THE EFFECTS OF ANTHROPOGENIC NOISE ON ANTI-PREDATORY BEHAVIOUR IN GREAT TITS

(Parus major)

Wildlife Ecology and Conservation (WEC)

An MSc. thesis research report by Whitney Loh (1049487)

WAGENINGEN
UNIVERSITY & RESEARCH

Project information:

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Contents

Abstract

Title: The effects of anthropogenic noise on anti-predatory behaviour in Great tits *(Parus major)*.

Background: Anthropogenic noise has detrimental fitness effects on wildlife fitness and body condition due to audio masking and increased predation risk. Birds are particularly susceptible as acoustic communication plays an important role in the reduction of predation risk. Great tits (*Parus major)* are a well-adapted urban bird species, that can be found in large numbers in urban environments, making them a suitable candidate for studying the effects of anthropogenic noise on anti-predator behaviour. By studying the impact of anthropogenic noise and audio masking of alarm calls on urban bird behaviour in different noise environments, this study aims to contribute to the development of effective conservation strategies for urban wildlife.

Aim: This study investigated the effects of anthropogenic noise and conspecific alarm calls on the behaviour of great tits. It was predicted that noise pollution would reduce anti-predator behaviour, feeding behaviour, and decrease visitation rate.

Organisms: Great tits (*Parus major)*

Place of research: Wageningen University of Research campus, Wageningen, Netherlands

Methodology: This study investigated the effects of experimentally-added conspecific alarm calls (presence vs. absence), experimentally-added anthropogenic noise (presence vs. absence), and feeder location (quiet vs. noisy) on the behaviour of great tits. A total of 20 bird feeders were used in the study, with 10 bird feeders placed in quieter locations and 10 feeders placed in noisier locations on Wageningen University campus. Data was collected on-site at sunrise during winter months at two feeders, one at a quiet site and one at a noisy location, whereby four audio treatments were broadcasted: (1) 'Alarm' (conspecific alarm calls only), (2) 'Anthro' (anthropogenic noise only), (3) 'AnthroAlarm' (a combination of anthropogenic noise and alarm calls), and (4) 'Control' (no additional sounds). Bird behaviour was also recorded using a video camera for further analysis.

Principle findings: This study found that audio type had a significant effect on the visitation rate of great tits ($p=0.01$), with a significant difference between 'AnthroAlarm' and 'Anthro' treatments (estimate = - 0.49), implying an audio masking effect of alarm calls by anthropogenic noise. It was found that great tits did not frequent feeders in quiet locations more than in noisy locations ($p=0.60$). There were no significant findings for the time spent at feeder, vigilance rates and seed consumption rates for feeder site and audio types.

Conclusions: These findings reinforce that great tits may be able to adapt to the presence of anthropogenic noise to some extent, as evidenced by their continued visitation to the feeders. Overall, this study contributes to our understanding of the potential effects of anthropogenic noise on wildlife behaviour and provides useful information for conservation and management efforts, such as the installation of noise barriers to limit noise emissions and creating noise-reduced habitats (Shannon et al., 2016). Future research could investigate the potential long-term effects of anthropogenic noise on the behaviour and physiology of birds and other urban wildlife as well as the impact of noise pollution on a variety of bird calls used for avian communication.

1 Introduction

Wildlife species in human-dominated environments face a range of novel disturbance factors, such as noise, light, chemical pollution, and direct disturbances from human activities (Grunst et al., 2021). Anthropogenic noise, in particular, has been found to have negative impacts on an individual's behaviour, physiology, and fitness (Kunc et al., 2016). For birds, these effects include physical damage to the auditory canal, stress responses, fight-flight responses, and avoidance behaviours (Ortega, 2012). Behavioural responses towards noise pollution can have significant consequences on factors like foraging, reproductive success and vocal communication, including interference with predator detection and potentially leading to population declines. (Ortega, 2012). Urban and suburban areas are predominantly affected by anthropogenic noise, with traffic noise being a major source of concern due to the rapid development of transportation networks and associated noise pollution (Grunst et al., 2021).

Traffic noise is a pervasive and stressful sensory pollutant for wildlife. The unpredictable nature of traffic noise can alter behaviour and cortisol levels in animals, leading to potential long-term changes in the hypothalamic-pituitary-adrenal (HPA) axis (Injaian et al., 2018). Traffic noise can also lead to sensory pollution in animals and interfere with their acquisition and response to critical auditory cues such as alarm calls (Grunst et al., 2021), making it a significant stressor for many species. Although animals may be able to adapt to some extent by changing their behaviour and becoming accustomed to disturbance, novel disturbance factors can overwhelm their coping mechanisms, disrupt adaptive physiology and behaviour patterns, and ultimately lead to declines in individual fitness and population stability (Grunst et al., 2021).

Noise pollution can have complex interactions with birds and natural sounds in their acoustic environment. Birds have evolved intricate acoustic signalling systems that enable them to communicate effectively by maximising the transmission distance of their sound for long-distance communication while minimising degradation and attenuation to avoid masking (Trujillo-Torres et al., 2021). For example, birds often sing at dawn to coincide with acoustic conditions that are most favourable for sound transmission when there is less wind noise and atmospheric fluctuations (Warren et al., 2006). However, elevated noise levels from anthropogenic sources can interfere with these natural sounds and disrupt the communication strategies that birds have evolved over time and negatively impact bird populations. Traffic noise pollution, for example, has been repeatedly associated with decreased species diversity and breeding densities (McClure et al., 2013). Noise pollution can cause audio masking, which occurs when biologically important sounds such as vocal communication or predator sounds, are obscured by loud noises Ortega (2012). This can interfere with bird territorial defences, mate attraction and communication signals such as begging, alarm or distress calls (Warren et al., 2006). Additionally, noise pollution can interfere with contact calls that aid in group cohesion, resulting in lost individuals or a breakdown in group structure (Ortega, 2012).

Noise pollution and audio masking have significant impacts on predator-prey interactions. Predators face decreased food-finding success because noise pollution can mask prey noises, making it difficult for them to locate food and thus affecting their survival and fitness (Halfwerk & Slabbekoorn, 2013). From the perspective of prey, noise pollution can have significant impacts on anti-predator behaviours, as many species rely on auditory cues to detect and respond to potential threats (Voellmy et al., 2014). Anti-predator behaviours which describes the responses of prey to predators (Lunn et al., 2022), such as mimicry, grouping, alarm calls, warning signals, and escape behaviour, have evolved in multiple species to reduce the risk of predation (Suzuki, 2014). For birds, alarm calls are a crucial component of their anti-predator response, enabling them to convey detailed information about predator risk and threat level to their conspecifics (Suzuki, 2014). However, audio masking from noise pollution can interfere with these conspecific alarm calls and increase the risk of predation due to a lack of awareness of dangerous situations that results in inappropriate responses to predation (Ortega, 2012). Furthermore, anthropogenic noise can act as a distractor by masking important auditory cues which can increase the predation risk of prey individuals. For example, when predator sounds such as footsteps, breathing, and rustling leaves are muffled by anthropogenic noise, the predator become harder to detect by prey individuals (Ortega, 2012).

1.1 Knowledge gap

The significant impact of noise pollution on predator-prey interactions highlights the need for further research to understand how anthropogenic noise pollution and audio masking affect anti-predator behaviours of urban birds. To bridge this gap, this study aims to investigate the effects of anthropogenic noise pollution and audio masking of conspecific alarm calls on the anti-predator behaviours of great tits, a well-adapted passerine species that are found in large numbers in both noisy urban areas and quiet forested areas (Krebs et al., 1978; Reijnen et al., 1995). By comparing the behavioural responses of birds in the two different noise environments, quiet and noisy, towards different experimentally added audio treatments, this study aims to contribute to the understanding of the effect of anthropogenic noise on bird behaviour in urban environments. The results of this study can assist in the development of effective conservation strategies for urban wildlife, such as measures to reduce the negative effects of noise pollution on bird populations.

1.2 Research questions & Hypothesis

1.2.1 Research questions

This has led to three research questions,

1) Does anthropogenic noise affect the **feeding behaviour** (i.e., time spent at the feeder, seed consumption rate) of great tits in response to alarm calls and whether this effect is mediated by the overall noise levels of a location?

2) Does anthropogenic noise affect the **vigilance behaviour** of great tits in response to alarm calls and whether this effect is mediated by the overall noise levels of a location?

3) Does anthropogenic noise affect the **visitation rate** of feeders by great tits in response to alarm calls and whether this effect is mediated by the overall noise levels of a location?

1.2.2 Hypothesis and predictions

My hypothesis is that the audio masking of conspecific alarm calls from anthropogenic noise pollution, which is a frequently cited mechanism for explaining how noise compromises communication (Zhou et al., 2019), may result in a reduction of anti-predator behavioural responses due to decreased awareness of predation risk. Furthermore, I hypothesise that this effect will be more pronounced in noisy locations due to an added noise effect. Figure 1 presents the audio masking effect on conspecific alarm calls on wild superb fairy wrens (*Malurus cyaneus)*, as illustrated by Zhou et al. (2019).

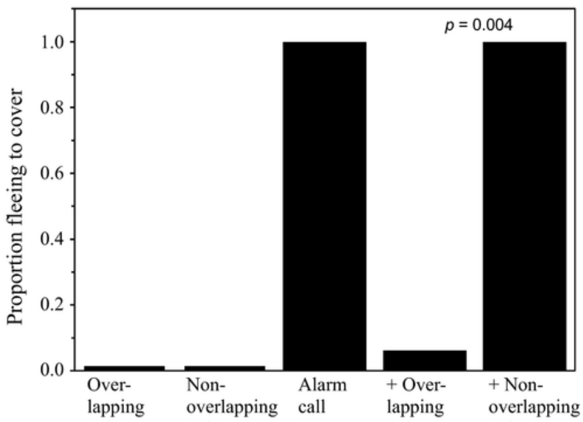

Figure 1 Audio masking effect affecting proportion of fleeing behaviour by wild superb fairy wrens, Malurus cyaneus (Zhou et al., 2019)

1) My predictions regarding the effects on feeding behaviour of great tits are as follows:
   i. Firstly, I predict that the presence of conspecific alarm calls ('Alarm' treatment) is likely to deter great tits from the feeder, as alarm calls serve as an effective warning to conspecifics for threat detection (McLachlan & Magrath, 2020), resulting in a shorter time spent at the feeder and a lower seed consumption rate.
   ii. Secondly, I predict that great tits will spend less time at the feeder and consume fewer seeds at feeders placed in noisy locations compared to quiet locations. A previous study by Klett-Mingo et al. (2016) found that feeding bouts of great tits were shorter during peak noise periods because the birds were likely to experience increased stress and reduced auditory perception due to audio masking. In addition, as the amplitude of background sound increases, acoustic communication tends to become more challenging for many species which is consistent with the concept of masking (Zhou et al., 2019). Therefore, I predict that this negative effect on feeding behaviour will be exacerbated in the presence of experimentally induced anthropogenic noise ('Anthro' treatment) due to

additional stressors that may result from an added noise effect. Hence, auditory cues necessary for predator detection may be further masked.

iii. Finally, in audio treatments with both added anthropogenic noise and alarm calls ('AnthroAlarm' treatment), I predict that audio masking of conspecific alarm calls by anthropogenic noise may result in a longer time spent at the feeder as compared to treatments with only alarm or only anthropogenic noise as the perceived predation risk may be reduced.

2) My predictions regarding the effects on vigilance behaviour in great tits are as follows:
   i. Firstly, I predict that the presence of conspecific alarm calls ('Alarm' treatment) is likely to elicit more vigilant behaviour in great tits, given that these calls serve as an effective warning for detecting potential threats detection (McLachlan & Magrath, 2020).
   ii. Secondly, I predict that great tits will display a higher vigilance at feeders placed in noisy locations compared to quiet locations. Klett-Mingo et al. (2016) showed that during peak noise levels, the proportion of time that great tits devote to vigilance was at its highest, and the duration of vigilance episodes were strongly correlated with noise levels. Moreover, I predict that the effect of increased vigilance by great tits in noisier locations will be exacerbated in the presence of experimentally induced anthropogenic noise ('Anthro' treatment) due to additional stressors that may result from an added noise effect .
   iii. Finally, in audio treatments with both added anthropogenic noise and alarm calls ('AnthroAlarm' treatment), I predict that audio masking of conspecific alarm calls by anthropogenic noise may result in a reduction of vigilance behaviour as compared to treatments with only alarm or only anthropogenic noise as the perceived predation risk may be reduced.

3) My predictions regarding the effects on the visitation rate of great tits are as follows:
   i. Firstly, I predict that the presence of conspecific alarm calls ('Alarm' treatment) is likely to deter feeder visitation by great tits, given that these calls serve as an effective warning for detecting potential threats detection and this prompts fleeing behaviour (Kalb et al., 2019; McLachlan & Magrath, 2020)
   ii. Secondly, I predict that great tits will frequent feeders in quiet locations more than feeders in noisy locations. As suggested by Halfwerk and Slabbekoorn (2013), noise pollution can act as a distraction and mask important auditory cues that can affect food-finding success. Hence, great tits might prefer to frequent feeders placed in quiet locations as auditory cues could be better perceived there. Additionally, due to the abovementioned mechanism and negative effects of noise, I predict that visitation rate of great tits to feeders will be low in the presence of experimentally induced alarm treatments ('Alarm' treatments) and experimentally indued anthropogenic treatments ('Anthro' treatments) as compared to control treatments.
   iii. Finally, in audio treatments with both added anthropogenic noise and alarm calls ('AnthroAlarm' treatment), I predict that audio masking of conspecific alarm calls by anthropogenic noise may result in a higher visitation rate as compared to treatments with only alarm or only anthropogenic noise as the perceived predation risk may be reduced from the reduced ability to detect auditory cues due to sensory degradation in great tits by anthropogenic noise (Shannon et al., 2016).

2 Methodology

2.1 Study species

The great tit (*Parus major*) is a well-adapted urban passerine species that defends established feeding territories and is found in large numbers in both relatively quiet forested areas and noisy urban areas, such as cities and highways (Krebs et al., 1978; Reijnen et al., 1995). As a key species in many behavioural studies, including breeding ecology and performance Artemyev (2008), great tits offer a diverse sample for studying the effects of anthropogenic noise pollution on anti-predator behaviour. Additionally, their preference for roosting in artificial nest boxes over natural cavities makes them easier to measure and monitor, as compared to many other species (Dulisz et al., 2021; Halfwerk & Slabbekoorn, 2013). Hence, they are an ideal candidate species chosen for this study.

Figure 2 Photo of great tit on branch (Canva)

2.2 Study site

The foraging behaviour of great tits was studied on the Wageningen University campus using a 2 x 2 x 2 experimental design (**Table 1**). The study investigated the effects of experimentally added conspecific alarm calls (presence vs. absence), experimentally-added anthropogenic noise (presence vs. absence), and feeder location (quiet vs. noisy) on the bird's foraging behaviour. A total of 20 bird feeders were used in the study, with 10 bird feeders placed in quieter locations (labelled k-t) and 10 feeders placed in noisier locations (labelled a-j) (**Figure 2**). To determine feeder locations, data that was previously collected by Pot (2022) was utilised, in which various audio recordings of noise levels were taken at different distances from road. He found that there was a positive relation between distances to road and the average amplitude of recordings, indicating loudness. Therefore, based on these findings, feeders that were placed in noisier locations were placed closer to roads whereas feeders that were placed in quieter locations were placed further away from roads. Furthermore, the presence of main roads were taken into account for the placement of feeders in noisier locations in whereby a higher volume of traffic and thus noise was anticipated.

Table 1 Experimental variables

Dependent variables	Independent variables	Options	Number	Variable type
• Feeding time • Visitation rate • Vigilance rate • Seed consumption rate	Anthropogenic noise	Yes/No	2	Discrete
	Alarm calls	Yes/No	2	Discrete
	Background noise at feeder site (Feeder site)	Noisy/Quiet	2	Discrete

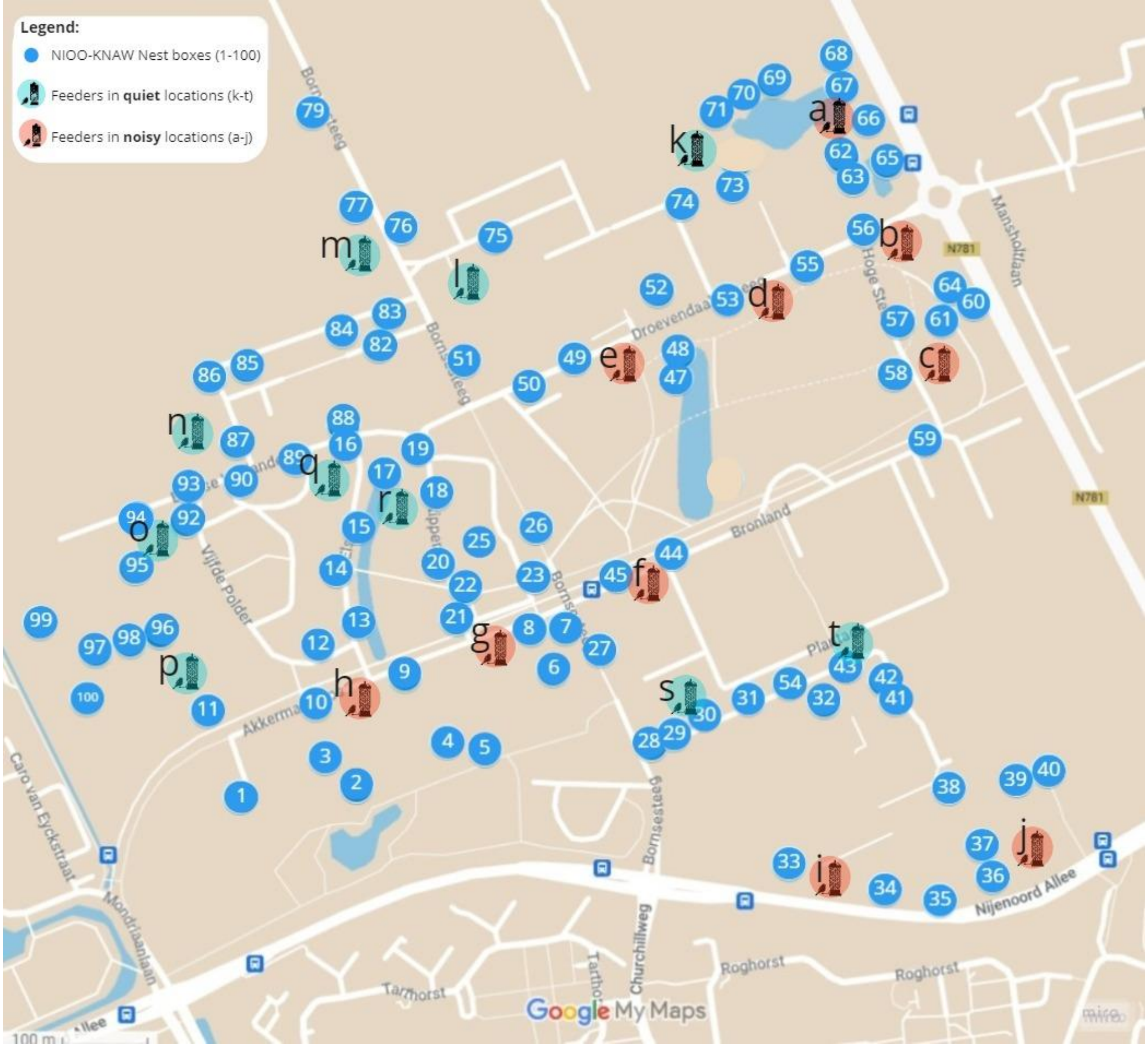

Figure 3 Map of the locations of quiet and noisy feeders on Wageningen University campus. Noisy feeders are highlighted in red and labelled a-j; Quiet feeders are highlighted in blue and are labelled k-t.

2.3 Study design

Following a 2-week habituation period, data (i.e., time spent at feeder, visitation rates) was collected on-site at two bird feeders (one at a quiet site and one at a noisy site) for a total of 120 minutes per day at sunrise during winter months, November-January. Each day, the two bird feeders were selected at random. At each of bird feeder, a pre-compiled audio treatment consisting of either anthropogenic noise only, alarm calls only, or a combination of both was broadcasted for 5 minutes using a bluetooth speaker that was located 10 meters away from the feeder (Mockford et al., 2011). During the experiment, bird foraging behaviour was recorded using a JVC EverioR Quadproof video camera that is placed 15 meters away from the feeder, enabling further analysis of vigilance rate and seed consumption rate. To collect observational data and to ensure the functionality of the camera, the experimenter was positioned 15 meters away from the feeder, behind the video camera.

2.3.1 Audio treatments

The study included four audio treatments, which are named ‘Alarm’(1), ‘Anthro’(2), ‘AnthroAlarm’(3) and ‘Control’(4). The ‘Anthro’ and ‘AnthroAlarm’ treatments were created by combining anthropogenic sounds and conspecific alarm calls. Anthropogenic sounds recordings were obtained from (*Pixabay*) and ranged from 0-3 kHz. Conspecific alarm calls recordings were obtained from (*Xeno-canto*) and ranged from 4-8kHz. To create the audio treatments, the sounds were compiled together using Audacity (Audacity 3.2.1), a sound editing software. In total, there are 7 different variations of each audio treatment.

During data collection, at each bird feeder, the selected pre-compiled audio treatment was broadcasted for 5 minutes at 68 dB using a MEGABOOM 3 Bluetooth speaker that was located 10 meters away from the feeder (Mockford et al., 2011). The audio treatment was repeated 3 times, with 10-minute breaks between treatments (**Table 2**). To prevent habituation of birds to sound within the audio treatments, new audio treatments were compiled every week. The details of the audio treatments are outlined in Appendix 2. The audio treatments are defined as follows: (1) Alarm– involves the playback of conspecific alarm calls, (2) Anthro– involves the playback of anthropogenic sounds, such traffic noises and bike bells, (3) AnthroAlarm– involves the playback of a combination of conspecific alarm calls and anthropogenic sounds and (4) Control– involves audio treatments with no added playback of conspecific alarms and anthropogenic sounds.

Table 2 Auditory playback set at each feeder site

Auditory playback set	
Quiet site	Noisy site
10 min rest	10 min rest
5 min of audio playback 🔊	5 min of audio playback 🔊
10 min rest	10 min rest
5 min of audio playback 🔊	5 min of audio playback 🔊
10 min rest	10 min rest
5 min of audio playback 🔊	5 min of audio playback 🔊
10 min rest	10 min rest
Total: 15 min of playback + 40 min of rest = 55 min	

2.4 Data analysis

2.4.1 Response variables

The statistical analysis for this study was performed using R studio (RStudio 2022.02.2 Build 485). There are four response variables in the study, which are ‘Time spent at feeder’(5), ‘Visitation rate’(6), ‘Vigilance rate’(7) and ‘Seed consumption rate’(8). These variables are defined as follows: (5) Time spent at feeder– This represents the amount of time that great tits spend at the feeder (in seconds), to perform either foraging or vigilance behaviour, (6) Visitation rate– This is defined by the number of great tit sightings/experimental duration (in minutes), (7) Vigilance rate– This is defined by the number of vigilance counts (represented by the number of head turns)/experimental duration (in minutes) and (8) Seed consumption rate– This is defined as the number of seeds picked up/experimental duration (in minutes).

2.4.2 Model selection
To ensure the validity of the model, normality and homoscedasticity checks were performed on the model using a QQ plot and a residuals scatterplot. If these assumptions were violated, transformation of the response variable was considered. In addition, Cook's distance analysis was performed to identify any influential values in the dataset. The threshold used for identifying influential values was calculated using the chi-squared distribution with a significance level of 0.5. Any observations with a Cook's distance greater than this threshold were considered influential. Further details on the specific R code and threshold values for each research question are outlined in Appendix 4.

A linear mixed model (LMM) was used for statistical analysis. Random effects were applied for feeder identity (Feeder ID) and session to account for multiple observations on the same feeder and to address variation across experimental sessions. The independent variables included were 'Feeder site' and 'Audio Type'. The significance of the independent variables were determined using hypothesis and statistical significance tests. The formulas for the models are as follows:

1) *Time at feeder ~ Feeder site * Audio Type + (1|Feeder ID) + (1| Session)*
2) *Visitation rate ~ Feeder site * Audio Type + (1 | Feeder_ID)*
3) *Log_Vigilance_rate ~ Feeder_site * Audio_Type + (1 | Feeder_ID) + (1 | Session)*
4) Seed consumption rate ~ Feeder site * Audio Type *+ (1 | Feeder_ID) + (1 | Session)*

Additionally, log transformation was performed for the response variable 'Vigilance rate' to improve linearity, and the data set was filtered to remove 0 values. This removes 'pick and go' behaviour, whereby birds land briefly on the feeder to pick up a seed and immediately flee the feeder, without looking around or exhibiting any vigilance behaviour (resulting in '0' vigilant behaviour in terms of vigilance counts). Hence, removing 0 values creates a better representation of vigilant behaviour in the dataset, as it focuses on vigilant counts using the number of head turns observed.

2.4.3 Post hoc analysis
Post hoc analysis was conducted to investigate if there were any significant effects within the audio treatments, using the emmeans() and pairs() function in R studio, with Tukey's adjustment to correct for multiple comparisons.

3 Results

3.1 Ambient noise and its interaction on the response of great tits to audio treatments

There was no interaction found between ambient noise and audio treatments for the time spent at feeder (χ^2=5.48, df=3, p=0.14), seed consumption rates (χ^2=1.71, df=3, p=0.43), vigilance rates (χ^2=1.78, df=3, p=0.41) and visitation rates (χ^2=1.22, df=3, p=0.75).

3.2 Responses to experimental audio treatments

Results from the experimental audio treatments showed no significant effect on the time spent at feeder (χ^2=0.11, df=3, p=0.99, Figure 4), seed consumption rates (χ^2=1.03, df=3, p=0.79, Figure 5) and vigilance rates (χ^2=7.70, df=3, p=0.053, Figure 6). Therefore, it appears that great tits were not affected by the type of audio being broadcasted for these response variables. However, there was a significant effect of audio treatments on the visitation rates of great tits (χ^2=10.90, df=3, p=0.01, Figure 7), indicating that the type of audio broadcasted affected their visitation behaviour.

Additionally, post hoc analyses revealed a significant difference in visitation rates between 'Anthro' and 'AnthroAlarm' treatment (estimate = -0.4865, SE = 0.155, df = 43.2, t-ratio = -3.134, p-value = 0.0158).

3.3 Influence of ambient noise levels on the behaviour of great tits

In the context of ambient noise levels, results indicated that great tits did not exhibit significant differences in behaviour between noisy and quiet locations. Specifically, there was no evidence that ambient noise levels affected the time spent at the feeder (χ^2= 2.14, df=1, p=0.14, Figure 4), seed consumption rates (χ^2=0.11, df=1, p=0.74, Figure 5), vigilance rates (χ^2=0.17, df=1, p=0.68, Figure 6) and visitation rates (χ^2=0.27, df=1, p=0.60, Figure 7). These results suggests that great tits do not spend less time at the feeder or consume less seeds in noisy locations compared to quiet locations. They also indicate that great tits did not display increased vigilance in noisy locations compared to quiet locations. Finally, the findings suggests that great tits do not frequent feeders that are placed in quiet locations more than in noisy locations.

3.4 Influential observations

Cook's distance analysis was performed on all response variables in the study, and no influential observations were found, indicating that none of the observations exerted a significant influence on the statistical model.

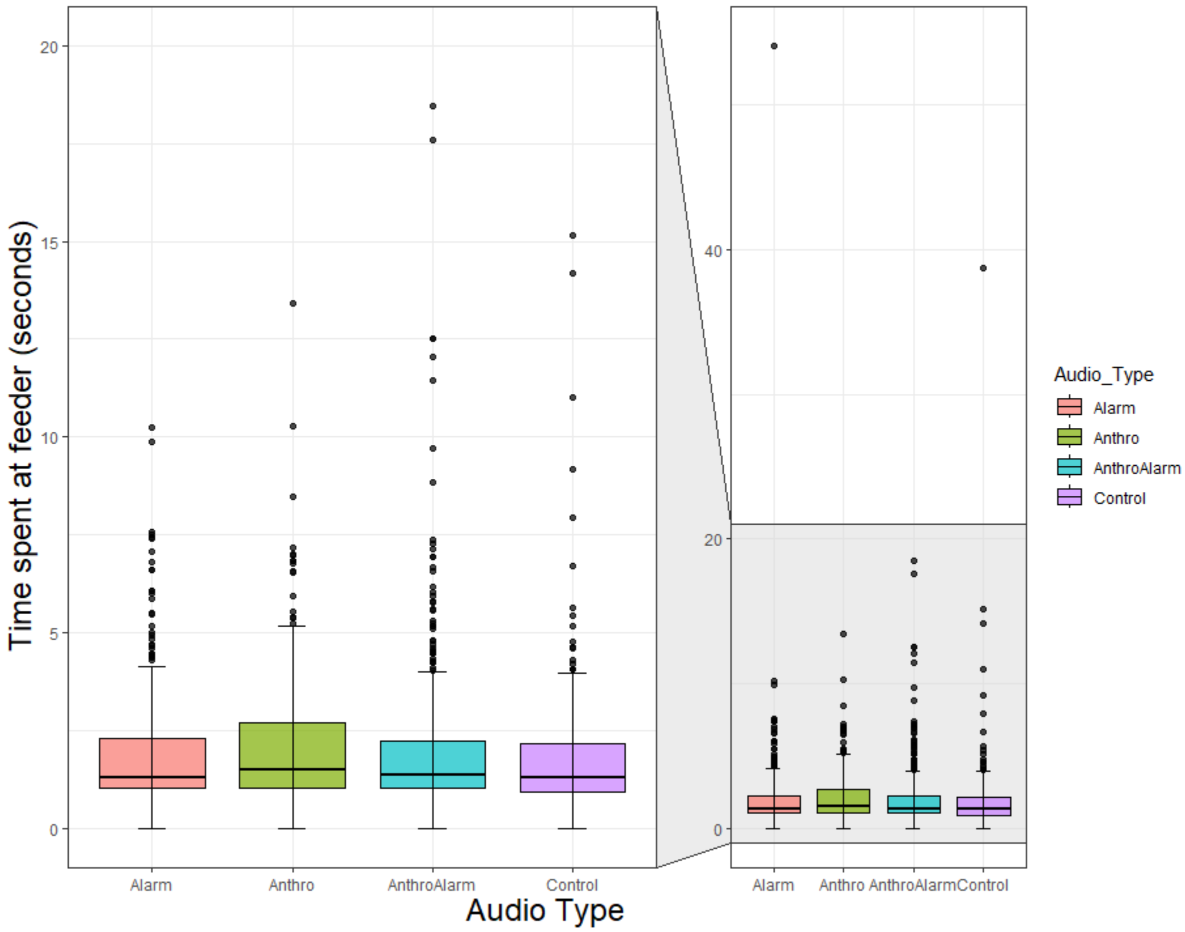

Figure 4 This figure demonstrated that the time spent at feeder by great tits across the different audio types was similar, with a median ranging between 1 and 2 seconds. Only two extreme outliers were observed, but the remaining data were scattered below under 20 seconds. After conducting an ANOVA, the effect of feeder site was found to be non-significant (χ^2=2.14, df=1, p=0.14). This indicates that whether a feeder site was noisy or not did not influence the time spent by great tits at the feeder.

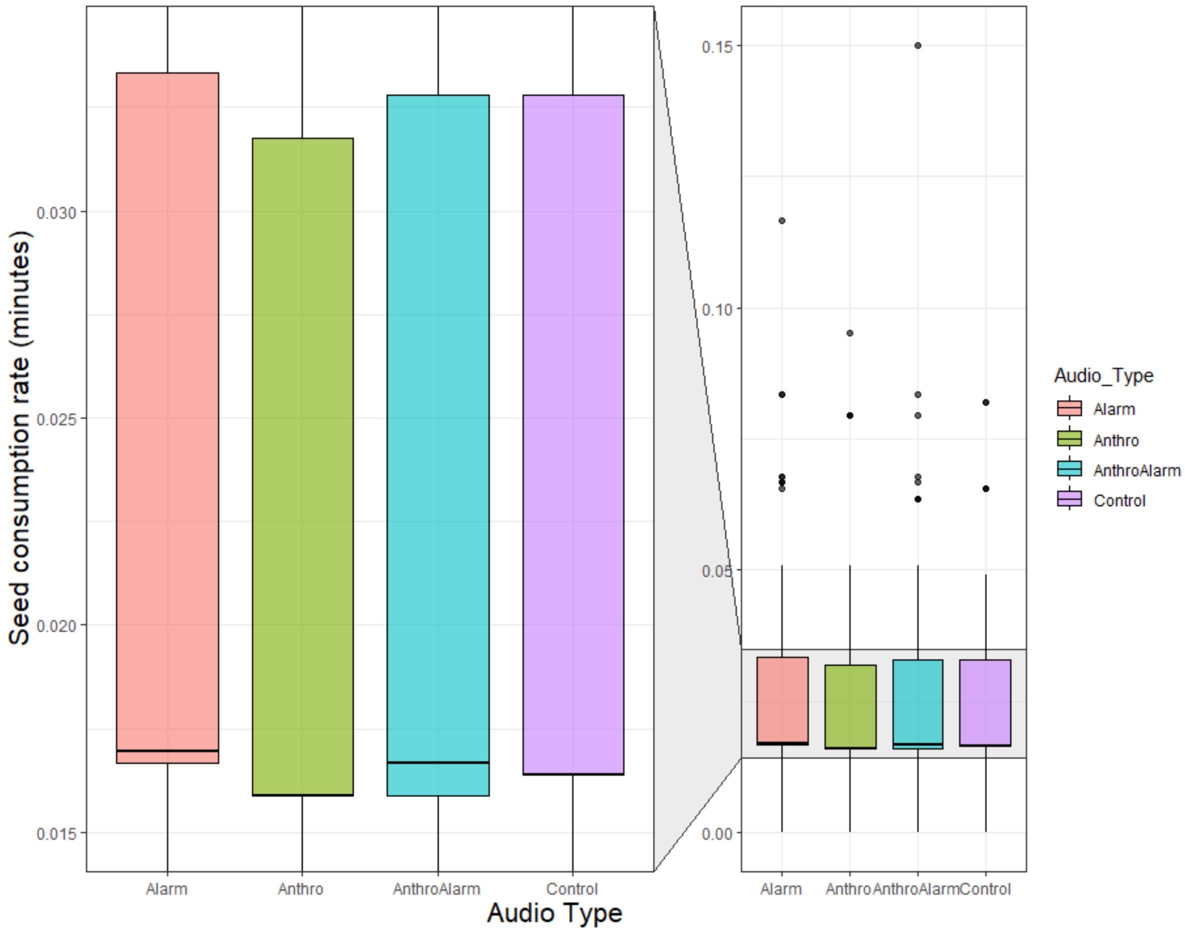

Figure 5 Seed consumption rates appear to be undifferentiated across the different audio treatments with medians clustered around 0.016-0.017 seeds per minute. After conducting an ANOVA, the effect of feeder site was found to be non-significant (χ2=0.11, df=1, p=0.73). This indicates that whether a feeder site was noisy or not did not influence the seed consumption rates of great tits at the feeder.

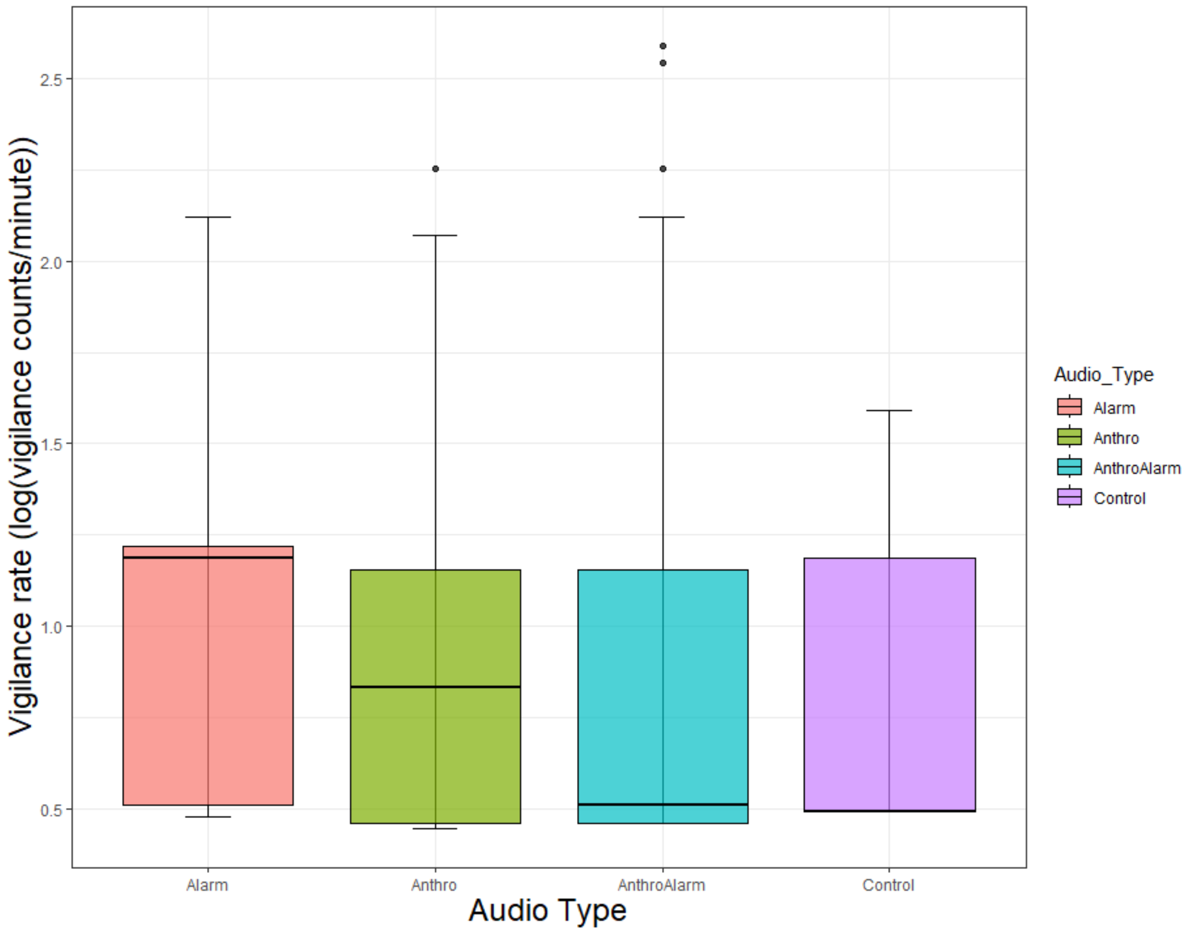

Figure 6 indicates that the medians of the log transformed vigilance rates for 'Alarm' treatment were higher than those for 'Anthro', 'AnthroAlarm' and 'Control', at 1.2 log(vigilant counts/minute), as compared to 0.7, 0.55 and 0.5 log(vigilant counts/min) respectively. After conducting an ANOVA, the effect of feeder site was found to be non-significant (χ^2=0.17, df=1, p=0.67). This indicates that whether a feeder site was noisy or not did not influence the vigilance rates of great tits at the feeder. .

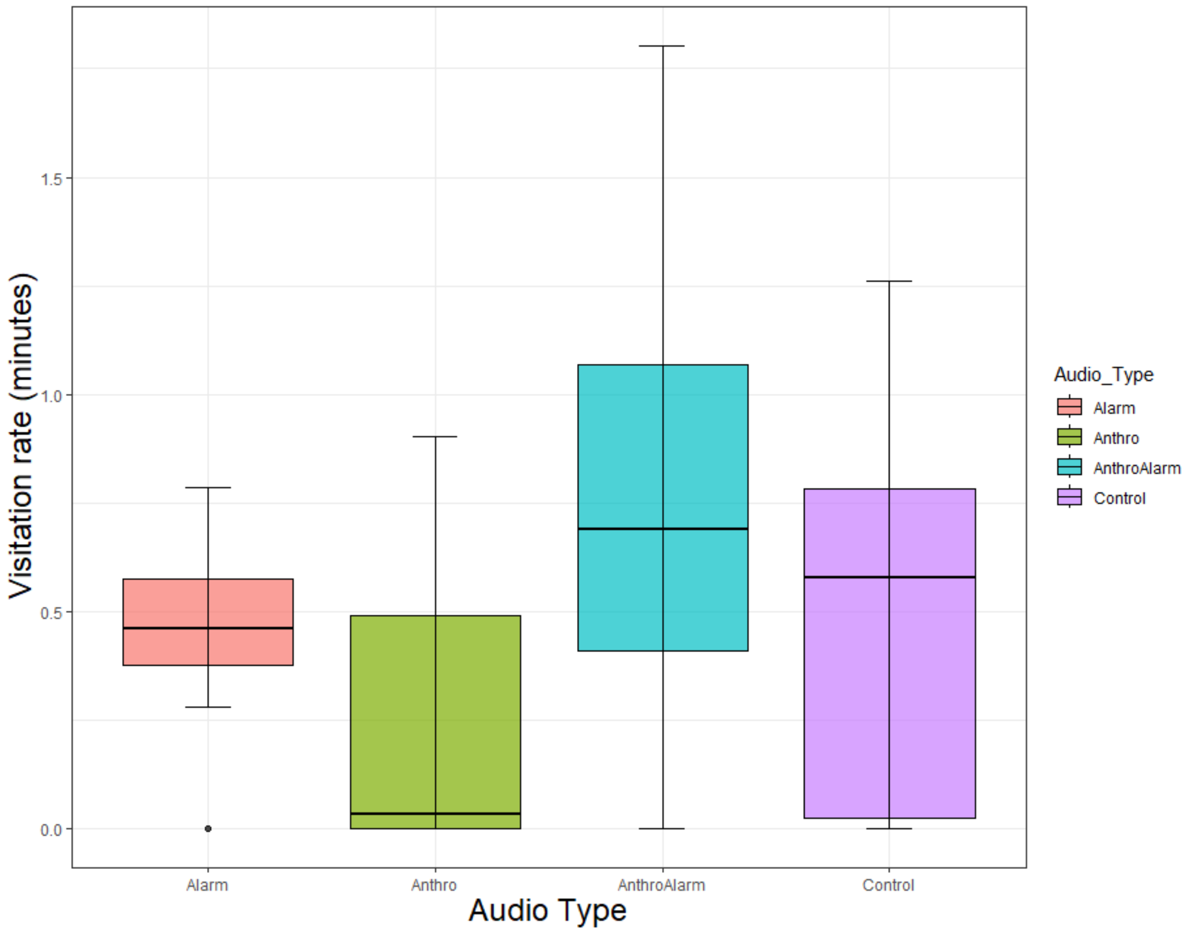

Figure 7 showed that 'Anthro' treatment results in the lowest visitation rate (0.05 sightings per minute), followed by 'Alarm' treatment (0.45 sightings per minute), 'Control' treatment (0.6 sightings per minute) and 'AnthroAlarm' treatment resulting in the highest visitation rate (0.7 sightings per minute). After conducting an ANOVA, the effect of feeder site was found to be non-significant (χ^2=0.27, df=1, p=0.60). This indicates that whether a feeder site was noisy or not did not influence the visitation rate of great tits.

4 Discussion

The aim of the study was to investigate the effects of experimentally induced anthropogenic noise pollution and audio masking of conspecific alarm calls on anti-predator behaviours of great tits such as feeding behaviour, vigilance and visitation rate of feeders in both noisy and quiet feeder locations. My hypothesis was that the audio masking of conspecific alarm calls from anthropogenic noise pollution, which is a frequently cited mechanism for explaining how noise compromises communication (Zhou et al., 2019), may result in a reduction of anti-predator behavioural responses due to decreased awareness of predation risk. The results showed that audio type had a significant effect on the visitation rate of great tits with an increased visitation rate for 'AnthroAlarm' treatment compared to 'Anthro' treatment, indicating that great tits respond differently to alarm calls with the inclusion of anthropogenic noise in their feeder visitation rates which suggests an anthropogenic noise masking effect on the response of feeder visitation rate by great tits. However, the study did not find support for a more pronounced audio masking effect in noisy locations as there were no significant findings for the time spent at feeder, vigilance rates and seed consumption rates across audio types for noisy and quiet locations. These findings suggest that audio masking can influence the anti-predator behaviours of great tits but no difference in effect was observed on their behaviour in different noise environments.

4.1 ***Lack of interaction between ambient noise and audio treatments***

The results of this study revealed that there was no significant interaction between ambient noise and experimental audio treatments on the response of great tits. This finding suggests that the response of great tits to the broadcasted experimental audio treatments was not influenced by the levels of ambient noise. However, this lack of interaction is contrary to previous studies that have reported that a significant interaction between ambient noise and audio treatments, in the form of traffic noise, which can impact the response of birds to acoustic signals in their production and perception of alarm calls (Templeton et al., 2016). Templeton et al. (2016) found that the exposure of great tits to experimental noise manipulation in laboratory conditions resulted in an increase in the amplitude of their mobbing alarm calls, but audio playback experiments conducted in the field resulted in masking effects by road traffic noise on alarm calls that obstructed the ability of great tits to perceive critical auditory signals.

A possible explanation for the lack of interaction between ambient noise and audio treatments was the differences in study design such as the use of laboratory or field conditions. Laboratory studies provide more controlled and standardised conditions which makes it easier to detect significant interactions between variables. On the other hand, field studies are much more complex and variable, which makes it difficult to detect significant effects. This was also observed in the study of Kleist et al. (2016) who found that the quality of noise in the field can significantly differ from laboratory-based or noise-simulated scenarios in field scenarios, which may affect experimental results. The comparisons of the results of this study to those of Templeton et al. (2016) revealed that neither studies found a significant interaction between ambient noise and experimental audio treatments in the field. This could suggest in field and real-life circumstances, the effects of ambient noise on the behaviour of birds may be less pronounced or more variable than in laboratory conditions. In addition, it is possible that the experimental design and audio treatments in both studies were not strong enough to elicit an interaction effect in the field. As a result, further research is required to draw conclusions about the interaction effects of ambient noise and audio treatments in field conditions.

4.2 ***Effects of experimental audio treatments on visitation rates***

The results of this study found that the type of audio broadcasted had a significant effect on the visitation rates of great tits. Specifically, audio treatments with both added anthropogenic noise and alarm calls (‘AnthroAlarm’ treatment) led to a lower visitation rate as compared to audio treatments with added anthropogenic noise (‘Anthro’ treatment). This finding suggests that great tits respond differently to different types of audio broadcasted, and that certain sounds may result in feeder deterrence.

4.2.1 Anthropogenic noise disrupts great tit visitation rates
It was predicted that the visitation rate of great tits to feeders will be lower in the presence of experimentally induced anthropogenic treatments (‘Anthro’ treatments) as compared to control treatments.

While the effects of experimentally induced anthropogenic noise were not statistically significant, post-hoc analysis revealed a possible trend suggesting that ‘Anthro’ treatments resulted in lower visitation rates as compared to ‘Control’ treatments (estimated mean difference = - 0.2646). This could be attributed to the negative associations that great tits have formed with anthropogenic noise in urban environments. Loud and sudden anthropogenic noises can startle birds, causing them to flee from feeding thus potentially reducing their foraging effort and fitness (Klett-Mingo et al., 2016). In addition, anthropogenic noises are often associated with strong negative experiences such as near-collisions, which can make great tits more adverse to such sounds (Lima et al., 2015). Due to the lack of statistical significances in the results, further research for a better understanding of the effects of anthropogenic noise on great tit visitation rates is needed.

4.2.2 Understanding the role of audio masking in avian communication
In audio treatments with both added anthropogenic noise and alarm calls (‘AnthroAlarm’ treatment), it was predicted that audio masking of conspecific alarm calls by anthropogenic noise may result in a higher visitation rate as compared to treatments with only alarm or only anthropogenic noise.

The results showed that the visitation rate was significantly higher in audio treatments with both added anthropogenic noise and alarm calls (‘AnthroAlarm’ treatment) when compared to audio treatments with only added anthropogenic noise (‘Anthro’ treatment) (estimate = - 0. 49), which could be attributed to an audio masking effect, in which anthropogenic sounds from the ‘Anthro’ component masks the ‘Alarm’ component, resulting in a lower risk perception and an increased exploration of the feeder by the birds. The results of my study aligns with the findings of Templeton et al. (2016), whereby a similar audio masking effect was found from anthropogenic traffic noises which significantly reduced the anti-predator responses of wild great tits to the audio playback of conspecific alarm calls. In addition, the combination of anthropogenic sounds and alarm calls in ‘AnthroAlarm’ treatments could have resulted in a complex and ambiguous soundscape, which may have initially attracted great tits to visit the feeder and eventually compelled them to feed due to the provision of an abundant and easily accessible source of food. This could be a plausible explanation for the higher visitation rates observed in ‘AnthroAlarm’ treatments, compared to ‘Anthro’ treatments.

For the interpretation of the audio masking effect shown results in this study, it is vital to consider two sides of the same coin. On one hand, a higher visitation rate in audio treatments with both added anthropogenic noise and alarm calls (‘AnthroAlarm’ treatments) suggests that great tits might be adapting to the presence of anthropogenic noise, and therefore increasing their feeding efficiency. This is particularly important in winter, where food availability are scarce. On the other hand, an audio masking effect might suggest that great tits are less able to perceive auditory cues due to the masking effect of anthropogenic noise which could increase their risk of predation. Therefore, further research is needed to understand the potential implications of audio masking effect on avian communication and fitness.

4.2.3 Importance of frequency overlap in audio masking
Another important factor to consider when investigating the impact of anthropogenic noise masking on bird vocalization is the overlap of frequency range between the anthropogenic noise and the acoustic signals (Brumm & Slabbekoorn, 2005). The extent of this overlap determines the amount of interference caused by masking (Slabbekoorn, 2013). Therefore, it is necessary to understand the extend of overlap to adequately assess the effects of anthropogenic noise on avian communication.

4.2.4 Variable effects of anthropogenic noise masking on bird calls
Bird vocalisations occur in a range of frequencies that conveys varying levels of information and urgency (Verboom, 2018). Anthropogenic noise, on the other hand, is biased towards lower frequencies which can mask lower frequency vocalisations, such as song types and begging calls (Halfwerk and Slabbekoorn (2013); (Pohl et al., 2012). Although my results suggest an audio masking effect on the visitation rates of great tits, it is important to note that they do not fully represent the entire range of bird calls nor do they account for the potential of anthropogenic noise to mask bird vocalisations. These findings suggests that great tits and other urban birds perceive and respond to different types of audio stimuli in complex ways and the potential impacts of environmental factors on their behaviour.

4.3 Effects of experimental audio treatments on vigilance and feeding behaviour

It was predicted that great tits will display increased vigilance in the presence of conspecific alarm calls ('Alarm' treatment) and experimentally induced anthropogenic noise ('Anthro' treatment).

The study found that the type of audio broadcasted did not have a significant effect on the vigilance of great tits. However, post-hoc analysis revealed a trend that shows that treatments with experimentally added alarm calls ('Alarm' treatments) resulted in a higher vigilance rate compared to control treatments (estimate=0.25). This is consistent with previous research indicating the importance of vocal alarm calls in predator vigilance for birds (Goodale & Kotagama, 2008). Additionally, treatments with experimentally added anthropogenic sounds ('Anthro' treatments) resulted in a relatively high vigilance rate compared to control treatments, aligning with previous studies by Merrall and Evans (2020) where it was shown that the exposure of anthropogenic noise resulted in increased vigilance, and that duration of vigilance episodes were strongly correlated with noise levels (Klett-Mingo et al., 2016). Although the difference in vigilance rate between the audio treatments was not statistically significant ($p=0.052$), the observed trends are notable and calls for additional research with a larger sample size to further confirm the findings to potentially give a greater insight of the effects of anthropogenic noise and audio masking on bird behaviour.

It was also predicted that the presence of conspecific alarm calls ('Alarm' treatment) and experimentally induced anthropogenic noise ('Anthro' treatment) will result in a shorter time spent at the feeder and a lower seed consumption rate.

Contrary to the hypothesis, the results of my study did not demonstrate a significant effect of audio type on the time spent at feeder or the seed consumption rate of great tits. Great tits spent a similar amount of time at the feeder and had a comparable seed consumption rate across different audio treatments. The lack of significances found in the study could be explained by various factors.

Feeders play a crucial role in determining important life history traits such as survivorship, phenology and fecundity of birds (Tryjanowski et al., 2016). In addition, studies have shown urban birds have a greater inclination towards neophilia[9] and can readily exploit unpredictable food resources present in urban environments that offer superabundant food sources, such as in feeders. Therefore, the tendency of urban great tits towards exploration and neophilia may have facilitated the exploitation of the novel food source (Tryjanowski et al., 2016). Hence, the audio treatments may not have had a significant impact on the feeding behaviour of great tits as they were highly motivated to feed from provisioned feeders during the winter months. In addition, to maximize fitness, individuals must accurately assess local predation risks and engage in foraging behaviours that optimize energy intake (Abdulwahab et al., 2019). Great tits may have prioritised feeding based on their risk assessment of experimental audio treatments, which did not pose any physical threats. Lastly, the effects of anthropogenic noise and alarm calls on ecological interactions among species can sometimes result in unexpected shifts which causes some species to become more abundant in noisy environments (Halfwerk & Slabbekoorn, 2013). Although the results of my study do not suggest a significant impact of experimentally introduced anthropogenic noise and alarm calls on the feeding behaviour of great tit*,* further studies with clearer distinctions of noise locations may be needed to confirm this finding.

4.4 Negligible impact of ambient noise on great tit behaviour

It was predicted that great tits will spend less time at the feeder and consume fewer seeds in feeders placed in quiet locations more than feeders placed in noisy locations. It was also predicted that great tits will display a higher vigilance, and frequent feeders placed in quiet locations more than in noisy locations.

The results of this study did not reveal any significant effect of ambient noise on the behaviour of great tits. Specifically, the time spent at feeder, seed consumption rates, vigilance rates and visitation rate of great tits were similar between noisy and quiet feeders. These findings imply that the impact of ambient noise on urban bird behaviour may be less significant than hypothesised. However, the lack of a significant difference between the two noise types could also be explained by a methodology flaw, specifically on the inadequate distinction of noise levels between noisy and quiet feeder sites. As both types of sites were present in an urban area, on the Wageningen campus, the difference in ambient noise level between the two types of sites might not be substantial enough to detect a significant interaction effect between feeder sites and audio types. Hence, further research with clearer noise distinctions could be needed to confirm this finding.

4.5 Implications for conservation and management

As urbanisation and its associated noise pollution continue to rise, it is becoming increasingly important to manage noise pollution in order to reduce the reduce the negative effects of anthropogenic noise on wildlife.

This study found that anthropogenic noise disrupts great tit visitation rates, affecting foraging efficiency and fitness, and that audio masking may have negative effects on great tits, such as an increased risk of predation. Therefore, conservation and management efforts should focus on the reduction of noise pollution in urban areas. A commonly suggested mitigation method is the installation of noise barriers, which have been implemented along roads to reduce noise levels for wildlife (Shannon et al., 2016). However, one of the drawbacks of noise barriers is that they can restrict wildlife movement, leading to further fragmentation of their habitats. Furthermore, barriers can be costly to install and maintain, and there may be less expensive noise mitigation strategies that have fewer costs for wildlife (Summers et al., 2011). Proposed alternatives for reducing traffic noise pollution include the limitation of traffic flow during sensitive life-history periods, lowering vehicle speeds, and improving road surface substates such that more noise and impact can be absorbed from vehicles (Shannon et al., 2016). Habitat management such as the use of green bridges can create a natural barrier to noise pollution, and mitigate negative effects of audio masking for sensitive species (Proppe et al., 2013). Songbirds are especially vulnerable to anthropogenic noise because communicate through acoustic signals (Proppe et al., 2013). Therefore, reducing noise pollution may improve habitat suitability for many songbird species, particularly those with songs that include lower-frequency songs, as anthropogenic noise has been suggested to reduce the density and reproductive success of bird species with lower frequency vocalisations (Proppe et al., 2013).

However, it is important to note that many noise mitigation methods can be costly and thus must be ecologically justified and effective (Duquette et al., 2021). As animals have species-specific sensitivities to changes in noise volume across the frequency spectrum, specific noise management guidelines must be developed based on a species' behavioural response to different types of sound (Duquette et al., 2021). Therefore, an updated synthesis of wildlife response to noise pollution, which involves the compilation of research studies on how wildlife respond to various types and levels of anthropogenic noise, would benefit future research and mitigation by providing a current understanding of the impacts of anthropogenic noise pollution on various wildlife and how it affects their behaviour and physiology. This information could be used to develop more effective and targeted noise mitigation strategies tailored to the specific needs of various species (Duquette et al., 2021).

5 Limitations and Recommendations

5.1 Vary amplitudes in audio treatments

As the study focused on a single amplitude, at 68 dB, it may not have fully captured the effects of noise on avian communication. Many bird species have demonstrated the Lombard effect[(12)], in which they adjust their calls by singing louder, longer, or higher in noisy areas to reduce the masking effect of noise (Brumm & Slater, 2006; Slabbekoorn & Peet, 2003). This adaptation, however, may have long-term behavioural consequences that affect predation risk. For instance, Halfwerk and Slabbekoorn (2013) discovered that long-term noise exposures inside of nest boxes had a negative effect on female response behaviour to singing males, which required males to venture out of safer zones to be heard by females, making them more vulnerable to aerial predators. Thus, varying amplitudes within audio treatments is recommended to gain a more comprehensive understanding of the effects of anthropogenic noise on avian communication.

5.2 Anthropogenic noise impacts beyond alarm calls:

The study only focused on alarm calls which encompasses higher frequencies that are between 4-8 kHz, and did not consider other bird calls with lower frequencies, such as begging calls and songs. During the breeding season, birds tend to increase the frequency of their song which may affect mate attractiveness (Gross et al., 2010; Slabbekoorn & Ripmeester, 2008). Hence, urban males may face a trade-off between singing songs that favour female attraction at a lower frequency, or singing songs that favour signal detection at a higher frequency (Gross et al., 2010; Slabbekoorn & Ripmeester, 2008). Future research should take these factors into consideration to better understand the trade-offs that birds face during the adaptation of their acoustic communication.

5.3 Inadequate feeder site locations

The study found no significant effects for the response variables in feeder site, which could be attributed to poorly defined noise locations on the Wageningen campus. To better understand the effects of noise on bird communication, future studies can focus on the experimentally induced audio types at targeted at lower frequency levels to gain a better understanding of the impact of anthropogenic noise on lower frequency vocalisations.

6 Conclusions

This study aimed to examine the effects of experimentally induced anthropogenic noise and conspecific alarm calls on the feeding behaviour, vigilance, and visitation rate of great tits in both noisy and quiet feeder locations. The results show that audio type had a significant effect on the visitation rate of great tits, but there were no significant findings for the time spent at feeder, vigilance rates, and seed consumption rates for feeder sites and audio types. These findings reinforce that great tits may be able to adapt to the presence of anthropogenic noise to some extent, as evidenced by their continued visitation to the feeders. In addition, this study provided experimental support for the audio masking effect of anthropogenic noise on urban wildlife. However, the extrapolation of the findings of this study to other species is dependent on a number of other factors such as the sensitivity of the species to anthropogenic noise, species' vocal frequency range and their behavioural plasticity and responses to noise (Slabbekoorn, 2013). Additional research is necessary to investigate the potential long-term effects of noise on the behaviour and physiology of these birds and other species in the ecosystem. It is also critical to investigate the impact of noise on other bird calls that are used for communication, including mate attraction calls.

In conclusion, the world is unlikely to become quieter (Halfwerk & Slabbekoorn, 2013). As a result, there is an urgent need to understand the potential problems that noise pollution has caused for many bird species and other urban wildlife. This knowledge will be essential for both scientific advancement and raising awareness (Halfwerk & Slabbekoorn, 2013). Overall, this study contributes to our understanding of the potential effects of anthropogenic noise on wildlife behaviour and provides useful information for conservation and management efforts with the hope that an increased understanding can help raise awareness and promote a greater appreciation and respect for natural ambient noise conditions.

7 Glossary

(1) Alarm– involves playback of conspecific alarm calls.
(2) Anthro– involves playback of anthropogenic sounds, such as traffic noises and bike bells.
(3) AnthroAlarm– involves playback of a combination of conspecific alarm calls and anthropogenic sounds.
(4) Control– An audio treatment with no added playback of conspecific alarms and anthropogenic sounds.
(5) Time spent at feeder– This represents the amount of time that *Parus major* spend at the feeder (in seconds), to perform either foraging or vigilance behaviour.
(6) Visitation rate– This is defined by the number of *Parus major* sightings / experimental duration (in minutes).
(7) Vigilance rate– This is defined by the number of vigilance counts (represented by the number of head turns) / experimental duration (in minutes).
(8) Seed consumption rate– This is defined by the number of seeds picked up / experimental duration (in minutes).
(9) 'Pick and go' – This represents a feeding behaviour whereby individuals fly to the feeder, pick a seed and instantly flee the site.
(10) Neophilia– response to novel stimuli with interest
(11) Lombard effect– involuntary tendency of speakers to increase their vocal effort to enhance the audibility of their voice.

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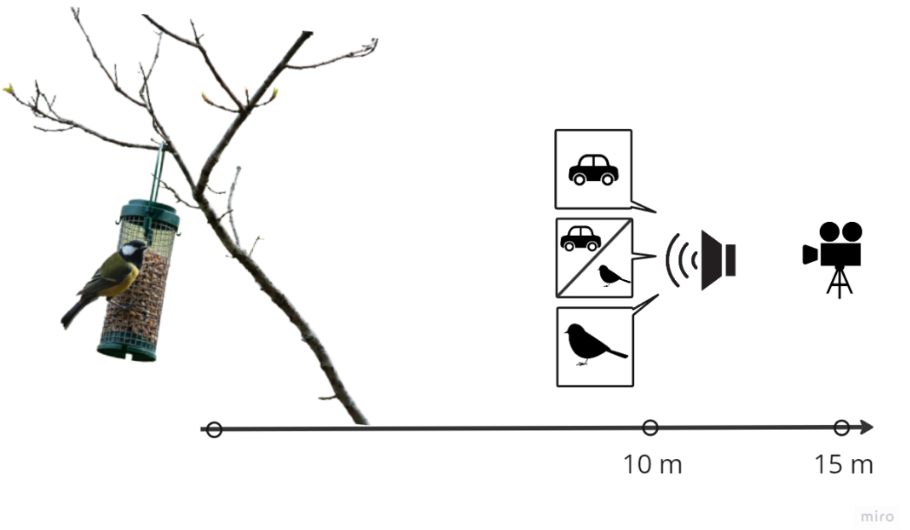
9 List of Appendices

Appendix 1: Conceptual model of experimental set-up.

This appendix describes the experimental set-up in which a pre-selected and compiled audio recording (consisting of either anthropogenic noise only, alarm calls only, or a combination of both) was played for 5 minutes at 68 dB (Mockford et al., 2011) using a MEGABOOM 3 Bluetooth speaker that was located 10 meters away from the feeder. The audio treatment was repeated 3 times, with 10-minute breaks between treatments. To prevent habituation of birds to sound within the audio treatments, new audio treatments are compiled every week. During the experiment, bird foraging behaviour was recorded using a JVC EverioR Quadproof video camera that is placed 15 meters away from the feeder, enabling further analysis of vigilance rate and seed consumption rate. The experimenter is seated behind the video camera to record observations and ensure the functionality of the video camera during data collection.



Appendix 2: Table of links for audio treatments used during data collection.

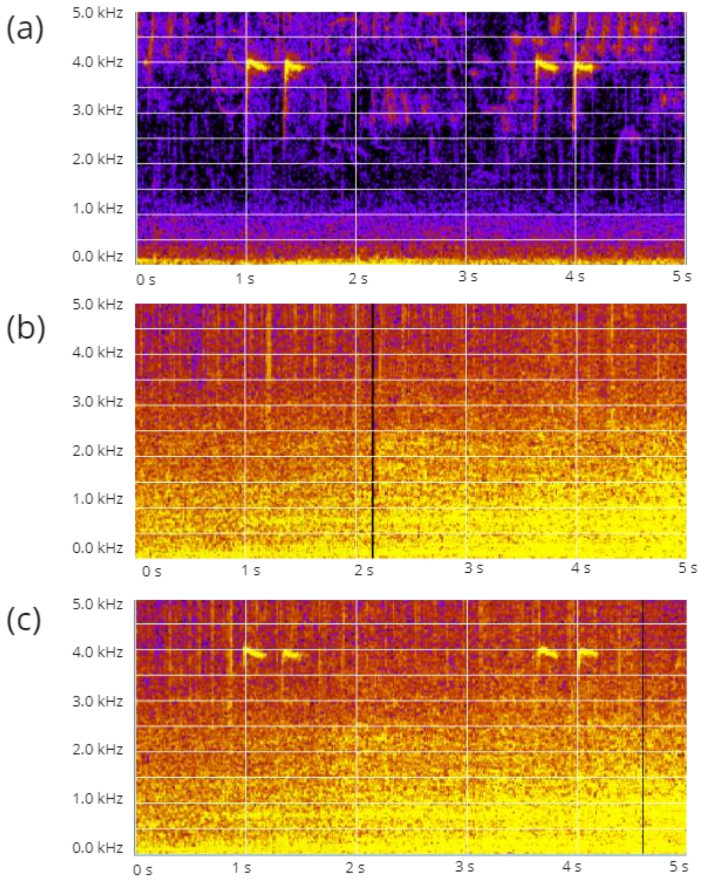
The table in this Appendix contains information on the week of data collection, the type of audio treatment used, and the corresponding link to the audio treatment. This table could be useful for readers who are interested in learning more about the specific audio treatments used in the study, or for those who may want to use the same treatments in their own research.

Week	Type of Audio Treatment	Link
1	Alarm	https://drive.google.com/file/d/1KXAENeC9ZdXkqkOha0YDlm5H3_fOqGra/view?usp=share_link
1	Anthro	https://drive.google.com/file/d/1igwrzBbz15LPOpxDZB6zLQgGe7AoQgUf/view?usp=share_link
1	AnthroAlarm	https://drive.google.com/file/d/1AwmyK69SGLkAQLJFidgdY4lataBWDfgz/view?usp=share_link
2	Alarm	https://drive.google.com/file/d/1scpv9G81P_AUu4UKKikckwGUVsK1APHs/view?usp=share_link
2	Anthro	https://drive.google.com/file/d/1Q63puurzsfqg6YkRDrb7vG5NdGuS4QuI/view?usp=share_link
2	AnthroAlarm	https://drive.google.com/file/d/1gn_UGNlA9yhQ0OmE0cn-Jll9gm18QTyp/view?usp=share_link
3	Alarm	https://drive.google.com/file/d/1Nm497g8HPz0MxkrjtIdWO-3EM4x7_GAF/view?usp=share_link
3	Anthro	https://drive.google.com/file/d/1Nm497g8HPz0MxkrjtIdWO-3EM4x7_GAF/view?usp=share_link
3	AnthroAlarm	https://drive.google.com/file/d/1FTxm9crekqbnFrB_nFyVQFiQrXrFe3Fu/view?usp=share_link
4	Alarm	https://drive.google.com/file/d/1W2bQMWORCfJnU4ZdF9lJgi6XnfTaKziN/view?usp=share_link
4	Anthro	https://drive.google.com/file/d/1W2bQMWORCfJnU4ZdF9lJgi6XnfTaKziN/view?usp=share_link
4	AnthroAlarm	https://drive.google.com/file/d/1R0wo7J9LVa3nbQGeiFS3Djuu9Yy95K83/view?usp=share_link

5	Alarm	https://drive.google.com/file/d/1nglXLOA0MfrBBWufwF8Y-xUw_JHjn2nU/view?usp=share_link
5	Anthro	https://drive.google.com/file/d/1IzpIYVVYrHItZUlRoftyvSCWQsbaDEum/view?usp=share_link
5	AnthroAlarm	https://drive.google.com/file/d/1yM7s3IAjwYyq0Tw0cx0ifdzjDDY4WuLz/view?usp=share_link
6	Alarm	https://drive.google.com/file/d/1lKDE4hYO6HQUX-zEnjH5y4XWrfklU9Vg/view?usp=share_link
6	Anthro	https://drive.google.com/file/d/1kELXON4L0ZcIdpoF_9_MB2DMZHzqeNY7/view?usp=share_link
6	AnthroAlarm	https://drive.google.com/file/d/1EuuSrfB4YPdneUpmcJSvESF42RpFMAd8/view?usp=share_link
7	Alarm	https://drive.google.com/file/d/1EuuSrfB4YPdneUpmcJSvESF42RpFMAd8/view?usp=share_link
7	Anthro	https://drive.google.com/file/d/1y3qEIHikMQjQvCSJaZY0VFfKtz92St03/view?usp=share_link
7	AnthroAlarm	https://drive.google.com/file/d/1AEpaUFVJNGkFDz0t2kvFUGU5PNMwUCLy/view?usp=share_link

Appendix 3: Spectrograph of audio recordings

This appendix presents a sample of spectrographs from audio treatments. Figure (a) represents conspecific alarm calls derived from (*Xeno-canto*), Figure (b) represents anthropogenic noise derived from (Pixabay) and Figure (c) represents a combination of anthropogenic and alarm calls, overlayed in Audacity (Audacity 3.2.1). The spectrographs are presented at 0-5 kHz for 0-5 s at a linear frequency scale and are generated using (Academo online spectrograph) in which the x axis represents time (seconds), the y axis represents frequency (kHz) and the amplitude is presented by brightness. These spectrographs provide a visual representation of the audio recordings used in this study and can serve as a visual representation of audio masking or noise distraction effect.



Appendix 4: R code for post hoc analysis and Cook's distance

Research Question 1 had a sample size of 1515 for the response variable, time spent at feeder. The threshold used for identifying influential values was 3.36 and it was calculated using the chi-squared distribution with a significance level of 0.5.

The following R code was used to carry out the post hoc analysis and Cook's distance for Research Question 1 for the response variable, time spent at feeder:

```
# Research question 1, Post-hoc tests ----
emmeans(model_time_at_feeder, ~ Audio_Type)
pairs(emmeans(model_time_at_feeder, ~ Audio_Type), adjust = "tukey")


# Research question 1, Cook's distance ----
cooks_distance_time <- cooks.distance(model_time_at_feeder, normalized = FALSE)


# Identify influential observations
cooks_cutoff <- qchisq(0.5, df = length(coef(model_time_at_feeder)) + 2)
influential_obs <- which(cooks_distance_time > cooks_cutoff)


# Print the influential observations
cat("Influential observations: ", influential_obs)
```

Research question 1 had a total of 583 observations for the response variable, seed consumption rate. The threshold used for identifying influential values was 3.36 and it was calculated using the chi-squared distribution with a significance level of 0.5.

The following R code was used to carry out the post hoc analysis and Cook's distance for Research Question 1 for the response variable, seed consumption rate:

```
# Research question 1, Post-hoc tests ----
emmeans(model_seeds, ~ Audio_Type)
pairs(emmeans(model_seeds, ~ Audio_Type), adjust = "tukey")

# Research question 1, Cook's distance ----
cooks_distance_time <- cooks.distance(model_seeds, normalized = FALSE)

# Identify influential observations
cooks_cutoff <- qchisq(0.5, df = length(coef(model_seeds)) + 2)
influential_obs <- which(cooks_distance_time > cooks_cutoff)

# Print the influential observations
cat("Influential observations: ", influential_obs)
```

Research question 2 had a total of 330 observations for vigilance rate. The threshold used for identifying influential values was 3.36 and it was calculated using the chi-squared distribution with a significance level of 0.5.

The following R code was used to carry out the post hoc analysis and Cook's distance for Research Question 2 for the response variable, seed consumption rate:

```
# Research question 2, Post-hoc tests ----

emmeans(model_vigilance_rate, ~ Audio_Type)

pairs(emmeans(model_vigilance_rate, ~ Audio_Type), adjust = "tukey")


# Research question 2, Cook's distance ----

cooks_distance_time <- cooks.distance(model_vigilance_rate, normalized = FALSE)


# Identify influential observations

cooks_cutoff <- qchisq(0.5, df = length(coef(model_vigilance_rate)) + 2)

influential_obs <- which(cooks_distance_time > cooks_cutoff)


# Print the influential observations

cat("Influential observations: ", influential_obs)
```

Research Question 3 had a sample size of 49. The threshold used for identifying influential values was 2.37 and it was calculated using the chi-squared distribution with a significance level of 0.5.

The following R code was used to carry out the post hoc analysis and Cook's distance for Research Question 3:

```
# Research question 3, Post-hoc tests ----
emmeans(model_visitation_rate), ~ Audio_Type)
pairs(emmeans(model_visitation_rate, ~ Audio_Type), adjust = "tukey")


# Research question 3, Cook's distance ----
cooks_distance_visit <- cooks.distance(model_visitation_rate, normalized = FALSE)


# Identify influential observations
cooks_cutoff <- qchisq(0.5, df = length(coef(model_visitation_rate)) + 2)
influential_obs <- which(cooks_distance_visit > cooks_cutoff)


# Print the influential observations
cat("Influential observations: ", influential_obs)
```

Appendix 5 Supplementary Table 1.
Linear mixed model summary using Type II Wald Chi-square test for effects of independent variables on the time spent at feeder. Feeder ID and session were added as random effects. Significant effects are reported in bold. Number of observations is n=1515.

Variable	X^2	df	p-value
Feeder site	2.67	1	0.10
Audio Type	0.07	3	0.99
Feeder site*Audio Type	5.48	3	0.14

Appendix 6 Supplementary Table 2.
Linear mixed model summary using Type II Wald Chi-square test for effects of independent variables on the seed consumption rates. Feeder ID and session were added as random effects. Significant effects are reported in bold. Number of observations is n=583.

Variable	X^2	df	p-value
Feeder site	0.09	1	0.76
Audio Type	0.99	3	0.80
Feeder site*Audio Type	1.71	2	0.43

Appendix 7 Supplementary Table 3.

Linear mixed model summary using Type II Wald Chi-square test for effects of independent variables on the vigilance rates. Feeder ID and session were added as random effects. Significant effects are reported in bold. Number of observations is n=330.

Variable	X^2	df	p-value
Feeder site	0.15	1	0.69
Audio Type	6.10	3	0.11
Feeder site*Audio Type	1.78	2	0.41

Appendix 8 Supplementary Table 4.
Linear mixed model summary using Type II Wald Chi-square test for effects of independent variables on the visitation rates. Feeder ID and session were added as random effects. Significant effects are reported in bold. Number of observations is n=49.

Variable	X^2	df	p-value
Feeder site	0.27	1	0.59
Audio Type	**10.46**	**3**	**0.015 ***
Feeder site*Audio Type	1.22	3	0.75

Appendix 9 Supplementary Table 4.
Post-hoc summary using emmeans() and pairs() on audio type after Type II Wald Chi-square test for effects of independent variables on the visitation rates. Significant effects are reported in bold. Number of observations is n=49.

Contrast	Estimate	SE	Df	t.ratio	p.value
Alarm-Anthro	0.21	0.15	43.5	1.35	0.54

Alarm-AnthroAlarm	-0.28	0.15	40.2	-1.89	0.25
Alarm-Control	-0.06	0.17	43.8	-0.33	0.99
Anthro-AnthroAlarm	**-0.49**	**0.16**	**43.2**	**-3.13**	**0.016 ***
Anthro-Control	-0.26	0.16	40.0	-1.67	0.36
AnthroAlarm-Control	0.22	0.16	40.2	1.40	0.51

Appendix 10 Supplementary Table 5.
Cook's distance threshold for each response variable.

Response variable	Cook's Distance Threshold
Time spent at feeder	3.36
Seed consumption rate	3.36
Vigilance rate	3.36
Visitation rate	2.37