REPORT ON EXPEDITION / PROJECT

Outcome (a minimum of 500 words):-

AUTHORS NOTE

The nature, aims, and approach of this expedition changed markedly from its initial intentions. Permissions from the local government and logistics changed in the months leading up to expedition. Thankfully, we were able to connect with a local conservation group – Green Gold Forestry – that enabled us to conduct a similar acoustic survey in the Peruvian Amazon. More importantly, we encountered many anthropogenic and logistical problems once we arrived in Peru. In an effort to conduct some meaningful science – both for ourselves and the local conservation area –we made the decision in the field to shift from investigating river barriers to monitoring methods, temporal, and spatial patterns in the soundscape of the remote Tachsa Cuarary in Northern Peru.

TEMPORAL TRENDS IN A TROPICAL SOUNDSCAPE

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INTRODUCTION

Ecoacoustics is a rapidly evolving field (Seur *et al.,* 2014). It combines many fields, including but not limited to bioacoustics and landscape ecology, to investigate "the collection of biological, geophysical and anthropogenic sounds that emanate from a landscape and which vary over space and time reflecting important ecosystem processes and human activities" [\(Pijanowski](https://link.springer.com/article/10.1007/s10980-011-9600-8#auth-Bryan_C_-Pijanowski-Aff1) *et al.,* 2011a). The combination of these sounds can be used to understand dynamics in the natural and anthropogenic worlds across different temporal and spatial scales (Pijanowski *et al.,* 2011b).

Acoustic signals can determine spatial biodiversity patterns and be informative in conservation (Grant and Samways, 2016; Lailo, 2007), especially with improving automated sound recordings and processing tools (Lailo, 2010). In a 6-year period, over 20 'acoustic indices' were created in attempts to quantify alpha and beta diversity in soundscapes (Sueur *et al.,* 2014). While these indices are far from direct biodiversity estimates, they can be used to monitor changes in an environment, and as precursors to change (Alocer *et al.,* 2022).

Passive acoustic monitoring (PAM), utilising automatic recorders, also has the potential to greatly increase the efficiency of monitoring and surveys, minimising costs and producing massive datasets with relative ease (Rajan *et al.,* 2018). However, as a burgeoning field, many questions remain, including the robustness of indices and patterns in the soundscape (Pijanowski *et al.,* 2011b).Characterising temporal and spatial patterns in soundscapes remains a priority, especially concerning temporal partitioning among diel phases (Fuller *et al.,* 2015; Pijanowski *et al.,* 2011b). Understanding these patterns, especially in site dependent contexts, can aid conservation biologists and land managers in understanding when and where communication takes place, and aid evolutionary biologists in answering questions regarding the evolution of acoustic niches.

The potential for PAM to aid in management and conservation is especially prominent in remote, difficult to access or survey areas, such as the biodiversity hotspot of the Amazon basin. Despite the importance of mitigating habitat degradation in the Amazon, its remoteness, political instability in the region, and surveying conditions constrain the ability for scientists to undertake research. This can make monitoring changes in biodiversity difficult and expensive. This can hinder conservation, especially for smaller conservation plots and teams. In this study, we

conducted a PAM survey to investigate temporal and spatial variation in the soundscape of a conservation allotment in the Northern Peruvian Amazon.

METHODS

Study system and period

We conducted the acoustic survey from 11-14/07/2023 (dry season) at three sites in the Loreto Region of the Peruvian Amazon. This area is located within the Green Gold Forestry (GGF) holdings on the Tacsha Curaray, (from -2.712607, -73.584963 to -2.770142, -73.564442; Fig. 1). The region is tropical and the closest estimate for precipitation is about 2940 mm annually, with mean yearly temperature at 25.6 °C (Climate-data.org; see Fig. 2). This area consists of sporadically placed local communities, small subsistence farming patches, and boat travel (small *peque-peque*'s and larger fast-boats) is a daily occurrence. The GGF group maintain the allotment and undertake local conservation initiatives in the area, but the majority of the land is unaltered.

Figure 1. Study area (left) and monitor locations (right).

Figure 2. Annual mean temperatures and rainfall in the region. Note that the study occurred in July (07). Data and Figure from climate-data.org.

Data collection

To investigate temporal patterns in the soundscape, we employed two Song Meter Mini 2AA (Wildlife acoustics TM) mono-directional units throughout the 4-day study period. Each morning, we paddled to and located the two monitors from the night before (collected between 11:00-13:00) and moved them to a new location in the afternoon (placed from 13:00-15:00). We looked for accessible banks, and placed monitors 3 m in from the river placed the monitors on tree trunks between 1 – 2 m (Whytock and Christie, 2017) above the ground or water on each side of the river facing away from the river (Fig. 3). We placed the monitors at three sites at least 1 km apart along the river, and rotated the two monitors among banks to control for any monitor effects.

Figure 3. Example monitor placement at chest height, 1-2m above the ground; roughly 3 m in from the bank.

We programmed the units using the iOS Song Meter Configurator app (v. 1.6) to record continuously (sampling rate: 44100 Hz, sample size: 16-bit, WAV format, left channel only; Bradfer-Lawrence *et al.,* 2019; Moreno-Gomez *et al.,* 2019), and divided recordings into hour long files for storage and analysis. Song Meter Mini units automatically recorded the temperature each hour and stored the data and recordings internally in WAV format on 64 GB Extreme PLUS SanDisk SD cards. The units have weatherproof cases and are powered by four AA cell batteries, which lasted the entirety of the sample period.

Acoustic analysis

We then inspected the spectrograms of each recording using RavenLite 2.0 to check for irregularities. We included only complete hour-long recordings in the analysis, excluding the incomplete initial and terminal recordings that included us setting and recovering the units. This also allowed for a 'settling time' in case we disturbed any of the calling biota.

To utilise multiple complementary indices (Sueur *et al.,* 2014; Bradfer-Lawrence *et al.,* 2019), we investigated temporal patterns using the Acoustic Evenness Index (AEI), Acoustic Diversity Index (ADI), and the Normalised Difference Soundscape Index (NDSI; see Box 1 for biological relevance and original publication). We chose these indices to provide complementary soundscape information, and have been reported as the most robust indices (Sousa-Lima *et al.,* 2018; Zhao *et al.,* 2019; Jorge *et al.,* 2018; Moreno-Gomez *et al.,* 2019; Fuller *et al.,* 2015; Bradfer-Lawrence *et al.*, 2019). Other indices are more sensitive to geophony and sonic abundance, and current literature is inconclusive with regards to their relationships with richness and diversity (Bateman and Uzal, 2021; Sousa-Lima *et al.,* 2018).

We used the 'multiple_sounds' function of the *soundecology* (v. 1.3.3) package to calculate the acoustic indices using the metrics in Box 1. Note that the bands for anthrophony and biophony vary from the values typically used in the literature. Upon visual inspection of the spectrograms, significant sources of biophony and anthrophony were found outside of the default frequency values(Fig. 4). We calculated all indices with a minimum threshold of -75 dB, from 0-22050 Hz, with frequency bins of 1 kHz.

Box 1.

Figure 4. Examples selections of high frequency anthrophony (boat motor, top panel) and low biophony (bottom panel) on the spectrograms.

Using the Astronomical Applications website of the US Naval Observatory (location: W073.21, S02.26, GMT-5, Astronomical Twilight and Sunrise/Sunset tables), we categorised each hour into the diel phase: day (07:00-17:59), night (20:00-04:59), or twilight (05:00-06:59 and 18:00- 19:59) to account for dawn and dusk choruses.

Statistical analysis

We fit linear mixed effects models (LMMs) using the R package "lme4" for each index against diel phase, and bank side, with temperature as a covariate and site and recorder as random effects. We dropped insignificant terms from models. We used the *emmeans* package (DF method: Kenward-

Roger, CI level: 0.95) to calculate least error means using these models. We inspected models for normality, and ran Type III Analysis of Variance (Satterthwaite's method, *lmerTest* v. 3.1-3). We included diel phase instead of hour in the model, because we anticipated (and observed when plotting raw data) a sigmoidal curve throughout the day (Bradfer-Lawrence *et al.,* 2019), which could not be accounted for in a linear mixed model. For illustrative purposes, we used `geom_smooth` Loess method (fewer than 1000 observations) and formula $= 'y \sim x'$ to illustrate the daily patterns with respect to hour. We conducted all analyses and data processing in R (Version 4.3.1).

RESULTS

Temporal patterns

Acoustic diversity, evenness, and normalised soundscape index varied among diel phases (See Tables 1-3 for full model outputs). The ADI was lowest during the day $(2.66 + / -0.03 \text{ SE})$ followed by twilight $(2.77 + / - 0.04 \text{ SE})$, and finally night $(2.94 + / - 0.03 \text{ SE})$; Fig. 5, Table 1). The NDSI was also lowest during the day $(0.47+/- 0.11 \text{ SE})$, followed by twilight $(0.90+/-00.12 \text{ SE})$ and night $(1.00 +/- 0.11 \text{ SE}; \text{Fig. 5}; \text{Table 2}).$ The only negative NDSI values (indicating encroaching anthrophony) occurred during the day. Nocturnal and twilight NDSI values remained close to 1 (no anthrophony). Acoustic evenness, in contrast was highest during the day $(0.43 + / -0.02 \text{ SE})$ followed by twilight (0.38+/- 0.02 SE) and night (0.26 +/- 0.02 SE; Fig. 5, Table 3). ADI, AEI, and NDSI varied as expected with temperature (see model outputs). Recordings on the west bank had lower evenness, higher diversity, and the NDSI was unaffected (dropped insignificant term dropped; see model outputs).

Figure 5. ADI, AEI, and NDSI throughout the day. Raw datapoints are shown, coloured by site. Lines are included for illustrated purposes and are smoothed Loess curves. The black points are the least square means for each diel phase: night (00:00), day (10:30 and twilight (18:30). Note that the twilight phase also includes the

dawn period. For all indices, daytime and night-time indices differed; twilight and daytime indices also differed for ADI and NDSI (see tables 1-3 for full model outputs.)

Table 1 - NDSI. Final model output from a generalised linear mixed model of NDSI with site and recorder as random effects. Linear mixed model fit by REML; t-tests use Satterthwaite's method (ImerModLmerTest). Marginal (.) and significant (*) values are noted. There were a total of 130 hour long recordings from three sites and 2 recorders.

Fixed effects

Table 2 - AEI. Final model output from a generalised linear mixed model of AEI with site and recorder as random effects. Linear mixed model fit by REML; t-tests use Satterthwaite's method (lmerModLmerTest). Marginal (.) and significant (*) values are noted. There were a total of 130 hour long recordings from three sites and 2 recorders.

Fixed effects

Table 3 - ADI. Final model output from a generalised linear mixed model of ADI with site and recorder as random effects. Linear mixed model fit by REML; t-tests use Satterthwaite's method (lmerModLmerTest). Marginal (.) and significant (*) values are noted. There were a total of 130 hour long recordings from three sites and 2 recorders.

Fixed effects

DISCUSSION

As expected, we found that daytime and night-time acoustic indices differed, with greater evenness and more anthrophony present in the diurnal soundscape. The soundscape in the Tacsha Curaray appears to follow a diurnal cycle, as expected. Clear differentiation between diel phases and strong diurnal patterns have been widely reported (Bradfer-Lawrence *et al.,* 2019). The indices imply greater unevenness in the diurnal soundscape (higher AEI and lower ADI). There may have been a greater acoustic richness (calling biota) during the nocturnal period, which increased evenness throughout the bands. This is consistent with personal observations (the team camped in the area); during the day, calls were more intermittent and frequency bands were more distinguishable. This contrasts with other studies in the region (Bradfer-Lawrence *et al.,* 2019). While it is possible that this reflects genuine differences in habitats and soundscapes, it may also be that different recording lengths are responsible. It seems unlikely that this would produce such an inverse relationship however. Standard errors for temporal patterns tend to stabilise after 120 hours of recording (Bradfer-Lawrence *et al.,* 2019), so it is likely that we have accurately characterised the temporal patterns in the study area.

Anthrophony was greater in the diurnal period, consistent with most studies (Bradfer-Lawrence *et al.*, 2019), presumably die to increased human activity, and therefore anthrophony, during daylight hours (Fullernce *et al.,* 2015). Throughout the sampling period, small motorised boats (*peque-peque*'s) could be heard throughout the day. These were prevalent in spectrograms and it is unsurprising that there was almost no anthrophony present during the nocturnal period (NDSI values close to 1). It is possible that increased anthrophony influenced the evenness of the soundscape during the day. It is more likely, however, that trends in evenness are driven by the biology and life history of the animals present in the area.

Interestingly, our data seem to indicate a trend with respect to spatial soundscape patterns. Because we only had 3 sites along the river and took measurements on either side of the river, site was included as a random effect rather than fixed effect. However, NDSI trends appeared become more pronounced with proximity to the camp and community (site 3). However, this apparent pattern is highly preliminary and should be investigated with proper replication along a gradient away from human settlements. This site replication seems particularly important when using the NDSI to investigate anthrophony as site had the largest deviation for this index.

In addition to potentially varying along the river, our data also indicate that soundscapes may vary across rivers. The soundscape on the west bank was more even (greater ADI, smaller AEI) than the east bank. The east bank lays between the Tacsha Curaray and the larger, heavily trafficked Rio Napo. It is possible that its proximity to this larger waterway influenced the evenness of the soundscape.

In general, much more replication is needed to characterise temporal and spatial patterns of the soundscape in the study area. This is especially true for characterising spatial patterns, only three sites were used with one monitor on either side of the river. Future work in spatial soundscape patterns should seek to replicate at the site level along, across, and ideally away from the river to better characterise the spatial layout of the soundscape with respect to human activity in the area. Future work should also aim to characterise site specific relationships between the indices used and species richness and diversity metrics to increase the applications of passive acoustic monitoring (Bateman and Uzal, 2021). Frequency bands used for anthrophony and biophony were difficult to characterise in this study. Biotic signals existed below and above traditional bands (2-8 kHz), so these needed to be amended. Traditional bands tend to be biased towards avian signals, but anurans and insects make up an important part of chorusing animals, especially in the tropics, and should be included (Zhao *et al.,* 2019). Motorised boats in the area also presented a challenge. The signals of these boats often exceeded the anthrophony band (0- 2kHz). However, the peak frequencies lay within the anthrophony band, so the error should not

be a fatal flaw of the study. Future work should focus on region specific frequency bands and the effects of variation in occupied frequencies for PAM.

Science in unfriendly environments

When undertaking research in remote areas, especially in LEDC regions or countries, logistics and people present one of, if the most important component of a study to consider. While planning this study, we planned on having over a week in the field. However, immigration issues, ATM scams, and hostile locals while traveling to and in the study, area presented us with delay after delay, compromise after compromise. These combined to limit our total sampling time from the expected 8 days to 4 days – a severe constraint to the study. The importance of dealing with people in these regions cannot be overstated; nor can it be ignored, both for the safety of field teams and the effects on time available for meaningful research and the quality of the produced data. While the team is pleased with the work we were able to do in the short period of time, the study design needed to be adjusted multiple times, up to days before we were set to depart for the study area. This compromised the robustness of the dataset and severely limited the conclusions we can draw at this time.

However, passive acoustic monitoring shows promise, especially in areas like these where it may be advisable to limit the amount of time spent in the field due to safety concerns. The ability to obtain data of this quantity with relative ease should be utilised, especially by groups such as GGF who seek to monitor and enhance remote areas in the Amazon. The quantity of data presents its own problems – what to do with all of it? While this study's main focus was to characterise and observe patterns in the soundscape of this study area, there is significant potential to utilise these practices and data to make meaningful advancements in the field of ecoacoustics. Future work should focus on investigating and distilling the relationships between the acoustic indices and other biotic indices; facilitating robust datasets automatically collected and easily analysed to efficiently monitor areas of conservation concern, especially in remote areas.

CONCLUSION

We detected strong diel cycles in evenness and prevalence of anthrophony the soundscape of the study area in Northern Peru. There was a clear differentiation between the diurnal and nocturnal soundscapes, with a more even nocturnal soundscape. The prevalence of anthrophony was greater in the diurnal soundscape, and this pattern may increase with proximity to human settlements.

Complications in the field severely limited our sample size and limited our conclusions with respect to spatial patterns in the area, but it may be that gradients along and across rivers in the Amazon should be considered for surveyed regions. Excess time should be allotted for when designing studies in remote, LEDC areas.

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