



Introduction

The ability to recognize oneself in a mirror is a sign of self-awareness, which is an important part of thinking, social learning, and complex cognitive behaviors. This skill involves brain areas responsible for higher thinking, like the prefrontal cortex. Self-recognition is impaired in individuals with autism spectrum disorder. While many animals are intelligent, only a few—such as some primates, several species of bird, and fish—have shown signs of self-recognition. The Mirror Mark Test is the “gold standard” for assessing the presence or absence of visual self-recognition.

Zebra finches are small birds known for their social communication and ability to learn and imitate songs. Scientists study these animals because of their vocal learning and social communication abilities. We sought to explore whether these birds also have the capacity for self-awareness. If they can recognize themselves, this could provide valuable insights into the evolution of self-awareness and how it relates to brain function. Furthermore, developing animal models for social disorders would facilitate treatment development.

Parishar et al. (2021) conducted the first test for visual self-recognition in zebra finches. They used the mirror mark test but did not find strong evidence for self-recognition. We sought to replicate and extend upon the prior research. Birds were exposed to a sequence of stimuli, first a female conspecific, then a male conspecific, followed by mirror exposure with a color mark placed above the left or right eye. We examined first whether the birds showed evidence of mark removal followed by analysis of spectrotemporal features of song in the three social conditions.

To explore this further, we analyzed the acoustic features of their songs. This analysis focused on three main aspects: spectral features, temporal features, and amplitude.

Method

Six adult zebra finches housed at Texas Christian University, following IACUC-approved protocols were investigated in this experiment. Birds were identified with colored leg bands and kept on a controlled light-dark cycle with *ad libitum* access to food and water. During testing, each bird will be placed in a divided cage setup allowing visual exposure to a mirror or social condition. The experiment spanned four days: a habituation phase, exposure to a male, female, or a mirror exposure phase. Birds were recorded twice daily. Mark-directed behaviors (e.g., cheek scratching) and social behaviors (e.g., songs, calls, mirror pecks) were measured.

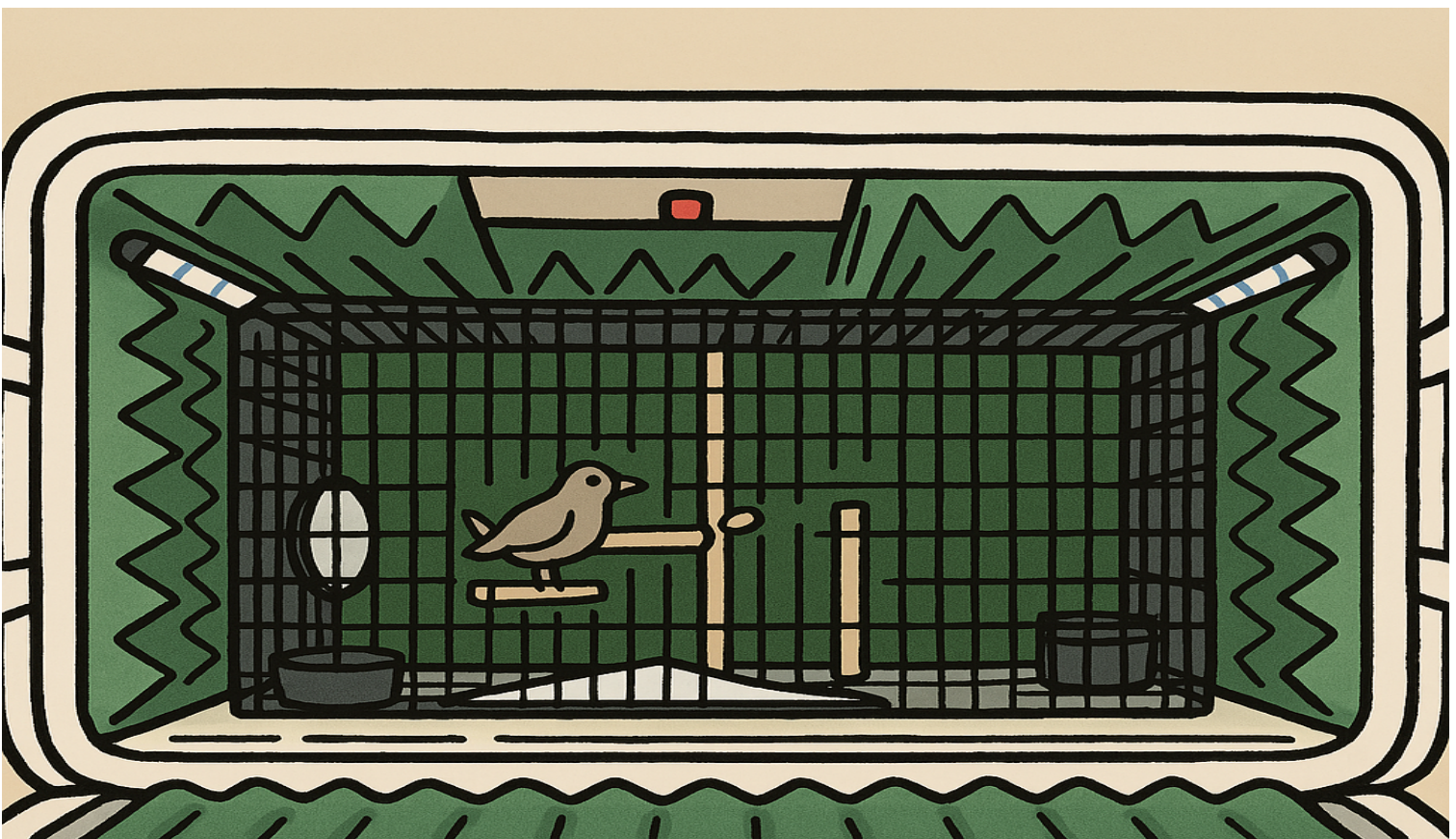


Figure 1. The experimental setup. A removable mirror is placed across from one perch or a conspecific placed in the adjacent cage with a perch allowing for interaction between both birds. Video recording allowed for coding of mark-directed actions, mirror orientation (facing vs. not facing), singing, and social interactions with a conspecific.

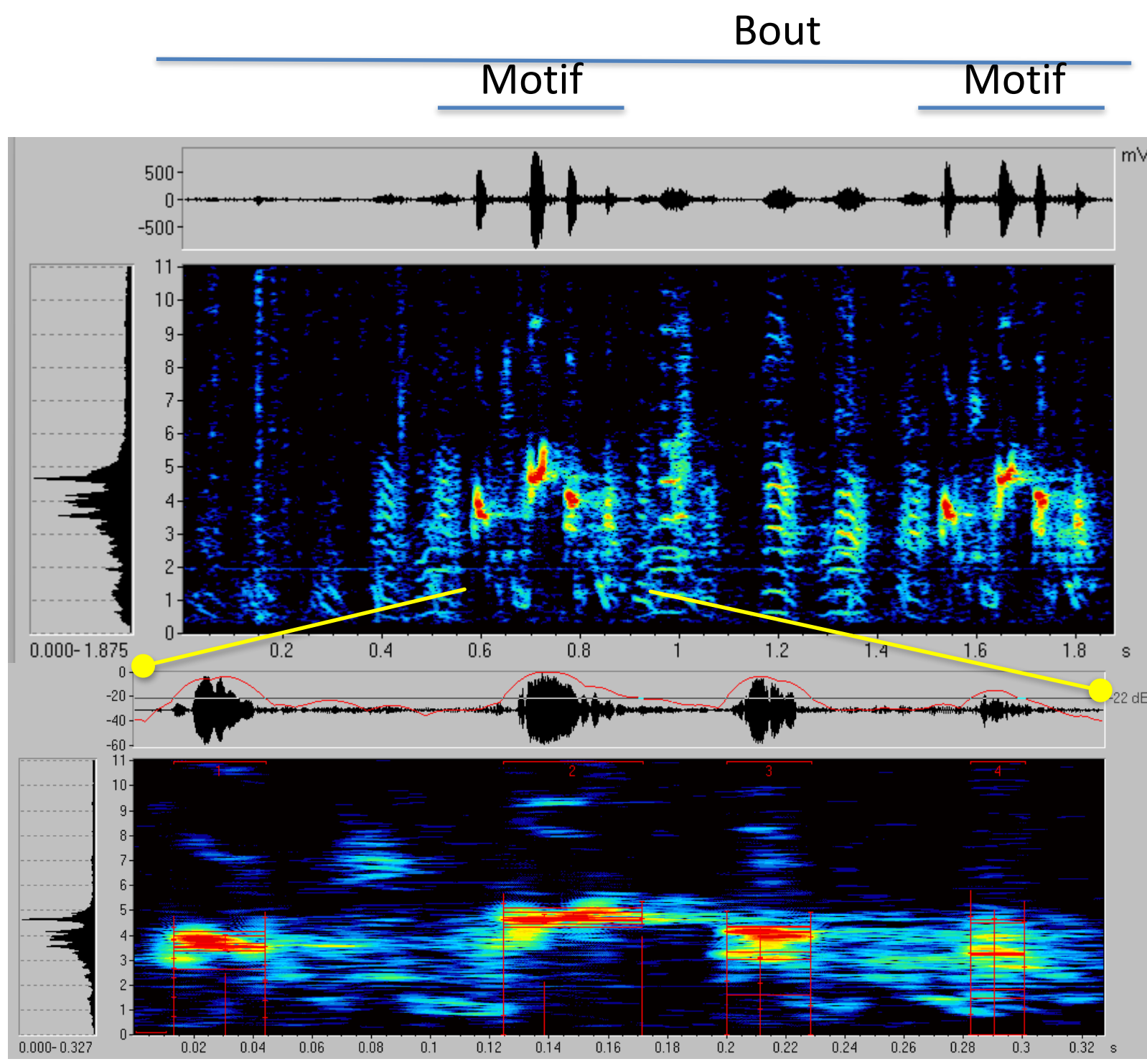


Figure 2. Acoustic analyses. A spectrogram display of an example bird's song. Time is on the X axis, frequency on the Y axis, and amplitude is color coded with higher amplitude sounds in red and darker colors showing lower amplitude sound. Left panels are mean spectrum showing relative amplitudes of the different frequencies. A song bout consists of one or more motifs. Syllables within the motif are amplitude segmented, and the duration, entropy (disorder), and amplitude were measured for each song syllable.

1. Do Zebra Finches Show Evidence of Visual Self-Recognition?

1a. No Mark Removal Behavior

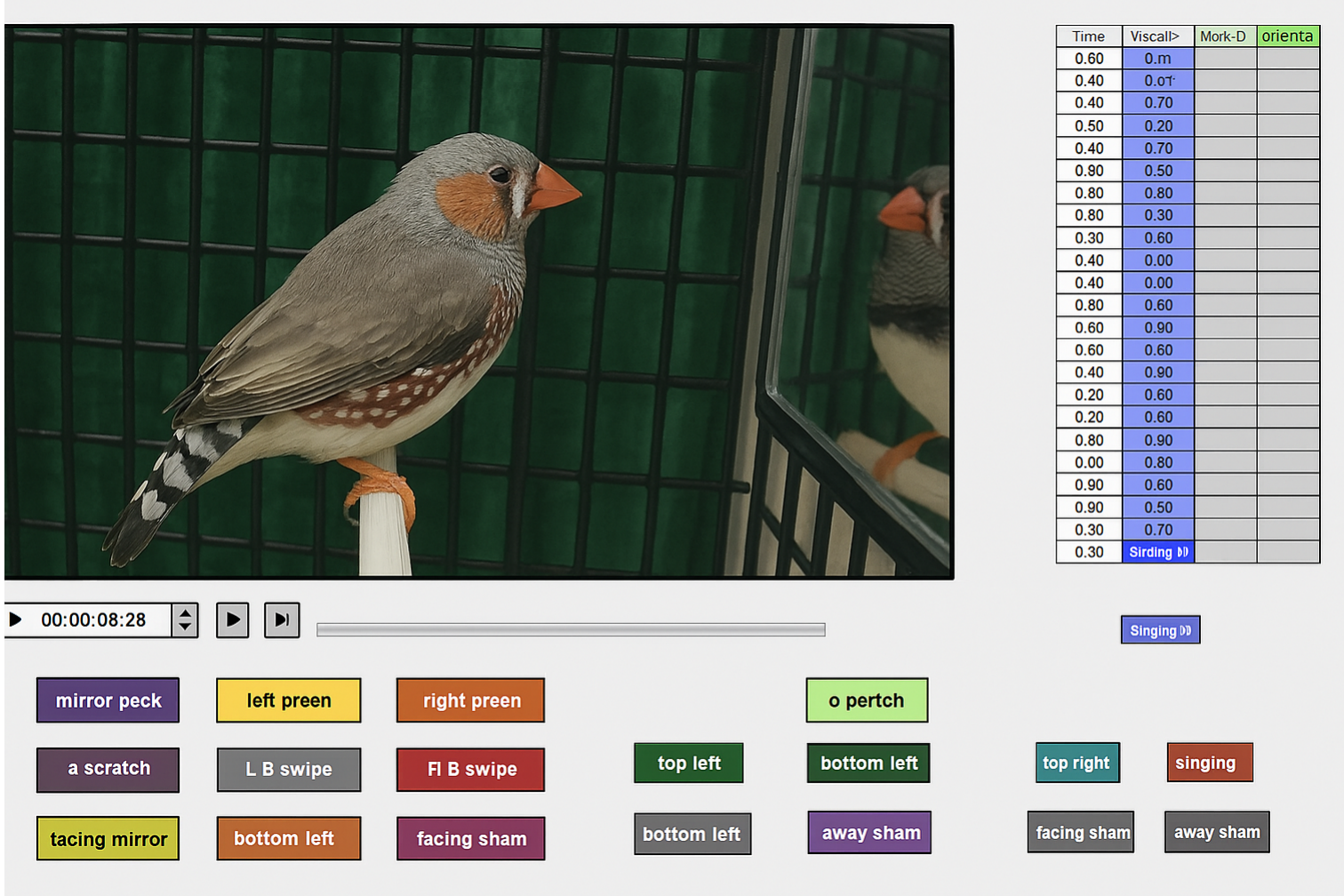


Figure 3. Data Coding. This coding platform is used to systematically label and track specific zebra finch behaviors during the experiment. Each colored button represents a distinct action or condition—such as mirror peck, scratch, call, song, or location in the cage (e.g., top left, bottom right). There are also buttons for facing direction (e.g., facing toward or away from the mirror) and whether the bird is perched adjacent to the mirror.

Visual self-recognition is defined as a bird scratching more on a marked side while facing the mirror. Thus is “mark removal behavior.” Analyzing vocalizations and mirror-directed actions also provides insight into social behaviors and awareness.

Four birds were tested with the mirror mark test. None showed any evidence of mark removal behavior.

2. Are there other indicators of visual self-recognition in zebra finches?

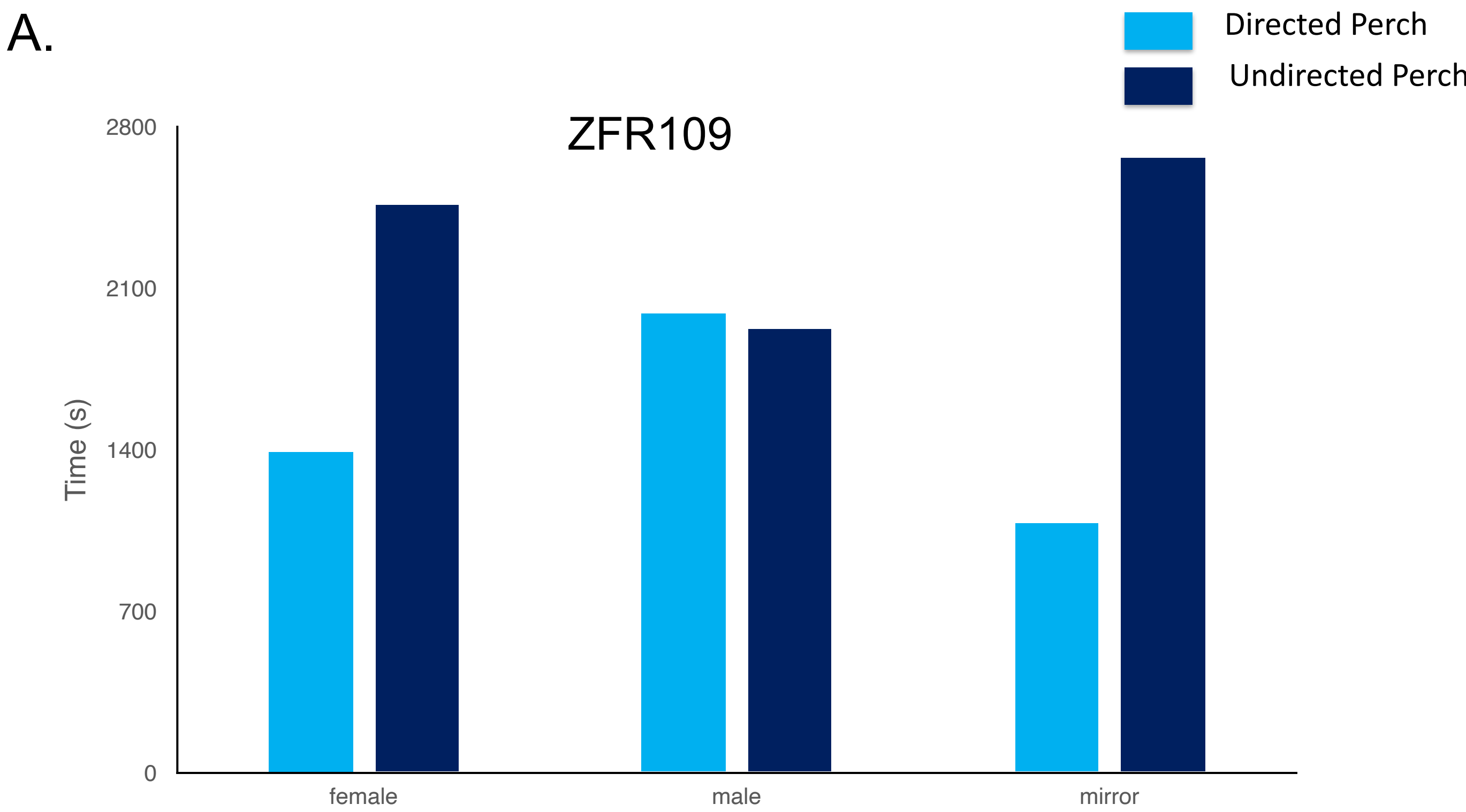


Figure 4. Male interactions dominated behavioral responses in this recording setup. In this example bird, the bird spent an equal amount of time on the perch facing the male conspecific and this was greater than what was observed in the female or mirror condition.

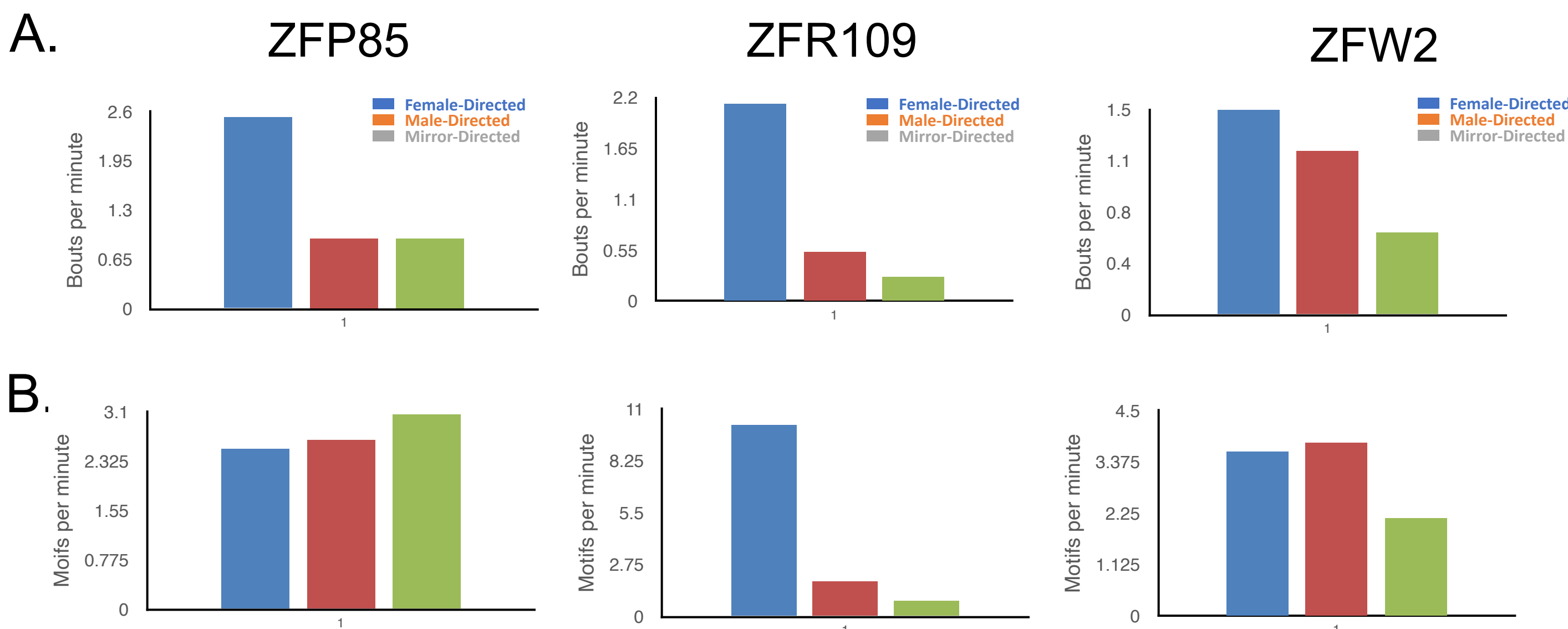


Fig. 5. Measures of singing quantity. A) The number of bouts per minute for the female conspecific was the greatest for all three birds that sang in all conditions. B) Surprisingly, several of the birds sang long duration bouts and produced as many motifs when singing to the female conspecific as they did when singing to a male conspecific.

3. Song Stereotypy Is Greatest in Male-Directed Singing

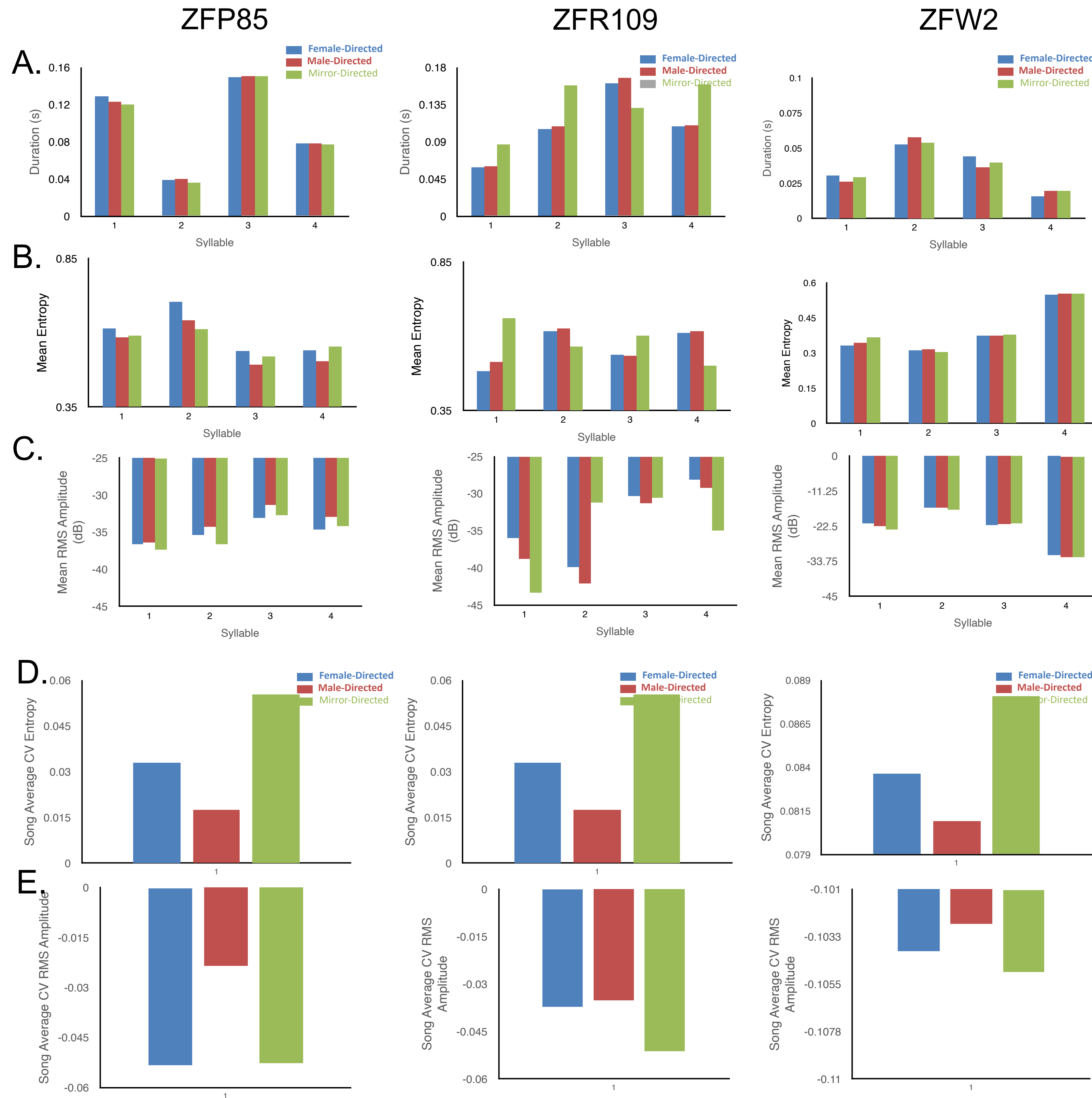


Fig. 6. Measure of singing quality. A) The temporal features of song were not dramatically different between conditions (for all graphs bars are syllable averages, circles individual repetitions). B) The entropy did not vary systematically between conditions, but note the reduction in individual variability during the male-directed singing compared to the mirror-directed song renditions. C) In general, the lowest amplitude songs occurred when birds were presented with a mirror, and the loudest song syllables were produced when a male or female bird was in the adjacent cage, indicating some level of self-recognition. Furthermore, the most spectrally variable song renditions occurred in the mirror-directed song and the least spectrally variable songs when presented with a male conspecific (D). E) The song average coefficient of variation for amplitude shows that the greatest performance variability occurs in the mirror condition and the most stereotyped song delivery when singing with a male in an adjacent cage.

Conclusions

- The birds in this study did not exhibit clear evidence of visual self-awareness using the mirror mark test. This replicates previous research (Parishar et al. (2021)).
- We observed how the songs of birds changed across the mirror, male, and female conditions. The results showed that the birds sang their most consistent, patterned songs when facing another male, but their songs became more variable and less predictable when facing a mirror. This suggests the birds may respond to their reflection differently than they do to real birds—possibly showing a basic awareness that the mirror image is not quite the same as another bird.
- Results suggests that self-recognition may not be as simple as an evident dichotomy, but rather something that occurs in continuum.
- Results also leads to a serendipitous discovery of putative territorial song in zebra finches, a colonial species that is not known to sing in intra-specific competition.

Acknowledgements: We thank Lindy Bledsue for expert animal care.