Assessment of a passive and active technique for anuran monitoring in a lowland secondary Bornean Forest, Sabah, Malaysia.

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Biological Sciences (Zoology) with a Professional Training Year

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Part A: PTY Reflection

The past year at the Danau Girang Field Centre has been an extremely rewarding experience. I selected this placement two years ago, but due to the COVID-19 pandemic the placement was postponed for a year. Despite this setback, this year given me skills I believe I wouldn't have obtained otherwise; I have gained confidence when interacting with a range of people and have realised I am capable of much more than I previously thought. Some of my favourite highlights from this year have included: educating local school children, showing visitors the wildlife in the jungle, being trusted to assist in veterinary procedures involving pangolins, leopard cats and monitor lizards and the subsequent VHF tracking of these individuals. Working on many projects has been one of my favourite aspects of DGFC, people's passion for conservation and their study is infectious and I have enjoyed learning about animals I had no prior knowledge of. I have also developed creatively by designing t-shirts for projects at the field centre and making GIS waypoint images for different projects.

Working on my project has been a major highlight for me. I have had the desire to work with frogs for a long time and this research has made me realise that the more information we have, the better we can protect this diverse taxon. I have enjoyed finding specialist species such as the Harlequin Tree Frog (*Rhacophorus pardalis*) and the Jade Tree Frog (*Rhacophorus dulitensis*) while also showing visitors as many species as possible, with the hopes of spreading knowledge and appreciation for frogs. Experiencing thousands of frogs breeding during the flooding was a unique experience and one I will not forget. This project has been a huge personal achievement and I hope I reflect this in my report.

This year has, of course, come with many challenges both from living at the centre and with my project. I am grateful for these as I learnt to think logically and look for solutions, however, this has impacted my project as widespread flooding and wildlife have meant I could not collect all my data. Despite this, my project has adapted and grown in many ways. Occasionally I have felt out of my depth with the vast amount of data or when learning a lot of new things in short spaces of time. This was stressful and frustrating at times, but this has shown me the reality of fieldwork and made me feel proficient enough to face these challenges. There are too many fascinating projects and encounters to list in 500 words, but the abilities gained from these are invaluable. Overall, I am very grateful for this year and all that it has taught me. Moving forward I feel far more capable both professionally and personally and it has reinforced my desire to pursue a career in research, zoology, and conservation.

Part B: Scientific Report

1. Abstract

Technological advancements can improve data collection, which is crucial when conserving areas of ecological importance. Sampling techniques require a comparison before the widespread use of new equipment. This study is the first to compare AudioMoth recorders to the standard transect-based active searches to detect anuran species in the Lower Kinabatangan, in the state Sabah in Borneo. The Lower Kinabatangan is home to a diverse range of anuran species, which differ from one another in many contexts. This diversity means it can be challenging to sample and monitor amphibian populations. Due to the loss of forest habitats in Borneo, it is necessary to know the status of each species. Frogs are bioindicators, so monitoring this taxon could aid in streamlining conservation efforts when habitats are disappearing at an alarming rate. Improving upon current monitoring techniques to minimise effort can help track the health of an entire ecosystem. The main aim was to compare the performance of AudioMoth recorders to the active searches typically done in the area. Sampling occurred from November 2022 to June 2023 in the Pin Supu Forest Reserve, at sites of varying forest types and stages of restoration, and a plantation site. An Audiomoth was deployed to record for a minute every five minutes, for each transects where active searches took place. PAM (passive acoustic monitoring) with AudioMoths detected a significantly lower number of species across the transects when compared to AS (active searches). A chi-squared test shows the diversity sampled by each technique didn't significantly differ, despite AS (15) capturing a greater number of species than PAM (11). This accuracy could be increased through extensive knowledge of local anuran vocalisations and increased sampling effort. Sampling continuously through to recording 1 minute every 10 showed no significant difference in detection accuracy. PAM could be a viable technique to estimate anuran species richness. The current deployment methodology and bioacoustics analysis led to a lower detection success of PAM to AS. Further research and different deployment methods could close this gap in performance. These findings indicate areas of improvement for PAM and what to consider when monitoring anurans in the Lower Kinabatangan rainforest.

2. Introduction

Declines in amphibian populations have been apparent since the 1980s across the globe (Wells 2007). Frogs face many threats, such as infectious diseases, habitat destruction and fragmentation, environmental pollution, and the introductions of alien predators and competitors (Stuart *et al.* 2004; Wake and Vredenburg 2008). These threats aggregate, creating pressures and stressors for amphibian populations (Wells 2007). Asia has an extremely high diversity of amphibians, with Southeast Asia having the highest diversity, with over 650 species of amphibians (Inger 1999). The lowland tropical rainforests of Malaysia, including those in Borneo, contain a high anuran species diversity, many of which are endemic to the island (Wells 2007). These areas interest conservation herpetologists due to this diversity, and many new species continue to be described (Wells 2007). However, globally, terrestrial biodiversity is declining mainly due to the agricultural and forestry sectors (Kok *et al.* 2018). In Malaysia, 50% of regional tropical deforestation has been due to oil palm expansion and is also associated with peatland draining and burning in Southeast Asia (Meijaard *et al.* 2020). The resulting habitat loss and reduction of wildlife corridors have harmed wildlife populations, and amphibians are no exception (Goossens *et al.* 2005; Pounds *et al.* 2006; Dinerstein *et al.* 2007; Gillespie *et al.* 2012; Abram *et al.* 2014). Frogs are susceptible to abiotic changes and are environmental degradation indicators due to various biological characteristics (Carroll 1999; Wells 2007). Sensitivities vary between species, and assessing the species composition of an area could provide insights into its environmental condition (Gillespie *et al.* 2012; Haryati and Dzati 2013; Scriven *et al.* 2018). It is well-documented that oil palm plantations support generalist anuran populations but far fewer forest specialists (Gillespie *et al.* 2012; Scriven *et al.* 2018). Forested areas have a higher relative species richness and support more arboreal and endemic species than oil palm plantations (Gillespie *et al.* 2012; Scriven *et al.* 2018).

Globally, amphibians have the highest proportion of data-deficient species (Bland *et al.* 2017). There is a lack of information on the status of many amphibians in Asia and little quantitative data compared to the extensive data sets in North America, Europe, and Australia (Wells 2007; Das *et al.* 2014). Previous studies to monitor tropical anurans used transect-based methodologies (Gillespie *et al.* 2012; Konopik *et al.* 2015; Scriven *et al.* 2018). Transect-based techniques for long-term monitoring come with several issues, mainly the onsite effort required and the site-specific challenges, including weather, widespread flooding, and hazardous wildlife (Melo *et al.* 2021). Acoustic monitoring is a non-invasive technique which allows researchers to collect large amounts of data continuously through a range of environmental conditions, and data collection is standardised (Skalak *et al.* 2012; LeBien *et al.* 2020; Ribeiro, *et al.* 2022). PAM can research species distributions, spatial and temporal dynamics, biodiversity, and the status of cryptic and elusive species without impacting animal behaviour (Hill *et al.* 2019; Melo *et al.* 2021; Revilla-Martín *et al.* 2021; Toenies and Rich 2021; Ribeiro, *et al.* 2022).

Although recording frog vocalisations is not a novel concept, the advancement of technology means PAM is increasingly affordable and accurate (LeBien *et al.* 2020; Revilla-Martín *et al.* 2021; Ribeiro, *et al.* 2022). Low-cost AudioMoth recorders perform comparably to higher-cost recorder units and have proven successful for many wildlife conservation monitoring projects (Prince *et al.* 2019; Toenies and Rich 2021). New technologies and their applications are being explored and improved (Revilla-Martín *et al.* 2021).

PAM also comes with challenges that vary depending on the research and requires consideration when selecting a long-term monitoring technique. Bioacoustics is an increasingly popular method, so comparisons to previous studies may be complex (Toenies and Rich 2021). The analysis of recordings is labour-intensive, may contain misidentifications, and environmental factors impact data collection (Barber-Meyer *et al.* 2020; Toenies and Rich 2021).

Frog vocalisations are a large part of the Bornean soundscape and vary from species to species (Inger *et al.* 2017). Vocalisations have a wide range of applications, from attracting and deterring other frogs to providing other frogs with environmental information such as rain or predators (Wells 2007; Inger *et al.* 2017). Different vocalisations have different call aspects, including duration, dominant frequency, volume, pulse repetition rate, and pulse “shape” (Inger *et al.* 2017). Species within a genus often share common call aspects (Inger *et al.* 2017). But variation exists even at an individual level (Davies and Halliday 1978; Arak 1983; Inger *et al.* 2017). Neighbours can recognise each other, and males attack others based on the pitch of calls, as this indicates their size (Davies and Halliday 1978; Arak 1983; Inger *et al.* 2017). Previous studies have used AudioMoths to record frogs (LeBien *et al.* 2020; Campos-Cerqueira and Aide 2021; Campos-Cerqueira *et al.* 2021; Hoffmann and Mitchell 2022; Ribeiro, *et al.* 2022). Due to the diversity and specificity of calls, local research on the native species is a requirement before selecting the most applicable technique. Comprehensive knowledge of anuran vocalisations in this area could lead to many other discoveries like undescribed species, previously unidentified calls, breeding patterns, and species ranges, as well as suggest improvements for PAM (Das *et al.* 2014). Inger et. al. (2017) recognises the importance of Bornean frog calls as it has ‘shown itself to be a rewarding avenue for further research’ and could create an extensive database.

Habitat loss and climate change are the main drivers of biodiversity loss and consequential extinctions, the preservation of secondary habitat could alleviate some of the pressures on native species (Sodhi *et al.* 2010; Kok *et al.* 2018). Reforestation is ongoing in the Lower Kinabatangan by the community initiative Kopel BHD and the Danau Girang Field Centre. Protection for amphibians and their habitats varies, and there needs to be clarity on the impacts of conservation efforts (Wells 2007). A thorough understanding of anurans could provide a representative indicator of the effects of this restoration. Therefore, finding an efficient data collection technique is of value. To effectively preserve this biodiverse taxon, the population status of amphibian species should be well defined, and the impacts of these conservation efforts well documented (Das *et al.* 2014). This study aims to evaluate the performance of AudioMoth recorders compared to the standard transect-based technique of active searches and the feasibility of long-term PAM.

3. Materials and Methods

3.1. Study Area

The Lower Kinabatangan River is in the Malaysian state of Sabah (5°10′–5°50′N; 117°40′–118°30′E) in northeast Borneo. It flows through a large flat floodplain (10-20m asl), mainly classified as an extreme lowland forest (Gillespie *et al.* 2012). Mean annual precipitation is between 2600 and 3600mm and temperatures range from 21°C to 34°C (Sooryanarayama 1995; Gillespie *et al.* 2012; Scriven *et al.* 2018). The river can rise 5m overnight with widespread rain upriver (Boonratana 2000). Wet periods include November to March with Northeast Monsoons and April to August with Southwest Monsoons (Sooryanarayama 1995; Das *et al.* 2014). Between the 1960s and 1995, this floodplain experienced widespread deforestation to make way for a range of plantations, oil palm plantations being the current dominator (Azmi 1998; Gillespie *et al.* 2012; Scriven *et al.* 2018). Due to this logging, the floodplain is almost entirely regenerating secondary forest, varying in levels of disturbance (Mcmorrow and Talip 2001). These secondary forests create a series of State Forest blocks or 'lots' amongst plantation monocultures, small villages, and agricultural land (Gillespie *et al.* 2012; Abram *et al.* 2014). These lots and protected forest blocks create the 27,960-ha Lower Kinabatangan Wildlife Sanctuary (LKWS), gazetted in 2005 by the State Government of Sabah (Gillespie *et al.* 2012; Abram *et al.* 2014). In addition to this reserve, there are 10,000ha of uncleared state and privately-owned forests and 15,000 ha of Virgin Jungle Reserves (VJRs) (Ancrenaz *et al.* 2004). These patches of remnant forest and protected areas form a partially fragmented corridor extending 70km from the coastal mangrove swamps upstream to the dry-land foothill forests (Gillespie *et al.* 2012).

3.2. Site Selection

Four study sites were selected, three forest sites: Laab Swamp (a peat swamp), Kaboi Stumping (a freshwater riparian swamp), Kaboi Lake (a freshwater swamp), and one oil palm plantation named Hillco. At these forest sites, the community initiative Kopel BHD, and the Danau Girang Field Centre are restoring the lost native forest. This restoration provides three forest ages; active restoration (aged between 1 and 4 years), restored forest (aged 5-20 years) and the natural secondary forest. The Pin Supu Forest Reserve acts as a link between lots 7 and 8 of the LKWS and is jointly managed by KOPEL and the Sabah Forestry Department. Transect length and arrangements at each site differ due to size restrictions and to avoid edge effects (Scriven *et al.* 2018). Laab Swamp contains transects of 100m, Kaboi Stumping transects are 200m, and Kaboi Lake contains two parallel 100m transects at each forest age. Comparisons between sites are not possible due to the variations in transect length and arrangement, habitat types, type of restoration technique, and time since restoration efforts began. Hillco contains a 200m transect, 100m in the oil palm plantation and 100m in the High Conservation Value (HCV) area, to sample the full range of anurans utilising the plantation habitats available.

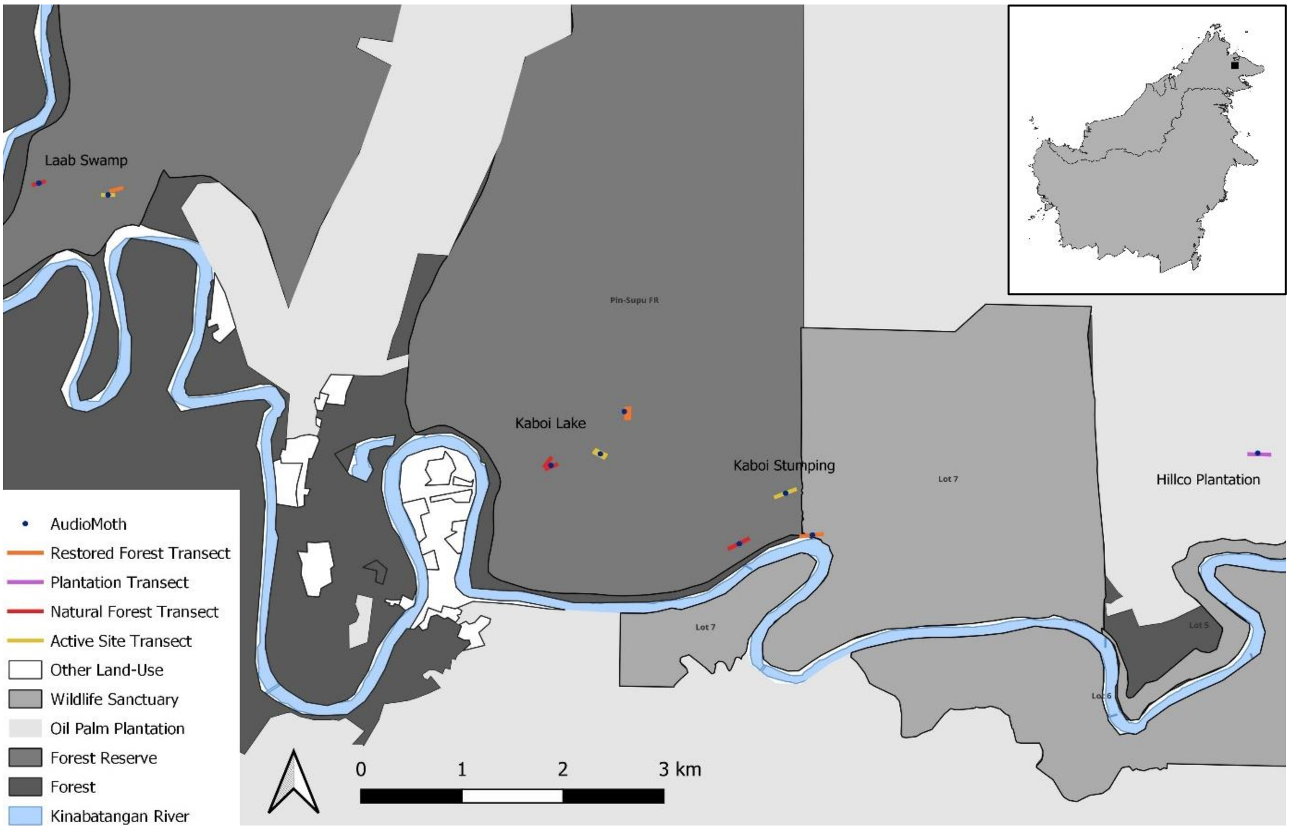

Figure 1 The Lower Kinabatangan Wildlife Sanctuary in Sabah, Malaysian Borneo. The study area contains forests, forest reserves, wildlife sanctuaries and the surrounding oil palm plantations. The ten transects and the associated AudioMoth placement are coloured based on the restoration process. The top right corner shows the map of Borneo, and the black circle shows the location of the study area. Made in the QGIS programme (Version 3.28.2) (QGIS Development Team 2022).

3.3. Anuran Sampling

3.3.1. Study Taxa

This floodplain contains 39 recorded frog taxa to date, and further research may uncover more species (Gillespie *et al.* 2021). Six families: *Bufonidae, Dicroglossidae, Microhylidae, Megophryidae, Ranidae, and Rhacophoridae*, and two taxa that are not yet formally described, which may be endemic to the region (Gillespie *et al.* 2021). These frogs are highly diverse in ecological range and reproductive strategies, utilising various microhabitats from leaf litter to forest canopies (Gillespie *et al.* 2021). Taxa reproduce in ephemeral puddles, swamps, streams, and tree hollows or through direct development (Gillespie *et al.* 2021). Most species specifically adapted to their respective forest habitat and are dependent on those areas (Gillespie *et al.* 2012; Scriven *et al.* 2018). Due to deforestation and degradation, these species have faced significant range contractions (Gillespie *et al.* 2012; Scriven *et al.* 2018). Distinct communities of non-forest frog species exist in oil palm plantations and human-modified habitats (Gillespie *et al.* 2021). This community is mainly generalist species with lower sensitivities to microclimatic and microhabitat alterations, particularly regarding their reproductive strategies (Gillespie *et al.* 2012; Scriven *et al.* 2018). As the plantations expand and species assemblage differs, sampling techniques must be tested in these areas (Gillespie *et al.* 2012; Abram *et al.* 2014; Scriven *et al.* 2018). Vocalisations, habitat use, and conspicuousness are species-specific and impact detection success (Inger *et al.* 2017; Köhler *et al.* 2017; Gillespie *et al.* 2021). Therefore, a range of species and habitats are included in this study to provide a representative, unbiased comparison of techniques (Heyer *et al.* 1994). Monitoring is ongoing, and anuran inventories have been conducted in this region (Gillespie *et al.* 2012; Scriven *et al.* 2018). However, this paper aims to identify whether technological advancements can improve data collection and anuran monitoring.

3.3.2. Active Surveys (AS)

Data collection occurred between November 2022 to June 2023. Sampling twice in the wet and dry seasons aimed to capture seasonal and temporal variation, resulting in four rounds of 40 censuses (Gillespie *et al.* 2012). A census contained four consecutive nights of data collection at each transect (Gillespie *et al.* 2012). The rounds were a minimum of 23 days apart on any single transect. Surveys started from 1830h-2200h to coincide with a high level of anuran activity (Konopik *et al.* 2015). At sites with multiple transects, the order of transects sampled rotated each night to reduce temporal effects and consequential systematic bias. Two or more observers searched acoustically and visually for anurans along a transect for a survey (Gillespie *et al.* 2012). Within a range of 2m on either side of the transect, leaf litter, logs, understory vegetation and tree trunks were examined with torches from the transect start coordinate (Gillespie *et al.* 2012; Scriven *et al.* 2018). All transects had a standardised pace and maximum time limits (30 minutes per 100m). Identification followed nomenclature by Inger *et al.* (2017) and Gillespie *et al.* (2021).

3.3.3. Passive Acoustic Monitoring (PAM)

Passive Acoustic Monitoring (PAM) meant placing an AudioMoth device (software version 1.8.1.) in a position where its range covered the same area as the transect. Each transect had an AudioMoth however, at the Laab active and restored transect, a single device recorded data for both transects. The typical range for an AudioMoth is 200m for ecosystem-wide monitoring, and the area available at Laab is limited, so one device picked up data for both transects (Rainforest Connection 2023). Recorders were secured to trees 1.5m from the ground in Rainforest Connection waterproof cases. Methods followed the standard procedure for monitoring in this area and for studies involving AudioMoths and anurans (LeBien *et al.* 2020; Campos-Cerqueira and Aide 2021; Campos-Cerqueira *et al.* 2021; Ribeiro, *et al.* 2022). Calls were recorded for 1 minute every 5 minutes, for at least the duration of a survey; the sample rate was set at 48 kHz on a medium gain (30.6 dB) as is used for anuran PAM studies (LeBien *et al.* 2020; Campos-Cerqueira *et al.* 2021; Hoffmann and Mitchell 2022; Ribeiro, *et al.* 2022). Data collection occurred at the same time and location for both techniques. The same observers completed the species count comparison of the two techniques. Analysis included Raven Pro (version 1.6.4), as it is often used to analyse anuran vocalisations and aids in identifying vocalisations (Köhler *et al.* 2017; Melo *et al.* 2021). Calls of known species were matched to the recordings using information from Inger *et al.* 2017 and the knowledge of local experts.

3.4. Optimum Sampling Rate

Limiting factors of long-term device deployment include data storage, power supply and hardware durability (Wood *et al.* 2023). Lower sampling rates can alleviate power and storage constraints but may lead to fewer detections (Wood *et al.* 2023). Identifying the optimum sampling rate included continuous recordings from round two. As each transect had four nights of data, random selection chose the night used in the analysis. This totalled 238 minutes of data analysis. Sampling rates covered a range of times (shown in Table 1). A count of the number of species found for each sampling rate started at the first minute. From here, only minutes included in the sample rate pattern contributed to the total number of species for a recording.

3.5. Detection Range

To ensure a fair comparison, the distance range of AudioMoths needs to adequately cover the transect length for the opportunity to detect the same anurans in the area. In the forest surrounding the Danau Girang Field Centre (study site at 05° 24' 48'' N, 118° 02' 16'' E), three AudioMoths placed at a height of 1.5m (LeBien *et al.* 2020) at the end of a 125m transect recorded sounds played at the distances 0m, 25m, 50m, 75m, 100m, and 125m. Frog calls from a range of common species (*Indosylvirana nicobariensis, Kaloula baleata, Kurixalus chaseni, Pulchrana glandulosa, Zhangixalus dulitensis*) played from an iPhone 13 mini (model number MLK43B/A) facing the speaker north at maximum volume and half volume. Vegetation density was measured using a density stick at each set distance to correlate the impact on detection distance (Köhler *et al.* 2017; Rainforest Connection 2023). The AudioMoths faced north, east, south, and west repeating the same procedure on the same transect for each orientation.

3.6. Habitat Parameters

Frogs are sensitive to slight changes in environmental conditions, therefore climatic effects are incorporated to reduce bias (Heyer *et al.* 1994). Temperature and humidity regulate the vocal activity period for many species and an EasyLog USB (Version 7.7.0.0) recorded both every 5 minutes (Wells 2007). Many anuran species in the Lower Kinabatangan depend on waterbodies for basic survival and reproduction (Inger *et al.* 2017). The frequency and duration of reproduction events vary between species and are often triggered by weather conditions such as heavy rains

(Wells 1977; Heyer *et al.* 1994). Weather observations and available aquatic sites were recorded for each survey due to this dependence. Vegetation variation influences the available microhabitats and thus anuran diversity (Vitt *et al.* 1990; Gillespie *et al.* 2015; Scriven *et al.* 2018). Data analysis investigated the effect of vegetation structure, density, and percentage cover for each transect. Supplementary Information Appendix 5 - 6 provides further details. Variations in environmental conditions also affect bioacoustics due to differential excess attenuation and reverberation on vegetation (Köhler *et al.* 2017). The effect of these variables requires consideration when selecting a sampling technique. (Köhler *et al.* 2017). The number of species detected by each sampling technique was correlated with these ecological variables.

3.7. Data Analysis

R studio (Version 2023.06.1) and Microsoft Excel generated statistical analysis and plots (RStudio Team 2023)(Microsoft Corporation 2023). The accepted significance was at a p-value of 0.05. Shapiro-Wilk normality tests tested the normality of data sets before further analysis. Pearson’s Chi-Squared test directly compared total species detection by AS and PAM, AS and PAM diversity indices, and different sampling rates against the continuous recording. Further comparison of AS and PAM utilised the following tests: a Wilcoxon signed rank test with continuity correction, an asymptotic two-sample Kolmogorov-Smirnov test, and a Spearman’s Rank correlation Rho. In RStudio, the vegan package (Oksanen *et al.* 2022) calculated diversity indices of Shannon and Simpson’s diversity indices. The packages iNEXT (Hsieh *et al.* 2022) and ggplot2 (Wickham *et al.* 2023) generated sample-based based rarefaction curves (Figure 4). Sample-based rarefaction curves interpolated and extrapolated the data for a) species richness, b) Shannon diversity, and c) Simpson’s diversity to see the impact of increasing the number of individuals on species diversity. The average number of total species detected by each sampling technique doubled to create the endpoint for the sample-size-based plots (888). Pearson's product-moment correlation assessed the correlation between average density and average frog detection at maximum volume, and Kendall's rank correlation tested at half volume. The effects of environmental heterogeneity resulted in thirteen environmental variables (see Appendix 9). The continuous and categorical variables produced a visual comparison of techniques and sites. The package cluster generated the cluster plots (Maechler *et al.* 2022). To identify any significant factors, Poisson generalised linear models statistically compared each sampling technique to the 13 environmental variables.

4. Results

Widespread flooding, adverse weather, and hazardous wildlife meant only 107 censuses out of the proposed 160 were sampled. Sampling occurred a minimum of once in the wet and dry seasons for each transect. Three censuses were possible for most transects. Only 66.9% of the proposed sampling could occur. This percentage highlights the challenges of obtaining large, standardised data sets in the study area. For a direct comparison, each transect census included sampling with both techniques. Taxonomical identification to species level on the genus *Microhyla* was not always possible. Two groups classified the individuals found to avoid inaccuracies. *Microhyla* with three digits (M3) or four digits (M4) on their front feet. This grouped species with this description from Gillespie *et al.,* 2021 and Inger, 2017. Species-level identification was possible for all other individuals.

4.1. Anuran Sampling

4.1.1. Plots and Statistical Tests

On average, AS detected four species per transect with a range from 0 to 8 species detected and an outlier of 9, as shown in Figure 2. On average, AudioMoth recorders detected two species and had a range of 0 to 5. The data from both sampling techniques do not show a normal distribution (Shapiro-Wilk normality test results for AS (W = 0.91266, p = 3.02e-06) and PAM (W = 0.92227, p = 9.979e-06). A chi-squared between the number of species detected by AS and PAM at each transect showed a significant difference (X-squared = 94.624, df = 45, p = 2.18e-05). Therefore, AS detected species at a significantly higher frequency over the sampling period than PAM. As the data is non-parametric, a Wilcoxon signed rank test with continuity correction was used and showed a significant difference between the two sampling techniques (V = 4355, p = 2.364e-16). Therefore, the population mean ranks obtained by each technique significantly differ. An asymptotic two-sample Kolmogorov-Smirnov test also found a significant difference between the two sampling techniques (D = 0.46729, p = 1.425e-10). The two samples coming from the same distribution is the null hypothesis. This result rejects the null hypothesis, indicating PAM currently cannot replace AS and get the same results. A Spearman's Rank correlation Rho tested the strength of association between two variables and showed a significant value (S = 116669, p = 4.127e-06) proving the techniques produce significantly different results. A rho value of 0.4285304 also suggests no significant relationship between the two variables.

4.1.2. Species Diversity and Rarefaction Curves

Each sampling technique had Shannon and Simpson's diversity indices calculated. A Pearson's Chi-squared test on these values showed that the diversity indices captured by each technique were not significantly different (X-squared = 702, df = 676, p = 0.2369). Figure 3 shows the total species detected by each technique during the sampling period. This study found 16 species in total. AS detected 15 species, five unsampled using PAM. PAM detected 11 species, one unsampled by AS. Detection varied between species, the two species with the highest detection rate for both techniques were *Kurixalus chaseni* (155) and *Pulchrana glandulosa* (159). The two most frequently sampled species were *Chalcorana megalonesa* (81) and *Pulchrana glandulosa* (80) for AS and *Kurixalus chaseni* (80) and *Pulchrana glandulosa* (79) for PAM.

To show the impact of increasing the number of individuals recorded on the diversity values, the sample-sized based rarefaction curves interpolated and extrapolated the data to an endpoint of 888 individuals (Figure 4). Species richness, Shannon diversity and Simpson's diversity curves plateaued for AS, suggesting complete sampling of dominant species, and further sampling would not cause an increase in these diversity indices. The curves for AS have a narrow confidence interval showing the estimate is stable and shows a degree of certainty with this sampling method. The Shannon and Simpson's diversity curves also plateaued for PAM and have a small confidence interval. Therefore, they are not assumed to increase with further sampling. The species richness curve for PAM did not plateau and could suggest a lack of recording for some species. Unlike the other curves, the PAM species richness curve shows a wide confidence interval indicating the estimate has a relatively higher degree of uncertainty with this sampling method.

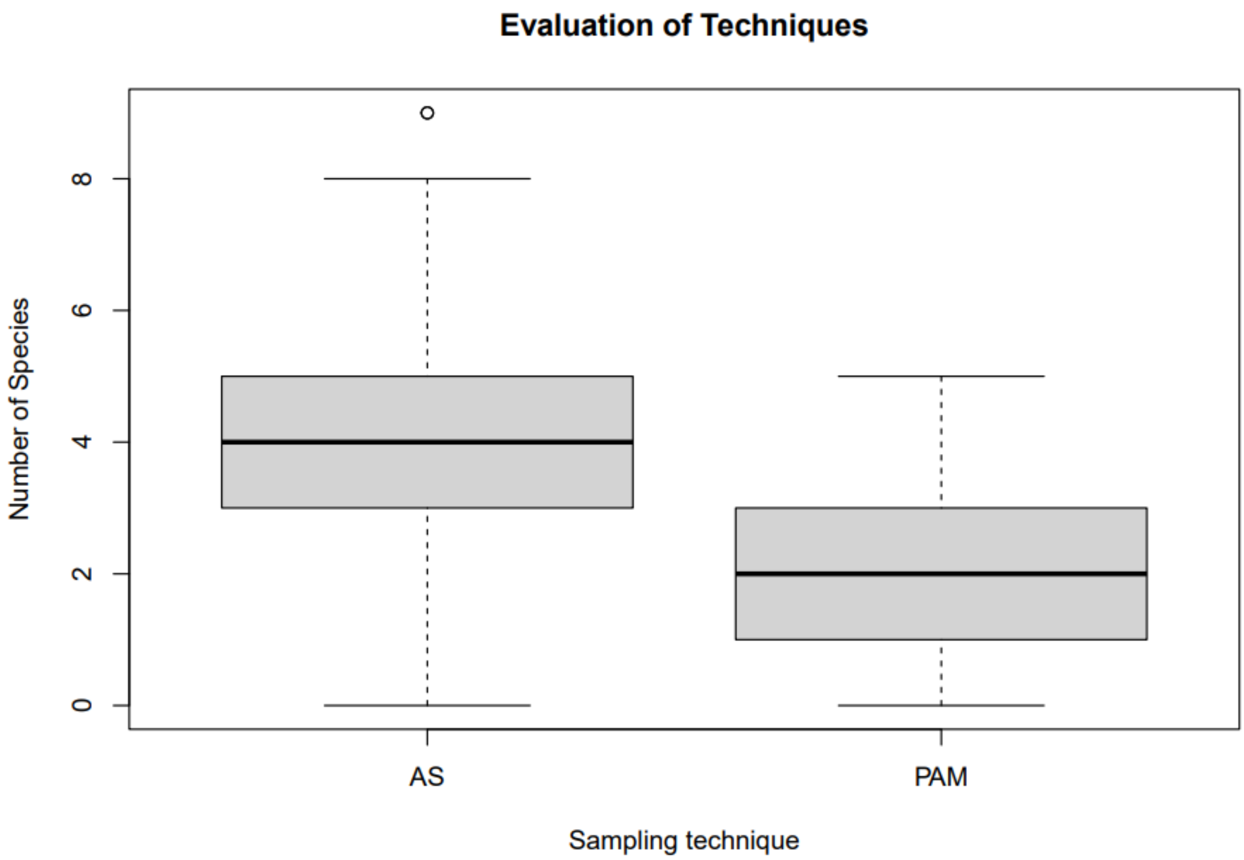

Figure 2: A boxplot of number of species detected at each survey. This plot shows the average, quartiles, and range for the number of species detected by each sampling technique. AS contains an outlier (9). The interquartile ranges of both techniques show minimal overlap.

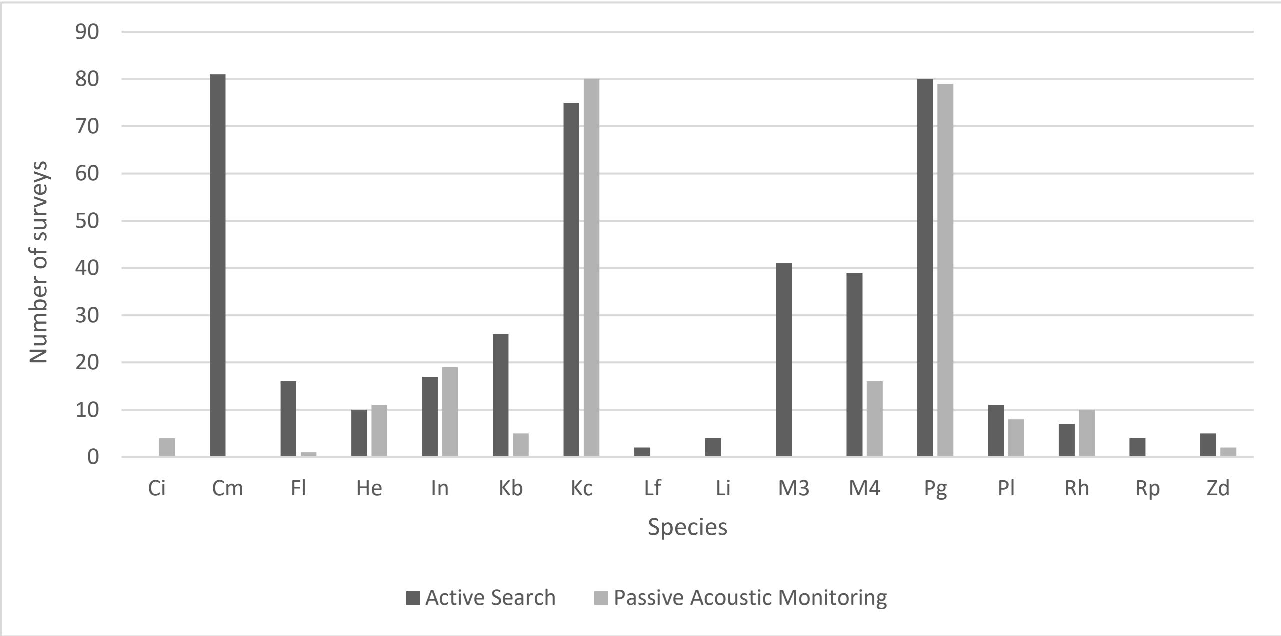

Figure 4: The species detected by each sampling technique and the frequency of surveys that detected each species. The species list is as follows: Ci (Chiromantis inexpectatus), Cm (Chalcorana megalonesa), Fl (Fejervarya limnocharis), He (Hylarana erythraea), In (Indosylvirana nicobariensis), Kb (Kaloula baleata), Kc (Kurixalus chaseni), Lf (Limnonectes finchi), Li (Limnonectes ingeri), M3 (Mircohyla genus with three fingers), M4 (Mircohyla genus with four fingers), Pg (Pulchrana glandulosa), Pl (Polypedates leucomystax), Rh (Rhacophorus harrissoni), Rp (Rhacophorus pardalis), Zd (Zhangixalus dulitensis). Nomenclature from Gillespie field guide. Produced in Microsoft Excel (Microsoft Corporation 2023).

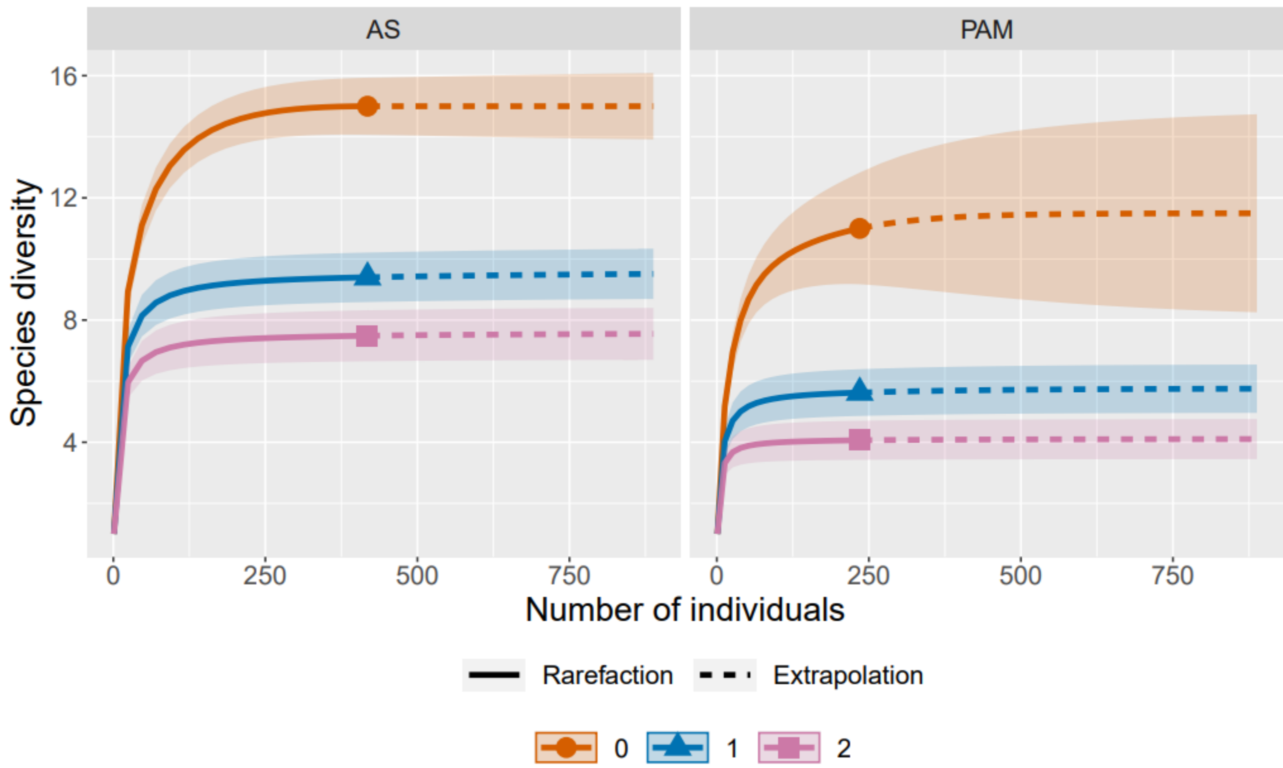

Figure 3: A plot with rarefaction and extrapolation curves showing the number of individuals and species diversity for each sampling technique. The curves show how the number of individuals impacted species diversity and the continued impact as the number of individuals increased to an endpoint of 888 individuals. The key is as follows: AS: Active Surveys, PAM: Passive Acoustic Monitoring, 0: Species richness, 1: Shannon diversity, and 2: Simpson diversity. The number of recorded individuals varies for each sampling technique: AS (418), PAM (235). Either side of each line shows the confidence intervals.

4.2. Optimum Sampling Rate

Detection accuracy does decrease at sampling rates of 8 and 10 minutes and suggests extended periods between sampling lowers detection accuracy. Statistical tests compared the detection accuracy of the continuous recording (the expected value) to the different sampling rates (the observed value). Pearson's Chi-squared tests did not get a p-value below the 0.05 threshold, so detection accuracy does not significantly change with a sampling rate range of continuous to a minute every 10 minutes.

4.3. Detection Range

The distance from the AudioMoth and the counts of frog calls detected are negatively correlated; counts decrease with the distance increased, as shown in Figure 5. Frog calls played at maximum volume have greater detection distances than calls played at half volume. A Pearson's product-moment correlation test showed no significant correlation between average vegetation density and average frog detection at maximum volume ($t = 0.30446$, $df = 4$, $p = 0.776$). Further calculations show a 2% correlation between the two variables. The average calls detected at half volume showed a non-parametric distribution. The average number of calls detected at half volume compared to average vegetation density produced a p-value of 1 ($z = 0$, $p = 1$) using Kendall's rank correlation. As both these tests resulted in no correlation between plant density and frog call detection, another variable may be responsible for the detection range. Values extrapolated at a confidence of 0.05 gave the maximum distance where 95% of calls were heard (Figure 5). Averaged from the three AudioMoth devices, the detection distance was 19.5m at maximum volume and 2.1m at half volume. These values input into the following equation gave the area covered by the AudioMoth devices:

$$\pi r^2$$

The ranges covered by the AudioMoth devices included 1194.59m^2 at maximum volume and 13.85m^2 at half volume for common species calls.

Table 1: Contains the Pearson's Chi-squared test results of each sampling rate. Sampling rates and corresponding recording pattern x: y where x equates to the minutes recording and the y is the minutes not recording before repeating the pattern. Total Incidences of Species Detected refers to the number of species recorded at each transect combined to create a total. Detection accuracy compares the number of species detected by each sampling rate to the number detected on the continuous recording. X-squared, df (degrees of freedom) and p-value are the results of Pearson's Chi-Squared test. This test compared the Incidences of Species Detected at each transect of each sampling rate individually to those detected by the continuous recording.

Sampling Rate (minutes)	Recording Pattern (x: y)	Total Incidences of Species Detected	Detection Accuracy	X-squared	df	p-value
2	1:1	21	100.00%	0	9	1
3	1:2	21	100.00%	0	9	1
5	1:4	21	100.00%	0	9	1
8	1:7	20	95.24%	0.17571	9	1
10	1:9	16	76.19%	0.7378	9	0.9998

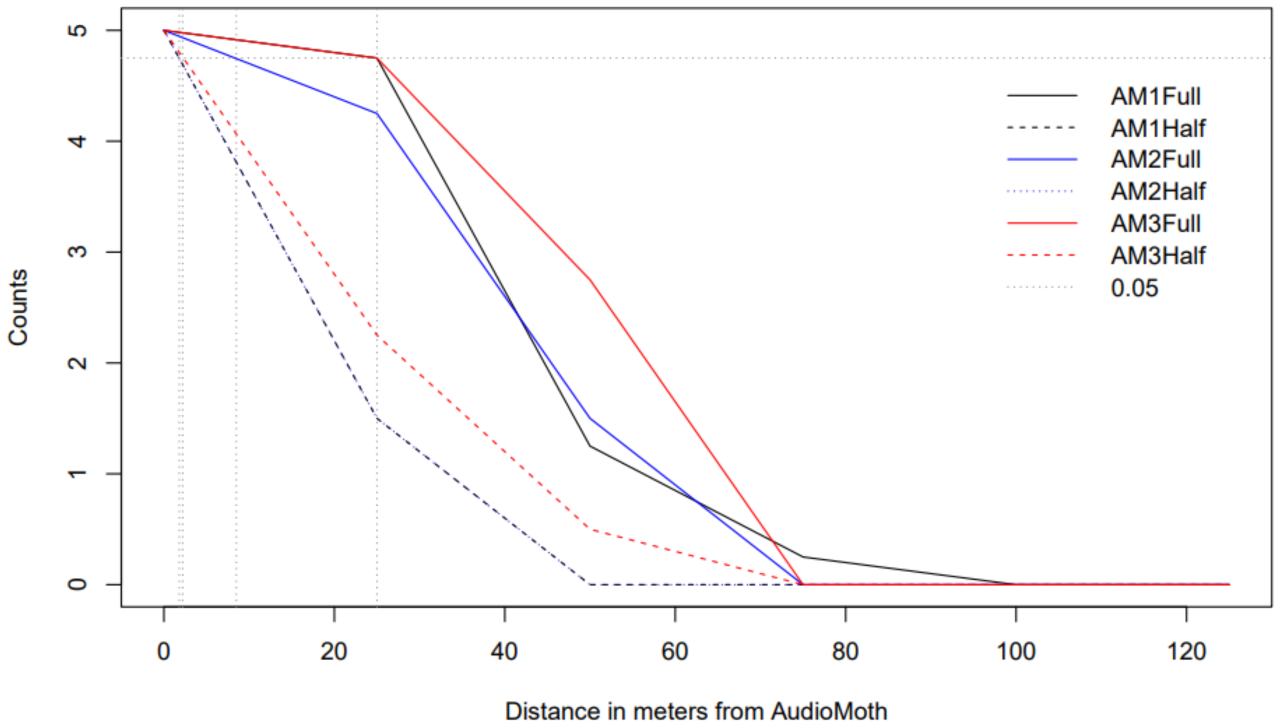

Figure 5: Graph shows the correlations between frog counts detected and distance from the AudioMoth. AM1, 2 and 3 refer to a different AudioMoth device. Counts refer to the number of frog calls detected. 0.05 represents the 95% accuracy used to infer the distances of call detection at maximum and half volume.

4.4. Habitat Parameters

Cluster analyses used Gowers distance for ecological variables (Figure 6 and Appendix 8-9). Techniques collected data simultaneously at the same transects, so both techniques had a complete overlap when compared (Appendix 7). Comparison of sites uses the two components with the highest variance (Components 1 and 2) and shows forest sites cannot be distinguished at different stages of the reforestation process as a range of sites are close together or overlap (Figure 6). However, PC1 appears meaningful in distinguishing between plantation and forest environmental conditions. A Poisson GLM on AS and the 13 environmental variables showed no significant values (Appendix 9), indicating these variables had no significant impact on the number of species found. The environmental variables also did not significantly impact PAM performance except for water availability which significantly impacts the number of species found (Pr(>|z|) value 0.00109).

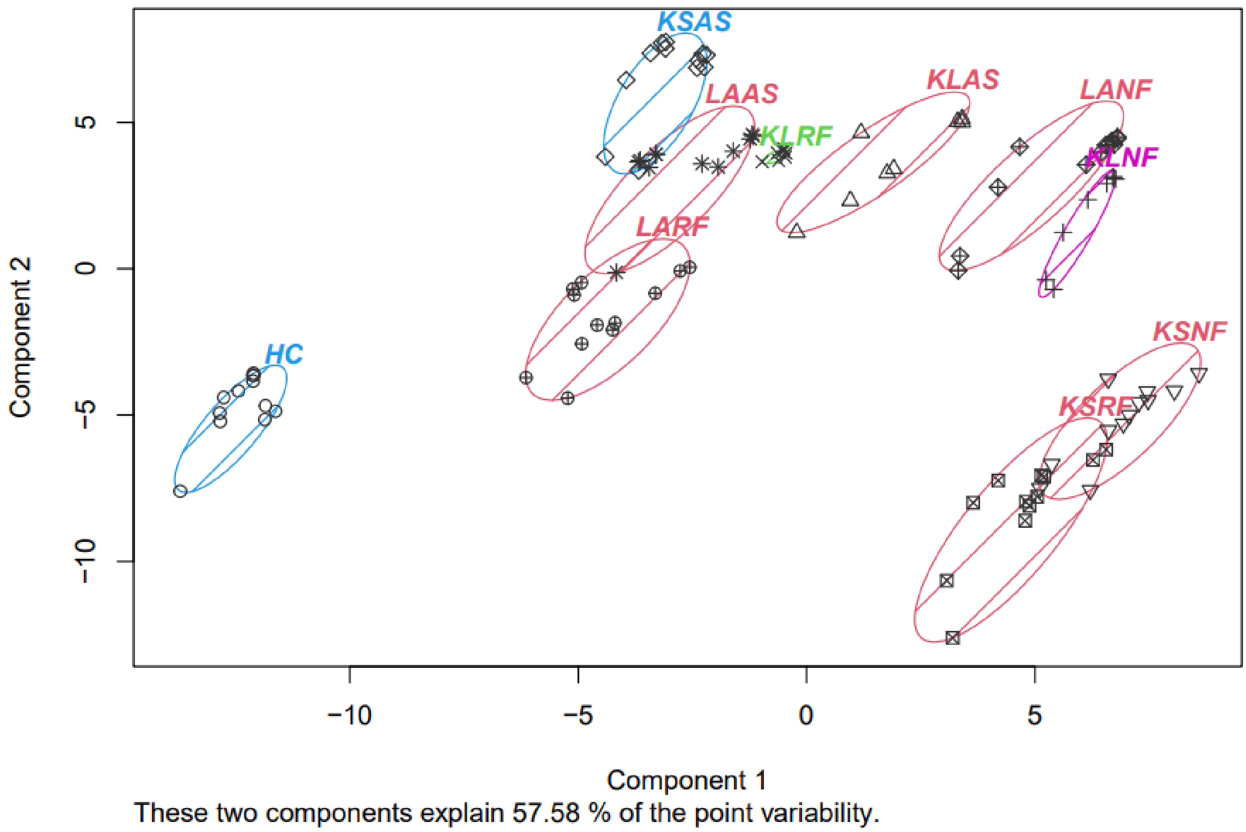

Figure 6: A cluster plot to show the sites when plotting with Components 1 and 2. These components show the highest variation across the dataset. PC1 appears meaningful in distinguishing between plantation and forest environmental conditions. Neither component can distinguish between forest sites based on the reforestation process. Sites in different reforestation stages are close together and show overlap. Due to a lack of surveys for KL (Kaboi Lake transects), direct site comparisons in this area are not possible. Ellipses' colour shows their density, starting with light blue, light green, red and purple, increasing in density.

5.0. Discussion

This study is the first to compare the efficiency of AudioMoth recorders compared to the standard transect-based active searches for the anuran species found in the Lower Kinabatangan; and to address the feasibility of PAM for future research. Technological advancements can revolutionise data collection and, therefore, maximise efficiency when conserving areas of ecological importance (Wood *et al.* 2023). Comprehensive and standardised data will aid in streamlining conservation efforts in critical habitats (Heyer *et al.* 1994). Identifying improvements can get us closer to higher efficiencies and accuracies when sampling. This study has quantified the efficiency of active searches and AudioMoth acoustic recorders.

5.1. Anuran Sampling

The number of species obtained using AS and PAM significantly differed in a range of tests indicating PAM cannot replace AS to get the same results with this sampling scenario. AS significantly performed better than PAM on species detection across the transects. Other studies have also found AS to be a sensitive sampling technique and still required in long-term monitoring programs, especially to detect those not vocally active (Parris *et al.* 1999; Boullhesen *et al.* 2021). The sampling scenario was biased towards AS, with the bioacoustics only being analysed during AS, to directly compare the techniques. A previous study investigated the effect of sampling scenarios between AS and PAM in Brazilian savanna wetlands, and when sampling was biased towards AS, it also performed better (Melo *et al.* 2021). However, under a similar or biased-towards- PAM sampling effort, PAM detected more species (Melo *et al.* 2021). Under different sampling methodologies and efforts, PAM may outperform AS, as in other studies (Melo *et al.* 2021).

The diversity sampled by each technique didn't significantly differ, despite AS capturing more species than PAM, and these results suggest they both target the same species. PAM could be a viable technique to estimate anuran species richness in the Lower Kinabatangan floodplain. Similar conclusions have been made in other parts of the world (Boullhesen *et al.* 2021; Melo *et al.* 2021). Some species calls are undocumented such as *Microhyla perpava,* and 5 out of the 16 species detected by AS did not have calls available online to compare to. Undocumented calls and incomplete databases of reference vocalisations hinders PAM accuracy. Further research into species-specific vocalisations may improve performance. So, during sampling, species without recorded vocalisations found calling were identified and recorded, including *Kaloula baleata.*

Results show that AS had reached the required sampling effort to capture the diversity found in this region. Despite this, other species near the transect area included: *Fejervarya cancrivora, Nyctixalus pictus,* and *Polypedates macrotis;* suggesting AS has reached an asymptote for the transect area, but sampling other areas or incorporating an opportunistic approach could capture a wider range of species. The results for PAM showed that a sufficient sampling effort was not reached and further sampling is required to reveal the full potential of PAM. Increased sampling with PAM does not require increased onsite effort, but it can, however, require increased bioacoustics analysis programs and computer methods (Haryati and Dzati 2013). Analysis of anuran vocalisations tested different machine learning and analysis techniques, such as automatic syllable segmentation (Haryati and Dzati, 2013; Wood et al., 2023). Automatic syllable segmentation can improve detection rates for anuran calls in Malaysia, but it is species-dependent, and detection did not increase with species also contained in this study (Haryati and Dzati 2013). Manual segmentation is as effective when analysing certain species, but automatic segmentation is a useful tool that can reduce analysis time (Haryati and Dzati 2013). Online analysis programmes require a stable internet connection, and the premade tools did not include many local species. Only manual analysis was possible due to a limited internet connection and time constraints. Investigating the performance of different types of bioacoustics analysis may show an increase in performance for PAM. Future goals include the development of a program customised to detect the calls of local frogs in the Lower Kinabatangan.

The challenges of accessing remote areas apply to active and passive surveys, but flooding and hazardous wildlife are the main constraints to data collection in this area (Wood et al., 2023). Without these hazards, these areas are accessible, so time is the pressure. Increased sampling effort and duration would overcome issues and could detect the temporal variation in activity across species (Melo *et al.* 2021). Acoustic devices could be left out in the field to collect data during times of inaccessibility. Longer deployment times would generate bigger data sets even with a lower detection rate. Technological malfunctions of the devices did occur and led to the resampling of a site. Although this was a rare occurrence, devices require testing before deployment.

Bioacoustics is suggested to fill in gaps for data-deficient taxa (Wood *et al.* 2023). Only PAM detected *Chiromantis inexpectatus* during this study. The vocalisations matched published descriptions, and confirmation was given by Samsir Laimun (Gillespie *et al.*, 2021). This species described in 2014 is of Least Concern (IUCN SSC Amphibian Specialist Group, 2018; Matsui, Shimada and Sudin, 2014). Its population size, status, distribution, life history, ecology, and threats are unknown (IUCN SSC Amphibian Specialist Group 2018). This arboreal frog is difficult to sample as it mainly resides in the upper limits of trees and only comes down to water bodies to breed (Matsui *et al.* 2014). Known distributions include the Lower Kinabatangan floodplain in the inundated ultra-low rainforest and the Maliau Basin Conservation Area, which is not easily accessible (Matsui, Shimada and Sudin, 2014; Gillespie et al., 2021). Chiromantis inexpectatus is a biogeographically significant species, as this genus was never expected on the island (Matsui *et al.* 2014). Recorders detected *Chiromantis inexpectatus* vocalisations on four occasions, twice in May and twice in June. Rain occurred on or around the days of detection.

These findings support information that males descend from the canopy and call on top leaves after rain (Matsui *et al.* 2014; Gillespie *et al.* 2021). Vocalisations were only detected in the natural forest sites suggesting *Chiromantis inexpectatus* avoids human altered habitats such as plantations, as is observed in many arboreal specialist species (Gillespie *et al.* 2012; Scriven *et al.* 2018). PAM has provided some insights into this important but largely unknown species. Further sampling may answer many of the unanswered questions. Other canopy-dwelling species, such as *Kurixalus chaseni* and *Rhacophorus harrissoni,* had a higher detection rate when using PAM*.* This reinforces the idea that the most suitable sampling technique will depend on the study taxa and aims (Heyer *et al.* 1994). PAM can aid in reducing data deficiencies for canopy-dwelling species that are often inaccessible (Wood *et al.* 2023). Previous studies have shown sampling effort is associated with species detection during monitoring programs (Melo *et al.* 2021), and PAM has the potential to improve these parameters and maximise detection rates (Melo *et al.* 2021). Technological developments, different sampling regimes and comprehensive knowledge of local vocalisations could result in PAM becoming the preferred monitoring technique to assess overall trends and indicate the health of ecosystems.

5.2. Optimum Sampling Rate

The results from this study show that detection success does decrease with lower sample rates, starting from a 1:7 recording pattern and beyond. However, the sampling rate can be modified as there are no significant differences in the detection success of each sample rate. The detection accuracy of identification systems varies between species (Haryati and Dzati 2013). The accuracy of different sampling rates may also be species-dependent due to variations in call aspects (Inger et al. 2017). Due to the species-specificity, maximising device efficiency requires tests on the target taxa. Device deployment in the study area has been as long as 79 days. This used 118GB of the 128GB microSD card. Device storage is of concern, but the battery remained functional. These tests found lowering the sampling rate could enable longer deployment times without significantly impacting detection success. Therefore, a wider sampling distribution could lead to higher detection rates as breeding events, regardless of duration, are more likely to be sampled (Melo *et al.* 2021).

5.3. Detection Range

The detection range of calls played at half volume decreased by 17.4m compared to maximum volume calls. This indicates low amplitude calls have lower detection probabilities and may be misinterpreted as a lack of low-amplitude anurans, this correlation has also been reported by studies on other taxa (Goerlitz 2018). AudioMoths cover a larger area than the transects when calls are at maximum volume but not as the volume decreases. With a maximum radius of 19.5m, device placement for the transect at the Laab restored site is insufficient to capture the vocalisations in this area. PAM at this site did not detect any species not also detected by AS and suggested the same community of frogs were targeted, potentially due to the small size of Laab. However, this may not be the case for other sites. Device placement should be investigated before deployment to adequately cover the study area, considering the amplitude of the vocalisations.

5.4. Habitat Parameters

The oil palm plantation was distinguishable from forest sites using PC1 (Figure 6), but forest sites at different stages of reforestation were not. This supports the concept that a distinct community of generalist species reside in plantations due to different environmental conditions (Gillespie *et al.* 2012; Scriven *et al.* 2018). As forest sites at different stages of reforestation were not distinguishable, another technique is required to show the environmental conditions of an area. Active restoration sites had ground vegetation removed around saplings, which may have influenced the habitat data recorded. Increasing the frequency of habitat assessments could increase accuracy, but this was not possible with the timescale and resources available. Bioindicators may show the effects of restoration efforts with higher accuracy, highlighting the need for research into suitable sampling techniques (Gillespie *et al.* 2012; Haryati and Dzati 2013; Scriven *et al.* 2018). Although both sampling techniques were identical for components 1 and 2, the GLMs found a difference between the two techniques. The availability of aquatic sites significantly impacts the number of species detected with PAM but not for AS. Many anuran species depend on water bodies for reproduction, and these breeding events allow detection by PAM due to the consequential vocalisations (Heyer *et al.* 1994; Wells 2007; Inger *et al.* 2017). This would have a lesser effect on AS as species can be detected visually without breeding vocalisations, and include voiceless anuran juveniles and females (Boullhesen *et al.* 2021). The other variables were not significant for either of the sampling techniques. This suggests deployment in a variety of habitat and environmental conditions is an option. Adverse weather may mask frog calls and reduce the detection success in PAM studies (Parris *et al.* 1999). Requiring adequate field conditions for AS meant this did not have a large effect.

5.5. Conclusions

The development and growing accessibility of technology has the potential to reduce data deficiencies worldwide (Melo *et al.* 2021; Wood *et al.* 2023). Overall, this study found AS is significantly better than PAM at detecting species in the Lower Kinabatangan rainforest. With no significant difference in total biodiversity indices either technique can target the anuran community found in the Lower Kinabatangan rainforest. However, PAM can detect arboreal species missed by AS; therefore, a combination of the techniques can provide more accurate representations of the overall species diversity at study sites. PAM could provide information on endemic, elusive, and novel species, such as *Chiromantis inexpectatus,* before biodiversity declines (Gillespie *et al.* 2012; Abram *et al.* 2014; Scriven *et al.* 2018). Reducing the sampling rate did not cause a significant loss in accuracy, and lower rates can prolong deployment. Detection distance is dependent on the amplitude of vocalisations. Identifying the most suitable sampling technique requires in-situ testing due to the species-specific vocalisations, site-specific challenges, and environmental variation. Further research into a comprehensive data base of local frog calls, the impact of increasing device deployment times or developments in bioacoustics analysis, the preferred method of ecological monitoring could be PAM (Melo *et al.* 2021).

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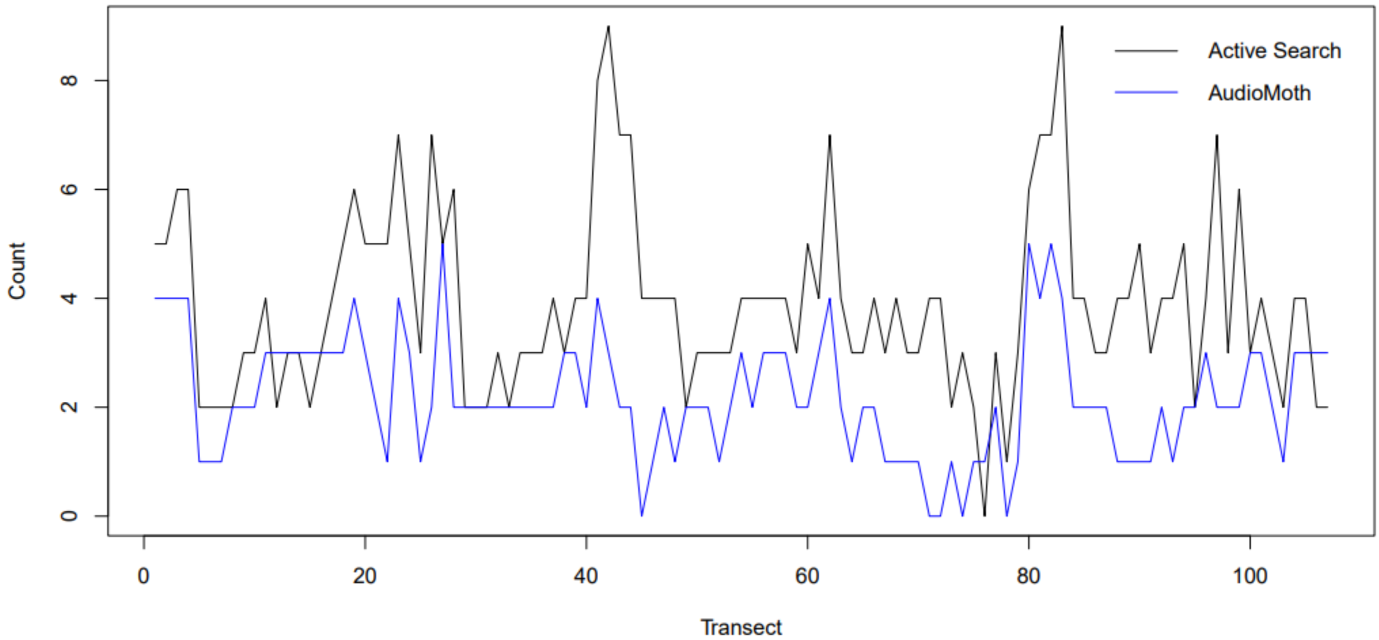
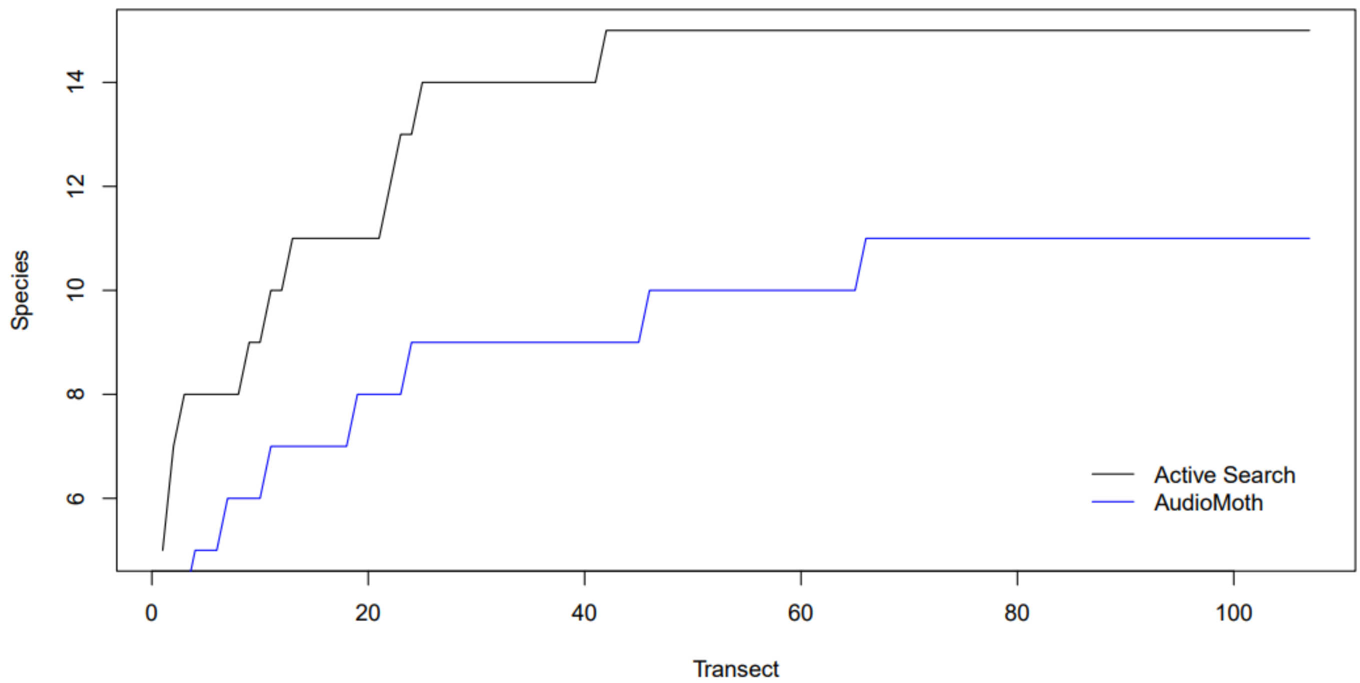
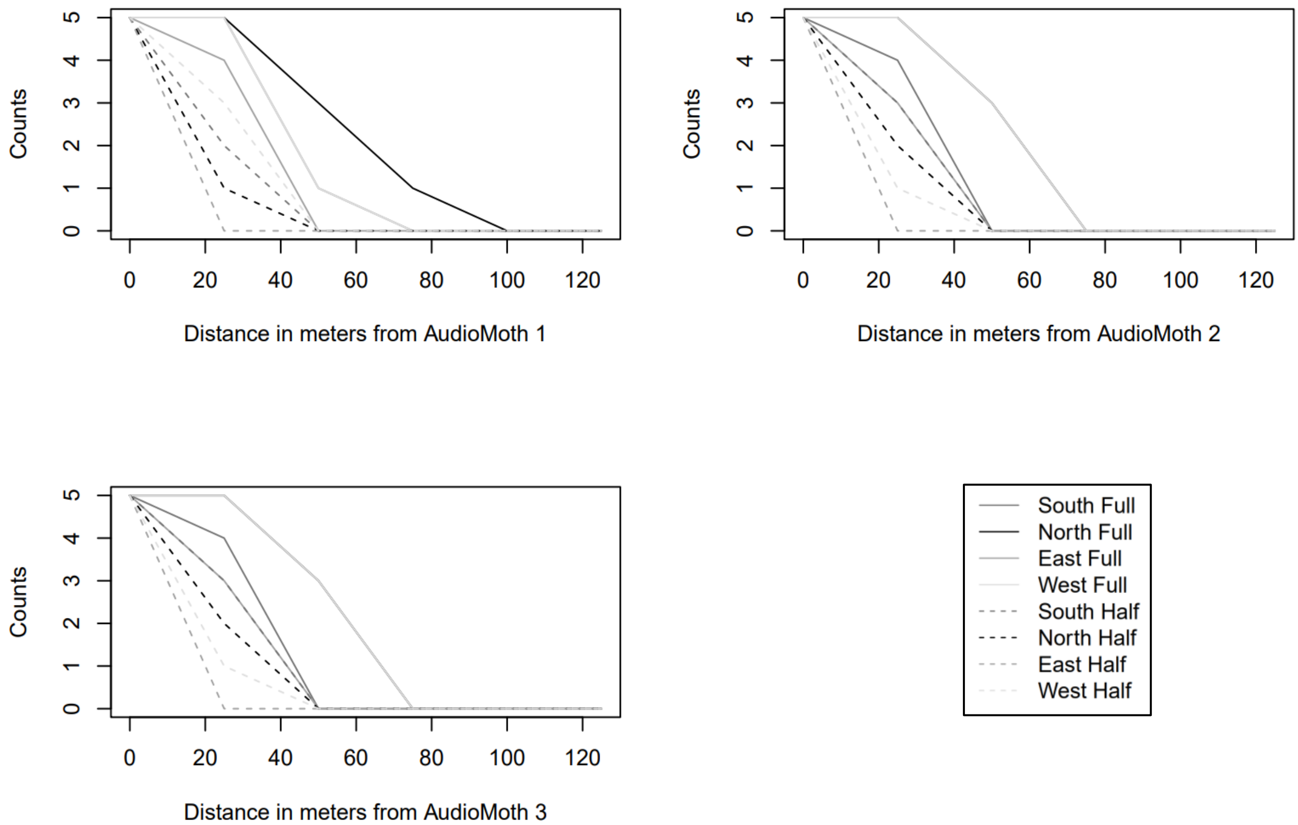
Supporting information



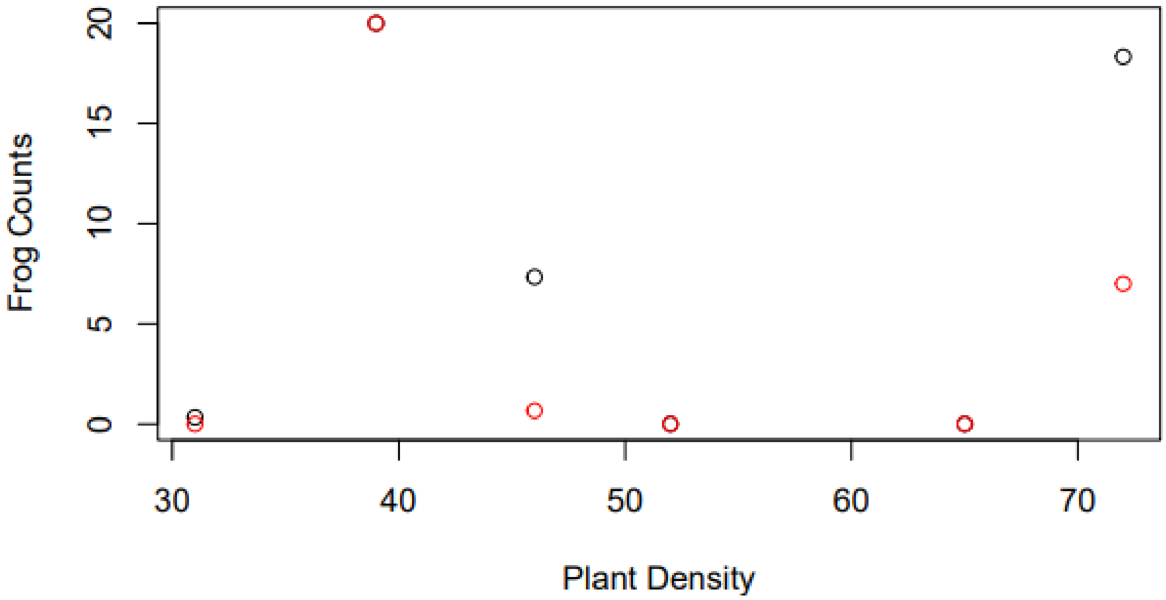
Appendix 1: A plot showing the number of species detected (Count) at each transect survey (Transect) by each sampling technique.



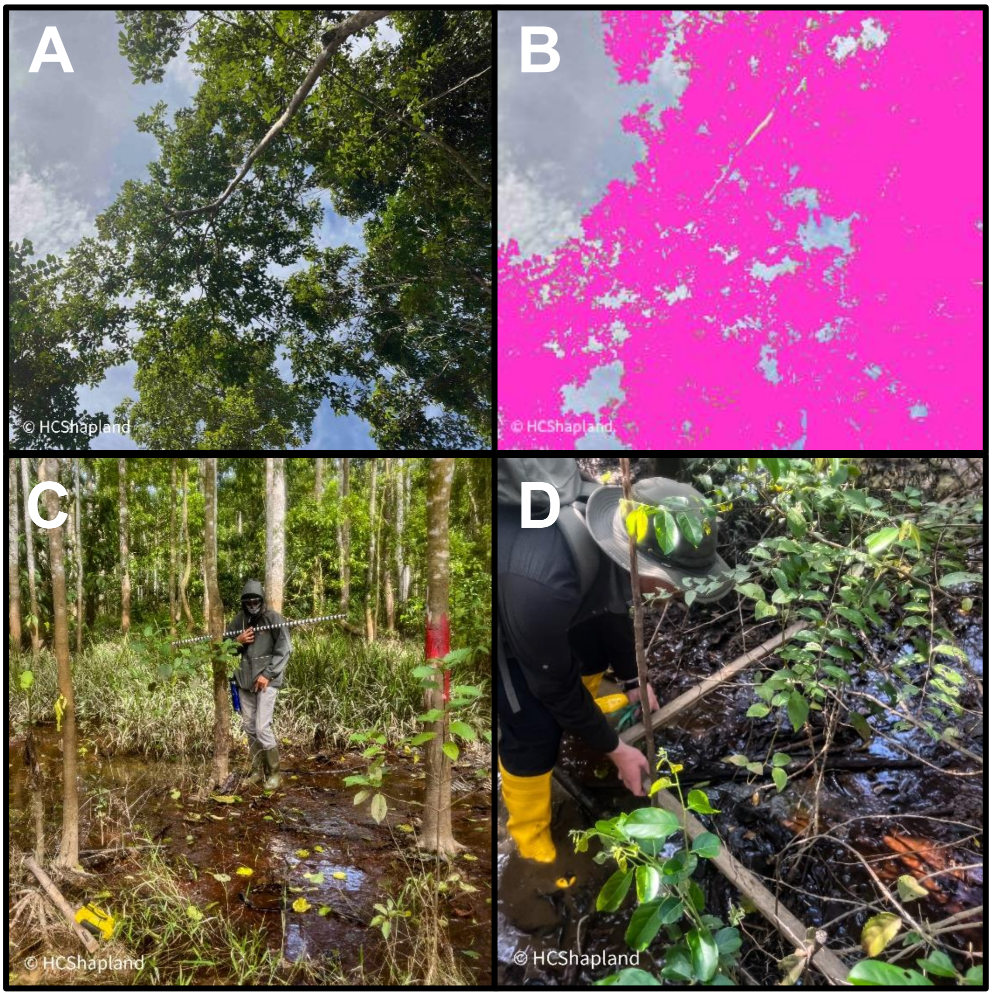
Appendix 2: A rarefaction curve to show the impact of increasing sampling effort on the number of species. The curve levels off at 42 for AS, and 66 for PAM.



Appendix 3: Plots to show the counts of frog calls as distance increases for three AudioMoth devices during the same test. The test was repeated for each compass orientation the AudioMoth devices faced (north, east, south, and west). Full refers to full volume and Half refers to half volume, the two volumes at which the frog calls were played.



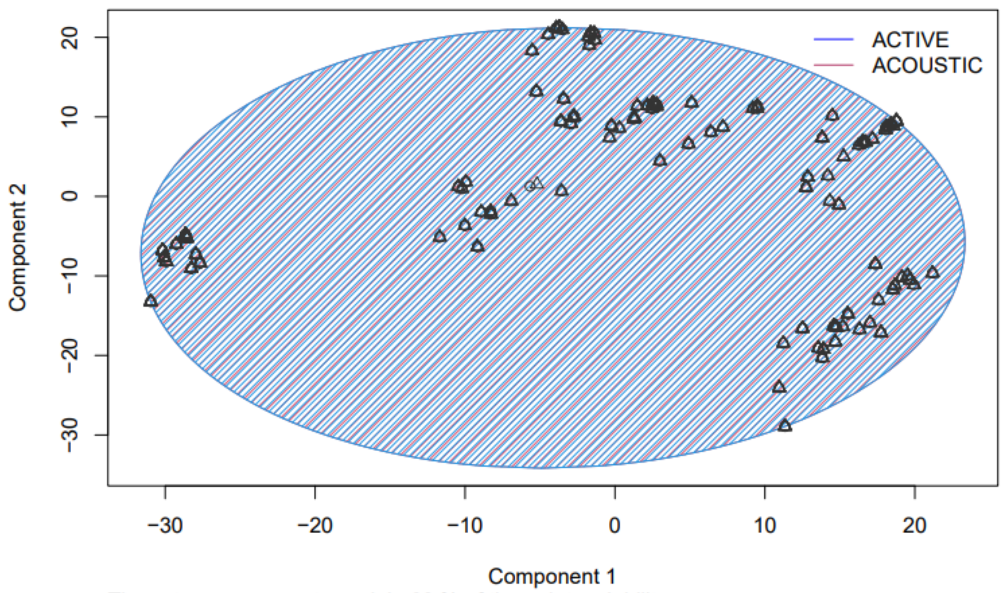
Appendix 4: a graph to show the relationship between the frog call count and plant density. This shows no correlation between frog counts and plant density. Ave1 = black, red = Ave2. Although the r is 0.15, the p-value is 0.776 which is higher than 0.05 and thus not significant. The 2% correlation was calculated using: (0.1504952) ^2 = 0.0226.



Appendix 5: Images outlining the habitat assessments completed at each transect. 8 m2 quadrats were randomly selected for each transect. Two quadrats for 100m transects and four quadrats for 200m transects. At the centre, north, east, south, and west points within the 8 m2 quadrat a series of measurements were taken within a 1m2 quadrat. Image A shows the canopy when calibrated with the gyroscope to standardise the images. Image B shows the canopy after it is filled in using the brush tool and sensitivity was increased to 100. Images A and B show steps required to calculate the canopy cover percentage using the CanopyApp (University of New Hampshire 2018). Image C shows the setup of using the density stick, the stick is then held horizontally, and the black stripes counted to calculate the vegetation density at knee and chest height for a given 1m2 quadrat. The number of black strips counted is doubled and then subtracted from 100 to get the percentage density of a 1m2 quadrat. Image D shows the 1m2 quadrat used to estimate the percentage cover of different vegetation types such as: ground cover, leaf litter, vines, ground vegetation and low vegetation. The understory depth is measured using the tallest vegetation found in the 1m2 quadrat. All the trees within the 8 m2 quadrat were counted and classified into ranges utilising diameter at breast height (DBH) including: ≤10, 11-20,21-30, 31-40, 41-50, ≥50. All measurements were completed by the same individuals to ensure standardisation between quadrats. Measurements from all the quadrats were average to produce one value in each category for a transect.

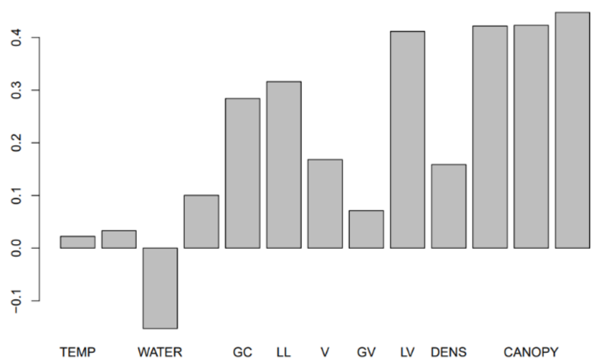
Appendix 6: Table outlining the scale used to rank the available aquatic sites (the water variable). On each survey a rank of 1-6 was given based on the environmental conditions of the transect.

	Description		
Scale	Ground	Low ground (ditches)	Puddles
1	Dry	Dry	Absent
2	Dry	Contain water	Absent on the transect
3	Saturated with water	Contain water	1-3inches deep
4	Flooded (1-3inches deep)	Contain water	3-6inches deep
5	Flooded (3-6 inches deep)	Contain water	>6 inches deep
6	Flooded >6 inches deep	Contain water	>6 inches deep

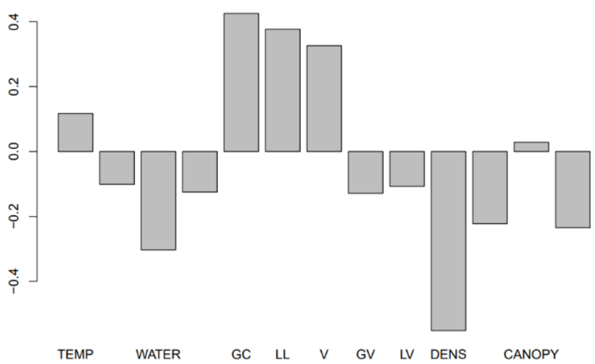


Appendix 7: A clustered analysis showing the overlap of techniques. This shows the variables with the largest influence of 66% (PC1 and PC2) influence both techniques equally.

8A



8B



Appendix 8: Graph 8A and 8B to see how variables contribute to PC1 and PC2 respectively. Variables include temperature, humidity, water, weather, ground cover, leaf litter, vines, ground vegetation, lower vegetation, density, depth of vegetation, canopy cover, trees.

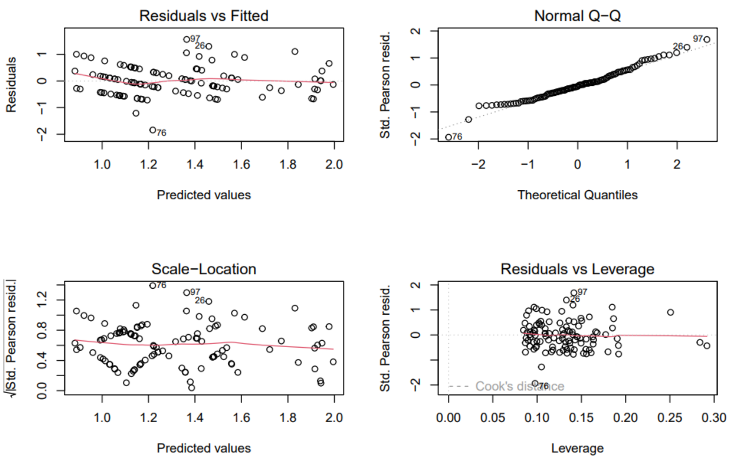
Appendix 9: Table A and B displaying the results and model values from the Poisson GLM models on both the AS and PAM techniques. Table A shows there are no significant Pr(>|z|) values for AS and only one for PAM. Water is shown to be significant to 0.001 with a value of 0.00109 for PAM. Table B shows PAM has a lower AIC and pseudo. R2 score suggesting a better fit. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

A.

Technique Coefficients	AS				PAM			
Variables	Estimate	Standard Error	z value	Pr(>|z|)	Estimate	Standard Error	z value	Pr(>|z|)
(Intercept)	-1.57829	5.285678	-0.299	0.765	5.909546	7.174905	0.824	0.41014
TEMP	-0.00542	0.055139	-0.098	0.922	-0.03283	0.074888	-0.438	0.66114
HUMIDITY	0.021027	0.018792	1.119	0.263	0.004023	0.024489	0.164	0.86951
WATER	0.025985	0.048941	0.531	0.595	0.211828	0.064833	3.267	0.00109 **
WEATHER	0.052549	0.109403	0.48	0.631	-0.07416	0.158823	-0.467	0.64053
GROUND COVER	-0.01851	0.021556	-0.859	0.39	0.010298	0.032545	0.316	0.75168
LEAF LITTER	0.029018	0.034027	0.853	0.394	-0.01908	0.048054	-0.397	0.69135
VINES	0.009412	0.132822	0.071	0.944	0.140726	0.206808	0.68	0.49621
GROUND VEGETATION	0.015218	0.015087	1.009	0.313	-0.00807	0.022617	-0.357	0.72132
LOWER VEGETATION	-0.01164	0.04765	-0.244	0.807	0.047184	0.063809	0.739	0.45963
DENSITY	0.055771	0.084494	0.66	0.509	-0.06425	0.109333	-0.588	0.55674
DEPTH	-0.00161	0.047303	-0.034	0.973	-0.05179	0.065249	-0.794	0.4274
CANOPY	0.0008	0.043759	0.018	0.985	-0.05347	0.061849	-0.864	0.38732
TREE	-0.26368	1.239753	-0.213	0.832	1.203389	1.708512	0.704	0.48122

B.

	Degrees of freedom	AS	PAM
Null deviance	106	74.775	67.879
Residual deviance	93	34.369	34.588
AIC		399.27	333.65
Number of Fisher Scoring iterations		4	4
pseudo. R2		0.54036	0.49044



Appendix 9: Residual tests from the Poisson GLM, both for AS and PAM.