

Investigating the Use of Conspecific Playback in Eliciting Movement and Vocal Response in the Lemur Leaf Frog *Agalychnis lemur*

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ABSTRACT

Conspecific playback has been shown to encourage the movement of target species to previously unoccupied sites. Similarly, it has been used to assess areas for the presence of cryptic but vocal species by eliciting a vocalised response. Amphibians are one of the fastest declining groups of vertebrates, and conservation efforts are underway to halt and reverse these declines. For this study, two experiments were conducted on a population of Critically Endangered Lemur Leaf Frog (*Agalychnis lemur*) at Las Brisas Nature Reserve, Costa Rica. The first experiment investigated whether playback can be used to encourage *A. lemur* to move to new waterbodies. This was done by installing new waterbodies and using conspecific playback to attract *A. lemur* to these sites. The second experiment used playback at sites where *A. lemur* were known to be present to determine whether playback can elicit a vocalised response from non-calling males. By conducting a logistical regression model, it was found that conspecific playback did not encourage *A. lemur* to colonise the new waterbodies ($p = 0.1987$). A chi-square test of association found that conspecific playback did elicit a response from non-calling males at already occupied sites. ($p = 0.017$). The likelihood of getting a response from *A. lemur* after playback was 80.6% overall. The likelihood of getting a response varied between the waterbodies with tubs producing a 58% response rate, and ponds 91.6%. The findings of this study have the potential to have a strong conservation value in the management and conservation of *A. lemur*. This is especially true in using conspecific playback to identify the presence of *A. lemur*. However, more work is needed to understand *A. lemur* behaviour which in turn can contribute to the *in-situ* conservation of this species.

KEYWORDS: lemur leaf frog *Agalychnis lemur*, amphibian conservation, playback, conspecific attraction, breeding behaviour, bioacoustics

INTRODUCTION

Playback is the use of pre-recorded calls which can be used to encourage conspecifics to move into previously unoccupied sites (James et al. 2015). With many species declining and absent from their known historical ranges, *in-situ* conservation efforts are often based on restoring habitats with the hope re-colonisation will occur. However, with many species, conspecific attraction is what leads to individuals moving into an area (Smith and Peacock 1990). Therefore, with newly restored unoccupied habitat, the likelihood of natural re-colonisation with no intervention is slim. Conspecific playback has been used widely in avian conservation, with several studies highlighting the success of the technique (Ward and Schlossberg 2004; Ahlering et al. 2010).

Amphibians are one of the fastest declining vertebrate groups in the world (Ortega-Andrade et al. 2021), and conservation efforts are underway to halt and reverse these declines, including habitat restoration. Studies have shown that several amphibian species are attracted to conspecific vocalisations, and are social species, with many coming together to breed (James et al 2015; Schwartz and Wells 1985). With improving ethics and understanding the fragility of this group of species, more passive hands-off conservation and research work is being developed and carried out.

Conspecific playback has been used in a handful of recent studies on vocal amphibian species to encourage movement and dispersal. James et al. (2015) used playback to change the distribution of the Green and Gold Bell Frog (*Litoria aurea*) in already colonised ponds, whilst Testud et al. (2020) broadcast amphibian breeding calls to encourage the use of passages under railways by frog and newt species. Buxton et al. (2015) found that Cope's Gray Tree Frogs (*Hyla chrysoscelis*) colonised new breeding ponds with chorus playback present significantly quicker compared to ponds with no playback.

Similarly, conspecific playback has also been used to assess whether a target species is present in an area by eliciting a response to the playback. This has been used for several vocal anuran species. Bozzell (2012) found that the use of playback significantly increased the detection rates of the Spring Peeper (*Pseudacris crucifer*) compared to only using visual survey techniques. Many of these species are nocturnal and difficult to locate using more traditional techniques such as visual surveys and are often easily disturbed, causing the males to cease calling (Bozzell 2012).

The Lemur Leaf Frog (*Agalychnis lemur*) (Boulenger 1882) is a Critically Endangered species of leaf frog found in Costa Rica, Colombia, and Panama (Skelton 2012). Their historic range in Costa Rica used to stretch from the northeast of the country through to the border with Panama, however, there are now only three known sites in the country where they are found (IUCN SSC Amphibian Specialist Group 2020). Like many anuran species, there is not one outstanding effect causing their species to decline, instead, there are several factors (Solís et al. 2008). Habitat loss and fragmentation are large contributing factors to their decline and current low numbers in Costa Rica (IUCN SSC Amphibian Specialist Group 2020). Chytridiomycosis is also believed to be a factor in their decline, however, this species appears to be more resilient to the disease than most other species, which may explain why these small populations still exist (Woodhams et al. 2006). Very little is known about their ecology. According to the EDGE of Existence Programme, *A. lemur* is phylogenetically and evolutionarily distinct (EDGE of Existence 2023). A study by Gray (2011) identified mitochondrial DNA differences between *A. lemur* in Costa Rica and those in Panama. By being genetically distinct from the Panamanian population, it highlights the importance of conserving the remaining small populations in Costa Rica.

The survival of this species may also be of use to the pharmaceutical industry due to peptides found in the skin. Some peptides found in *A. lemur* skin have been linked with the potential

use of treating Type 2 diabetes (Abdel-Wahab et al. 2008), as well as having anti-cancer and anti-bacterial properties (Conlon et al. 2007).

This species is a good candidate for the methods used in this study as previous observation suggests that male vocalisation is used to show ownership of territories, therefore it is potentially used to attract both sexes (Savage 2002).

Firstly, this project aims to investigate the use of conspecific playback and whether it can be used to accelerate the re-colonisation of newly installed waterbodies by *A. lemur*. It is hypothesised that re-colonisation of the waterbodies by *A. lemur* will happen faster when conspecific playback is used compared to waterbodies where conspecific playback is not used. A logistical regression model will be used to test this.

Secondly, it aims to establish whether playback can be used to detect *A. lemur* by eliciting a response from individuals who are not vocalising. It is hypothesised that there will be an increase of male vocalisation after conspecific playback is played. A chi-square test of association will be used to test this.

To test these hypotheses, two experiments were carried out. One focused on using playback to investigate whether it can attract *A. lemur* to new waterbodies, therefore expanding their range. The second used playback to investigate whether it can elicit a vocalised response from non-calling *A. lemur* males.

MATERIALS AND METHODS

Ethical approval for this project was granted by the University of Bristol Animal Welfare and Ethics Board (UIN/23/018).

The Study Site

The study occurred at Las Brisas Nature Reserve (10.06915, -83.62957) in the Central Volcanic Range of Limon Province, Costa Rica. It has an elevation range of 650-1030m above sea level and comprises a variety of habitats including primary, early succession, and secondary forest, ponds, and streams (Las Brisas Nature Reserve 2022). It also contains several artificially installed tubs and ponds throughout the site. No specific number of waterbodies has been found, however, a survey of the site with another researcher identified over 80 waterbodies. This is one of only three confirmed sites in Costa Rica known to have a population of *A. lemur* (IUCN SSC Amphibian Specialist Group 2020).

The Study Species

A. lemur is a small nocturnal species that is typically found in primary forests with an elevation of between 400-1600m. They are between 30-45mm in length and weigh between 2-4g, with the females being larger (Kubicki 2004). During the day they appear lime green in colour with brown spots, allowing them to blend into the foliage as they rest, and during the night when they are active, they are brown (Skelton 2012).

Data Collection

Data collection for both methods took place between 15/06/2023-14/07/23. For Method A, data collection took place every day, and for Method B, every other day. Data was not gathered when there was persistent torrential rain. This was due to the difficulty in hearing playback over the rain, and to ensure the safety of the observers. It was the rainy season when this research took place. Four days were missed due to persistent torrential rain (20/06/2023, 26/06/2023, 01/07/2023, and 06/07/2023). This resulted in a total of 26 days where data was collected for Method A, and 12 days for Method B. Data for each method was gathered once daily between the hours of 19:00 and 22:00.

Playback Stimuli

Stimuli for the playback were recorded prior to the start of data collection on-site at Las Brisas using a Sennheiser directional recorder and a Marantz recorder, model PMD661. The recordings were cleaned using Audacity 3.3.3 (Audacity Team 2023). These recordings were used for both methods. After cleaning, the recordings were downloaded onto a phone. A playlist was created, allowing the recordings to be put on repeat and shuffled. The phone was connected to a JBL Flip 5 Portable Speaker, allowing for the sound to be played at each site. The call characteristics of *A. lemur* have previously been described by Emmett et al. (2020). Recordings were made from this site to guarantee that the stimulus is from the same population being studied.

Method A – Movement to New Waterbodies by Using Playback

To investigate whether conspecific playback can encourage *A. lemur* to inhabit new waterbodies, six new tubs were installed at Las Brisas Nature Reserve. This method was carried out for 26 days with six sets of observations taken each day, resulting in a sample size of 156.

Three food storage barrels were purchased and then cut in half by the seller to create the six water tubs used in this study. The tubs were blue in colour due to being the only available barrels that did not previously contain chemicals. Each tub was between 43 cm and 50 cm in height. A hole was also drilled into each tub to prevent water from reaching the top of the tub, as *A. lemur* lay egg masses on the side of tubs or overhanging leaves (IUCN SSC Amphibian Specialist Group 2020). An illustration of how each site was set up can be seen in *Fig. 1*. The tubs were placed along two separate trails in the reserve that had no tubs previously: ‘Ficus’ and ‘Laguitos’. A diagram of the trails and their proximity to each other can be seen in *Fig. 2*.

An adjacent trail running along the bottom of the two study trails had a known population of *A. lemur* which occupy artificially installed tubs and ponds.

The six tubs were subject to one of two conditions. Three tubs were randomly selected to be control tubs (C1, C2, C3). The other three tubs were subject to playback (P1, P2, P3).

Playback involved the playing of multiple pre-recorded stimuli. The phone and speaker were placed in a dry bag to provide protection from rain and left hanging above the ground on nearby branches close to the tubs. Playback was played for 30 minutes at each site every night. Prior to playback being deployed each night, a 1-minute visual scan survey (VSS) was conducted at all six water tubs for the presence of *A. lemur*. Playback was used between 19:00 and 22:00. Emmett et al. (2020) stated times between 19:00 and 21:00 as the peak call time of *A. lemur*, suggesting them to be most active between these times. *A. lemur* was recorded as being either present or absent. The weather, temperature (°C), playback start time, and observation start time were also recorded. The weather was recorded as either dry, after, or rain. See the full description of terms in *Table 1*. The order of the three tubs playback was used at each night was randomised to reduce any bias.

Method B – Playback Response Survey

To investigate whether conspecific playback can elicit a vocalised response from non-calling *A. lemur* males, playback was used around known occupied sites. This method was carried out for 12 days with three sets of observations taken each day, resulting in a sample size of 36.

On each night this method was carried out, three sites were randomly selected. These sites were in the form of either a tub or a pond. They were selected on the basis that *A. lemur* is known to occupy the site, but are not heard vocalising at the time of observation. Before playback is deployed, a 1-minute visual scan sample is taken to note down any visible

individuals. The weather and temperature are also recorded. At each site, the playback is played for 10 minutes. The playback used for this experiment is the same used in Experiment A. All lights are turned off to mitigate the impact the observer has on the frogs. After the 10 minutes is complete, the observer listens for 2 minutes for *A. lemur* responses. Vocalisations are marked as being either present or absent, and the response time after playback is also recorded. This is recorded in inclinations of 10 seconds. The first starts at 0 seconds, meaning calls were heard immediately after the playback was turned off.

Statistical Analysis

Before any statistical analysis was done, playback recorded as either 'Yes' or 'No' was changed to a numerical format where '1' = 'Yes', and '0' = 'No'. Similarly, this was done for whether the frogs were present or not with '1' = 'Present', and '0' = 'Absent'.

Logistic regression analysis was performed on the data collected for Experiment A. These models are used to predict whether the presence and absence of *A. lemur* are influenced by selected factors: the day, and the presence or absence of playback. A logistic regression was also carried out to investigate whether playback was influenced by the weather. The analysis was carried out in R 4.2.2 (R Core Team 2022) using the lme4 package (Bates et al. 2015).

Experiment B analysis was carried out using R 4.2.2 (R Core Team 2022) and Jamovi (the Jamovi project 2023) in the form of a chi-square test of association. This was done to assess whether there was a correlation between frog number and response time. It was also done to assess whether frogs were more likely to call if they were located by either a tub or a pond.

The data collected for this project will be available to access from the University of Bristol Repository.

RESULTS

There was a sample size of 156 analysed for Method A. A logistical regression model showed that the presence of *A. lemur* was not influenced by day, $X^2(1, N = 156) = 1.9758, p = 0.1598$. A logistical regression model also showed that the presence of *A. lemur* was not influenced by conspecific playback, $X^2(1, N = 156) = 1.6521, p = 0.1987$. It was also found that the influence of playback was not significantly dependent on the weather ($p = 0.579$).

Fig. 3 shows the days in which *A. lemur* was present and absent at each of the six tubs.

Throughout the 26 days, no frogs were present at P2, C2, or C3. For P3 and C1, there was one day out of the 26 where *A. lemur* was present. P1 was the only tub to have multiple days where *A. lemur* was present (8).

For Experiment B, 36 lines of data were analysed. A sum function on Excel found that the likelihood of getting a vocalised response from *A. lemur* after the use of conspecific playback is 80.6%. A chi-square test of association was conducted using Jamovi (The Jamovi project, 2023) to investigate the relationship between the use of conspecific playback (present/absent) and vocalisation response (call/no call) by *A. lemur*. Overall, there was a significant result, $X^2(1, N = 36) = 5.67, p = 0.017$.

Comparing the two waterbody types, tubs, and ponds, showed that there was a 58% probability that using playback at tubs would result in a vocalised response. With ponds, there was a 91.6% probability that using playback would illicit a vocalised response from *A. lemur*. Overall, of the 12 playbacks used at tubs, seven resulted in a response. Of the 24 playbacks used at ponds, 22 resulted in a response (*Fig. 4*).

Of the 36 waterbodies sampled, vocalised responses were observed at 30 of them. The response times after playback was stopped were all recorded as either 0 seconds or 10 seconds.

A chi-squared test of association was performed to investigate whether the number of frogs observed before playback was used correlated with the response time. It was found to have no significant difference, $X^2(3, N = 30) = 0.855, p = 0.836$.

Fig. 5 shows the response time (seconds) in relation to the number of frogs visually observed before the start of playback.

Out of a total of 30 responses recorded, 13.3% responded after 10 seconds, with 86.6% responding after 0 seconds, meaning that vocalisation was heard immediately after conspecific playback was stopped.

DISCUSSION

In the first part of this study, playback was found to not influence the presence of *A. lemur*. Biological factors out of the control of the researchers could provide reasons for this result such as the natural behaviour of the species. The increased human presence in the area by the researchers could have led to increased disturbance of the surrounding environment which also has the potential to have caused changes in behaviour (Garner et al. 2008). Despite it being documented that some species are attracted to new waterbodies by conspecific vocalisations (Buxton et al. 2015), there is a suggestion that visual and olfactory mechanisms may also play a role in amphibians detecting new waterbodies (Sinsch 1990).

It has to be considered that *A. lemur* may not have moved to these newly installed waterbodies due to them being artificial structures. However, throughout the reserve, artificial tubs, including several that are blue, have been observed to have *A. lemur* present and breeding.

Already established ponds and tubs being used by *A. lemur* at this site are artificial, and during the study period, it was observed that none of these waterbodies dried up. It could simply be assumed that already established and plentiful breeding areas for this species reduced their need to expand looking for new sites. This was suggested as a potential reason why the American Toad (*Anaxyrus americanus*) in a similar study did not occupy new ponds.

A consideration not accounted for in this study is whether the frogs could hear the calls from their original sites, and how far the species travel to find new waterbodies. Few studies have been published on the movement of anuran species, however, a study on the dispersal of neonate Pacific Tree Frogs (*Pseudacris regilla*), through a mark-recapture method found that some travel over 230 meters to a new pond (Jameson 1956). Similarly, Vos et al. (2000) found that the European Tree Frog (*Hyla aborea*) can travel long distances to new waterbodies, with the mean dispersal distance being 1,469 meters. Both these species are in the *Hylidae* family, which *A. lemur* is also part of. Therefore, it can be suggested that the 50-meter distance used in this study is sufficient, however, as mentioned previously, due to the lack of ecological and behavioral knowledge on this species, the suitability of the distance can only be assumed. However, even if it is possible that this species can travel over 50 meters to find new waterbodies, it is unclear whether individuals will orientate to the playback and move towards it over this distance.

Studies by Swanson et al. (2007) and Christie et al. (2010) on the Cope's Gray Treefrog (*Hyla chrysoscelis*) and the Gray Treefrog (*Hyla versicolor*) found that females orientated to conspecific male vocalisation over shorter distances. *H. chrysoscelis* orientated to calls from up to 40 meters away, and *H. versicolor* up to 32 meters. Therefore, it is possible that although the dispersal distance may be over 50 meters, the species may not be encouraged to move toward the playback at such a distance.

The placement of the speakers may also have influenced the distance in which the conspecific playback was broadcast. Dense vegetation may have prevented the sound from traveling as far, meaning that some individuals may not have heard the calls (Boullhesen et al. 2021).

Results from the individual tubs show that P1 was the only tub to have more than one day where *A. lemur* were recorded as being present. With playback and day determined to not be significant in influencing the presence of *A. lemur*, other factors that were not accounted for may have influenced their presence at this site and their absence at others. Several studies have investigated surrounding vegetation and its impact on influencing colonisation and breeding (Cline and Hunter 2014; Vasconcelos et al. 2009). Although all tubs had vegetation in close proximity to the surface of the water, the species and amount of coverage were not considered. With P1 and C1 being on a separate trail from the other four tubs, the general surroundings may have also had an impact. The researcher observed that this trail in general had a much higher anuran species richness than the second trail, suggesting this area may have been more suitable for anurans overall.

The second part of this study was to assess whether the use of conspecific playback at known *A. lemur* sites would elicit a vocalised response from non-calling males. Here there was a statistically significant result, meaning the use of conspecific playback did appear to gain a response from non-calling males.

Experiment B suggests that conspecific playback can be used to efficiently survey for the presence of *A. lemur*. It also shows that surveys between 19:00 and 21:00 are a suitable time to conduct studies on *A. lemur*. This further supports the peak calling and surveying time put forward by Emmett et al. (2020).

With only 4 known sites in Costa Rica, this method could be used in new areas to survey for the presence of *A. lemur*. One drawback in using this method to survey for a new population

is that a separate group of animals may not recognise the playback. The captive population of *A. lemur* at Bristol Zoo has been observed to produce a different call than the population at Las Brisas. Passos et al. (2021) showed that captive Golden Mantella Frogs (*Mantella aurantiaca*) responded differently to wild and captive conspecific playback, with the responses to playback from a wild population reducing over generations in captivity. This could also have an impact if any new reintroductions take place in areas where *A. lemur* is already present as if the introduced individuals do not recognise the calls of already present individuals, they may not respond and breed.

It was found that there was a higher probability of getting a response from individuals located at the ponds compared to the tubs. This could be due to the ponds providing a more suitable breeding habitat for *A. lemur*. However, more research is needed in this area to identify the optimum breeding conditions for this species.

This study was conducted during the wet season, and so, despite this species calling year-round, they may call more during this time (Savage 2002). They may not respond as frequently during the dry season, which must be considered if this detection method is used outside the wet season.

Furthermore, there was no control used when this method was deployed, meaning that there is the potential for the vocalised responses to be triggered by a different factor. If this method is to be made more reliable, the author suggests that a more robust survey method be developed that involves a control.

Recommendations

Method A had no apparent impact on the movement of *A. lemur* to new waterbodies. It would not be recommended to use this method with this species. Although this method was not

effective in the case of *A. lemur* it is not to say it does not have the potential to work with other anuran species.

The initial Method B study provided promising results; however, a more robust method is needed. To potentially have a strong conservation impact on this species in the future, especially in identifying the presence of new populations, a more in-depth study focusing on strengthening this method is recommended. This method also has the potential to be developed to study known populations, by providing more knowledge on their breeding behaviour.

In general, more work is needed in this area to understand the movement and breeding behaviours of *A. lemur* as well as their response to conspecific cues in the wild.

CONCLUSIONS

The use of conspecific playback as a passive method to encourage the movement of anuran species has been used successfully with other species, however, there were no significant results to show this was the case with *A. lemur*.

It is also concluded through this initial study that conspecific playback does elicit a response from male *A. lemur*. This allows for the detection of the species in areas even if they are not in sight or calling.

There is little published literature on this species, so any new investigations into their ecology and behaviour have the potential to provide greater understanding. Knowledge gained from these studies provides more guidance to shape the *in-situ* management and conservation of these critically endangered and important species in future projects.

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FIGURES

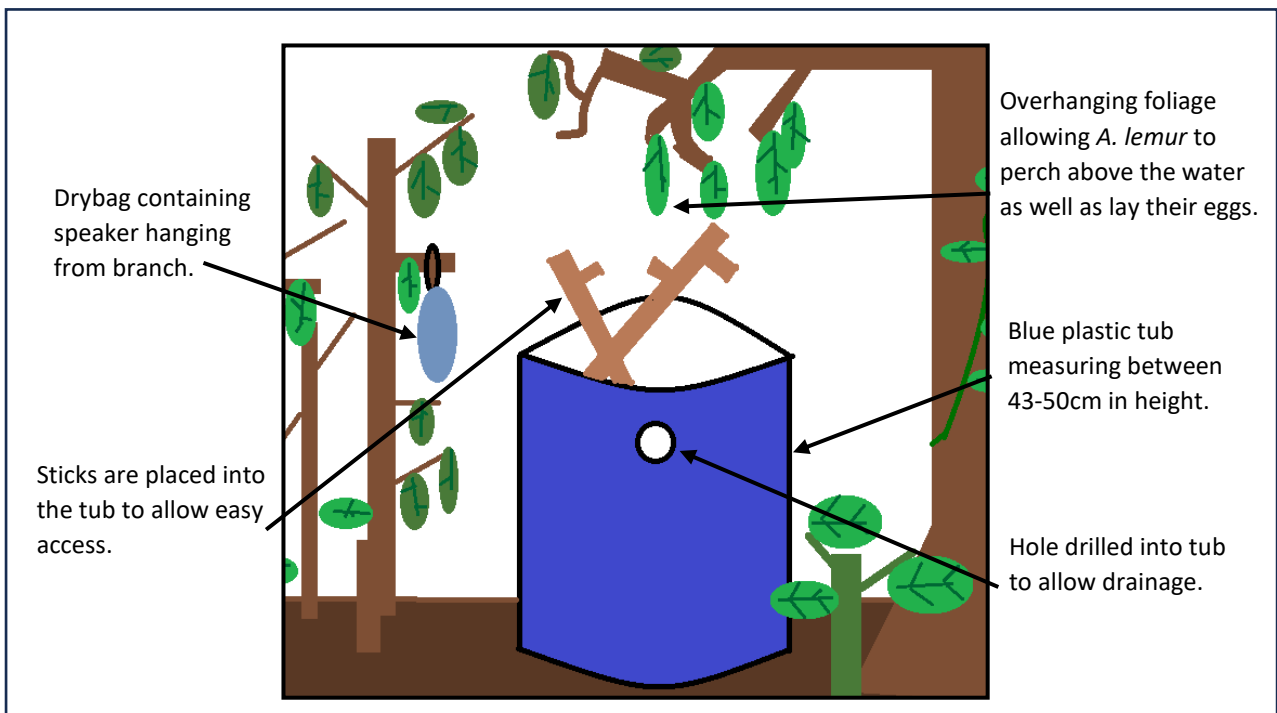


Figure 1: Shows a labelled illustration of the setup of the six tubs used in the field. All six tubs followed the same set up, however only the three playback tubs had the drybag and speaker present. Image created by the author.

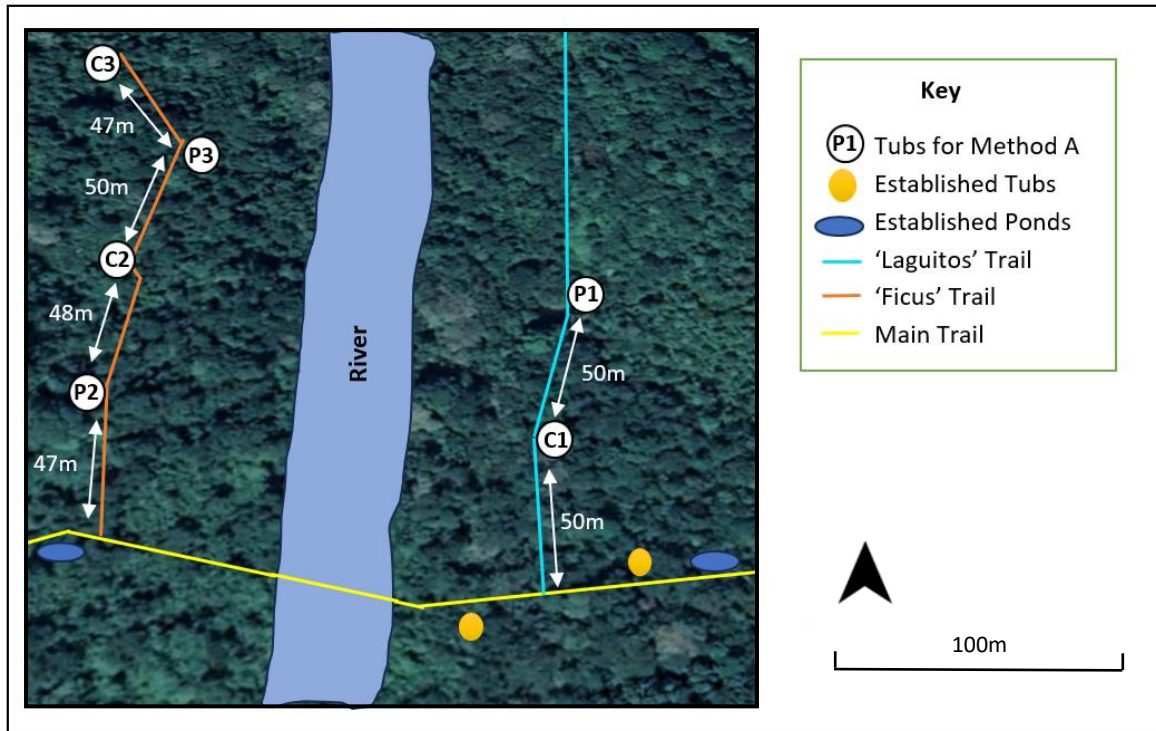


Figure 2: Shows a labelled diagram with key of the study area for Method A. Tubs used for Method A are labelled with a number and a letter: C = control tub, P = playback tub. The main trail extends further than what is shown in this diagram. In total, there are over 80 established tubs and ponds present along the main trail. Background image from Google Earth (Google Earth 7.3 2023)

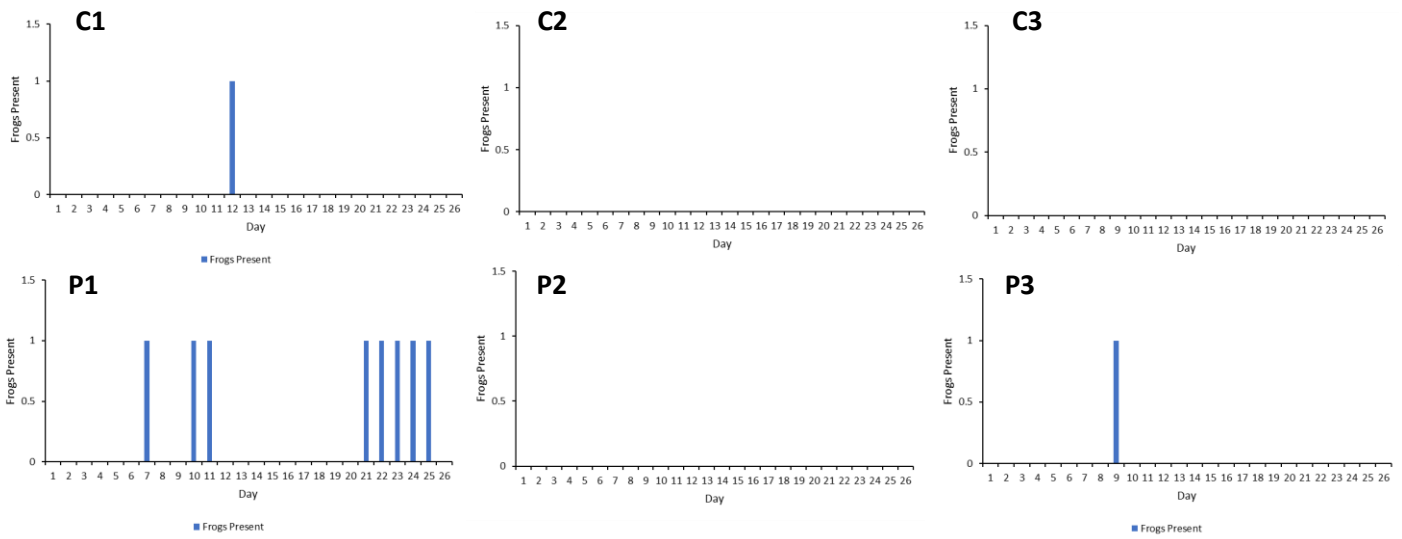


Figure 3: Shows when *A. lemur* were present and absent at each of the six tubs. On the y-axis, 1 means present, and 0 means absent. The control tubs are shown and named in the top three graphs, whilst the lower three graphs show the tubs with playback.

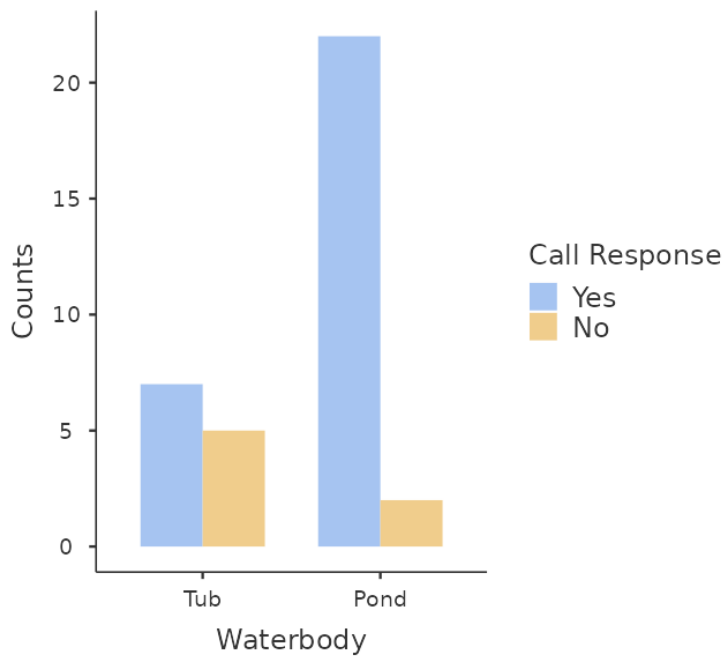


Figure 4 : Graph showing the number (counts) of call responses recorded at each waterbody type. N = 36. Created using the Jamovi project (2023).

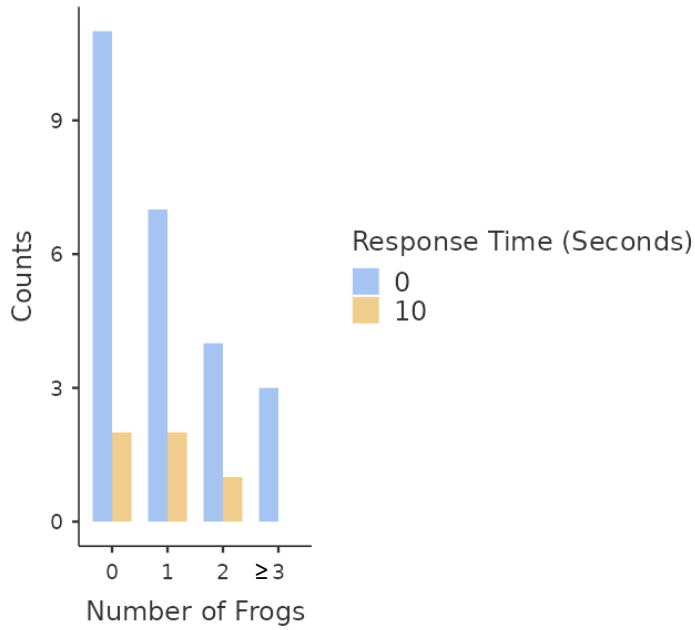


Figure 5 : Shows the number (counts) of response times (seconds) for the number of frogs observed. The number of frogs were observed and recorded before the start of playback. N = 30. Created using the Jamovi project (2023).

TABLES

Table 1: Description of weather terms

| Dry | After | Rain |
|---|---|-----------------------------------|
| There is no precipitation, and the ground is dry. | There is no precipitation, and it has previously rained before the survey takes place. The ground is still wet. | It is raining during the surveys. |