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An ecoacoustic approach to understand the effects of human sound on soundscapes and avian communication

Alexander R. Schindler, Jacob E. Gerber and John E. Quinn

Department of Biology, Furman University, Greenville, SC, USA

ABSTRACT

Anthropogenic sound disrupts animal communication, but little research has both quantified how this disruption changes the soundscape and connected these changes to shifts in avian behaviour. We examined the effects of anthropogenic sound on soundscapes and avian vocalisation and flight behaviour. We recorded Carolina Chickadee (*Poecile carolinensis*) vocalisations, tested if vocalisations differed with varying levels of anthropogenic sound and overall variation in soundscape, and observed behavioural responses to differing vocalisation playbacks to test the functionality of possible vocalisation shifts. We found that anthropogenic sound disturbance predicted change in soundscape indicators. Vocalisation frequency was higher in areas of high disturbance but did not differ with changes in soundscape. Vocalisation frequency shifts did not provide a functional advantage, possibly explaining the lack of vocalisation changes in response to disturbed soundscapes. These analyses demonstrate that anthropogenic sound does change the surrounding soundscape, but the interaction between these changes and animal behaviour is multidimensional.

Key policy insights

- By quantifying the effects of sound from roads on the surrounding soundscape, we demonstrated how anthropogenic sound disturbance impacts the surrounding ecosystem, not just the immediate site of the generated sound.
- Increasing anthropogenic sound disturbance affected avian vocalisation. Birds vocalised at a higher frequency in the presence of higher anthropogenic sound disturbance.
- Vocalisation frequency shifts did not provide an advantage in overcoming acoustic masking from roads.
- The assessment of vocal responses to anthropogenic sound needs to extend to include complex assessments of behaviours. Without these behavioural assessments we may not be able to properly evaluate the effects of anthropogenic sound disturbances on avian communication and behaviour.

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Acoustic masking; anthropogenic disturbance; avian vocalisation; Carolina Chickadee; ethology; road

Introduction

Humans have a long history of acting as ecosystem engineers, altering both ecosystem structure and process (Smith 2007). Human managed landscapes are now more abundant than land unaltered by humans (Foley et al. 2005) across agricultural (Tilman et al. 2001; Galloway et al. 2008), forest (Trumbore, Brando, and Hartmann 2015) and aquatic systems (Scheffer, Carpenter, and de Young 2005). These anthropogenic modifications have disrupted various species' abilities to carry out basic behaviours necessary for communication, reproduction and survival. For example, the construction of roads restricts bat movement (Bennett and Zurcher 2013), pollution of aquatic environments alters fish behaviour and sensory function (Braun 2015) and exposure to chemical toxins has caused adverse development and reproductive effects in alligators, fish and birds (Harrison, Holmes, and Humfrey 1997).

One under-recognised change is the increase in anthrophony, or human-generated sound. Recent work shows that anthropogenic sound pollution stimulates physiological responses in animals including effects on cardiovascular health, the neuroendocrine system, sleep and cognition and the immune system (Kight and Swaddle 2011; Francis and Barber 2013). Behaviourally, human sound can negatively impact an individual's ability to effectively communicate reproductive and territorial messages (Lillis, Bohnenstiehl, and Eggleston 2015). Human sound can disrupt alarm calls (Lohr, Wright, and Dooling 2003), foraging cues (Luo, Siemers, and Koselj 2015) and contact calls (Ernstes and Quinn 2016) for

CONTACT John E. Quinn john.quinn@furman.edu 3300 Poinsett Hwy, Greenville, SC 29613, USA
Supplemental data for this article can be accessed here.

group cohesion, and may limit calls regarding habitat decisions. Acoustic masking, a process in which anthropogenic sound 'masks' an organism's communicative sounds, can negatively impact an organism's detection of both prey and predators (Barber, Crooks, and Fristrup 2010). In particular, acoustic masking decreases an animal's ability to properly communicate by overlapping the animal's vocal frequency range with background sound of a similar frequency range. When faced with acoustic masking from anthropogenic sound, many species modulate both frequency and amplitude of their vocalisation to reduce the effects of masking (Brumm 2004; Kight and Swaddle 2015; Oden et al. 2015).

Prior research on the effects of acoustic masking on avian species has focussed on behavioural responses to only some factors of the soundscape. New tools have recently become available to study all the acoustic components of a landscape, allowing research to parallel landscape ecology, which focusses on spatial patterns of corresponding ecological processes (Pijanowski et al. 2011; Villanueva-Rivera et al. 2011). When studying these spatial patterns, landscape ecologists find it more beneficial to study the landscape as a whole rather than a single landscape feature. Similarly, studying only isolated sounds keeps us from more fully understanding broader ecological processes associated with sound.

Studying all the components of the soundscape is important to understanding these processes and the spatial and temporal relationships between them (Rodriguez et al. 2014; Pieretti et al. 2011). Soundscapes are also a reflection of community behaviour, and studying the entire soundscape gives us valuable insight into these community behaviours and interactions over traditional practice of only looking at an individual's communication (Farina, Pieretti, and Piccioli 2011; Ruppé et al. 2015; Buxton et al. 2016). Extending from simple measurements of power most often used in past research, acoustic sound indices have been developed to quantitatively measure the soundscape (summarised in Quinn et al. 2018). Together, these soundscape indices give us a means to quantitatively examine communicative behaviours of entire animal communities over a given area, as well as measure changes in these behaviours in different environmental conditions. However, there has been little prior application of these indices collectively as measures of community behaviour or as predicators of animal behaviour. Additionally, to date, no studies have examined if or how soundscapes affect songbird vocalisation.

In this study, we used soundscape indices to examine how the soundscape affects frequency of Carolina Chickadee (*Poecile carolinensis*) vocalisations. We studied the 'chick-a-dee' alarm call given by this species. This call is used in a variety of social functions, including situations of alarm, contact calls for pairs and flocks and in coordinating group movements (Ficken, Ficken, and Witkin 1978). When used in situations of alarm, this call encodes information such as predator size and level of threat and signals other chickadees to group together in a 'mobbing' behaviour to drive the predator away (Baker and Becker 2002; Templeton, Greene, and Davis 2005). Acoustic masking from anthropogenic sound therefore may have negative impacts on the function of this call to signal the 'mobbing' behaviour.

Furthermore, while changes in bird vocalisation in response to anthropogenic sound have been monitored, few studies have tested the functionality of these vocalisation shifts (Brumm and Bee 2016). This gap prevents us from determining if the vocalisation shifts occur as a mechanism to overcome acoustic masking, or if they are occurring due to some other reason and do not have a functional advantage. To test for functional advantages of potential vocalisation shifts in overcoming acoustic masking from anthropogenic sound disturbance, we also monitored behaviour of Carolina Chickadees and Tufted Titmice (*Baeolophus bicolor*), a bird species known to flock with chickadees. We hypothesised that anthropogenic acoustic disturbance will (1) result in differences in the soundscape quantified by the acoustic indices calculated, (2) result in shifts of vocalisation frequency and amplitude to overcome the effects of acoustic masking and (3) that these vocalisation shifts change response times to alarm calls. Specifically, we hypothesise that the soundscape in areas of high anthropogenic disturbance will be less diverse and complex, resulting from a decrease in biological activity and an increase in anthropogenic activity. We believe this will be quantified by lower values of biophony, Acoustic Diversity Index (ADI), Acoustic Complexity Index (ACI), Normalised Difference Soundscape Index (NDSI) and Bioacoustic Index (BAI), and higher values of anthrophony and Acoustic Evenness Index (AEI) in sites near roadways. We also hypothesise that the minimum vocalisation frequency of the chickadee will be higher at sites near roadways, as well as at sites of high anthrophony, low biophony and low acoustic complexity and diversity, and that higher frequency chickadee alarm calls will decrease the alert response time of both chickadees and titmice to these alerts in areas near roadways.

Methods

Study species

We focussed on the behaviour of two species of bird: Carolina Chickadee (*Poecile carolinensis*) and Tufted

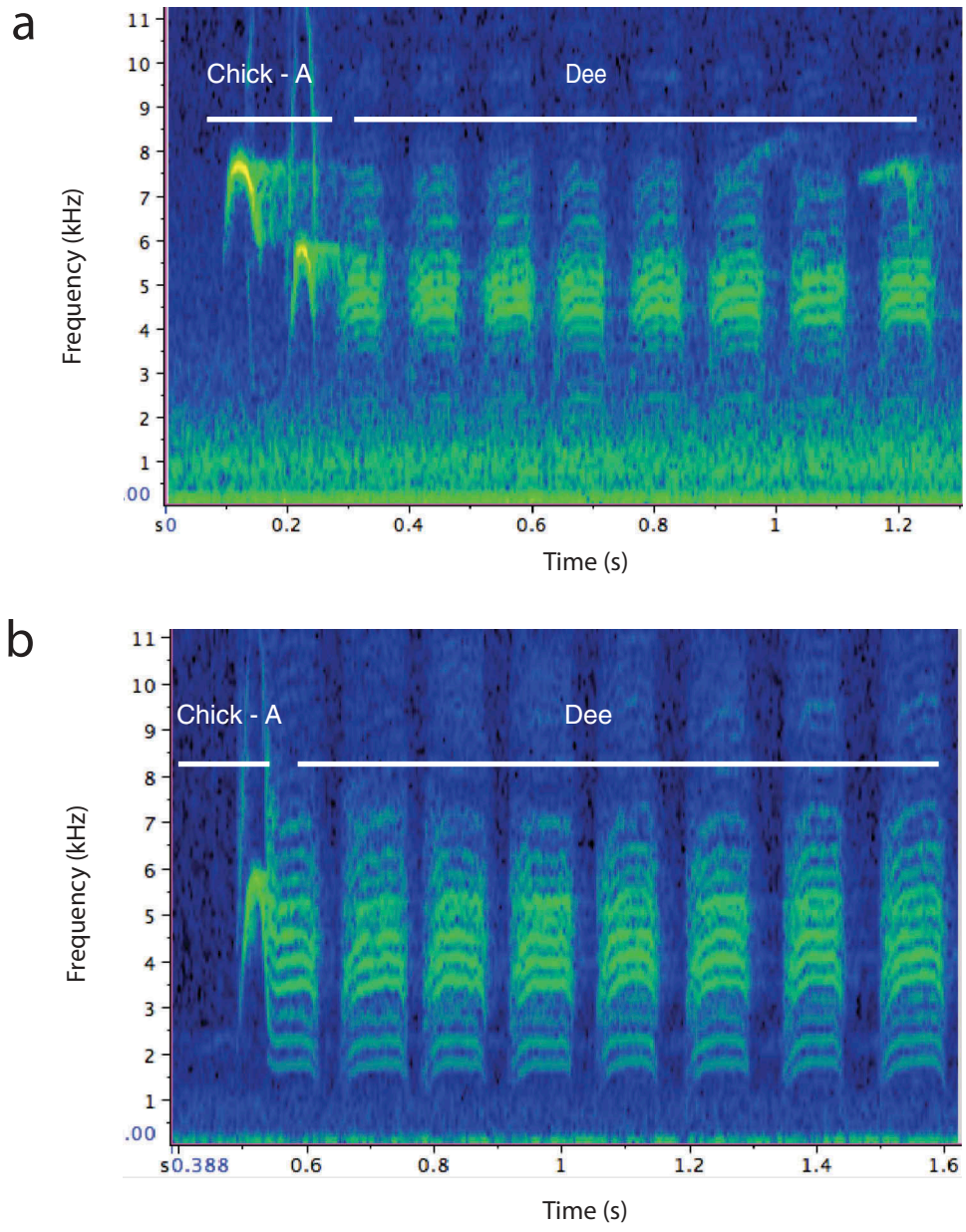

Figure 1. Spectrograms of Carolina Chickadee alarm call vocalisations, generated in Raven Pro (Raven Pro: version 1.5 2014). The *x*-axes are time in seconds and the *y*-axes are the frequency in kHz. The brightness of colour on a blue-green colour scale represents the intensity (i.e. power, measured in decibels). The beginning marks of upside-down U shapes are the 'chick-a' part of the call, while the following stacks of chevron-shaped bands are the 'dee' portions of the call. (a) A spectrogram of the call from a loud site used for behavioural analyses and (b) the spectrogram of the call from a quiet site used for behavioural analyses. Recorded with SM2 + automated recording units in Greenville, SC, in the winter of 2016.

Titmouse (*Baeolophus bicolor*). We focussed on Carolina Chickadees when examining the effects of soundscape on bird vocalisation. This species was ideal because they frequent feeders in flocks during the nonbreeding season and have distinct calls and vocal habits. The 'chick-a-dee' call of the Carolina Chickadee (Figure 1) is also ideal for the study because it is easily identified in recordings and spectrograms and has distinct minimum and maximum frequencies that can be identified on a spectrogram (Oden et al. 2015). Here we focus on the minimum frequency of the vocalisation as it has been shown to be the most likely to shift and is most clearly measured given our acoustic sampling rate (16 kHz). Chickadees are considered a 'sentry' bird in bird communities, and their alarm calls often alert other avian species in the community to predation threats. Changes in chickadee vocalisations

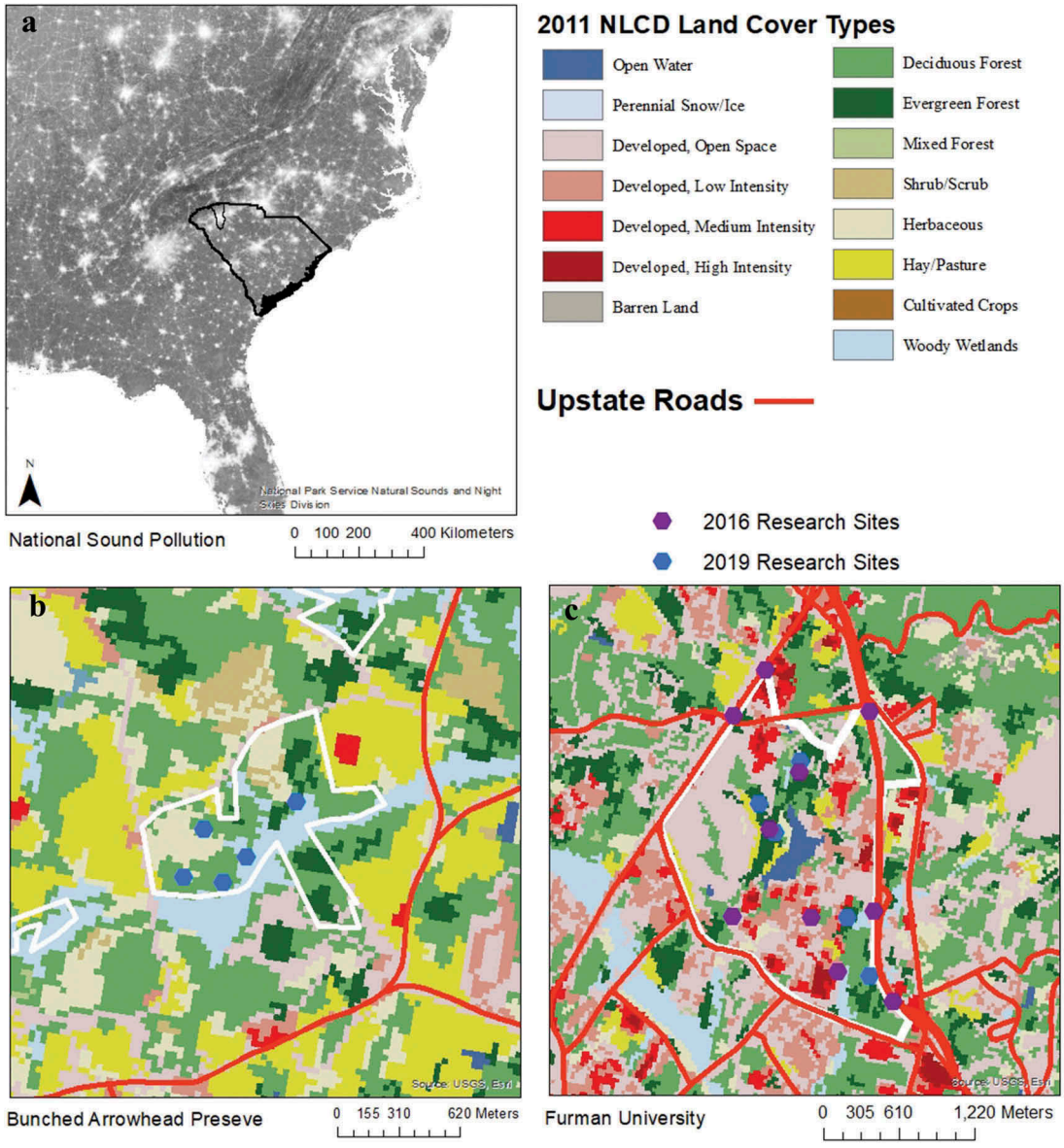

Figure 2. A map of the research location sites in Greenville Co, SC.

therefore have potential to impact entire avian communities, making them good candidates to extend from vocalisation to communication. We studied both Carolina Chickadees and Tufted Titmice when examining behavioural changes. Like Carolina Chickadees, Tufted Titmice frequently flock around feeders during the non-breeding season.

Study sites

We observed sites at Furman University campus and Bunched Arrowhead Heritage Preserve, located in Greenville, South Carolina (Figure 2). Within each area recording locations and behaviour observations were a minimum of 400 m apart in order to ensure recordings from different flocks at each site. To avoid confounding effects of landscape structure on sound transmission, all sites were located at the edge of deciduous, evergreen or mixed forest patches. In 2016 we selected five site locations in areas near high traffic roads (i.e. high anthropogenic sound disturbance) and five in areas we perceived to have little anthropogenic sound. In 2019 we selected an additional five sites in high anthrophony areas and five sites in areas with low anthropogenic sound. We used a handheld sound recording device (PMD661MKII Handheld Solid State Recorder 2008) to record a 1 min interval of sound at each site. We then analysed each sound recording in Raven Pro version 1.5 (Cornell Lab of Ornithology 2014) and recorded the average decibel level for the

recording at each site. Using a t-test, we determined that the average decibel level was significantly louder at the sites we perceived to have high anthropogenic sound (near large roads) than sites we perceived to have low anthropogenic sound (away from roads) ($P < 0.05$).

Acoustic sampling and vocalisation analysis

We recorded chickadee vocalisations, along with the surrounding soundscape, in each of the 10 sites, for a period ranging from 10–12 days in early February in 2016. At each site, we hung a bird feeder containing black oil sunflower seeds from a stand about 1 m off of the ground. We used SM2+ automated recording units (Song Meter SM2+ 2013) to record both chickadee vocalisations and the surrounding soundscape in WAV file format. We hung the recorders about 1 m off of the ground and within 1 m of each bird feeder. This was done to reduce the error/variation in recordings as a function of distance between the bird and the ARUs. The recorders were set to record at a sampling rate of 16 kHz for 10 min on the hour for every hour between 0600 and 1000 EST. Morning sampling times were chosen in order to sample when chickadees were most likely to be active. We sampled in 10 min intervals in order to mimic general bird point count sampling practices to maximise detection probability (Quinn et al. 2011). The total number of sound samples per site ranged from 50 to 95.

To analyse the soundscape of each site, we used the tuneR package (Package tuneR 2018) in R version 3.4.1 (R Development Core Team 2017) to read the sound files. We then used the soundecology (Package Soundecology 2015) package to obtain values of anthrophony, which we defined as sound occurring in the 1–2 kHz range, and biophony, which we defined as sound occurring in the 2–8 kHz frequency range, for each sound file. Analysis of a large number of recordings from many sampling locations in past research supports these characterisations of biophony and anthrophony frequency ranges (Gage, Napoletano, and Cooper 2001; Gage and Napoletano 2004; Kasten et al. 2012). We also used the soundecology package to obtain values of NDSI, ACI, ADI, AEI and BAI for each sound file. Through use of this package, we calculated NDSI with the equation NDSI = (biophony – anthrophony)/(biophony + anthrophony). We calculated ACI by measuring the amount of variation in intensity of sounds. We calculated ADI by dividing the spectrogram into bins, taking the proportion of the signals in each bin above a threshold, and applying the Shannon diversity index to these bins. We calculated AEI in the same way as ADI but applied the Gini index of evenness instead of the Shannon index. We calculated the BAI by calculating the area under each curve including all frequency bands associated with the dB value that was greater than the minimum dB value for each curve. Due to sound recorder malfunctions, soundscape data was not collected from two of the predetermined loud sites (loud sites 2 and 5). The results of this soundscape analysis were seven soundscape index values for each of the 50–95 recordings at each site. In order to avoid pseudo replication, we scaled the data down, averaging all values for each index for each site and calculated the standard deviation for each index for each site. Thus, site became the unit of study for subsequent analyses.

To analyse the chickadee vocalisations, we uploaded each file into Raven Pro and calculated the minimum, maximum and delta (difference between the minimum and maximum) frequencies of the 'dee' portions of the chickadee alarm calls using the power spectra method described by Zollinger et al. (2012) in order to reduce bias in frequency measurements. This method allows for reliable measurements of frequency and avoids the errors in frequency measurement common with using spectrograms (Ríos-Chelén et al. 2017). After identifying the minimum and maximum frequencies of the dee portion following Zollinger et al. (2012), we also recorded the average and peak power (i.e. amplitude, measured in decibels) of the call in this frequency range. While field recordings can have bias in these measures, the aforementioned standardisation in distance from feeder to ARU limited this bias. We focussed analysis on the 'dee' portions of the vocalisations because these are the portions of the call that vary the most with different predation dangers, and therefore contain a large functional component of the calls (Templeton, Greene, and Davis 2005). We then averaged all the maximum frequencies, minimum frequencies, delta frequencies, average powers and peak powers for each site in order to account for the possibility of recording the same chickadee multiple times. Variability in the recordings was somewhat limited by the fact that the recordings were all taken in close proximity to the feeders.

Behavioural response analysis

We recorded Carolina Chickadee and Tufted Titmouse (a local con-specific) behaviour in response to playbacks of chickadee alarm calls at each site between late February and early April in 2019. We selected multiple calls from the perceived loud sites (high anthropogenic sound) and from the perceived quiet sites (low anthropogenic sound) that had similar power levels and number of 'dees' in the call. Multiple calls were selected to reduce the effects of pseudo-replication in the call. The calls from the loud site had a higher minimum and

maximum frequency in the 'dee' portion of the call than the call from the quiet site (Figure 1). We set a small, portable Bluetooth speaker (JBL Flip 4) on top of the bird feeder. We used a GoPro camera (GoPro HERO 3+ 2013) on a tripod, set about 1 m away from the feeder to video record all bird behaviour. After setup, we then sat 8–10 m away. We waited 10 min to allow bird behaviour to return to normal and not be influenced by our presence. We then waited for either a Carolina Chickadee or a Tufted Titmouse to land on the feeder and played the chickadee alarm call from the quiet site on a loop until the bird left the feeder. We continued to do this each time one of the two bird species landed on the feeder for an hour. We repeated this same protocol at each site for both the loud and quiet calls, never testing the same site twice in the same day.

We used the Premiere Pro CC and Audition CC (Premiere Pro CC 2016; Audition CC 2016) computer programs to analyse the videos. In response to the playback call, each bird turned its head in the direction of the sound before flying away from the feeder. We considered the end of the head turn of a bird to be the moment of its 'alert' to the alarm call and the moment the bird's feet were no longer touching the feeder to be the moment of its 'flight response.' Using Premiere Pro CC, we were able examine each frame of the video recordings and identify the exact frame of the video each of these behaviours occurred. We then recorded the time in the recordings of the identified frames. We did not record alert response times from birds that were on the backside of the feeder, and therefore out of view of the camera. Using Audition CC, we were able to view a spectrogram of the audio of the videos. Using the spectrogram, we recorded the time each playback of the chickadee alarm call occurred. We then calculated the difference between the alert response and flight response times and the playback call times. We averaged the times it took each bird to exhibit each behaviour for each bird species in each site in response to each type of playback call, keeping site as the study unit.

Statistical analysis

It was not possible to record data blind because our study involved the observation of focal animals in the field. To assess the effects of anthropogenic sound disturbance on the soundscape of our sites, we used a principal component analysis (PCA) of correlation matrices to reduce dimensionality of the average values of the seven soundscape indices using the FactoMineR package (Package FactoMineR et al. 2018) in R. We scaled and centred variables prior to analysis. We used the resulting scree plot to determine that retaining two principal components (PCs) was sufficient (82.3% of variance explained) for our data. We similarly conducted a PCA of the scaled and centred standard deviations of all seven of the soundscape indices and retained the first two PC axes (84.6% of variance explained). We then plotted sites on the first two PC axes for both PCAs. This allowed us to visually examine the differences in sound profiles of each site and look for differences in soundscape between sites near and far away from road sound disturbances.

To conduct the vocalisation analysis, we first tested if the presence of anthropogenic sound disturbance had an effect on chickadee vocalisations. We ran a series of ANOVAs comparing the site-averaged values of all measured vocalisation characteristics (minimum frequency, maximum frequency, delta frequency, average power, and peak power of 'dee' vocalisation frequencies) in response to the predetermined location type described above (high anthropogenic and low anthropogenic sound sites ($P < 0.05$)). We then used the PCA results to test whether the soundscape as a whole had an effect on chickadee vocalisation. We used the factor loadings of the first two PC axes of the PCA of the average soundscape index values to calculate factor scores of soundscape for each site. We used these factor scores for each of the two PCs as new variables that summarised the soundscape as a whole. We then used a series of linear regressions of all measured vocalisation characteristics in response to the factor scores to test if soundscape had an effect on chickadee vocalisation frequency or power. We also used a series of linear regressions of the standard deviation of all measured vocalisation characteristics in response to the factor scores to test if soundscape had an effect on the variation of chickadee vocalisation frequency or power. We used the factor loadings of the first two PCs of the PCA of the standard deviation of soundscape index values to calculate the factor scores of soundscape variation for each site. We used these factor scores for each of the two PCs as new variables that summarised variation of the soundscape as a whole. We then used a series of linear regressions of the average and standard deviations of all measured vocalisation characteristics to test if variation in soundscape had an effect on frequency, power or variation of frequency or power of chickadee vocalisation. We used the correlation coefficient between each factor score and each soundscape index to interpret factor scores.

When conducting the bird behaviour analysis, we conducted a two-way ANOVA with an interaction term of chickadee alert time in response to playback call type, location type, and an interaction between playback call and location types to test the functionality of

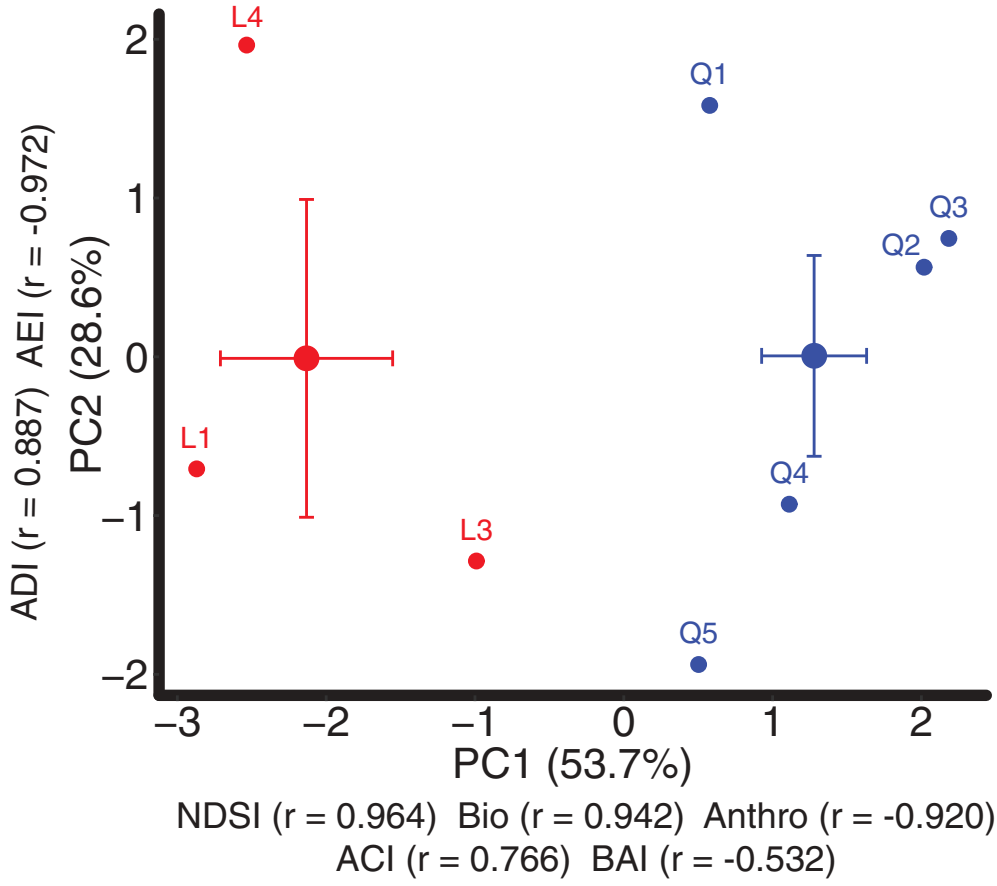

Figure 3. Sites plotted based on average soundscape indices values on the first two principal component axes from the principal component analysis (PC1 and PC2). Amount of variation in data represented by each principal component axis is denoted as a percentage. Sites of low anthropogenic sound disturbance are labelled in blue and sites of high anthropogenic sound disturbance are labelled in red. Unlabelled points are the group centroids with error bars denoting the standard error. We used the correlation coefficient between each factor score and each soundscape index to interpret factor scores. Sites within each location type differed in PC2 value, while sites differed between location type in PC1 value.

each playback call type. We conducted a two-way ANOVA with an interaction term of chickadee flight response time in response to playback call type, location type, and an interaction between playback and location call types. We did the same tests for alert time and flight response time for Tufted Titmice. We conducted all statistical analyses in R version 3.4.1 (R Development Core Team 2017).

Results

Soundscape analysis

We averaged the values of each soundscape index for all recordings in each location and calculated the standard deviation for all recordings in each location (Appendix 1). When examining both PCA plots of sites along the first two PC axes, we observed grouping of the sites by anthropogenic sound disturbance level, and the centroids of the groups are distinctly separated (Figures 3 and 4). Sites with similar levels of anthropogenic sound disturbance differed in ADI and AEI, as well as in standard deviation of ACI and BAI (PC2 in Figures 3 and 4). Sites with low levels of anthropogenic sound disturbance had increased values of NDSI, biophony, ACI, and decreased values of anthrophony and BAI, as well as increased standard deviations in NDSI, biophony, anthrophony, ACI, ADI and AEI (PC1 in Figures 3 and 4).

Vocalisation analysis

We averaged all of the minimum frequencies of the chickadee 'dee' vocalisations at each site and calculated the standard deviation between the minimum frequencies for all vocalisations for each site. We repeated this for the maximum frequencies, delta frequencies, average power, and peak power of the 'dee' vocalisations (Appendix 2). The minimum frequencies of vocalisations were significantly higher in areas of high anthropogenic sound disturbance than in areas of low anthropogenic sound

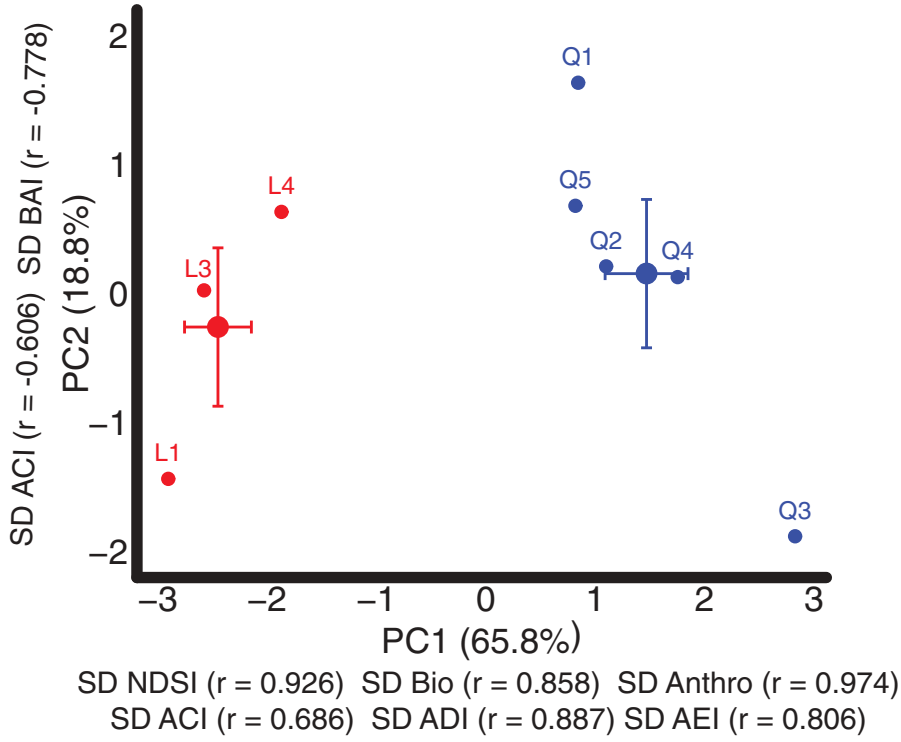

Figure 4. Sites plotted based on standard deviation of soundscape indices values on the first two principal component axes from the principal component analysis (PC1 and PC2). Amount of variation in data represented by each principal component axis is denoted as a percentage. Sites of low anthropogenic sound disturbance are labelled in blue and sites of high anthropogenic sound disturbance are labelled in red. Unlabelled points are the group centroids with error bars denoting the standard error. We used the correlation coefficient between each factor score and each soundscape index to interpret factor scores. Sites within each location type differed in PC2 value, while sites differed between location type in PC1 value.

disturbance (F = 6.059, P = 0.049) (Figure 5). There was no significant difference in the maximum (F = 0.017, P = 0.901) or delta (F = 1.064, P = 0.342) frequencies of vocalisations in response to anthropogenic sound level. There was also no significant difference in the average (F = 0.520, P = 0.498) or peak (F = 0.348, P = 0.577) power of vocalisations in response to anthropogenic sound level.

We found no significant differences in any vocalisation characteristic and either of the PCA variables for average soundscape value (P > 0.05) (Appendix 3). Minimum frequency did decrease with an increasing value of the variable for standard deviation of soundscape index value associated with the first PC axis (i.e. with increasing standard deviation of NDSI, biophony, anthrophony, ACI, ADI, and AEI) (P = 0.027) (Appendix 4). We did not find significant differences in any other vocalisation characteristic and either of the variables for standard deviation of soundscape value (P > 0.05) (Appendix 4).

Behavioural analysis

We averaged the alert response times for each playback call type for each location for each bird species and calculated the standard deviation of alert response times for each playback call type for each location for each bird species. We calculated the average and standard deviations of flight response times in the same way. We found that titmice alert time ranged from 2 ms to 12 ms and titmice flight response ranged from 6 ms to 212 ms. Chickadee alert time ranged from 1 ms to 20 ms, while flight response ranged from 11 ms to 76 ms. We found that chickadee and titmice alert times were significantly faster in response to high frequency calls (F = 11.823 P = 0.009, Figure 6), but there were no other significant effects of location on alert time (F = 1.169, P = 0.311) or of the interactive effect of call type and location type on alert time (F = 2.079, P = 0.187). There were no significant results when considering the effects of call type on flight response (F = 3.290, P = 0.103, Figure 6), the effects of location on flight response (F = 1.383, P = 0.270), or the interactive effects of call type and location type on flight response (F = 1.104, P = 0.321).

Discussion

We found that anthropogenic sound disturbance did have measurable impacts on the acoustic habitat of

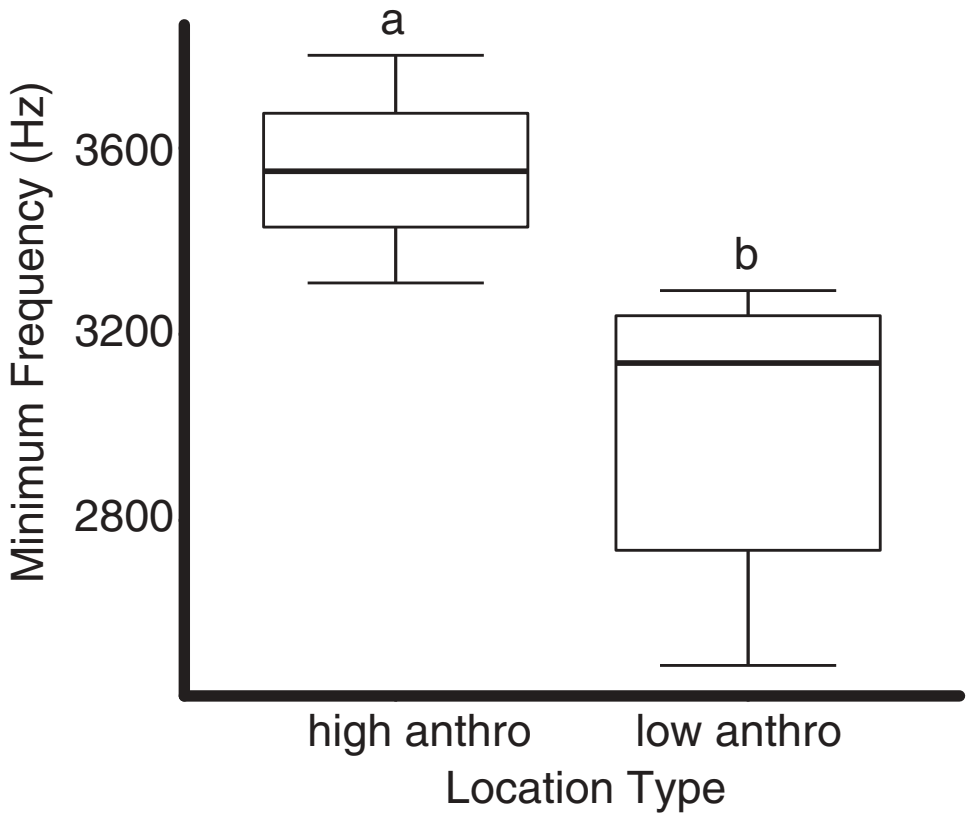

Figure 5. Boxplot of minimum frequency of the 'dee' portion of Carolina Chickadee vocalisations in response to high or low anthropogenic sound disturbance at site locations. Chickadee vocalisations had higher minimum frequencies in sites with high anthropogenic sound disturbance than vocalisations in sites with low anthropogenic sound disturbance.

chickadee. We also found that chickadee vocalisations did differ in response to levels of anthropogenic sound disturbance, but not in response to the soundscape as a whole. We found that shifts of chickadee vocalisation frequency did not translate to a behavioural advantage, highlighting a need for study of entire soundscapes paired with behavioural analyses to better understand if potential vocalisation shifts are a viable mechanism for overcoming the harmful effects of acoustic masking.

Soundscape analysis

We found that anthropogenic sound disturbance did have measurable impacts on the soundscape in our study sites. Upon visual examination of plots of our sites along the first two PC axes in both the soundscape and variation in soundscape PCAs, we observed distinct clustering by site type. There was some soundscape variation within site type, including variation in ADI and AEI, as well as in standard deviation of ACI and BAI. However, we also observed variation between sites of high and low anthropogenic sound disturbance, including decreased values of NDSI, biophony and ACI, increased values of anthrophony and BAI as well as decreased standard deviations in NDSI, biophony, anthrophony, ACI, ADI and AEI in sites with high anthropogenic sound disturbance. It is not surprising that our sites near roads had higher levels of anthropogenic sound, measured as anthrophony, as a result of increased traffic sound. Anthropogenic sounds are also characterised as having low levels of acoustic complexity, leading to decreased acoustic complexity values for the site as a whole (Pieretti, Farina, and Morri 2011). Prior research demonstrated high correlation between the BAI and avian abundance (Boelman et al. 2007). It is therefore likely that high levels of anthropogenic sound at locations near roadways reduce fitness for organisms in some way, such as by decreasing propagation and perception of sounds necessary in organisms' foraging, anti-predator behaviour, reproductive success and community structure (Barber, Crooks, and Fristrup 2010). Decreases in BAI and biophony, biologically generated sounds, may be a result of organisms choosing to avoid areas of high anthropogenic sound to limit these negative effects. The changes of anthrophony and biophony are further evident in the decrease of NDSI in sites with high anthrophony, as NDSI is calculated using anthrophony and biophony, and is often used as an indicator of

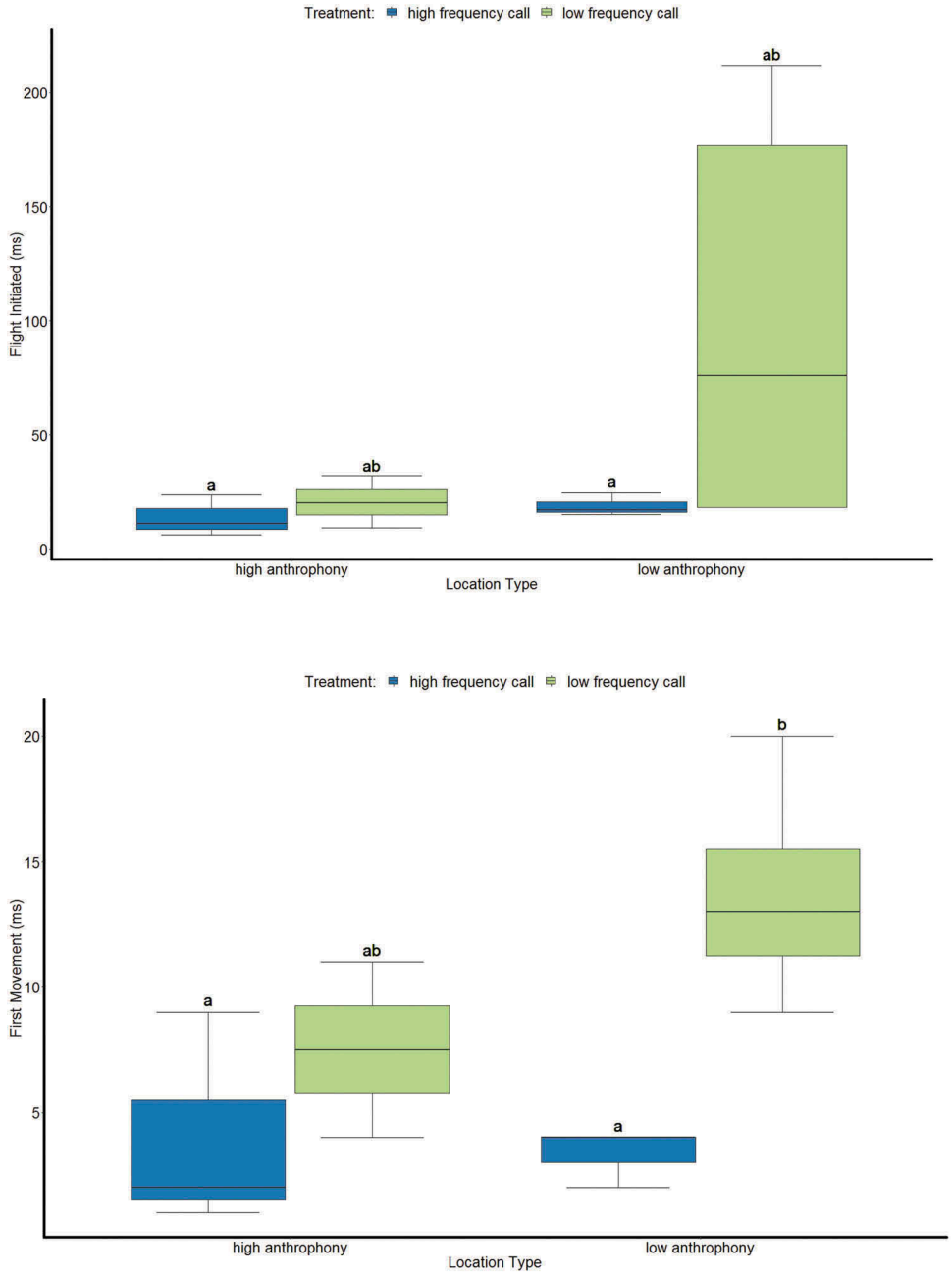

Figure 6. Boxplot of the Carolina Chickadee and Tufted Titmouse alert time in response to (a) treatment type (high or low frequency playback calls) and (b) treatment type and location type (high or low anthropogenic sound disturbance).

anthropogenic sound disturbance. From the decreases in variation in most of our soundscape indices associated with high anthropogenic disturbance, it is also likely that disturbances from road sound result in more monotonous soundscapes. Future research of each of these soundscape measures individually is needed to better understand the biological value and implications of each index, as well as to better define when each index is appropriate. This would allow us to better understand the mechanisms for soundscape change in response to disturbances.

Vocalisation and behavioural analyses

We found that chickadee vocalisations did differ in areas of different anthropogenic sound disturbance, but not in association with differing soundscapes as a whole. Results of prior research have been inconclusive as to whether or not birds do alter their vocalisation to overcome the effects of acoustic masking in areas with high anthropogenic sound disturbance. About half of previous studies have shown vocalisation frequency shifts in bird vocalisations to higher frequencies (Brumm 2004; Kight and Swaddle

2015; Grace and Anderson 2015) in response to acoustic masking. The Black-capped Chickadee (*Poecile atricapillus*), a closely related species to the Carolina Chickadees we studied, is among those that increase vocalisation frequency (Oden et al. 2015). We did find a difference between vocalisation frequency and level of anthropogenic sound, similar to other work in the region looking at Brown-headed Nuthatch (*Sitta pusilla*), Carolina Wren (*Thryothorus ludovicianus*) and Northern Cardinal (*Cardinalis cardinalis*) (Ernstes and Quinn 2016; Quinn unpublished data). However, we did not find clear differences between vocalisation characteristic and the soundscape values as a whole. We did find a difference between the minimum frequency of calls and the first PC axis for variation in soundscape. It is therefore possible that variation in anthropogenic sound disturbance impacts chickadees, something previously unstudied in vocalisation analyses, though it may also be that the soundscape measures do not add new information. The seemingly contradictory results between our analyses using traditional sound metrics of power and analyses using newer soundscape indices further adds question as to whether or not altering vocalisation frequency is a strategy utilised by birds to overcome acoustic masking. It is therefore possible that other environmental or behavioural factors are causing vocal changes in other species, rather than acoustic masking. This could also mean that some frequency changes are non-functional by-products of other behaviours. For example, the increasing of vocalisation amplitude to increase propagation of calls can lead to increases in call frequency that is not related to masking by anthropogenic sound (Brumm and Bee 2016).

Few studies actually measure the functionality of observed and recorded vocalisation frequency shifts. When we measured the functionality of high and low frequency chickadee vocalisations at sites of high and low anthropogenic sound, we found that chickadee alert times were slower in response to low frequency calls. In both cases, results were regardless of anthropogenic sound levels. Thus, there appears here to be no adaptive advantage to vocalisation frequency shifts to higher or lower frequencies. If there is in fact no advantage to vocalisation frequency shifts, birds could be responding to acoustic masking in different ways. For example, birds and other species could be leaving the area altogether in pursuit of less disturbed areas, which could help explain the changes in soundscape observed in the broader soundscape analysis discussed above.

Conclusion

Anthropogenic sound disturbance is causing increasingly negative consequences on both natural areas and urban systems. However, these effects on natural ecosystems are still largely described by a narrow set of measures (e.g. power of sound disturbance and frequency of vocalisation). From this soundscape analysis, we can conclude that anthropogenic sound does have significant impacts on the surrounding soundscape. However, the interaction between a changing soundscape and animal behaviour is quite complex. Previous studies have mixed results linking change in bird vocalisation frequency to anthropogenic sound disturbance. In this study, we found a chickadee vocalisation frequency shift associated with high anthropogenic sound disturbance. However, we did not find similar vocalisation shifts in response to changes in the soundscape as a whole. The inconsistency of results in this and previous studies (Brumm 2004; Kight and Swaddle 2015; Grace and Anderson 2015) calls to attention the need to test behaviours for functional advantages in overcoming anthropogenic disturbances. In our results we found that vocalisation frequency shifts did not increase the effectiveness of the communication in the presence of anthropogenic sound disturbances from roads. Birds in our study area are likely utilising different behavioural changes to overcome the negative effects of acoustic masking.

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Data availability statement

The datasets developed and analysed during the current study are available from the corresponding author on reasonable request.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Notes on contributors

Alexander R. Schindler is currently a doctoral student and research assistant at the University of Missouri. His research interests include addressing how global change affects wildlife populations and communities. He studies both individual

decision-making and population-level processes to understand how anthropogenic changes in the environment drive changes in animal abundance and behaviour.

Jacob E. Gerber is currently an undergraduate Biology student and research assistant. His research interests span from understanding ecological systems using acoustic technology, large-scale data analytics and data mining, text-mining in the humanities, and modern consciousness research. He approaches research through a broad liberal arts perspective grounded in his multidisciplinary experiences.

John E. Quinn is an Associate Professor of Biology at Furman University. His research and teaching emphasise concerns related to biodiversity conservation and sustainability; in particular, avian ecology, conservation in managed ecosystems at local and global extents, and sustainability for landscape planning. Currently his research focus is on how agricultural and urban landscapes and soundscapes in Upstate South Carolina can be managed to conserve biodiversity and enhance ecosystem services and the governance tools to reach community goals.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. No IACUC was required as the study organisms were free-living vertebrate wildlife and the study presented no potential to cause harm or materially alter the behaviour of the animals.

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