



Male and female signaling behavior varies seasonally during territorial interactions in a tropical songbird

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Abstract

In many tropical birds, both sexes use conspicuous vocal signals during territorial interactions. Although a growing number of studies examine male and female signals in the context of coordinated vocal duets, the use of vocal signals by both sexes in non-duetting species is poorly documented, even though these species are more numerous than duetting species. Furthermore, few studies of tropical non-duetting species test for seasonal variation in signaling behavior. We studied season-specific and sex-specific variation in signaling behavior of a tropical resident songbird, the Rufous-capped Warbler (*Basileuterus rufifrons*), by conducting a playback experiment where we simulated conspecific territorial intruders producing three types of vocalizations (male songs, female calls, or a “pair” with simultaneous male songs and female calls) and a heterospecific control. We repeated playback during the pre-breeding and breeding seasons. Response intensity to playback varied with season and sex of the focal birds. During the pre-breeding season, both sexes showed strong physical approach responses and vocal responses to all conspecific intrusions, especially paired intrusions. During the breeding season, males responded strongly to all conspecific treatments, whereas females showed little response. Although females primarily used calls in response to conspecific playback, many females also sang, especially during the non-breeding season. Our results therefore suggest that both male and female signals are used for shared territory defense, but that the contributions of each sex to territory defense vary seasonally. Our results also contribute to our understanding of the evolution of combined male and female signaling during territory defense.

Significance statement

In resident tropical animals, both males and females often use conspicuous signals during territorial interactions with conspecifics. Seasonal and sex-specific variation in vocal behavior of tropical resident birds during these territorial interactions has received little research attention. We conducted a playback experiment to investigate season- and sex-specific variation in signaling behavior of tropical Rufous-capped Warblers (*B. rufifrons*). In the pre-breeding season, both sexes responded strongly to all conspecific intrusions, especially paired intrusions, by approaching the playback speakers and vocalizing. In the breeding season, males actively defended the territory alone whereas females rarely responded. Our results suggest that both sexes of Rufous-capped Warbler use vocal signals for shared territory defense, although the contributions of each sex to territory defense differ seasonally.

Keywords Female signals · Female song · Mate competition · Rufous-capped Warbler · Stereo playback experiment · Territory defense

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Introduction

Many animals use conspicuous vocal and visual signals to communicate, including complex vocalizations and brightly colored ornaments. Across taxa, these signals are known to function in mate attraction, competition for mates, and defense of territorial resources (Bradbury and Vehrencamp 2011). Traditionally, sexual selection on males to attract and compete

for mates was thought to be the primary mechanism driving the evolution of conspicuous traits (reviewed in Kraaijeveld et al. 2007; Tobias et al. 2012). In many tropical birds, however, both males and females produce similar vocal and visual signals, and both sexes defend territories throughout the year (Stutchbury and Morton 2001). Furthermore, recent analyses show that male ornaments in dimorphic species are not necessarily sexually selected (Candolin and Tukiainen 2015). An alternative view suggests that female signals, including vocalizations and bright coloration, function in competition among conspecifics for territorial resources (West-Eberhard 1983; Tobias et al. 2012).

Most research on female signaling in birds has focused on duetting species, where both members of a breeding pair sing in a coordinated manner (Hall 2004; Dahlin and Benedict 2014). When duets are played to territorial pairs, birds often show strong coordinated responses to territorial intrusions by producing more duets and staying close together (e.g., Hall and Peters 2008; Mennill and Vehrencamp 2008; Benedict 2010; Dahlin and Wright 2012; Koloff and Mennill 2013). A growing body of evidence suggests that duets are multi-functional signals and that birds use duets primarily in cooperative territory defense, mate-guarding, and maintaining contact between pair members (reviewed in Douglas and Mennill 2010; Dahlin and Benedict 2014; Tobias et al. 2016). In contrast, the function of female vocal signals in non-duetting species has received little study, in spite of the fact that 84% of all bird species do not produce duets (Tobias et al. 2016). Many non-duetting tropical resident species also defend shared territories year-round (e.g., Freed 1987). Females of these species often use calls only (e.g., Adelaide's Warblers, *Setophaga adelaidae*; Staicer 1996) or both calls and songs that are not produced as duets (e.g., Banded Wrens, *Thryophilus pleurostictus*; Hall et al. 2015) during territorial interactions.

In territorial resident birds that breed seasonally, both male and female signals might serve different functions across the seasons (e.g., Fedy and Stutchbury 2005; Gill et al. 2007). Most studies have focused on temperate zone species where territory defense is performed primarily by males during the breeding season (reviewed in Catchpole and Slater 2008). In tropical resident species, however, both sexes may be involved in territory defense and these behaviors may persist throughout the year (Stutchbury and Morton 2001). Previous studies conducted on tropical resident duetting birds during both the breeding and non-breeding seasons indicate that territorial responses are season- and sex-specific (Fedy and Stutchbury 2005; Gill et al. 2007, 2008; Odom et al. 2017). In White-bellied Antbirds (*Myrmeciza longipes*), both males and females show high levels of aggression to intruders and respond more intensely during the non-breeding season, providing evidence that both sexes contribute to shared resource defense (Fedy and Stutchbury 2005). In Buff-breasted Wrens (*Cantorchilus leucotis*), both sexes show high aggression to

rival females and pairs in both seasons, suggesting both territory defense and female-female competitive functions for their signals (Gill et al. 2007, 2008). Remarkably few studies have quantified seasonal variation in vocalizations of males versus females for resident, non-duetting bird species (Logue 2005). Furthermore, although both duetting and non-duetting species often use calls for territory defense (e.g., Staicer 1996; Neudorf and Tarof 1998), few playback studies have presented both songs and calls to territorial pairs to compare conspecific responses to these signals (Sandoval et al. 2013).

In this investigation, we studied Rufous-capped Warblers (*Basileuterus rufifrons*), non-duetting tropical resident songbirds with conspicuous male and female vocal signals, using an experimental approach to evaluate whether these animals exhibit season- and sex-specific variation in signal use. We evaluated season- and sex-specific variation in territorial behavior in the context of three non-mutually-exclusive hypotheses of signal function: (1) territory defense, (2) intrasexual competition for mates, and (3) mate attraction (Kraaijeveld et al. 2007; Tobias et al. 2011). If birds use their signals primarily for territory defense, we predicted that male and female vocal signals would be used throughout the year in response to territorial intrusions, and we predicted that both sexes would respond strongly to both intersexual and intrasexual conspecific intruder signals (Tobias et al. 2011; Dowling and Webster 2016). Conversely, if birds use their signals primarily for intrasexual competition for mates, we predicted that these signals would be used more frequently during the breeding season and that males and females would both respond more strongly to intrasexual versus intersexual signals (Tobias et al. 2011). Finally, if birds use their signals primarily in mate attraction, we predicted they would be used more at the start of the breeding season and that males and females would respond more strongly to intersexual versus intrasexual signals (Tobias et al. 2011).

Methods

Study species

Rufous-capped Warblers are tropical resident songbirds distributed from southern AZ and northern Mexico to northern Colombia and Venezuela, from sea level up to 3000-m elevation (Curson 2010). They are common foliage-gleaning insectivores in semi-open habitats such as second-growth forest and shade coffee plantations (Perfecto et al. 2004; Jedlicka et al. 2006; Morrison et al. 2010; Morrison and Lindell 2011) as well as mature neotropical dry forests (Stiles and Skutch 1989). Pairs typically defend territories year-round (Jedlicka et al. 2006), but may also participate in mixed-species foraging flocks during the non-breeding season (Hutto 1988). Both sexes have bright, sexually monomorphic plumage (Curson

2010). In our study population, males sing regularly and females sing only rarely (ADD pers. obs.), but both males and females produce “chip” calls (Fig. 1; females produce these calls more often than males) and pair members often vocalize simultaneously (i.e., the male produces songs or calls, while the female produces calls). Females appear to produce calls in response to their partner’s songs, a behavior observed in other tropical resident warbler species (Staicer 1996).

Playback design

We conducted playback experiments from April to June 2015 in Sector Santa Rosa of the Área de Conservación Guanacaste in northwestern Costa Rica (10° 51' N, 85° 36' 30" W). Santa Rosa is characterized by neotropical dry forest habitat with a pronounced dry season from approximately December to May (the onset of the rainy season varies from late April until early June) and a rainy season from approximately May to November. Rufous-capped Warblers begin breeding activities at the start of the rainy season (Stiles and Skutch 1989). We therefore conducted pre-breeding season trials on territorial pairs of warblers at the end of the dry season (April 28–May 22; $N = 25$ pairs received playback in the pre-breeding season; the rainy season began on June 3 in 2015) and breeding season trials at the beginning of the rainy season (June 8–23; $N = 21$ pairs received playback in the breeding season). We conducted all trials between 0630 and 1100 CST, a time window that coincides with the morning period of peak vocal activity, while avoiding overlap with the dawn chorus when males typically sing at a higher rate.

We used a stereo playback design, broadcasting male and female vocalizations from separate speakers, to provide a natural simulation of a territorial intrusion by two individuals (reviewed in Douglas and Mennill 2010). We presented three treatments of conspecific vocalizations to pairs of territorial warblers to simulate different numbers and sexes of intruders: (1) male treatment, of a male song from one speaker; (2) female treatment, of a female call from one speaker; and (3) pair treatment, of a male song and female call broadcast

simultaneously from two separate speakers. In addition, we presented (4) a one-speaker control treatment, of male vocalizations of Long-tailed Manakins (*Chiroxiphia linearis*), a sympatric non-competitor bird species that is common at our study site. Each pair received one treatment on each of four successive days using a factorial design with randomly selected order of stimulus presentation. Each set of treatments was repeated for each pair in both the pre-breeding and breeding seasons. To minimize time-of-day effects on response strength, trials for each pair took place within 30 min of the same start time across days.

We tested the same pairs during both the pre-breeding and breeding periods, whenever possible, to account for individual variation in response (as in Gill et al. 2007; Akçay et al. 2014); 16 of 25 subject pairs received playback in both periods. For the remaining five pairs, a different male defended a given territory during the pre-breeding and breeding periods. In seven pairs tested, the female was not banded, so it is possible that the female changed between periods and we did not detect this change. We captured one or both pair members using mist-nets and conspecific playback and gave each bird a unique color-band combination to allow for individual identification during trials, since both sexes look alike. We waited at least 10 days between the capture date and start of playback trials for all birds and used different conspecific stimuli from the playback trials while luring birds into mist nets, thereby minimizing the effect of previous experience on playback response. We determined the sex of individuals based on the presence of a cloacal protuberance for males (from April 15 through July) and a brood patch for females (in June and July). We also confirmed the sex of each bird behaviorally during territorial observations, based on continuous song during the dawn chorus for males, and observations of nest-building and incubation activity for females.

We placed two speakers (model: FoxPro Scorpion TX200) 5 m apart and 1 m above the ground near the focal pair’s territory center (as in Mennill 2006; Koloff and Mennill 2013); this allowed us to simulate a pair of birds engaging in a territorial intrusion. We chose the speaker locations based on 60-min territorial observation periods of each pair in early April. We placed small pieces of flagging tape at 1- and 5-m distances away from each speaker to aid the two observers in estimating the distance of birds from the speakers during trials. All stimuli were broadcast at 88 dB(A) SPL (amplitude measured at 1 m from the speaker with a Casella CEL-240 sound level meter; Casella CEL Inc., Buffalo, NY, USA). This amplitude is a natural level for warbler vocalizations (e.g., Hof and Hazlett 2010) and comparable to the natural volume of Rufous-capped Warbler vocalizations heard in the field.

Trials consisted of a 5-min playback period followed by a 5-min post-playback observation period. During each trial, two observers sat together 15–20 m away from the playback speakers and recorded the trial with a Marantz PMD660

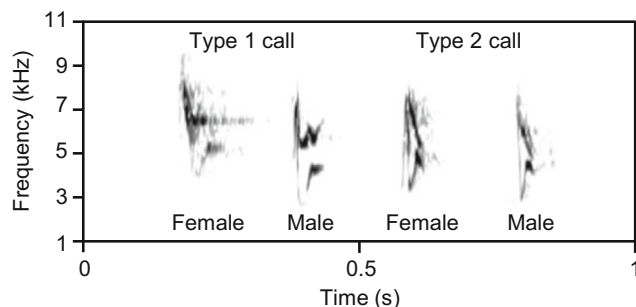


Fig. 1 Sound spectrograms of male and female Rufous-capped Warbler “chip” calls of two types. Male and female calls are similar in structure, but male calls have a lower average minimum frequency than female calls

digital recorder and an Audiotechnica AT8015 directional microphone. Two observers were necessary to accurately track the behavior of the male and female of each pair separately during the playback trials. Each observer dictated the horizontal and vertical distance of one bird from the speaker and other physical behaviors. We included any pair's response in our analysis as long as one or both individuals approached within 10 m of either speaker during the 5-min playback. For the conspecific treatments, if there was no response during this 5-min period and the focal birds were not seen or heard in the territory, we assumed that they did not detect the playback (territories can be > 100 m across; ADD pers. obs.). We then waited 2 min and played the 5-min stimulus again. If there was still no response, or if a neighbor also responded to the playback by approaching within 10 m or interacting with the focal birds during the trial, we repeated the trial on the following day. If there was still no response on the second day, we considered the pair to be non-responsive to that treatment. To calculate the proportion of responses by males and females to each playback treatment, we used only the final trial conducted for a specific treatment type and pair of birds.

We recorded both physical and vocal responses from males and females separately during each trial. We analyzed the following four physical approach responses: (1) number of flights over each speaker; (2) closest approach to speaker (m); (3) latency to approach within 10 m of the speaker; and (4) time spent within 10 m of the speaker. We analyzed the following four vocal responses: (1) number of songs; (2) number of calls; (3) latency to first song; and (4) latency to first call. For trials where males sang at least one song, we also tested whether song duration and number of syllables per song differed by treatment or season. We did not statistically compare female song duration or number of syllables per song by treatment or season because of the small available sample size. These response measures have been used in other playback studies on songbirds and are associated with strong territorial responses (e.g., Akçay et al. 2013; Hof and Podos 2013). Since identification of color-banded individuals was central to our study and the playback stimuli were audibly distinct to the observers, we were unable to use blinded methods for data collection.

Playback stimuli

We created playback stimuli from high-quality recordings of male songs and female calls we collected from color-banded male and female Rufous-capped Warblers from the study population in 2013–2014. We used male songs and female calls because they were the most common vocalizations recorded from each sex during naturally-occurring conspecific territorial interactions (ADD pers. obs.). We used Audition 3.0 software (Adobe, San Jose, CA, USA) to filter recordings with a high pass filter of 1000 Hz and to normalize the amplitude of

the final playback files to -1 dB. All stimuli were from birds with territories at least 200 m away from the playback subject's territory and therefore presumably unfamiliar to the focal birds prior to the playback experiment. We used a different set of playback stimuli for each pair whenever possible to avoid pseudoreplication (McGregor 1992), although we were limited by the number of high-quality recordings of confirmed-identity animals. For male stimuli ($N=20$), we used four different song types from each male, presented alternately at a natural daytime song rate of 6 songs/min. Since males typically alternate between three and five song types during daytime song bouts (ADD unpubl. data), the use of multiple song types per bird was more natural than repeating only one song type. For female stimuli ($N=9$), we used one chip call repeated at a natural call rate of 15 calls/min. We calculated natural song and call rates from a subset of daytime 2013–2014 focal recordings from Santa Rosa ($N=10$ each for males and females). We constructed pair stimuli using one male and one female stimulus file broadcast from separate speakers, which simulated the vocal behavior of Rufous-capped Warbler pairs observed during naturally occurring territorial interactions (A. Demko pers. obs.). For control stimuli ($N=15$), we used long-tailed Manakin songs we recorded from Santa Rosa, broadcast at a rate of 9 songs/min. We used the same stimuli for each pair during both seasons to ensure that variation in response strength across seasons was unrelated to differences in acoustic properties of the playback stimuli used. In the cases where partnerships changed on a given territory between seasons, we used the same stimuli previously used at that territory.

Although both male and female Rufous-capped Warblers produce chip calls, sex-specific differences in the frequency of these calls and the behavioral context of their use should allow sex identification of the caller by territorial pairs during the female and pair treatments. To confirm sex-specificity of chip calls, we conducted a paired comparison of structural variation in this call type between 10 mated warbler pairs, and found significant sex differences (Fig. 1). Male calls had a minimum frequency that was on average, 604 Hz lower than the minimum frequency of females (mean \pm SD males: 3237 ± 455 Hz, females: 3841 ± 626 Hz; paired t test, $t_9 = 3.55$, $P = 0.006$), although neither maximum frequency ($t_9 = 1.44$, $P = 0.18$) nor note duration ($t_9 = -0.15$, $P = 0.89$) differed between the sexes. Songs and calls are known to convey different messages across bird species (Catchpole and Slater 2008), and therefore, it is possible that the type of vocalization (song versus call) is confounded with the sex of the treatment type (male versus female). Therefore, different responses to the treatment types should be interpreted primarily in the context of seasonal effects rather than sex-specific effects, pending further studies comparing responses to the same vocalizations across sexes (e.g., testing responses to male vs. female songs and male vs. female calls).

Male versus female song comparison

After discovering that females sang in response to playback stimuli (see Results), we compared male and female Rufous-capped Warbler songs. We measured songs from ten males (ten songs each randomly selected from the pool of high-quality spontaneous songs available from focal recordings) and ten females (1–18 songs each from the best-quality songs available on playback recordings). The males and females selected for this analysis were ten of the mated pairs tested in our study. We measured seven acoustic variables on each song: song duration, number of syllables per song, number of syllable types, syllable diversity (number of different syllable types divided by the total number of syllables per song), maximum frequency, and minimum frequency. We defined a syllable as the smallest continuous trace on a spectrogram comprising a song (Catchpole and Slater 2008), and we defined a syllable type as a syllable with specific spectro-temporal properties that is different from other such syllables. ADD identified all syllable types based on their frequency, shape, and duration by visual comparison of spectrograms. A second observer naive to the sex and identity of the individual birds repeated the syllable classification for a subset of 50 songs (35 male and 15 female), and we then compared the scores of both observers using two inter-observer reliability coefficients modified from Illes (2015):

$$\text{Score 1} = 1 - ((a/b)/b) \quad (1)$$

$$\text{Score 2} = 1 - ((c-b)_{\text{absolute value}}/b) \quad (2)$$

a was the absolute value of the mean difference between ADD and the second observer's syllable count for each song; b was the mean of ADD's syllable type count per song; and c was the mean of the second observer's syllable type count per song. Both observers had high consistency in syllable classification, with similarity scores of 99.6% for score 1 and 97.1% for Score 2.

We then calculated average values within individual birds to produce a single value for each variable, and ran two sample t tests to compare these variables between sexes. We collected frequency measurements in Avisoft-SASLab Pro (version 5.2.09; R. Specht, Berlin, Germany) from the power spectrum of each song at a threshold amplitude of -20 dB from the peak amplitude. This method is the recommended method in order to standardize measurements across recordings with differing signal-to-noise ratios (Zollinger et al. 2012; Brumm et al. 2017). Power spectra were produced with a Hann window with FFT size of 512 and frequency resolution of 62.5 Hz. We collected temporal measurements manually from spectrograms in Raven Pro v. 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY) with the following settings: Hann spectrogram window with FFT size of 512, time resolution of 2.9 ms, and frequency resolution of 86.1 Hz.

Analyses

We used Syrinx PC software (J. Burt, Seattle, WA, USA) to annotate the observers' narration of physical responses by the birds, as well as the vocalizations produced by the birds, on the recordings for each playback session, producing a time-stamped record of the behaviors and vocalizations of each playback subject. We analyzed physical approach responses and vocal responses separately, in order to quantify differences in use of visual and vocal signals in this species. Since many of the response variables were correlated, we used principal components analysis (without rotation) on the extracted variables to produce uncorrelated composite variables (McGregor 1992). For males, the distance of closest approach was log-transformed prior to analysis to improve linear relationships between the response variables according to the assumptions of principal components analysis (Quinn and Keough 2002). To incorporate the two-speaker pair treatment in this analysis, we used the strongest response of each individual to either speaker for each physical response variable. Since male and female speakers were only 5 m apart, we considered the target area for stimulus detection to be similar for the one-speaker and two-speaker treatments, particularly in relation to the large territories of our study species.

For physical approach responses, the first principal component (PC1) explained 73.0% of the overall variation for males and 75.7% for females. For both sexes, the PC1 loadings corresponded to a shorter latency to approach, a closer distance of approach, more time spent within 10 m of the speakers, and more flights over the speakers (Table 1); we refer to this PC1 score as "physical approach response." For vocal responses, PC1 explained 51.1% of the overall variation for males and 63.4% for females. For males and females, the PC1 loadings corresponded to a greater number of songs and calls produced and a shorter latency to first song and first call; we refer to this PC1 score as "vocal response." PC2 explained 40.7% of the variation in males and 27.3% of the variation in females. For both sexes, PC2 loadings corresponded to more

Table 1 Summary of correlations between physical approach response variables for first principal component in two separate principal components analyses, one for male responses to playback, and one for female responses to playback

	Male PC1	Female PC1
Eigenvalue	1.71	1.74
Percentage of variation (%)	73.0	75.7
Latency to approach within 10 m	-0.55	-0.55
Closest approach	-0.53	-0.52
Time spent within 10 m	0.55	0.54
Number of flights over speaker	0.34	0.37

calls and longer latency to first song, as well as fewer songs and a longer latency to first call (Table 2).

Using the PC1 scores as the response variables for both physical approach and vocal responses, we ran linear mixed models using the lme4 package in R. We ran models separately for males and females with fixed effects of playback treatment (four levels: male, female, pair, or control) and season (two levels: pre-breeding or breeding) and pair identity as a random effect to account for repeated sampling of the same individuals. We initially included a fixed effect of treatment presentation order, but this effect was non-significant (all $P > 0.09$), so we excluded it from final analyses. To estimate P values for fixed effects, we conducted likelihood ratio tests comparing models with each combination of additive and interactive fixed effects. To quantify significant fixed effects, we conducted post hoc analyses using the glht function in the multcomp package in R.

To account for multiple comparisons in t tests and post hoc tests, we used the false discovery rate correction with an adjusted α value based on the number of comparisons used in the test (Benjamini and Hochberg 1995). We conducted all analyses using R v.3.3.1 (R Development Core Team 2016). Values are presented as mean \pm SE unless otherwise specified.

Data availability statement The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Results

Overall playback response

Both sexes of Rufous-capped Warbler responded to playback of conspecific songs and calls by approaching the speakers and vocalizing, and response intensity varied with season and sex of the focal bird. In the pre-breeding season, both males and females responded (i.e., approached within 10 m of the playback) to the majority of conspecific treatments (males 95%, females 73%). Both sexes had similar response rates to pair and female treatments (chi-squared test: pair: $\chi^2_1 = 2.4$, $P = 0.12$; female: $\chi^2_1 = 1.9$, $P = 0.17$; Fig. 2), although

females responded less often than males to the male treatment ($\chi^2_1 = 5.2$, $P = 0.02$). In the breeding season, males responded to the majority of conspecific treatments (78% overall), whereas females responded little (29% overall; pair: $\chi^2_1 = 11.8$, $P < 0.001$; female: $\chi^2_1 = 7.7$, $P = 0.005$; male: $\chi^2_1 = 6.10$, $P = 0.01$; Fig. 2). Both sexes responded little to the heterospecific control stimuli (Fig. 2).

Physical approach responses

Males showed strong physical approach responses to all conspecific treatments during both seasons, showing the strongest response to the pair treatments (Fig. 3a, b). There were significant effects of treatment (likelihood ratio test: $\chi^2_3 = 111.8$, $P < 0.001$) and season ($\chi^2_1 = 17.0$, $P < 0.001$) on responses to playback for males. Males responded more strongly to all conspecific treatments than to the control (post hoc tests: all $P < 0.001$; Table 3a). They responded significantly more to pair than female treatments (mean \pm SE: estimate = 0.63 ± 0.21 , $z = 2.9$, $P = 0.02$), although responses did not differ between pair and male treatments (estimate = 0.47 ± 0.21 , $z = 2.2$, $P = 0.12$) or male and female treatments (estimate = 0.16 ± 0.21 , $z = 0.7$, $P = 0.88$). There was no significant interaction effect between treatment and season (likelihood ratio test: treatment \times season: $\chi^2_3 = 7.3$, $P = 0.06$), indicating that males showed similar responses to each treatment type during both seasons (Fig. 3a, b).

Females showed strong physical approach responses to conspecific treatments during the pre-breeding season but responded very little during the breeding season (Fig. 3c, d). There were significant effects of treatment (likelihood ratio test: $\chi^2_3 = 41.2$, $P < 0.001$) and season ($\chi^2_1 = 22.8$, $P < 0.001$) on responses to playback for females. There was also a significant interaction between treatment and season ($\chi^2_3 = 11.1$, $P = 0.01$), indicating that females differed significantly in their response strength to each treatment type across seasons. In the pre-breeding season, females responded more strongly to all conspecific treatments than to the Control treatment (all $P < 0.001$; Table 3b, Fig. 3c). They responded significantly more to pair than male treatments (estimate = -1.25 ± 0.41 , $t = -3.1$, $P = 0.01$), although responses did not differ between pair and female treatments (estimate = -0.89 ± 0.41 , $t = -2.2$,

Table 2 Summary of correlations between vocal response variables for first and second principal components in two separate principal components analyses, one for male responses to playback, and one for female responses to playback

	Male PC1	Male PC2	Female PC1	Female PC2
Eigenvalue	1.43	1.28	1.59	1.05
Percentage of variation (%)	51.1	40.7	63.4	27.3
Number of songs	0.62	-0.33	-0.46	0.59
Number of calls	0.19	0.70	-0.51	-0.48
Latency to first song	-0.65	0.24	0.53	-0.42
Latency to first call	-0.40	-0.58	0.50	0.49

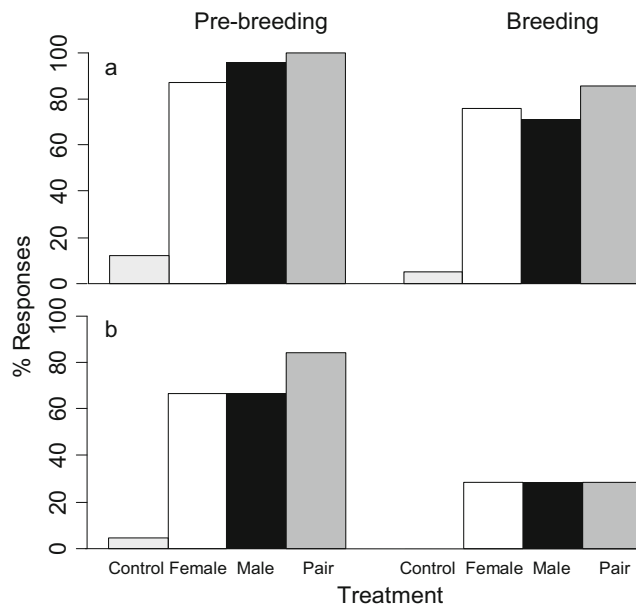


Fig. 2 The responses of Rufous-capped Warbler males (a) and females (b) to four playback treatments (heterospecific control, conspecific female calls, conspecific male songs, or both conspecific female calls and male songs) during the pre-breeding season (left) and the breeding season (right). Males responded to all conspecific treatments during both seasons, while females responded primarily in the pre-breeding season

$P = 0.14$) or male and female treatments (estimate = 0.36 ± 0.41 , $t = 0.9$, $P = 0.82$). In contrast, during the breeding season, physical approach responses were uniformly low and did not

differ significantly between conspecific treatments and the control treatment (all $P > 0.05$; Table 3b, Fig. 3d).

Vocal responses

Males showed strong vocal responses to all conspecific treatments during both seasons, but the relative response strength to each treatment type differed seasonally (Fig. 4a, b). For vocal responses, there was a significant interaction between treatment and season (likelihood ratio test: $\chi^2_3 = 10.2$, $P = 0.02$). In the pre-breeding season, males had a stronger vocal response to all conspecific treatments than to the control (all $P < 0.001$; Table 4a). They responded more to pair than to female treatments (estimate = -1.03 ± 0.24 , $t = -4.3$, $P < 0.001$), although the vocal response during pair and male treatments (estimate = -0.41 ± 0.24 , $t = -1.7$, $P = 0.31$; Fig. 4a) and male and female treatments (estimate = -0.62 ± 0.24 , $t = -2.5$, $P = 0.05$) did not differ. During the breeding season, males had a stronger vocal response to conspecific treatments than to the control treatment (all $P < 0.001$; Table 4a), although vocal response did not differ among conspecific treatments (all $P > 0.05$; Table 4a, Fig. 4b).

Male song duration differed significantly by season (likelihood ratio test: $\chi^2_1 = 22.8$, $P < 0.001$) but not by treatment type ($\chi^2_2 = 0.95$, $P = 0.62$). Males sang significantly longer songs in the breeding season (mean \pm SD = 2.1 ± 0.4 s) than in the non-breeding season (mean \pm SD = 1.7 ± 0.4 s; post hoc

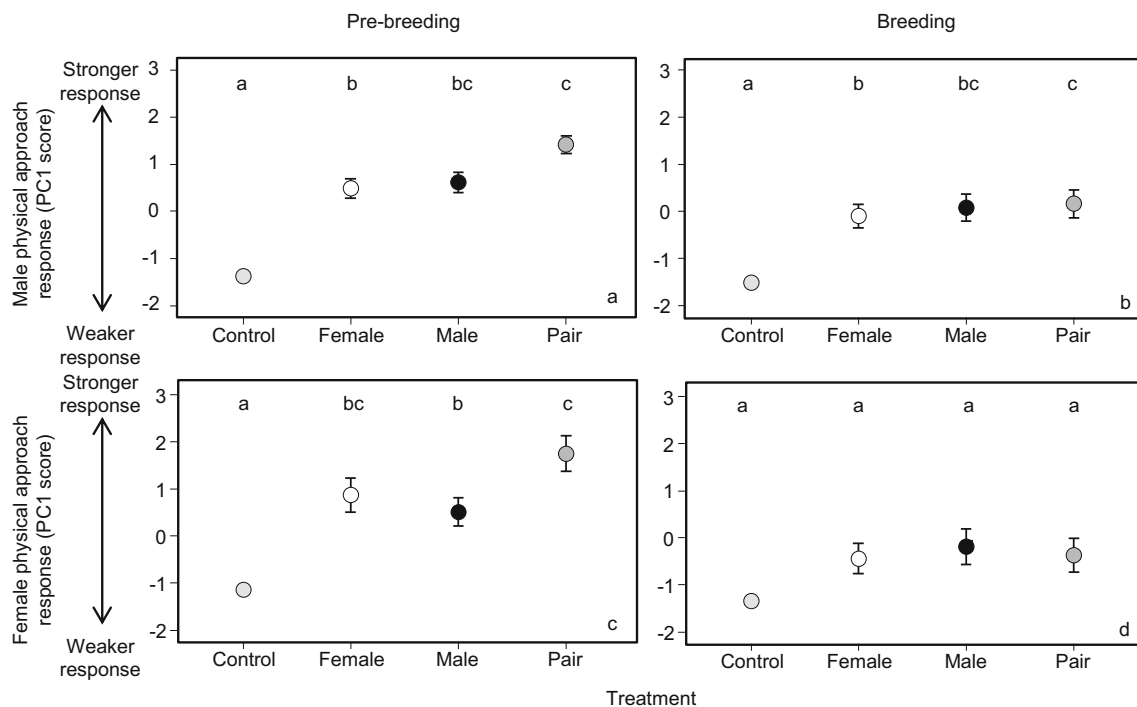


Fig. 3 Male Rufous-capped Warblers responded more strongly to pair treatments than to female treatments during both the pre-breeding (a) and breeding (b) seasons. Females responded more strongly to pair treatments than to male treatments during the pre-breeding season (c) and responded

weakly to all conspecific treatments during the breeding season (d). A higher PC 1 score indicates stronger response intensity to playback. Error bars indicate SE of PC 1 scores, and different letters above bars denote statistical significance

Table 3 Linear mixed model and post-hoc comparisons results of physical approach response (PC1) for male (a) and female (b) Rufous-capped Warblers in response to playback; significant effects (after false discovery rate correction) are highlighted in bold

a. Male physical approach response (PC1)			
Full LMM	χ^2	df	P value
Treatment	111.8	3	<0.001
Season	17.0	1	<0.001
Treatment \times season	7.3	3	0.06
Post hoc comparisons	Estimate \pm SE	z value	P value
Both seasons			
Control–female	1.66 \pm 0.21	7.7	<0.001
Control–male	1.82 \pm 0.21	8.5	<0.001
Control–pair	2.29 \pm 0.21	10.7	<0.001
Female–male	0.16 \pm 0.21	0.7	0.88
Female–pair	0.63 \pm 0.21	2.9	0.017
Male–pair	0.47 \pm 0.21	2.2	0.12
b. Female physical approach response (PC1)			
Full LMM	χ^2	df	P value
Treatment	41.2	3	<0.001
Season	22.8	1	<0.001
Treatment \times season	11.1	3	0.011
Post hoc comparisons	Estimate \pm SE	t value	P value
Breeding season			
Control–female	–0.90 \pm 0.44	–2.0	0.17
Control–male	–1.14 \pm 0.44	–2.6	0.05
Control–pair	–0.97 \pm 0.44	–2.2	0.13
Female–male	–0.25 \pm 0.44	–0.6	0.95
Female–pair	–0.07 \pm 0.44	–0.2	1.0
Male–pair	0.18 \pm 0.44	0.4	0.98
Pre-breeding season			
Control–female	–2.01 \pm 0.41	–4.9	<0.001
Control–male	–1.65 \pm 0.41	–4.0	<0.001
Control–pair	–2.90 \pm 0.41	–7.1	<0.001
Female–male	0.36 \pm 0.41	0.9	0.82
Female–pair	–0.89 \pm 0.41	–2.2	0.14
Male–pair	–1.25 \pm 0.41	–3.1	0.014

comparison estimate = -0.35 ± 0.07 , $z = -5.0$, $P < 0.001$). The number of syllables per song also differed significantly by season (likelihood ratio test: $\chi^2_1 = 23.6$, $P < 0.001$) but not by treatment type ($\chi^2_2 = 0.96$, $P = 0.62$). Males sang songs with significantly more syllables in the breeding season (mean \pm SD = 15.9 ± 3.9) than in the non-breeding season (mean \pm SD = 12.5 ± 3.7 ; post hoc comparison estimate = -3.39 ± 0.68 , $z = -5.0$, $P < 0.001$).

Females showed strong vocal responses during all conspecific treatments during the pre-breeding season, but responded little during the breeding season (Fig. 4c, d). For female vocal responses, there was a significant interaction between

treatment and season (likelihood ratio test: $\chi^2_3 = 18.3$, $P < 0.001$). In the pre-breeding season, females responded more strongly to all conspecific treatments than the control treatment (all $P \leq 0.001$; Table 4b). They also responded more to pair versus male treatments (estimate = 1.08 ± 0.34 , $t = 3.1$, $P = 0.01$) and pair versus female treatments (estimate = 1.21 ± 0.34 , $t = 3.5$, $P = 0.003$), although their vocal responses to male versus female treatments did not differ (estimate = 0.13 ± 0.35 , $t = 0.4$, $P = 0.98$; Fig. 4c). During the breeding season, female vocal responses were low and did not differ significantly between conspecific treatments and the control treatment (all $P > 0.05$; Table 4b, Fig. 4d).

Female song

We recorded female song, previously undocumented in Rufous-capped Warblers, during many conspecific playback trials. Over one third of females in the study (36%) sang at least one song in response to playback, with female song recorded during 20% of pre-breeding and 6% of breeding season trials. Female songs were similar to male songs in minimum frequency and syllable structure (Table 5, Fig. 5). Female songs, however, were shorter, had a higher maximum frequency and broader bandwidth, contained fewer syllables and syllable types, and had a lower syllable diversity than male songs (Table 5).

Discussion

Recent studies comparing male and female signaling in diverse animal taxa suggest that conspicuous signals are used by both sexes not only to attract and compete for mates but also to compete with conspecifics for territories (e.g., Robinson and Kruuk 2007; Watson and Simmons 2010; Tobias et al. 2011; Cain and Langmore 2015; Tibbetts et al. 2015). In Rufous-capped Warblers, a tropical resident songbird, we found both season- and sex-specific responses to simulated territorial intrusions. In the breeding season, males were more responsive than females to all conspecific treatments. This pattern of response resembles that of temperate bird species (Catchpole and Slater 2008) and other tropical resident species in that males responded more strongly than females during the breeding season (e.g., Busch et al. 2004). In the pre-breeding season, however, responses were more equal among the sexes; at this time of year, both male and female Rufous-capped Warblers responded strongly to all conspecific intruders, as has been observed in previous studies of tropical birds (e.g., Fedy and Stutchbury 2005; Gill et al. 2007, 2008). Males also sang longer songs overall during all conspecific treatments in the breeding season compared to the pre-breeding season. The strong seasonal variation in response to territorial intrusions by Rufous-capped Warblers highlights

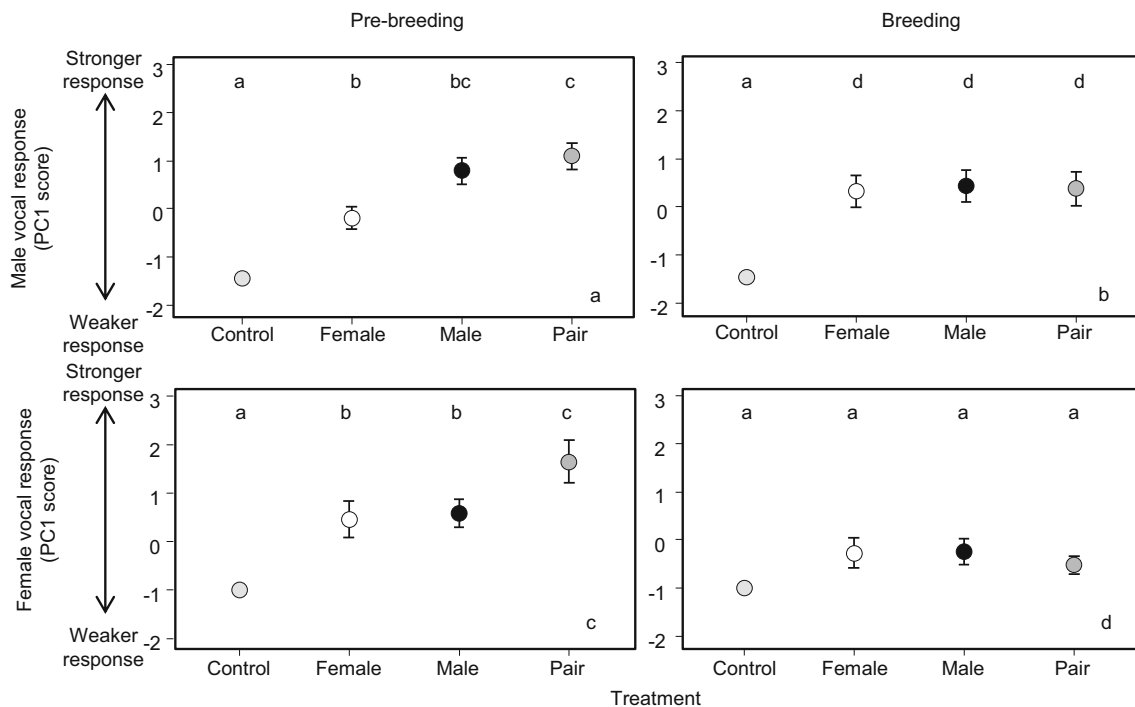


Fig. 4 Males had stronger vocal responses to pair treatments than to female treatments during the pre-breeding season (**a**), but responded strongly to all conspecific treatments during the breeding season (**b**). Females had a stronger vocal response to pair than to male and female treatments during the pre-breeding season (**c**), but responded little to all

conspecific treatments during the breeding season (**d**). A higher PC 1 score indicates stronger response intensity to playback. Error bars indicate SE of PC 1 scores, and different letters above bars denote statistical significance

the importance of conducting behavioral studies across different seasons and breeding stages to gain a more thorough understanding of signal function in tropical resident animals.

During the pre-breeding season, both male and female Rufous-capped Warblers showed strong physical approach and vocal responses to all conspecific treatments and responded most strongly to the pair treatment. Our results suggest that shared territory defense is an important function of this species' vocal signals (Tobias et al. 2011; Dowling and Webster 2016). Other studies on tropical resident songbirds have also found strong responses by both sexes to paired intrusions during the non-breeding season (Gill et al. 2007, 2008) and stronger responses overall during the non-breeding season than the breeding season (Fedy and Stutchbury 2005). Defense of territorial and food resources may be critical during the non-breeding season, particularly at our neotropical dry forest study site, which experiences an extended dry non-breeding season when food resources for warblers are especially scarce. Similarly, ecological resource defense during the non-breeding season is a proposed function of female signaling in stripe-headed sparrows (*Peucaea ruficauda*) at the same field site (Illes 2015) and white-bellied antbirds at another site with a comparable climate (Fedy and Stutchbury 2005). Higher population density during the non-breeding season related to scarcity or patchiness of food resources could also increase the intensity of territorial defense behavior during this

period (e.g., Wicklund and Village 1992). Furthermore, we conducted our study during the pre-breeding season, when intensity of territory and mate defense may be greater than earlier in the non-breeding season, so it could be useful to conduct further studies well outside of the breeding season in order to gain a deeper understanding of any differences between non-breeding and pre-breeding territorial behavior (e.g., Odom et al. 2017). Regardless of the drivers of territory defense during the non-breeding and pre-breeding periods, both male and female Rufous-capped Warblers likely use vocal signals to defend territorial resources from conspecifics.

During the breeding season, only males showed a strong response to conspecific intruders by vocalizing and approaching the speakers. This pattern of response was also found in the tropical resident Rufous-collared Sparrow (*Zonotrichia capensis*), in which males responded more strongly than females to intruders of both sexes during the breeding season (Busch et al. 2004). The low female response we observed may be related to breeding stage, because 75% (6 of 8) of females with monitored nests in our study were nest-building, egg-laying, or incubating during the trial period, and females are the primary nest-builders and sole incubators in this species (Stiles and Skutch 1989; unpubl. data). Our results therefore refute the intrasexual mate competition hypothesis, since males responded strongly and females responded very little towards all intruders during

Table 4 Linear mixed model and post-hoc comparisons results of male (a) and female (b) vocal responses (PC1) of Rufous-capped Warblers to playback; significant effects (after false discovery rate correction) are highlighted in bold

a. Male vocal response (PC1)			
Full LMM	χ^2	df	P value
Treatment	115.7	3	<0.001
Season	9.5	1	0.002
Treatment \times season	10.2	3	0.017
Post-hoc comparisons	Estimate \pm SE	t value	P value
Breeding season			
Control–female	–1.34 \pm 0.26	–5.1	<0.001
Control–male	–1.37 \pm 0.26	–5.3	<0.001
Control–pair	–1.41 \pm 0.26	–5.4	<0.001
Female–male	–0.03 \pm 0.26	–0.1	1.0
Female–pair	–0.07 \pm 0.26	–0.3	0.99
Male–pair	–0.04 \pm 0.26	–0.2	1.0
Pre-breeding season			
Control–female	–1.31 \pm 0.24	–5.4	<0.001
Control–male	–1.93 \pm 0.24	–8.0	<0.001
Control–pair	–2.34 \pm 0.24	–9.8	<0.001
Female–male	–0.62 \pm 0.24	–2.5	0.05
Female–pair	–1.03 \pm 0.24	–4.3	<0.001
Male–pair	–0.41 \pm 0.24	–1.7	0.31
b. Female vocal response (PC1)			
Full LMM	χ^2	df	P value
Treatment	35.4	3	<0.001
Season	21.1	1	<0.001
Treatment \times season	18.3	3	<0.001
Post-hoc comparisons	Estimate \pm SE	t value	P value
Breeding season			
Control–female	0.72 \pm 0.37	1.9	0.22
Control–male	0.74 \pm 0.37	2.0	0.19
Control–pair	0.47 \pm 0.37	1.3	0.58
Female–male	0.03 \pm 0.37	0.1	1.0
Female–pair	–0.25 \pm 0.37	–0.7	0.91
Male–pair	–0.27 \pm 0.37	–0.7	0.88
Pre-breeding season			
Control–female	1.44 \pm 0.35	4.1	<0.001
Control–male	1.57 \pm 0.35	4.5	<0.001
Control–pair	2.65 \pm 0.34	7.7	<0.001
Female–male	0.13 \pm 0.35	0.4	0.98
Female–pair	1.21 \pm 0.34	3.5	0.003
Male–pair	1.08 \pm 0.34	3.1	0.01

the breeding season, rather than showing stronger same-sex responses. Although female Rufous-capped Warblers contributed less to territory defense during the breeding season than did males, further studies examining territorial behavior across multiple breeding stages will be

useful for comparing seasonal roles of both sexes in territory defense (e.g., Dowling and Webster 2016).

Male Rufous-capped Warblers showed strong responses to all conspecific treatments during the breeding season, whereas they responded less to the Female treatment than to pair and male treatments during the pre-breeding season. Since the focal males were all paired and many of their female mates were incubating on nests during the breeding season, it is possible that males were responding strongly to all conspecific intruders in the absence of territory defense by their mates during the breeding season. Conversely, males may have been signaling more to the playback-simulated female during the breeding season. Our finding that males sang longer songs with more syllables during the breeding season than during the non-breeding season suggests that they modify their singing behavior depending on the breeding status of their mate. In many songbird species, males increase song complexity during their female mate's pre-fertile or fertile period (e.g., Ballentine et al. 2003; Zhang et al. 2015). Another explanation is that males approached and vocalized more to females in order to solicit extra-pair copulations. Rufous-capped Warblers in our study population live at high densities, suggesting that extra-pair mating may occur at high rates. Longer songs are also associated with increased extra-pair paternity and decreased loss of within-pair paternity for males in at least one other songbird species (Willow Warblers, *Phylloscopus trochilus*; Gil et al. 2007). However, any such interpretations should be made cautiously, because extra-pair paternity rates are currently unknown for Rufous-capped Warblers (Macedo et al. 2008). Although the results of our study do not support the mate attraction hypothesis for male vocal signals, future detailed vocal analyses will investigate how male Rufous-capped Warblers modify their song structure and singing behavior relative to breeding status.

Our study was the first to document female song in Rufous-capped Warblers. Although calls were the most common vocalization used by females in response to playback, over one third of females in our study population also produced song during playback trials. Female song is now known to be more common in birds than previously thought (Odom et al. 2014) and was likely overlooked in the past in species such as the Rufous-capped Warbler in which both sexes look alike and male and female songs are similar in acoustic structure. A recent comparative analysis of female trait evolution in the Parulidae revealed that female song likely evolved independently in different genera, suggesting that it may serve different functions across species (Najar and Benedict 2015). Occasional female song early in the breeding season is reported for at least 13 temperate zone parulid warbler species (e.g., Taff et al. 2012; Matthews et al. 2016). Pair bond formation is the proposed function in at least four temperate-breeding species: Wilson's Warbler (*Cardellina pusilla*; Gilbert and Carroll 1999), Common Yellowthroat (*Geothlypis trichas*; Taff et al.

Table 5 Comparison of seven acoustic variables of male and female Rufous-capped Warbler songs; all values are mean \pm SD. Significant *t* test results are highlighted in bold

Acoustic variable	Males (<i>n</i> = 10)	Females (<i>n</i> = 10)	<i>t</i> test	<i>P</i> value
Maximum frequency (kHz)	8.06 \pm 0.23	8.70 \pm 0.34	4.97	< 0.001
Minimum frequency (kHz)	3.28 \pm 0.17	3.23 \pm 0.32	-0.44	0.67
Bandwidth (kHz)	4.77 \pm 0.29	5.47 \pm 0.40	4.45	< 0.001
Song duration (s)	2.37 \pm 0.26	1.26 \pm 0.28	-9.18	< 0.001
Total number of syllables	17.57 \pm 2.73	7.23 \pm 1.83	-9.96	< 0.001
Number of syllable types	14.24 \pm 2.53	4.81 \pm 1.64	-9.88	< 0.001
Syllable diversity	0.81 \pm 0.05	0.68 \pm 0.16	-2.31	0.04

2012), Prothonotary Warbler (*Protonotaria citrea*; Matthews et al. 2016), and Prairie Warbler (*Setophaga discolor*; Nolan 1978) and is also a possible function of female song in Rufous-capped Warblers. Territory defense is a more likely function of female song in our study species, as in duets produced by other tropical species (e.g., Hall and Peters 2008; Benedict 2010; Dahlin and Wright 2012; Koloff and Mennill 2013) and non-duet female songs used by temperate and tropical songbirds (Hall et al. 2015; Krieg and Getty 2016). We documented the majority of female song during the pair treatment in the pre-breeding season, to which both males and females responded strongly by vocalizing at high rates and approaching the speakers closely. Furthermore, females sang in our study population in other situations where a territory defense function is likely: (1) in response to continuous playback of conspecific songs and calls during capture attempts; (2) spontaneous song on the territory when unaccompanied by the male; and (3) during the dawn chorus in the first week of the breeding season (ADD pers. obs.). Further studies testing seasonal responses of both male and female Rufous-capped Warblers to female song playback would be useful to thoroughly investigate the function of this signal.

Our study revealed seasonal variation in the responses of Rufous-capped Warblers to territorial intrusions, wherein pairs typically responded together during the pre-breeding season and males typically responded alone in the breeding season. We found that males primarily used song during shared territory defense against both single and paired conspecific intruders, whereas females primarily used calls. The similarity of this response pattern to other temperate warbler species is consistent with the proposed temperate origins of the Family Parulidae and other related families (reviewed in Barker et al. 2015) and provides important evidence for further investigation into the evolution of duetting behavior and female song in this clade. We also found that males and females may make different use of similar signals, such as song, depending on season or breeding status. Male Rufous-capped Warblers used song to defend territories during both seasons, whereas females primarily used song during the non-breeding season. Indeed, shared male and female signals in other species, such as complex song in Superb Fairy-wrens (*Malurus cyaneus*; Cain and Langmore 2015) and bill color in American Goldfinches (*Spinus tristis*; Murphy et al. 2014), are also used differently by males and females. Species-specific levels of female competition may also affect

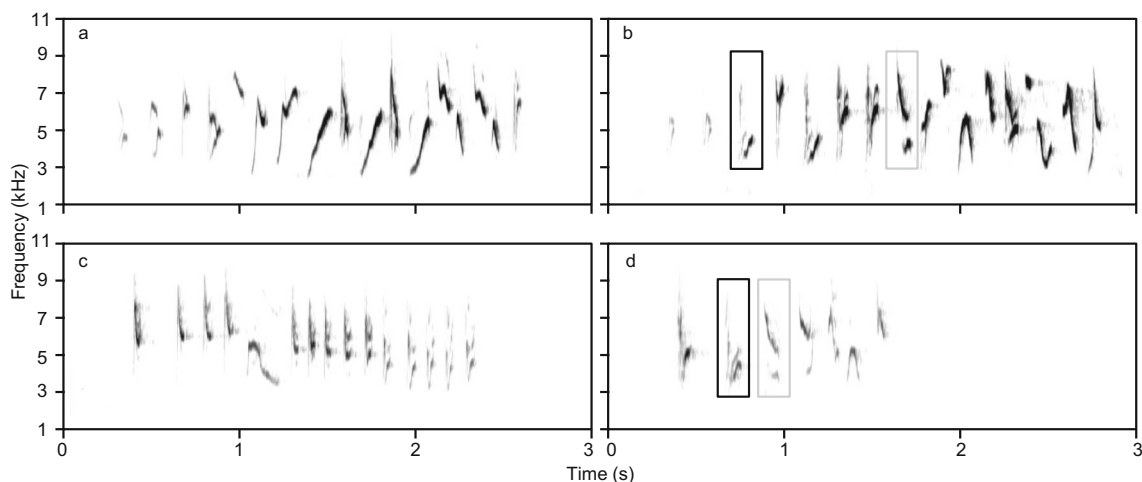


Fig. 5 Sound spectrograms of male (a–b) and female (c–d) Rufous-capped Warbler songs from two mated pairs (a/c and b/d) in Santa Rosa, Costa Rica. Male and female songs share a similar syllable structure

and organization. Examples of syllable types shared by males and females are enclosed in boxes for male song (b) and female song (d)

female signaling behavior (Colombelli-Négrel 2016), although this idea requires further investigation in our study species and other tropical resident species. Overall, our research provides support for the shared territory defense hypothesis for both male and female Rufous-capped Warblers. In addition to paralleling results of conspecific intrusion studies on other tropical species (e.g., Fedy and Stutchbury 2005; Gill et al. 2007, 2008), our study demonstrates that seasonality influences the territorial behavior of both sexes in a year-round resident songbird. It also highlights that females of a non-duetting tropical songbird participate in shared territory defense as effectively as duetting species, by using vocal signals such as calls and songs not produced as duets. Future studies incorporating playback of multiple types of male and female vocalizations (such as male calls and female songs), or using visual models to experimentally test responses to visual signals, will be useful to further understand the relative importance of vocal and visual signals and seasonal variation in use of such signals, in the territorial behavior of tropical animals.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures using animals were authorized under permits from the University of Windsor Animal Care Committee (AUPP-13-15), the Government of Costa Rica (MINAE), and the Area de Conservación Guanacaste.

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