Chapter 3

How to best quantify anthropogenic noise exposure? A case study comparing a manual analysis to the bioacoustics index NDSI score in the Tamshiyacu Tahuayo Community Regional Conservation Area

3.0 Abstract

As human expansion continues to occur at unprecedented rates there has been an increase in global environmental noise levels. These higher levels of man-made noise were previously thought to mainly be concentrated in urban settings, however, anthropogenic noise is now also becoming prevalent in rural and remote areas. This chapter provides the first comparison of a manual analysis versus the bioacoustics index, Normalised Difference Soundscape index (NDSI) score, at their ability to catalogue and measure anthropogenic noise disturbance. I catalogued the change in anthropogenic noise levels across the landscape, by comparing the levels of anthropogenic disturbance inside a reserve in Peru to that outside the reserve boundaries including a nearby community and ecotourism lodges. I compared the results of a manually catalogued acoustic analysis method with the NDSI score from the 'soundecology' R package in audio recordings obtained using an Audiomoth. I found that for a finer scale of anthropogenic noise differences the human conducted analysis was more accurate and appropriate for that setting. This was also the first study to alter the abiotic and biotic frequency boundaries for an NDSI index and I postulate that when the frequency boundaries are known it is the superior method of NDSI analysis as it provides a more nuanced look at the soundscape in an area. This chapter highlights the limitations and advantages of both measurement techniques.

3.1 Introduction

Anthropological pressures are triggering ecosystem changes on a global scale which have resulted in global wildlife population declines (Mitchell *et al*., 2020). The five main anthropogenic causes of anthropological pressures linked to biodiversity change are climate change, exploitation of natural resources, pollution, changes in habitat, and introduction of invasive species (Bowler *et al*., 2020). Due to this largescale and rapid change, biodiversity assessments have become an ever more urgent task (Pereira *et al*., 2013). With this need to monitor and quantify this change, conservationists have been searching for cost-effective, easy to use and scalable ways to monitor biodiversity (Anderson, 2018; Mitchell *et al*., 2020). It is also equally important to monitor the pressures that are threatening biodiversity as they can act as the bridge between the socioeconomic driving forces behind the pressures and the biological impacts they cause (Spangenberg, 2007; Jones *et al*., 2013).

In terrestrial habitats this disruption came from industrial pursuits and air, rail and road transportation (Barber *et al*., 2010). While in marine habitats an influx in shipping transportation, oil and gas exploitation and sonar operations contribute to changes in the soundscape (Hildebrand, 2009). This incursion on the natural soundscape by anthropogenic noise can cause physiological stress in animals (Francis and Barber, 2013), can mask acoustic signals (Radford *et al*., 2014), and even cause shifts in habitat use (Rako *et al*., 2012). These impacts are the reason why it is essential to study and monitor anthropogenic noises and their influences on ecosystems and wildlife in order to reach a better understanding of how we can mitigate the effects they are having or a larger biodiversity level (Stem *et al*., 2005; Spangenberg, 2007; Merchant *et al*., 2015; Pieretti and Danovaro, 2020).

Ecoacoustics, the study of environmental sound, has become a popular method for quantifying biodiversity as the common methodologies used in these studies are more cost-efficient and rapid than other more traditional surveys (Bradfer-Lawrence *et al*., 2020). The soundscape (the sounds present at a given place at a given time) is comprised of three elements: the geophony, sounds from natural processes (i.e. rain and wind); the anthrophony, sounds that are produced by humans and man-made machinery; and the biophony, sounds made by non-human animals (Pijanowski *et al*., 2011). The characteristics of the soundscape can reflect changes in landscapes which is why they are being used to assess disturbance impacts on wildlife (Pijanowski *et al*., 2011). Open-source audio recorders that are cheap and can be deployed in the field for weeks on end are now widely available and an increasingly popular method of data collection (Bradfer-Lawrence *et al*., 2019). These audio recordings can be described with acoustic indices which are automatically generated and used as effective measures of biodiversity. These acoustic indices describe the soundscape by using the amplitude and frequency of the audio in recording files. Soundscape approaches to conservation research are increasingly used in the context of landscape-scale problems and across a variety of habitat types, such as assessing species diversity in tropical environments (Mammides *et al*., 2017) and marine ecosystems (Harris *et al*., 2016).

There are many indices that are available to statistically describe the distribution of acoustic information related to the biodiversity in a recording (Mitchell *et al*., 2020). Those most commonly used are those that measure the complexity and richness of ecological communities (Mitchell *et al*., 2020). Others measure specific features of soundscapes like anthropogenic disturbance (Kasten *et al*., 2012) or acoustic dissimilarity (Sueur *et al*., 2008). The indices could replace traditional field collection techniques used to assess disturbance impacts on wildlife, which are labour-intensive and not as effective when working with elusive species (Doser *et al*., 2020). Zwart *et al.* (2014) compares traditional transect surveys with results obtained from bioacoustic recorders and found that recorders are better at detecting nightjars (*Caprimulgus europaeus*). Other studies have compared bioacoustic indices and other methods to identify whether they are suitable alternatives but less research has been conducted on the efficacy of bioacoustics indices to measure pressures on biodiversity (Pieretti and Danovaro, 2020).

This study will take an in-depth look at one soundscape index and compare it to more laborious traditional methods for quantifying levels of anthropogenic noise in a habitat. I focus on the Normalised Difference in Soundscape Index (NDSI) score which computes a ratio between anthropogenic and biological noise by assuming the sounds found in the 1-2 kHz frequency band are solely anthropogenic sounds and the sounds found in the 2-11 kHz band are only biological sounds, the score is given on a scale of -1 to 1(Kasten *et al*., 2012). The NDSI is commonly used in combination with other acoustic indices when conducting rapid species richness and abundance assessments (Bradfer-Lawrence *et al*., 2020). For example, Doser *et al*. (2020) used the NDSI score in conjunction with other common acoustic indices as a time and cost-efficient methodology for an assessment of disturbance impacts on biodiversity in a logged forest in northern Michigan. It is used in combination as most studies utilising bioacoustics indices use all the indices in order to have an encompassing idea of the soundscape in an area. In this chapter I choose to focus singularly on this index because I was solely interested in the amount of anthropogenic noise present in the soundscape and the NDSI index is the only bioacoustics index that focuses on quantifying anthropogenic noise. However, despite the widespread use of the

NDSI index, its comparison to the actual amount of anthropogenic noise present in a file has not been made. This is most likely because the NDSI index provides a ratio of the biological versus anthropogenic noise in a file rather than just purely the amount of anthropogenic noise present.

Here I provide a comprehensive analysis of how effective the NDSI score is at quantifying levels of anthropogenic noise as compared to a traditional manual analysis, using soundscapes recorded in and around a protected area in Peru. This is the first direct comparison of its kind and allows for a direct assessment on the strengths and weaknesses of this index and in what scenarios it is best suited. My first aim is to compare the NDSI score with a traditional manual method for estimating the amount of anthropogenic noise in a soundscape. To test this, I quantified anthropogenic noise across multiple sites using the two methods at the same location and time of day, then tested whether the NDSI score could predict the number of minutes of anthropogenic noise present in a 30-minute sound file. I also compare the mean NDSI score per site to the total amount of anthropogenic noise that was catalogued there, to see if the NDSI score can detect overall presence of anthropogenic noise disturbance over time in an area. My second aim is to see what factors are associated with a higher presence of anthropogenic sounds. I hypothesise that location and time of day will impact the amount of anthropogenic sound, with the highest levels being found in the late morning (due to when people in the local community start their day) and outside of the reserve limits. Lastly, my third aim is to see what factors are driving changes to the NDSI score of a soundscape. I hypothesise that outside the reserve NDSI scores will be lower (indicating higher levels of anthrophony) and that there will be higher NDSI scores in the early morning and late afternoon to reflect bird choruses.

3.2 Methods

3.2.1. Study site

The research was conducted in Área de Conservación Regional Comunal Tamshiyacu Tahuayo reserve and sounding area, located in north-eastern Peru. The reserve was demarked a conservation protected area in 1991 (Newing and Bodmer, 2003), subsistence hunting is regulated and the hunting of primates is illegal (Hurtado-Gonzales and Bodmer, 2004). It is located in the Amazon flood basin and undergoes monomodal flooding. Only one tour operator has built accommodation inside the reserve limits, Amazonia Expeditions. Their main lodge is based outside of the reserve limits and close to the El Chino community, this is where most of their clients stay. This is where the majority of anthropogenic noise in the area comes from as it is the main tourist hub as well as a habitation site. With a concentration of people comes an influx of anthropogenic noises such as motorboats (the only source of transport in the area), construction, and general sounds that come from human presence (such as people talking and music). Inside the reserve the tourist accommodation is much smaller so there is less anthropogenic noise generated than at the main lodge, there is still motor boat traffic as the river that goes through the reserve also serves as another connection point to the main Amazon river. It is also travelled by people who live in the surrounding communities (like El Chino) for the collection of wood and other resources in the reserve which is regulated as this is a communal protection zone.

3.2.2 Experimental procedure

One autonomous sound recorder (Audiomoth, Open Acoustics) was placed near the main feeding tree of 23 pygmy marmoset groups in the dry season. Thirteen of the groups were outside of the reserve and ten were inside the reserve (Figure 3.1). The Audiomoths were placed by pygmy marmoset groups to establish the levels of anthropogenic noise disturbance each group was exposed to; this information will then be used in Chapter 4 to see how the amount and type of anthropogenic noise impacts pygmy marmoset communication. The Audiomoths were programmed to record continuously for 24 hours over a 2-day period in August-September 2019. They were configured to record continuous 30-minute WAV files at a sampling rate of 48 (kHz) with a medium gain.

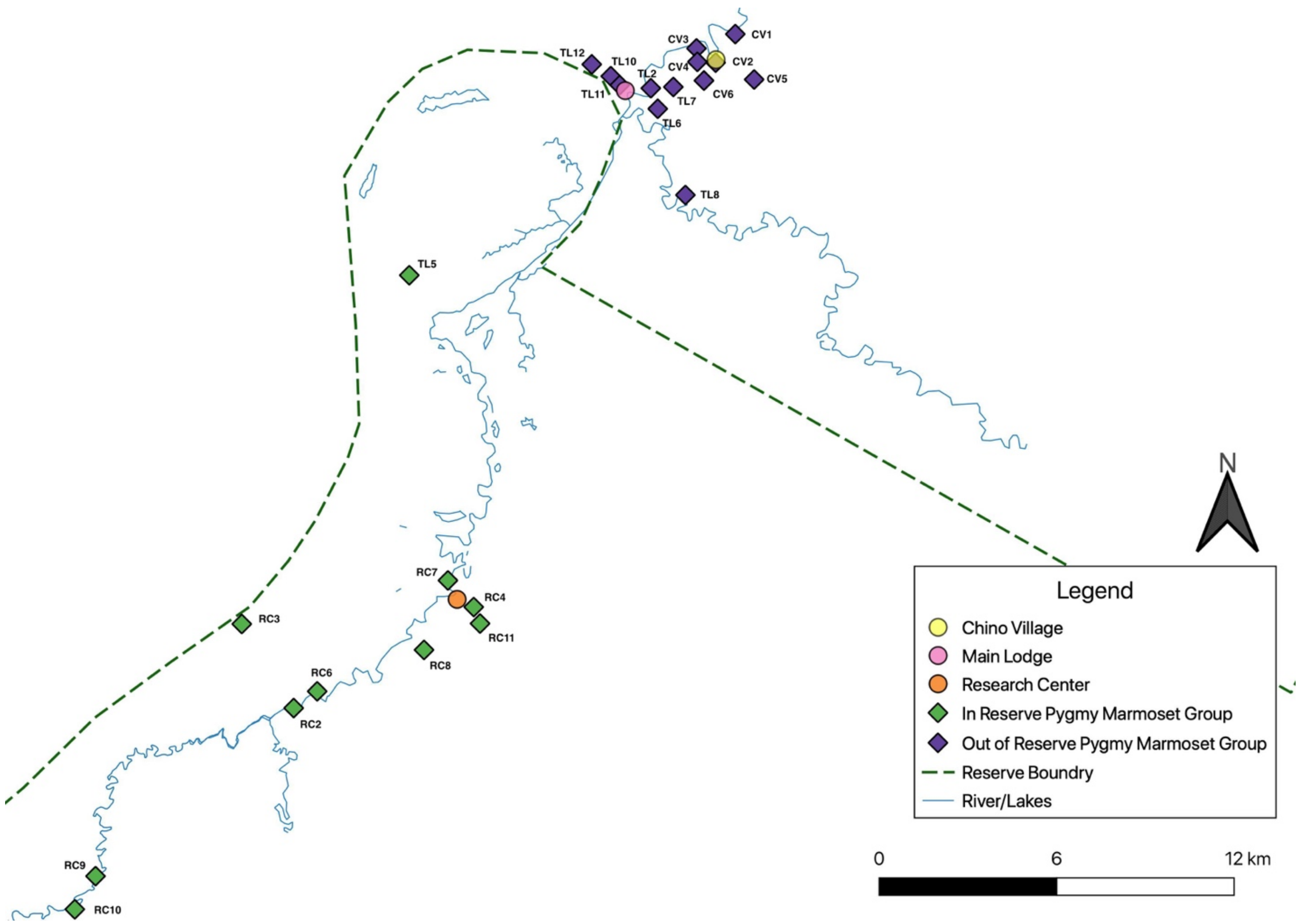

Figure 3.1 A map of the locations of the marmoset groups where the Audiomoths were placed in August-September 2019 with green diamonds denoting a group located inside the reserve and a purple diamond representing a group located outside of the reserve boundary.

3.2.3 Data extraction

3.2.3.1 Manual Analysis for Anthropogenic Noise

Once the data was collected the WAV files were processed in Adobe Audition. Twelve hours (from 05:30 to 17:30) were analysed for anthropogenic noise for each day of recordings, so the total recording time analysed for each location was 24 hours. The thirty-minute WAV files were viewed in Audition, cataloguing any anthropogenic noise recorded (in number of seconds heard).

The anthropogenic noise was categorised as music, talking, motors, and tools and sounds included in these categories are shown in Table 3.1. When heavy rain was encountered in the recording the files were not included in the analysis (n = 105), as heavy rain can skew the results of bioacoustic indices (Sánchez-Giraldo *et al.*, 2020). The files were then separated into different time of day categories with files recorded between 06:30-09:30 being classified as early morning; late morning as 09:30-12:30; early afternoon as 12:30-15:30; late afternoon as 15:30-18:30.

The experiment underwent and was approved by the Royal Holloway's ethical review process as some of these marmoset groups are in the local community, El Chino, and therefore will pick up human conversations. Only the length of conversations was inputted into the dataset not the context of them and the recordings are kept on password-protected hard drives ensuring their privacy. Permission by the landowners was also sought before the audio recorders were placed near their homes.

Table 3.1 Categories of anthropogenic noise.

Anthropogenic Noise category	**Sounds included**
Music	Any music played, both live or over speakers
Talking	Both by people living in Chino and tourists from the ecolodge
High Motor	Motor boats and generators 6 kHz and above
Medium Motor	Motor boats and generators between 1-5 kHz
Low Motor	Motor boats and generators from 0-1 kHz
Tools	Chainsaws, hammering, building, etc.

3.2.3.2 NDSI Score

The NDSI score ranges from 1 to -1, with 1 representing a biologically rich soundscape (low anthropogenic noise, high biological sounds) and -1 representing a human-dominated soundscape (high anthropogenic noise, low biological sounds). The R package 'soundecology' (Villanueva-Rivera *et al.*, 2018) was used to quantify the NDSI score for the recordings used in the manual analysis described above.

The NDSI score is calculated as NDSI = $\frac{\beta-\alpha}{\beta+\alpha}$

β denotes the sum of the 1 kHz binned normalized Welch Power Spectral Density (PSD) (Welch, 1967) from 2–11 kHz and α is the normalized PSD of the 1–2 kHz region (Kasten *et al*., 2012). With 2-11 kHz denoting biological sounds and 1-2 kHz denoting abiotic (anthropogenic) sounds (Kasten *et al*., 2012).

I decided to run the two NDSI analyses, one with unaltered and one with altered parameters as this data is going to be used to infer exposure to anthropogenic noise disturbance levels for different pygmy marmoset groups (Chapter 4). Therefore, I wanted to use the data I collected to inform a set of NDSI parameters. Which is why I expanded the abiotic boundary to reflect the frequencies I had found in my manual analysis and to expand the biotic boundary to encompass the frequencies of the pygmy marmoset calls. The altered parameter frequencies were set to be 0.1 - 2.5 kHz to denote abiotic noise and 2.5 - 23.9 kHz to denote biological sounds.

3.2.4 Statistical Analysis

As the NDSI score is bound on a scale of 1 to -1, I scaled the data according to the following formula, (NDSI +1)/2, in order to be able to run statistical tests (as done in Fairbrass *et al*., 2017; Bradfer-Lawrence *et al*., 2020; Ross *et al*., 2021).

The statistical program RStudio version 1.1.456 (R Core Team, 2020) was used to run a Spearman's rank correlation analysis between the NDSI score calculated for a file versus the total time of anthropogenic noise catalogued in the file. As well as a simplified comparison between the average NDSI score per site and the total amount of anthropogenic noise catalogued at said site.

I ran a generalized linear mixed effects model (GLMM) with a Poisson distribution, as the data was not normally distributed, using the glmmTMB package (Brooks *et al*., 2017), to distinguish if location (in/out of the reserve) and time of day (early and late morning and early and late afternoon) was a predictor for the number of total hours of anthropogenic noise documented using manual analysis. This was then further broken down into a GLMM with a Poisson distribution, as the data was not normally distributed, for total motor sounds and zero inflated GLMMs from the glmmTMB package (Brooks *et al*., 2017) on the specific anthropogenic sound types (low, medium, high motor; tools; talking). The zero inflated GLMMs were used due to the high presence of zeros in these datasets and to fit the assumptions of the model. Music was excluded from the analysis as it contained too many zeros in the dataset for the model to run. I used Bonferroni corrections for the zero inflated GLMMs run on the specific sound types to adjust the alpha value for multiple testing, resulting in 0.05/6 = 0.0083 as the new significance threshold.

A generalized linear mixed effects model with a beta error structure from the glmmADMB package (Fournier *et al*., 2012; Skaug *et al*., 2013) was used to discern if location (in/out of the reserve) and time of day (early and late morning and early and late afternoon) was a predictor for the NDSI score, this was run on both NDSI analyses (unaltered and alerted parameters). The beta error structure was used because the data was not normal and had been transformed.

3.3 Results

A total of 999 files were analysed, 413 from 10 locations inside the reserve and 586 from 13 outside the reserve boundary. At the 10 locations inside the reserve 13 hours of anthropogenic noise was catalogued over 24 hours, versus 157 hours at the 13 locations outside the reserve (Figure 3.2). The location (CV2, Figure 3.1) with the highest total anthropogenic noise catalogued, 31 hours, was in a local community. The location (RC3, Figure 3.1) with the lowest total anthropogenic noise catalogued, 4 minutes, was in the reserve boundary, far from the main river on a trail to an oxbow lake. The most common anthropogenic sound encountered was low motor which across all locations was recorded for 85 hours and the least heard sound was high motor which was only present for 4 hours (Figure 3.2).

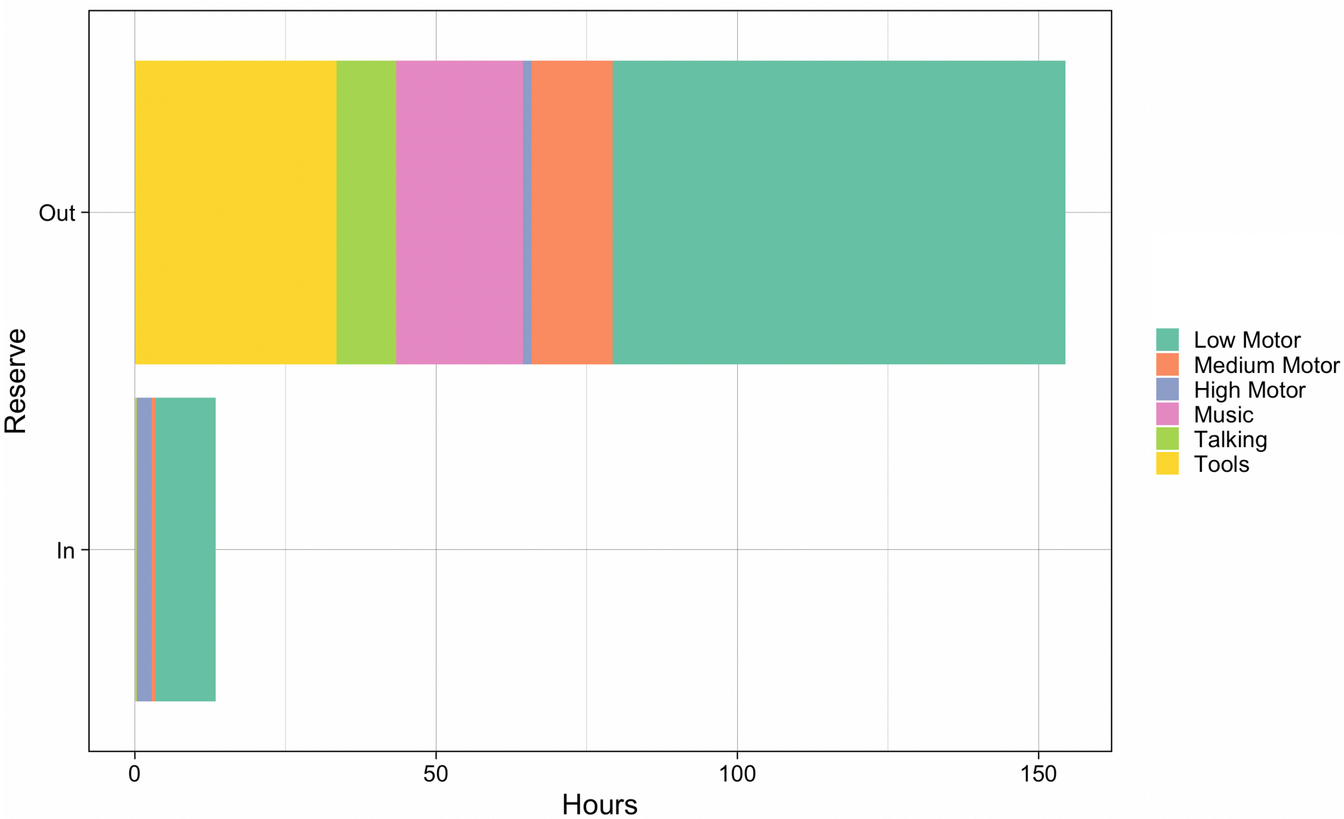

Figure 3.2 A visual breakdown of the number of hours of each sound type was catalogued during the aural analysis at the 10 locations inside and 13 locations outside of the reserve boundary limits, N=999 audio files.

<u>3.3.1 Is it worth customising the biotic and abiotic frequency thresholds?</u>

The NDSI score was significantly different between the not changed and changed parameter analysis, with the non-changed scores skewing more towards 1 indicating more biological sound present than anthropogenic noise (Wilcoxon test, V= 473634, $p<0.001$, N=999 audio files). However, there is a positive correlation between the changed parameter NDSI score and the not changed parameter NDSI score (Spearman's rank correlation, S= 24580228, rho= 0.852, $p<0.001$, N=999 audio files).

<u>3.3.2 Are the NSDI score and aural analysis comparable?</u>

There was a negative correlation between the altered parameter NDSI score and the number of minutes of anthropogenic noise present in a file (Spearman's rank correlation, S= 207874131, rho= -0.211, p<0.001, N=999 audio files). A negative correlation was also found between the unaltered NDSI parameters score and the number of minutes of anthropogenic noise present in a file (Spearman's rank correlation, S= 184831737, rho= -0.112, p<0.001, N=999 audio files). With files that had a higher NDSI score having a low total number of hours exposed. However, there was no correlation between the average NDSI score for a site and the total number of hours of anthropogenic noise catalogued at the site across both analyses (Spearman's rank correlation; Figure 3.3a Altered NDSI parameters, Figure 3.3b Unaltered NDSI parameters).

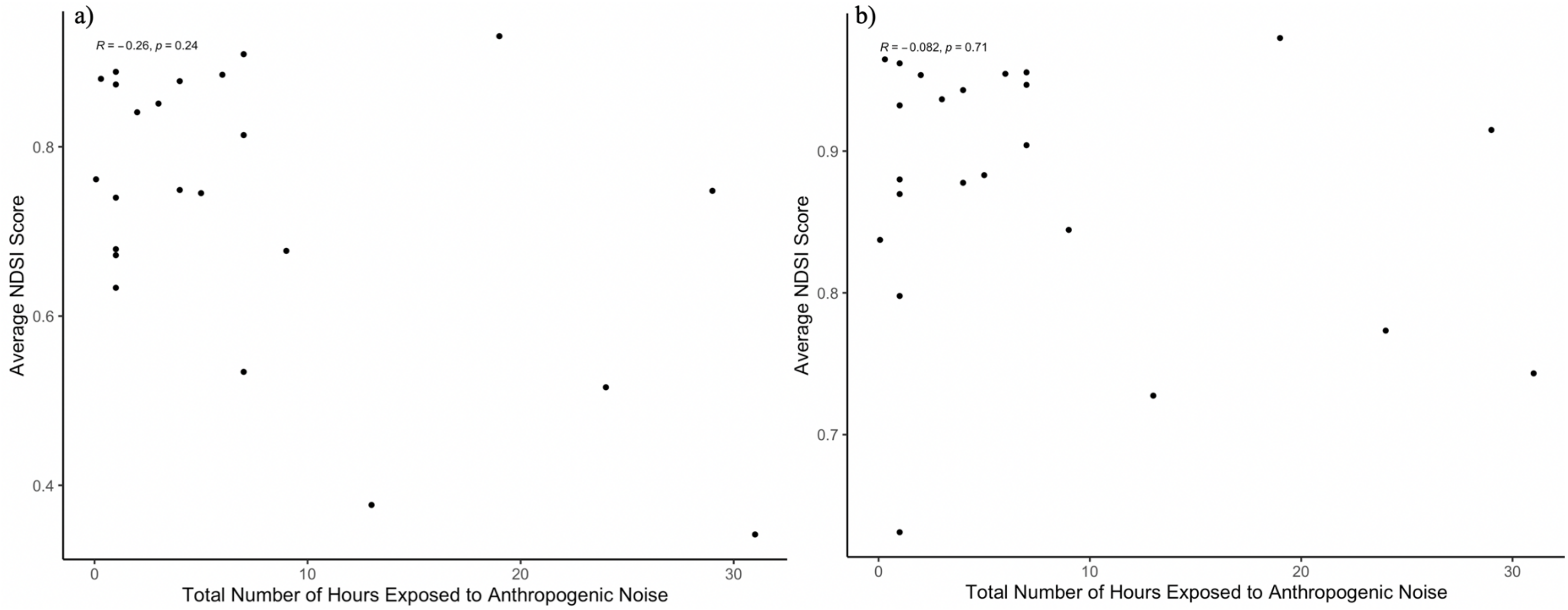

Figure 3.3a) Correlation of the average NDSI score at a site and the total hours of anthropogenic noise catalogues for the altered parameters. b) Correlation of the average NDSI score at a site and the total hours of anthropogenic noise catalogues for the unaltered parameters.

3.3.3 What factors drive a higher presence of certain anthropogenic sounds?

Anthropogenic noise was more prevalent outside of the reserve boundary (GLMM, estimate= 2.245, z= 5.698, p<0.001, N=184 totals for times of day; Figure 3.2) and significantly less inside the reserve as compared to outside in the late morning (GLMM, estimate= 0.157, z= 5.401, p<0.001, N=184 totals for times of day; Figure 3.4), early afternoon (GLMM, estimate= 1.795, z= 6.000, p<0.001, N=184 totals for times of day; Figure 3.4) and the late afternoon (GLMM, estimate= -0.091, z= -2.941, p<0.01, N=184 totals for times of day; Figure 3.4). The models R^2 was 0.979 conditional and 0.582 marginal.

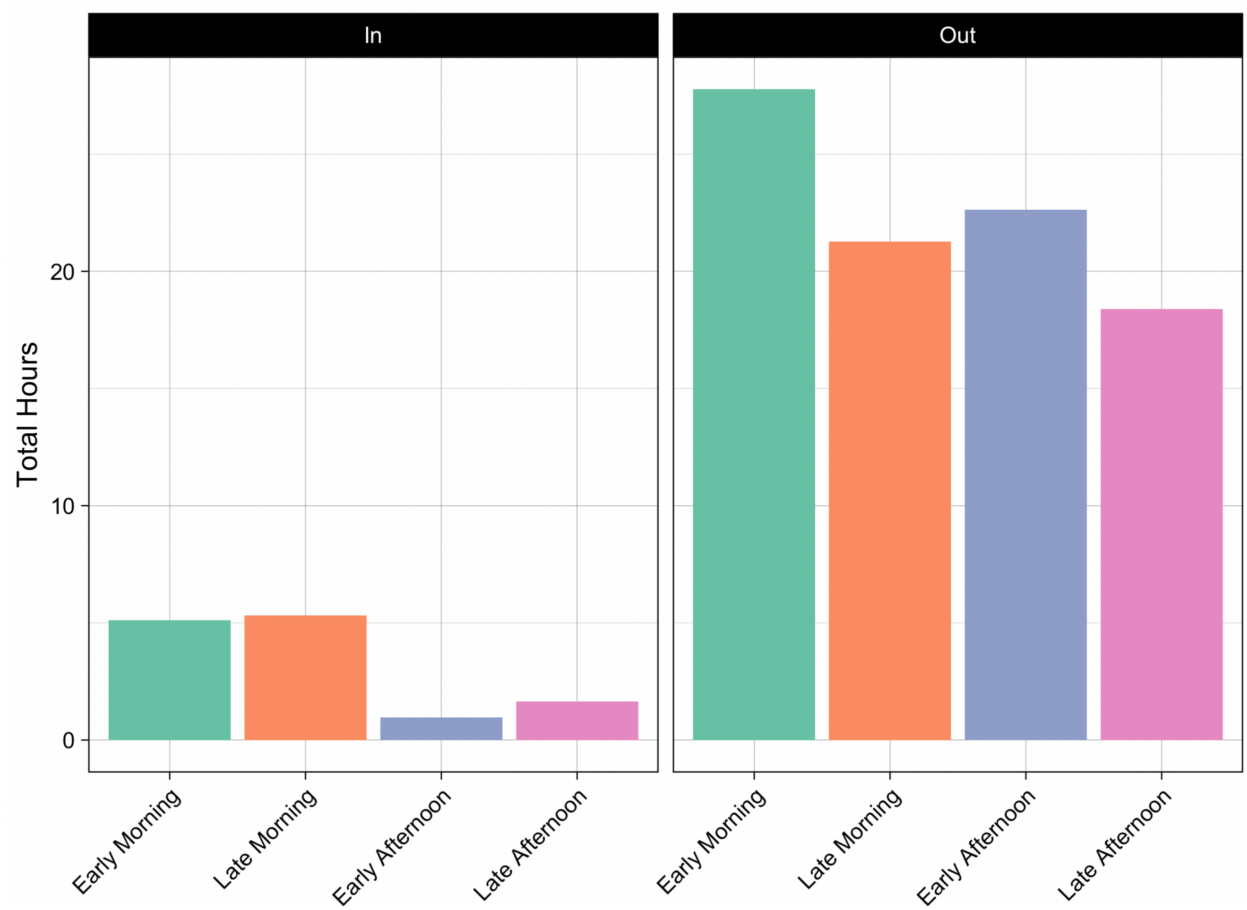

Figure 3.4 A breakdown of the amount of total anthropogenic noise heard in and out of the reserve across the four times of day (early morning: 06:30-09:30; late morning: 09:30-12:30; early afternoon: 12:30-15:30; late afternoon: 15:30-18:30) in hours, N=999 audio files.

A further breakdown of this by sound found that the presence of motor sounds was higher outside of the reserve (GLMM, estimate= 2.03, z= 5.210, p<0.001, N=184 totals for times of day ;Figure 3.5) and across all times of day outside of the reserve (GLMM; early morning, estimate= 0.332, z= 73.780, p<0.001; late morning, estimate= 0.120, z=25.410, p<0.001; early afternoon, estimate= 5.743, z= 19.630, p<0.001; late afternoon, estimate= -0.1.63, z= -32.150, p<0.001; R^2 was 1.0 conditional and 0.551 marginal, N=184 totals for times of day; Figure 3.6). However low motor was the only subset of motor sound type to be significantly more present in recordings outside of the reserve (zero-inflated GLMM, estimate= -2.232e+00, z= -2.859, p=0.004, N=184 totals for times of day; Figure 3.5), the models R^2 was 0.872 conditional and 0.537 marginal. Tools were also significantly more present in recordings out of the reserve boundary (zero-inflated GLMM, estimate=2.691, z=-3.437, p<0.001, N=184 totals for times of day; Figure 3.5), the models R^2 was 0.805 conditional and 0.437 marginal. However, there was no link between time of day or location for the likelihood of encountering talking, medium motor or high motor (Table 3.2; Figure 3.6).

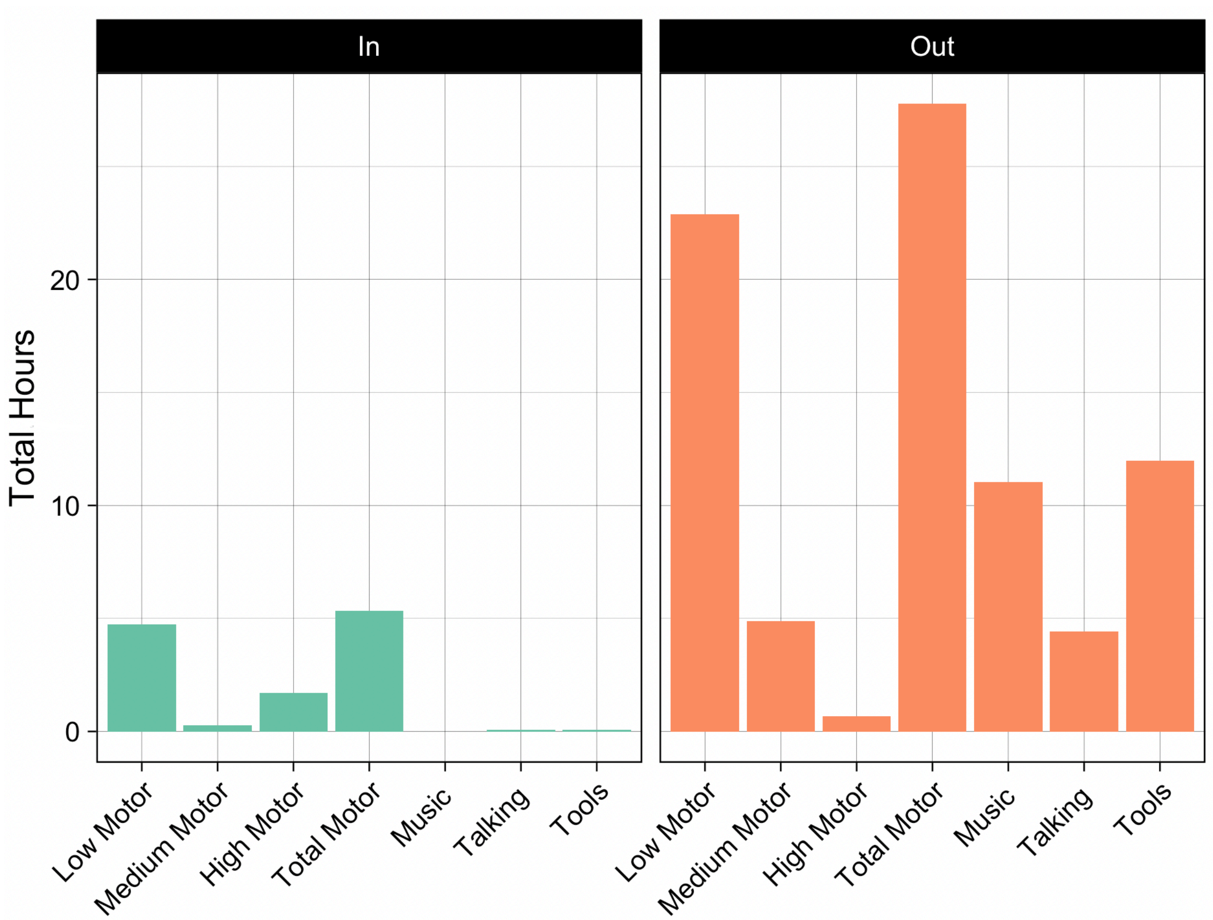

Figure 3.5 A breakdown of the amount of total anthropogenic noise heard in and outside of the reserve boundary by sound type in hours.

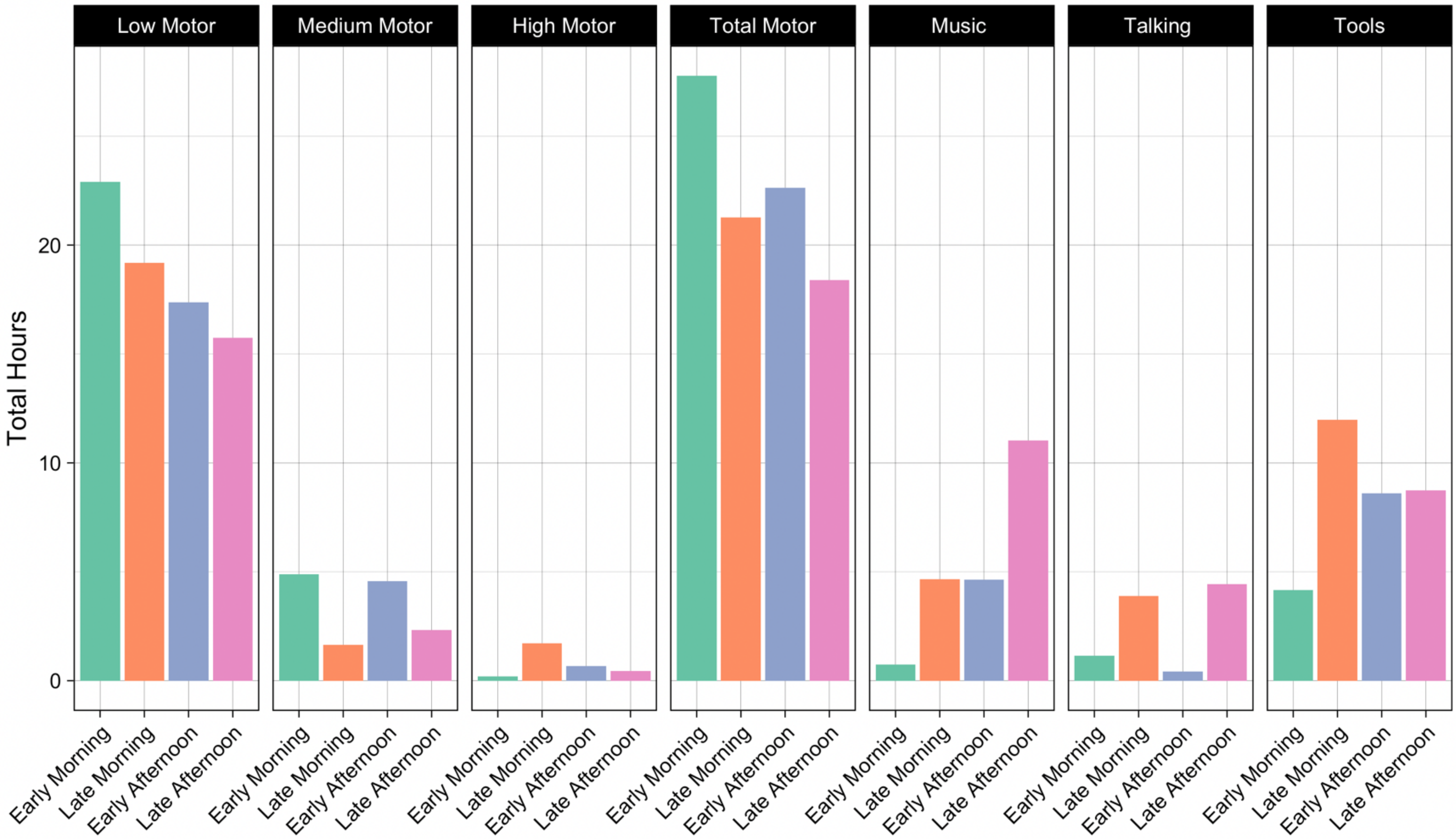

Figure 3.6 A breakdown of the amount of total anthropogenic noise heard across the four times of day (early morning: 06:30-09:30; late morning: 09:30-12:30; early afternoon: 12:30-15:30; late afternoon: 15:30-18:30) by sound type in hours.

Table 3.2 The results of the zero-inflated model GLMMs (not including the conditional model) from the full sound breakdown that showed that location and time of day did not influence the amount of the medium motor, high motor or talking present. The significance threshold for these results is $p<0.0083$. The anthropogenic noise in the files in the time of day breakdown were collated to create one total per each time of day per group resulting in, N=184 totals for times of day.

Sound	Intercept	Estimate	Standard Error	Z value	P value	Conditional R^2	Marginal R^2
Medium Motor	Location (Reserve Out)	-1.263	1.219	-1.036	0.300	0.737	0.133
	Time of Day (Early Morning)	-0.240	0.719	-0.334	0.739	0.737	0.133
	Time of Day (Late Afternoon)	0.780	0.732	1.065	0.287	0.737	0.133
	Time of Day (Late Morning)	0.238	0.719	0.332	0.740	0.737	0.133
High Motor	Location (Reserve Out)	-1.751	1.949	-0.898	0.369	0.874	0.008
	Time of Day (Early Morning)	1.345	1.221	1.102	0.271	0.874	0.008
	Time of Day (Late Afternoon)	0.636	1.138	0.558	0.577	0.874	0.008
	Time of Day (Late Morning)	-2.713	1.460	-1.859	0.063	0.874	0.008
Talking	Location (Reserve Out)	-2.450	1.137	-2.156	0.031	0.843	0.002
	Time of Day (Early Morning)	1.237	0.824	1.501	0.133	0.843	0.002
	Time of Day (Late Afternoon)	0.640	0.802	0.798	0.425	0.843	0.002
	Time of Day (Late Morning)	-0.001	0.807	-0.001	0.999	0.843	0.002

3.3.4 What factors drive changes to the NDSI score of a soundscape?

NDSI scores from the altered parameters did not differ inside or outside the reserve depending on location (Figure 3.7) but they did by time of day (Figure 3.8). With recordings having an NDSI score closer to 1 when they were produced in the early morning (GLMM, estimate= 0.3110 z= 4.10, $p<0.001$, R^2 was -0.900 conditional and -0.077 marginal, N=999 files) and the late afternoon (GLMM, estimate= 0.3490, z= 4.47, $p<0.001$, R^2 was -0.900 conditional and -0.077 marginal, N=999 files).

NDSI scores from the unaltered parameters dataset also did not differ inside or outside the reserve however the scores did differ across time of day (Anova, chi2= 48.222, $p<0.001$, N=999 files; Figure 3.8). With recordings produced in the early morning (GLMM, estimate= 0.2598, z= 3.39, $p<0.001$, R^2 was -0.563 conditional and -0.069 marginal, N=999 files) and the late afternoon (GLMM, estimate= 0.4554, z= 5.67, $p<0.001$, R^2 was -0.563 conditional and -0.069 marginal, N=999 files) once again having an NDSI score closer to 1.

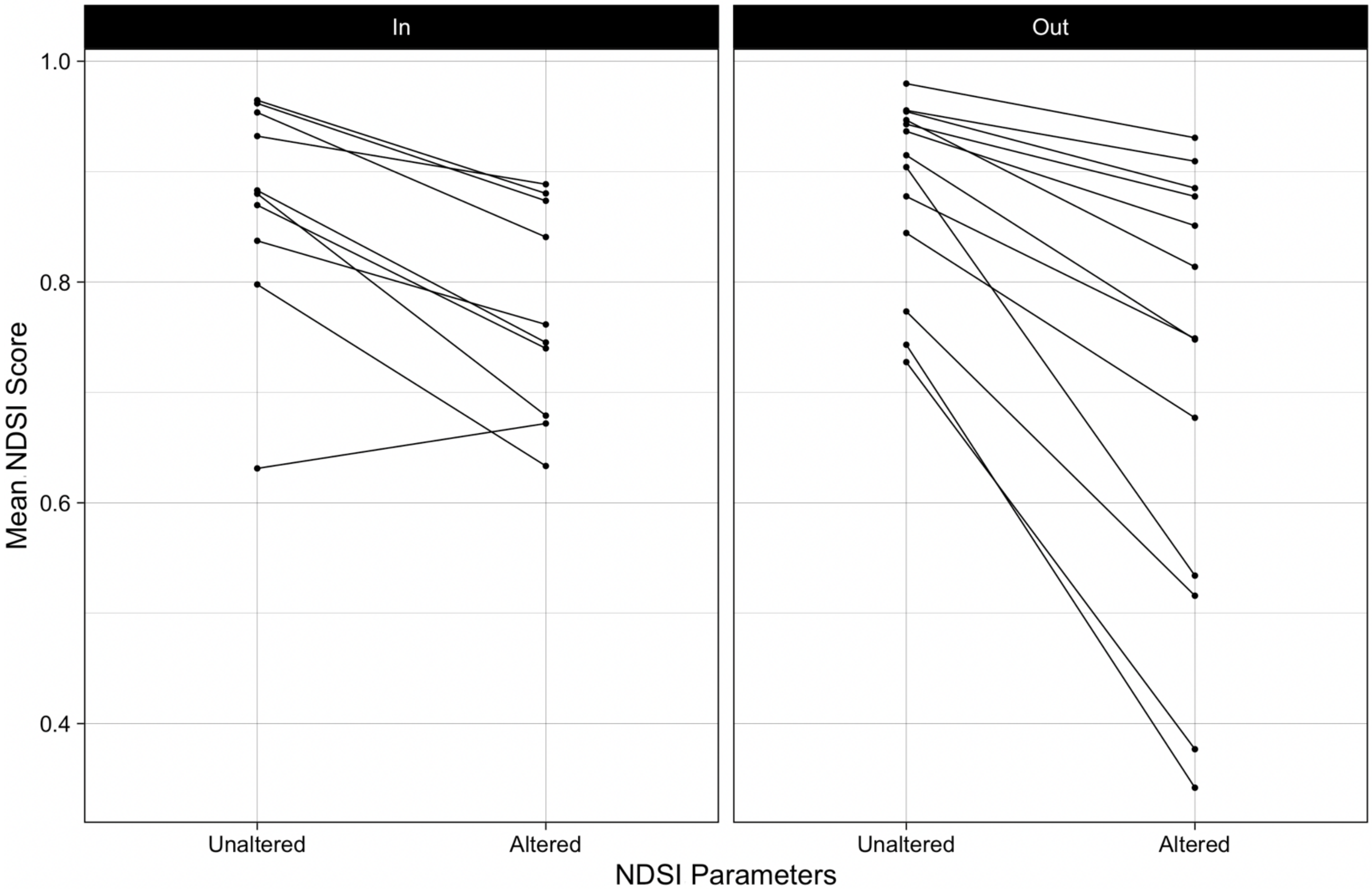

Figure 3.7 The mean untransformed NDSI score for each location, in and out of the reserve boundary, across all the recordings and for both the unaltered and altered NDSI parameter analyses, N= 46 mean NDSI scores.

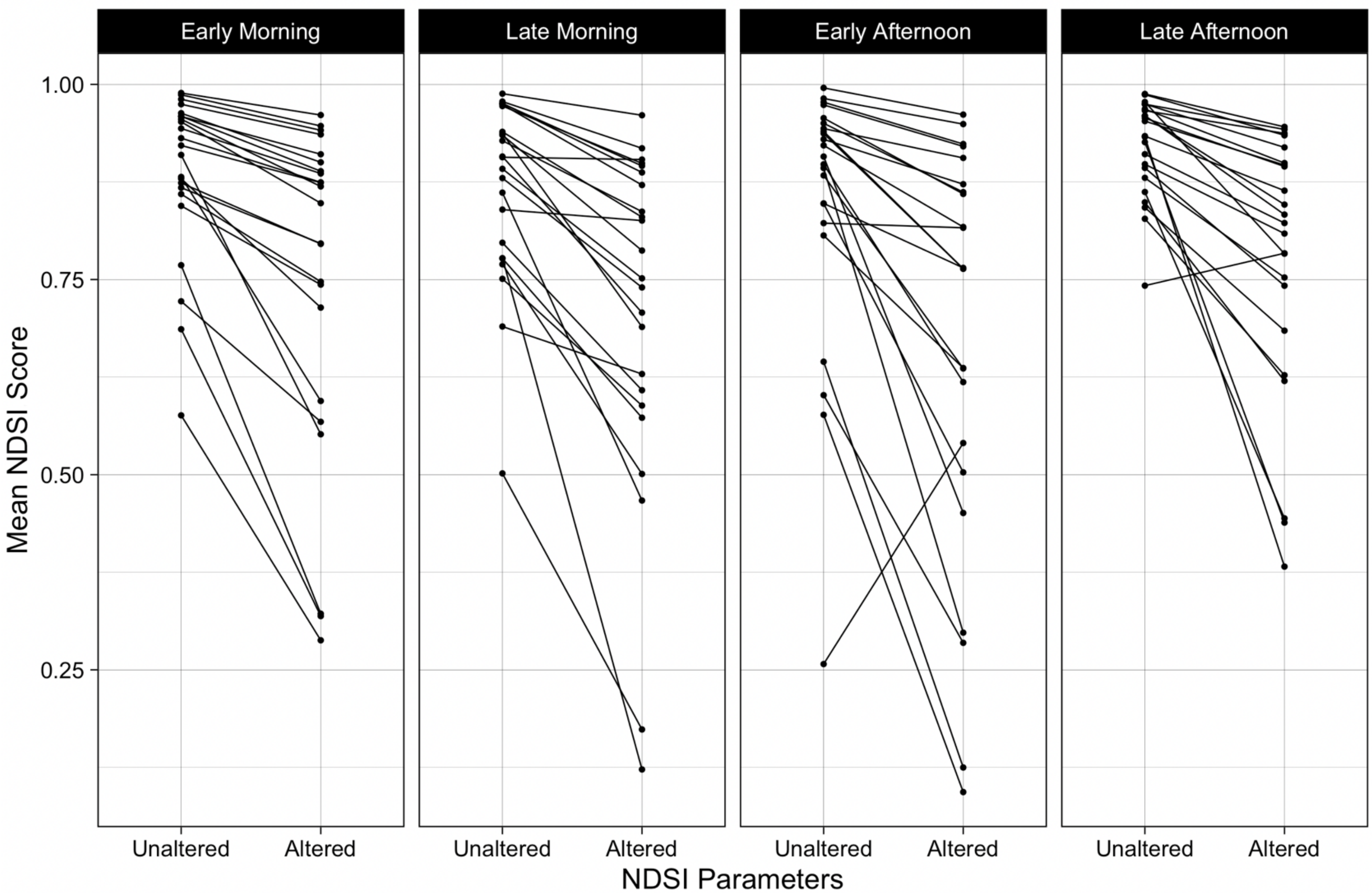

Figure 3.8 The mean untransformed NDSI score for each location for the four different times of day and for both the unaltered and altered NDSI parameter analyses, N= 184 files.

3.4 Discussion

I predicted that there would be a correlation between the amount of anthropogenic noise found during the manual analysis and the NDSI score computed, i.e. that files with lower NDSI scores would have a higher total amount of anthropogenic noise present, and this was found. However, I conclude that the NDSI score of a file cannot be used to indicate the amount of anthropogenic noise present at a site in general, as the NDSI score did not reflect the total amount of anthropogenic noise catalogued when correlated with the mean NDSI score for the site. Although these two measurements (manual analysis and NDSI score) could never be a true one to one replacement for one another as the NDSI score is a reflection on the ratio between the biotic and abiotic sounds in a file. A reason for this could be that different anthropogenic noises exert different bias on this acoustic index as found by Fairbrass *et al*. (2017), who found that human speech caused lower than expected NDSI values. Devos (2016) found that the NDSI score cannot reliably detect when biotic and abiotic sounds occur simultaneously. The NDSI values for a singing blackbird in conjunction with a house sparrow are similar to that of solely the blackbird, in contrast to the NDSI score of the blackbird and a train which gives a high negative value, indicating that in this case the anthropogenic noise is the dominant sound present in the soundscape (Devos, 2016). So, this could also be a factor in the differing NDSI scores across a landscape.

I predicted both measures would show higher anthropogenic noise outside the reserve, so more total minutes and lower NDSI scores. This was expected as two major sources of anthropogenic noise (the main lodge and El Chino) are outside the reserve limits. There was more anthropogenic noise, especially motor boats, outside of the reserve in the morning, perhaps as this is when people are starting their working day and using their motor boats to travel to work. Low motor sound was significantly more present outside of the reserve, it was the most prevalent motor type and there is a higher density of boats near places of habitation explaining its high presence. Interestingly, I did not find the same pattern for the NDSI scores, as there was no significant difference between inside and outside the reserve in both NDSI analyses. Which was noteworthy as I had excepted the ratio to measure a higher biotic noise and lower anthropogenic noise in the reserve limits, leading to lower NDSI scores. I found the early morning to have a higher presence of anthropogenic noise but these recordings had higher NDSI scores for both analyses and in the late afternoon which also had higher NDSI scores for both analyses. This could occur because even through there is higher anthropogenic noise at these times there could also be more biotic sounds due to dawn and dusk choruses, pushing the ratio to show higher biological noise causing the NDSI score to be closer to 1. NDSI scores have shown temporal variation, with Devos (2016) finding spikes in biophonic noise activity at sunrise and sunset. I did not expect to find that both NDSI analyses found the same trends, as while there was a correlation between the two scores, the file's NDSI score was significantly different after the parameters were altered. This could be that while the scores differed significantly that the scores still reflected this temporal shift in when animal noises are more prevalent than anthropogenic ones, which would explain why the scores were still correlated.

This is the first in depth performance review for the NDSI score versus a manual analysis. While other studies have focused on using the NDSI score in situ (Fuller *et al*., 2015) or how one can use it to measure disturbance (Devos, 2016) no one has then compared these scores to the actual amounts of anthropogenic noise present in the soundscape in the file analysed. The results indicate that the NDSI score cannot be used as a replacement for manual analysis if the aim is to quantify the amount of anthropogenic noise present in an audio file. Although in most of the literature the use of the NDSI score is to correlate with species richness in areas (Fuller *et al*., 2015; Mitchell *et al*., 2020) and to see temporal behavioural patterns (Devos, 2016; Ritts *et al*., 2016). It has also been used to gather information on landscape attributes as the score has been found to be related to different site traits (biocondition score, extent of transport systems, patch size, conservation area) (Fuller *et al*., 2015). The use of the NDSI to detect temporal differences was supported in this study, so I believe it is useful for providing insight into the trends of levels of abiotic versus biotic sound throughout the day in a specific location. This fits with how the majority of the current literature is using the NDSI score as it relates to species richness and abundance and using the score as more of a reflection of shifts in activity patterns for both animals and people (Ritts *et al*., 2016).

I found that while altering the parameters for the frequency thresholds did impact the NDSI score it did not influence the patterns of how the score fluctuated. Therefore, I conclude that altering the parameters is best if one knows the anthropogenic noise limits in an area and the frequency of a study species vocalisation, as it allows for a more targeted analysis for the study question. I could not find another instance in the literature where the NDSI score had been altered from the standard set frequency bands. Leaving the parameters unaltered can be a limitation as its excluding frequency bands (infrasonic and ultrasonic) from the analysis which is then omitting a portion of biophony and anthrophony, so one must consider this when interpreting the results if leaving the parameters unaltered (Ritts *et al*., 2016). This is supported by Ross *et al.* (2021) who used the standard parameters and tested the performance of acoustic indices in three sonic conditions (wind or rain, human-related noise, and insect stridulation). They found that the NDSI score performed reasonably well and was insensitive to all three sonic conditions, showing that the parameters perform well in a variety of different sonic conditions, so altering the parameters should not affect this aspect of the score.

There were limitations to this study as the Audiomoths were placed near pygmy marmoset groups and were not randomly placed across the study site. Having a more systematic grid placement system could have helped discern locational differences between the reserve and the surrounding areas. As most of these sites were close to the main river system which sees a higher density of anthropogenic noise (motor traffic) which could be skewing results and not allowing for a more discernible difference in the breakdown of anthropogenic noise and NDSI score to be seen.

It is also pertinent to use recommendations for how to design your soundscape study like those set out by Bradfer-Lawrence *et al*. (2019) and Mitchell *et al*. (2020) to make sure one is gathering the correct data needed to address the research question. While these studies focus on bioacoustics on the whole and how the data can be used to extrapolate information on biodiversity it is also important to be monitoring the pressures that are driving changes in biodiversity. Future avenues for streamlining this analysis would be to develop machine learning algorithms. Through the expected increased use of machine learning algorithms, the analysis of large-scale acoustic data with a focus on anthropogenic noise disturbance will become more widespread in the literature. This will mean that the ramifications of noise disturbance will be better understood and clear, targeted conservation efforts can be made to mitigate them. Acoustic detection algorithms have been used to collect evidence of poaching in a nature reserve in Belize by detecting gunshots in audio files (Prince *et al*., 2019). Studies like Prince *et al*. (2019) show that the implementation of acoustic detection algorithms can further extend the use of low-cost open source methodologies to create new and more time efficient avenues for conservation researchers on a large-scale.

Bioacoustic indices are revolutionising how we assess an environment however it is important to understand the limitations of these indices and in what cases they are the most appropriate. This study supports the use of the NDSI score to predict temporal changes in the levels of anthrophony and biophony in a soundscape and concludes that it should not be used to provide an accurate look at the actual amount of anthropogenic noise present. However, the manual process needed to get a comprehensive breakdown of the level of anthropogenic noise disturbance in a site is still a laborious and time-consuming process (Pimm *et al*., 2015). Through advances in machine learning algorithms, methods for automated acoustic analysis of anthropogenic disturbances will hopefully become the standard and rapid method utilised for monitoring and informing the effects of anthropogenic disturbances on wildlife and ecosystems.

3.5 References

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