

## Short-term effects of traffic noise on avian dawn chorus in a neotropical cloud forest

*Efecto de corto plazo del ruido por tráfico sobre coros de aves en un bosque nuboso neotropical*

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### Abstract

Road construction has been shown to have detrimental effects on animal populations. Noise pollution is suggested to be one of the most influential disturbances caused by roads on bird species because acoustic masking can decrease their communication abilities. There are many variables associated with roadways that could confound the effects of road noise on biodiversity. The

objective of this study was to isolate the effects of road noise on the avian dawn chorus in El Silencio de Los Ángeles Cloud Forest Reserve, San Ramón, Costa Rica, by utilizing playback. Over the course of six days, avian dawn choruses were recorded in the presence and absence of traffic noise playback between 05:00 and 06:00. The recordings were analyzed using Raven Pro 1.5 software and measured for total bioacoustic time of four focal species — Black-headed Nightingale-Thrush (*Catharus mexicanus*), Slaty-backed Nightingale-Thrush (*Catharus fuscater*), Gray-breasted Wood-Wren, and Black-faced Solitaire (*Myadestes melanops*) — as well as the intensity and complexity of the dawn chorus — as shown through average sound pressure ( $\mu\text{Pa}$ ) and entropy (bits). We found no significant differences in bioacoustic time among any focal species but did find that average entropy increased and average sound pressure decreased in playback trials between 3–12 kHz. This suggests that birds are affected by road noise playback and respond by either increasing their call frequency (kHz) or vacating areas with road noise. These data show the immediate responses of the bird populations to acoustic disturbances; thus, we suggest that future studies should investigate the long-term effects of road noise on bird communication using these new methods. Continued road expansion in the tropics due to urbanization and globalization is a prominent threat to tropical wildlife, therefore, it is imperative that the effects of roads are examined to aid in habitat conservation.

**Keywords:** Road noise, playback technique, bioacoustic time, biophony.

## **Resumen**

La construcción de carreteras ha demostrado tener efectos perjudiciales en las poblaciones animales. Se sugiere que la contaminación acústica es una de las perturbaciones más influyentes sobre las especies de aves debido a que el enmascaramiento acústico puede disminuir la

habilidad de comunicación. Existen muchas variables asociadas con las investigaciones en carreteras que podrían confundir los efectos del ruido sobre la biodiversidad. El objetivo de este estudio fue aislar efectos del ruido de la carretera sobre el coro de aves del amanecer en la Reserva del Bosque Nuboso El Silencio de Los Ángeles, San Ramón, Costa Rica, mediante la utilización de la reproducción acústica. Durante seis días, se grabaron coros de aves del amanecer en presencia y ausencia de la reproducción del ruido del tráfico entre las 05:00 y las 06:00. Las grabaciones se analizaron con el programa Raven Pro1.5 y se midió el tiempo de bioacústica total de cuatro especies focales: Zorzal Cabecinegro (*Catharus mexicanus*), Zorzal Sombrío (*Catharus fuscater*), Soterrey de Selva Pechigrís (*Henicorhina leucophrys*), y el Solitario Carinegro (*Myadestes melanops*). También se midió la intensidad y complejidad del coro del amanecer por medio de la presión de sonido promedio ( $\mu\text{Pa}$ ) y la entropía (bits). No encontramos diferencias significativas en el tiempo de bioacústica en las especies focales, aunque la entropía promedio aumentó y la presión de sonido promedio disminuyó en las frecuencias entre 3 y 12 kHz durante la reproducción del ruido. Esto sugiere que las aves son afectadas por el ruido de la carretera, y pueden responder ya sea aumentando la frecuencia del canto (kHz) o evitando las áreas con ruido. Estos datos muestran respuestas inmediatas en las aves ante estos disturbios. Por lo tanto, sugerimos que estudios futuros deberían investigar los efectos del ruido a largo plazo sobre la comunicación de las aves utilizando métodos similares a los nuestros. La continua expansión de carreteras en los trópicos debido a la urbanización y la globalización es una amenaza importante para la vida silvestre tropical, por que es imperativo investigar los efectos de las carreteras para ayudar en la conservación de estos hábitats.

**Palabras Claves:** Ruido de carretera, técnica de reproducción acústica, tiempo de bioacústica, biofonía

## Introduction

Soundscapes are important, dynamic elements existing within ecosystems that are used to describe the relationship between the landscape and the composition of sounds. They can be used to describe the health of an ecosystem because changes in soundscape warrant behavioral responses and adaptations (Pijanowski *et al.* 2011a, 2011b). Many species, especially anurans and birds, rely on sound for general communication, territory defense, and courtship interaction; thus, soundscapes are crucial and limiting factors of the distribution and diversity of an ecosystem (Halfwerk *et al.* 2011). Birds are dominant components of biological soundscapes and can act as good indicators for the overall health of an environment in order to inform conservation efforts (e. g. Arévalo and Newhard 2011; Francis *et al.* 2009).

As many tropical countries develop infrastructure, urban sprawl — and the associated increase in roadway connectivity — fragments much of the natural landscape (Laurance *et al.* 2002). While the natural soundscape of an ecosystem has both abiotic and biotic sounds — such as insects, rainfall, wind, or streams — that birds must compete with, the addition of anthropogenic traffic noise adds an artificial variable that can negatively affect bird communities (Brumm 2004; Halfwerk *et al.* 2011). Low-frequency noise pollution (<3 kHz) like traffic noise, has been shown to reduce nesting success, body condition of migratory warblers, avian species diversity and abundance, as well as degrade forest habitat (Francis *et al.* 2009, 2011a). Birds that vocalize at similar frequencies as road noise are subject to acoustic masking wherein vocalizations are more difficult to detect (Klump 1996; Patricelli and Blickley 2006). Birds that are masked by traffic noise exhibit three responses: changing their vocal behavior — by increasing their average frequency, amplitude, or repetition of vocalization — failing to communicate effectively, or avoiding habitat adjacent to roadways (Brumm and Zollinger 2013;

Rheindt *et al.* 2003). Failure to adapt to masking pressure has major biological implications such as a decrease in reproductive success through female fidelity, a decrease in the probability of males being sexually selected, and a decrease in avian occupancy of habitat in close-proximity to roads (Halfwerk *et al.* 2011; Goodwin *et al.* 2011). Bioacoustic time of different species, which is synonymous to song duration, can also be disrupted by traffic noise. To date, no previous studies have investigated the effects of road noise on the bioacoustic time in birds, but some studies have shown that frogs have reduced calling times in the presence of anthropogenic noise (Vargas-Salinas *et al.* 2014). A decrease in the contribution of a species to the biological soundscape can be assessed through the reduction of bioacoustic time — meaning that an individual is not performing its acoustic repertoire effectively (Ey and Fischer 2009).

Most studies attempting to examine the effects of noise on bird populations are often conducted in the presence of confounding variables: i.e., habitat fragmentation, edge effects, road mortality, chemical pollution, etc. Additionally, these studies could have been influenced by temporal effects from migration, seasonality of resource distribution, landscape heterogeneity, and limited sample sizes. In this study, we seek to isolate the variable of road noise by utilizing experimental playback. Despite the high biodiversity and vulnerability of the neotropics, there are little to no studies regarding the effects of road noise on bird populations, let alone the effects of road noise playback. This study can help to inform management decisions by providing information regarding how roads will affect adjacent bird communities.

Our objective was to isolate the effects of anthropogenic road noise on the dawn chorus in a tropical cloud forest as reflected by overall bioacoustic complexity and four focal species that are abundant within the soundscape of our field site in El Silencio de Los Ángeles Cloud Forest Reserve, San Ramón, Costa Rica: the Black-headed Nightingale-Thrush (*Catharus*

*mexicanus*), Slaty-backed Nightingale-Thrush (*Catharus fuscater*), Gray-breasted Wood-Wren (*Henicorhina leucophrys*), and Black-faced Solitaire (*Myadestes melanops*). Some portion of the songs of all selected focal species fall below the 3 kHz threshold; we therefore hypothesize that all focal species are subject to some degree of acoustic masking while vocalizing. We then predict that their bioacoustic times will decrease in sites with anthropogenic noise due to decreased communication abilities. Additionally, we hypothesize that community composition will change during dawn choruses exposed to playback to reflect species with higher call frequencies ( $\geq 3$  kHz) due to masking pressure. We expect this to be represented by a change in the complexity (measured via entropy) and intensity (measured via sound pressure) of dawn choruses exposed to playback. Therefore, we predict that average entropy and sound pressure will be lower during playback choruses at lower frequencies.

## **Methods**

### *Study Site*

Data was collected from April 11 to 16, 2019, at the El Silencio de Los Ángeles Cloud Forest Reserve (10° 12' 7.2" N, 84° 29' 2.4" W), which is located 12 km North of San Ramón, Alajuela, Costa Rica. The reserve is approximately 800 ha and 1,100 m in elevation. The main study area in question is found within primary and secondary premontane tropical cloud forest (Holdridge 1967). This life zone receives an average of 4,000–8,000 mm of rainfall annually. Approximately 6 km South of El Silencio de Los Ángeles is Highway 702, which is a major highway connecting the San José Metropolitan Area to La Fortuna and Arenal Volcano — one of Costa Rica's most popular tourist destinations (Matarrita-Cascante *et al.* 2010). There is currently an ongoing construction project to build Ruta Nacional 35 that will branch off from

San Ramón and further increase the roadway connectivity between the Central Valley and the Caribbean. This highway will directly border the East side of El Silencio de Los Ángeles Reserve — posing an imminent conservation concern for the bird communities found there.

### *Playback treatment*

We recorded traffic noise of Highway 702 using a Sennheiser ME 64 directional microphone (Sennheiser, Connecticut, USA) connected to a Marantz PMD661 MKII handheld solid state recorder (Marantz Professional, Rhode Island, USA) for 15 minutes from 15:25 to 15:40. The Marantz unit gain-dial was set to 4.5 to avoid vehicle pass-by events from distorting the recording. The recordist stood 5 m away from the road edge and held the microphone 1.2 m above the ground directly parallel to the road, so that the audio file could display an accurate doppler effect. Using an CEM DT-8851 handheld sound-level meter (CEM Instruments, West Bengal, India) calibrated at 94 dB and set to A-weight and fast response, we measured both the background level-equivalent (LEQ) every 30 seconds and maximum sound pressure level of each vehicle pass-by event while recording. Vehicles travelled at approximately 40 km/h, a common speed limit throughout the area, producing an average pass-by sound pressure level of 76.1 dB and an average background LEQ of 56.5 dB. These values are akin to other studies using experimental noise playback, and are also within the threshold at which road noise negatively affects bird density (Reijnen *et al.* 1997; Barber *et al.* 2011). We repeated this recording four times to create a 60 minute audio file to be used in our playback experiment. Given the length of the playback experiment, and considering that the birds of El Silencio de Los Ángeles are rarely exposed to anthropogenic road noise, and that the playback file contains natural variation in vehicle pass-by events, it was unlikely that birds would habituate to the recording. The created

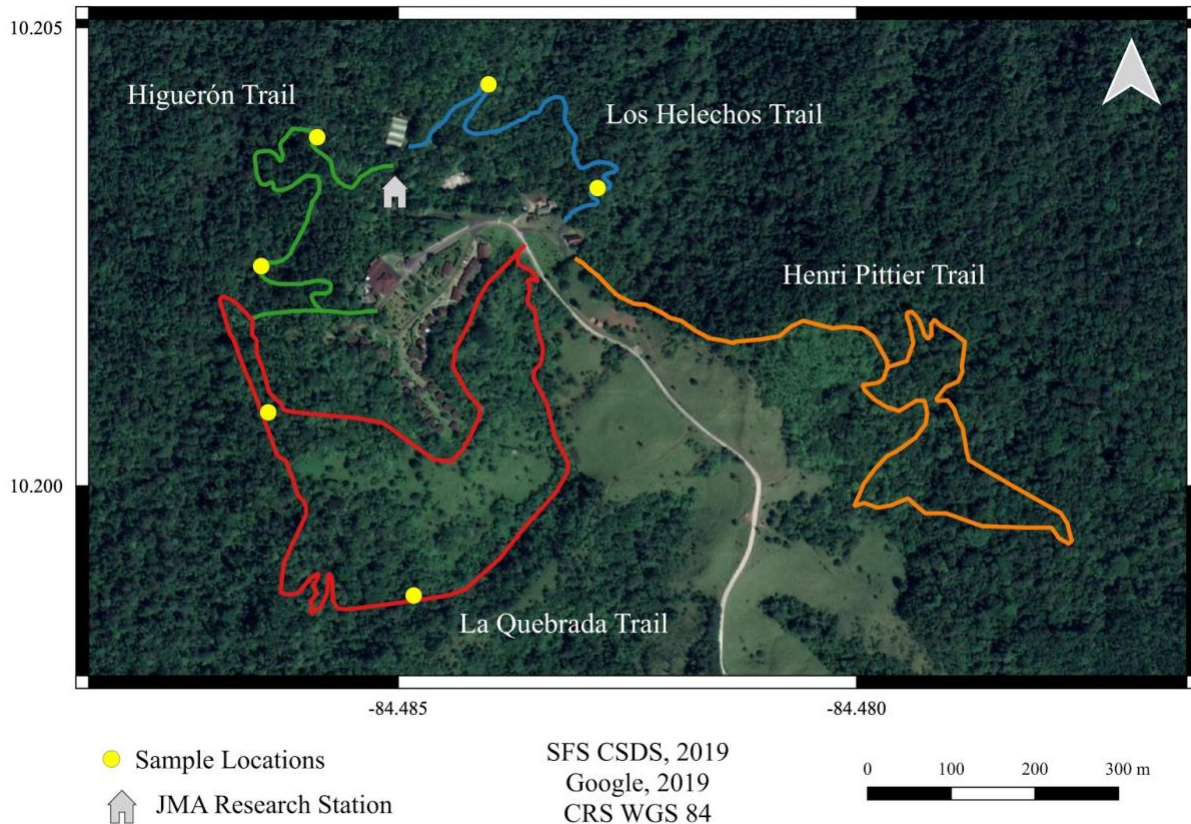
file represents the most accurate depiction of possible future road noise that would be introduced to El Silencio de Los Ángeles soundscape if the construction of Ruta Nacional 35 were to be completed.

### *Playback experiment*

We established six survey points along three different trails within the El Silencio de Los Ángeles Reserve: the Higuieron Trail, Los Helechos Trail, and Quebrada Trail (Figure 1). All trail systems were located in the same cloud forest habitat type and we made sure there were no apparent differences in forest structure surrounding the points, such as forest gaps. Each of the six points was surveyed twice, once for the control trial and once for the playback trial.

However, due to equipment limitations, we could only survey two points each morning. A set of two points was surveyed for two successive mornings before recording a new set of points, in which the autonomous recording units (ARUs) and speaker were swapped after every morning of recording. All points were located at least 150 m apart to ensure that different bird individuals were sampled. Playback and control points that were occurring on the same morning were located at least 300m apart to ensure that the playback would only influence the intended location.





**Figure 1.** Map of the trail systems and sampling locations at El Silencio de Los Angeles Cloud Forest Reserve, San Ramón, Costa Rica.

At each point, we secured one SONG METER SM2+ (Wildlife Acoustics Inc., Massachusetts, USA), equipped with an omnidirectional microphone and set to record at mono, 16-bit depth, and 44.1 kHz, and one Anchor AN-30 speaker (Anchor Audio Inc., California, USA) tied to a tree 1.2 m above the ground. The speaker was pointed in the direction of and was located 15 m away from the ARU at all points. We played back the road noise sound file using an iPhone 6S (Apple Inc., California, USA).

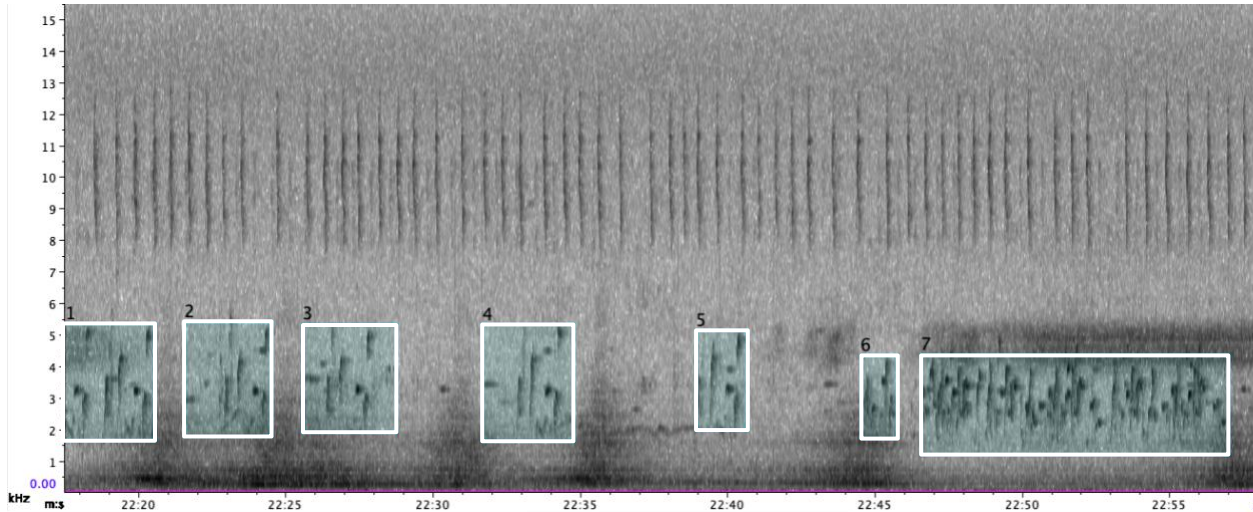
We manipulated the source level of the speaker to match the actual level of traffic noise observed at Highway 702. We stood 5 m in front of the speaker (the same distance we stood

from the road edge when recording the sound file), pointed the same sound level meter with the same settings at the center of the speaker, and adjusted the volume until the maximum sound pressure level of the first vehicle-pass event of the sound file matched what we observed in the road. In doing so, we ensured that the speaker played the same background LEQ of Highway 702 as we have observed in the field.

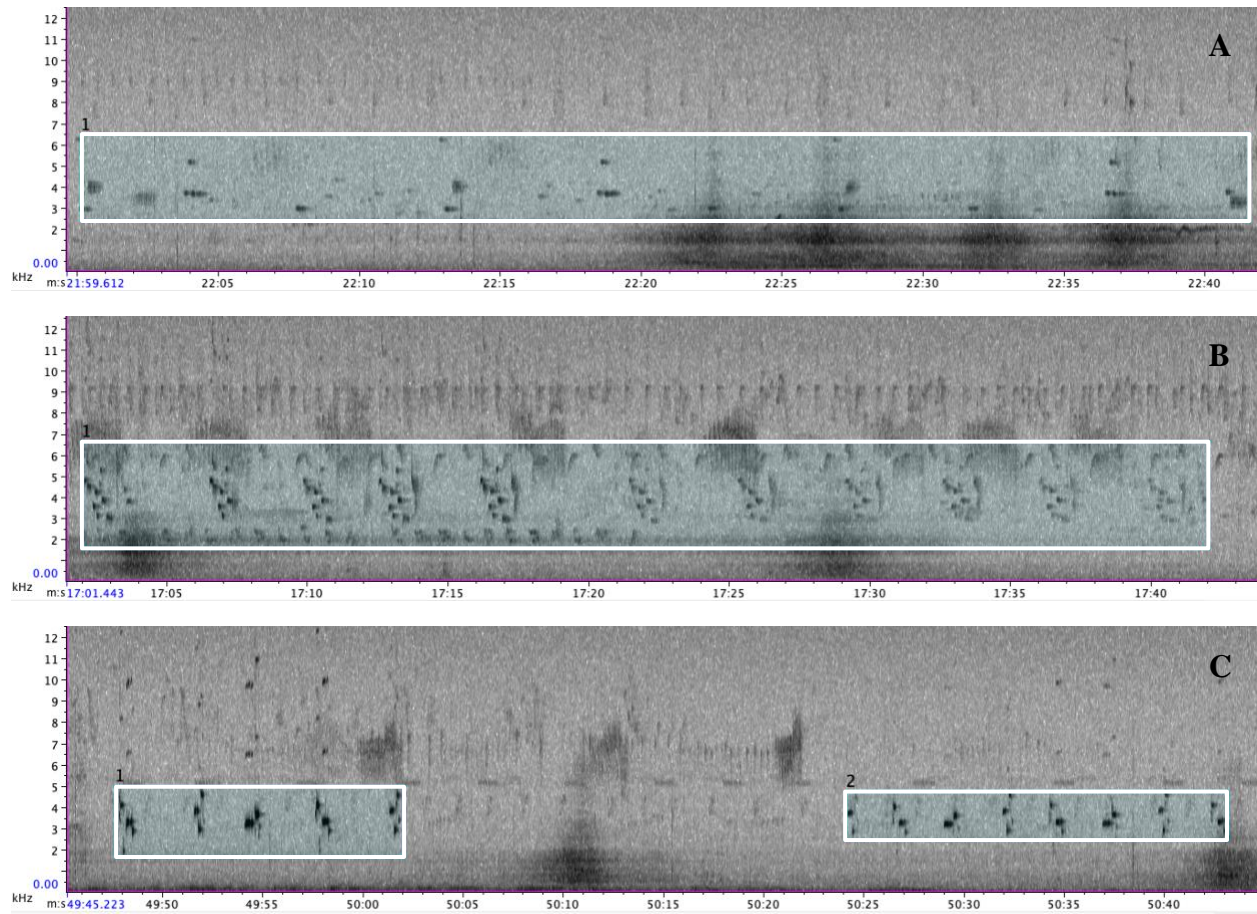
ARUs recorded dawn choruses from 05:00 to 06:00 each morning. This time span encompassed the peak level of dawn chorus intensity of El Silencio de Los Ángeles, which is typically observed around 05:30 and trails off sharply after 05:45.

#### *Measuring bioacoustic time*

To measure bioacoustic time, both the playback and control dawn chorus recordings were processed using the Raven Pro 1.5 software. When opening Raven, we set page size to 2 minutes, measured bioacoustic time by selecting from the beginning of the first note to the end of the last note vocalized in a consecutive song. If a vocalization was split by the page boundaries, the selection would cease at the 2 minute increment mark, and a new selection would be started on the following page where the vocalization began again. Natural pauses during a species' dawn chorus were incorporated into the measurement. However, when the natural cadence of a song was interrupted for any reason, the measurement was stopped at the last part of the song and a new selection was started when the song commenced again (Figure 1–2). If there were multiple individuals calling in the same recording, only the individual with the best-defined spectrogram marks, and thus the clearest song, was measured.



**Figure 2.** Gray-breasted Wood-Wren (*Henicorhina leucophrys*) song selection and sonogram in Raven. Song shows clear interruptions in its natural cadence, thus multiple selections were made. In this sonogram, the songs seem to be disrupted by road noise, as they do not overlap with vehicle pass-by events. This individual was recorded at 5:22 am on April 14, 2019, in the El Silencio de Los Ángeles Cloud Forest Reserve, San Ramón, Costa Rica.



**Figure 3.** Example call analysis in Raven for 3 focal species. Boxed sections of notes indicate how bioacoustic time was recorded in the A.) Black-faced Solitaire (*Myadestes melanops*), B.) Black-headed Nightingale-Thrush (*Catharus mexicanus*), and C.) Slaty-backed Nightingale-Thrush (*Catharus fuscater*). Different sections were made only when it was obvious that it was not a natural pause in the song. These examples were recorded in 2019 on April 13th, 11th, and 15th respectively at El Silencio de Los Ángeles Cloud Forest Reserve, San Ramón, Costa Rica.

### *Measuring Community Effect*

We measured the changes in the overall acoustic structure of the dawn chorus by opening the entire dawn chorus recordings into the Raven platform, created two 60-min selections — one from 0–3 kHz and the other from 3–12 kHz — and measured the average entropy (bits) and

average power (dB). As decibels are logarithmic units, it is incorrect to take the average of decibel measurements (Symes *et al.* 2016). We converted the “Average Power (dB)” outputs given by Raven to their raw sound pressure levels ( $\mu\text{Pa}$ ) using the following formula. We set the reference pressure ( $P_{reference}$ ) to 20  $\mu\text{Pa}$  as it is the threshold for human hearing:

$$L_{dB} = 20 \log_{10} \left( \frac{P_{rms}}{P_{reference}} \right) \rightarrow P_{rms} = P_{reference} \left( 10^{\frac{L_{dB}}{20}} \right)$$

$L_{dB}$  = “Average Power (dB)” in Raven

$P_{reference}$  = 20  $\mu\text{Pa}$  (threshold for human hearing)

$P_{rms}$  = Sound pressure of dawn chorus

Measurements within the same frequency band were then compared between playback and control recordings for each site. We use average entropy and sound pressure here as a proxy to measure dawn chorus intensity and complexity. Higher entropy, as it measures spectrogram complexity, would indicate more vocalizing species and individuals, and higher sound pressure, as it measures spectral strength, would indicate birds vocalizing at closer distances (Charif *et al.* 2010). To our knowledge, this is the first attempt to use Raven selections across entire dawn chorus recordings in order to infer anthropogenic impact at the community-level.

These frequency band selections were made with the knowledge that the majority of the energy of typical traffic noise falls between 0–3 kHz with a peak of around 1 kHz (McClure *et al.* 2013), and bird species are rarely capable of vocalizing higher than 12 kHz (Anon, 2019). Entropy nor power measurements in Raven can distinguish between ambient noise and bird sounds; and since the energy of traffic noise is assumed to be much higher than bird vocalizations, spectrogram measurements below 3 kHz are expected to be skewed by the impact

of traffic noise. Selecting from 3–12 kHz allows us to exclude most, if not all, of ambient traffic noise and only compare bird vocalizations present in between those frequencies. Bird vocalizations present below 3 kHz can still be compared statistically because we played back a standard sound file for all trials. However, these values represent the entropy and sound pressure of the dawn chorus in addition to the ambient traffic noise. These frequency band selections also allow us to separately measure and compare vocalizations of species that would be subject to masking (<3 kHz) and those that would not (>3kHz) — allowing us to evaluate the impact of traffic noise on the masked and unmasked bird communities independently.

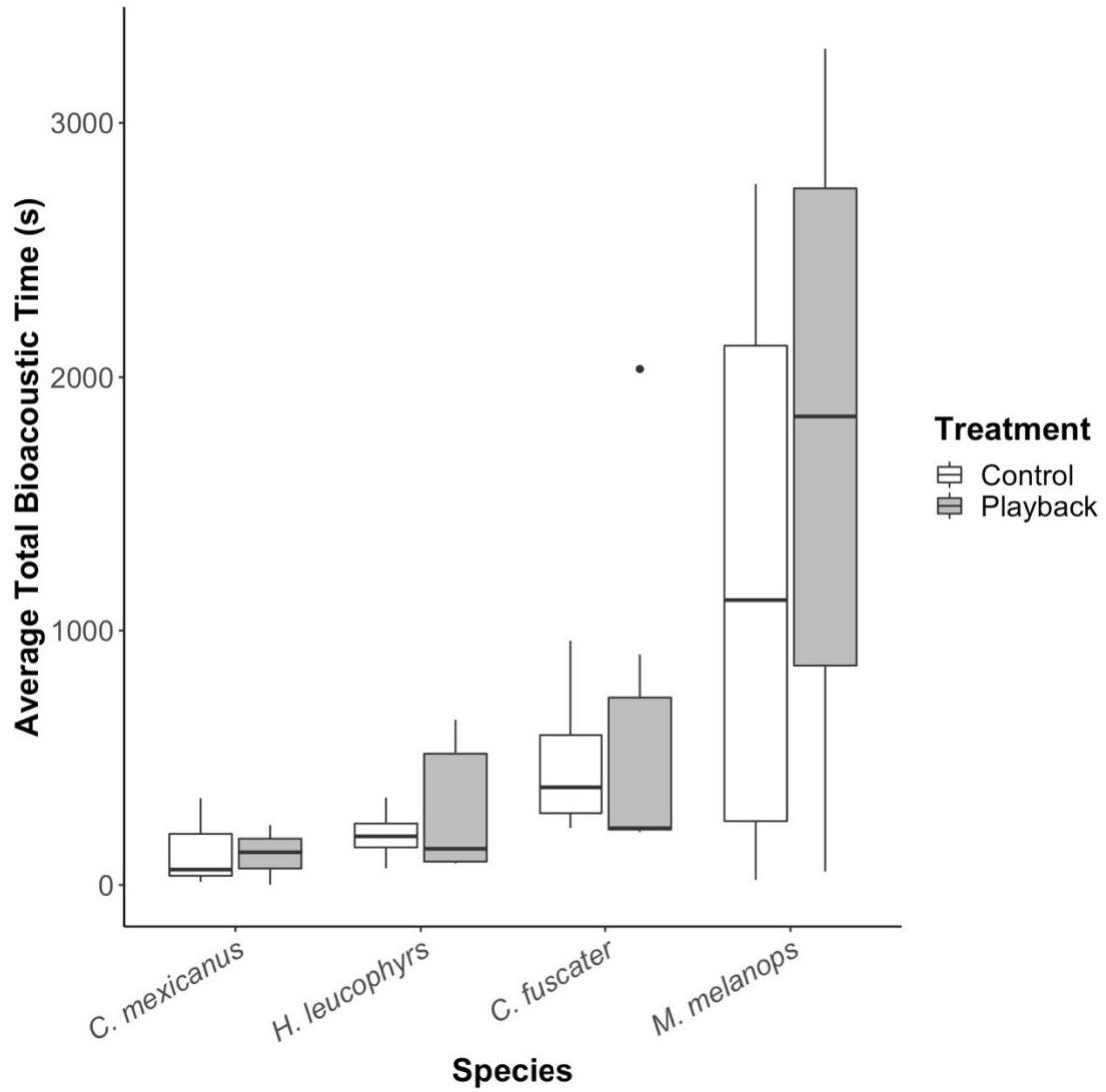
### *Statistical Analysis*

We summed bioacoustic time observed in each dawn chorus recording for each focal species and compared control and playback values at each site using a generalized linear model (GLM) in the R computing environment. Since we did not use any visual means to assure that territorial birds were present and stationary during the recording time, any mornings that had a sum of zero bioacoustic time for a species were excluded from the analysis. In a similar way, we compared average entropy and sound pressure values of control and playback dawn chorus recordings at each site using a *t*-test. Data that deviated from the normal distribution via a Shapiro-Wilk's normality test were compared using a non-parametric Mann-Whitney U-test.

### **Results**

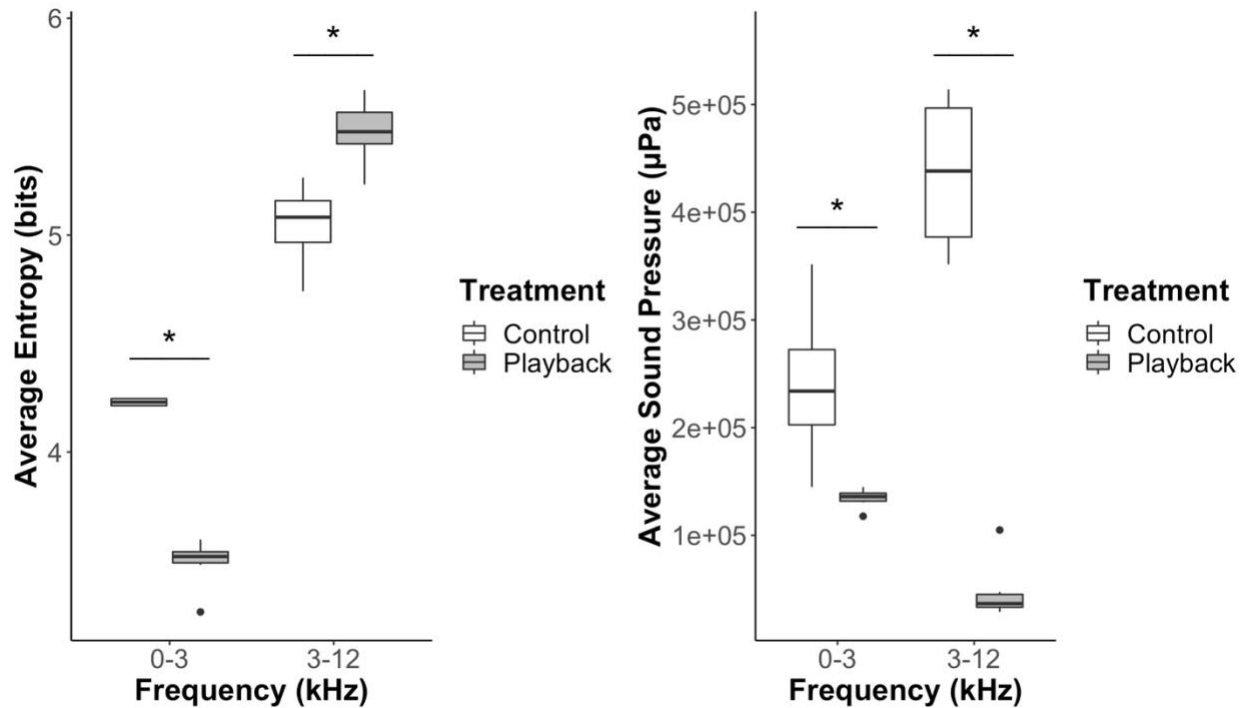
We analyzed a total of 10 hours of dawn chorus recordings (n = 4 control, n = 6 playback) using Raven. We found no significant differences between the bioacoustic time of playback and control sites in any of the four focal species (GLM, Gamma distribution:  $P =$

0.621; Figure 4). In the 0–3 kHz frequency band, both average entropy (Mann-Whitney:  $z = 2.459$ ,  $P = 0.0139$ ) and average sound pressure ( $t = 3.164$ ,  $P = 0.0139$ ) were significantly higher during control dawn choruses than during dawn choruses exposed to traffic noise playback. In the 3–12 kHz frequency band, average sound pressure was significantly higher during control than in playback trials (Mann-Whitney:  $z = 2.452$ ,  $P = 0.0142$ ); however, average entropy was significantly lower in control trails ( $t = 3.711$ ,  $P = 0.00595$ ; Figure 5).



**Figure 4.** Total bioacoustic time during control and playback dawn choruses averaged across all sites. Data was collected between April 11 and 16, 2019 in El Silencio de Los Ángeles Cloud Forest Reserve, San Ramón, Costa Rica.





**Figure 5.** Box plots of average entropy average sound pressure during control and playback dawn chorus recordings obtained in El Silencio de Los Angeles Cloud Forest Reserve, San Ramón, Costa Rica. \* denotes statistical significance.

We hypothesized that there would be a reduction in bioacoustic time in our four focal species in response to road noise playback. Additionally, we hypothesized that the community composition of the dawn chorus would change to include more individuals that vocalize at  $\geq 3$  kHz. We found that there was no significant change in bioacoustic time in any of our focal species (Figure 4). However, our results did support our hypothesis that dawn chorus community composition changes when exposed to playback to reflect species with higher call frequencies ( $\geq 3$  kHz) due to masking pressure.

## Discussion

Considering this is could be the first study to examine the effects of road noise playback on bioacoustic time in birds, we expected to observe significant changes because previous studies have demonstrated a reduction in bioacoustic time in other animals that rely on vocal communication (Vargas-Salinas *et al.* 2014). In our study, we observed large variation in bioacoustic times. Before excluding null data, there were several instances ( $n = 5$ ) where focal species were detected during control, but were not detected during playback trials. Feint-singing birds that could be detected on spectrograms at control sites, could potentially go undetected in playback trials as traffic noise forced these individuals farther away from the microphone. This supports the hypothesis that the immediate response of some bird species is not to change the duration of their vocalizations, but rather to move farther away from the affected area. Similar results were also seen by Goodwin and Shriver (2010) as they observed that the Yellow-billed Cuckoo (*Coccyzus americanus*) and White-breasted Nuthatch (*Sitta carolinensis*) were ten times less likely to be found in areas affected by traffic noise than quiet habitats in Virginia, USA. However, it is important to acknowledge the species-specific effects of road noise. Little is known about the acoustic behavior of our four focal species, although some research has examined the duetting of Gray-breasted Wood-Wrens.

The Gray-breasted Wood-Wren is a monogamous, territorial bird that relies heavily on its song and it has been shown to coordinate its song with potential or already-established mates to make a precisely-timed duet (Dingle and Slabbekoorn 2018). Dingle and Slabbekoorn (2018) describes that Gray-breasted Wood-Wrens have two-types of duetting behavior: an antiphonal duet — which requires a high degree of coordination and precise timing — and a jumbled duet — which does not require as much coordination. To perform an antiphonal duet Gray-breasted

Wood-Wren pairs must listen closely to each other; therefore, a distracting, loud sound — such as road noise — could disrupt this duet and hinder communication (Lohr *et al.* 2003). However, when making selections in Raven we did not observe any antiphonal duets, only more jumbled duets. As these duets are messy by nature and do not require as much coordination between partners, this could explain why we did not observe any significant differences in the bioacoustic time of this species. In the future, there could be a high potential for traffic noise to affect the antiphonal duets of the Gray-breasted Wood-Wren.

Our results of the community analysis can be most reasonably explained using the following rationale: 1) traffic noise playback heavily influenced the entropy and sound pressure measurements at low-frequencies, 2) birds respond to traffic noise playback by fleeing the immediate area, and 3) birds respond by raising the average frequency of their vocalizations. In the 0–3 kHz band, average entropy was significantly higher in control dawn choruses than in playback choruses. As entropy in Raven measures spectrogram complexity, this finding may demonstrate the spectral influence of the low-frequency traffic noise in playback dawn chorus recordings. Road noise has a spectrally simple sound profile, and thus more vehicle pass-by events are likely to have a negative effect on average entropy by diluting the complexity of bird vocalizations. Likewise, average sound pressure was also significantly higher in control dawn choruses compared to playback choruses within 0–3 kHz. At playback sites, road noise would be assumed to increase the average sound pressure, which represents the spectral density of both bird vocalizations and traffic noise, of a dawn chorus recording because vehicle pass-by events are typically louder than bird vocalizations (McClure *et al.* 2013). Average sound pressure levels of our playback are similar to those seen in other studies demonstrating the negative effects of road noise on biodiversity (Reijnen *et al.* 1997; Barber *et al.* 2011). Therefore, it is clear that our

playback file strongly impacted the soundscape. However, vehicle pass-by events may not be spectrally dense — especially compared to spectral marks of birds vocalizing at close-range. Thus, playback may actually have a negative influence on average sound pressure.

Although the traffic noise sound profile could have influenced the low-frequency results, birds reacting to traffic noise playback by increasing their average vocalization frequency and vacating the immediate area are stronger arguments that are supported by the majority of our community analysis data (Lohr *et al.* 2003). Within the 0–3 kHz band, both average entropy and average sound pressure are lower in playback dawn choruses. This would suggest that less birds are vocalizing within this frequency range. As per our bioacoustic data, it is unlikely that birds stop singing during these dawn choruses. As the spectrogram density directly correlates with distance from the sound source (Charif *et al.* 2010), it is more likely that birds flew out of range of the microphone and thus decreased the observed entropy and sound pressure of the recording. This relationship is also supported in the 3–12 kHz band where sound pressure decreases within playback choruses. Previous studies have demonstrated that birds will avoid areas with road noise likely due to masking pressure (Francis *et al.* 2011b; Slabbekoorn and Peet 2003).

An additional response that could be contributing to the observed relationships is that a significant amount of individuals raised their average vocalization frequency to overcome masking pressure. Entropy decreased in playback trials between 0–3 kHz, but increased between 3–12 kHz — meaning that it is likely that individuals originally vocalized below 3 kHz and were forced to increase their vocalization above 3 kHz to avoid acoustic masking. This result is also supported by a decrease in average sound pressure within the 0–3 kHz band during playback trials, indicating there are fewer birds singing in this range. Changes in bird vocalization frequency due to anthropogenic noise have been documented by numerous other studies and

support our findings (Francis *et al.* 2011b; Slabbekoorn and Peet 2003). A potential reason that average entropy increased but average sound pressure decreased during playback trials between 3–12 kHz could be due to a combined effect of birds increasing their frequency, thus increasing the spectral complexity above 3 kHz, but vocalizing at farther distances, thus decreasing the spectral density. Since we saw no change in the bioacoustic time of our focal species, but did observe significant differences in the average entropy and sound pressure during playback dawn choruses, our results suggest that the immediate reaction of birds to traffic noise is to raise their average frequency and vacate the affected area rather than change their vocal duration. These findings have never been demonstrated on such a short time-scale. However, it is unlikely that all bird individuals changed their behavior in both of these fashions. It is more reasonable to expect that traffic noise playback affected different species within the community in different ways. Some species could have responded by increasing their average vocalization frequency to escape masking pressure, while other species — that were unable to change their vocal behavior — could have increased their distance from the offending sound source (Lohr *et al.* 2003).

To date, an experimental road noise playback study has never before been conducted in the neotropics. Other studies analyzing the effects of anthropogenic road noise are conducted in the presence of roads and provide limited information regarding the effects of noise pollution produced by roads (McClure *et al.* 2013; Francis *et al.* 2009). McClure *et al.* (2013) states that it is imperative for studies to isolate the effects of road noise so that confounding variables from roads cannot exacerbate effects from noise pollution seen on avian populations. Additionally, we know of no other studies using the same approach to demonstrate the effects of road noise also demonstrate a change in acoustic community composition.

The bird community of El Silencio de Los Ángeles has never before been exposed to the pressures of road noise on the soundscape; therefore, it is unlikely we observed any habituation effects to road noise by recorded individuals. According to Katti and Warren (2004), research focused on acoustic communication provides some of the clearest explanations of how organisms adapt to their environments. Therefore, we suggest further studies be conducted in the El Silencio de Los Ángeles area — as well as other neotropical sites — to determine the long-term effects of road noise on bird communication. Additional large-scale playback research is needed to generalize observed results to a broader scale (McClure *et al.* 2013). This is especially critical in the neotropics where there is already a low basal data set. We demonstrated that traffic noise had no immediate impact on bioacoustic time, but collecting data over a longer time could reveal potential differences not demonstrated in this study. We recommend that future research efforts also explore long-term species distributional changes and changes in habitat quality, which is most easily accomplished through direct surveying of individuals (i.e. point counts and mist-netting) (Ware *et al.* 2015). These research directions will inform the growing pool of research conducted in the neotropics to help in determining the impacts of introduced roads on bird populations and aid in decision making regarding road implementation and conservation strategies. To minimize the effects of roads, we suggest the implementation of live barriers to separate roads from surrounding habitats, which would decrease the effect of noise on the surrounding area (Slabbekoorn and Ripmeester 2008). The speed limit of roads bordering forests can also be reduced to minimize negative consequences on the surrounding forest (Slabbekoorn and Ripmeester 2008; Arévalo and Newhard 2010). With additional research and cautious road planning, the impact of the growing road system in the neotropics could be mitigated and aid in biodiversity conservation.

## **Conclusions**

It is important to preemptively document the effects of anthropogenic road noise on bird communities in an unfragmented landscape to establish a baseline knowledge without the influence of confounding variables such as the presence of a physical road. The fact that we found no significant effect on bioacoustic time, but did find significant effects in the spectral complexity and density of dawn choruses, suggests that different bird species can be affected in different ways. It is possible that birds can react by increasing their vocalization frequency or vacating affected areas to escape acoustic masking. Since our results only show the immediate responses of bird species to anthropogenic noise, we believe that this experiment should be replicated in order to gain information about the long-term effects of noise pollution to be used for conservation efforts and urban planning.

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## Appendix

Appendix 1. Average total bioacoustic time for four focal species and significance values

Species	Trial	n	Average Total Bioacoustic Time (s) $\pm$ 1 SD	P-value
<i>Henicorhina leucophrys</i>	Control	4	197.51 $\pm$ 114.19	0.4828
	Playback	6	296.67 $\pm$ 266.27	
<i>Catharus fuscater</i>	Control	4	487.61 $\pm$ 330.72	0.6806
	Playback	6	634.84 $\pm$ 737.87	
<i>Catharus mexicanus</i>	Control	4	137.64 $\pm$ 177.70	0.9023
	Playback	6	121.42 $\pm$ 117.27	
<i>Myadestes melanops</i>	Control	4	1254.85 $\pm$ 1301.56	0.6233
	Playback	6	1759.09 $\pm$ 1448.36	

Appendix 2. Average sound pressure and entropy of dawn chorus and significance values

<b>Frequency Band</b>	<b>Trial</b>	<b>n</b>	<b>Average Entropy (bits) <math>\pm</math> 1 SD</b>	<b>P-value</b>	<b>Average Sound Pressure (<math>\mu</math>Pa) <math>\pm</math> 1 SD</b>	<b>P-value</b>
0–3 kHz	Control	4	4.23 $\pm$ 0.02	0.0139	241090 $\pm$ 85361	0.0139
	Playback	6	3.49 $\pm$ 0.12		134208 $\pm$ 9359	
3–12 kHz	Control	4	5.04 $\pm$ 0.22	0.00595	435528 $\pm$ 79141	0.0142
	Playback	6	5.48 $\pm$ 0.15		47903 $\pm$ 28652	