olinkiewicz and Osiejuk 2003; Liu 2004; Gorestskaia 2004; Yoon et al. 2012). For example, in a removal experiment with Chipping Sparrows (*Spizella passerina*), males greatly reduced their dawn singing rate when all of their conspecific neighbours were removed, and increased their song output when those neighbours were subsequently returned (Liu 2004). Male Corn Buntings (*Emberiza calandra*), conversely, showed higher song output when they had one neighbour compared to two or more neighbours, suggesting that males with many neighbours spend more time engaged in physical interactions with other males or listening to singing of their neighbours (Olinkiewicz and Osiejuk 2003). In Chaffinches (*Fringilla coelebs*), repertoire size was negatively correlated with number of territorial neighbours (Slater 1981). Although varying results were found across different species, most previous studies share a common theme: they focus solely on male birds in temperate locations. Many questions in bird behaviour that have been addressed only in north-temperate bird species need to be revisited in tropical and south-temperate taxa, and in birds where females sing and exhibit different life histories and behaviours.

We studied how variation in the number of territorial neighbours impacts vocal behaviours of Rufous-and-white Wrens (*Thryophilus rufalbus*), a species that defends year-round territories in the Neotropics and exhibits both female song and vocal duets. Females and males are monochromatic but can be distinguished by morphometric features, behaviour, and voice (Mennill and Vehrencamp 2008). Both female and male Rufous-and-white Wrens sing with eventual variety, repeating a given song type many times before switching to a new song type (Mennill and Vehrencamp 2005). Females sing less often than males and display a smaller repertoire of song types. Females usually sing at a higher average frequency than males, and their songs usually contain fewer trill syllables (Mennill and Vehrencamp 2005). Both sexes create duets by responding to their partner's songs, although females create duets more often than males (Mennill and Vehrencamp 2005). Independent song rate and duet responsiveness are highest in females during the pre-breeding season, and in males when their breeding partners are fertile (Topp and Mennill 2008). Playback experiments reveal that duets are especially common during territorial encounters (Mennill 2006; Mennill and Vehrencamp 2008). Anecdotally, in a previous study of Rufous-and-white Wrens, a male with no conspecific neighbours within 1.0 km was observed producing fewer songs than other males with neighbours (Topp and Mennill 2008). Rufous-and-white Wrens provide a special opportunity to compare intersexual differences in vocal behaviours, and the effects of number of neighbours on the vocal behaviour of both sexes.

Based on 17 years of field recordings of Rufous-and-white Wrens living in fragmented dry forests of northwestern Costa Rica, we tested the hypothesis that female and male vocal behaviours are influenced by the number of territorial neighbours. We predicted that with an increase in the number of neighbours, we would find differences in the following four features: (1) independent song rate (i.e., the song rate for solo songs plus the first song in a duet), (2) duet responsiveness (i.e., the proportion of their partner's songs that a bird responded to, turning their partner's song into a duet), (3) repertoire use (i.e., the number of song types sung divided by the number of songs recorded), and (4) song-type switching rate (i.e., how often birds switched to a new song). We also tested the hypothesis that male and female vocal behaviours would vary with time of day and time of year, in keeping with widely-recognized patterns in this and other species. We predicted that we would find temporal variation in the aforementioned four features of singing behaviour for both females and males.

Methods

Study system

Between 2003 and 2019, we studied a population of Rufous-and-white Wrens living in Sector Santa Rosa of the Área de Conservación Guanacaste (10°52′N, 85°36′W) in northwestern Costa Rica. We collected data between April and July of each year, which coincides with the onset of heavy rains and the breeding activities for this population (Topp and Mennill 2008; Woodworth et al. 2018). Each year, our team captured and banded birds, monitored breeding activities, and collected recordings in ~ 240 ha of forest in Sector Santa Rosa. On a daily basis, we traveled through the study site, detecting all birds based on their vocalizations and by observing their unique combinations of coloured leg band. We surveyed for wrens by returning to the same areas where wrens held territories in previous years. We found new territories by exploring mature forests within the study site and listening for wrens.

The Área de Conservación Guanacaste is the result of a decades-long conservation effort to protect the fragmented remains of the mature dry forest, and regrow the surrounding forests (Allen 2001). Within Sector Santa Rosa of the Área de Conservación Guanacaste, patches of mature primary forest exist in a matrix of regenerating forest; the mature semi-evergreen forest fragments are home to a resident population of Rufous-and-white Wrens (Fig. 1). In Sector Santa Rosa, Rufous-and-white Wrens are only found in primary forest and patches of older regenerating forest (50 + year old forest; Owen et al. 2020), and most pairs in the study population nest in primary forest patches (Osmun and Mennill 2011). The size of this population, and therefore the number of wrens within each forest patch, varies each year with annual survival, with a heavy influence of changes in temperature (Woodworth et al. 2018). In less fragmented areas of Costa Rica, Rufous-and-white Wrens occur at higher densities with higher numbers of neighbours (Graham et al. 2018). Based on these observations, we make the assumption that forest fragmentation influences the number of neighbours from which Rufous-and-white Wrens defend territories. Rufous-and-white Wrens provide a compelling species for this study because they defend year-round territories from conspecifics in mature forest fragments of Sector Santa Rosa. Most of these mature forest patches are home to one or more breeding pairs of Rufous-and-white Wrens, resulting in wrens with different numbers of neighbours.

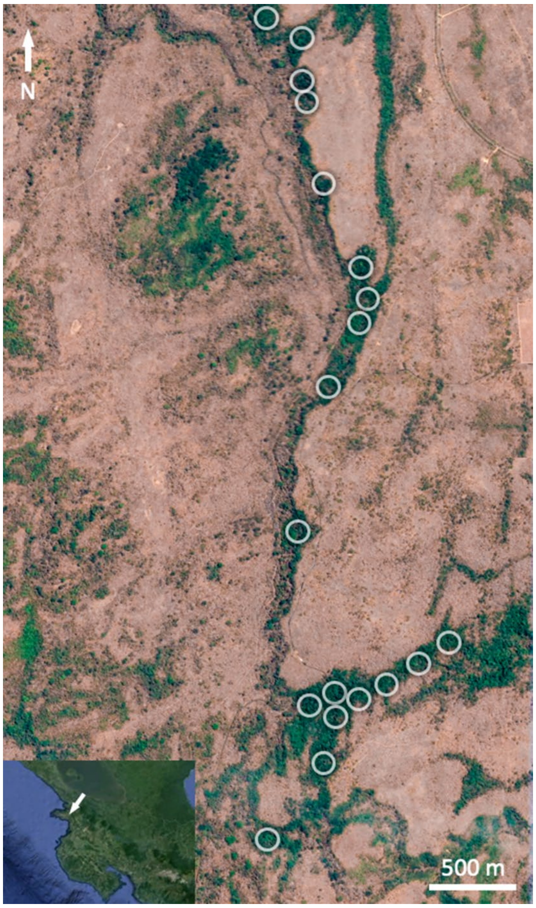


Fig. 1 Map of Sector Santa Rosa of the Área de Conservación Guanacaste, Costa Rica, with locations of Rufous-and-white Wren territories for a single year represented in circles. Imagery is from the end of a dry season in May 2013. Mature forest fragments are visible in dark green. Inset map shows the location of the study site within Central America (colour figure online)

As in previous studies of this population, we define "neighbours" as pairs of wrens with territory boundaries less than 50 m apart, and we define "territory boundaries" as areas where two or more neighbouring pairs were observed having vocal or physical interactions (Battiston et al. 2015). Rufous-and-white Wrens defend large territories, often with undefended space between them, therefore it is unlikely that wrens could hear or respond to neighbours with non-adjacent territories (Mennill and Vehrencamp 2008). Across the 17 years of this study, we found pairs of wrens with zero to four neighbours. Most pairs of wrens in our study population had one or two neighbouring pairs, and several had zero or three neighbouring pairs, whereas four neighbouring pairs was rare. We focused our analyses on a subset of all available data, to have a similar representation of birds with 0, 1, 2, or 3 + neighbours, limited by the number of pairs with zero and three and four neighbours. We chose to analyze birds with three and four neighbours together (i.e., three or more neighbours) given that we rarely found birds with four neighbours.

Recording techniques

To study the vocalizations of Rufous-and-white Wrens, our research team recorded wrens on their territories, visiting each territory at least once every two weeks, and more often whenever possible. We recorded and observed each pair for ~ 1 h during the early morning hours (0500 to 0700 h; sunrise occurs at ~ 0515 h). Wrens were recorded using two approaches. First, we recorded wrens on their territories by following animals and using digital recorders (Sennheiser MKH70 or ME67) and shotgun microphones (Marantz PMD660 or PMD670; 22,050 Hz sampling rate, 16-bit encoding accuracy, WAVE format). In some recordings, playback was used, or the recordist whistled to imitate Rufous-and-white Wren song, to draw birds near to observe colour bands; given that playback influences song rate (Mennill 2006; Mennill and Vehrencamp 2008), we excluded any sections of recordings where playback or imitation was evident. If a period of playback or whistling was followed by an hour of silence from the recordist, we included the subsequent recordings in our analysis (previous research has confirmed that response to playback by Rufous-and-white Wrens decline to baseline levels at intervals less than 1 h; Mennill and Vehrencamp 2008). Approximately 73% of recordings in this study were collected through in-person focal recordings.

In addition to focal recordings, we also used passive acoustic monitoring to collect recordings of wrens. Passive acoustic monitoring equipment was placed within a pair's territory, usually in the approximate center of the pair's territory or near a nest. Passive acoustic monitoring equipment varied over the 17 years: in 2003 and 2004, recordings were collected used eight-channel microphone arrays (details in Mennill et al. 2006; Mennill and Vehrencamp 2008); from 2007 to 2010, recordings were collected using automated recorders with elevated omni-directional microphones (Sennheiser ME62) connected to solid-state digital recorders (Marantz PMD670; details in Mennill and Vehrencamp 2005); and from 2011 to 2019 recordings were collected using autonomous recorders (Song Meter models SM1 and SM2 + ; Wildlife Acoustics Inc. Concord, Massachusetts, USA; details in Mennill et al. 2012). No automated recordings were collected in 2005 and 2006. Our team collected autonomous recordings at all hours of the day, however, the majority of data used in our analyses are from the morning hours (Harris et al. 2016). Approximately 27% of recordings were collected using passive acoustic monitoring.

Song analysis

We analysed songs and duets of 45 pairs of Rufous-and-white Wrens. We chose 90 unique individuals for vocal behaviour comparisons, and included at least one pair from each of the 17 years of the study. All pairs had at least 2 h of audio recordings, and the average ± SE total recording length was 13.1 ± 2.5 h ($n = 45$ pairs).

We analyzed recordings using SYRINX-PC sound-analysis software (J. Burt, Seattle, Washington). Each Rufous-and-white Wren has a unique repertoire of song types, and for each bird, we built a library of sound files representing all of the song types for that individual (as in Harris et al. 2016). Song types can be differentiated on the basis of fine-structural features on the sound spectrograms (Mennill and Vehrencamp 2005). Due to differences in female and male song structure (Fig. 2), the sex of singing birds can be identified from the recordings as well. For each recording, individual birds were identified by visualizing their songs on a sound spectrogram, and each song was annotated with the individual's identity (i.e., the unique colour band combination as dictated by the recordist) and the song type. For each wren, we counted the number of independent songs they produced (i.e., solo songs or songs where a bird sang and then its partner responded, turning the song into a duet). If a wren sang within one second of its mate, we deemed this to be a duet and we counted the number of male-created duets (i.e., duets where the male sang in response to a female song, turning her song into a duet) and female-created duets (i.e., duets where the female sang in response to a male song, turning his song into a duet), as in previous studies of duetting in this population (Mennill and Vehrencamp 2005; Topp and Mennill 2008). When duets involved more than one song from the male or the female, we considered only the first contribution of each bird to the duet in our analysis of number of duets created.

Our analysis of vocal behaviour focused on four features of singing behaviour for each sex: independent song rate, duet responsiveness, repertoire use, and song-switching rate. We calculated independent song rate by dividing the number of independent songs per recording by the total length of the recording (measured from first song to last song). We calculated duet responsiveness by dividing the number of duet-creation songs for each bird by the total number of songs sung by its partner (i.e., total number of opportunities to create a duet). We calculated repertoire use by counting the total number of unique song types used in a single recording and dividing by the total number of songs (both

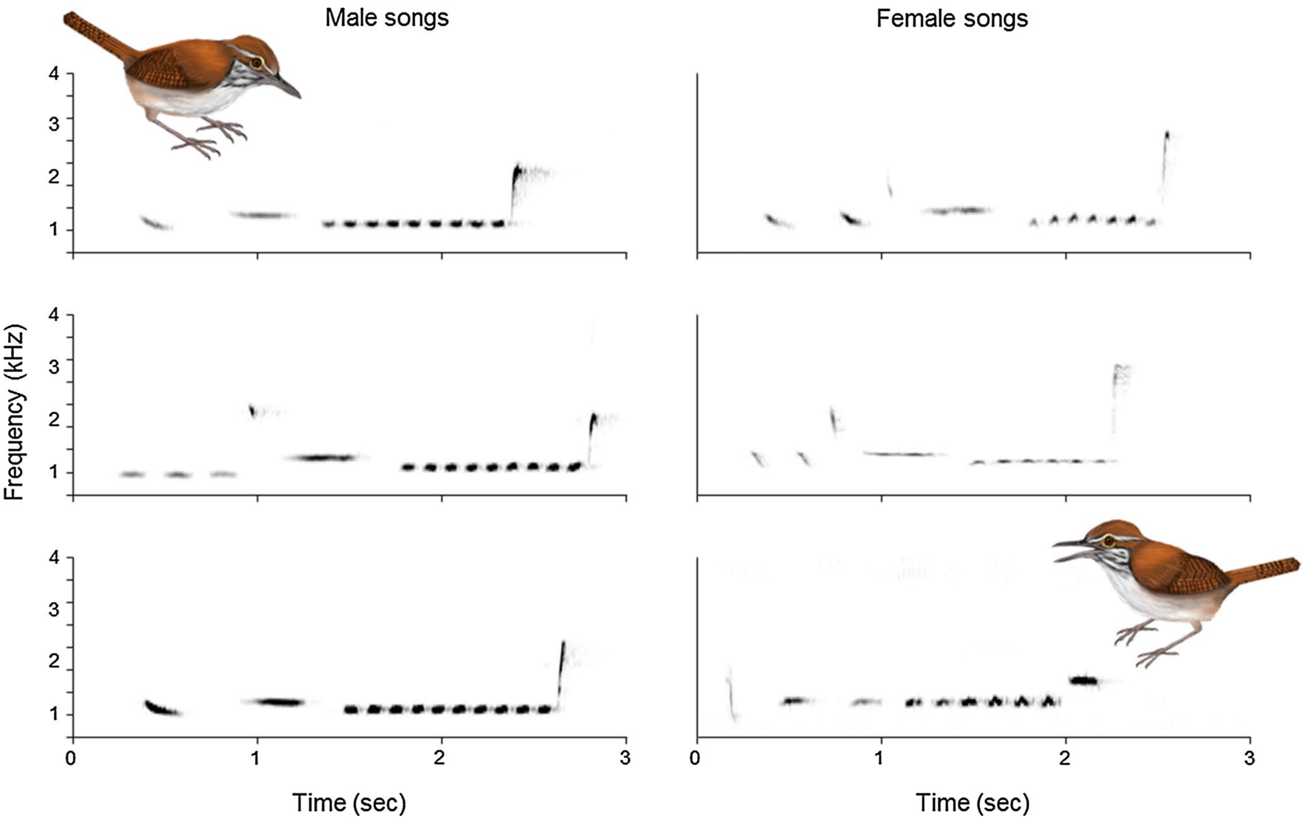


Fig. 2 Sound spectrograms of the songs of Rufous-and-white wrens, contrasting three male songs (left) and three female songs (right). Male songs are longer, with more trill notes, and lower frequency components than female songs (Mennill and Vehrencamp 2005)

solo and duet songs) produced by that individual during that same recording. Finally, we calculated song-type switching rates by dividing the total number of song type changes in a recording by the total number of independent songs (Molles and Vehrencamp 1999).

Statistical analyses

We conducted all analyses in R v3.6.1 (R Development Team 2019). We created eight linear mixed models (four for each sex, for the four variables listed above) using R package ‘lme4’ (Bates et al. 2015). Dependent variables were independent song rate, duet responsiveness, repertoire use, and song type switching rate. Our four dependent variables each accounted for different aspects of wren singing behaviour, and were not highly correlated ($r < 0.5$). All models included fixed effects of number of neighbours, year, time of day, and ordinal day. We included pair identity as a random effect. We chose to include time of day and ordinal day as fixed effects because previous research on this population showed that male and female vocal behaviours were influenced by time of day and time of year (Topp and Mennill 2008). We also included year as a fixed effect because of the documented influence of climate cycles and extreme weather events on survivorship within our study population over the years (Woodworth et al. 2018). We used R package ‘lmerTest’ to obtain P-values for our models (Kuznetsova et al. 2017).

Previous research on this population of wrens showed substantial sex differences in vocalization rates and repertoire sizes (Mennill and Vehrencamp 2005). Given the remarkable differences in the vocal behaviour of females and males, we felt it was inappropriate to compare the sexes within the same models. To confirm that the sexes exhibit substantial differences, we created four additional and simplified linear mixed models comparing females and males for each of the four response variables. These four models compared the sexes for each of our four independent variables, without any fixed effects (we included the random effect of pair identity). These models confirmed that females and males exhibit very different vocalization rates (Table 1), further justifying the importance of analyzing females and males separately in our final analyses.

Given that female Rufous-and-white Wrens vocalize less often than males, and that females create more duets than males (Mennill and Vehrencamp 2005), our data exhibited an inflation of zero values. We used Tukey’s Ladder of Powers (Tukey 1977) in R package ‘rcompanion’ (Mangiafico 2016), a transformation technique for addressing violations of assumptions including normality of residuals and equality of variances. We used Levene’s Tests and variance inflation factors from R package ‘car’ (Fox and Weisberg 2019) to assess variance equality and collinearity. We visually assessed Q–Q plots of residuals for normality, and we plotted residuals by fitted values to evaluate homoscedasticity.

To test our hypothesis that vocal behaviour is influenced by number of neighbours, we first examined our data using ANOVA on our eight models with R package ‘car’ (Fox and Weisberg 2019). To test our hypothesis that vocal behaviour would change with time of day and time of year, we first examined our models using ANOVA, and then assessed results from our linear mixed models to determine the direction of the effect. We conducted post-hoc tests on any models showing differences in neighbour groups from the ANOVA using R package ‘emmeans’ (Lenth et al. 2020).

Results

Based on repeated recordings of 45 pairs of Rufous-and-white Wrens collected over a 17-year period, we found that several aspects of singing behaviour varied with time of year and time of day, and one singing behaviour varied with number of conspecific neighbours (Table 2; Fig. 3). We found limited support for the hypothesis that vocal behaviour changes with the number of neighbours for wrens living in a fragmented forest; for females, song-type switching rates change with different numbers of neighbours (Table 2; Fig. 3). Tukey–Kramer post-hoc tests showed differences

Table 1 Results from linear mixed effects models examining relationships between four response variables, (independent song rate, duet responsiveness, repertoire use, song-type switching rate) by sex (male and female)

		Estimate	SE	t	p
Independent song rate	Intercept	5.3	4.1	1.3	0.2
$R^2_m = 0.4$; $R^2_c = 0.4$	Sex	107.3	5.2	20.6	< 0.001
Duet responsiveness	Intercept	− 0.5	0.05	− 9.1	< 0.001
$R^2_m = 0.3$; $R^2_c = 0.4$	Sex	1.2	0.07	15.8	< 0.001
Repertoire use	Intercept	0.6	0.06	9.8	< 0.001
$R^2_m = 0.3$; $R^2_c = 0.3$	Sex	− 1.1	0.07	− 14.4	< 0.001
Song-type switching rate	Intercept	0.2	0.2	1.5	< 0.001
$R^2_m = 0.09$; $R^2_c = 0.2$	Sex	1.2	0.2	7.3	< 0.001

Random effect of pair ID is included in each model. Marginal and conditional R^2 values are provided for each model

Table 2 Results from eight linear mixed models examining relationships between four response variables (independent song rate, duet responsiveness, repertoire use, song-type switching rate) by number of neighbours for both male and female Rufous-and-white Wrens using ANOVA

		Males				Females			
		F	*p*	Estimate	SE	*F*	*p*	Estimate	SE
Independent song rate	Number of neighbours	0.9	0.4			1.2	0.3		
	Year	0.03	0.9	0.002	0.01	0.5	0.5	− 0.01	0.01
	Ordinal day	46.5	< 0.001	0.01	0.002	7.7	0.006	− 0.007	0.003
	Time of day	36.3	< 0.01	− 0.3	0.05	0.6	0.5	− 0.05	0.06
		$R^2_\mathrm{m} = 0.24$; $R^2_\mathrm{c} = 0.29$				$R^2_\mathrm{m} = 0.05$; $R^2_\mathrm{c} = 0.2$			
Duet responsiveness	Number of neighbours	0.1	0.9			2.0	0.1		
	Year	0.4	0.5	− 0.01	0.01	0.6	0.4	− 0.01	0.02
	Ordinal day	0.9	0.4	0.003	0.003	40.8	< 0.001	− 0.01	0.002
	Time of day	0.8	0.4	− 0.06	0.07	0.005	0.9	− 0.004	0.06
		$R^2_\mathrm{m} = 0.01$; $R^2_\mathrm{c} = 0.04$				$R^2_\mathrm{m} = 0.2$; $R^2_\mathrm{c} = 0.3$			
Repertoire use	Number of neighbours	0.3	0.8			1.0	0.4		
	Year	2.8	0.1	0.03	0.02	0.6	0.4	0.005	0.002
	Ordinal day	18.5	< 0.001*	− 0.01	0.002	4.6	0.03	0.005	0.002
	Time of day	1.5	0.2	0.07	0.06	0.4	0.6	− 0.03	0.06
		$R^2_\mathrm{m} = 0.08$; $R^2_\mathrm{c} = 0.2$				$R^2_\mathrm{m} = 0.05$; $R^2_\mathrm{c} = 0.1$			
Song-type switching rate	Number of neighbours	0.5	0.7			2.9	0.03		
	Year	0.7	0.4	− 0.1	0.01	3.0	0.08	− 0.02	0.01
	Ordinal day	1.8	0.2	− 0.002	0.002	0.7	0.4	− 0.002	0.002
	Time of day	2.1	0.2	− 0.06	0.05	2.2	0.1	− 0.08	0.06
		$R^2_\mathrm{m} = 0.03$; $R^2_\mathrm{c} = 0.2$				$R^2_\mathrm{m} = 0.07$; $R^2_\mathrm{c} = 0.07$			

Results from regression are provided for fixed effects of year, ordinal day and time of day only. Random effect of pair ID is included in each model. Marginal and conditional R^2 values are provided for each model

at 0.95 confidence levels in one comparison: female wrens with one neighbour had lower song-switching rates than those with two neighbours ($p = 0.02$). No other comparisons yielded results supporting the hypothesis that vocal behaviour varies with number of neighbours.

We found that females and males exhibited different time-of-day and time-of-year effects. Male independent song rate and repertoire use were positively related to time of year (Table 2; Fig. 4), such that males sang more songs and used a larger proportion of their vocal repertoire as the breeding season progressed. Male independent song rate showed negative relationships with time of day (Table 2; Fig. 5), such that males sang less often as the day progressed. Male duet-responsiveness and song-type switching rates were not influenced by time of day or time of year.

Females showed different patterns of temporal variation in independent song rate and duet responsiveness compared to males. Female independent song rate and duet responsiveness were negatively related to time of year, such that females reduce their song output and duet-responsiveness as the breeding season progressed (Table 2; Fig. 5). Females showed a similar pattern to males in their repertoire use; repertoire use by female wrens increased with ordinal day, such that females, like males, use a greater proportion of their song repertoire as the breeding season progressed (Table 2; Fig. 5). Female song-type switching rate was not influenced by time of day or time of year.

Discussion

We tested the influence of time of day, time of year, and variation in number of neighbours on vocal behaviour of Rufous-and-white Wrens. Although we did not find that male song or duetting behaviour was influenced by number of neighbours for either females or males, we found an effect on female singing behaviour: females with more neighbours switch song types at higher rates. We conclude that the number of territorial neighbours has no effect on male vocal behaviours, but an effect on song-type switching rates in female Rufous-and-white Wrens. We also found that female and male vocal behaviours differ with time of day and time of year.

Many studies on temperate birds have suggested that vocalization rates change with different numbers of

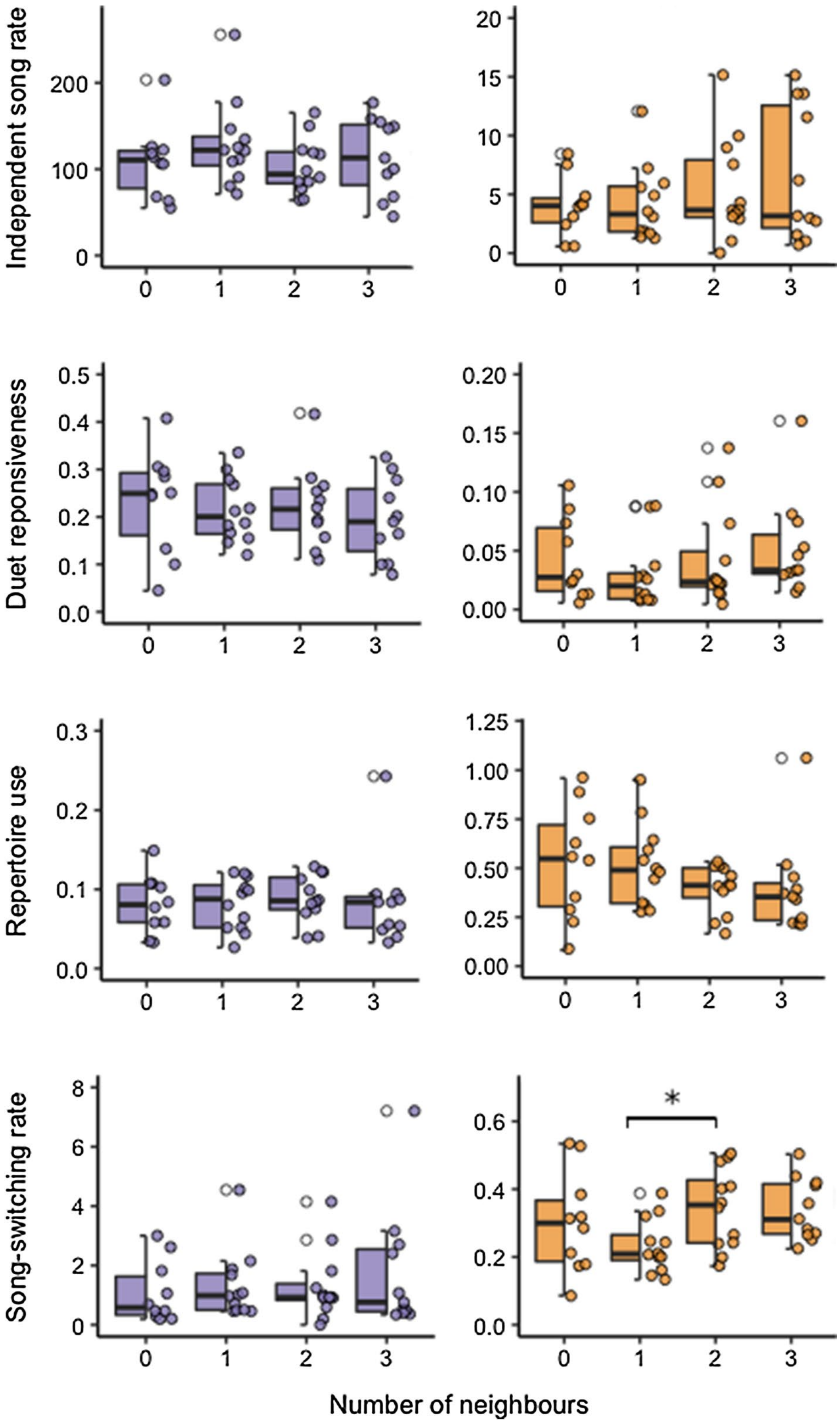


Fig. 3 Male (left, purple) and female (right, orange) Rufous-and-white Wren vocal behaviour versus the number of conspecific neighbours. Boxplots summarize the mean and interquartiles ranges for each vocalization rate in all females and males in the study sample. Individual points represent mean vocalization rates per bird. Asterisk denotes comparisons where there was an effect with $p < 0.05$; open circles represent boxplot outliers (colour figure online)

neighbours, although the patterns vary across taxa (Olinkiewicz and Osiejuk 2003; Liu 2004; Gorestskaia 2004; Yoon et al. 2012). In Chipping Sparrows (*Spizella passerina*; Liu 2004), Willow Warblers (*Phylloscopus trochilus*; Gorestskaia 2004), and Orange-crowned Warblers (*Oreothlypis celata*; Yoon et al. 2012), vocalization rates increased with higher population density and increased numbers of neighbours. In contrast, Corn Buntings showed a decrease in song output with more neighbours (Olinkiewicz and Osiejuk 2003). Similar to our observations in

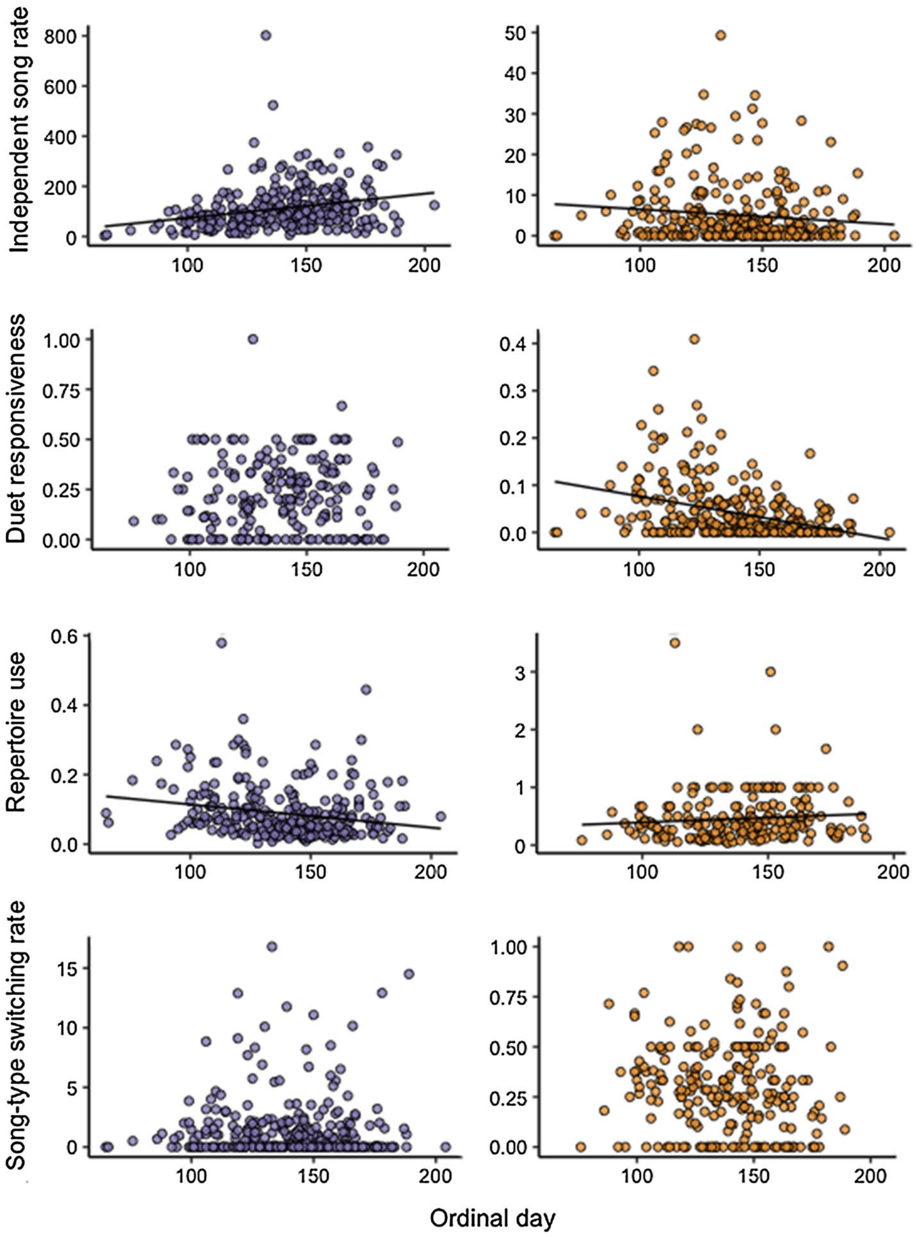
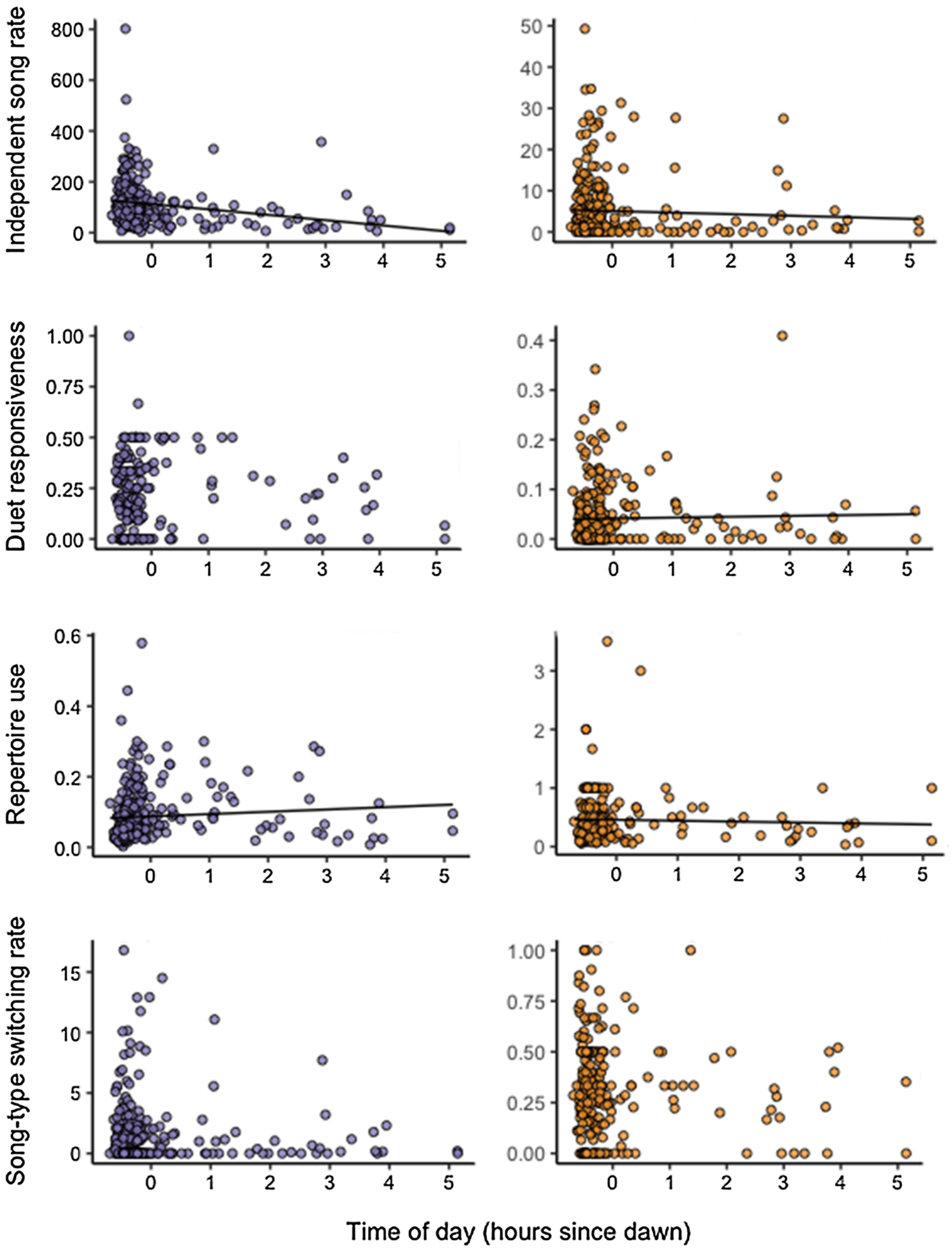


Fig. 4 Male (left, purple) and female (right, orange) Rufous-and-white Wren vocal behaviour versus ordinal day. Day 100 is April 10 (April 11 in leap years). Line of fit is shown for any plots where there was an effect with $p < 0.05$ (colour figure online)

male Rufous-and-white Wrens, House Wrens (*Troglodytes aedon*; Wilson and Bart 1985) and Black-throated Blue Warblers (*Setophaga caerulescens*; Sillett et al. 2004) showed no effect of population density or neighbours on vocalization rates. Our research highlights the importance of continued research on tropical species, including revisiting questions concerning bird behaviour that have only been addressed with temperate or migratory species.

Female Rufous-and-white Wrens showed higher song-type switching rates with higher number of neighbours, particularly in a post-hoc comparison of females with one neighbour versus two neighbours. Previous research has found that song-type switching can play a role in aggressive signalling in male songbirds (Searcy and Beecher 2009; Deoniziak and Osiejuk 2020), and may be associated with song-type matching interactions (Vehrencamp 2001; Akçay et al. 2013), although there has been very little study of song-type switching behaviour in female birds. If higher rates of song-type switching are associated with aggressive interactions with neighbours, and given that we found some evidence that female wrens have higher song-type switching rates with more neighbours, then we suggest that female wrens may be using song-type switching in territorial defence against conspecifics. We did not observe the same pattern in male wrens, and therefore this explanation does not apply to both sexes, raising the idea that the sexes may show different responses to living with different numbers of neighbours. Our results emphasize the increasingly popular

Fig. 5 Male (left, purple) and female (right, orange) Rufous-and-white Wren vocal behaviour versus time of day. Time of day is expressed in hours since 0500 h. Line of fit is shown for any plots where there was an effect with $p < 0.05$ (colour figure online)



idea that female behaviour needs to be addressed in studies of bird behaviour, which has traditionally focused solely on male birds (Topp and Mennill 2008; Odom et al. 2014; Riebel et al. 2019). Our results also suggest there is a need to test hypotheses in females and males separately, and that it is important not to assume that behaviours from both sexes will be influenced in the same way by external factors. Our results corroborate previous findings that male and female Rufous-and-white Wrens exhibit dramatic differences in singing behaviour, not just in song output, but also in duet responsiveness, repertoire use, and song-type switching rate (Mennill and Vehrencamp 2005; Table 1). Previous research on female song has shown stronger physical responses by females to playback of conspecific females rather than males (Mennill and Vehrencamp 2008; Krieg and Getty 2016), but similar levels of vocal responses to both sexes. We recommend future research into the role of neighbours should focus on disentangling the effect of male and female neighbours on female vocal behaviours.

There are a number of possible explanations for the results we observed. Given that male and female vocalizations serve multiple functions, it is likely that the diverse functions of both male and female song (including mate attraction or communication between mates) complicate or obscure any effects of territorial neighbours. Over the 17 years of study on this population of wrens, our research team has mainly collected data in the weeks before and following the onset of the breeding season, when male and female wrens are focused on breeding activities. Because songs and duets serve multiple functions (Hall 2004; Catchpole and Slater 2008), it is possible that the effects of neighbours are masked by the influence of other breeding activities (e.g., attracting an extra-pair mate, or communication between mates). Studying vocal behaviour in these wrens at other times of year could shed light on whether neighbours influence vocal behaviours in the non-breeding season. Another possibility is that Rufous-and-white Wrens do not respond strongly to familiar neighbours, a phenomenon known as the "dear enemy effect" (Temeles 1994), although one previous experimental study on this population suggests that Rufous-and-white Wrens do not exhibit this effect (Battiston et al. 2015). In the same study, the authors examined aggressive non-vocal behaviours of Rufous-and-white Wrens to neighbours, such as distance to closest approach (Battiston et al. 2015). Rufous-and-white Wrens may use non-vocal behaviours in territorial disputes differently than vocalizations. Therefore, future research on the effect of neighbours might consider including non-vocal behaviours.

We observed relationships between singing behaviours and both the time of day and the time of year for both females and males. For males, independent song and song-type switching rates decreased later in the day, while independent song rate and repertoire use increased as the season progressed. In females, independent song rate and duet responsiveness were highest earlier in the season, while repertoire use was highest later in the season. Similar to previous research on Rufous-and-white Wrens in this population, we found that independent song rate had a positive relationship with time of year in male wrens, and a negative relationship in female wrens (Topp and Mennill 2008). Previous research on female song, including in Rufous-and-white Wrens, has shown that female song reaches its highest level before the start of the breeding season (Topp and Mennill 2008). For males, this peak in independent song rate occurs later in the year at the onset of the breeding season when the female becomes fertile (Topp and Mennill 2008). We did not find relationships with time of day or year and song-type switching, and in males, we did not find that duet responsiveness was related to time of day or year. However, we did observe that females were less likely to respond to male songs to form duets later in the season, a phenomenon that probably reflects the increasing attention that females devote to nesting and parental care later in the year (Topp and Mennill 2008). Despite an overall reduction in vocal output by females, we found that female wrens increased their repertoire use with time of year, exhibiting more song types later in the year. Later in the year, females may produce more song types during the period when young birds are learning to sing. More research into repertoire use by females is needed to better understand this relationship.

Habitat fragmentation has been shown to have different effects on population density or territory size in different bird species and functional groups (Hansbauer et al. 2008). Generalist and edge species tend to increase in population size following fragmentation, while forest specialist species generally decline (Bender et al. 1998). The impacts of habitat fragmentation on population sizes in birds is complex, therefore, we can improve understanding of how habitat change influences social behaviours by investigating more closely the effect of neighbours or population density on these behaviours. Due to the fragmented nature of mature tropical dry forests in Costa Rica, Rufous-and-white Wrens share suitable habitat patches with zero to several neighbours. Although most vocal behaviours were not influenced by the number of neighbours in our analysis, we did find that female song-type switching was higher in birds with more neighbours. Therefore, habitat fragmentation may be indirectly influencing singing behaviour in female wrens through population dynamics. If habitat fragmentation, or future forest regeneration, influences Rufous-and-white Wren density in this population, we expect that some wren vocal behaviours, particularly in female wrens, could be affected. By studying behaviours of female and male animals living in changing habitats, we can better inform conservation initiatives while increasing our understanding of how different sexes respond to habitat changes.

In this paper, we emphasize the importance of analyzing female and male behaviours separately due to considerable differences in some behaviours between sexes. We found an effect of neighbours on females but not males, and differences in responses to time of day and time of year suggesting that female and male wrens respond differently to environmental and social factors. We recommend future research consider separate analyses of female and male behaviours, especially when those behaviours are on substantially different scales.

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Declarations

Conflict of interest The authors declare there are no conflicts of interest.

Ethical approval Our research was authorized under permits from the Animal Care Committee of the University of Windsor and MINAE permits from the Government of Costa Rica.

Availability of data and material All data are available upon reasonable request.

Code availability R code for analyses are available upon reasonable request.

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