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Partners coordinate territorial defense regardless of distance between paired intruders in a duetting bird

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ABSTRACT

Year-round territorial defense is crucial for reproductive success in many tropical birds. In these species, partners often coordinate their songs in duets to defend a shared territory. In this scenario, the pair members may behave according to different motivations and interests, or through essentially cooperative strategies. However, few studies have examined whether sex-specific roles in leadership and cooperation vary with the sex and spatial arrangement of intruders. The aim of this study was to experimentally investigate how the distance between intruders influences partner coordination in territorial defense in the Rufous Hornero (*Furnarius rufus*). We conducted playback experiments simulating intruder duets broadcast either from adjacent speakers (0.1 m apart) or from spatially separated speakers (~12 m apart). We recorded the number, duration, and latency of songs, as well as the latency to approach within 20 m and 5 m of the speakers and the time spent by each individual within 5 m of them. Partners showed strong coordination across all approach and vocal behaviors, jointly approaching a single speaker when the speakers were spatially separated. Pairs responded more cautiously when the speakers were adjacent; however, responses did not differ according to the sex simulated by each speaker. Notably, when females led the vocal response, pair defense was biased toward female intruders. Overall, our findings support the joint territory defense hypothesis, highlighting the importance of pair cooperation and coordination in Rufous Hornero territorial defense.

1. Introduction

Tropical birds exhibit a range of strategies to defend their territory, including joint defense of year-round territories by pair members (Fedy and Stutchbury, 2005; Tobias et al., 2016; Arashanapalli and Freeman, 2026). Nevertheless, the specific strategies employed by each member of a pair to defend common territories is not yet fully understood (Wheeldon et al., 2020; Jones et al., 2022). This includes whether they possess distinct motivations and interests (Logue and Gammon, 2004; Quinard and Cézilly, 2011), or if their strategies are essentially cooperative (Koloff and Mennill, 2013; Moody et al., 2025). In many cases, males and females often exhibit sex-specific aggression, targeting intruders of the same sex (Logue and Gammon, 2004; Cain et al., 2011; Quirós-Guerrero et al., 2017). In contrast, in some other species, aggression is not directed solely at same-sex intruders, but rather involves a more cooperative response, with partners attacking the same target together (joint territory defense) (Schuppe et al., 2016; Ippi et al., 2017; York et al., 2019). This cooperative behavior can be found especially in birds that produce duets (Logue, 2005; Hall and Peters, 2008a; Templeton et al., 2011; Quirós-Guerrero et al., 2017), that is, when there are two individuals, typically a male and a female, that sing together in a highly coordinated manner (Hall, 2009; Douglas and Mennill, 2010). This behavior may be facilitated by the close proximity of the pair during territory defense (Jedlikowski and Rek, 2025).

Duets might have multiple functions associated with different contexts. It may signal partnership against territorial intruders (Hall, 2000; Mann et al., 2003; Dahlin and Benedict, 2013). It can also function for intrapair communication, assisting in reproductive synchrony between pair members and facilitating spatial cohesion during a coordinating territorial response (Logue and Gammon, 2004; Mennill and Vehrencamp, 2008; Hall, 2009; Quirós-Guerrero et al., 2017). When used as a response to territorial intruders, duets may occur even if there is a division of labor, in which each sex plays a specific role—such as directing aggression toward one of the sexes more intensely (Levin, 1996; Templeton et al., 2011). However, it is more likely that duetting is associated with a unified response, where both sexes coordinate their

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aggression toward the same target, jointly defending the territory (Hall and Peters, 2008b; Dahlin and Wright, 2011; Speelman et al., 2025). The duet coordination is thought to be highly relevant, as well-coordinated duets can signal a strong commitment to maintaining the pair bond (an intrapair signal related to the attention given to the partner), the high motivation to defend the territory, and the coalition quality formed by the pair members (if coordinated duets signal quality attributes of the pair such as proximity, pair bond duration, and territory tenure) (Hall, 2000; Marshall-Ball et al., 2006; Hall and Magrath, 2007; Quirós-Guerrero et al., 2017; Diniz et al., 2021; Stewart and Kelley, 2025).

For collective territorial defense to occur, one individual must initiate the agonistic response toward intruders, while the partner coordinates its behavior in synchronization with the leader (Mares et al., 2012; Colombelli-Négrel, 2016). Consequently, joint territorial defense can occur even if the leader consistently directs its response toward only one individual of an intruding pair (Mennill, 2006; Colombelli-Négrel, 2016). This would occur if the male, for instance, led the aggressive response by rapidly approaching a solitary intruding male, while the female followed him to maintain response cohesion (as observed in the Variegated Fairywren *Malurus lamberti*) (Colombelli-Négrel, 2016). In such a scenario, the male's proximate motivation could be territorial defense against same-sex individuals (Templeton et al., 2011) or the defense of the pair bond (Dowling and Webster, 2017a). The female's proximate motivation instead could be commitment to the partner (Templeton et al., 2013), while ultimate adaptive benefits may arise from joint territorial defense (Colombelli-Négrel, 2016).

Within this framework, the Rufous Hornero (*Furnarius rufus*), is a species that performs duets in response to territorial intruders year-round, with a peak during the breeding season (Diniz et al., 2018, 2020; Mentesana et al., 2020). In this species, duetting may serve multiple functions, including year-round collective territorial defense and the mutual defense of the pair bond (Diniz et al., 2018, 2019, 2020). Rufous Hornero pairs exhibit a cautious territorial response to duets with a high degree of overlap of female and male songs, which might suggest the presence of more threatening intruders (Diniz et al., 2021). Partners also tend to exhibit greater vocal overlap during duetting when responding to playbacks of duets with preserved rhythmic coordination. This suggests that, in addition to the degree of vocal overlap, rhythmic coordination also plays a critical role in territorial conflict resolution (Diniz et al., 2021). This may occur because coordinated and more overlapping duets could signal a high level of pair commitment during territorial disputes (Diniz et al., 2021; Mentesana et al., 2024). However, it remains unclear what specific roles each sex plays in territorial interactions and the duets produced in this context. For instance, it is unknown whether the sexes of Rufous Hornero pairs have divergent interests during conflicts with intruding pairs. Furthermore, it is unclear how the spatial distribution of intruders influences the behavioral and acoustic cohesion of the territorial partners (Diniz et al., 2020).

A previous study conducted during the non-breeding season demonstrated that Rufous Hornero partners coordinate territorial defense in response to simulated intrusions by solitary females or males (solo playbacks) and by pairs in close proximity (duet playback via a single speaker). These findings support the joint territory defense hypothesis (Diniz et al., 2020). However, females spent less time in territorial vigilance and pair coordination was lower specifically in response to a solitary male. This suggests that females may be more inclined to defend the territory—or cooperate with the male in its defense—against other females (the sex-biased territorial defense hypothesis) (Templeton et al., 2011; Diniz et al., 2020). By contrast, females did not respond more strongly to a solitary female intruder than to a paired intrusion, as would be expected if they were primarily defending their position within the pair (the mate-guarding hypothesis) (van den Heuvel et al., 2013; Diniz et al., 2020). Furthermore, since the coordination of the aggressive response to simulated territorial intrusions may be, in part, a consequence of both partners approaching the same speaker (Rogers et al., 2004; Douglas and Mennill, 2010), the previous study did not evaluate whether one or both sexes can lead the response to the playback (Diniz et al., 2020). One way to bridge these gaps is to evaluate the response of pair members to territorial intrusions by partners that are spatially separated, simulated by a dual-speaker playback design. This approach allows for an investigation into how each individual directs its approach and cooperates with its partner during the aggressive response to intruders of each sex producing a duet (Rogers et al., 2004; Mennill, 2006; Douglas and Mennill, 2010).

In this study, we investigated whether the spatial arrangement of intruding pairs influences acoustic leadership, intensity, and coordination of the aggressive response in territorial Rufous Hornero pairs. To this end, we conducted a dual-speaker playback experiment simulating duets of intruding partners across two treatments: one with speakers placed close together (0.1 m) and another with speakers placed far apart (~12 m) (Fig. 1). According with the joint territorial defense hypothesis, we expected to find a coordinated physical (proximity between the focal pair) and vocal (songs) response against the simulated intrusion, regardless of the spatial arrangement of the simulated intruders (Mennill, 2006; Hall and Peters, 2008b; Hall, 2009; Douglas and Mennill, 2010; Koloff and Mennill, 2013). Furthermore, in accordance with this hypothesis, we expected partners to respond more intensely to the duet of simulated intruding partners when speakers were placed close together rather than far apart. This is because the former would represent a greater threat to territory ownership due to their spatial cohesion during the intrusion (Hall and Magrath, 2007; Hall and Peters, 2008b).

Alternatively, if the pair partitions territorial defense tasks (the division of labor hypothesis) (Templeton et al., 2011), we expected a reduction or absence of physical or vocal coordination in response to intruders placed far apart. Under this hypothesis, the apparent coordination in territorial defense against paired intruders in close proximity may simply reflect partners approaching together, with each directing aggression toward a different intruder (Mennill and Vehrencamp, 2008). In contrast, in response to intruders placed far apart, individuals might separate, with each individual being more aggressive against a different intruder. This could occur regardless of sex or against the sex that represents a greater threat, supporting the sex-biased territorial defense hypothesis and/or mate-guarding hypothesis (Mennill and Vehrencamp, 2008; Templeton et al., 2011; Weng et al., 2012).

2. Materials and methods

2.1. Study area and population

We conducted fieldwork between April and June 2025 at the campus of the Universidade Estadual Paulista (UNESP) and its surroundings in Rio Claro, Brazil (22°23′50″ S, 47°32′35″ W). The identification of nests and territories was carried out through active searches for nests on utility poles and trees, as well as by locating territories based on the pairs' spontaneous songs. The study was conducted midway through the Rufous Hornero non-breeding season, when territories are occupied by adult pairs with or without delayed-dispersal juveniles from the previous breeding season (Fraga, 1980; Diniz et al., 2018).

The Rufous Hornero population in the study area was partially banded as a result of ongoing banding activities by the Laboratório de Ecologia de Aves (LECAVE). Bird capture and banding activities by LECAVE are authorized by the Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAVE, license no. 3362/10) through the Chico Mendes Institute for Biodiversity Conservation (ICMBio, SISBIO authorization no. 45434-5), and were carried out months prior to the playback experiments to ensure that handling stress would not interfere with territorial defense responses. Rufous Horneros were captured using mist nets (12 m × 3 m, 15 mm mesh) and banded with unique combinations of CEMAVE metal bands and plastic color bands, or with a metal band only. Banding even a single individual within an adult pair

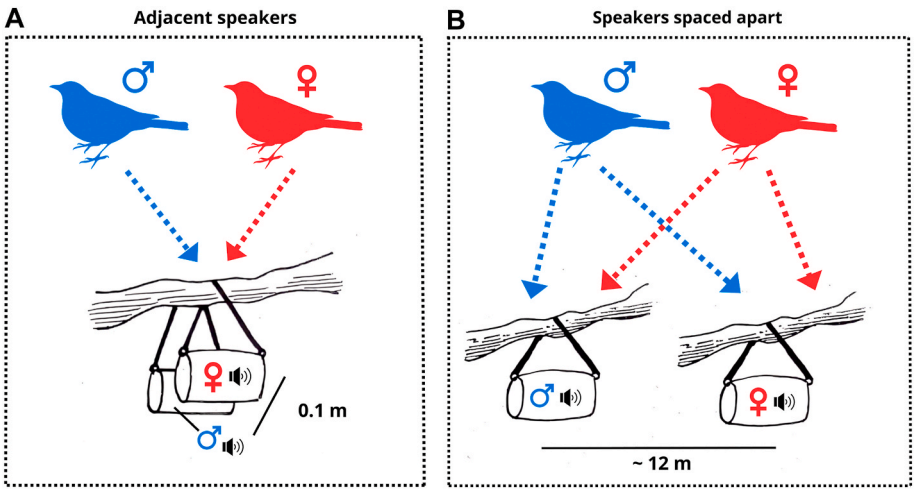


Fig. 1. Experimental design showing the distance between speakers simulating duet playbacks of territorial intruders in two settings: adjacent (**A**) and spaced apart (**B**). Dashed lines indicate the potential approach trajectories toward the speakers for each sex.

facilitated sex differentiation. Furthermore, since it is possible to differentiate the sexes of unbanded adult pairs based on their unique song types (Roper, 2005), we could identify individuals even without bands.

2.2. General procedures

We conducted a playback experiment (i.e., song broadcasting) by exposing 25 adult pairs of Rufous Horneros (16 without and nine with juveniles, see Results) to simulated territorial intrusions by conspecific pairs. Specifically, each adult pair was subjected to two treatments: (1) intruding partners placed far apart (~12 m) and (2) intruding partners placed close together (0.1 m). This territorial intrusion was simulated using playback of duets from an unfamiliar pair broadcast from within the focal pair's territory. Because juveniles rarely initiate group singing, although they may join parent-initiated songs, we focused on adult territorial responses and assumed that juveniles had little or no influence on responses to the playback treatments or the sex of the simulated intruder (Diniz et al., 2018, 2020). When territories contained delayed-dispersal juveniles, we identified the adult female and male as the individuals showing the strongest, leading, and most coordinated responses to the playback, applying this classification retrospectively to the pre-playback period (Diniz et al., 2018, 2020).

2.3. Playback stimuli

The playback stimuli consisted of overlapping female and male solos, creating an artificial duet (Diniz et al., 2021). We adopted this approach because male and female songs cannot be accurately extracted from natural duet recordings due to their partial overlap in time and frequency (Roper, 2005; Diniz et al., 2021). Although this method may reduce the fine-scale coordination between partners that characterizes Rufous Hornero duets (Laje and Mindlin, 2003), territorial pairs respond similarly to playbacks of artificial and natural duets (Diniz et al., 2021). In addition, Rufous Horneros use the same song type in both solos and duet contributions, although duet contributions are slightly longer and contain more elements (Diniz et al., 2018, 2020, 2021).

To prepare the stimuli, we used a dataset of solo songs recorded from a Rufous Hornero population at the Universidade de Brasília's Darcy Ribeiro campus (Diniz et al., 2018, 2020, 2021), located ~734 km from the study population. Thus, the tested birds were unfamiliar with these songs. Rufous Horneros belong to a non-vocal-learning clade (ten Cate, 2021) and show no evidence of geographic vocal dialects. Consistent with this, a previous study found that variation in individual duet contributions was greater among pairs than between populations separated by ~820 km (Diniz et al., 2024), suggesting that geographic variation was unlikely to influence responses to the playback stimuli. We selected ten female and ten male solo songs of high acoustic quality, produced by distinct individuals. All solos were recorded in WAVE format (sampling rate: 48 kHz, bit depth: 16-bit) using a Marantz PMD660 equipped with a Sennheiser ME66-K6 directional microphone.

Background noise in each solo recording was filtered in two stages (Diniz et al., 2021). First, we applied a high-pass filter to remove acoustic energy below 500 Hz in each solo using Raven Pro 1.6.5 software. Subsequently, we used the noise reduction tool in Audacity software (version 3.3.3) to remove general background noise (Noise Reduction: 20 dB; Sensitivity: 6; Frequency Smoothing: 3). The peak amplitudes of the solos were then normalized to 0 dB for both females and males (Diniz et al., 2021), again using Audacity.

The playback order for female and male solos was randomized separately. We created artificial stereo duets by combining the first randomized male solo (Track 1) with the first randomized female solo (Track 2), the second female solo (Track 1) with the second male solo (Track 2), and so forth. The first five artificial duets were initiated by the male (the randomly selected sex), while the final five duets were initiated by the female. The latency between the initiator and the responder was set at 0.29 s. This value corresponds to the mean answering latency observed in highly overlapping simultaneous songs in Rufous Hornero duets (Diniz et al., 2021).

To create each stimulus, the same artificial duet was repeated five times, with 60-s silent intervals between repetitions and after the final one. Additionally, we added a 60-s silent interval to the beginning of the stimulus to ensure that any Bluetooth playback delay occurred before the first duet. Excluding this initial silent interval, stimulus duration was 5.48 ± 0.07 min (mean ± SD; range: 5.40–5.63 min). Variation among stimuli reflected differences in the duration of the artificial duets (mean ± SD = 5.73 ± 0.84 s; range: 4.85–7.55 s). The playback stimuli were exported in WAVE format (sampling rate: 48 kHz; bit depth: 16-bit). The presentation order of the playback stimuli (1 to 10) was randomized for playback to the birds. Because we had 10 stimuli, after the experiment was conducted with the first 10 pairs, the stimuli were repeated in the same order for the subsequent pairs.

2.4. Playback experiment

We conducted a playback experiment using two numbered speakers (JBL Xtreme 3; dynamic frequency response: 53.5 Hz to 20 kHz), to simultaneously broadcast a different solo song from each sex, thereby

forming an artificial duet (Fig. 1). Two treatments were applied to each pair: 1) a duet with speakers spaced apart (mean ± SD = 11.62 ± 2.05 m; range = 8.80 to 16.00 m; hereafter, the distant-speaker treatment); and 2) a duet with adjacent speakers (0.1 m; hereafter, the close-speaker treatment). These distances represent the natural variation in spacing between Rufous Hornero partners while duetting. Partners typically sing in close proximity (median = 0.76, Diniz et al., 2020), although they occasionally duet more than 10 m apart (P. Diniz, unpublished data). The distant-speakers treatment also used inter-speaker distances comparable to those in previous playback studies of other duetting bird species (10 m: Molles and Waas, 2006; Dowling and Webster, 2015; Quirós-Guerrero et al., 2017; Wheeldon et al., 2021; 12-15 m: Rogers et al., 2004; 16 m in Mennill and Vehrencamp, 2008). We conducted the trials using the nearest available perches (trees or poles) to the active nest, with both treatments sharing one non-systematically selected perch. Because suitable speaker locations and bird perches varied among territories, the distance between speakers in the distant-speakers treatment varied slightly among trials.

The same stimulus was used for both treatments (close and distant speakers) within each pair, whereas distinct stimuli were used between different pairs. Additionally, to ensure experimental balance, we balanced the playback of songs for each sex across speakers: female songs were broadcast 27 times in Speaker 1 and 23 times in Speaker 2. Each adult pair received both treatments with an interval of at least 2 days (mean ± SD = 5 ± 3 days, maximum = 14 days) to reduce the likelihood of habituation or sensitization to the stimulus (Dong and Clayton, 2009). The order of treatments was nearly counterbalanced across pairs: 14 pairs were first exposed to playback from close speakers and subsequently to playback from distant speakers, whereas 11 pairs were first exposed to playback from distant speakers. The order of exposure to treatments was also reversed for the stimulus. Specifically, if Stimulus 1 was initially presented in the close-speaker treatment, we subsequently used it in the distant-speaker treatment during its second repetition. We conducted a median of 3 experimental trials per day (range: 1–4). These playbacks were performed in the morning, at an average time of 10:03 (ranging from 8:27 to 11:43).

Each experiment consisted of three phases: (a) pre-playback (5 min), (b) during the playback (mean = 5.48 min; see Section 2.3, Playback stimuli), and (c) post-playback (5 min). Before initiating these phases, speakers were positioned on perches, which consisted of either tree branches or fence posts. Additionally, we determined which sex would be simulated by each speaker. During the 5-min pre-playback phase (a), we also recorded the vocalizations (number of songs for each individual) of the focal pair to establish a baseline prior to playback. Subsequently, in the second phase (b), the duet playback stimulus was broadcast with pre-defined intervals between repetitions, as previously described. To achieve this, we used VLC Media Player 3.6.5 to broadcast audio in WAVE format. We initiated the stimulus even without prior localization of the individuals, due to the difficulty of locating them within territories that often include buildings as visual obstacles. Consequently, the playback broadcast prompted the individuals of that territory to approach the experimental site and/or vocalize, thereby allowing for the localization of the focal pair.

At each stage, we recorded vocalizations and narrated behaviors using a Zoom F3 or Marantz PMD660 recorder equipped with a Sennheiser ME67-K6 microphone (WAVE format, sampling rate: 44.1 kHz, resolution: 24 or 32 bits). We narrated approach latency and the time spent within 20 m and 5 m of each speaker for both adult females and males during the playback (b) and post-playback (c) stages. The 20 m and 5 m radii were visually estimated using landmarks as reference points and by agreement between two researchers (GML and PD). In addition, we also recorded narrations of which adult sex initiated and which responded to each song (thereby forming a duet), as well as the total number of songs produced by each individual.

Audio recordings were analyzed using Raven Pro 1.6 software. For each adult sex, we quantified approach and vocal behavior during the combined playback and post-playback periods because Rufous Hornero pairs typically remain near the speakers and continue singing after conspecific song playback (Diniz et al., 2020), whereas no bird approached within 20 m of either speaker and few sang during the pre-playback stage (see Results). For each adult sex, we quantified the following approach behavior variables during the combined playback and post-playback periods: (1) '20-m latency': the time elapsed from the onset of playback until the individual approached within 20 m of the loudspeaker; (2) '5-m latency': the time elapsed from the onset of playback until the individual approached within 5 m of the loudspeaker, similarly to prior research (Dowling and Webster, 2015); and (3) '5-m time': the total time spent within a 5-m radius of each loudspeaker. Quantifying focal pair behavior at these distinct spatial scales was necessary to differentiate a general entry into the surrounding experimental environment (within 20 m of the speakers) from a targeted approach toward the loudspeakers themselves (within 5 m). Additionally, for each sex, we quantified the following individual vocal response variables during the combined playback and post-playback periods: (1) 'song latency': time elapsed between the onset of the playback and the first song (whether sung as a solo or as part of a duet or chorus); (2) 'songs': the number of songs produced (including solos and contributions to duets or choruses); (3) 'song duration': the duration of songs (solos and contributions to duets or choruses); and (4) 'initiated songs': the number of vocalizations initiated by the individual (including initiated solos, duets, or choruses). These vocal response variables were quantified for the close-speaker and distant-speaker treatments but not separately for each speaker in the distant-speaker treatment, as in many cases songs could not be objectively assigned to one of the two speakers. Duets and choruses were defined as events in which two individuals and three or more individuals, respectively, coordinated their songs by singing simultaneously (Hall, 2009). While 'songs' represent overall song output, 'initiated songs' represent leadership in duets and choruses and in vocal response to the playback (Logue and Krupp, 2016).

2.5. Statistical analyses

To perform the statistical analyses, we used generalized linear mixed models (GLMMs; glmmTMB package v1.1.14) (Brooks et al., 2017) and Pearson's correlations followed by Fisher's tests (base functions) in the R environment v4.5.3 (R Core Team, 2026). Accordingly, the analyses were organized into four stages: (a) response to the treatments (close vs. distant speakers); (b) correlation between partners in their behavioral responses; (c) response to each speaker (i.e., the sex of the intruder); and (d) the association between vocal behavior and approach bias toward the simulated intruder's sex, measured as the difference in time spent within 5 m of the female and male speakers.

For the treatment comparison stage (a), a GLMM was used for each of the following response variables: '20-m latency', '5-m latency', '5-m time', 'song latency', 'songs', and 'song duration'. In these models, we included treatment (close or distant speakers), the focal individual's sex, and the interaction between these variables as fixed predictors, while pair and stimulus identities were included as random intercepts in the models.

For the correlation between partners in their behavioral responses (b), we performed analyses using Pearson's correlations. Specifically, '20-m latency', '5-m latency', '5-m time', 'song latency', 'songs', and 'song duration' (averaged per pair) were used as variables to assess correlations between partners (sexes). These Pearson correlations between variables were tested between partners for each treatment (close and distant speakers). Furthermore, we performed Fisher's z-tests to determine whether there were differences in partner behavioral correlations between treatments, where non-significant results ($p > 0.05$) indicate an absence of difference between treatments.

To analyze the response to each speaker (i.e., the sex of the simulated intruder) (c), we used a GLMM only for the response data from the distant-speaker treatment. In this model, the response variable was the

time spent within 5 m of a speaker, which better reflects bias toward one speaker than approach latency because birds may switch between speakers after their initial approach. Thus, each focal individual contributed two observations per trial: time spent near the speaker simulating the female intruder and time spent near the speaker simulating the male intruder. Predictor variables included the sex simulated by the speaker, the sex of the focal individual, the sex that initiated the duet stimulus (with half of the stimuli initiated by females), and the interactions between the sex simulated by the speaker and each of these variables. The inclusion of the playback initiator's sex as a fixed effect, and its interaction with the sex simulated by the speaker, is important because individuals may bias their approach toward the speaker representing the initiating sex, as reported for Magpie-lark (*Grallina cyanoleuca*) (Rogers et al., 2004). Additionally, pair and stimulus identities were included as random intercepts. Because no population-level bias in time spent near male versus female speakers was detected (see Results), we performed a post hoc analysis to test whether this reflected similar within-pair responses (i.e., pairs spent similar amounts of time near both speakers) or variation among pairs (i.e., some pairs spent more time near the male speaker, whereas others spent more time near the female speaker). This model had the same structure as the previous GLMM but additionally included the speaker's simulated sex as a random slope within pair identity, allowing the effect of speaker sex on time spent near each speaker to vary among pairs.

Regarding the association between vocal behavior and approach bias toward a specific speaker (i.e., the intruder's sex) (d), we performed the analysis using three GLMMs exclusively for the response data from the distant speakers. Specifically, we built a model for each of the three response variables: 'songs', 'songs initiated', and 'song duration' (averaged per pair). As noted above, these variables could not be objectively attributed to either speaker and were therefore quantified as the overall response to the distant-speaker treatment. In these models, the predictor variables included (i) the difference between the time spent within 5 m of the speaker simulating the female intruder and the time spent within 5 m of the speaker simulating the male intruder (with time spent within 5 m of either speaker interpreted as an index of approach bias), (ii) the focal individual's sex, and (iii) the interaction between these variables. Finally, pair and stimulus identities were included as random intercepts in the models.

In the GLMMs, error distributions varied according to the nature of the response variable (continuous: Gaussian; continuous with many zeros: Tweedie (log link); counts: Poisson, Conway-Maxwell Poisson, or Negative Binomial). Model residuals were evaluated using the DHARMa package v0.4.7 (Hartig, 2024). Likelihood ratio tests (Type II Wald χ^2) were performed using the Anova function from the car package v3.1-5 (Fox and Weisberg, 2019), and standardized model coefficients were obtained using the model_parameters function (standardize = "basic") from the parameters package v0.29.0 (Lüdecke et al., 2020).

3. Results

3.1. Experimental groups

We conducted playback experiments in 25 territories: 16 contained only an adult pair, while the remaining nine included one or two juveniles in addition to the adult pair. Based on previous studies (Fraga, 1980; Amorim et al., 2024), we assume these juveniles were the most recent brood (2024) of one or both adults. In two of the territories containing juveniles, these individuals were present on only one of the days. In total, there were 63 individuals, consisting of 50 adults from 25 pairs and 13 juveniles. As only 13 of these were banded, the predominant method of sex identification was based on sex-specific song types (Roper, 2005).

We collected data in 50 experiments, consisting of two different treatments in each of the 25 territories. Of these 50 experiments, in 40 instances no individual from the pair sang during the 5 min of silence preceding the first artificial duet playback. This indicates that the behavior observed during and after the playback (see below) was in response to the experimental stimuli. In total, we recorded 40 solo songs, 124 duets, and 28 choruses. In only one experiment did one of the individuals (male) from the pair fail to approach the experiment area (within 20 m of at least one of the speakers). In three experiments, one of the members of the focal pair appeared only during the post-playback period (defined as within 5 min after the end of the five artificial duets).

3.2. Partner coordination

Rufous Hornero partners showed high coordination across all measured behaviors, ranging from physical approach to the speakers to singing patterns. Regarding their approach toward simulated intruders, pairs showed a high correlation in approach latency, regardless of the treatment (close or distant speakers), at both the 20 m distance from the speakers (Fisher's z-test: $z = -0.65$, $p = 0.52$; Fig. 2A) and for the 5 m distance from the speakers (Fisher's z-test: $z = -0.11$, $p = 0.91$; Fig. 2B). Furthermore, we found a similarly high correlation between partners in the time spent within 5 m of the speakers in both treatments (Fisher's z-test: $z = -0.64$, $p = 0.52$; Fig. 2C). Singing behaviors were also highly correlated between partners. Regardless of the treatment, partners showed strong correlations in song latency (Fisher's z-test: $z = 1.04$, $p = 0.30$; Fig. 3A), total number of songs (including solos and contributions to duets and choruses) (Fisher's z-test: $z = 0.11$, $p = 0.91$; Fig. 3B), and song duration (Fisher's z-test: $z = 0.08$, $p = 0.94$; Fig. 3C).

3.3. Response to treatments

Pairs approached within 5 m of at least one speaker more quickly in the distant-speaker treatment than in the close-speaker treatment; that is, for both sexes, approach latency to the speakers (5 m) was lower when the speakers were distant (Table 1, Fig. 2E). In contrast, the other physical approach responses to the speakers (approach latency to 20 m, Fig. 2D; time spent within 5 m of the speakers, Fig. 2F) (Table 1), as well as singing responses (song latency, Fig. 3D; total number of songs, Fig. 3E; and song duration, Fig. 3F) (Table 2) did not differ between treatments.

3.4. Response to intruder sex

On average, among pairs, the time individuals spent within 5 m of the speaker simulating a female intruder did not differ from the time spent within 5 m of the speaker simulating a male intruder (Table 3). However, we observed significant variation among pairs. The model that included the random slope for speaker 'sex' and pair identity showed a better fit (AIC: 890) than the model including only pair identity as a random intercept (AIC: 917; likelihood ratio test, $\chi^2 = 31.89$, df = 2, $p < 0.0001$). In line with this variation, approximately half of the pairs remained closer to the speaker simulating a female intruder, while the other half remained closer to the speaker simulating a male intruder (Fig. 4). Moreover, most pairs stayed closer to one speaker than to the other (Fig. 4). This bias was not related to which speaker broadcast the song of the duet initiator, as indicated by the absence of a significant interaction between speaker sex and initiator sex (Table 3, Fig. 4).

3.5. Vocal behavior and response to intruder sex

The total number of songs (solos plus contributions to duets and choruses) and their duration were not associated with the approach bias (time spent within a 5 m radius toward the speaker simulating a female intruder relative to the speaker simulating a male intruder; Table 4, Fig. 5A–C). However, we found that the number of songs initiated (i.e., solos plus duets and choruses in which the individual was the first to start singing) varied with the approach bias depending on the focal individual sex (Table 4). Sub-models, each including only one of the focal

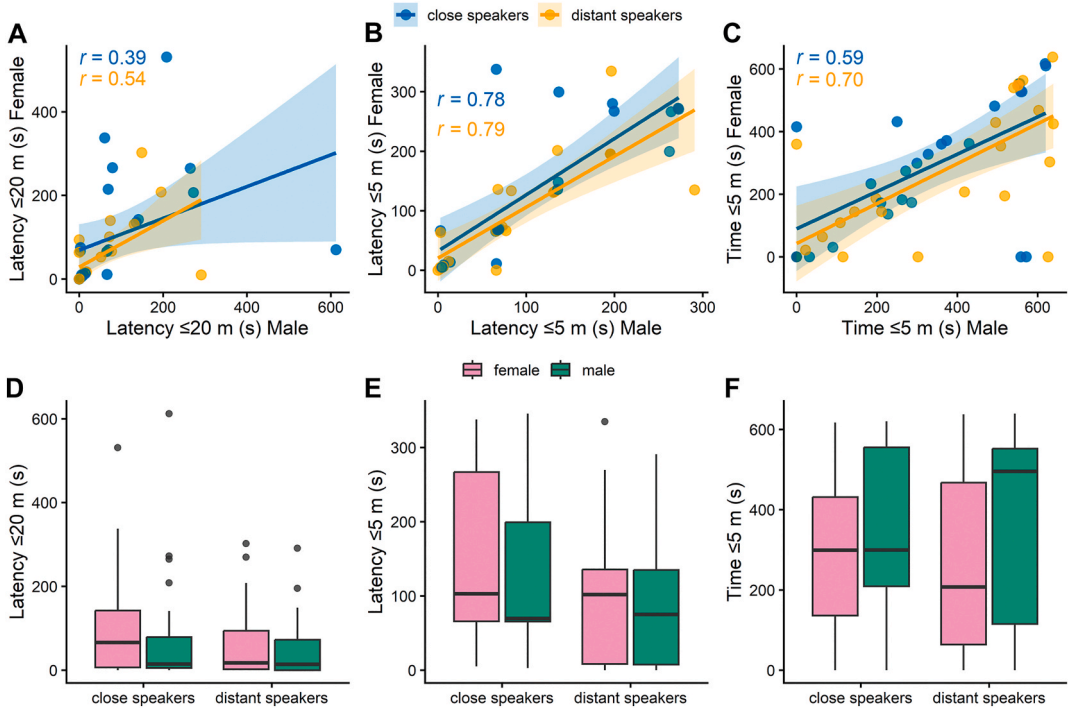


Fig. 2. Variation in intensity and coordination of the Rufous Hornero physical approach to speakers simulating close (0.1 m) and distant (~12 m) territorial intruders. Panels 'Latency ≤20 m' (**A**,**D**) and 'Latency ≤5 m' (**B**,**E**) describe approach latencies to a 20 m and 5 m radius of at least one speaker, respectively, following playback onset. Additionally, 'Time ≤5 m' (**C**,**F**) represents the time spent within a 5 m radius of at least one speaker during the playback and post-playback periods. In **A**–**C**, lines and shaded areas represent, respectively, estimates and standard errors derived from simple linear models (for illustration purpose only).

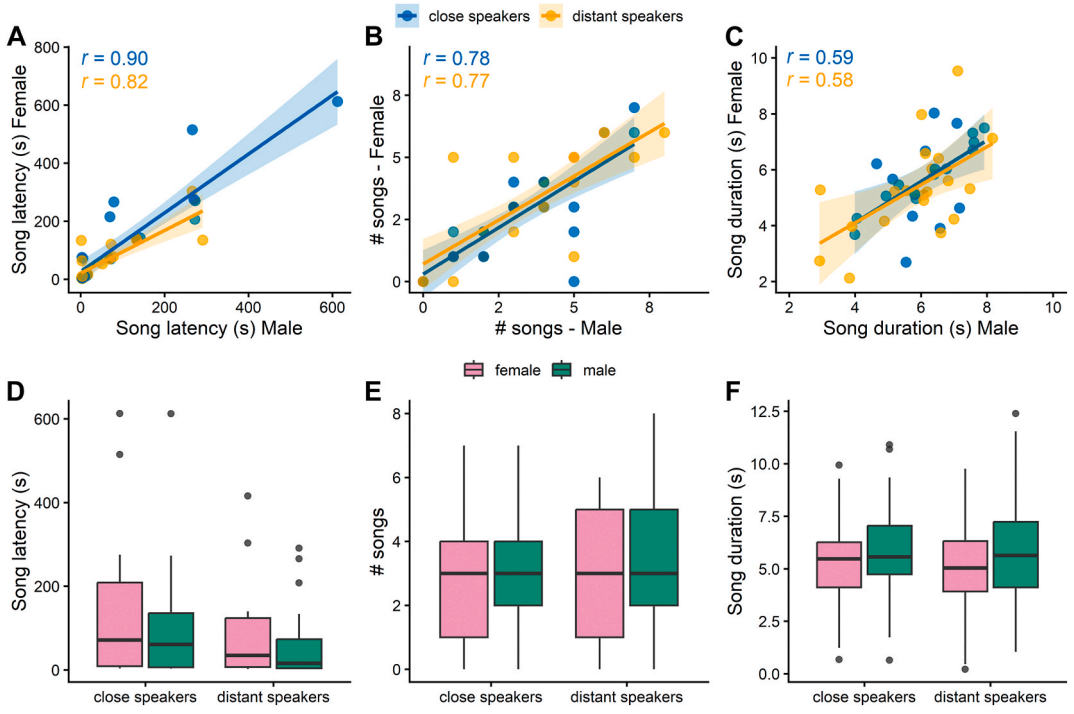


Fig. 3. Variation in the singing response of Rufous Horneros to speakers simulating close (0.1 m) and distant (~12 m) territorial intruders. Specifically, 'Song latency' (**A**,**D**) indicates the latency to sing following playback onset. '# songs' (**B**,**E**) represents total number of songs (complete solos or contributions to duets or choruses) during the playback and post-playback periods. Finally, 'Song duration' (**C**,**F**) displays the mean (**C**) or raw (**F**) duration of each song (complete solos or contributions to duets or choruses) during these same playback and post-playback periods. In **A**–**C**, lines and shaded areas represent, respectively, estimates and standard errors from simple linear models (for illustration purpose only).

sexes, showed that in pairs that spent more time near the speaker simulating a female intruder, females initiated more songs (Conway-Maxwell Poisson model; $\beta \pm SE = 0.62 \pm 0.21$, $z = 2.84$, $p = 0.003$, Fig. 5B).

Table 1
Variation in the intensity of Rufous Hornero physical approach to speakers simulating close and distant territorial intruders, according to generalized linear mixed models (GLMM). Values are presented as $\beta \pm$ SE. Bold type indicates a significant effect based on likelihood ratio tests (type II Wald χ^2).

Parameter	Latency ≤20 m	Latency ≤5 m	Time ≤5 m
Sex [male]	−0.11 ± 0.17	−0.08 ± 0.10	0.07 ± 0.11
Treatment [distant speakers]	−0.20 ± 0.18	**−0.24 ± 0.11**	−0.02 ± 0.11
Sex [male] × Treatment [distant speakers]	0.02 ± 0.22	0.02 ± 0.13	0.06 ± 0.13
SD (stimulus identity)	0.29	0.00	0.00
SD (pair identity)	0.69	1.01	0.33

SD = standard deviation of the random effects. Latency 20 m and Latency 5 m: latency to approach within a 20 m and 5 m radius, respectively, of at least one of the speakers after the onset of playback. Time 5 m: time spent within a 5 m radius of at least one of the speakers during the playback and post-playback period. Error family: Tweedie. Reference values: female (sex) and close speakers (treatment).

Table 2
Variation in the singing response of Rufous Horneros to speakers simulating close and distant territorial intruders, according to generalized linear mixed models (GLMM). Values are presented as $\beta \pm$ SE. Bold type indicates a significant result based on likelihood ratio tests (type II Wald χ^2).

Parameter	Song latency	# songs	Song duration
Sex [male]	−0.13 ± 0.15	0.17 ± 0.16	**0.19 ± 0.16**
Treatment [distant speakers]	−0.26 ± 0.17	0.12 ± 0.17	−0.19 ± 0.16
Sex [male] × Treatment [distant speakers]	0.09 ± 0.19	−0.09 ± 0.23	0.16 ± 0.22
SD (stimulus identity)	0.00	0.00	0.16
SD (pair identity)	1.06	0.47	0.30

SD = standard deviation of the random effects. Song latency: latency after the onset of playback. # songs: total number of songs (complete solos or contributions to duets or choruses) during the playback and post-playback periods. Song duration: duration of each song (complete solos or contributions to duets or choruses) during the playback and post-playback periods. Error family: Tweedie (Song latency), Poisson (# songs), or Gaussian (Song duration). Reference values: female (sex) and close speakers (treatment).

Table 3
Variation in the approach of Rufous Horneros to two distant speakers (~12 m), with each simulating a territorial intruder of a different sex, according to generalized linear mixed models (GLMM). Values are presented as $\beta \pm$ SE.

Parameter	Time ≤5 m
Intruder sex [male]	−0.28 ± 0.26
Initiator sex [male]	−0.15 ± 0.20
Focal individual sex [male]	−0.14 ± 0.20
Intruder sex [male] × Initiator sex [male]	0.29 ± 0.25
Intruder sex [male] × Focal individual sex [male]	0.02 ± 0.25
SD (stimulus identity)	0.13
SD (pair identity)	0.00

SD = standard deviation of the random effects. Time ≤5 m: time spent within a 5 m radius of one of the speakers during the playback and post-playback periods. 'Intruder sex': sex of the stimulus broadcast from a given speaker. 'Initiator sex': sex of the stimulus from the speaker that initiated the artificial duet. 'Focal individual sex': sex of the focal individual responding to the playback. Error family: Tweedie. Reference values: female (sex of the intruder, focal individual, and playback initiator).

4. Discussion

In all behaviors, both including physical approach and singing, the responses of males and females within pairs were highly correlated (Figs. 2 and 3). Specifically, when one individual approached the speaker, the other followed; similarly, when one began to sing, the partner joined in, creating duets, and so forth. This result is consistent

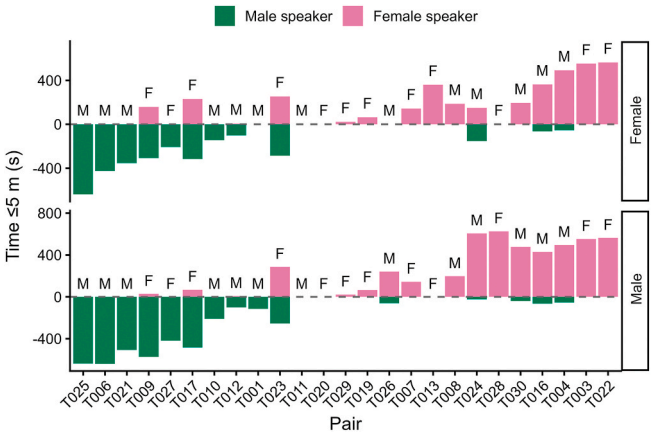


Fig. 4. Inter-pair variation in the approach of Rufous Horneros to two distant speakers (~12 m), each simulating a territorial intruder of a different sex. 'Time ≤5 m' represents the difference between the time spent within 5 m of the speaker simulating a female intruder and the time spent within 5 m of the speaker simulating a male intruder. Positive values indicate an approach toward the female intruder speaker, while negative values indicate the opposite. Letters above the bars indicate the sex of the playback initiator (F = female, M = male).

Table 4
Relationship between vocal response and approach bias toward the speaker simulating a female intruder during a simulated territorial intrusion by distant partners (~12 m), according to generalized linear mixed models (GLMM). Approach bias represented by the difference between the time spent within 5 m of the speaker simulating a female intruder and the time spent within 5 m of the speaker simulating a male intruder. Values presented as $\beta \pm$ SE. Bold type indicates a significant result based on likelihood ratio tests.

Parameter	# songs	Initiated songs	Song duration
Approach bias	0.14 ± 0.14	**0.75 ± 0.27**	−0.06 ± 0.14
Focal individual sex [male]	0.05 ± 0.05	**0.43 ± 0.15**	**0.16 ± 0.08**
Approach bias × Focal individual sex [male]	−0.09 ± 0.09	**−0.65 ± 0.25**	0.02 ± 0.14
SD (stimulus identity)	[1]	[2]	[1]
SD (pair identity)	0.66	0.00	0.06

SD = standard deviation of the random effects. # songs: total number of songs (complete solos or contributions to duets or choruses). Initiated songs: total number of complete solos, and initiated duets and choruses. Song duration: duration of each song (complete solos or contributions to duets or choruses). The three vocal behaviors were computed for the period encompassing the playback and post-playback periods. Error family: Conway-Maxwell Poisson (# songs), Negative Binomial (initiated songs), or Gaussian (song duration). Reference value: female (sex of the focal individual). Stimulus identity was removed from the model because it led to convergence issues[1] or errors in the likelihood ratio test[2].

with previous studies of Rufous Horneros and other tropical and temperate duetting, which have reported a high correlation in territorial defense responses between partners (Logue, 2005; Hall and Peters, 2008a; Ippi et al., 2017; Diniz et al., 2020; Mentesana et al., 2020; Liu et al., 2023), consistent with the joint territorial defense hypothesis (Hall, 2009).

The Rufous Hornero pair members responded in a coordinated manner and independently of the sex of the simulated intruders, both in terms of physical approach and singing behaviors (Tables 1 and 2, Figs. 2 and 3). This finding supports the joint territorial defense hypothesis (Hall, 2009), under which predicts that sexes should not respond individually to different speakers, but rather to respond together to the same ones. Furthermore, these results contradict the competing division of labor hypothesis (Templeton et al., 2011), which posits that individuals would respond less coordinately and/or respond to different speakers.

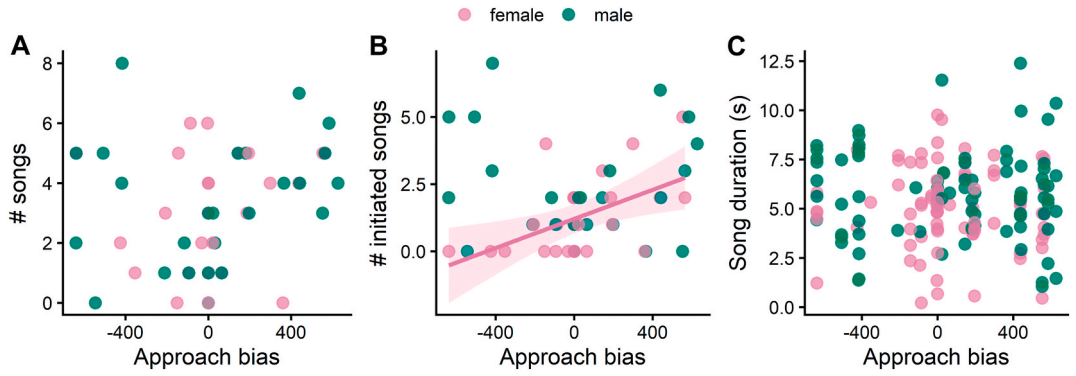


Fig. 5. Relationship between vocal response and the approach bias toward the speaker simulating a female intruder during a simulated territorial intrusion by distant partners (~12 m). The approach bias is represented by the difference between the time spent within 5 m of the speaker simulating a female intruder and the time spent within 5 m of the speaker simulating a male intruder. Positive values indicate an approach toward the speaker simulating a female intruder, while negative values indicate an approach toward the speaker simulating a male intruder. '# songs' (**A**): the total number of songs (complete solos or contributions to duets or choruses). '# initiated songs' (**B**): the total number of songs initiated (complete solos, or initiated duets or choruses). 'Song duration (s)' (**C**): the duration of each song (complete solos or contributions to duets or choruses). The three vocal behaviors were computed for the period encompassing the playback and post-playback periods. The lines and shaded areas represent, respectively, estimates and standard errors derived from simple linear models (for illustration purpose only).

Although there is some evidence for sex-specific division of labor in territory defense in a few duetting species (Levin, 1996; Mennill and Vehrencamp, 2008; Templeton et al., 2011), our data reinforce the notion that cooperative defense is more prevalent in duetting species (Logue, 2005; Quirós-Guerrero et al., 2017). Dual-speaker playback experiments across duetting species should help determine whether year-round territoriality and long-term pair bonds, as in Rufous Horneros (Diniz et al., 2018; Amorim et al., 2023; Diniz, 2025), have favored the evolution of joint territory defense over division of labor territory defense (Tobias et al., 2016; Arashanapalli and Freeman, 2026).

Rufous Hornero pairs had a longer approach latency to within 5 m of the close speakers compared to the distant ones; that is, they approached within 5 m of the distant speakers more quickly (Table 1, Fig. 2E). The faster approach to distant speaker could be due to the distant speakers having a larger acoustic range, as they were positioned approximately 12 m apart. However, the pairs did not differ in their latency to reach the experimental site, given that the approach latency to within 20 m of the speakers was equal across both treatments. Therefore, we suggest that the most appropriate interpretation for our results is that close intruders represent a greater threat and thus elicited a more cautious response from the focal individuals. Such closer proximity between the speakers may enhance the perceived overlap between songs (by minimizing the relative delay between signals) while signaling a highly coordinated invading pair (Jedlikowski and Rek, 2025), thereby eliciting a more cautious response from the Rufous Horneros. This result corroborates a previous study indicating that Rufous Hornero pairs are more cautious when responding to intruders with a high degree of song overlap (Diniz et al., 2021). However, this same finding conflicts with our initial prediction, which suggested that the response to close speakers would be more intense than to distant speakers. Therefore, a more cautious response to threatening stimuli seems to better align with studies on the Rufous Hornero, in contrast to research on other species in which close speakers stimulate a more intense response (Hall and Magrath, 2007; Hall and Peters, 2008b).

Regarding the response to the simulated sexes across the different speakers, on average, at the population level, individuals spent a similar amount of time near each speaker. This result is consistent with patterns reported for the Riverside Wrens (*Cantorchilus semibadius*) (Quirós-Guerrero et al., 2017) and Barred Antshrikes (*Thamnophilus doliatus*) (Koloff and Mennill, 2013), but contrasts with the sex-biased territorial responses observed in Rufous-and-white Wrens (*Thryothorus rufalbus*), where individuals preferentially approach same-sex members of intruding pairs (Mennill, 2006; Mennill and Vehrencamp, 2008). Despite similar time spent near each speaker at the population level, individual-level analyses revealed that Rufous Horneros typically remained close to only one speaker (Fig. 4). This bias was not directed toward the speaker representing the sex of the playback initiator (Table 3, Fig. 4), as would be expected if horneros approached and remained closer to the first intruder (regardless of sex), as observed in magpie-larks (Rogers et al., 2004). Alternatively, this pattern could reflect differences between territorial partners in approaching the speakers (e.g., if the female arrived first, she might approach the female speaker), but partners typically approached together (median = 0.38 s of difference between partners; Fig. 2A,B). A third possibility is that the first bird approached the speaker closest to its initial location, which was not observed or measured. However, birds likely heard both speakers and often arrived at or moved toward the more distant speaker during the >5 min playback. Finally, the bias toward a given speaker might reflect among-pair variation in initiative or leadership during territorial responses. Future studies should investigate the role of leadership in sustaining the territorial response effort toward intruders, as well as determine whether factors such as personality traits (Sasaki et al., 2018), body size (Reebs, 2001), and territory tenure (Flack et al., 2012) contribute to leadership roles within pairs.

Overall, males and females exhibited no significant difference in their response to the sex of the intruders (Table 3, Fig. 4). This finding aligns with our expectations, given that Rufous Hornero is a species characterized by high intraspecific territorial competitiveness, rare extra-pair copulation, low divorce rates, and pairs that tend to remain together for years (Diniz et al., 2019; Amorim et al., 2023). Consequently, territorial behavior and duet singing in this species appear to be better supported by the joint territorial defense hypothesis (Hall, 2009) rather than mate guarding (Templeton et al., 2011; Diniz et al., 2020), particularly during the non-breeding season. Nevertheless, we observed a notable pattern in female behavior: those that spent more time near the speakers simulating female intruders also initiated a higher number of songs (Table 4, Fig. 5B). This finding aligns with a previous study showing that females spent more time in territorial surveillance in response to simulated female intruders (either alone or paired) than to male solo intruders (in experiments using a single speaker) (Diniz et al., 2020). Together, these findings support the sex-biased territory defense hypothesis (Templeton et al., 2011; Diniz et al., 2020), suggesting that even when partners jointly defend their territory, individuals may preferentially defend it against same-sex rivals (Templeton et al., 2011), and that female-female competition is an important factor in the evolution of female signaling traits overall (Diniz et al., 2019).

We acknowledge the possibilities that the results of this study may or

may not remain constant or be generalizable throughout the year (Zapata and McEntee, 2023; Guo et al., 2020). This study was conducted during the non-breeding, when low competition for immediate mating opportunities favors cooperative defense of territorial resources and the pair bond (Diniz et al., 2018, 2020). During the breeding season in austral spring and early summer (September to December), individuals might exhibit divergent signaling strategies (Diniz et al., 2018; Demko and Mennill, 2018) and interests driven by competition for immediate mating opportunities (Andersson and Iwasa, 1996; Appleby et al., 1999; Fedy and Stutchbury, 2005; Dowling and Webster, 2017a; Demko and Mennill, 2018). For example, during the mating period, sexes might respond more intensely to simulating intruders of their own sex, consistent with the division of labor and/or mate guarding hypotheses (Templeton et al., 2011; Diniz et al., 2020). Alternatively, males may remain close and vocally responsive (answering female-initiated songs to create duets) to its social mate to physically and acoustically guard this mate (Dowling and Webster, 2017a, 2017b; Diniz et al., 2018), thus reducing the chance of female engages in extra-pair copulations (Diniz et al., 2019; Diniz and Biagolini-Jr, 2021). Nevertheless, even during the breeding season, social selection may favor the cooperative defense of the common territory and the pair bond against paired intruders (Mentesana et al., 2020), with no discrimination regarding the sex of each intruder (Dowling and Webster, 2015). Further research replicating our study during the breeding season could shed light and clarify these possibilities.

We acknowledge that using solos to construct artificial duets may not reflect the precise fine coordination of natural Rufous Hornero duets, which are slightly longer, contain more elements, and preserve rhythmic alignment between partners (Laje and Mindlin, 2003; Diniz et al., 2018, 2020, 2021). However, this approach was necessary because the male and female contributions overlap in both time and frequency, making it impossible to isolate them for playback through separate speakers while preserving their natural rhythmic alignment. Despite this limitation, Rufous Horneros have been shown to respond similarly to natural and artificial duets (Diniz et al., 2021). For future studies on antiphonal duets, we suggest isolating the contribution of each individual to better match the natural characteristics of the duet.

5. Conclusions

Pairs responded in a highly coordinated manner across all vocalization and physical approach behaviors toward the simulated intruders. Furthermore, they exhibited a more cautious response to the treatment where speakers were close together, showing a greater latency to approach within 5 m of the speakers when they were near each other than when they were far apart. Taken together, these results corroborate the joint territorial defense hypothesis, suggesting that (1) even when intruder pairs are distant from each other, partners prioritize a coordinated response over division of labor; and (2) duets performed by spatially close intruder pairs may be a greater threat to territory ownership.

Individuals exhibited the same behaviors and at the same intensity levels, regardless of the sex simulated by the speakers. Pairs exhibited a pattern of investing their time in only one of the speakers; however, females from pairs that spent more time near the speakers representing female intruders led the acoustic response to the playbacks. These results also support the joint territorial defense hypothesis but suggest that when females lead the territorial response, they direct it toward female intruders (sex-biased territory defense), potentially due to the dual threat of losing both their partner and their territory.

CRediT authorship contribution statement

Gabriel M. Lopes: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Isadora Z. Santieff:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Marco A. Pizo:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Pedro Diniz:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics statement

Bird capture and banding activities were conducted by LECAVE under permits from the National Center for Bird Conservation and Research (CEMAVE, permit: 3362/10) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio, SISBIO authorization: 45434-5). Furthermore, all experimental protocols were approved by the Institutional Animal Care and Use Committee (CEUA) of the Institute of Biosciences at UNESP – Rio Claro Campus (Decision No. 17/2025).

Data availability

All playback stimuli WAV files, data, and R scripts required to reproduce the analyses presented in this manuscript are permanently archived in Mendeley Data (DOI: 10.17632/s34gj3gwjm.1).

Declaration on the use of generative AI technologies

During the preparation of this work, the authors used ChatGPT to assist with debugging and R coding. In addition, Google Gemini was used for translation from Portuguese to English and for improving the clarity and cohesion of the text. After using these tools, the authors carefully reviewed and edited all content as necessary and take full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pedro Diniz reports financial support was provided by the São Paulo Research Foundation. Isadora Zavan Santieff reports financial support was provided by Coordination for the improvement of Higher Education Personnel. Marco Aurelio Pizo reports financial support was provided by National Council for Scientific and Technological Development. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.avrs.2026.100442.

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