A guide to Surveying and Monitoring diurnal frogs in PPBio's RAPELD grids and modules in the Amazon.

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Why monitor diurnal frogs?

Frogs are a good group to monitor because, their sensitive permeable skin is extremely sensitive to subtle changes in temperature and humidity in their environment, as well as to contact with pollutants.

Although most species of frogs are nocturnal, a reasonable number of diurnal species can be found in any forest area in the Amazon region. These species are usually small and can be found on the forest floor, where feed, vocalize and reproduce.

Diurnal frogs have a wide range of reproductive modes and search for specific environments to lay their eggs or tadpoles. Thus, environmental variations reflect differences in the quality and availability of suitable sites for reproduction. There will usually be related variations in species composition between one location and another. The distribution of species in their study area may be affected by the loss of these locations or by changes in their micro-climate. Most species can not disperse over very large distances, and many have a restricted distribution. This makes the diurnal frogs a very good group to be used in complementary analyzes of conservation units in bio-geographical studies, or any ecological analysis involving beta-diversity.

The vocalization of species allows us to perform surveys in a very similar way to those that ornithologists apply in surveys of bird species, recording the species through visual and auditory searches, without requiring a large investment in equipment or traps.

Despite the advantages of working with diurnal frogs, remember that they they represent only a small portion of the total species of frogs found in a certain area. Thus, for a complete survey of the diversity of amphibians any region, the following protocol must be complemented by night-time species surveys, to be discussed at another time. For now, this protocol suggests a quick and economical way to get useful data for this diverse group of animals which are highly sensitive to environmental variations, natural or induced, without, at the end, the surveyers being "buried" by a huge number of species to identify.

How does the RAPELD system work?

The grids and RAPELD modules are standardized trails and permanent plots. Figure 1 shows a schematic map of a RAPELD grid system. The long straight lines represent trails, which are usually 5 km long and separated by 1 km, and the red lines are the uniformly distributed permanent plots. This type of arrangement can be modified (e.g. into modules containing less transects) according to the study objectives or logistical conditions, provided that the standard distances between trails and sample plots are maintained.

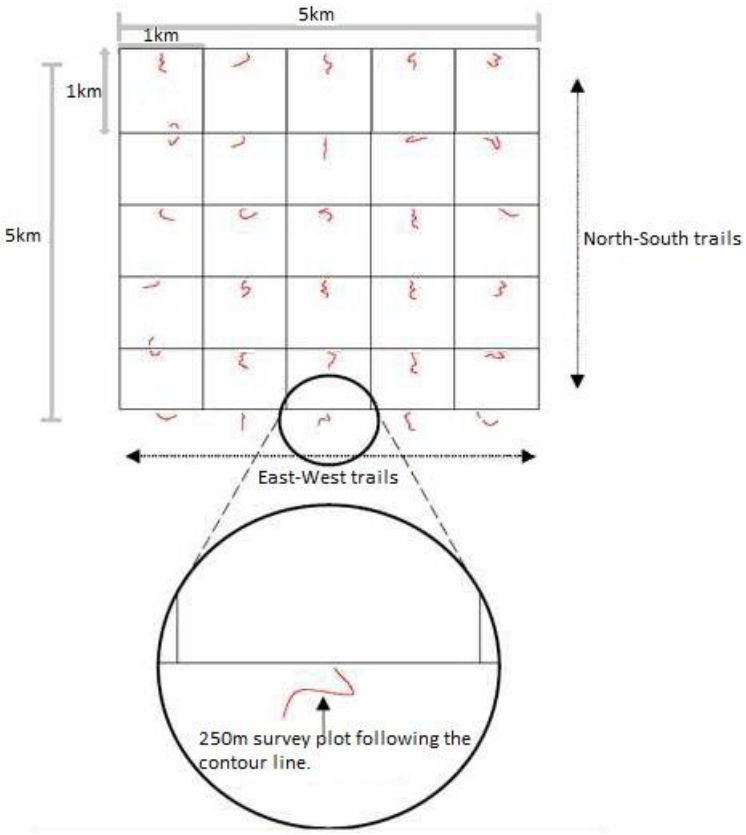


Figure 1. Example of a RAPELD grid system, showing the straight trails and evenly distributed plots (red lines).

The trails are marked by numbered pickets, every 50 meters, containing platelets with the track name and the distance along the track (Fig. 2).

Figure 2. Example of a Figure 3. The picket has an trail marked with pickets. aluminium tag stamped with trail name, and position in meters (3000m in this *example).* Images: Julio Valley

RAPELD has several types of permanent plots, but here we only consider the uniformly distributed plots locaed every kilometer along the trails. These plots have no fixed shape because they follow the same contour as the trail (Fig. 4). The center line of the plot consists of 25 straight segments, 10m in length, marked by numbered pickets, all at the same contour. All position measurements are made relative to the center line. The center line, should not be confused with auxiliary lines auxiliary, used to demarcate the displacement corridor or sampling areas.

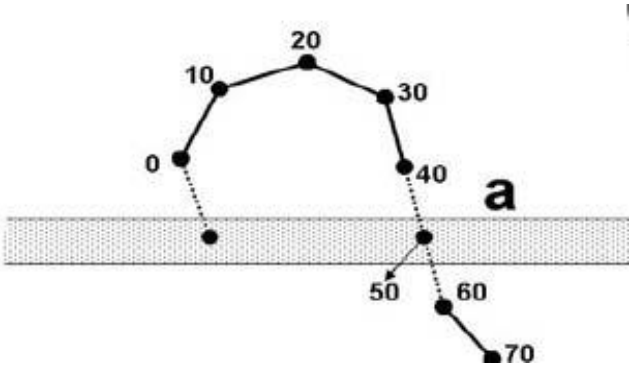


Figure 4. Plot following the contour with 10m straight segments. (Only the first 70m of the plot is illustrated).

Design: Fabricio Baccaro

When is it best to collect Anurans?

A common planning error in survey work monitoring anuran species is the poor selection of collection times. Few frog species reproduce throughout the year. Weather conditions and suitable sites for most species to lay eggs are better during the rainy season in each region. Therefore, seasonality should be taken into account when we plan a schedule of monitoring campaigns. The peak rainy season in the study area is the recommended time for carrying out field work. If there are financial and logistical resources for doing more survey the first survey should start at the beginning of the rainy season, one at the peak of the rainy season (I.e. the month of greatest historical rainfall) and at the end of the rainy season. This allows for the detection of species with short breeding seasons. Vocalizations that males emit to attract females during the breeding season mean that most species will be much easier to detect. As already mentioned, most diurnal species are small and cryptic and difficult to distinguish from their environment so surveys based on records obtained during the dry season tend to be very unreliable.

Another important issue is that some species vocalize at different times during the day. In the following protocol we suggest doing the sampling in the morning. If you also plan to also take samples in the afternoon, you must be prepared to deal with the identification of a greater number of species such as we will comment below.

Knowing the target group.

Before starting any fieldwork, seek as much information about the species occurring in your area of study as you can. Use the pictures and sound files available on the Internet or in the literature to mount an

album and make collections of reference sounds. If possible, perform a preliminary survey along the 5 km trails (or grid), recording, capturing and photographing as many species as possible. Use the material obtained to start a reference collection, associating photos of each species to their vocalizations (Fig. 5). Photos and sonograms (visual representations of vocalizations) can be combined with photos, and can be used in the field, assisting in the identification of species (see "Resources extras "on page 17 to find out how to get sonograms).

If there is doubt as to the precise identification of the species at this stage, to name morphotypes is enough. Use the name of the genus or family to name each species, followed by the epithet "Sp" (eg Allobates sp1, Allobates sp2, sp3 Allobates, Dendrobatidae sp1,

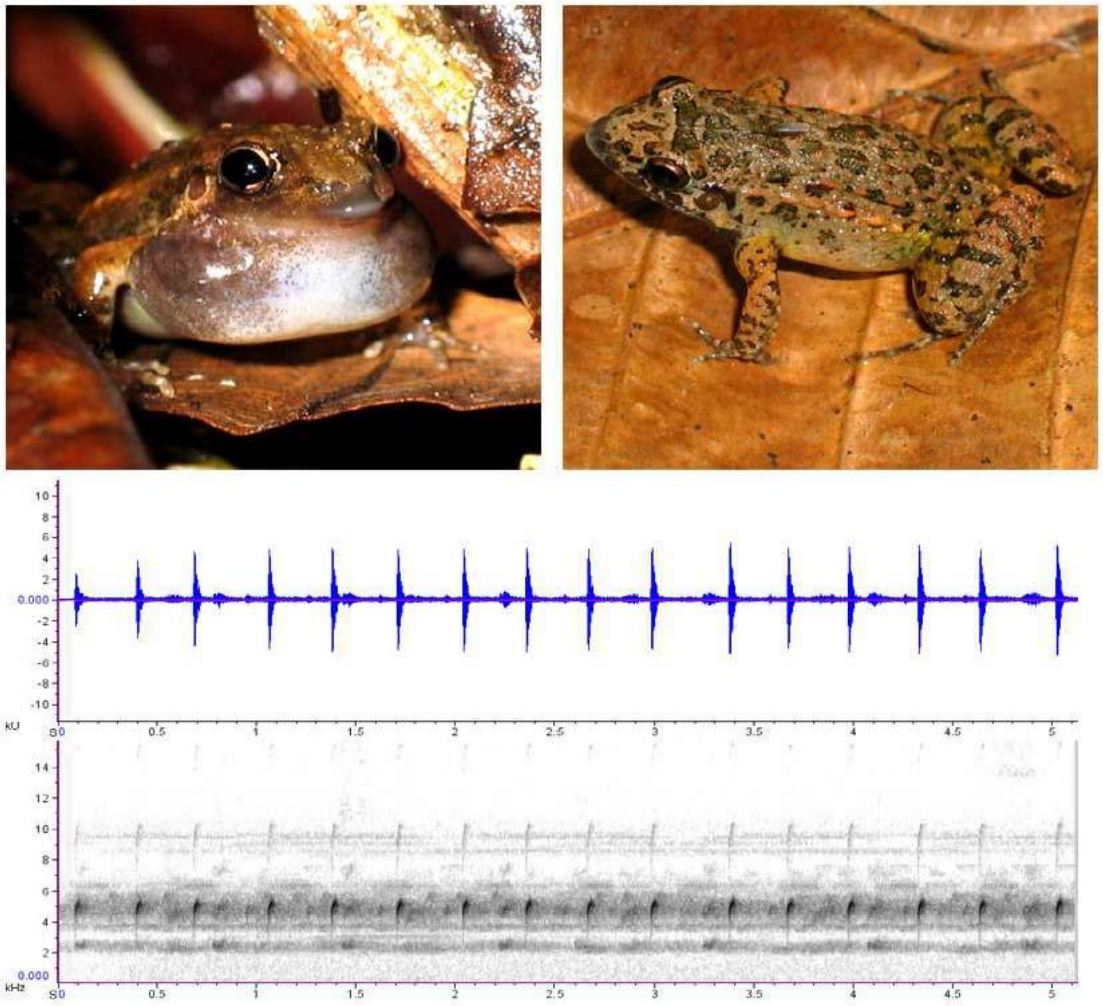


Figure 5. Page from a reference collection of photos and vocalizations of an unidentified species (Adenomera sp.). Photos: Walter Hödl and Pedro Ivo Simoes.

The reference material can be printed, placed in a binder with plastic sheets and taken to the field to assist with species identification.

In areas with a great diversity of species and without previous studies, it is very difficult to predict which species will be found, and can seem complicated to memorize the the sounds of the many species available on the internet. However, some genera or groups of diurnal species are common in anuran surveys in the Brazilian Amazon (Figs. 5, 6).

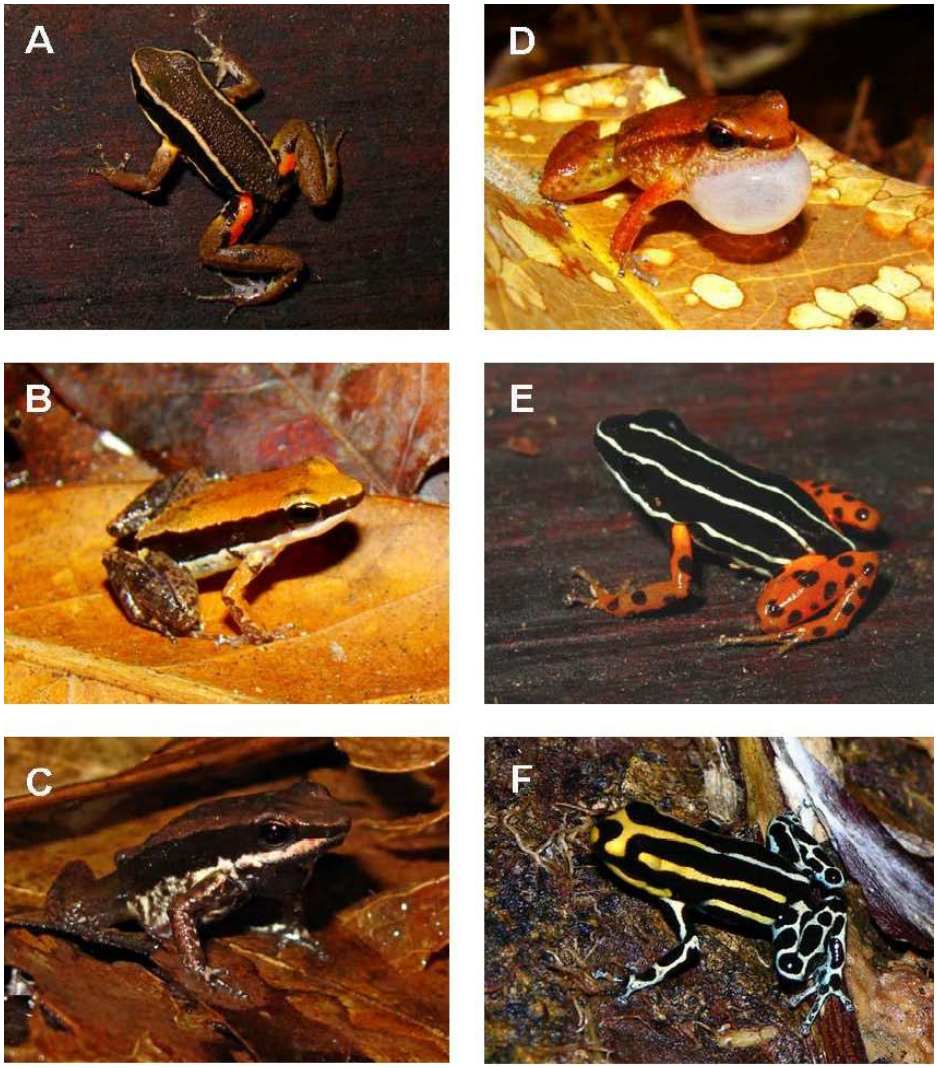


Figure 5. Six species representing genera of diurnal frogs common in the Brazilian Amazon. A) Allobates femoralis (Aromobatidae Family), colorful, nontoxic, widely distributed in the Amazon; B) Allobates sumtuosus (Family Aromobatidae), a species found in the Ducke Reserve nr. Manaus, AM; C) Allobates nidicola (Aromobatidae Family), a species whose embryos develop in a gelatinoud nest over the soil; D) anomaloglossus stepheni (Aromobatidae family), another species whose embryos do not depend on water bodies to develop; E)Adelphobates quinquevittatus (Dendrobatidae Family), endemic poisonous dendrobatid from Rondônia; F) Ranitomeya toraro(Dendrobatidae Family), a semi-arboreal dendrobatid also poisonous, widely distributed in the Brazilian Amazon. Photos: Pedro Ivo Simões.

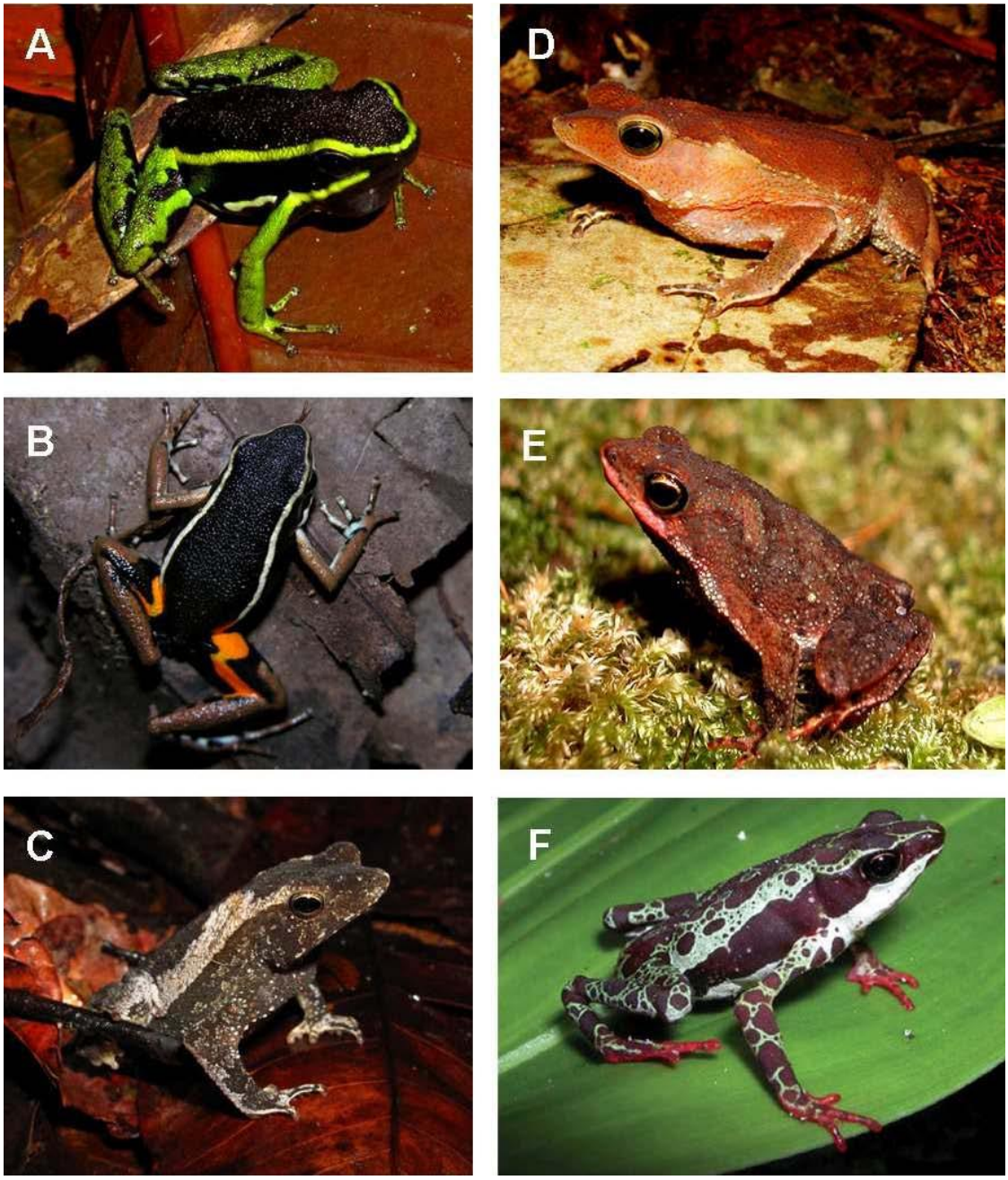


Figure 6. Six species representing diurnal frogs genera common in the Brazilian Amazon. A) Ameerega trivittata (Dendrobatidae Family), widely distributed in the Amazon; B) Ameerega picta (Dendrobatidae Family)a disjointed geographical distribution, between French Guiana and Rondonia; C) Rhinella gr. margaritifera (Bufonidae family), species not yet described,belonging to a species complex known as leaf-frogs; D) Rhinella Proboscidea (Bufonidae family), a described leaffrog, found on the outskirts of Manaus, AM; E) Amazophrynelladraft (Bufonidae family), a species to riparian areas; F) Atelopusspumarius (Bufonidae family), the harlequin frog; the genus has been the subject of conservation programs. Photos: Pedro Ivo Simões (A-D) and Lima et al. 2006 – Guide to the Frogs of Reserva Ducke (E and F).

Initially your search for information should emphasize the following groups:

Aromobatidae family: Genus Allobates and Genus anomaloglossus;

Dendrobatidae family: Genus Ameerega, Genus adelphobates, Genus Dendrobates and Genus Ranitomeya;

Bufonidae Family: genus Atelopus, Genus Dendrophryniscus Rhinella, genus (species of the margaritifera complex);

Some groups of species of nocturnal or crepuscular habits possibly vocalize in late afternoon or after heavy rain (Fig. 7).

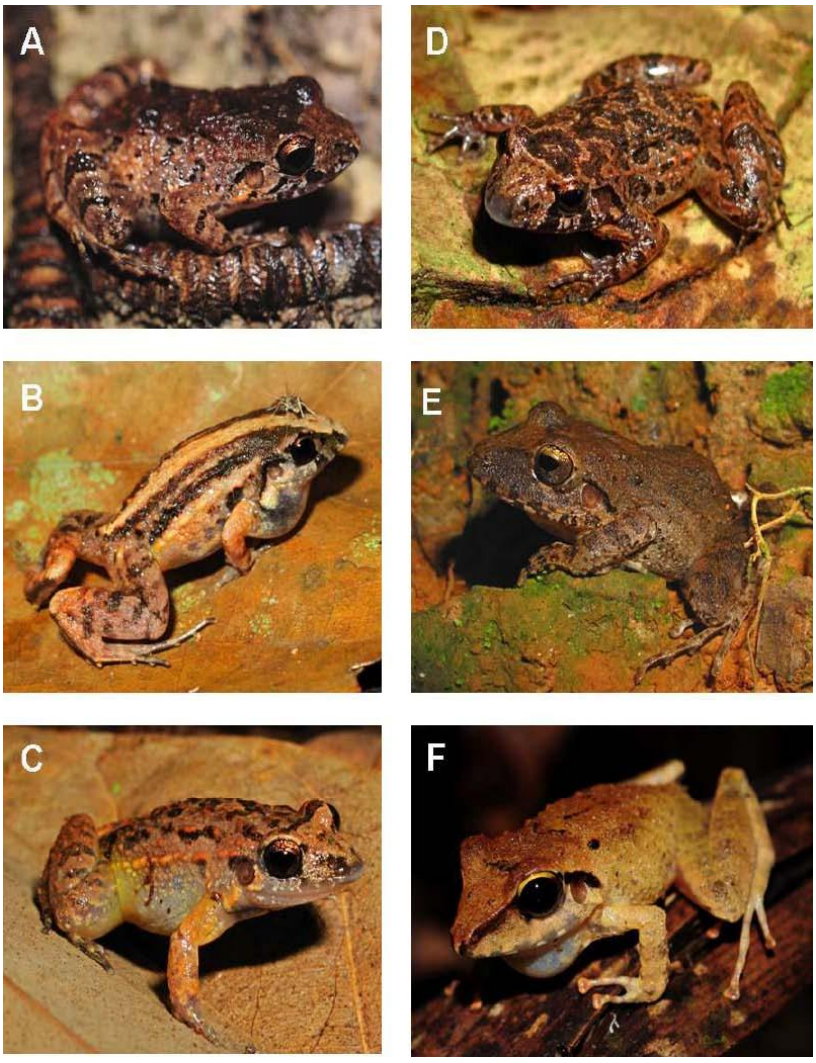


Figure 7. Primarily nocturnal species that vocalize towards the end of the afternoon, or during the day after heavy rains. A) Adenomera andreae (Family Leptodactylidae), forest environments; B) Adenomera hylaedactyla, a species found in open, environments; C)Adenomera hylaedactylus (Leptodactylidae family), with another color pattern;D) Adenomera sp. (Leptodactylidae Family), a species not yet described, very similar to previous species; E) Prisitmantis fenetratus (Strabomantidae Family) typically found on the banks of the Madeira River; F) Pristimantis fenestratus (Family Strabomantidae), Ducke Reserve, in Manaus, probably belongs to a different species found on the Madeira River. Photos: Pedro Ivo Simões.

If the survey includes sampling during the afternoon, you should also familiarize yourself with species of these genera.

Leiuperidae family: genus Pesudopaludicola

Leptodactylidae family: genus Adenomera (marmoratus group)

Strabomantidae family: genus Pristimantis

Try to listen to sound files several times and study photos of species of these groups to used to the types of vocalization and their appearance. same kind of species tend to have reasonably similar vocalizations.

Fieldwork Preparation

The materials needed to survey work and monitor diurnal frogs is relatively straight-forward:

-A digital sound recorder;

Can be pocket-sized with built-in microphone. You could also opt for a more sensitive professional recorder with external microphone to improve the range and sound quality.

In any case, the ideal recorder will be able to record in WAV format. MP3 format is useful for identifying species, but suffers information loss because due compression and impairs detailed analysis of vocalizations and precludes, for example ,description of vocalizations in a scientific paper or reliable comparisons with the vocalizations of individuals recorded in other regions.

-Digital camera;

For images of the observed or collected animals. Records help us recover reliable information on the coloration and external anatomy of the animal. The camera can be a model compact or professional. The important thing is having a good macro function, or a macro lens, the case of professional cameras. A built-in flash is also essential, because in the forest natural lighting is often very weak.

-Transparent plastic bags (5L);

For transporting animals to base camp if it hasn't been possible to identify them. It is advisable to carry a large number of them per outing (> 100 units) and reuse them whenever possible.

Additional material includes:

-A suitable thermometer to measure the temperature of the air;
-A wristwatch;
-Permanent markers;
-Pencil and eraser;
-Clipboards;
-Traps made from clear plastic bottles with the bottom cut off;
-A large cloth bag;
-A head torch;
-Compass or GPS;
-An umbrella. Useful for photographing or record animals during light or moderate rain.

Before leaving for the field, make sure that you have the necessary worksheets to record data (there are models available at https: //ppbio.inpa.gov.br).

These include:

-Metadata forms that describe information about the time, place and people involved in the survey;

-The datasheets on which you will write down the frog survey data. (Annex I)

The number of datasheets should be sufficient for the number of surveys that you intend to carry out. That is, one for each surveyed plot multiplied by the number of times you are going to survey the plots (at least three times is ideal, as described below).

Do not forget to take a map of the grid or module, and maps of central lines of the plots. The map of the survey plot central line is important because sometimes there are breaks when the contour line crosses a stream or road. The maps for your area of study should be available on the PPBio website at https: //ppbio.inpa.gov.br or on the website specific to your project.

For safety reasons, the surveys are usually made in pairs. When more than one team is working simultaneously, each team must carry with them the essential materials listed above.

Field Survey

The first step is to find the permanent plot. Walk the trail to the picket which marks the location of the survey plot (normally a multiple of 500 meters). The start (picket zero) is generally ten meters from the right side of the track on the same contour as the trail picket.

Before you start sampling, and before entering the plot, turn on the thermometer and wait for the temperature on the thermometer to stabilize. The thermometer sensor should not touch the ground, you, or anything you've been carrying.

Note the temperature of the air and the time of the start of the survey. Make sure you have some plastic bags, the small trap, the recorder and the camera are in readily accessible places. Finally, your hands should be clean and must not have any trace of insect repellent insects or other lotions. Frogs' skin is very sensitive, there is a good chance they will die after contact with the residues of such products.

Diurnal frogs are detected visually and aurally along each of 10m segments that constitute the RAPELD plot (Fig. 8). A movement corridor is usually delimited by a line parallel to the center line of the plot. Staying within the corridor, we avoid trampling organisms studied by other researchers, such as seedlings, herbs, fungi, etc.

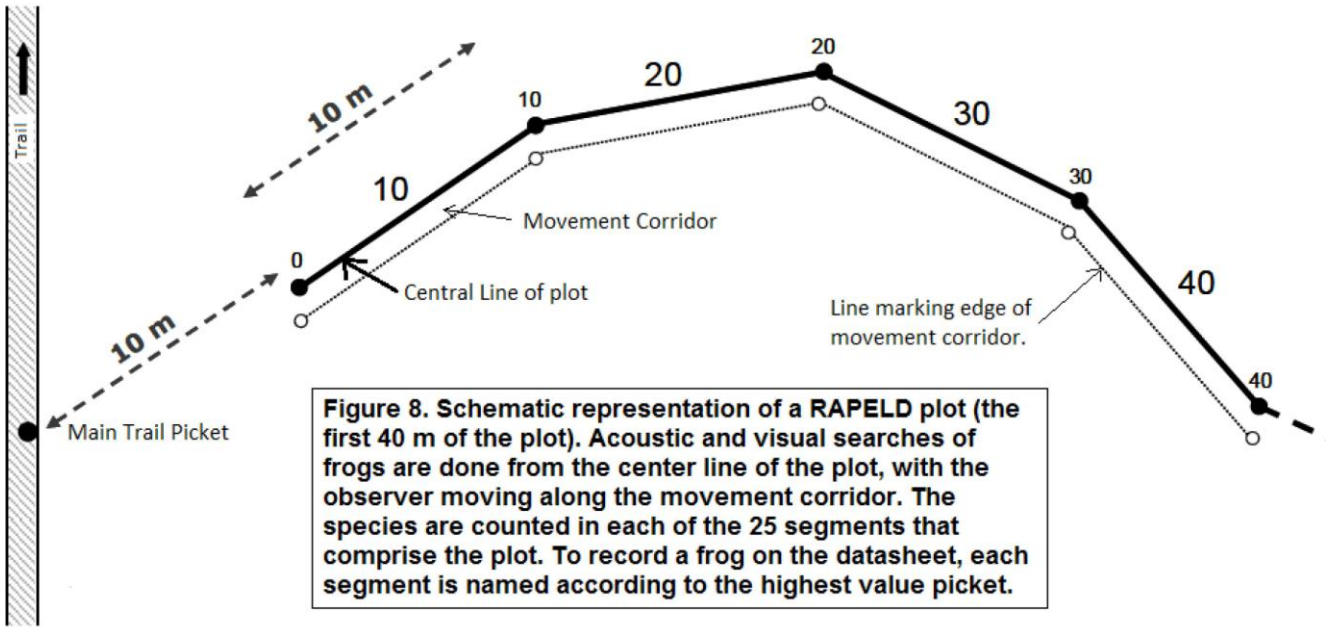


Figure 8. Schematic representation of a RAPELD plot (the first 40 m of the plot). Acoustic and visual searches of frogs are done from the center line of the plot, with the observer moving along the movement corridor. The species are counted in each of the 25 segments that comprise the plot. To record a frog on the datasheet, each segment is named according to the highest value picket.

Picture: Pedro Ivo Simões.

Monitoring begins with the observer moving slowly along the middle of the first 10 m segment, from the zero picket. Along the way, the observer should be attentive to the ground, looking for individuals jumping their way. When the 5m mark has been reached, stop for 2 minutes and listen for calls. Note on the data sheet all the species that have been detected since the beginning of the picket up until this point. Record whether the detection was visual or aural.

After the two-minute listening interval, the observer must move to the picket at the end of the 10m segment again recording any previously undetected. The procedure is repeated throughout the following segments to the end of the plot.

Importantly, the number of species in each plot is estimated by its presence in each segment. Thus, if more than one individual of the same species was seen or heard in the same segment, they are still counted as a single record. The same individual must also not be registered in more than one segment. Take special care with individuals who are vocalizing at very large distance from the center line of the plot, so that it is not recorded in more than one segment. (see illustration of the method in Annex II).

At the end of each plot, the observer should note the time at the end of the sampling and the datasheet should contain a list of the species in each segment. The relative abundance of each species in the plot may be represented as the number of segments in which it was detected. Thus, for standard plots 250

m long, this value may range from 0 to 25. Alternatively, the data can show frequency as a percentage.

Recording and capturing frogs during a survey.

If a frog call does not identify a species a recording needs to be made. Remember not to trample on small organisms outside the movement corridor as this may affect subsequent biodiversity surveys.
Try to approach the animal that is vocalizing carefully, so that you don't scare and cause it to go into hiding. Get close enough to ensure there are no other animals vocalizing nearby that will cause confusion later. Take care with the recording volume – it should be neither too loud not too quiet. Information on how to control the recording volume should be in the instructions for the device. At the end of recording, record the record number, the time, the temperature of the air, date and location of the plot (including plot number, track, module, conservation unit name, city or state). Duplicate this information with a written record so that the data to be recovered more easily. If possible, record details that will help to identify the species, such as probable genus or family or information about their appearance or micro-habitat (e.g. "anuran about 2 cm long, light brown staining on the back with a lateral stripe dark brown on the sides, white vocal sac, found vocalizing on the leaf litter, an area of primary forest, distant at least 200 m from the nearest stream "). Try to capture and photograph the animal being recorded, whenever possible. There is only a small chance that an expert will be able to identify a species from a recording alone.

With some practice, you should be able to capture all frogs with a small hand-trap. It can be positioned directly above the animal and lowered rapidly or held on the ground, with the opening towards the animal. With the other hand, try to make the animal jump into the bottle. A head torch can help to see the animals in low-light situations. Newly obtained recordings can be used to find individuals who are hiding in the leaf litter or in holes. Many species of diurnal frogs are territorial and males usually will fight with other males of the same species that vocalize within their territory. Playing the recorded vocalizations at a natural volume natural can be used to attract individuals who hid during the capture procedure (refer to this technique as playback).

Some species of diurnal frogs are (very) poisonous, especially those have very striking color, contrasting black with some very light color, such as yellow, white, light green, red or blue. Handle these species with care and wash hands as soon as possible. Never touch eyes or mouth after handling one of these animals, until hands have been washed under running water.

Keep collected individuals in plastic bags with some damp leaves inside, in a shady location. With a marker-permanent, write on each bag information about the location (grid/module, trail, plot) and time (date, start time) the survey title, as well as information about the recording, if any.

Never leave the animals in the sun. If you need to travel long distances, put plastic bags inside a large cloth bag, and soak it with cold water constantly, until it reaches the base or campsite.

After recording and/or capturing an animal, always return immediately to where the survey was interrupted.

Comments on the collection and care of data.

For security reasons, surveys are usually made by two people. However, many statistical analyzes are made on the premise that the data have been collected by a single observer. So, if a field assistant observed an animal that the observer did not detect, this must wait until the end of the survey of the segment. Any data obtained in this way should be noted separately. These records can be considered "casual encounters" or integrated into the raw database, provided that a separate column is created containing data about who was responsible for each record.

If the second observer has training in anuran surveys identification can be done by both. If this is the case, it is important that each observer has their own data sheet and don't inform each other until the end of the survey of the plot. The database should also contain information about who did what.

To obtain estimates of the probability of detection and population in plots or modules, it is important to have some data on the viewer's detection efficiency. Therefore, it is recommended that each plot, is sampled at least three times by the same observer. Thus, one can obtain a reasonable measure of how much effort is required for a given observer to detect a species that occurs in a particular survey unit.

The abundance of a named species using this method is extremely dependent on the experience and perception ability of the observer. Thus, if the same investigator is responsible for the survey in all the plots the abundance estimates are equivalent.

If for some reason the survey has been done by multiple teams and you see very large differences in the estimated number of individuals in the same plot for different observers, it is recommended that only the presence or absence data is used for the statistical analyses that are required.

Upon returning from the field, the raw data digitally scanned and immediately entered into the spreadsheet program of your choice Keep copies of this data in various media (such as external hard drives, CDs, email or DropBox). Every time the data changes spreadsheet, redo all copies, keeping all the backup files updated.

Finally, deposit the original scanned spreadsheets and forms into free access digital data repository. This will allow the data to be used in comparisons with other databases constructed from standardized surveys.

Useful websites for taxonomic revision

During the last two decades, molecular and behavioral data have begun to be used quite frequently to assess the evolutionary relationships between frog species. Previously, these relationships were mainly based on external morphology. This new information has resulted in taxonomical rearrangements in the way the species are classified better, reflecting their family relationships.

Because of these rearrangements, the team responsible for the survey should take care to tabulate and provide the data collected in the field, especially if the species identifications were based on relatively old guides. The team also needs to verify whether individuals of the same species have been given different scientific names because different observers have used different references.

Some websites are very useful for this. To find out the current scientific name of a species, its most recent and possible synonyms, use the website Amphibian Species of the World:

Taxonomy, systematics and current nomenclature of amphibian species:

Amphibian Species of the World - American Museum of Natural History
(Http://research.amnh.org/vz/herpetology/amphibia/)
For a list of frog species that occur in Brazil, see Sociedade Brasileira de Herpetologia's website
List of Brazilian amphibians: Brazilian Herpetological Society
(Http://www.sbherpetologia.org.br/checklist/anfibios.htm)
To verify that the geographical distribution of a species coincides with your area of study, and the conservation status of the species, see the red list of the IUCN amphibians:

Geographical distribution of species and conservation "status":

International Union for Conservation of Nature and Natural Resources - IUCN
(Http://www.iucnredlist.org/initiatives/amphibians)

See also:

Software for acoustic analysis:
The Raven program, developed by the Laboratory of Ornithology at Cornell University is one of the most popular and complete alternative for studies involving vocalizations. Raven Lite version 1.0 contains the necessary resources to build sonograms. It is free and can be downloaded from the laboratory website (Http://www.birds.cornell.edu/brp/raven/RavenOverview.html). Full versions of program are paid, but students may request temporary licenses at low prices.

The sonograms (Fig. 9) are three-dimensional graphics that visually represent the sounds. From them, an expert can verify characteristics such as duration and intervals, frequency of vocalization, or parts of it, and make comparisons with other vocalizations that have been already described, aiming at correctly identifying the species. The sonograms can also be printed and used as supplementary information to field guides, or a survey reference collection.

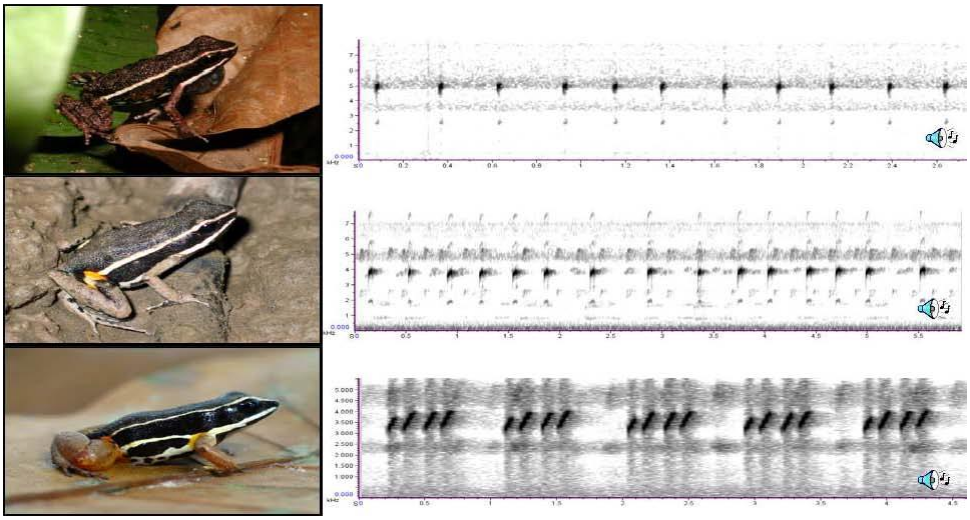


Figure 9. The sonograms of three species of diurnal frogs found in the Brazilian Amazon, with very similar markings and morphology (from top to bottom: Ameerega hahneli, Ameerega pictish, Allobates femoralis). Photos: Albertina P. Lima and Walter Hödl.

Sonograms are three-dimensional graphs showing information about the length (X-axis), frequency (Y-axis) and the volume or sound pressure (whose amplitude is shown in shades of gray; the louder the sound, the darker the color. White areas indicate intervals or frequency bands (silent). Although visually similar, the sonograms help us to distinguish the three species without great difficulty. All sonograms were generated in Raven 1.2 program.

A collection of sounds of Amazonian frogs:

Since 2011, the Program for Research in Biodiversity (PPBio) has operated an online collection of vocalizations of species of Amazonian frogs, named Sapoteca. On the website, each species can be listed by its scientific name or photo. In addition to vocalizations there are also videos and sonograms (.jpg format), which can be used in field guides, to help the identify individuals in nature. Since its creation, a special effort has been made to information on specimens coming from the type locality of each species, allowing for unambiguous comparison with exemplars collected or recorded elsewhere. The Sapoteca can be accessed directly by the link https: //ppbio.inpa.gov.br/sapoteca/paginainicial.

Laboratory procedures

In surveys conducted in remote areas where there are no guides or species reference collections, all and any types of biological information can further assist in the identification of individuals.

A detailed description of laboratory procedures goes beyond the scope of this document.
However, here are some general recommendations:
Before collecting any kind of biological material plan the laboratory procedures. Always consult a specialist in herpetology and check that the suggested protocols follow the rules accepted by Reptiles National Center for Research and Conservation and Amphibian ICMBio (RAN / ICMBio) and the ethics board of their institution, if any. Never collect any material without having authorization from SISBIO / ICMBio. Today, it is essential to obtain a sample of muscle or liver tissue of all samples that are collected and intended for scientific collection as a testimony. This tissue can be used in genetic analysis that will assist in clearly identifying the species or, for example, in monitoring or management programs impacts on the genetic diversity of a species or population over the long term.

The tissue should be stored in suitable tubes (cryogenic or Eppendorf tubes) filled with ethyl alcohol (> 95%) and labeled to tie it to the original exemplars. It is essential to use a unique number (field number), to link the fieldwork to a recording, the tissue sample and the specimen deposited in the museum or collection.

In addition, all specimens or samples in a zoological collection, must have the geographical coordinates of their origin. It is up to the managers and curators of collections to ensure that the correct audit trail documentation is maintained.

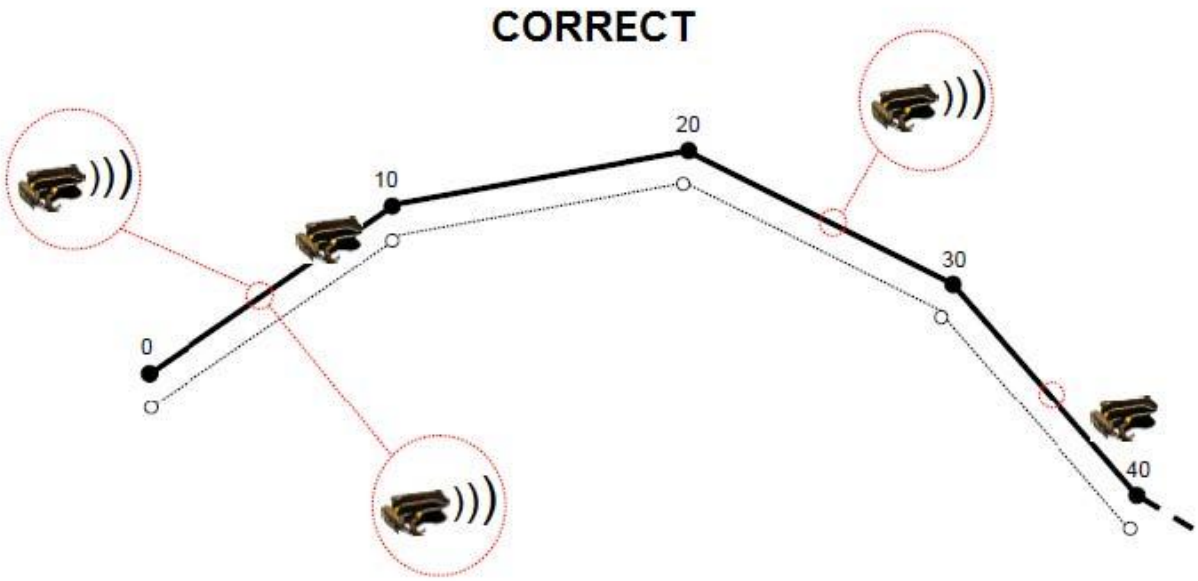
Annex I Example of a datasheet for surveying diurnal frogs using a RAPELD plot.

Date: Time: Air Temp/humidity: Team: Module: Trail: plot:

Species	Seen	Heard	Segment	No collected	# total	# recorded	Obs.

Annex II – Diurnal frog survey using RAPELD. Visual and acoustic detection. Correct and Incorrect detection.

Small red circles denote the observer's position at the time of recording an individual in calling activity. The table shows a (partial) standardized data sheets for surveying frogs through the proposed protocol.



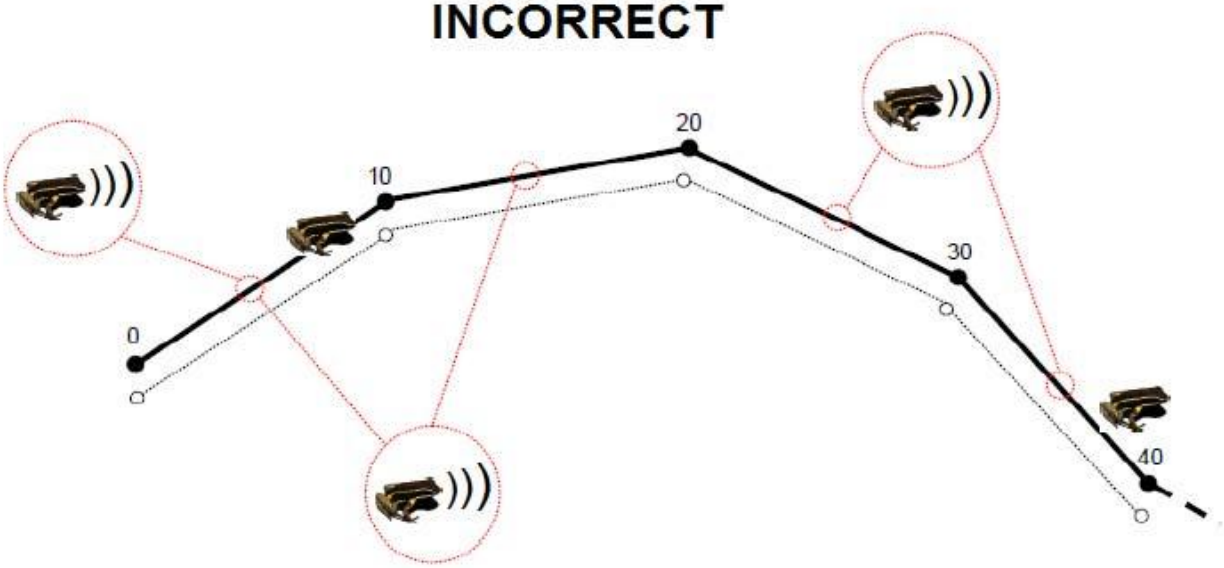
Species	Heard	Seen	Segment
Allobates femoralis	X	X	10
Allobates femoralis	X	0	30
Allobates femoralis	0	X	40

a) The species is recorded in the segment where it was detected;

b) Only the presence of the species is recorded, not how many. X or 0 = yes or no.

c) The detection type is recorded in a heard or a seen column.

INCORRECT



Species	Heard	Seen	Segment
Allobates femoralis	2	1	10
Allobates femoralis	1	0	20
Allobates femoralis	1	0	30
Allobates femoralis	1	1	40

a) The same individual is counted twice.

b) The observer has tried to estimate the number of individuals that he heard.

c) The count is continuous, not binary.