Manual on Methods for Monitoring Biodiversity in Panama

Edited by

Carolina Puerta-Piñero, Raymond E. Gullison, Richard S. Condit

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Editors

Dr. Carolina Puerta-Piñero. Instituto de Investigación y Formación Agraria y Pesquera de Andalucía (IFAPA). Camino de Purchil s/n. 18004. Granada. Spain. email: cpuertapinero@gmail.com

Dr. Raymond E. Gullison. Hardner & Gullison Associates. Box 202, 7163 Lancrest Terrace. Lantzville, BC. V0R 2H0. Canada. email: ted@hg-llc.com

Dr. Richard S. Condit. Instituto Smithsonian de Investigaciones Tropicales. 9100 Panama City Pl. Washington DC 20521-9100. USA. email: conditr@gmail.com

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About the authors

Paul Angermeier

Dr. Paul L. Angermeier is a research scientist and Professor in the US Geological Survey stationed at the Virginia Cooperative Fish & Wildlife Research Unit at Virginia Tech, Department of Fish and Wildlife Conservation. His main research interests include the ecology and conservation of stream fishes, the provision and use of aquatic ecosystem services, and assessments of environmental quality based on resident biota.

Richard Condit

Dr. Richard Condit has been Staff scientist at the Smithsonian Tropical Research Institute, Panamá, for 22 years. His interests in populations of wild animals and plants are broad, including marine mammals, birds, microbes, and most often forests and trees; and natural history and field guides about all these organisms have been a staple of his experience. He has written 160 scientific papers, a book on the methods behind measuring and mapping tree plots, and a guide to the trees of Panama.

Raymond Gullison

Dr. Raymond E. (Ted) Gullison is an Honorary Research Associate at the Centre for Biodiversity Research at the University of British Columbia and a consultant to non-profits, governments, foundations and corporations on biodiversity management.

Roberto Ibáñez

Dr. Roberto Ibáñez is Director of the Amphibian Rescue and Conservation Project in Panama, Smithsonian Tropical Research Institute (STRI). Recently, he has been STRI's research associate, part-time professor at the Universidad de Panamá, and professor during the Panama Field Study Semester of McGill University. Roberto participated for several years in a series of projects focused on monitoring and conservation of biodiversity in the Panama Canal watershed. Roberto has published a guide to the amphibians of the lowlands of central Panama. He was also an active supporter of The Research and Analysis Network for Neotropical Amphibians (RANA).

Patrick Jansen

Dr. Patrick A. Jansen is research associate at the Smithsonian Tropical Research Institute, Panama, and assistant professor at Wageningen University, the Netherlands. His primary interests are interactions between vertebrate and plants, such as seed dispersal and herbivory. He has 25 years of research experience in tropical forests. Jansen coordinates the Vertebrate Program of the Smithsonian Institution Global Earth Observatory (SIGEO), a global network of forest plots, as well as Smithsonian's contribution to the Tropical Ecology Assessment and Monitoring (TEAM) network, including extensive camera trapping in tropical forests.

Carolina Puerta-Pinero

Dr. Carolina Puerta-Piñero is a researcher in the Institute of Agricultural Research and Training (IFAPA, Granada, Spain). Her scientific interests are broad, ranging from plant-animal interactions within populations to the application of landscape approaches, to the study of how communities and ecosystems dynamics change over time and space under global change scenarios including study systems in temperate and tropical forests.

Rolando Pérez Rolando

Bh. Rolando Alberto Pérez Mendieta works at the Center for Tropical Forest Science, Smithsonian Tropical Research Institute. He has been in charge of leading and supervising forest censuses in 1995, 2000, 2005 y 2010 within the 50 ha plot in Barro Colorado Island, Republic of Panama. Recently he has visited, worked and supervised studies in plots of tropical forests in several countries (Panamá, Brazil, Ecuador, Colombia, Perú and USA).

James Roberts

Dr. James H. Roberts is a Research Associate in the Department of Fish and Wildlife Conservation at Virginia Tech University. His research seeks a greater understanding of the dispersal ecology, metapopulation dynamics, community regulation, and conservation genetics of stream fishes, as well as the development of novel, non-invasive techniques for sampling fish populations. Over the past 15 years, he has sampled fishes throughout North America using a variety of techniques.

Douglas Robinson

Dr. W. Douglas Robinson works at the Department of Fisheries and Wildlife, Oak Creek Lab of Biology, Oregon State University, Corvallis, Oregon USA. He studies the dynamics of bird communities and how birds respond to environmental change. He has studied birds in the tropics since 1992, including evaluations of how forest fragmentation influences birds in Panama and Costa Rica. He has surveyed birds in Panama every year for the last 21 years.

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SECTION 1

Chapter 1. Introduction

Raymond E. Gullison, Richard S. Condit, Carolina Puerta-Piñero

Why monitor biodiversity?

Biodiversity is the variety that exists in living things, including variation at the genetic, species, ecosystem and landscape levels. As part of the natural world, biodiversity is important in its own right, but it also provides humans with essential ecosystem services, such as contributing to the regulation of water quality and quantity, provision of bush meat and timber, and acting as a source of genetic diversity for food crops and medicines.

There are many reasons to survey and monitor biodiversity. These reasons include:

- To understand patterns of biodiversity at different spatial scales, ranging from individual sites to across entire countries.
- To understand how natural and anthropogenic stressors such as climate change, land use change, and industrial development influence biodiversity.
- To understand the effectiveness of different types of management actions taken to enhance or protect biodiversity.

One of the most important places to monitor biodiversity is protected areas. Those areas act as safeguards for protecting biodiversity, as well as serving as "ecological controls". By comparing biodiversity outside of parks to relatively intact biodiversity within national parks, we can better understand the impacts of human actions on the environment.

The role of this manual in supporting biodiversity monitoring in Panama

At present, Panama, like many countries, does not have a standardized credible methodology for monitoring biodiversity. The lack of a standardized methodology creates at least two significant problems. The first problem is that the lack of guidance on robust monitoring protocols and survey design leads to the collection of information that cannot fulfill the objectives for which it was collected. For example, if surveys to support an environmental assessment are not designed in a rigorous manner, they may fail to detect sensitive species or ecosystems that may require special mitigation. The second problem is that the lack of a consistent methodology across sites prevents the direct comparison of biodiversity information from different sites, and therefore hinders or prevents an understanding of the patterns of biodiversity at larger spatial scales. For example, if the abundance of bird species is monitored differently at different protected areas, it may not be possible to combine the information to say which protected area has populations with the highest viability, and is therefore most important in protecting the species.

Our hope is that this manual will provide a standardized methodology for biodiversity monitoring, and as a result, help to improve the quality and consistency of work done at individual sites, as well as the ability to compare biodiversity at different sites. In this way, it will enhance the understanding biodiversity in Panama and improve the scientific basis for its management.

Here are some specific examples of how different user groups might benefit from adopting the biodiversity monitoring methods that are described in this manual:

Governments: governments are potentially the greatest beneficiaries of adopting standardized and robust methodologies for monitoring biodiversity. These methods can be used to help environmental ministries understand the diversity and distribution of biodiversity across their territories, and to support integrated land use planning that helps to conserve these species, while also meeting social and economic objectives.

There are many other ways that governments will benefit from adopting the methodologies described in this manual.

- The methods in this manual can be used to help evaluate how well protected area managers are maintaining biodiversity within protected areas.
- They can be used to support environmental assessments and mitigation planning for large industrial projects such as mines or energy developments.
- These methods can be used to inform many other problems faced by decision makers, such as the management of large predators, the development of forest management plans for timber, and greenhouse gas inventories in different land use and land cover types.

Scientists: scientists will benefit from standardized biodiversity monitoring methodologies because it increases the quality of their own data, and improves its comparability with data collected by others.

Other groups, such as science-based NGOs, naturalists, and interested private land-owners, can also benefit from the deployment of the methods described in this manual.

The approach taken here

With this manual we have taken the approach of providing a simple set of core, meaningful biodiversity monitoring protocols, along with straightforward guidance for survey design (deciding on where to measure biodiversity in the landscape). The methods described in this book can be used to conduct initial baseline studies or surveys of biodiversity at a site. Periodic re-measurement of the same sites will help to understand how biodiversity is changing over time. Following the methodology will allow the computation of biodiversity metrics, such as the absolute or relative abundance of species, the diversity of assemblages or communities of species, and the structural diversity and biomass of forests. We felt that developing a pragmatic approach for monitoring biodiversity was better than prescribing a highly technical and comprehensive approach, which would be challenging to implement and not feasible for biologists without a strong technical background.

The basic components of our approach are:

- The focus of this manual is biodiversity in forested habitats. Nevertheless, many of the protocols described here will work in other types of natural habitats, or in degraded forests. Consultation with local experts will assess the adequacy of these methods in non-forest habitat and ensure that they are adjusted if necessary.
- In this first delivery, we have selected taxonomic groups (birds, large mammals, amphibians, reptiles, trees, and fresh water fish) that form a credible and practical basis for monitoring species and community diversity.
- We provide a complete description of the monitoring protocol in non-technical terms so that it is understandable to someone who is not a specialist in the field.

- We provide general rules of thumb for survey design (the number and distribution of survey points in a landscape) without making the approach overly technical.

Each chapter has been written by a recognized expert in the field, all with experience in Panama or similar sub-tropical and tropical habitats. Chapter 2 discusses issues that are common to monitoring all of the taxonomic groups addressed in this manual. In Section 2 of the manual, Chapters 3 – 7 describe taxon-specific protocols for birds, fishes, large vertebrates, amphibians and reptiles, and plants respectively. Chapter 8 provides recommendations for managing and analysing the collected data. Each chapter attempts to:

- Recommend specific monitoring protocols;
- Discuss challenges related to species identification and handling;
- Describe the training required for staff to implement the methodology in the field;
- Identify logistical issues; and finally,
- Make recommendations for the collection and storage of data.

The manual concludes by presenting analytical methods that can be used to calculate a set of core species and community metrics useful for describing the results of the monitoring. References and resources are listed at the end of the manual for readers who desire a more technical treatment of the methodologies described in the manual.

Limitations of this manual and future directions

As mentioned above, the intent of the manual is to provide a practical set of core biodiversity monitoring protocols that can be used for a diversity of purposes in forest habitats. However, there are some applications for which this general methodology will not be suitable, and the reader will need to seek guidance elsewhere. The following topics are not covered by this manual:

- Generating reliable estimates of the abundances of rare/inconspicuous species – such species are too rare or cryptic to be reliably detected with the generalist methodologies described in this manual, and require tailored methodologies designed specifically to their needs.
- Detailed guidance on conducting power analyses – general guidelines are given to ensure that sufficient samples are taken at a site, but a detailed statistical treatment is beyond the scope of the manual and the basic protocols.
- Biodiversity monitoring protocols for other taxa – methodologies for many other diverse and potentially important taxonomic groups are not included in this manual, as for invertebrates, herbaceous plants and fungi.
- Biodiversity monitoring protocols for rare or sensitive habitats – methodologies are not provided for habitats such as swamps, marshes, and disturbed areas.
- The human use of biodiversity – this manual does not include protocols for directly measuring anthropogenic pressures on biodiversity such as hunting, collection for the pet trade, and logging.
- Comprehensive description of possible post hoc statistical analyses – this manual describes how to calculate basic and meaningful metrics from survey data, but does not provide a comprehensive and detailed technical treatment of all possible metrics that could be employed.

- Methodologies for assessing biodiversity below the level of species – the focus of this manual is the study of species, communities (assemblages of species), and forest structure. It does not provide guidance on the study of genetic diversity, for example.

These topics may be addressed in future versions of this manual. We encourage feedback from users of this manual so that future revisions can better meet their needs.

Chapter 2. Core Methods for Establishing The Monitoring Project

Richard S. Condit, Raymond E. Gullison, Carolina Puerta-Piñero

1. Introduction

In this chapter we cover several aspects of the methods required for the entire project, extending across surveys of all taxonomic groups. This includes the selection of sampling locations, since we consider it important to – as much as possible – sample trees and vertebrates at common sites in the forest. The exception to this goal is the monitoring of large vertebrates, which must extend over much larger areas than other taxonomic groups we cover.

Our program for positioning a network of survey sites begins by considering forests areas that are fairly homogeneous environmentally - that have only modest variation in climate or elevation (including precise recommendations about how much variation is modest). These would be areas usually less than 20,000 ha (or 200 km^2), though in some cases could be as large as 50,000 ha (500 km^2) in unusually large homogeneous areas. In such a study area, a network with a minimum of six and up to a maximum of 15 survey locations should be chosen following the precise guidelines outlined below. Surveys of plants, arboreal birds, primates, stream fauna, reptiles, and amphibians will be undertaken at each of those sites. The survey of large vertebrates, however, will extend across the entire study area.

This chapter also discusses personnel requirements across the entire program, including supervisors and coordinators of all the work, and some core information about taxonomic needs. Section 2 of this guidance manual includes chapters that provide detailed survey methods for individual taxonomic groups, expanding on topics covered in this chapter and adding detailed field methods.

2. Site Selection

It is our recommendation that surveys of all of the taxonomic groups be executed at a common set of sites; but of course it is impossible to census precisely the same parcel of land for fish, birds, trees, and jaguars. Our intent is to define sampling sites as plots of 1 km^2, and for each taxonomic group to be sampled within. There are three principal reasons for joint sampling in this manner: 1) field work and transportation will be more efficient if all teams work in the same area; 2) results from different groups can support one another, for example, tree canopy height and density might be relevant to fish and frogs; and 3) long-term changes through time of all the groups are measured at the same locations, so the impact of disturbances can be assessed across taxa. We recognize, however, that there are detailed considerations of different groups that may require some sampling sites where only a subset of the taxonomic groups are studied.

Because tropical forests of Panama are rich in biodiversity, replicated sampling sites are needed. The difficult problem is that many species are uncommon or rare, and therefore not encountered in great numbers. In order to capture statistically meaningful results about abundances of much of the community and to document the presence of many rare species, minimum sample sizes must be considered with care.

Even in small areas of forest (< 5000 ha) in fairly homogeneous terrain, a minimum of six sites should be surveyed for trees and vertebrates. In much larger areas, covering > 10,000 hectares and variation in elevation and climate, the minimum number of sites must be 20-30. Even that many will be insufficient for the largest forest blocks in Panama, those covering >

250,000 ha. Surveys and censuses to be done at each of these sites will be covered in detail in the relevant chapter in Section 2. This chapter provides guidelines about how to locate the sampling sites across a region.

A. Preliminary needs for site selection

Good maps and Geographic Information Systems (GIS) tools are widely available in Panama, and careful selection of survey sites requires understanding the terrain and vegetation types. A topographic map of the entire area is needed, with roads (any accessible by 4WD), streams, and human settlements included. A map of land-use that indicates forest and agricultural land must be overlaid on this.

Two additional chores are subsequently important, since most existing maps will lack these elements. First, trails through the forest should be mapped by walking with a hand-held GPS. Second, any permanently-flowing streams that are not found on the principal topographic map should be added. In very large forest blocks, it may be unfeasible to map every stream or trail. The alternative, then, is to review our sampling recommendations below first, then map a randomly-chosen subset of streams and trails from across the region. These two final features need to be merged in the main map or GIS.

B. A random grid of sampling sites in small forests

Consider first a proposed study area of forest covering < 5000 ha that is relatively homogeneous in terms of elevation and climate and includes only one major river drainage. All forest streams should be mapped, and the smallest and largest with permanent flow identified. In such an area, effective surveys of species present could be accomplished with six sites placed in such a way that no two are within 1.5 km of each other.

If there is one stream, sites should be placed at random intervals along it, spaced by > 1.5 km; with two streams, three sites should be placed on each, etc. The locations should be chosen by looking at a map, before going in the field. After choosing the initial locations on streams, a companion site must be selected for each of the six. This second site must be > 500 m but < 1000 m perpendicular distance from the stream and the sampling site. Each site should be chosen by generating a random side of the stream (ie, left or right when facing downstream) and a random number between 500 and 1000. Again, this is done on a map without going into the field (see Figure 2.1 for an example).

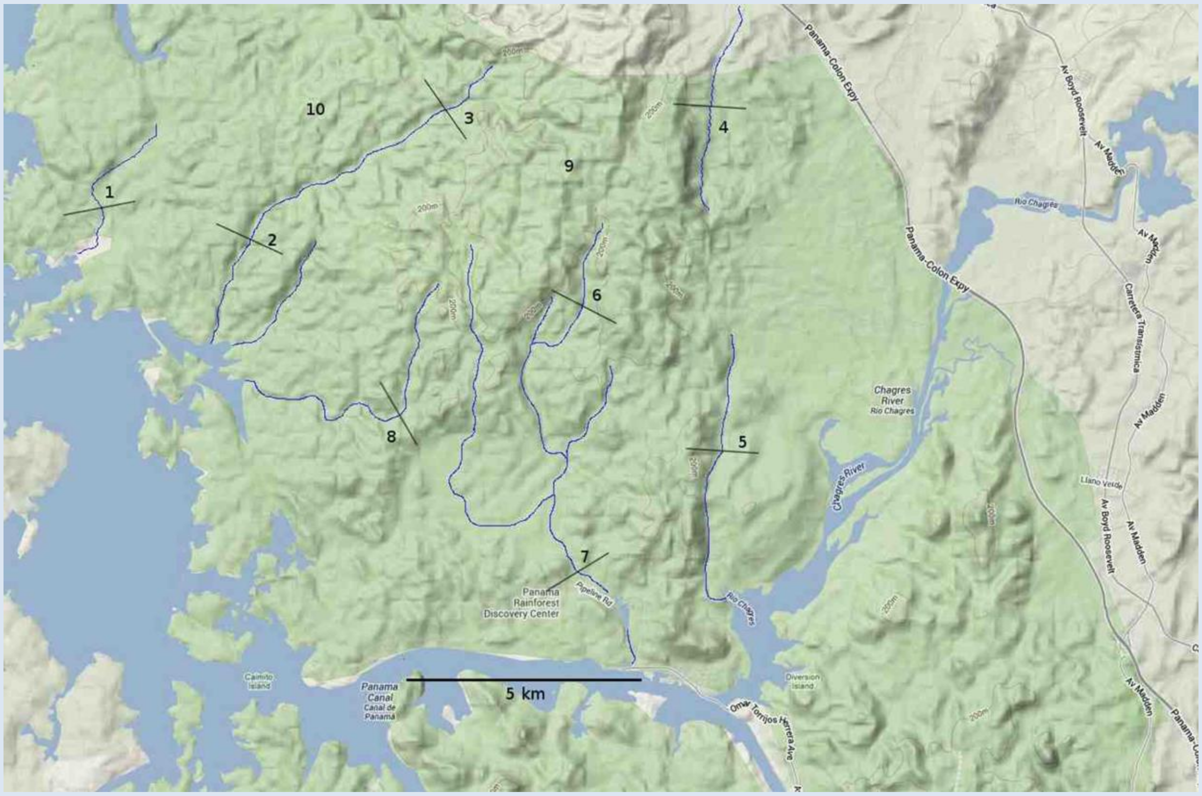


Figure 2.1. Arrangement of sampling sites in a small forest. The hypothetical sampling is designed to cover 10,000 ha of forest in Parque Soberanía. The background map shows the terrain of the area. Given 10,000 ha to sample, we recommend eight sampling sites arranged near stream valleys (sites numbered 1 to 8), and two ridge sites > 1 km from streams (9 and 10). Five major streams are mapped: sites 2 and 3 are on one stream, and sites 6 and 7 another, in both cases adjacent transects are separated by at least 1.5 km. The other major streams each have one site. The black lines are 2 km long, perpendicular to streams, and form the basis of the surveys for trees, birds, amphibian/reptiles, and fish. Details of plot and transect arrangement near those 2 km lines are given in respective chapters about each group. The cameras aimed to census large mammals would be placed on a grid across the entire forest area on the map.

When sites are chosen following this arbitrary algorithm, there is a possibility some might be impossible to access or outside the study area. If so, the random selection must be repeated, until a suitable site is found.

Stream surveys for fish, amphibians, and reptiles will be carried out at each of the six stream locations. The six companion sites will form the basis of additional terrestrial surveying for trees, arboreal birds, primates, and terrestrial amphibians and reptiles (see Figure 2.2 for a hypothetical example and Table 2.1 for a recommended timetable for fieldwork). Details of how censuses of each group are done will be provided in the respective chapters of Section 2.

Table 2.1. Recommended timetable for biodiversity monitoring in Panama. Grey cells indicate the months recommended for fieldwork for different taxonomic groups. To the extent possible, the overall project should begin in January, coinciding with the start of the dry season. In order to facilitate this start date, selection of study sites and the organization of office space should be completed before the start of the project (ideally November-December).

	Month												
Taxonomic Group	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**Estimated duration of fieldwork per site**
Birds (Chapter 3)				■	■								15-30 days
Fish (Chapter 4)	■	■	■					■	■	■	■		1-5 days (2 times per year)
Mammals (Chapter 5)	■	■	■	■									1-2 months
Amphibians and reptiles (Chapter 6)	■	■	■		■	■		■	■		■	■	10-12 days (4 times per year)
Woody vegetation (Chapter 7)	■	■	■	■	■	■							1-2 weeks

Camera traps for censusing large vertebrates such as cats, deers, and tapirs must be placed over a much wider area than a single sampling site. Since a typical set of 60-100 cameras should span about 10,000 ha (100 km^2), a single camera network will be placed spanning 10 of the local sites. Details on the camera network are given in Chapter 5.

C. Larger forest sites and important heterogeneity in Panama

In proposed study areas larger than 5000 ha, environmental heterogeneity must be considered. There are three principal axes of variation in Panama's forest that must be taken into account: elevation, watersheds, and distance from the Pacific coast. The last is a surrogate for rainfall, since rainfall in Panama increases substantially moving from the Pacific toward the Caribbean. For each of the three gradients, important zones must be defined.

Environmental gradients in Panama

Elevation. There is a reasonably consistent trend across many taxonomic groups in Panama for species communities to change at about 750 m and 1500 m above sea level. While not entirely consistent, this works fairly well as general rule in guiding site selection and leads us to recommend dividing any forest area into three zones based on elevation: 0-750 m, 750-1500 m, and > 1500 m. Note that there are not many forest blocks in Panama spanning all three zones, unless they are very large national parks.

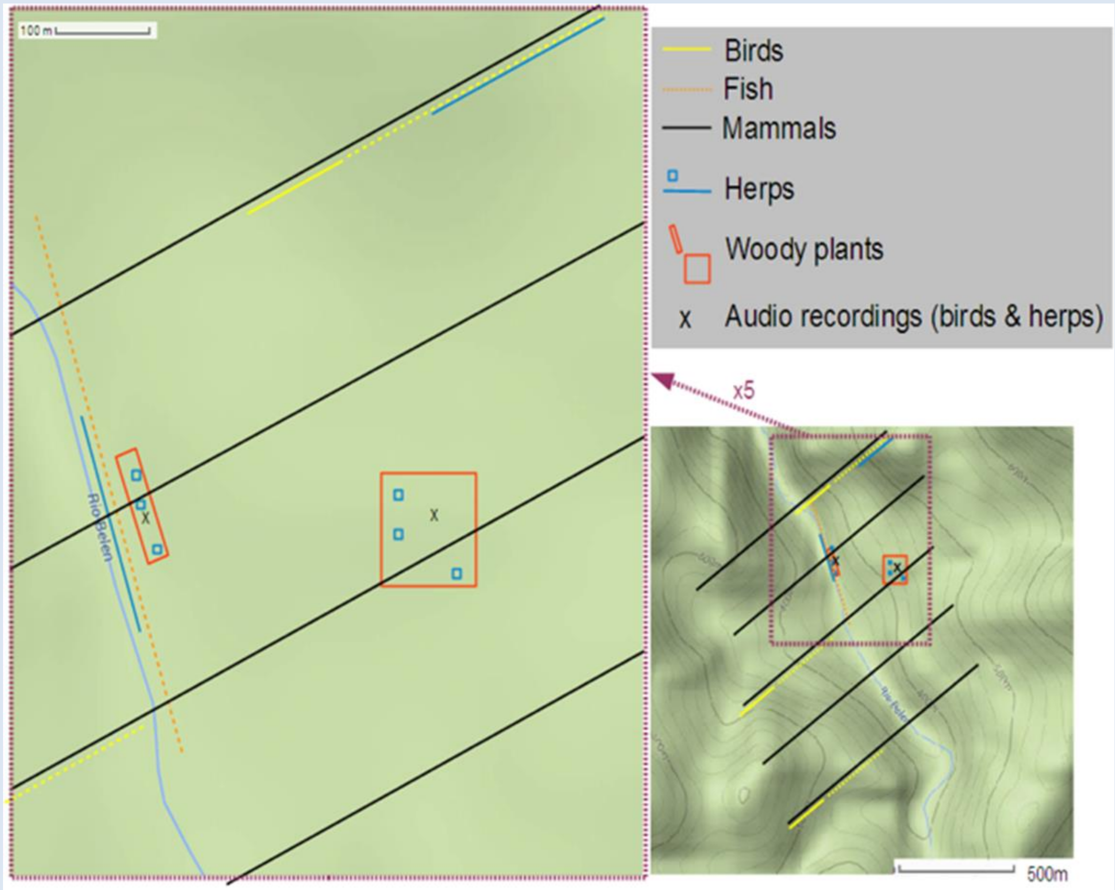


Figure 2.2. An example of a hypothetical sampling site including the spatial arrangement of the field procedures for all the taxa. For a spatial arrangement of camera traps please see Chapter 5.

Watersheds. Defining important river drainages is an important axis for variation in aquatic species. There are three considerations: First, any river drainages on opposite sides of the continental divide must be separated as two sampling zones. Second, if a forest area includes two major rivers that separately meet the ocean, their basins should be considered separately. Lastly, any two major rivers in the lowlands with a mountain ridge > 750 m elevation between them should be treated as separate zones. Criteria for identifying 'major rivers' are somewhat arbitrary, and local biologists with experience in the area must decide. Only large forest blocks in Panama will cover more than one drainage based on these criteria.

Table 2.2. Sample size in homogeneous forest. Core samples are 1 km square units covering aquatic and terrestrial groups. Ridge samples are far from streams and include only terrestrial groups.

Area	Core samples	Ridge samples
<5000	6	0
5000-10,000	8	2
10,000-20,000	10	2
20,000-50,000	15	4

Rainfall. A simple surrogate for rainfall within any forest block in Panama is distance from the Pacific Ocean. Any forest zone with edges spanning a range > 10 km from the Pacific coast should be divided into subsets. Here, forest zone refers to a division based on criteria 1 (Elevation) and 2 (Watersheds), that is, the entire study region has already been divided into elevation zones and watersheds. After these zones are defined, find *Dmin* as the distance from one edge of a forest zone to the Pacific (i.e. where the forest is closest to the Pacific). Then find *Dmax* as the distance from an opposite edge of the same forest zone to the Pacific (i.e. where the forest is furthest from the Pacific). If *Dmax-Dmin* > 10 km, then the zone spans important variation in rainfall, and it should be divided further, into smaller zones.

Medium-sized forest without heterogeneity

The first consideration for larger areas is forest blocks > 5000 ha but which lack any of the heterogeneity that was described in the previous section. In Panama, this will usually mean < 20,000 ha, because larger areas will encompass heterogeneity. With 5000-10,000 ha, there should be eight sampling sites, and for 10,000-20,000, ten sites (Table 2.2, core samples). In the event that homogeneous areas much larger are chosen, more sites can be added (Table 2).

Valley-ridge catena

In sampling forests much larger than 5000 ha, an additional environmental axis must be added to the considerations at this point. This axis is the gradient from valley bottom to ridge crest, and is relevant only for terrestrial communities. The sampling unit defined in paragraph 1.2 of this chapter is 1 km squared and extends 1 km away from streams; and in mountainous areas in Panama, such sampling would exclude high ridges that might be several kilometers from the nearest stream. When there are such ridges, 2-4 terrestrial sampling sites should be chosen at random near the ridge tops (Table 2, ridge samples). In areas < 5000 ha, we assume the core samples < 1 km from streams adequately cover terrestrial variation, so no ridge samples will be needed.

Heterogeneous forests

If the region of forest under study is heterogeneous by the criteria of elevation, watersheds, and distance to the Pacific, sampling needs to be stratified following Table 1. The rules of Table 1 lead to a zonification: the whole forest region is divided into smaller zones, and these zones need to be mapped and their areas estimated. For individual zones < 50,000 ha, the number of samples in each can be drawn from Table 2.

A gradient of accessibility

Before selecting the sites in each zone, following the rules illustrated in **Figure 2.1**, one additional aspect must be considered. A crucial factor in understanding the natural biota of Panama is the impact of people who hunt large vertebrates or harvest wood or other plants. The fact of the matter is that forests near settlements and roads are altered. Conversely, regions where large vertebrate communities and higher forest biomass might approach the undisturbed state are remote, far from roads.

We must strive to sample these remote communities; and by definition, inaccessible forests are difficult for even biologists to reach. In most cases, they require an entire day of travel on foot (or perhaps horseback) over terrain that is likely difficult. Sampling a large number of remote sites is thus impractical – too costly and time-consuming. But avoiding these remote sites entirely will cause a major bias in the sampling, omitting the areas where an intact biota is most likely to be found.

The balance we recommend is to locate approximately 15% of the sample sites in forests that are > 10 km from any road or settlement. If there are no forests > 10 km from roads, then the criterion would be relaxed to a minimum distance of 5 km from roads. Obviously, it is possible that sites in Panama have no such remote forest (none > 5 km from a road), and in these circumstances, samples should be placed as much as possible at varying distances from roads. Maps of forest, roads, and major trails must be used to stratify samples by accessibility.

Summary on site selection in larger forests

Within each zone, the assigned number of locations is then located along streams, with companion sites for each, following the recipe illustrated in Figure 2.1, calculating the number of samples in each zone using Table 2. In large areas in which roads are sparse and accessibility difficult, the gradient of accessibility must be taken into account, and the rules of Figure 1 must be applied relative to the location of roads. If randomly chosen points include too many close to roads, or too many far from roads, new selections should be made to generate a good balance of remote sites with sites that are easy to access.

In very large areas in Panama with several environmental zones, the total number of recommended samples might exceed 30 or even 50. For example, Darien National Park would have to be divided into eight different zones, several of which are > 20,000 ha, leading to a recommendation of 90 sampling sites. Realistically, the Darien would in fact require sampling of that magnitude to be covered well, but for practical reasons, this would have to be divided into several sub-projects.

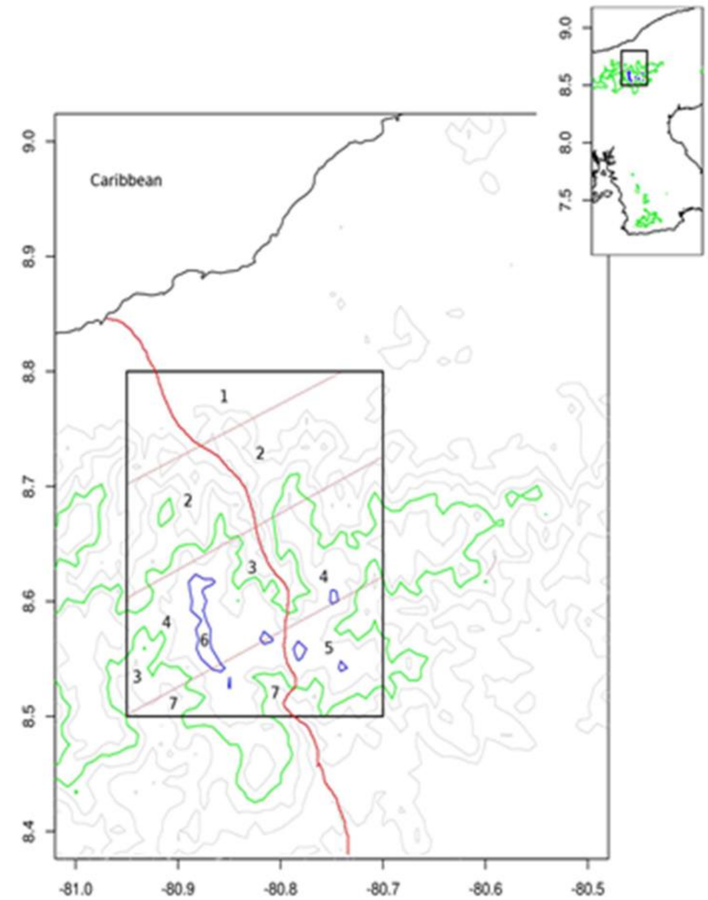


Figure 2.3. Sampling zones across a large, heterogeneous forest region. The map shows a hypothetical proposal for sampling a rectangular area of 82,000 ha in Panama, straddling the Veraguas-Colon border (as shown in inset). Axes are labeled with latitude and longitude. The blue topographic line is at 1500 m ASL, the green at 750 m ASL, defining three elevation zones (lowlands, < 750 m; mid-elevation, 750-1500 m; high elevation, > 1500 m). Parallel red lines divide the region into sections based on distance from the Caribbean coast; the lines are at 5, 15, and 25 km from the coast. Seven sampling zones are indicated: 1) lowlands 5-15 km from the coast; 2) lowlands 15-25 km from the coast; 3) lowlands 25-35 km from the coast; 4) mid-elevation 25-35 km from the coast; 5) mid-elevation > 35 km from the coast; 6) high elevation; 7) low elevation > 35 km from the coast. The red curve is a hypothetical road, and some portions to the northeast and southwest of the sampling rectangle are > 10 km from the road. Based on Table 1, we would propose six sampling sites in each of the zones 1, 3, 5, 6, 7, plus ten sampling sites in the large zones 2 and 4. A total of eight sites in zones 2, 3, 4 should be deliberately placed > 10 km from the road. The remaining sites can be 0.5-5 km from the road. The total number of suggested sites would be 50 by this scheme, but zones 3, 6, and 7 are small, and the elevation just barely exceeds 1500 m ASL. It would thus be reasonable to reduce sampling in those zones considerably.

3. Basic Taxonomic Methods

In Panama's forests, rich in species diversity, the task of correctly identifying specimens cannot be taken lightly. A substantial investment in training in taxonomy is necessary, and sufficient time must be devoted to assure that specimens encountered during censuses are properly identified. It must be assumed that some specimens will need to be collected and returned to a laboratory for further study to assure accurate identification. This will be especially true for plants, but also relevant for vertebrates.

Taxonomists making collections in Panama forests need to carefully follow standards for vouchering species identifications. For plants, there are precise guidelines: at least one specimen of every species should be collected in triplicate and placed in an herbarium in Panama. If possible, the laboratory running the monitoring project should keep its own collection of plants, and a third specimen may be needed to send overseas to confirm identification. For vertebrates, photographic records should suffice in most cases: each species of fish, frog, small mammal, and where possible, bird, should be photographed in triplicate. The camera trapping program captures photos of larger vertebrates as part of the core protocol. For small birds impossible to photograph, recordings of vocalizations might stand as vouchers, if this is possible.

The field biologists responsible for identification should have prior experience with the taxonomic group on which they will work. Ideally, they will already have experience collecting specimens and handling them in the laboratory (as pressing plants, preserving fish, recording bird song). Before the project starts, a complete list of trees and vertebrates known in the vicinity of the study site should be compiled, based on published sources and online references. For birds and plants, the taxonomists need to subsequently spend several weeks studying these potential species prior to the census work. Details of this training will be covered in sections on individual taxonomic groups in Section 2 of this manual.

Tropical taxonomy can also easily run afoul of errors in Latin names. It is crucial to validate all names used against published checklists mentioned above. This avoids routine spelling errors that can plague long species lists, and it also helps to make certain that the name used is the valid, currently accepted name for the taxon found in Panama. The taxonomic authority must be recorded and validated for each name as well. The best sources for species names will be provided in the sections on individual taxonomic groups in Section 2.

4. Personnel and Training

To ensure a successful biodiversity inventory, we recommend two people take on supervisory roles for all the teams. One of these would be the project director, ideally a biologist with broad experience with field work, data management, and data analysis. This person is most likely a trained scientist who has a keen interest in the outcome of the project and will be the responsible party. He or she may be a scientist who is simultaneously involved with other projects, but who can focus a good deal of attention on the forest monitoring. We assume this director's position is a scientist already in place, whose salary is covered by a participating institution.

The second supervisory position is a field and laboratory coordinator. This person is responsible for details of logistics: making sure equipment, supplies, and transportation are organized and teams are in place. The coordinator will be in the field most days, evaluating each of the teams and assisting in certain important roles, and thus should be an experienced technician with a background in biology and taxonomy. A good strategy could be to have the project coordinator serve also as a chief taxonomist, responsible for much of the identification (mostly plants, since vertebrate taxonomy will not pose many difficulties); this person might thus also be responsible for organizing and identifying plant specimens that must be returned to the laboratory for further study.

Other aspects of training cover the methods specific to the monitoring of each taxonomic group, and these will be covered in individual sections of the taxon-specific chapters. The director and coordinator should be involved in designing and executing the necessary training sessions prior to the field work, including arrangement of participation by outside experts during training where possible.

5. Permits and Legislation for the Republic of Panama

This document was drafted with assessment and support of the Protected Areas and Wildlife Directorate, Department of Biodiversity and Wildlife – National Environmental Authority (Autoridad Nacional del Ambiente, ANAM), Republic of Panama.

Current Legislation

It is highly advised that scientists review current Panamanian legislation before starting any biodiversity monitoring activities within the country. Laws referring to the wildlife of Panama may be read and downloaded from the website of the National Environmental Authority (http://www.anam.gob.pa/ from 2006 onward and from http://www.asamblea.gob.pa/main/LegispanMenu/Colecci%C3%B3nDigitaldeGacetasOficiales.aspx for previous years. The most relevant legislation regarding the protocols described in this manual may be found in:

- **Act 41, of 1998** "General Environmental Act of the Republic of Panama", G.O. (23578).
- **Act 24 of 1995** "By means of which the Republic of Panama's wildlife legislation is established and other provisions", G.O. (22801).
- **Decree Nº43** "Which regulates Act 24 of June 7, 1995 and other provisions", G.O. (25091).
- **Resolution AG-0138-2004**, "Which approves the procedures manual of the National Environmental Authority (ANAM) for actions regarding Panamanian wildlife", G.O. (25381).
- **Decree of April 25 – 29, 2009**. "Which regulates Article 71 of Act 41 of July 1, 1998, Environmental General ". (G.O. 26272)

Scientific permits

The following is a summary of the steps necessary for requesting scientific permits:

- 1º Collection request (see form in Annex 2.1) **Note**: All collection requests must be supported by a Panamanian counterpart institution and national researchers must participate in all project activities.
- 2º Letter of commitment (see form in Annex 2.2)
- 3º Informed Prior Consent. This form is required if access is requested to special-status areas (concessions or private areas), private lands, indigenous territories or communities, or a Metropolitan Natural Park. Additionally, for research to be carried out at Coiba National Park, the Coiba Scientific Committee (by ways of the ANAM liaison) must be consulted. The Informed Prior Consent form must be presented after performing an assessment of the resource to be researched (internal procedure, ANAM) (see form in Annex 2.3)
- 4º Short resume (See form in Annex 2.4)
- 5º Copy of ID or passport of each member of the research team.

- 6º Payment for fiscal stamps (B/ 8.00 as of January 2014).

The remainder of this chapter includes the original Spanish forms translated into English in order to help researchers understand their obligations. However, in all cases, scientists should submit forms to the Panamanian authorities in Spanish. In case of any differences in understanding between the Spanish forms and their English translations, the Spanish version should be assumed to be correct.

Forms should be submitted to the UNARGEN offices, Biodiversity and Wildlife Department – Directorate of Protected Areas and Wildlife; or to the relevant regional office, from which it will be resubmitted to the UNARGEN office for assessment of the request and issuance of the permit, if deemed appropriate. It is highly recommended that applicants present all documentation at least <u>60 working days before the planned project start-up date.</u>

Once research concludes, the researcher in charge must submit a **collection report** and **copies of any publications** that originate from the study, preferably in digital format, to the ANAM offices, Directorate of Protected Areas and Wildlife, Biodiversity and Wildlife Department , UNARGEN, located at Plaza Albrook, local 17, Panama (as of Jan. 2014).

The places approved for **Reference Collections** (as of Jan. 2014) are the University of Panama: Museum of Invertebrates, Museum of Vertebrates and Herbarium, and Autonomous University of Chiriquí. Collected samples must be tagged with the following information:

Scientific name:____ (Latin)

Family: ____________________________

Date: _________________________________

Site of collection:

Province: ___________________________, District_______________________________

Village: _______________________, Community:___________________________

Precise location: ____

Coordinates (latitude and longitude in decimal degrees): __________________________, ______________________________

Export and import permits

If exporting biological samples outside of the country is required, an Export Permit should be requested (see sample form in Annex 2.5). Prior to issuing the Export Permit, the research team must submit a collection report, a note certifying the delivery of the samples and the **Agreement of Material Transfer** (Annex 2.4) signed by one of the two Parties involved. If importing biological samples *into* the Republic of Panama is necessary, an Import Permit should be procured (see sample form in Annex 2.5). It is highly recommended that the Export/Import Permits be requested <u>at least fifteen days prior</u> to the date required. This is due to the fact that sometimes the samples involved are CITES samples requiring special permits that take some time to prepare.

Annex 2.1 Collection Request Form

DIRECCIÓN DE ÁREAS PROTEGIDAS Y VIDA SILVESTRE

UNIDAD DE ACCESO A RECURSOS GENÉTICOS

Solicitud de Acceso a Recursos Genéticos y Biológicos

Request to Access Genetic and Biological Resources

PARA USO OFICIAL DE LA UNARGEN FOR OFFICIAL USE ONLY			
Solicitud No.			
Fecha:		Firma del Funcionario que recibe	
PARA USO DEL SOLICITANTE *Please fill out the form below IN SPANISH*			
NOMBRE/TITULO DEL PROYECTO DE INVESTIGACIÓN *Name/Title of Research Project*			
NOMBRE DEL SOLICITANTE *Applicant's name*	*Name of PI who has a research project approved*		
Nacionalidad *Nationality*			
Número de cédula o pasaporte *ID or Passport Number*			
Dirección permanente *Permanent Address*			
País *Country*		Provincia *State/Province*	
Distrito *City*		Apdo.Postal *PO Box*	
Teléfonos *Telephone*			

Correo electrónico E-mail	
INVESTIGADOR EXTRANJERO RESPONSABLE *International Researcher in charge*	*If applicable (This only applies if the PI will not travel to Panama)*
Dirección Permanente *Permanent Address*	
Teléfonos *Telephone*	
Correo electrónico *E-mail*	
INVESTIGADOR NACIONAL RESPONSABLE *National Researcher in charge*	*If applicable (Name of a Panamanian researcher responsible of the project in Panama)*
Dirección Permanente *Permanent Address*	
Teléfonos *Telephone*	
Correo electrónico *E-mail*	
INSTITUCIÓN QUE RESPALDA/CONTRAPARTE EN PANAMÁ *Responsible Institution in Panama*	
Dirección Permanente *Permanent Address*	
Persona de contacto *Contact Person*	
Teléfono *Telephone*	
Correo electrónico *E-mail*	

DATOS SOBRE EL PROYECTO DE INVESTIGACIÓN

Research Proposal

1. INTRODUCCIÓN A LA INVESTIGACIÓN

Introduction

Brief information about the research

2. OBJETIVOS DE LA INVESTIGACIÓN

Objectives

A clear description of the research objectives. These objectives should describe your research project and not focus on the specific items that you are seeking permission to collect.

3. ESPECIFIQUE EL GÉNERO, ESPECIE, FAMILIA Y CANTIDAD DEL RECURSO PARA EL CUAL SE SOLICITA EL ACCESO

Specify family, genera, species, and quantity of samples

Nombre común *Common Name*	Nombre científico *Scientific Name*	Cantidad *Quantity*	Descripción *(Peso, volumen, tamaño)* *Description*

a. Colecta: *Collecting*	*Mark if applicable*
b. Marcado: *Marking*	*Mark if applicable*
c. Observación *Observing*	*Mark if applicable*
d. Otros: (especificar)	

Others

4. SITIO EXACTO DONDE SE UBICA EL RECURSO. *(Cuando se trate de un Área Protegida bajo administración externa de la ANAM, será requisito indispensable presentar el permiso de acceso al área)*

Collecting sites (In the case of protected areas outside of ANAM's jurisdiction, an additional letter is required to access the area)

a. Fuera de Áreas Protegida: Provincia/distrito/sitios/coordenada/ *Sites outside protected areas: Province/district/locality/Lat-Long*	
b. Área Protegida: Provincia/distrito/sitios/coordenada *Sites inside protected areas: Province/district/locality/Lat-Long*	*Be specific if you are planning to go to Pipeline Road or Old Gamboa Road*
c. Otras (especificar) *Others*	

5. METODOLOGÍA A UTILIZAR

Methodology

6. CRONOGRAMA DE TRABAJO

Research time line

a. Etapas previstas para las colectas y/o el análisis de muestras:

Phases for sample collection and analysis

Indicate dates or periods when you intend to do collection. You can put exact dates or periods of time (weeks/months). ANAM does not issue permits for a period of more than six (6) months.

Ex: "March 21, 2012-Sept. 01, 2012"

b.Transferencias previstas a centros internacionales- socios de la investigación:

If samples will be exported, please provide name of collaborator and institution who will receive samples

7. JUSTIFICACIÓN/IMPORTANCIA/IMPACTO DE LA INVESTIGACION

Justification/Importance/Impact of research

8. BIBLIOGRAFÍA

Literature cited

9. POSIBLES RIESGOS DE IMPACTO AMBIENTAL O CULTURAL QUE SE DERIVEN DEL ACCESO O EXTRACCIÓN CONTINUA DEL RECURSO

Potential risks, environmental or cultural impacts from accessing and collecting the samples

You must indicate potential environmental risks and/or cultural impacts where the research is taking place

10. ¿DE QUÉ MANERA LA INVESTIGACIÓN CONTRIBUYE A LA SOSTENIBILIDAD DE LOS ECOSISTEMAS Y CONSERVACIÓN DEL (LOS) RECURSO (S)?

How does this research contribute to the sustainability and conservation of resources?

11. ¿CONOCE USTED DE OTROS PROYECTOS EN PANAMÁ O A NIVEL INTERNACIONAL CON OBJETIVOS SIMILARES?

Do you know of other projects with similar objectives in Panama or overseas?

12. ¿CUAL ES EL MONTO DEL TOTAL DEL PROYECTO?

Total project budget

You must indicate total amount of the project even if not for commercial purposes

13. ¿CUENTA USTED CON EL CONSENTIMIENTO LIBRE INFORMADO PREVIO (CLIP)?

Do you have a valid Informed Consent (IC)?

The informed consent (IC) is the document that authorizes you to work at various sites. The Visitors Services Office (VSO) will provide the IC for the following sites: Pipeline Road, Old Gamboa Road, Parque Natural Metropolitano, Reserva Forestal Fortuna and Parque Municipal Summit.

For indigenous areas, you need an IC from the chief of the Comarca. For private properties, you need IC from the owner of the property. Research in BCI does not require an IC.

14. EQUIPO DE INVESTIGADORES NACIONALES E INTERNACIONALES PARTICIPANTES

List of national and international researchers. It is mandatory to include a Panamanian citizen researcher

Nombre *Name*	Cédula/Pasaporte *ID/Passport Number*	Nacionalidad *Nationality*	Institución *Institution*

15. CURRÍCULUM VITAE EN ESPAÑOL (incluir CV de todos los investigadores)

Curriculum vitae in SPANISH (include CV's for all researchers)

16. Formato a seguir para presentación del curriculum vitae (CV)

CV Format

Nombre *(Name)*

Dirección *(Address)*

Educación *(Education)*

Experiencia Profesional *(Professional experience)*

Lista de publicaciones *(List of publications)*

17. COPIA DE CÉDULA/PASAPORTE (DE TODOS LOS INVESTIGADORES)

Copies of ID or passport (for all researchers in the permit)

18. Presentar copia digital/escaneada de cédula (en el caso de investigadores panameños) o pasaporte (en el caso de investigadores extranjeros)

Please attach digital/scanned copies of ID's (for Panamanian researchers) or passports (for foreign researchers)

19. PUBLICACIONES RELACIONADAS DE LOS INVESTIGADORES (ADJUNTAR PDF, RESUMEN EN ESPAÑOL)

Publications related to the project (Please attach PDF copies of papers published by researchers related to the project)

20. CARTA DE COMPROMISO (FIRMADA EN ORIGINAL (bajar documento aquí)

Nota: para la elaboración del cronograma de trabajo, tomar en consideración que la UNARGEN contará con un plazo de cuarenta y cinco (45) días hábiles a partir de la fecha de recibo de la solicitud para dar respuesta a las solicitudes de permisos de acceso. Período que comprende toda la tramitación de la solicitud hasta el otorgamiento o denegación del permiso. La UNARGEN hará los esfuerzos necesarios para darle respuesta a su solicitud de la manera más rápida posible dependiendo del tipo de permiso solicitado, de la complejidad de la investigación a realizar, de que la información suministrada por los investigadores esté correcta y del volumen de solicitudes presentadas

---

Declaro bajo juramento que la información suministrada es verdadera.

Note: *Research timeline must take into consideration 45 working days required by UNARGEN to provide an answer to permit requests (grant or deny). UNARGEN will strive to have a reply for your request as soon as possible depending on the type of permit, research complexity, as well as accuracy of information provided and depending upon the volume of permit requests that have been received and require processing.*

I declare under oath that the information I have provided is true.

Annex 2. 2 Letter of Commitment Form

DIRECTOR OF PROTECTED AREAS AND WILDLIFE, NATIONAL ENVIRONMENTAL AUTHORITY (ANAM), E. S. D.:

I, _____**[name and family name]**__________, of ___**[nationality]**__ associated with **[name of company/institution/center]** ___ID/Passport Nº __________ in charge of the research study titled ____________**[Project title]**______, collection request Nº______, which conforms to the requirements for the entry of genetic material, hereby declare that all genetic and/or biological material, products and/or the results of the research will be used for scientific purposes only and, under no circumstance, will be used for financial or economic gain without previous notification to ANAM and its due authorization.

I hereby commit to:

- Acknowledge the unalienable rights of the Panamanian authority over all resources collected for the sole purpose of the proposed research.
- Acknowledge, in all publications, invention patents or other intellectual property instruments, that the samples are of Panamanian origin.
- Acknowledge the rights of the state of Panama as co-participant to the benefits accrued through the process.
- Release the State of Panama from any responsibility for damages caused to third parties' property stemming from the access permit granted.
- Submit a detailed report of the collection before requesting an Export permit, or annex the report to the request.
- Submit annual reports about compliance with contracts signed with the State, third parties, indigenous or local communities, or with parties under special appointment.
- Submit note of delivery to museum or herbarium (regardless of the sample being accepted) as a requisite for the Export permit.
- Submit the Final Report in Spanish in digital format and two hard copies.
- Coordinate with UNARGEN the reimbursement for the genetic material.
- Request authorization to the National Environmental Authority for any other activity not described in the access request.

Failure to comply with the clauses and commitments established herein will cause temporary or permanent cancellation of the access permit and will result in denial of all future requests.

____

(Signature)

____

(ID or passport #)

Annex 2. 3 Informed Prior Consent Model Form

[Note: this form is compulsory for working in areas of special concern (concessions or private areas), private farms, villages or indigenous communities, Natural Metropolitan Park and Coiba National Park, as well as in other areas under special restrictions.]

Panama, __ of ___ of 2,0 _______

[Information about the person and address of contact]

Dear __[Information about the person requesting the IPC]________

Regarding the project **[write title of project exactly as it appears in request]** permit request, to be developed by the main researcher **[Name and ID/Passport #]**, we hereby grant a permit of access to our area **[concession, village, or other]** for said purpose.

[Indicate responsibilities of the researchers while studying the area, as well as the type of work he/she expects to carry out therein.]

Brief description of the area and use for which consent is granted	
Responsibilities assumed by the researchers during the study	
1.	
2.	
3.	
Etc. (add/eliminate rows as necessary)	

Signature

Note: if the study is to be carried out inside a village, the permit should be signed by the village head.

Annex 2.4 Material Transfer Agreement Form

This Material Transfer Agreement is subscribed between: Provider, general data, contact information and Receiver, general data, contact information, and its specific purpose is for sample transfer (description of material to be transferred), from here on referred to as **the Material**.

The Material will be provided by (provider) to (receiver) under these conditions:

1. The material will be used by the receiver for the sole purpose of carrying out the following scientific experiments.................................... **[Description of the investigation]**...................................., and the main researcher is Dr. ....
2. The material cannot be transferred in part or whole by the receiver to third parties without specific, written authorization from the provider, after consulting with ANAM. Any requests regarding ***the material*** must be submitted to the provider. The receiver will guarantee at all times the use of the material according to the present document.
3. The receiver will not assume ownership of the material and will not pursue Intellectual Property Rights on it, its parts or genetic components described in the form provided. The receiver will not pursue Intellectual Property Rights for related information received.
4. ***The material*** cannot be used for commercial or exploitation purposes without specific, written consent from the provider.
5. The receiver will inform the provider of the results obtained by using the material, will deliver to all relevant parties drafts or final versions of all publications describing any and all studies carried out with the material, and will acknowledge the provider's assistance in the final document.
6. No party will share with third parties, under any circumstance whatsoever, scientific information, data, applications, reports, and final results related to the results of the study carried out under this agreement, as long as the information has not been previously made public.
7. Clause 6, above, does not consider any data owned by the receiver before subscribing to this agreement and that are not subject to confidentiality, that had been made public before subscribing to this agreement or that were made public by the receiver after subscribing to the agreement, or that were provided to a third party not related to the Parties, as long as the Parties have no intent of inquiring why the third party made them public.
8. Once the study under this agreement is complete, the remaining ***material*** will be returned to the provider or destroyed, after submitting written information to the provider.
9. The results of the tests and information generated under this agreement are the intellectual property of the Parties, which will each determine, after duly informing the other party, how and when they will be used, according to current legal guidelines. All publications must state that the material is of Panamanian origin.
10. If a financially exploitable discovery originates from this agreement, regardless of who is the owner of the intellectual property or patent, both Parties will negotiate, in good faith, a marketing agreement that will include the distribution of benefits, including those corresponding to the State of Panama.
11. This Material Transfer Agreement does not constitute a permit, and it does not grant any rights on the material provided to the receiver.
12. The public information and confidentiality clauses of this Agreement will remain active beyond the life of the Agreement.
13. Dr.. .... and Dr.. .... are the representatives assigned by the Parties for all matters related to communications and notifications and the implementation of this Agreement.

This Material Transfer Agreement will be in effect for a period not exceeding two (2) years from the date of subscription.

Representative [Entity A]: Representative [Entity B]:

Signature: ……………………..	Signature: ……………………..
Name: ……………………	Name: ……………………
Position: ……………………..	Position: ……………………..
Date: ……………………..	Date: ……………………..

Anexo 2.5 Plantilla permiso de Importación

DIRECCIÓN DE ÁREAS PROTEGIDAS Y VIDA SILVESTRE

UNIDAD DE ACCESO A RECURSOS GENÉTICOS

Solicitud de Importación de Recursos Genéticos y Biológicos

Request to Import Genetic and Biological Resources

<table>
<tr><td colspan="6">PARA USO OFICIAL DE LA UNARGEN
FOR OFFICIAL USE ONLY</td></tr>
<tr><td>Solicitud No.</td><td colspan="2"></td><td colspan="3" rowspan="2">Firma del Funcionario Receptor</td></tr>
<tr><td>Fecha</td><td colspan="2"></td></tr>
<tr><td colspan="6">PARA USO DEL SOLICITANTE
Please fill out the form below IN SPANISH</td></tr>
<tr><td colspan="6">Nombre del Proyecto de Investigación
Name/Title of Research Project</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td>Nombre del Solicitante
Applicant’s name</td><td colspan="5"></td></tr>
<tr><td>Nacionalidad
Nationality</td><td></td><td>Número de cédula/ pasaporte
ID or Passport Number</td><td colspan="3"></td></tr>
<tr><td>Dirección permanente
Permanent Address</td><td></td><td>Correo electrónico
E-mail</td><td colspan="3"></td></tr>
<tr><td>País
Country</td><td></td><td>Ciudad/Estado
State/Province</td><td colspan="3"></td></tr>
<tr><td>Teléfonos
Telephones</td><td></td><td>Apdo. Postal
PO Box</td><td colspan="3"></td></tr>
<tr><td>País de origen de las muestras / Country of origin of samples
País de procedencia de las muestras / Country of procedence of samples</td><td colspan="5"></td></tr>
<tr><td>Nombre de la persona responsable.</td><td colspan="5"></td></tr>
</table>

Person responsible for sending the samples	
Dirección Permanente Permanent Address	

Teléfonos: Telephones		Correo electrónico: E-mail	
País: Country		Ciudad/Estado: State/Province	

Apdo. Postal: PO Box	

DATOS SOBRE LA IMPORTACIÓN DEL RECURSO Information on genetic resources to be imported

Propósito de la importación: Purpose of the import	
Puerto de entrada Port of entry	
Medio de transporte Mode of transportation	

Especifique el recurso para el cual se solicita el permiso de importación. (Puede incluir o eliminar tantas filas como sea necesario). List of the samples (genetic resource) that will be imported (Add as many rows as necessary)

Nombre común Common name	Nombre Científico Scientific name	Cantidad Quantity	Descripción (Tipo de muestra/ Peso o volumen o tamaño/vivo o muerto) Description

Fecha prevista para la importación: Approximate date for importing samples

Nota: para la elaboración del cronograma de trabajo, tomar en consideración que la UNARGEN contará con un plazo de cuarenta y cinco (45) días hábiles a partir de la fecha de recibo de su solicitud para dar respuesta a sus solicitudes de permisos de acceso. Período que comprende toda la tramitación de la solicitud hasta el otorgamiento o denegación del permiso. La UNARGEN hará los esfuerzos necesarios para darle respuesta de la manera más rápida posible, dependiendo del tipo de permiso solicitado, de la complejidad de la investigación o actividad comercial a realizar, de que la información suministrada esté correcta y del volumen de solicitudes presentadas.

Para la realización de la transferencia debe contar con un Acuerdo de Transferencia de Materiales con la institución receptora.

<u>Note:</u> Research timeline must take into consideration 45 working days required by UNARGEN to provide an answer to permit requests (grant or deny). UNARGEN will strive to have a reply for your request as soon as possible depending on the type of permit, research complexity, as well as accuracy of information provided and depending upon request volume.

PLEASE PRINT THE FOLLOWING PAGE, SIGN IT, SCAN IT AND SUBMIT IT VIA EMAIL ALONG WITH THIS APPLICATION (IN WORD FORMAT, NOT PRINTED) TO:

Declaro bajo juramento que la información suministrada es cierta y verdadera

I hereby declare that all information provided is true.

____

Firma del solicitante o Responsable en Panamá

Applicant's signature

C.I.P o Pasaporte: ____

ID or Passport

SECTION 2

Chapter 3. Protocol for Monitoring Birds in Panama Forests

W. Douglas Robinson

1. Introduction

The avifauna of Panama is very rich, with nearly 900 species having been reported from the country. About 550 of those species occur in forests. The variety of lifestyles is impressive. Many species are residents, spending their entire lives in Panama. Others are long-distance migrants that breed in North America and spend the north temperate winter in Panama, or pass through Panama on their way to and from South America. A few species breed in Panama and spend their non-breeding season in South America. This variety of lifestyles is also accompanied by large differences among species in how detectable they are.

Counting birds is messy business. Unlike trees, birds are constantly on the move, small, and easily overlooked. Some sing, which improves our ability to detect them, but the propensity to sing can mislead us into thinking birds are easy to count. The possibilities for error are even greater in tropical habitats. Not only can vegetation be so dense that it is hard to see birds more than a few meters away, but the variety of lifestyles of birds is very large. For example, a territorial species may be regularly detected within its home range and may sing every morning to announce its presence to conspecific neighbors. Such species are easier to count. But many other species do not defend territories and do not sing regularly. Many wander across the landscape searching for fruit or nectar. Some are predators, so announcing their presence is counterproductive. The wide variety of lifestyles of birds in the tropics means that one has to take great care to evaluate how detectable a species is at any given place and time. Modern methods of counting birds gather data to help us measure detectability so that we can adjust our estimates of abundance.

2. Methods of Sampling

Data to gather during species inventories

Given the challenges of surveying birds in tropical forests, it is recommended that efforts to inventory species be combined with methods that allow estimation of abundances as well. This protocol explains how to combine line transect and point count sampling for estimating abundances of diurnal birds. From these same data, we can use species accumulation curves and richness estimators to evaluate the completeness of our species inventories.

The method utilizes transects with distance estimation and it utilizes point counts. Transects are lines or trails walked while counting birds. Transects can vary in length from 200 to 2000 m depending on accessibility and terrain. All birds detected are identified and the distance away from the observer at the time of identification are recorded, therefore we call these *transects with distance estimation*. Point counts are counts of birds while the observer is stationary. These stationary point counts also include distance estimation. Each bird detected is identified and then its distance away from the observer is estimated. Stationary point counts are 8 minutes long. Each 8-minute time period is divided into four 2-min time periods. For each bird detected, whether it was detected or not in each 2-min time period is noted; this kind of data collection is called *time-of-detection data*. With line transects and these two kinds of stationary point count data collection, we will have 3 ways to estimate abundances of diurnal birds. Nocturnal birds are not included, but similar protocols could be used at night, if desired. Effective surveys of nocturnal birds generally also require the addition of call playbacks, which are not discussed here.

This protocol is designed based on a few principles. It can be modified as particular circumstances warrant changes, but should follow these basic parameters: 1) point counts should be located no closer than 200 m apart; 2) line transects should be located no closer than 400 m to other line transects. Tropical bird communities usually have many rare species, so areas larger than 100 ha generally must be surveyed to estimate abundances of most bird species in a community. Therefore, if a goal of surveys is to estimate abundances of most species, then a sample of at least 50 points should be surveyed. For line transects, assuming the species with the shortest detection distances can be heard to a maximum distance of about 50 m, a minimum of 10 km of transects should be surveyed to produce estimates of abundance for most species in a community.

Line-transect sampling

Observers should walk each transect at a pace that takes them 100 m in 5-10 min, depending on the difficulty of terrain.

Each bird detected should be noted. Each line of the data sheet is an individual bird. When possible, try to note the location of each individual bird only once during a particular line transect survey on a given day. It can be very difficult to know for certain if the same bird is being detected from multiple points or from adjacent parallel line transects, especially early in a survey season when the observer is still learning the pattern of distribution of birds within the survey area. When in doubt, include the bird in the data. Once the observer has completed surveys, the data can be adjusted to better reflect improved knowledge of the locations and abundances of such species. If conspecific flocks or pairs of birds are seen at the same location, the number of individuals can be noted in the comments column.

Species names should follow standard conventions. Observers should use scientific names or English names because these names are standardized. (Spanish names may be used, but they are not officially standardized. If Spanish names are used, it is critical to include with the metadata a spreadsheet that lists the Spanish, English and scientific names of all species encountered). A short-hand six-letter code can be used on the data sheet to indicate the species while counting birds. Six-letter codes for scientific names usually include the first 3 letters of the genus name and the first 3 letters for the specific epithet. For example, *Chilan* is *Chiroxiphia lanceolata* (Lance-tailed Manakin). As part of the metadata for surveys of each plot, observers should provide a spreadsheet with the English and scientific names as well as the short-hand codes used during the surveys. The currently accepted names of American birds are archived at www.aou.org.

We will use distance sampling to allow calculation of densities from the observations, using the software program DISTANCE (http://www.ruwpa.st-and.ac.uk/distance/). Inputs required are, for each observation, location of the observer on the transect (GPS coordinates), and direction and distance to each identified bird. Direction is necessary because the software calculates perpendicular distance of the bird from the transect, not from the observer. Modified versions of the calculations are used when estimating abundance from points so that the distances from the stationary observer are included. Therefore, it is important to note direction from transects. Direction from points is important as well because the locations of the birds can be used later for spot-mapping.

The direction away from the observer of each bird when it is first detected should be noted on the data sheet. We will use a 16-direction system instead of getting an exact compass direction to each bird. One can use compass bearings if the number of birds being detected is low, however in most cases the number of birds is high and it takes too long to get compass bearings. This 16-direction system will provide sufficiently exact information in most situations.

- N north
- NNE north northeast
- NE northeast
- ENE east northeast
- E east
- ESE east southeast
- SE southeast
- SSE south southeast
- S south
- SSW south southwest
- SW southwest
- WSW west southwest
- W west
- WNW west northwest
- NW northwest
- NNW north northwest

For all birds visually detected, it is best to use a laser rangefinder to measure the distance of the bird from the observer while the observer is on the transect. Do not measure the perpendicular distance of the bird from the transect unless the bird is directly 90 degrees away from you as you stand on the transect. The software can calculate each bird's distance from the transect based on the direction you record and the linear distance between you and the bird at the time you detect the bird.

For birds that are not seen, estimate the distance from you to the location where you believe the bird to be. It is best if you can attempt to discern which tree a bird is at and then measure the horizontal distance from you to that tree. The distance to estimate, for seen and heard birds, is the distance along the ground to the location where the bird is thought to be. If a bird is in the canopy, measure the distance from you to the base of the tree where the bird is located, not the distance from you to the canopy.

When it is not possible to discern with any confidence which tree a bird is in (this should be the case most of the time), estimate to within 10 m the distance you think the bird is away from you. Use these 10 m intervals up to 100 m away. For farther birds, use 25 m intervals up to 200 m. If birds are more than 200 m away, you can use wider intervals at your discretion.

A very important aspect of distance estimation is detection of birds along the line transect; that is, at distance zero. It is important to always keep an eye on the transect ahead of you as you walk slowly. Birds detected on the transect should be entered as distance zero, even if you detect them at some distance ahead of you. This is because birds typically move away from us, even if we are moving slowly. So the chances of ever having a bird at distance zero directly overhead of you is very, very small. Yet, the equations in software program DISTANCE work best when some detections are at 0 meters. If birds are noticed ahead of you, but not on the transect, you should still estimate the distance and the direction away from you at the time the bird was first detected.

A caution about distance estimation: This is messy business. A bird can sound farther away if it is whispering a song or even if it simply moves behind a tree or turns its back to the observer. It will take lots of practice to acquire skills at estimating distance, and even with lots of practice the probability of making errors is still nontrivial. All observers should spend time listening to birds and comparing their estimates of how far birds are away with measured distances, so observers can refine their abilities to estimate distances. One way to do this is to look for birds that are singing along trails. Estimate distances to birds you hear singing ahead of you along a trail, then pace off the distance until you see the bird, and compare your estimate with the measured distance. With practice, observers can get quite good at estimating distances of unseen birds, but we should always keep in mind that these are just estimates and the chances of error are non-zero.

Next, note the <u>cue used to detect</u> each bird (singing, calling, visual, referred to as S, C, and V, respectively). Birds detected flying past the observer or over the transect should be noted as well, but indicate 'f' (of "flight") in the cue column. Individual birds can be detected with multiple cues. All cues used should be noted. So an individual bird could have cues such as 'scv' for a bird that sang, called, and was seen. A bird with cues 'fv' was one seen flying past. Observers should use their best judgment when deciding what sounds constitute a song versus a call. In general, songbirds that advertise their presence with territorial song are singing. Birds giving simple alarm calls, group contact calls, or vocalizing in a manner that is not advertising for mates or for occupancy of territories can be denoted as calls. Woodpeckers drumming on trees should be noted only if the drumming is so distinctively different from drumming sounds of other species that you are certain of the species identity. They can be noted with the letter 'd' in cues.

Then, note the <u>time</u> in this format: 17:15 (24-hr notation). If a transect was measured and laid out in advance (for example, if every 25 m is flagged), then the observer should note the time when each marked flag is passed. This allows calculation of how quickly the observer was moving so that differences among observers may be quantified. Otherwise, note the time about every 5 min, and also the GPS coordinates whenever you note a change in time.

The <u>transect locations</u> should be noted in the next columns. Every time you pass a grid marker, note it on the data sheet. Use GPS coordinates (decimal degrees) whenever possible.

In comments, you can record information that you think is relevant to improve understanding of the data you have been recording. This might include noting a number of birds if a flock of conspecifics is present. If multiple species are in a flock, you should make sure that each species gets its own line in the data and that you note the number of each species in the flock in the comments cell for that species. A valuable comment could be an indication that a bird was seen carrying nest material or you found a nest at a particular location. Data relevant to understanding how many birds were present and what they were doing is useful in the comments column.

In summary, during line transects you should walk slowly along the transect noting each bird detected, its direction and linear distance from you, the cues you used to detect each bird, the observer location, and the time. Aim to complete 100 m every 5-10 min. This means a typical 1000 m transect can be done in 50 to 100 min, but our method will extend the time because we will embed 8-min point counts within these transects. Therefore, it will take about 2-3 hours to finish a 1-km point-transect route, depending on ease of access, terrain and the number of birds being detected.

Point counts

Point counts are stationary counts where the observer stands at a location for 8 minutes. All birds are identified, their direction and distance are measured in the same manner described for line transects, and the detection cues used for each bird are also noted.

Points are located at 200 m intervals along transects. Although this protocol recommends adding point counts to transects, point counts alone may be appropriate in certain situations. The observer needs to decide if point counts are sufficient depending on the questions being asked. For example, if the objective is to estimate changes in abundance of bird species over time, then point counts without transects might be the best choice. This is because point counts are exactly repeatable over time. Observers go to exactly the same location and stand there for exactly the same amount of time. Transects are not exactly repeatable. Different observers walk at different speeds and may move in ways that disturb birds differently. Stationary point counts are best for evaluating changes in numbers of birds over time.

If estimating abundance across a survey area is the main goal, points can be counted while line transects are being run. One observer can start each survey route with a point count. When that point is finished, the observer can walk and survey for birds the 200 m of transect up to the next point, then conduct the next point count. It is important that all birds are included on the transects and the points. That is, if a point count is done and then a transect is walked to the next point, any bird detected while the transect is being surveyed, even if it was first detected during the point count, should be included in the transect data. This is not double-counting birds because the point count data and the line transect data will be analyzed separately.

The data collected during point counts are just like those collected during transect counts, except that one additional component is added to points. These are the time interval data.

Each 8-min point count will be divided into four 2-min time intervals. For every bird detected at any time during the 8-min count, either a 1 or a 0 should be entered into each of the 2-min interval columns. If an individual bird is detected within a 2-min interval it gets a 1. If that bird is not detected, it gets a 0. This generates a "capture-recapture" history within each point count for each bird, which allows calculations of detection probabilities. This method requires that observers keep track of all birds detected during a point count throughout the entire 8 min. A bird detected in the first 2-min interval will get a 1 noted in the first 2-min interval column, then the observer needs to check in on that bird in each subsequent 2-min interval and write another 1 if it is still detected (still vocalizing or being seen) or a 0 if it is no longer being detected. Some birds might get detected in the first interval then missed in one or more subsequent intervals before being detected again in the last interval. Other birds will not be detected in the first interval, but then get detected in a later interval. That is, they will get a 0 in the first interval, and then if detected in minute 3, for example, would get a 1 in the second interval. See the example data sheet for an illustration.

The time needs to be noted only at the start of each point count. Use the same time format that was used for line transects: e.g, 17:15.

The GPS location should be noted in the data row for the first species detected at each point count. It can be copied down for all the birds at that point count when data are entered later into a computer.

Comments of relevance are similar to those discussed in the line transect section of this protocol.

This combination of line transects with distance estimation and point counts with distance estimation and time intervals will provide 3 estimates of the abundance of each bird species. The fourth estimate will come from mapping the locations of all birds detected during transect surveys and point counts. Since direction and distance of each bird from the observer is noted, we will then use those data to map occurrences of each species. Interpretation of the spot maps will be best done by the observers who collected the data because those observers will know how to evaluate whether or not certain clusters of birds were likely to be different birds or the same birds moving around the study area.

Number of sites

The number of samples required to estimate bird abundance is difficult to gauge. At a minimum, it is recommended that at least 50 points or 10 km of transects be surveyed in Panama forests. These sample sizes should provide sufficient data to estimate abundances for more than 75% of species present. It will be difficult to estimate abundances very precisely for all species, so categorical estimates (for example, rarely detected, very rarely detected) may be the best option when time and funds are limited.

Mist-netting

The capture of birds with mist nets is a popular and frequently used method. Mist nets may be useful when a project objective is to create a list of the bird species known to inhabit a location. Some bird species that are difficult to detect during transect and point count surveys may actually be quite common. Small fruit- and nectar-eating birds that do not have territorial songs can comprise a substantial fraction of the avian community; mist nets can reveal that those inconspicuous species are frequently captured. However, mist nets should not be used to produce estimates of abundance of birds because of many biases. Remsen and Good (1996) summarize many challenges. Nevertheless, mist nets may have some advantages depending on exactly what the project goals are. For example, if one wishes to study the long-term demography of understory bird species at a site or collect blood and feathers for genetic analyses, mist nets can be very useful. For details on methodology and best practices, the reader should consult Ralph and Dunn (2004).

Automated acoustic sampling

A rapidly growing opportunity to study sounds of birds in tropical forests involves use of automated recording stations (Brandes 2008, Celis-Murillo et al. 2012). Microphones and digital recording devices are placed at selected locations and left to record sounds of birds while observers are away working in other locations. For bird studies, the current utility of this technology includes detection of species that rarely vocalize because the recorders can be set to record sounds for long periods of time. Another benefit is that distance sampling and other methods used to estimate abundance of birds rely on accurate measures of the probability of detection of birds. If sounds are the primary cues used to detect a species, then recorders can provide information on the rate at which songs or calls are produced. These rates can then be used to calculate the probability of hearing a bird species during survey intervals of relevant lengths (e.g., the chance of hearing a bird during an 8-minute stationary count). They can also be used to improve of our understanding of how the vocalization rates change with time of day and time of year.

For the purposes of this protocol for studying forest birds of Panama, it will be most effective to combine efforts to record birds with the efforts to record frogs. Therefore, the reader is referred to the section on automated acoustic recording in the frog protocol to see further details.

3. Species Identification and Handling

A common goal of many studies of tropical bird communities is to generate a list of all the species present. The first order of business in doing so is to be clear about what taxonomic authority on species and species' names is being used. For Central and South American birds, the authority most often used is the American Ornithologists' Union, which has official checklists of the birds for North America and South America (www.aou.org). These lists are updated annually and incorporate revisions to species' names. Field guides will help with identification criteria for most species, but the names in field guides can become out of date rather quickly, so the AOU checklists should be consulted as the most up-to-date authority on scientific and English species' names. Spanish names are not standardized, so it is best to use scientific or English names when collecting data. When reporting results of species inventories, it is important to cite which authority and which version of the checklist was used.

Observers should understand that accuracy of identification is most important, not generation of long lists of species. Therefore, the criteria for including rarely detected species on inventory lists should be carefully discussed beforehand. Every attempt should be made to acquire photographic evidence or voice recordings of very rare species so future researchers can have greater confidence that identification of rare species was accurate. If photos or voice recordings cannot be obtained, observers should make extensive notes and preserve those along with the data collection forms. Records of rare species, along with photos and field notes, can be archived at eBird (ebird.org). Recordings of songs and calls can be archived at www.xeno-canto.org.

4. Personnel Training

To conduct effective species inventories, one needs appropriately trained observers. Because many tropical bird communities are very rich with species and many species may be detected by voice alone and never actually seen, one needs highly skilled observers to conduct reliable species inventories. The best observers are the ones who have an excellent memory for bird sounds and who have been studying intensively the birds of an area for a minimum of 6 months. This does not mean the observer started bird watching more than 6 months ago; it means the observer has undertaken intensive fieldwork and focused on learning on all the songs and calls of the species expected to occur within the study area, for a minimum of 6 months. Ornithologists who are especially qualified at identification of birds by sound can sometimes learn a community in less than 6 months. Online resources (xeno-canto.org, for example) and DVDs of regional bird sounds (Birdsongs of Costa Rica, for example) can accelerate the learning process. In an ideal world, observers would be asked to pass quizzes on bird sounds and visual identification of bird images prior to conducting species inventories in an area. It is also recommended that survey designs always include more than one observer to reduce the chances that incomplete knowledge by any one observer affects the survey results.

The design of the survey will dictate how many surveyors are needed. At a minimum, sites should be surveyed by two different individuals, if those individuals have extensive knowledge of bird identification by sight and by sound. Such individuals are rare. Very few people know all the sounds made by forest birds of Panama, but some bird tour guides are quite talented with sounds. Yet, observers also need enough experience to be able to estimate distances of birds away from points or line transects. Observers should have a minimum of 6 months of extensive field experience surveying tropical forest birds. The experience could be from countries other than Panama, but even experienced observers should have at least 2 to 3 weeks of daily field time to become acquainted with birds in the survey area prior to beginning point and line transect counts.

5. Estimated Budget (to date) and Timing for Panama

Timing and sequence of surveys

All surveys should begin no earlier than 30 min prior to sunrise and should not extend beyond 4 hours after sunrise. How late surveys should go varies across locations. In some climates, birds get quiet much earlier in the day and surveys should not go past 3 hours after sunrise. In other, usually cooler climates, surveys can continue to 4 hours after sunrise.

Surveys should be completed on days with little or no rain or wind. If rains occurred before dawn, sometimes it will not be possible to conduct useful surveys that morning because of noise from canopy drip. Use your best judgment regarding whether or not you can hear birds effectively at least 100 m from you.

Surveys are best done in the month preceding and including the start of the primary breeding season of birds. This tends to be the time when birds are more likely to be vocalizing, and are therefore easier to detect. In Panama, surveys should be completed between 15 March and 15 June. The best 6 weeks for surveys is 1 April to 15 May.

Each transect and point should be visited a minimum of 3 times and, preferentially, 4 to 6 times if estimates of abundance are required. If simple inventories of species in a very large area are needed, then it may be better to sample many sites once rather than fewer sites multiple times.

Run each route of point-transects in opposite directions at least once whenever possible. This is especially important in some lowland tropical bird communities where many species vocalize only within 30 min of dawn.

Labor—how many days, people?

The number of observers and the length of the study depend on the questions being addressed. A common “stopping rule” is to finish surveys when the observed number of bird species is 95% or more of the estimated number of species (See Chapter 8 for a discussion of species accumulation curves and estimating the number of species at a site).

For estimating abundances of birds, one needs at least 40 detections of each species with distance estimates and time-interval data. This means that the decisions that must be made include how many species the observers wants estimates of abundance for. It will be difficult to obtain 40 observations of rare species, so one will never obtain quantitative abundance estimates for all bird species.

Equipment and costs

- Binoculars ($600 or more)
- Laser range finder ($200)
- Clipboards (aluminum covered to protect data from rain; $33)
- GPS ($150 or more)
- Copies of data forms ($100)
- Pens ($10)
- Compass ($50)
- Field clothes (personal choices)
- Satellite phone (for safety if in remote areas; $500)
- Cell phone (if in areas with cell coverage; can use personal phone)
- Maps of study area ($50)

6. Data Output

Metadata needs to be carefully gathered and archived along with the data. Data and metadata should be archived in Avian Knowledge Network (www.avianknowledge.net). The following items should be preserved with details in the metadata for every bird survey project.

Metadata and Archival Process

General Metadata

- Data owner contact information
- Data key words
- Citations for relevant protocols used
- Definitions for database fields/columns
- Data quality and completeness
- Data accessibility and sharing information

Geospatial Information

- Geodetic datum (WGS 1984)
- Geographic coordinate system (decimal degrees)
- Coordinates for line transects
- Coordinates for point count points
- Coordinates for individual detection locations from spot mapping
- Site boundary polygons (archived as JPEG maps and ArcGIS Shapefiles)
- List of site names and abbreviations
- Descriptions of habitats
- Descriptions of information regarding how to access sampling sites

Observation information

- Definitions for detection types (visual, song, call, drumming, fly by)

Bird related

- List of scientific names (consult www.aou.org)
- List of common names
- List of abbreviated species codes

All data and metadata collected in conjunction with this research should be digitally archived in secure locations such as the Avian Knowledge Network. Examples of databases are provided in Figure 3.1.

A.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	species code	direction	distance	scvf	Lat	Long	Time	Date	Observer	Comments	Scientific Name	Study site	State	Country
2	rcma	n	0	v	9.133511	-79.720665	6:33	4/14/2014	WDRobinson	female overhead	Pipra mentalis	Pipeline Road	Panama	Panama
3	rcmm	s	17	vc	9.133511	-79.720665	6:33	4/14/2014	WDRobinson		Baryphthengus martii	Pipeline Road	Panama	Panama
4	lesg	ssw	55	s	9.133511	-79.720665	6:33	4/14/2014	WDRobinson		Hylophilus decurtatus	Pipeline Road	Panama	Panama
5	lesg	nne	80	sc	9.133511	-79.720672	6:34	4/14/2014	WDRobinson		Hylophilus decurtatus	Pipeline Road	Panama	Panama
6	miki	n	150	vf	9.133511	-79.720678	6:35	4/14/2014	WDRobinson	flying north	Ictinia mississipiensis	Pipeline Road	Panama	Panama
7	dwaw	s	17	vc	9.133422	-79.720701	6:38	4/14/2014	WDRobinson		Microropias quixensis	Pipeline Road	Panama	Panama
8	wfla	s	18	vs	9.133422	-79.720708	6:40	4/14/2014	WDRobinson		Myrmotherula axillaris	Pipeline Road	Panama	Panama
9	cbab	ene	47	s	9.133422	-79.720721	6:41	4/14/2014	WDRobinson		Myrmeciza exsul	Pipeline Road	Panama	Panama
10	cbab	e	50	s	9.133422	-79.720801	6:48	4/14/2014	WDRobinson		Myrmeciza exsul	Pipeline Road	Panama	Panama

B.

	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	scvf	1-2	3-4	5-6	7-8	Lat	Long	Time	Date	Observer	Comments	Scientific Name	Study site	State	Country
2	v	1	0	0	0	9.133511	-79.720665	6:33	4/14/2014	WDRobinson	female	Pipra mentalis	Pipeline Road	Panama	Panama
3	vc	1	1	1	0	9.133511	-79.720665	6:33	4/14/2014	WDRobinson		Baryphthengus martii	Pipeline Road	Panama	Panama
4	s	1	1	1	1	9.133511	-79.720665	6:33	4/14/2014	WDRobinson		Hylophilus decurtatus	Pipeline Road	Panama	Panama
5	sc	0	1	1	1	9.133511	-79.720665	6:33	4/14/2014	WDRobinson		Hylophilus decurtatus	Pipeline Road	Panama	Panama
6	vf	0	0	0	1	9.133511	-79.720665	6:33	4/14/2014	WDRobinson	flying north	Ictinia mississipiensis	Pipeline Road	Panama	Panama
7	vc	1	1	1	1	9.133422	-79.720058	6:48	4/14/2014	WDRobinson		Microropias quixensis	Pipeline Road	Panama	Panama
8	vs	1	0	1	0	9.133422	-79.720058	6:48	4/14/2014	WDRobinson		Myrmotherula axillaris	Pipeline Road	Panama	Panama
9	s	0	0	1	1	9.133422	-79.720058	6:48	4/14/2014	WDRobinson		Myrmeciza exsul	Pipeline Road	Panama	Panama
10	s	0	0	1	0	9.133422	-79.720058	6:48	4/14/2014	WDRobinson		Myrmeciza exsul	Pipeline Road	Panama	Panama

Figure 3.1. Examples of databases for data collected from linear transects **(A)** and point counts **(B)**.

4. Standardized Methods for Sampling Fishes in Streams of Panama

James H. Roberts and Paul L. Angermeier

1. Introduction

The purpose of this chapter is to outline methods for standardized sampling of stream fishes in Panama. For the purposes of this document, we define “stream fishes” to mean any fish species (classes *Petromyzontida, Chondrichthyes, Sarcopterygii*, and *Actinopterygii*) that spends any portion of its life in freshwater streams that are shallow enough to be sampled by wading. This definition includes species that are lifetime residents in fresh water, as well as those that migrate between fresh and salt water over the course of a lifetime (i.e., anadromous, catadromous, and amphidromous species). We anticipate that the methods described herein can be applied to any wadeable stream in Panama to produce comparable data on fish distribution and abundance.

Fishes are the most speciose group of vertebrates, with approximately 32,500 species known worldwide (FishBase: www.fishbase.org; search conducted 9 June 2013). Freshwater habitats are disproportionately speciose; despite containing only 0.01% of the world’s water, freshwater habitats harbor 41% of the world’s fish diversity (Helfman et al. 2009). In Panama, streams and rivers are the most abundant freshwater habitats. Panama is known to harbor 222 species of freshwater fish, including 29 endemic species (FishBase: www.fishbase.org), making fishes a substantial component of Panama’s biodiversity overall. Monitoring spatiotemporal patterns of fish diversity therefore complements initiatives to monitor diversity of other, terrestrial taxa.

Besides their intrinsic value as contributors to biodiversity, fishes also have high socioeconomic value for food, sport, pets, and cultural icons. For example, Golder Associates (2010) found that a significant number of local people in the Donoso District of north-central Panama rely upon freshwater fish for subsistence. Particularly important species included *Brycon chagrensis*, *Gobiomorus dormitor*, *Agonostomus monticola*, and *Joturus pichardi*. Monitoring the abundance and stock structure of such exploited species would allow inferences about the status of fisheries and their ability to meet human needs.

Spatiotemporal monitoring of fish community structure also can provide information on the ecological integrity of stream ecosystems. Certain taxonomic and functional groups of fishes are highly sensitive to increases in environmental stressors such as sedimentation, eutrophication, and chemical pollution (Fausch et al. 1990). Changes in the abundance of these fish groups relative to more tolerant groups can indicate elevated levels of one or more stressors. Because fishes are the only major vertebrate group restricted to aquatic habitats, use of fishes for environmental assessment complements assessments based on terrestrial taxa.

As described above, potential goals of a stream-fish monitoring project range from biodiversity monitoring to environmental assessment to detailed assessment of the status of socioeconomically valuable species (e.g., those supporting fisheries). It is important to note that no single sampling protocol is optimal for all of these objectives. For example, estimates of species occurrence may be sufficient for answering general questions about biodiversity, but insufficient for environmental or fisheries assessments, which often require estimates of abundance or biomass. Our goal herein is to outline a sampling protocol that is scientifically robust, rapid, cost-effective, and reasonably effective for addressing a range of potential objectives that require data on fish distribution and abundance.

2. Methods of Sampling

It is impossible to draw spatial inferences about an entire stream or watershed, or temporal inferences about an entire year, from a single sampling event at a single site. However, each sampling site and event should provide a good representation of fish population and community characteristics within a given stream reach (i.e., alternating sequence of riffle-pool habitats; see Frissell et al. 1986) within a given time of year. As such, a site should be long enough to encompass the daily activities of most members of the fish community. In other words, individuals should not emigrate from or immigrate into a site over short time periods. Furthermore, sites should be long enough to represent a variety of habitat types, allow for the detection of rare and sparsely distributed species, and provide asymptotically accurate representations of community composition (Lyons 1992). The site length necessary to meet these criteria is a positive function of stream size, which can be approximated by mean wetted stream width. Based on the results of previous studies, we recommend that site length be set at 40 times the mean stream width, with a minimum length of 100 m and a maximum of 500 m (Lyons 1992; Angermeier and Smogor 1995). For example, sites that are an average of 2, 5, and 15 m wide should be 100, 200, and 500 m long, respectively. An important implication of this convention is that the same site likely will have different wetted widths during the wet and dry seasons, and site lengths must be adjusted accordingly. A final consideration in the designation of site dimensions is that, if possible, site endpoints should coincide with natural barriers to fish movement such as waterfalls, debris dams, or shallow riffles, or with man-made barriers such as culverts or dams. This reduces the likelihood of fish emigration from the site during sampling, which can be substantial (Price and Peterson 2010). This same effect can be achieved through the use of block nets at the up- and downstream endpoints of sites.

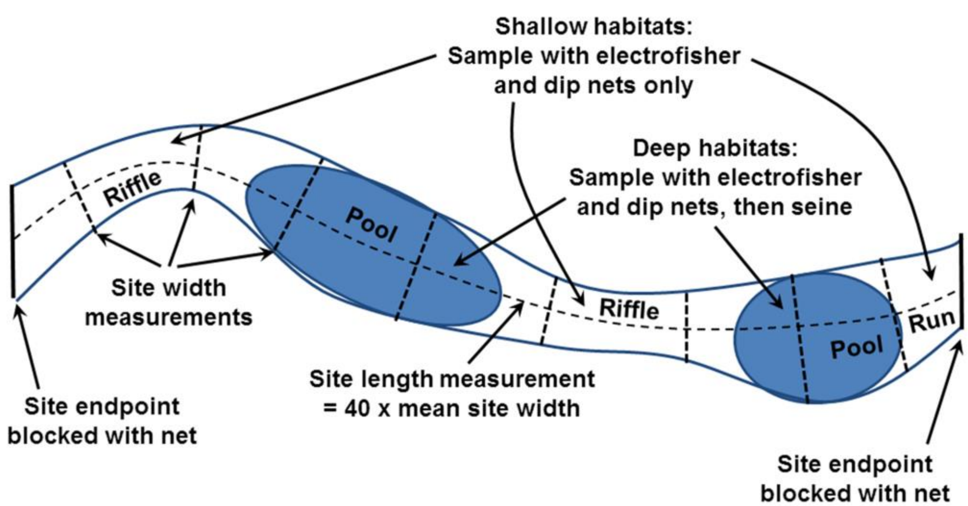


Figure 4.1. Diagram showing distribution of spatial sampling points along the length of a river

A combination of sampling methods is necessary to effectively sample fishes in Panamanian streams (Figure 4.1). In particular, we recommend a combination of direct-current backpack electrofishing with dip nets in all habitats (i.e., riffles, runs, and pools) (Figure 4.2), augmented by additional seining in deep areas (i.e., slower runs and pools). This combined approach ensures that each method is used in the habitat type in which it is most effective, and is similar to the approach employed by the Tennessee Valley Authority for sampling wadeable North American streams (Charles Saylor, Tennessee Valley Authority, personal communication).

Figure 4.2. An example of a field crew using backpack electrofishing and submerged nets to stun and collect fish. Photo credit: Brett Ostby.

On the day of sampling, the site endpoints should be delineated by flagging tape, and if block nets are to be used, these should be put in place (Figure 4.3). Investigators should minimize traffic through the stream reach prior to sampling and wait to begin sampling until disturbed silt and increased turbidity has dissipated. Beginning at the downstream end of the site, the backpack electrofishing crew should proceed upstream, sampling fishes in each sequential channel unit (riffle, run, or pool) that they encounter. The entire crew should move slowly in unison upstream, with the person operating the electrofisher sweeping the anode slowly from side to side (i.e., extending the electrical field across a 4-m-wide area), and the netters immediately netting any fish that are stunned and placing these in a bucket of water. The anode and cathode should remain underwater and the electrofishing current should be applied continuously for the duration of the sampling pass. All netters should scan the electrical field carefully for cryptic, benthic fish that have been stunned on the stream bottom. In swift water, such fishes are effectively captured by holding dip nets flush against the stream bottom. For safety and netting effectiveness, all personnel should wear polarized sunglasses. In addition, all personnel must wear insulated, non-breathable waders.

Optimal electrical settings for the electrofisher depend on conditions including fish size, water depth, and water conductivity. Most modern electrofishers have a “quick setup” feature that automatically adjusts the voltage, frequency, and duty cycle to reasonable levels that can then be fine-tuned by the investigators. As a starting point, we suggest 60 Hz pulsed DC with a 15% duty cycle and 250 volts. In low-conductivity water, we suggest 30 Hz pulsed DC with a 45% duty cycle and 500 volts (Rabeni et al. 2009). Besides being better in low-conductivity situations, lower frequencies also are more effective at capturing catfish (order *Siluriformes*). In any event, investigators should carefully observe the behavior of stunned fish and adjust voltage upward or downward as needed to achieve an electrostatic response (i.e., swimming toward the anode) but avoid tetany (i.e., involuntary, rapid contractions of muscles) or death.

Immediately after each deep habitat unit (pool or slow run; hereafter simply "pool") is sampled by electrofishing, the electrofisher and dip nets should be set aside and the pool then sampled via seining, in an attempt to capture any fish that remain. To seine, two persons each hold a seine braile and, beginning at the upstream end of the pool, drag the seine briskly downstream through the pool, perpendicular to the stream bank. During this "haul", seiners must move quicker than the velocity of the water, to prevent the seine from inverting. It is crucial that the leaded line maintain full contact with the stream bottom throughout the haul. A third person walks behind the seine and lifts the leaded line slightly if it hangs on an obstruction. At the end of the haul, the seine should be beached by dragging it onto an obstruction-free bank. Prior to starting a haul, seiners should carefully plan their path through the pool and decide where to end the haul. Each haul should be long enough to sample the length of an entire pool, or 15 m long, whichever is shorter. If multiple hauls are required to sample the length of a pool, seiners should attempt to separate these hauls by a natural habitat break. If the stream is ≤ 3 m wide, one seine should be used to sample the entire width of the stream. If the stream is 3-6 m wide, one seine should be used to sample whichever side of the stream is more favorable for seining effectiveness. If the stream is ≥ 6 m wide, two seines can be used simultaneously to sample both sides of the stream. In any case, each seine haul should sample a unique area (i.e., not overlap with another haul). Fishes captured during seining should be held in buckets separately from those captured during electrofishing, as these two sets of data will be separately recorded. The total number of seine hauls conducted per site should be recorded. Following the seining of a pool, the crew then resumes electrofishing in the next upstream habitat unit.

Figure 4.3. An example of a field crew blocking a pool. Photo credit: Margarethe Brummermann

Following the completion of a sampling pass through the site, block nets (if used) should be checked for any fish that have washed downstream and become impinged against the net. Separate data should be recorded for the electrofishing and seining catches, so that these data can be analyzed separately. Every individual fish should be identified to species, so that a list of fish species present at that site can be made. We further recommend that each identified fish be counted, so that the proportional abundance (PA) or catch-per-unit-effort of each species can be calculated. PA provides far more biological information than presence-absence data alone, particularly when the focus is on environmental or fisheries assessments, thus we feel that the benefits of counting fish outweigh the additional time costs.

3. Species Identification and Handling

Periodically, fish in buckets should be transferred to a larger holding container to await processing. This could be either a solid container on the stream bank that is filled with stream water and outfitted with an aerator, or a flow-through container that is left in the stream. In either case, a lid is recommended to prevent fishes from jumping out of the container. Care should be taken not to overload fishes into containers and to monitor the condition of fishes to make sure that oxygen levels in containers are sufficient to minimize stress to captured fishes.

Every individual fish should be identified to species, so that a list of fish species present at that site can be made. After identification and recording, a photograph of each species should be taken, for vouchering purposes. Any species that is difficult to identify, unexpected at that site (excluding protected species), or cannot be identified in the field should be euthanized in a 100-mg/L solution of buffered tricaine methanesulfonate (sold as "Finquel" by Argent Chemical Laboratories, Inc. of Redmond, Washington), vouchered in a 10% solution of buffered formalin, and later transferred to a 70% solution of ethanol for storage, safe handling, and positive identification. All other individuals should be returned alive to the stream, re-distributed evenly throughout the site.

4. Personnel Training

We recommend that at least one member of each sampling crew participate in a professional course on electrofishing. Such courses are offered by the American Fisheries Society (www.fisheries.org), United States Fish and Wildlife Service (http://nctc.fws.gov/), and Smith-Root (Vancouver, Washington; http://www.smith-root.com/services/training). This crew member should then train all other members of the crew.

5. Estimated Budget and Timing for Panama

Timing of sampling

Most standardized sampling methods for stream fishes have been developed for temperate streams (e.g., Bonar et al. 2009), where fishes typically are not sampled between late fall and early spring because water temperature is low, stream flows are high, fish are quiescent and difficult to capture, and little recruitment occurs (Matthews 1998). Tropical streams exhibit an entirely different pattern of seasonal variation, with year-round recruitment and little seasonal change in stream temperature or fish activity, despite dramatic seasonal variation in stream flows between wet and dry seasons (Lowe-McConnell 1975). Due to these differences, it is unclear which time of year is optimal for sampling tropical stream fishes. Such sampling is easiest during the dry season, because lower stream volume concentrates fish in smaller, shallower areas and lower turbidity makes fish more visible to samplers, both of which increase capture efficiency (Angermeier and Karr 1983). However, dry-season samples provide only a partial picture of biodiversity and ecological conditions at sites. Some migratory fish species occupy wadeable streams only during the wet season and would be unaccounted for in dry-season samples (Golder Associates 2010). Furthermore, different fish species reproduce during different seasons (Kramer 1978), and if accounting for the presence of on-site reproduction is important to study objectives (e.g., as an assessment of the capacity of a site to provide breeding habitat or recruits), such reproduction would go unaccounted for if sampling were conducted during only one season.

Based on these considerations, we conservatively recommend that sampling bouts for a given study be conducted in both wet and dry seasons, in order to fully describe annual patterns in biodiversity and ecological condition at sites. Thus, season is an additional sampling stratum. If, after a certain amount of preliminary sampling has been conducted, it turns out that wet- and dry-season samples are highly redundant (i.e., result in very similar estimates of species richness, relative abundances, ecological condition scores, etc.), then the investigator may decide to conduct subsequent sampling during only one of these seasons, likely during the dry season. However, based on the results of previous surveys, such redundancy is unlikely (Lowe-McConnell 1975; Kramer 1978; Golder Associates 2010). In any event, investigators should not pool data or findings across seasons, unless subsequent research demonstrates that it is appropriate to do so. To avoid confounding effects of streamflow, it is important to explicitly describe in data, reports, and publications the season and streamflow conditions in effect during sampling, be consistent in the timing of sampling within studies, and draw comparisons between studies only when the timing of sampling is consistent enough to do so.

Time needed to complete surveys

In our experience, the standardized protocol described above should take a trained sampling crew approximately four hours per site to complete on average, including site demarcation, fish sampling, fish processing, and simple habitat and water-quality data collection. This estimate is based on our 50+ years of combined experience sampling fishes in first- through fifth-order streams. However, the exact time needed to survey a given site will vary depending on site width and length, crew size, and fish density, among other factors.

For electrofishing we recommend a battery-powered or gasoline-generator-powered DC backpack electrofisher, equipped with a 2 m-long fiberglass anode pole with a round anode and safety switch and a 2 m-long braided steel cathode (“rattail”). Fishes should be captured in dip-nets with 6.4-mm mesh and 2 m-long wooden or fiberglass handles.

Each backpack electrofisher requires two to three people to operate; one person wears the backpack and carries a dip net, while an additional one to two people carry dip nets and buckets in which to deposit captured fish. This setup costs approximately USD $10,000 per electrofisher (including the electrofisher, electrodes, battery, charger, and nets). The number of electrofishers necessary to effectively sample a site depends on stream size; in a wide stream, fishes easily evade a single electrofisher by swimming outside of the electrical field. Rabeni et al. (2009) recommended one backpack electrofisher and two to three personnel for every 4 m of stream width. Thus, in a stream 16 m wide, four backpack electrofishers and eight to twelve personnel are needed. For seining, we recommend a 3-4 m-long by 2 m-tall flat seine (no bag), with 6.5 mm mesh, floats on the top line, and lead weights on the bottom line spaced at 15 cm intervals. The seine can be attached to any type of braile (pole) that is long enough and sturdy enough to pull the net. This setup costs approximately USD $150 per seine. Up to two seines can be operated simultaneously (in wide streams), each operated by three people. The site endpoints should be delineated by flagging tape, and if block nets are to be used, these should be put in place.

6. Data Output

Recommended standard fish metrics

Investigators should extract a set of standard metrics from the field fish data and present metric values in tables of reports and publications (See Figures 4.4- 4.6 for some examples of databases). From these standard metrics, a variety of additional metrics could be derived post hoc by the investigator, or by other investigators. Each metric should be presented separately for each gear type (electrofishing versus seining) at each site. In other words, if 30 sites are sampled, 60 values should be presented for each metric. Standard metrics include the following:

- A list of all fish species captured
- The number of fish species captured (i.e., species richness)
- The total number of individuals of each species that were captured (i.e., catch)
- The PA of each species based on electrofishing, calculated as catch divided by the length of the site (fish km-1)
- The PA of each species based on electrofishing, calculated as the catch divided by the area (length times mean width) of the site (fish ha-1)
- The CPUE of each species based on seining, calculated as catch divided by the number of seine hauls made (fish haul-1).
- Recommended environmental data collection

Besides fish data, the recording of other site characteristics would help investigators interpret variance in fish distribution and abundance, and possibly explain variance in sampling efficiency among sites. At a minimum, we recommend that investigators record the following types of supplementary data at each site during each visit:

- Latitude, longitude, and elevation (m) of the upstream and downstream site endpoints, and the geodetic datum upon which these coordinates are based
- Site length (m) and mean width (m)
- Water temperature (°C), pH, conductivity (µs cm-1), and turbidity (NTU)
- Electrofisher settings that were used (voltage, frequency, and duty cycle) and the number of seine hauls that were made
- Descriptive notes about sampling conditions, habitat characteristics, the presence of unusual features such as man-made barriers or pollutant discharges, and other noteworthy biological observations such as spawning activity, the presence of sores or deformities in fish, or the presence of dead fish.

Depending on study objectives and available resources, it also may be prudent to collect additional data on stream discharge (m^3 s^{-1}), water quality (e.g., dissolved oxygen [mg L^{-1}], metal concentrations), instream habitat (e.g., substrate size, embeddedness, woody debris), or riparian condition (e.g., extent and type of vegetation). If habitat measurements are to be collected, we recommend use of the U.S. Environmental Protection Agency's "Rapid Bioassessment Protocol" for physical habitat measurement in high-gradient streams (see Chapter 5 and Appendix A in Barbour et al. 1999; available at http://water.epa.gov/scitech/monitoring/rsl/bioassessment/).

	A	B	C	D	E	F	G	H
1	**Collection #**	**Sampling date**	**Site name**	**Double-visited site?**	**Visit #**	**Capture method**	**Fish species**	**Number captured and released**
2	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Gobiomorus dormitor*	3
3	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	12
4	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Agonostomus monticola*	2
5	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Seine	*Gobiomorus dormitor*	10
6	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Seine	*Gephyrocharax intermedius*	26
7	JR072113-001	21 July 2013	Uvero 2	No	1	Backpack electrofisher	*Gobiomorus dormitor*	2
8	JR072113-001	21 July 2013	Uvero 2	No	1	Backpack electrofisher	*Brycon chagrensis*	15
9	JR072113-001	21 July 2013	Uvero 2	No	1	Backpack electrofisher	*Hyphessobrycon panamensis*	8
10	JR072113-001	21 July 2013	Uvero 2	No	1	Backpack electrofisher	*Agonostomus monticola*	30
11	JR072113-001	21 July 2013	Uvero 2	No	1	Backpack electrofisher	*Aequidens coerulecpuntatus*	1
12	JR072113-001	21 July 2013	Uvero 2	No	1	Seine	*Gobiomorus dormitor*	7
13	JR072113-001	21 July 2013	Uvero 2	No	1	Seine	*Brycon chagrensis*	27
14	JR072113-001	21 July 2013	Uvero 2	No	1	Seine	*Atherinella chagresi*	2

Figure 4.4. An example of a database on a fish community.

	A	B	C	D	E
1	Collection #	JR071913-001	JR072013-001	JR072113-001	JR080313-001
2	Sampling date	19 July 2013	20 July 2013	21 July 2013	03 August 2013
3	Site name	Hoja River upper	Hoja River upper	Uvero 2	Petaquilla 6
4	Double-visited site?	Yes	Yes	No	No
5	Visit number	1	2	1	1
6	Waterbody	Hoja River	Hoja River	Uvero River	Petaquilla River
7	River Basin	Caimito	Caimito	Caimito	Petaquilla
8	District	Donoso	Donoso	Donoso	Donoso
9	Province	Colon	Colon	Colon	Colon
10	Country	Panama	Panama	Panama	Panama
11	Site downstream latitude	8.947677	8.947677	8.937677	8.977677
12	Site downstream longitude	-80.474428	-80.474428	-80.484428	-80.444428
13	Site upstream latitude	8.949246	8.949246	8.939246	8.979246
14	Site upstream longitude	-80.481034	-80.481034	-80.491034	-80.451034
15	Site downstream elevation (m)	50	50	35	25
16	Site upstream elevation (m)	53	53	38	28
17	Site length (m)	248	248	412	500
18	Site mean width (m)	6.2	6.2	10.3	13.8
19	Electrofisher settings (volts, frequency, duty cycle)	500 V, 30 Hz, 45%	500 V, 30 Hz, 45%	500 V, 30 Hz, 45%	500 V, 30 Hz, 45%
20	Electrofishing time (minutes)	220	215	203	199
21	Number of seine hauls made	6	6	14	17
22	Block nets used?	Yes	Yes	Yes	No
23	Weather conditions	sunny, 25 ° C	sunny, 24 ° C	cloudy, 22 ° C	rainy, 20 ° C
24	Stream discharge ($m^3 s^{-1}$)	0.8	0.8	2.4	not measured; baseflow
25	Water temperature (° C)	22	23	24	25
26	Turbidity (NTU)	2.1	1.9	4.8	6.6
27	pH	6.6	6.7	7.1	7.2
28	Specific conductivity (μs cm^{-1})	43.2	43.5	70.2	74.4
29	Dissolved oxygen (mg L^{-1})	9.4	9.2	8.1	9.6
30	Rapid habitat assessment score	186	not measured	154	132

Figure 4.5. An example of a database on site characteristics.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Collection #	Sampling date	Site name	Double-visited?	Visit #	Capture method	Fish species	Total length (mm)	Wet mass (g)	Abnormalities	Part collected for aging	Old tag present?	New tag given?	Comments
2	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Gobiomorus dormitor*	74	25	none	otoliths	No	Yes	
3	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Gobiomorus dormitor*	104	35	none	otoliths	No	Yes	
4	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Gobiomorus dormitor*	43	14	none	otoliths	No	Yes	
5	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	135	45	none	scales	No	Yes	
6	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	193	64	none	scales	No	Yes	
7	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	95	32	none	scales	No	Yes	
8	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	200	67	Red lesion on side	scales	No	No	Preserved
9	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	125	42	none	scales	No	Yes	
10	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	131	44	none	none	No	Yes	
11	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	193	64	none	none	No	Yes	
12	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	87	29	none	scales	No	Yes	
13	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	126	42	none	scales	No	Yes	
14	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	75	25	none	scales	No	Yes	
15	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	32	11	none	scales	No	Yes	
16	JR071913-001	19 July 2013	Hoja River upper	Yes	1	Backpack electrofisher	*Brycon chagrensis*	146	49	none	scales	No	Yes	

Figure 4.6. An example of a database on focal fish species.

Chapter 5. Methods for the Inventory and Monitoring of Large Mammals

Patrick A. Jansen

1. Introduction

Large mammals – here defined as medium- to large-sized mammals, with a body mass >1 kg – are a key component of tropical forest communities, providing important ecosystem services. They influence vegetation in multiple ways, such as through pollination, seed dispersal, seed predation and herbivory, through redistribution of nutrients and physical alteration of the abiotic conditions, and by controlling abundances of natural enemies. Large mammals can thus have a strong influence role in the composition and structure of vegetation, which is apparent for example in forest-grassland transitions mediated by large herbivores.

Figure 5.1. An example of a photograph captured by a camera trap on a pipeline in Soberanía National Park, Panama.

Large mammals are disproportionally affected by humans. Land use change and hunting particularly threaten scores of species with local or even global extinction. Loss of large mammals due to hunting or other causes can have cascade effects for other vertebrate species, and ultimately for the species composition and structure of the forest. The combination of a disproportionate importance for ecosystems and high vulnerability makes large vertebrates, particularly the large predators and primates, strong indicators of ecosystem health. Trends in relative abundance of species over time might serve as an indicator of levels of disturbance that would not be detected using remote sensing tools. Data on long-term trends in richness, abundance, and density are of great value for conservation policy. Large mammals are also charismatic, a property that can be used to engage a broad audience in conservation.

There is surprisingly little quantitative information about how mammal communities respond to local, regional, and global threats, especially in the tropics where most biodiversity is found. Fortunately, the development of surveys with camera traps, which photograph mammals as they pass in front of infra-red sensors, has partially solved this problem. In this chapter, we outline two standardized protocols for the survey of large mammals: (1) large-scale arrays of camera traps to survey terrestrial large mammals; (2) line transects to survey primates and other diurnal arboreal mammals. Together, these protocols cover all large mammals, except for the nocturnal arboreal species. All protocols are well-established, published, field-tested standardized procedures.

2. Methods of Sampling

Protocol 1. Camera trapping of terrestrial mammals and birds

Camera traps have emerged as a standard for surveying communities of large terrestrial mammals (see Figure 5.1 for an example of an image from a camera trap). Arrays of camera traps can be considered as sensor networks to detect and monitor variation of mammal relative abundances in space and time, where the rate and the proportion of points at which species are photographed can be used as an indicator for their abundance. Moreover, under certain conditions and assumptions, photographic rates can be used to estimate population densities.

The proposed protocol is based on the camera-trapping protocol of the Terrestrial Ecology Assessment and Monitoring network (TEAM; www.teamnetwork.org). The TEAM protocol was designed by world specialists for tracking the changing state of mammal communities, and is currently the best-available protocol for surveying terrestrial mammal communities in tropical forests. The TEAM protocol was designed to optimize the probability of photographing an adequate sample of tropical forest terrestrial mammal and bird species. It is intended to monitor changes in the community of terrestrial vertebrates, not to monitor the abundance of individual species. The sampling design is suited to detect species with large ranges, such as jaguars, as well as species with smaller home ranges, such as terrestrial birds and small carnivores. Data can be used to assess properties and trends of the community, such as structure, species, and functional diversity. The protocol has been systematically implemented at 16 tropical sites in Africa, Asia and Latin America, ranging from continuous to fragmented forests, where it is used for annual surveys.

The protocol deploys camera traps in systematic grids with randomized starting points, rather than subjective or ease-of-access placement, with standardized settings, and without bait. Thus, they capture animals that coincidentally move in front of the cameras. This is an important difference from protocols that attempt to maximize encounters with species by placing cameras subjectively at locations of high use by animals, e.g. trails, mineral licks or water holes, or attempt to attract animals to the camera location with lure.

To ensure that different people collect data in the same way, despite differences in field conditions and work cultures, the procedures for implementing the TEAM protocol have been described in great detail in a monitoring protocol implementation manual (TEAM Network 2011). The procedures and forms in the TEAM protocol will work for the protocol outlined here.

Sampling design for camera trapping

Camera traps are deployed at fixed points in large-scale arrays. The number of points should be 60-90 per national park, which is the number needed for detecting rare and wide-ranging species with reasonable likelihood (TEAM Network 2011). Two thirds of the points are arranged in one or more grids, with fixed interspacing between points while one third of the points are arranged along trails (especially designed to monitor large cats, see below). The preferred interspacing is 1414 m, which corresponds to a density of 0.5 point per km^2. At this density, the grid(s) sample 120-180 km^2 of forest. If the total area to be surveyed is too small to harbour 60-90 points at this density, then the interspacing can be reduced to 1000 m (i.e., 1 point per km^2) or 707 m (i.e., 2 points per km^2). If the sampling is focused on different parts of the area of concern, e.g., different watersheds, then each part will receive the same share of the total number of points. The grids need not be rectangular and can be laid out with respect to attributes of the terrain, e.g., as to avoid difficult terrain.

Large cats, such as jaguar (*Panthera onca*) and puma (*Puma concolor*), tend to occur in very low densities with very large home ranges, and are therefore relatively infrequently captured by grid-based camera trap surveys. This is why one third of the survey points will be arranged to specifically survey this taxa. These surveys take advantage of the distinct preference of most cats to follow trails. Cats will be surveyed using pairs of camera traps that are placed along established trails. The cameras are placed on opposite sides of the trail, facing each other, in order to simultaneously photograph both flanks of any animal that passes. This allows for building a “Facebook” of individuals, with separate photos of both flanks, which allows individual labeling and recognition on subsequent footage. These cameras will also capture poachers and other people that use the trails, and may thus give an idea of the hunting pressure. A fixed distance must separate neighboring cameras, preferably 1,414 m, corresponding with the spacing of the camera-trap grid network. This ensures that the deployments are independent. If the trail system has been mapped and imported into the Geographical Information System, then target locations for deployment can be pre-generated and loaded onto the GPS receiver. The setup of the cameras for cats (one third of the total amount of points) is the same as for the camera trap grid, except that (a) the locations chosen are trails, and (b) two cameras are used per location, placed along the trail, at opposite sides. Place each camera perpendicular to the trail at approximately 2 m from the trail and ensure that the camera has a clear view. Avoid placing the cameras exactly opposite to each other, because the flashes may otherwise interfere. Rather, put the second cameras 2-5 m up or down the trail with respect to the first. Make sure to camouflage the cameras, because trails are likely used by poachers and other people who might tamper with the camera or steal it.

The field crew uses the tracking function of the GPS receiver to record the actual route followed and the movement speed (this information can be used to optimize future navigation to the points). At a point density of 0.5 per km^2, a crew can do 2-8 points per day, depending on the difficulty of the terrain.

Once the target point is reached, the crew chooses a sampling point for camera deployment in the immediate vicinity, preferably within 25 m. A suitable location has (a) a stem with 20-50 cm diameter that is suitable for mounting a camera (thinner stems are too easy to cut with a machete to steal the camera, thicker stems cannot be rounded with a single cable lock); (b) a clear view at knee level of ca. 5-10 m in a certain direction, preferable the north (to have photographs without backlight). In practice, small game trails and other natural openings in the understory vegetation are best suited; and (c) A distance >25 m from rivers or other water bodies. Important: The crew should <u>not</u> purposely select specific locations with high animal activity, such as salt licks, water holes and burrows (unlike in protocol 2).

Figure 5.2. The PC900C Cellular HyperFire Professional HO Covert IR (Photo from Reconyx website).

Each sampling location is labeled with a unique ID code, with format 'ABC-1-23'. The element 'ABC' is a three-letter code for the park, '1' is the number of the camera-trap array, and '23' is the two-digit number of the camera trap point within the array. The code 'DON-03-12' would thus refer to the 12^{th} point of the third array in Donoso. The GPS coordinates of the deployment location are recorded by taking a waypoint with a GPS receiver. The waypoint is named with the location code, using the keypad of the receiver. At least 100 GPS readings are averaged before the coordinates are saved; they must be accurate enough to relocate the sampling point years later. For security, the coordinates must also be written down on a form, along with properties of the location. If the sampling location is to be re-used, such as for long-term monitoring, the stem should be given a metal tag of the same type used for tagging trees (see chapter on tree monitoring).

Camera preparation – Each sampling point receives one high-quality camera trap, preferably Reconyx Hyperfire PC900 (Reconyx Inc., Holmen WI, USA) (Figure 5.2). This camera has a fast trigger speed (1/10^{th} sec.) and the ability to take multiple photos upon each trigger of its passive infrared sensor

(PIR). Colour photographs are taken during the day, black-and-white photographs illuminated by infrared LED flash during the night. The Hyperfire PC900 has a nearly invisible flash (although cats appear to still see the flash or hear the mechanics of the camera). The trap is powered with 12 high-end rechargeable NiMH batteries, preferably Sanyo Eneloop XX (SANYO Electric Co., Ltd., Moriguchi, Osaka, Japan). Each camera is given the same settings. The 'label' programmed the should be the ID code of that point; this code will appear in the lower left corner of each photograph taken, so each image is directly linked to a location. Make very sure that the correct date and time are programmed.

Mounting – The camera trap is mounted to the focal tree with a high-end cable lock that is passed through the housing, preferably Master Lock © Python Adjustable locking cables, available with camouflage print (180-cm x 8 mm) or as heavy-duty (10 mm). These cables are difficult to cut. When there is a high risk of vandalism and theft, it is advisable to enclose the camera in a steel security case with a pad lock; parts of the camera can still be vandalized, but the memory card with photographs (of the offender!) cannot be accessed. Make sure to carefully label and store the keys to all locks, to avoid problems when recovering the camera. The camera is mounted close to the ground, with the camera lens at 30-40 cm from ground level. This must be verified with a tape measure, because cameras that are placed at greater heights will miss many small species.

Alignment –The camera must be aimed to open understory, without leaves, trees or debris blocking the 'view' of the sensor. It is allowable to minimally modify the vegetation to clear the view, e.g., removal of a few seedlings and course debris. Ideally, the camera faces the north, which yields the clearest images during daytime. The camera is aligned parallel to the ground so that the 'horizon' of the plane is in the centre of the image. The best way to verify this is to turn on the camera, have it take a few pictures, and then turn it off again. Then remove the memory card, put it inside a portable photo camera or image viewer, and check the images. Adjust the camera alignment as needed. Repeat this procedure until the camera is aligned perfectly.

Walk test and arming – The final steps of camera installation are to do a 'walk test' and to arm the camera. Put the camera in 'walk test' mode and measure at what maximum distance in front of the camera a person waving just above the ground is still captured by the sensor. This distance should exceed 5 m. Ideally, it exceeds 10-12 m, but this is rarely achieved in tropical moist forest. Measure the distance from the camera with a tape measure, and record it on the field form. Once the camera is properly installed, it can be armed and start monitoring. Walk in front of the camera to have it take some pictures of yourself, in order to create a photo record of the start of the deployment. Also record the date and time of arming on the field form.

Retrieval – The field crew should collect the camera no less than 30 days after placement. (Cameras can technically be left in the field for up to 3 months or more, but this will not be as informative as redeploying the equipment at another location after 30 days). Once the sampling point is relocated using the waypoint in the GPS, a field worker should walk in front of the camera to trigger it and thus create a photo record of the end time of the deployment. Turn off the camera to stop the triggering, and wrap for transportation to the base station. Once at the base station, turn the camera on again and use the menu to record the battery level and the total number of photographs taken. Write this information on the deployment form. Then, carefully remove the memory card and the batteries and clean / dry the camera, either for storage or for the next deployment. Dry the camera with opened case by placing it in a drying closet. Copy the photographs from the memory card onto an external hard disk.

Avoiding disturbance – While at the sampling point, field staff should be careful not to put their backpack and other gear on the ground because the smell may affect mammal behaviour. It is recommended to hang the gear in a tree. Also, avoid unnecessary walking in the view of the camera.

Make as little noise as possible, and do not stay at the point longer than needed for placement or collection of the camera.

Protocol 2. Line transect counts of primates and other arboreal mammals

Primates such as spider monkeys (*Ateles* spp.) tend to be heavily impacted by poaching, even in relatively pristine regions. At the same time, they are disproportionally important for tree recruitment, providing the necessary seed dispersal to numerous fleshy-fruited tree and liana species. Ground-based camera trapping is unsuitable for primates in the Neotropics, since most species are arboreal and come to the ground only infrequently. Here, we recommend distance sampling along line transects to survey primates and calculate estimates of absolute density, an approach widely used to survey primates and mammals in general. Distance sampling requires that the observer accurately estimates the distance to each animal at the time of observation and the angle of the observation from the transect line. These data are then used to calculate the distance of the animal from the transect (Figure 5.3), which is later used to correct for a declining detection probability of animals with distance from the transect, for each species separately. If the assumptions are met, these data then allow for estimation of population density.

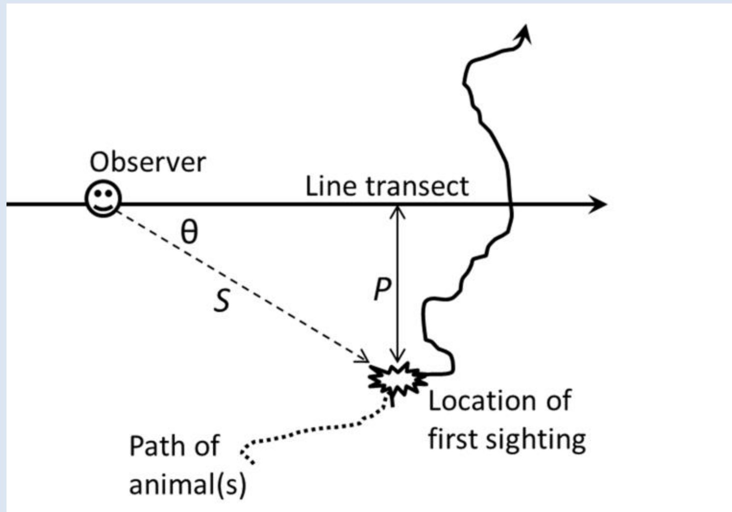


Figure 5.3. Principle of distance sampling. Upon sighting of an animal, the observer records his distance to the animal, *S*, and the observation angle with the line transect, θ. The distance of the animal from the transect, *P*, is then calculated as $P = S \sin\theta$.

The transect sampling follows the protocol of Lacher (2003) developed for the TEAM network. The transects sampling is conducted in multiple sites within the area of concern, overlapping with sampling plots for other protocols. At each site, the census is conducted using a fixed of number of parallel transect lines (e.g., 5) with fixed length (1 km) and fixed interspacing (e.g., 200 m). The lines must be walked in a specific fashion, as outlined in the methods section. The interspacing between lines is sufficient for each individual walking the transect to count independently, but there is a probability of double counting quickly moving groups.

Field procedures

The transects should be clear of debris, as noise may cause animals along the transect to flee before the observer gets close enough to detect them. If there are multiple observers, then observers must be rotated between different sites to cancel out potential observer biases.

Censuses should be done during calm weather, as rain and wind preclude reliable detection, and preferably during the early morning, as most diurnal animals are actively foraging during that time of the day. Transects should be walked by a single observer at an average speed of approximately 1,250 m/h.

The observer should record the code of the transect, the date, the start and end time of each walk, his name, and the weather conditions. Upon detection of an animal or group of animals, the observer records time, species identity, group size, group spread, the detection cue, the sighting location along the transect, sighting distance (S) using a range finder, and sighting angle (θ) using rangefinder binoculars or compass. Only record the perpendicular distance to the transect directly if that can be reliably estimated.

To meet the assumptions for density estimation, it is important that: (1) all animals on the transect line are detected; (2) animals are detected at their initial location, prior to any movement in response to the observer, and are not counted twice; (3) animals move slowly relative to the speed of the observer; (4) distances from the transect are measured accurately; and (5) detections are independent events. For this reason, observations must be done by one or two experienced observers.

3. Species Identification and Handling

Observations of cats should be identified not only to the species but also to the individual, each of which must be given a unique number or name. A library may be populated with photographs from the survey to help distinguish individuals.

4. Personnel Training

A single skilled person – preferably a zoologist – should oversee all work, manage the equipment, and compile the photographs and data. A spatial analyst is needed for compiling the spatial information into a Geographical Information System, generating the sampling design and access plan, preparing the GPS receivers, and compiling the spatial information collected during camera deployment (tracks and waypoints). Game wardens, field workers from other protocols, and other people with regular presence in the field should be involved in obtaining data on the presence of trails; these people can map trails with GPS receivers after brief instruction. The spatial analyst can then compile a trail map from this information. Field crews should consist of 2-3 people, for reasons of safety, with excellent physical condition and bush skills. Each crew should have one person who has received training in deployment of camera traps; the other members need no special training. Zoologists (professional or volunteer) are needed to process the photo material and identify the animals.

Protocol 2 can only be conducted by people with experience in distance sampling and knowledge of primates.

5. Estimated Budget and Timing for Panama

Camera deployment

One camera trap is deployed at each sampling point for a minimum of 30 days. With 60 sampling points, the total sampling effort will thus amount >1800 days. Deployment should happen during the driest season of the year for two reasons: to avoid camera problems related to humidity, and because the terrain tends to be better accessible to the field crew. Points can be sampled simultaneously or sequentially, by array, depending on the number of cameras that are available.

The major costs are involved in the field deployment of the camera trapping in protocol 1. Assuming a sampling-point density of 1 per 2 km^2 (1,414 m interspacing), a field crew can normally place two (range 1-4) camera traps per day. Collection of cameras is up to twice as fast, as the crew will know better how to access the sampling points, and as less work is needed at the points. In total, 60 points will require an estimated 90-115 days of field work (i.e., ca. 23 field days for two field crews of 2-3 people each). The actual amount of time needed will greatly depend on accessibility of the terrain on the location of the field station. Under certain circumstances, it may be necessary to camp in the field, which would require expanding the crew with porters.

Two people are recommended to run protocol 2. Total effort for protocol 2 is 30 mornings assuming 10 transects and 3 walks per transect. If it is possible to combine the primate surveys with bird monitoring and have them carried out by the same people, there is no additional cost to the bird monitoring.

Equipment

A survey of camera trapping with 60-90 points requires the following equipment (Table 5.1), assuming that 30 deployments will be done simultaneously (hence the total survey takes 2-3 rounds), and assuming that two field crews will be working simultaneously.

Table 5.1. Equipment needed for a camera trap survey of large terrestrial mammals according to protocol 1.

Equipment	Suggested model	Number [1]	Cost [2]
Camera traps	Reconyx Hyperfire PC900 (required)	30	$19500
Rechargeable AA batteries	Eneloop XX 2500 MAh	376	$1140
Security enclosure (optional)	Hyperfire security enclosure	30	$1500
Rain shield for camera (optional)		30	$300
Cable lock	Master Lock 8418KADCAM-TMB	30	$750
SDHC memory cards, ≥4GB, class ≥6	SanDisk Ultra 8 GB SDHC Class 10	60	$690
Battery charger	Titanium Smart Fast 16 Bay	4	$220
Handheld GPS receiver	Garmin GPSMAP 62	4	$1100
Batteries for GPS receiver + spares	Eneloop XX 2500 MAh	16	$50
Memory Card for GPS receiver	Sandisk 4GB Micro SDHC	4	$28
Pocket tape measure 3 m, metric		2	$15
Fiberglass tape measure 25 m, metric		2	$40
Waterproof two-way radio transceiver	Midland GXT1000VP4 36-Mile	2	$140
Waterproof flash light	Princeton Tec torrent flashlight	4	$150
Batteries for flash light + spares		64	$192
First aid emergency kit		2	$90
High-end computer + 22” monitor		1	$900
External hard disk USB 3.0, ≥500 GB	Seagate STBX500100	1	$50
Memory card reader USB 3.0	Transcend TS-RDF8K	1	$15
Waterproof paper for data sheets		1	
Pencils B (box)		1	
Compass		2	
Total			$26870

[1] Minimum number, without spares / replacements.
[2] Cost for purchase in USA, without discounts, without shipping costs + customs fees.

The equipment needed for the primate surveys is listed in Table 5.2, assuming that two observers simultaneously walk different transects.

Table 5.2. Equipment needed for a line transect survey of primates and other diurnal arboreal mammals according to protocol 2.

Equipment	Suggested model	Number [1]	Cost [2]
High-end waterproof binoculars 8-10x [3]	Leica 10x42 Trinovid	2	$3,000
Laser rangefinder [3]	Nikon ProStaff 3, Black	2	$300
Waterproof digital camera	Panasonic Lumix DMC-TS5D	2	$600
Handheld GPS receiver	Garmin GPSMAP 62	2	$350
Batteries for GPS receiver + spares	Eneloop XXX 2500 MAh	8	$20
Memory Card for GPS receiver	Sandisk 4GB Micro SDHC	2	$15
Compass	Suunto M-3 D/L Pro	2	$56
Water resistant field notebooks	Rite In The Rain Spiral - Level #313	2	$15
Waterproof paper for data sheets	Rite in the Rain - 200 sheets	1	$30
Pencils B (box)		1	$5
Total			$4,391

1 Minimum number, without spares / replacements.
2 Cost for purchase in USA, without discounts, without shipping costs + customs fees.
3 Binoculars and rangefinder are ideally combined, e.g. Leica Geovid 10 x 42HD Laser Rangefinder Binoculars @ $2,400 per unit. A low-cost alternative is the Bushnell 10X42 Fusion Arc 1600 @ $850 per unit.

6. Data Output

Recording camera locations

Upon setting up, the location of each camera trap should be carefully noted. Table 5.3 provides an example of data form for recording this information.

Table 5.3. Suggested camera trap setup field form (from Jansen et al. (2014)).

ID point	Latitude	Longitude	Start date + time	Person	ID camera	ID card	Key #	Notes

Photo processing

Camera trap surveys can generate large amounts of photographs that can be cumbersome to process. Various tools are available to facilitate the processing and storage of photographs, and turn them into meaningful data that can be analysed. We recommend use eMammal, an online processing and storage tool for camera trap photographs hosted by the Smithsonian Institution, available from late 2013 onwards. eMammal returns data files and provides data visualization and analysis tools. eMammal stores the data and underlying photographs in a secured environment where they are curated by the National Museum of Natural History.

Figure 5.4. A screenshot from an online tool for identification and management of camera trap photos.

Chapter 6. Methods for the Inventory and Monitoring of Amphibians and Reptiles

Roberto Ibáñez D.

1. Introduction

Despite its small territory, Panama contains a high diversity of amphibians and reptiles (see Figure 6.1 for some examples). The published numbers of native species of amphibians and reptiles known to occur in Panama are 206 and 244 species, respectively (Jaramillo *et al.* 2010, Hertz *et al.* 2012a). However, these kinds of published numbers become rapidly out of date as new species are discovered.

We do not intend to update these numbers on species richness here, but rather stress that the amphibians and reptiles of Panama are numerous and have a complex taxonomy. Therefore, they are a significant but often overlooked component of the vertebrate communities present in this country, and detailed observations and some caution are often needed to produce a species inventory of the herpetofauna from areas in Panama. Various online references maintain updated species lists and taxonomy of amphibians present in Panama: AmphibiaWeb (www.amphibiaweb.org) and Amphibian Species of the World (www.research.amnh.org/vz/herpetology/amphibia), and, for reptiles, The Reptile Database (www.reptile-database.org).

A.

B.

Figure 6.1. A. *Imantodes cenchoa* **B.** Pair of glass frogs (*Sachatamia albomaculata*) in nuptial embrace (male on top, female below).

In Panama, terrestrial and semi-aquatic amphibians and reptiles can be found in diverse habitats, from open grassy areas to closed canopy forests. They can be found from the ground to the canopy: in the soil, in the leaf litter, under or inside rotting logs, on vegetation such as shrubs and trees, under moss and bark of trees, inside epiphytic plants, along streams and rivers, and in puddles, ponds and swampy areas. Many frogs spend part of their lives in water, as a free-living larval stage or tadpole. A few amphibians and reptiles are mainly aquatic, such as the frog *Pipa myersi*, fresh water turtles (7 species), water snakes (*i.e., Hydromorphus concolor* and *H. dunni*), crocodilians (*i.e., Caiman crocodilus* and *Crocodylus acutus*) marine turtles (5 species) and sea snake (*i.e., Hydrophis platurus*).

2. Methods of Sampling

We present sampling methods that have proven effective in Panama and are also efficient in terms of labor and cost (*e.g.,* Ibáñez *et al.* 1995, 2002, Lips *et al.* 2006). They are adequate for forest amphibians and reptiles, including forest streams and ponds, but not species found underground or high in the forest canopy.

Methods for sampling primarily aquatic or marine species, such as freshwater and marine turtles, crocodilians and sea snakes, are not included either. In the case of amphibians, the sampling methods presented here are adequate for juveniles and adults, but not for larval stages.

To inventory the amphibians and reptiles of an area, day and nighttime sampling need to be conducted. Some species are best detected when they are active, while others are most easily detected during their time of inactivity when they are hidden or resting. The search should be focused on the ground, leaf litter, rotting logs, low vegetation, shrubs, trees and trunk and branches of large trees. At night, a headlamp or flashlight plus an adequate supply of charged batteries are a must, and carrying a second light source is recommended in case one fails.

The methods covered here are: (1) general non-constrained search at the survey locality, (2) visual encounter surveys along transects, (3) visual encounter surveys on quadrats or plots, and (4) audio recordings with automatic loggers. These methods can be used alone or combined to complement data. All of them can provide relative abundance data on the species present at the survey sites. The surveys of amphibians and reptiles should be conducted, at least, twice a year, *i.e.*, during the dry and wet season. However, initially, it is suggested to conduct surveys 3-4 times per year, especially in poorly surveyed localities. These surveys can provide data to determine future sampling effort at each location. Given the importance of the weather on the behavior, activity, and reproduction patterns of amphibians and reptiles, it is also highly recommended to record weather. In addition, due to the catastrophic effects that chytridiomycosis has on amphibian communities, obtaining data on the presence and distribution of the pathogen *B. dendrobatidis* is strongly suggested.

Two important considerations, valid for all methods presented here, are: (1) the recognition of different habitat types, and (2) keeping high quality data records. Usually an area is heterogeneous, presenting various habitat types (*e.g.*, closed canopy forest, secondary forest, clearings, grassy open areas, forest-open area transitions, streams, stream margins, and swamps). Therefore, sampling and record keeping should be carried out accordingly, treating them as distinct entities or units. Stratified sampling is recommended in such cases.

Site location coordinates should be taken using a GPS, using the latitude-longitude coordinate system and taking note of the datum (*e.g.,* WGS84, NAD27 Canal Zone). A few points should be taken for transects and quadrats. The elevation of sampling sites can be determined using a GPS, altimeter or from maps.

General non-constrained surveys

The main objective of this method is to record all species present at a survey locality, as in a complete species inventory (Scott 1994, Rueda *et al.* 2006, Raxworthy *et al.* 2012). However, in practice, this method aims to find the maximum number of species at the site as efficiently as possible. A second objective is to simultaneously obtain data on the abundance of individuals of a species. As they walk, observers visually survey all appropriate habitats and microhabitats for amphibians and reptiles. Often, observers give more priority to those that will give a larger species return, for example, stream margins and ponds. In addition, they move the leaf-litter, turn rocks and rotting logs on the ground, look inside holes on the ground and inspect bromeliads, holes and moss of tree trunks, searching for animals. Surveys are conducted during day and nighttime.

When an amphibian or reptile is sighted, the observer should identify it on the spot or capture it for identification. Once identified it is immediately released back at the place of capture. The number of individuals of each species seen should be recorded, and observers should avoid counting an individual more than once during a survey, for example, by searching in different areas, not surveying an area twice. The start and end time of the survey should be recorded, as well the name(s) of the observer(s), time when each animal was seen, and basic weather information. Observers should keep separate records according to the habitats being sampled (*e.g.,* forest, margin of stream, pond). These data will allow the calculation of sampling effort for each habitat type, expressed in person-hours. A measure of abundance can be presented for each species, based on a sampling effort unit (*i.e.*, number of individuals/person-hour). This allows comparisons between sites and habitats, as well as the ability to draw species accumulation curves. Furthermore, data obtained with this method can be used to estimate the number of species present at surveyed sites, by using the software EstimateS version 9 (http://viceroy.eeb.uconn.edu/estimates/).

Visual encounter surveys (VES) along transects

Transects of 200 m should be placed at the general survey sites (Chapter 2). The transect route should be marked with flagging material at least one day before the survey. Transects along stream margins should also be selected (Chapter 2). Transects are usually surveyed by one observer, who slowly walks along them while visually searching for amphibians and reptiles; care must be taken not to count the same individual twice. The width of transects is narrow, usually 1 or 2 m wide (*i.e.*, 0.5 m or 1 m to each side of the center line), because it is difficult to see animals farther away.

Surveys should be started at the same time of the day. Start and end time and location should be recorded, as well the name of the observer, and basic weather information. When an amphibian or reptile is sighted, the observer should try to identify it immediately. Often, it is required to capture the animal for identification. Once captured, additional data on the individual could be obtained, such as sex, life stage (juvenile, adult), snout-vent length and body mass. These and additional information about the individual (*e.g.,* activity, behavior, substrate, perch height) should be recorded. Its location along the transect and time when it was sighted should also be recorded. The animals must be handled as little as possible and released at the place of capture. Be aware that observers differ in their ability for sighting animals, and measures should be taken to reduce inter-observer biases. Visual encounter surveys also provide data on non-reproductive and non-calling individuals. Conducting surveys along transects will provide data on species presence and their abundance (*i.e.,* number of individuals/m, number of individuals/m^2, number of individuals/time searched). Surveys should be performed in the day and nighttime. Be aware that transect sampling underestimates species abundance or density, because not all individuals inside the transect area will be seen and counted.

Visual encounter surveys (VES) inside permanent quadrats

Three quadrats of 8x8 m should be placed inside the 1-ha forest plots. It is recommended to establish and demarcate the quadrats, by regularly marking the distance with flagging material along the perimeter and inside quadrats at least one day before the survey. When performing the survey, observers should slowly walk inside the quadrat while visually searching for amphibians and reptiles and turning over litter. They need to be careful not to count the same individual twice, and count individuals before they escape from the quadrat. Complete and paced quadrat surveys are preferred, but a fast time-constrained survey (*i.e.*, 1 hour of systematic search in a quadrat, not including handling time of animals) is a valid way to sample quadrats. Associated data on leaf-litter variables at each quadrat should be obtained. Commonly measured variables are leaf-litter depth, canopy cover, soil temperature, pH and moisture; vegetation data are available from the tree plot.

Surveys should be started at the same time of the day. Start and end time should be recorded, as well the names of the observers, and basic weather information. When an amphibian or reptile is sighted, the observer should try to identify it immediately. Often, it is required to capture the animal for identification. Once captured, additional data on the individual could be obtained, such as sex, life stage (juvenile, adult), snout-vent length and body mass. These and additional information about the individual (*e.g.,* activity, behavior, substrate, perch height) should be recorded. The time when it was sighted should also be recorded. The animals must be handled as little as possible and released at the place of capture. Observers differ in their ability for sighting animals and should take measures to reduce inter-observer biases. Conducting surveys in quadrat will provide data on species presence and their abundance, expressed in density per unit of habitat area (*i.e.,* number of individuals/m^2). Surveys could be performed in the day and nighttime, since species have different daily activity patterns. In Panama, there is a considerable assemblage of diurnal leaf-litter frogs to be surveyed. At night, it may turn out to be relatively difficult to systematically search a quadrat; however, nocturnal leaf-litter frogs are detected more easily.

Automated acoustic sampling

The use of automated recording systems (ARS) would greatly enhance the detection of species of frogs, since individuals of many species are easily heard but hard to find, or not seen at all. In addition, recorded digital audio files will be available for future use, reducing auditory misidentification of calls by observers and inter-observer biases. ARS are used to detect species-specific calls of anurans, allowing observers to determine the species present and to acoustically monitor their abundance at sites. ARS will allow an automated survey throughout the day for several days, being useful for establishing daily activity patterns in frog species. Remember that this method only samples reproductive calling male frogs, and it will not provide a complete frog species inventory. This acoustic technique for inventorying and monitoring frogs has not been used before in Panama, therefore will require a trial stage. Nonetheless, it could be very useful for monitoring species of the genus *Diasporus* (*e.g*., Drewry and Rand 1983) and will provide data on species presence and their abundance, expressed as number of individuals calling per recorded time (*i.e.,* number of individuals/h) or, more likely, by using a calling index, based on averaged calling activity levels.

ARSs could be placed the middle of transects, or quadrats in the plots, to collect complementary data on frog abundance. Using a random stratified design, three acoustic monitoring devices can be deployed per study area, such as one in the stream plot, one in the forest plot, and one in the linear transect. The placement of the devices is critical to avoid recording overlap between them. The devices should be set or programmed to make sound recordings according to a desired timetable. For example, record every hour for 10 minutes. Collecting related weather and environmental data is highly recommended, such as rainfall, relative humidity, light intensity, temperature, leaf wetness, and time of sunset and sunrise. Most of these can be recorded using environmental data loggers or a small automated weather station.

Digital audio files can then be analyzed manually and/or using software to detect and identify frog calls. These automated sound recording dataloggers, often called frogloggers, are commercially available. The acoustic monitoring devices are produced by Wildlife Acoustics (www.wildlifeacoustics.com) and Bedford Technical (www.froglogers.com) and are available with different configuration options and accessories. These devices can be deployed in the field and data can be periodically retrieved depending on the power and digital storage capacity. Software is available that can reduce the time-consuming manual analysis of sound recordings. Song Scope (www.wildlifeacoustics.com) and SoundID (www.soundid.net) software are used to detect and recognize species-specific calls. These require reference pre-recorded audio files of anuran advertisement calls of interest.

A digital library of anuran advertisement calls could be built by manually recording calling frogs as inventory surveys are being carried out. When making these recordings, the frog should be observed carefully, as it calls, to be absolutely sure that it is the calling individual. Body temperature of the calling individual or air, water or substrate temperature should be measured and recorded. In practice, air temperature will suffice. Voucher specimens of the recorded individuals should be collected.

3. Species Identification and Handling

An adequate identification of species of the individuals encountered is crucial when conducting surveys to inventory the herpetofauna of an area. Currently, two excellent books with keys to species known in Central America have been published. These books compile the species of reptiles (Köhler 2008) and amphibians (Köhler 2011) present in Panama and should be used as a basic reference for their identification. In addition, Savage's (2002) monumental book on the amphibians and reptiles of Costa Rica constitutes an extremely valuable resource, especially for species found in western to central Panama. The guide to amphibians of central Panama by Ibáñez *et al.* (1999) can also help identify the species in the area. These and, of course, all the specialized bibliography on amphibian and reptiles of Panama, *e.g.*, the genus *Rhadinaea* (Myers 1974), are essential tools that should be consulted for an appropriate identification of specimens.

Species identification could be complicated further by taxonomical changes, undescribed species, and a poor knowledge about some species. In the last few years, the taxonomy of amphibians and reptiles has changed at all taxonomic levels. More changes are expected, particularly at species level, in part due to the use of molecular techniques in systematic and phylogeographic studies in Panama. Based on their morphology and on molecular data, several specimens are known to be new species, but these species remain undescribed. Therefore, it is expected that more new species will be described in the near future. Some species have been described based on one or few specimens (*e.g.,* Myers 2003, Myers *et al.* 2012), thus variation in their characteristics is poorly or not known, presenting a potential problem for the identification of these species.

Special attention needs to be given to diagnostic morphological features and the color pattern throughout the life of individuals, in particular, the coloration of dorsal, ventral and concealed surfaces of frogs, color pattern of dorsal and ventral surfaces of lizards and snakes, and dewlap color of anole lizards. Taking notes on their color could help in the identification of individuals. With the advent and development of digital photography, it is easy to obtain good pictures. Today, there are several compact, waterproof and shock resistant camera models with macro capabilities and incorporated flash in the market. Photographic images can provide more precise information on color patterns and are faster than writing notes. In addition, these images may capture other characteristics that could help in the identification of the individual, for example, snout and body shape, presence of glands and tubercles, scale types, and many other morphological features. When in doubt about an individual, several shots (ventral, dorsal, hands and feet) can be taken and the image numbers can be associated to an observation or data. Therefore, the use a small digital camera in the field is highly recommended as an aide for species identification.

Often, calling males are heard before they are seen. Learning the most common calls can be valuable and can be facilitated by listening to recorded calls in audio media (*e.g.*, Ibáñez *et al.* 1999b), or by obtaining your own recordings and maintaining a collection of frog calls. Affordable, compact digital audio recorders with incorporated microphones are available. Recordings made at the standard Compact Disc audio format*, i.e.,* 44.1 kHz sample rate and 16-bit resolution per sample, and saved as WAVE audio file format will be more than enough for most work on frog vocalizations.

When completing the field forms, make notes or comments on those individuals that you are not certain about the species identification, and associate any image or recording you made to each individual. In some cases, it may be easy to capture the individual, place it inside a cloth or plastic bag and write a label (to associate it to an observation or data) for later identification, and once it is properly identified, release it back where it was found. Moisten the cloth bag or place some moist substrate inside the plastic bag to reduce water loss from the animal. Be careful to not expose directly the bag containing the individual to the sun, since the animal could easily overheat and die. If possible, take copies of basic books and/or keys to the field. These can be left in camp, and consulted shortly after your return from the field site. Remember to keep updated on the taxonomy and scientific names, using resources such as AmphibiaWeb, Amphibian Species of the World (Frost 2013), and The Reptile Database (Uetz and Hošek 2013).

Amphibians and reptiles are usually captured by hand. Even when trapping methods are used, at some point captured individuals are handled. A snake hook is helpful for collecting snakes, and a snake tong for venomous ones. Be careful when trying to collect snakes, determining first whether they are venomous or not. Avoid taking unnecessary risks by capturing and handling venomous snakes, especially if you are in an area with difficult access. Most venomous snakes can be easily identified from a photograph taken from a prudent distance. When capturing and handling amphibians and reptiles, you must have a good grip to prevent escape, but gentle enough not to harm the animal. Make sure that you do not have insect repellent on your hands before handling animals. As with other wild vertebrates, the humane treatment of amphibians and reptiles is considered an ethical, legal and scientific requirement. Therefore, there are guidelines for their use in field and laboratory research (Beaupre et al. 2004, AVMA 2013).

Given that several amphibians and reptiles could be misidentified, even by experienced herpetologists, the collection of a few voucher specimens is suggested. When collecting specimens, regulations of the Autoridad Nacional del Ambiente (ANAM) state that one specimen per species should be deposited in the Museo de Vertebrados de la Universidad de Panamá. Voucher specimens are useful for accurate identification and future verification, and also could be important for future studies. They are particularly valuable when collected from poorly surveyed areas. A maximum of 4 specimens per species, including individuals from both sexes, is often an acceptable number for ANAM. Unless you have a valid reason and a permit allowing it, refrain from collecting endangered or protected species. Voucher specimens require a unique numbered label and associated information, such as date and time of collection, name of collector, locality and any additional information. An appropriate method of euthanasia should be followed when a healthy or sick individual is going to be prepared as voucher specimen (Beaupre et al. 2004, Aguirre and Lampo 2006). More details on preparation of vouchers and collection specimens of amphibians and reptiles can be found in Pisani and Villa (1974), Reynolds *et al.* (1994), Lips et al. (2001), Simmons (2002), Reynolds and McDiarmid (2012) and Foster (2012).

In addition, the collection and archival of tissue samples from the voucher specimens will increase their scientific value. For example, liver and other tissue samples could be used for the identification of species with DNA Barcoding (Vonesh *et al.* 2010, Schulte 2012, Barcode of life 2013), and in molecular systematic studies that will increase our knowledge on the Panamanian herpetofauna. When extracting tissue, dissecting utensils should properly cleaned to avoid DNA cross contamination, and a careful liver tissue extraction is required to avoid cutting or removing the gall bladder, since the bile could interfere with DNA extractions and inhibit subsequent reactions. Once extracted, the tissue sample needs to be adequately preserved and/or frozen (Jacobs and Heyer 1994, Schulte 2012).

4. Personnel Training

Biologists and their assistants conducting an inventory need proper training. At the minimum, they should be fairly competent on searching and handling amphibians and reptiles, species identification, following the selected methods and protocols, using a GPS, recording data in field forms, data capturing, digital recording of calls, digital camera use, voucher preparation, biosecurity aspects, and safety issues, among others. Therefore, prior to any fieldwork, a training workshop covering these topics is strongly recommended. In addition, the personnel participating in the inventory, in any area in Panama, must have a research or collecting permit issued by the Autoridad Nacional del Ambiente (ANAM).

A preliminary list of possible species that are known or might be present in the area could be helpful. This list could be prepared from reports appearing in publications or unpublished documents from or near the area of interest, or based on the approximate distribution of species, particularly in the case of target species or a set of species. Estimates and information on the distribution of Panamanian species can be easily found online. For example, the IUCN's initiative on amphibians includes a range map for every species (IUCN 2013). This list could serve as a guide to the species that might be encountered during the inventory, allowing personnel to focus on how to distinguish those species.

Emergent infectious diseases and other known pathogens and parasites are a health threat to amphibians and reptiles. Therefore, biosecurity issues should be considered in any field study of amphibians and reptiles. One publicized emergent infectious disease is chytridiomycosis, caused by chytrid fungi of the genus *Batrachochytrium*. The amphibian pathogen *Batrachochytrium dendrobatidis* has drastically affected Neotropical amphibian communities, leading to the decline and disappearance in populations of many amphibian species (Lips *et al.* 2006, Lips and Mendelson 2008, Crawford *et al.* 2010). Pathogens and parasites have to be considered for at least two reasons: (1) possible infection of humans or other vertebrates, and (2) prevention or reduction of their spread to unaffected populations of amphibians or reptiles (Lips *et al.* 2001). All clothes and equipment should be cleaned and disinfected before moving from site to site (DAPTF 1998, Lips *et al.* 2001, Aguirre and Lampo 2006,). Guidelines to reduce the risk of transmission of pathogens among amphibian individuals within and between sites during field studies have been suggested by Aguirre and Lampo (2006), Phillott *et al.* (2010) and Green *et al.* (2010). These are also broadly applicable to reptiles.

Personnel will require adequate supervision throughout all aspects of the inventory project, both in the field and laboratory. Among the key aspects to be supervised are: proper species identification, attachment to established protocols, and verification of entered data. This will assure the quality of the data being collected and managed. Field surveys will generate large amounts and diverse sets of data (e.g., photographic images and digital audio files), and a data management system should be planned accordingly to collect, integrate, analyze and archive data in an efficient manner and to maintain a high level of quality.

Safety of all persons involved in the project should be considered at all times. Accidents can happen both in the field and laboratory. Personnel should be prepared. When in the field, if possible, carry a radio or satellite phone for communication, and bring a first-aid kit. Also, personnel should have an evacuation plan arranged beforehand, in case of an emergency. Basic first aid training is highly recommended.

5. Estimated Budget and Timing for Panama

An estimated annual budget for the first year is presented below covering the expenses to inventory two sites that will be sampled 4 times per year. Each sampling visit will last for 10 to 12 days. Extra days are considered in case scouting trips or more time at one site is needed. This budget is intended for sites with a moderately difficult access. It does not include any institutional overhead or boat or helicopter transportation. Therefore, this budget could vary according to the degree of difficulty and mode of transportation required to reach the sites.

Estimated budget for first year:

Personnel:	4 biologists @ $1000/month @ 12 months	$48,000
	XIII month @ 8.33%	3,999
	Pool benefits @ 29%	15,080
Subtotal		$67,079
Terrestrial transportation and field assistance:		
	Fuel @ $300/month for 12 months	3,600
	2 local field guides @ $30/day for 100 days	6,000
	3 local porters @ $20/day for 100 days	6,000
	4 horse rentals @ $15/day for 40 days	2,400
Subtotal		$18,000
Equipment:		
	1 vehicle (4x4)	$40,000
	2 digital audio recorders @ $750 ea	1,500
	2 microphones @ $1,250 ea	2,500
	3 Wildlife Acoustic Song Meter SM2+ @ $900 ea	2,700
	Sound editing and analysis software	2,000
	1 Laptop computer	1,800
	2 GPS units @ $500 ea	1,000
	Miscellaneous field equipment (cables, measuring tape, rulers, thermometers, dissecting kit, etc.)	1,500
Subtotal		$53,000
Camping gear:		
	Tents, hammocks, tarps, cooking utensils, waterproof bags and cases, headlamps, flashlights, ropes, etc	$ 3,500
Subtotal		$ 3,500
Supplies:		
	Food	$ 4,500

Batteries, field notebooks, waterproof paper, pens, markers, plastic bags, swabs, vials, ethanol, formalin, etc……………………………………………………….$ 3,500

Subtotal…………………………………………………………………………………………….$ 8,000

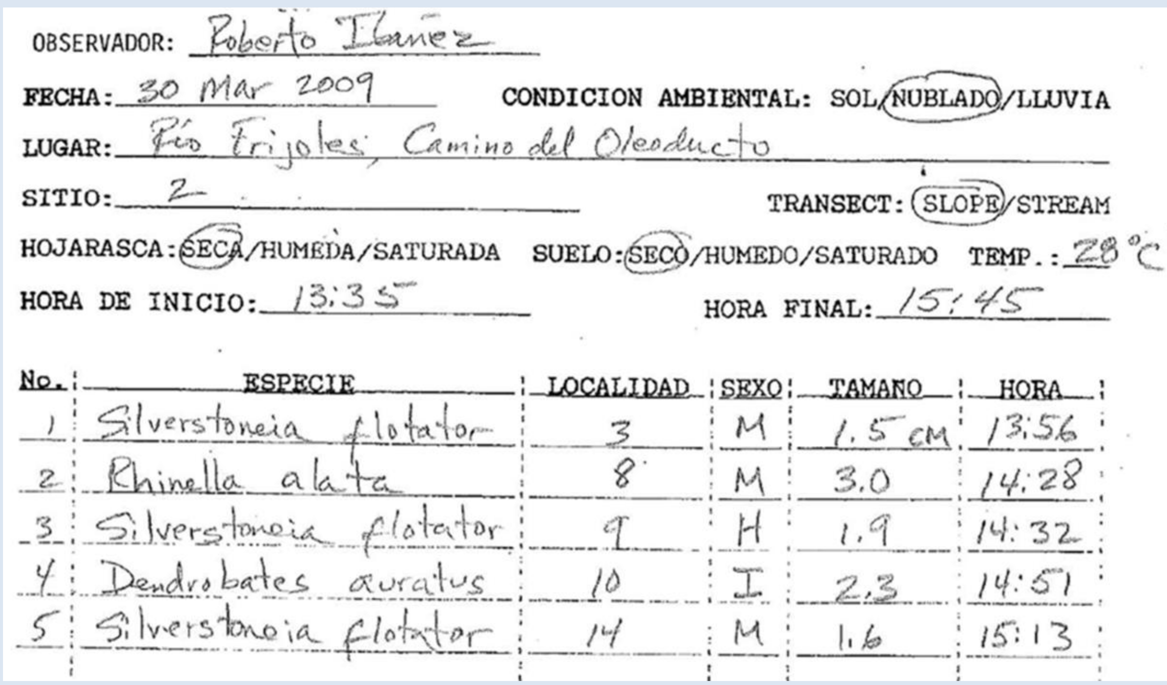
Unforeseen expenses ………………………………………………………………….$ 1,496

TOTAL…………………………………………………………………………………………$151,075

Timing: 4 samples per year that should fall in the dry season (January-March) and early (May-July), mid (August-September) and late (November-December) wet season. Alternatively, these can be carried out regularly every quarter, starting in January.

6. Data Output

We recommend checking Chapters 3 (Birds) and 4 (Fish) as examples to see how the data are presented in a digital form. Figure 6.2 shows an example of a field data collection form.



OBSERVADOR: Roberto Ibañez

FECHA: 30 Mar 2009 CONDICION AMBIENTAL: SOL/NUBLADO/LLUVIA

LUGAR: Río Frijoles; Camino del Oleoducto

SITIO: 2 TRANSECT: SLOPE/STREAM

HOJARASCA: SECA/HUMEDA/SATURADA SUELO: SECO/HUMEDO/SATURADO TEMP.: 28 °C

HORA DE INICIO: 13:35 HORA FINAL: 15:45

No.	ESPECIE	LOCALIDAD	SEXO	TAMANO	HORA
1	Silverstoneia flotator	3	M	1.5 cm	13:56
2	Rhinella alata	8	M	3.0	14:28
3	Silverstoneia flotator	9	H	1.9	14:32
4	Dendrobates auratus	10	I	2.3	14:51
5	Silverstoneia flotator	14	M	1.6	15:13

Figure 6.2. An example of a worksheet with field data taken at various locations.

Chapter 7. Methods for the Inventory and Monitoring of Woody Plants in Panama

Richard S. Condit, Rolando Pérez.

1. Introduction

This chapter describes scientific methods available for estimating diversity, species abundances, and biomass of trees in forests. The results provided include the number of species present, their exact abundances, total biomass of the vegetation, plus indices of diversity. Repeat censuses of the same location will then provide information on how the forest changes through time, and it will be possible to detect loss of important species or decline in forest biomass. The sampling method consists of square plots, 100 m x 100 m in size in the upland forest, and 20 m x 100 m near streams (smaller- sized plots can be used in small fragments of forest). In each plot, every tree ≥ 100 mm stem diameter is measured, mapped, and identified; in addition, tree saplings ≥ 10 mm are measured in a 10 m x 10 m subsection of the full plot. Methods for locating forest plots across the landscape were covered in Chapter 2. Here we provide details on how a plot is measured and identified.

2. Methods of Sampling

Tree plot census

Exact plot locations

In Chapter 2, we described methods for choosing locations of inventory sites from maps. This started with locating positions along forest streams for fish and frog census. A forest location was then chosen 500 - 1000 m from one side of the stream, using a random distance and direction. That will provide the first plot location, and a base stake must be placed at the spot, where the base stake becomes the southwest corner of the plot. From there, a precisely square plot of 100 x 100 m will be surveyed, with stakes placed at regular intervals to allow mapping of the trees.

A second, smaller plot should then be set up very close to the river. The base stake should be placed as close as possible to the selected stream location. The plot at the stream will be only 20 x 100 m in size, with the long axis parallel to the stream. One plot boundary should be as close as possible to the stream water, even intersecting with bends in the stream. When the two locations are chosen and the base stakes placed, precise GPS (in decimal degrees) coordinates of each must be recorded.

The plot survey

At this stage, a plot location has been established and a base stake has been placed in the ground. Additional stakes must now be placed at intervals of 20 meters, creating a grid within the plot that allows trees to be mapped precisely. The survey requires placing a device that measures distance, direction, and inclination on a tripod at one stake, then measuring ahead 20 m in a precise direction to place the next stake. Distance and inclination must be measured precisely.

The purpose of the surveying is to demarcate a plot where live trees will be counted. The survey team needs to take care not to cut or move anything. This may seem a trivial point, but many field workers are accustomed to moving through the forest with a machete, slicing away vegetation where there is no trail. This most certainly cannot be allowed inside the plot, indeed, I have on some occasions recommended that a rule be instituted forbidding machetes inside plots.

Surveying tools

An inexpensive and older alternative is a surveying compass. The compass consists of a large horizontal compass face and needle, on top of which sits a telescopic sighting device that can be rotated horizontally to take a bearing and vertically to measure inclination (Forestry Supplier sells a Sokkia surveying compass and tripod, catalog #37485 and #37487). Modern devices as of 2013 are laser rangefinders that accomplish all three measurements: direction, inclination, and distance, meaning the tape measure is no longer needed. A good rangefinder is the LacerAce 300 (MDL Lasersystems, http://www.mdl.co.uk/handheld_laser_systems/laserace-300/index.html). Just like a surveying compass, the rangefinder must be mounted on a tripod, and the tripod should have a plumb bob hanging below to allow it to be positioned directly above a stake. Whether using a surveying compass or a laser rangefinder, the methods are essentially the same. The distance, direction, and inclination must be measured exactly between two of the stakes on the 20-meter grid across the plot. With either tool, two sighting poles are necessary, each approximately 2 m in height with tape measures attached at identical heights on each. One pole should have a leveling bubble or plumb bob so that it can be held precisely vertically. The second is a target, and has a flat piece of wood with white cardboard marked with a large and brightly colored X. Rubber straps or ropes are also needed for pulling and tying vegetation out of sighting lines.

There are also much more sophisticated and expensive surveying devices known as 'total stations' which may be used if available. The procedure for surveying remains the same, but the total station automatically records all distances and inclinations and carries out calculations of the entire topography.

Grid markers at 20 m corners

The mapping grid is intended to be permanent, so that it can be relocated (at least for several years). In most circumstances, PVC can be used as markers: 1.2 m long by 50 mm in diameter, with a 1.5 mm wall thickness. If possible, the PVC stake should be pounded all the way into the ground, until its top is flush with the leaf litter, with a metal tag wired to the top of the stake by drilling a small hole through the PVC. The stake number should be etched into the tag. This tag will allow the stake be located beneath litter or mud using a metal detector. The sunken stake makes a long-lasting marker, and is also out of sight and out of the way. Of course, these permanent, sunken stakes should only be used with whatever permission is necessary to work in the forest.

During the census work, a temporary PVC stake should be inserted at each 20x20 m corner for high visibility; painting the stake bright orange is useful. Two or three pieces of bright orange flagging, about 1 m long, should then be hung from tree branches around each stake. This makes the 20 m corners easy to find from a distance. The coordinates of the 20x20 m quadrat should be marked on the flagging and on the top of the stake (the coordinate system is described below). These marking flags and stakes will not last long. Forestry Suppliers sells various flexible, colored, plastic flagging material.

Grid markers at 5 m corners

These will be placed the day tree enumeration begins. They are not intended to be permanent, though this depends on local preferences. Smaller PVC stakes, 1 m long with a 13 mm diameter and 1.5 mm wall, suffice, or even painted wood if they will not be left long. It is important that they be readily visible, and it is useful if they are distinguishable from the 20 m stakes.

Surveying a line

In describing the survey below, we refer to the measuring device as a rangefinder, though a surveying compass can be substituted. The stake from which a measurement is begun is called the origin, and the next stake to be placed is the target.

To survey a single line means placing a series of stakes at precisely 20 m intervals along a straight line. At least three, and possibly four people are needed. One person (Surveyor A) handles the rangefinder, setting it directly above a grid marker using the tripod and plumb bob and aiming it in the chosen compass direction. Two assistants move vegetation aside to allow a sighting line from the surveying compass to a point exactly 20 m ahead. Saplings and lianas can be bent aside and tied in position with rubber straps or ropes. Vegetation must not be cut! Flagging can be used to mark the division lines of the plot (Figure 7.1).

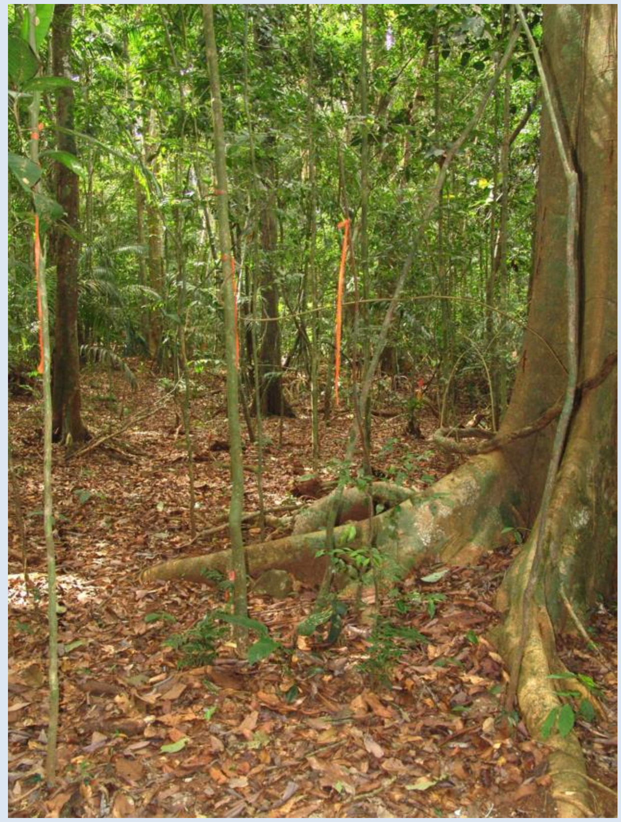


Figure 7.1. Orange flagging tape marking the division lines of the plot.

In the square plot of 100x100 m, the first survey line starts at the base stakes and moves precisely northward. Later, sightings due east, west, or south will also be used. The streamside plot, however, does not need to be on exact cardinal directions and should instead be approximately parallel to the stream. This means choosing an exact compass bearing for the long (100 meter) axis of the plot, calling it M^o, where *M* means the plain plot axis. All sighting lines in the plot will be exactly 90^o or 180^o from this main axis, so the four numbers *M*, $M\text{-}90^o$, $M\text{+}90^o$, and $M\text{+}180^o$ form the four plot axes.

Surveyor A then measures the height above the ground of the rangefinder using one of the sighting poles. Surveyor B holds the other sighting pole vertical, with the target conspicuous, and moves around until a location 20 m due north is located. Surveyor A finds the pole with the laser. Then B indicates with his or her finger the height on the second pole that matches the height of the rangefinder. Shining a flashlight on the target (or finger) can help in dark forest.

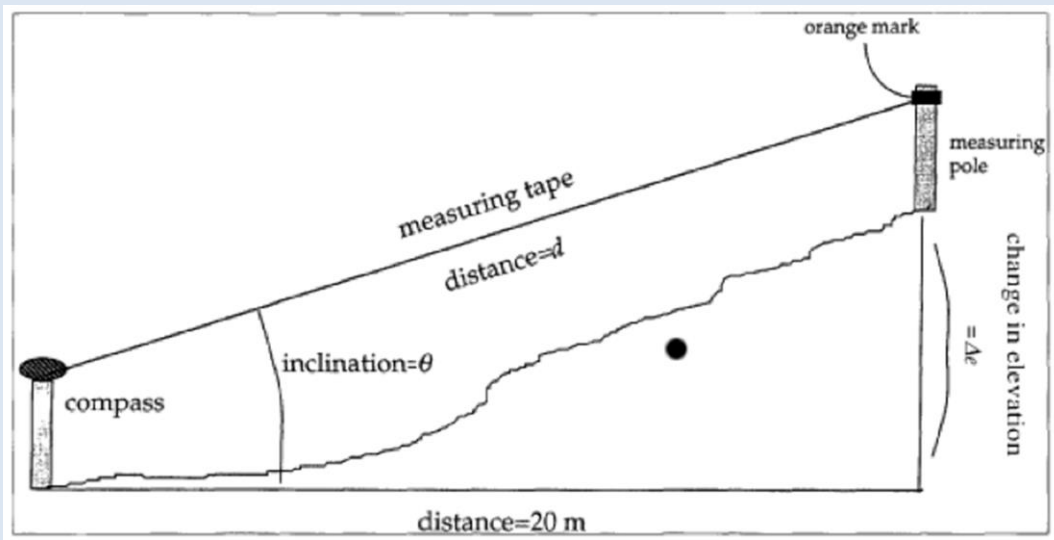


Figure 7.2. Surveying on an incline. The distance measured is *d*, parallel to the ground, and must be > 20 m, as given in the Annex 7.1. If using a laser rangefinder, it sits where the compass is indicated, and no measuring tape is needed.

Surveyor A must locate the target with the laser, then note the distance and inclination (vertical angle). If the inclination is non-zero, a distance correction is necessary, because the 20 m must refer to horizontal distance (Fig. 7.2). The distance *d*, parallel to the ground, is longer than 20 m, and is found from the formula

$$d = \frac{20}{\cos\theta} \qquad \text{Eq. 7.1}$$

where θ is the inclination. For example, if $\theta = 8^{o}$, *d* is 20.2 m. See the appendix at the end of this chapter for the distance correction for half-degree increments from 0.5^{O} to the 30^{O}. A copy of this table is needed in the field. With the preliminary reading of θ, a corrected value for *d* is read from the table. Surveyor B moves the sighting pole back to precisely *d* meters, and surveyor A must relocate the pole (asking B to move to the side if necessary) and read again. If changed, a new *d* must be read from the table and the pole moved again and resighted. When the inclination no longer changes, B places a permanent marker at the spot directly below the target (using the level to make sure the pole is vertical).

Surveyor A records the horizontal distance and the inclination to the final position exactly, plus the bearing (which is one of the plot axes, as already determined), and the numerical designation of the two stakes (the numbering system is described below). It is important to record the inclination as negative for downward slopes and positive for upward; this is easy to forget! Also, the stake numbers must be recorded in the correct order -- first the origin stake, where the rangefinder stood, and second the target, toward which the laser was aimed.

Now advance to what was the target stake and place the rangefinder there. During training, an important subsequent step is the *back-check*. From the second stake, take a sighting back to the first stake and confirm the direction (now 180^o opposite), distance (identical to the first measurement), and inclination (the exact negative of the first). Back-checking should be standard until the personnel are consistent with their results. It can then be omitted.

A parallel line

A second line should be started 20 meters from the first, and the two lines surveyed in parallel. This helps reduce error, as the distance between the two lines can be checked. Imagine instead surveying one line a long distance through forest, then returning to the start to place a second line parallel. Obviously, the two lines will drift, ending up either too close or too far apart. The goal is to avoid that drift using side-checks: sightings between the two parallel lines. In larger plots, I recommend three parallel lines for the initial axes.

To place the parallel line, return to the base stake after the first stake (20 m north) is placed and turn east; place another stake exactly 20 m to the east. Then move to the east stake and place a fourth stake north of it, completing a square of four stakes. Figure 7.3 shows three parallel lines and four stakes per line; in Figure 7.4, the four initial stakes are those numbered 0000, 0001, 0100, and 0101.

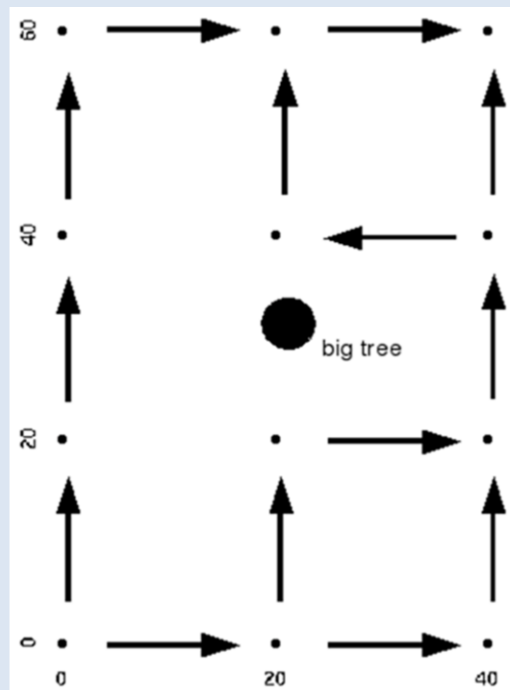


Figure 7.3. Hypothetical survey of a section of six 20 x 20 m quadrats. Each arrow indicates a survey measurement, with the arrow indicating direction. The big tree had to be surveyed around.

The first stake placed (0000) and the last stake placed (0101) should be exactly 20 m apart, but this has not been measured. Taking the measurement of direction, inclination, and distance between those two stakes is a *side-check*. It should confirm that the bearing between them is exactly $M+90^o$, that it is exactly perpendicular to the main axis, and that they are 20 m apart when corrected for inclination (correcting with the table of cosines in the Appendix). If there are substantial errors, the surveyors should return to the other stakes and try for improvement.

During training, and for the very first stakes placed, side-checks should be done for every new pair of stakes. One the surveyors gain experience, they will learn how accurate they are and know how often side checks are necessary. The rule should be to strive for 5 cm precision in distance and 0.15^o in inclination, but this is probably the margin of what is possible with this kind of equipment. If all measurements stay within 10 cm and 0.25^o, this should be sufficient.

In plots of 100x100 meters, maintaining this accuracy should not be a problem. Once experienced, the surveyors should set eight stakes in two parallel lines (up to 60 meters from the original stake), then perform a side-check between the two lines. If they are within the accepted precision, finish the lines out to 100 meters, then do one more side-check between the ultimate pair. This means two side checks between each pair of lines.

Whenever a side-check is done, record the measurements! These can be used in creating the topographic map, and they produce a record on survey accuracy.

Subsequent lines

From then on, single additional lines are added, with two side-checks per line, until the plot is finished. That is, after the first two parallel lines, the third line of stakes is set from start to finish, using the second line for side-checks, etc. (Fig. 7.3).

Obstacles

Unfortunately, in a real forest, large trees block some sighting paths. When one of the lines is blocked, it will be necessary to place a new stake from one of the parallel lines. Likewise, side-sightings may be blocked, so it is never possible to do side-checks at every intended stake. As long as errors can be kept small enough that side-checks are only necessary at 40-60 m intervals, this is not a problem. There will inevitably be some stakes that cannot be sighted from any of the four adjacent stakes. Here, a sighting from a diagonal stake must be used, with the distance adjusted to 28.3 m. Intermediate sightings to the mid-point of a quadrat (14.14 m) might be necessary in this circumstance.

Another difficult circumstance is when the position of a new stake is occupied precisely by a tree. If the trunk has a diameter < 40 cm, we recommend putting the stake as close as possible to its real position; at this diameter, it will be within 20 cm of the correct position. For larger trees, the PVC stake should simply be attached to the trunk with a nail, and the flagging hung as always to make it visible. In either case, the stake should not be used as an origin of sightings to other stakes, as this would compound the error. If a large rock occupies a stake's position, paint can be used to mark the location. Figure 7.3 shows a fictional but plausible surveying record for a portion of the initial axes.

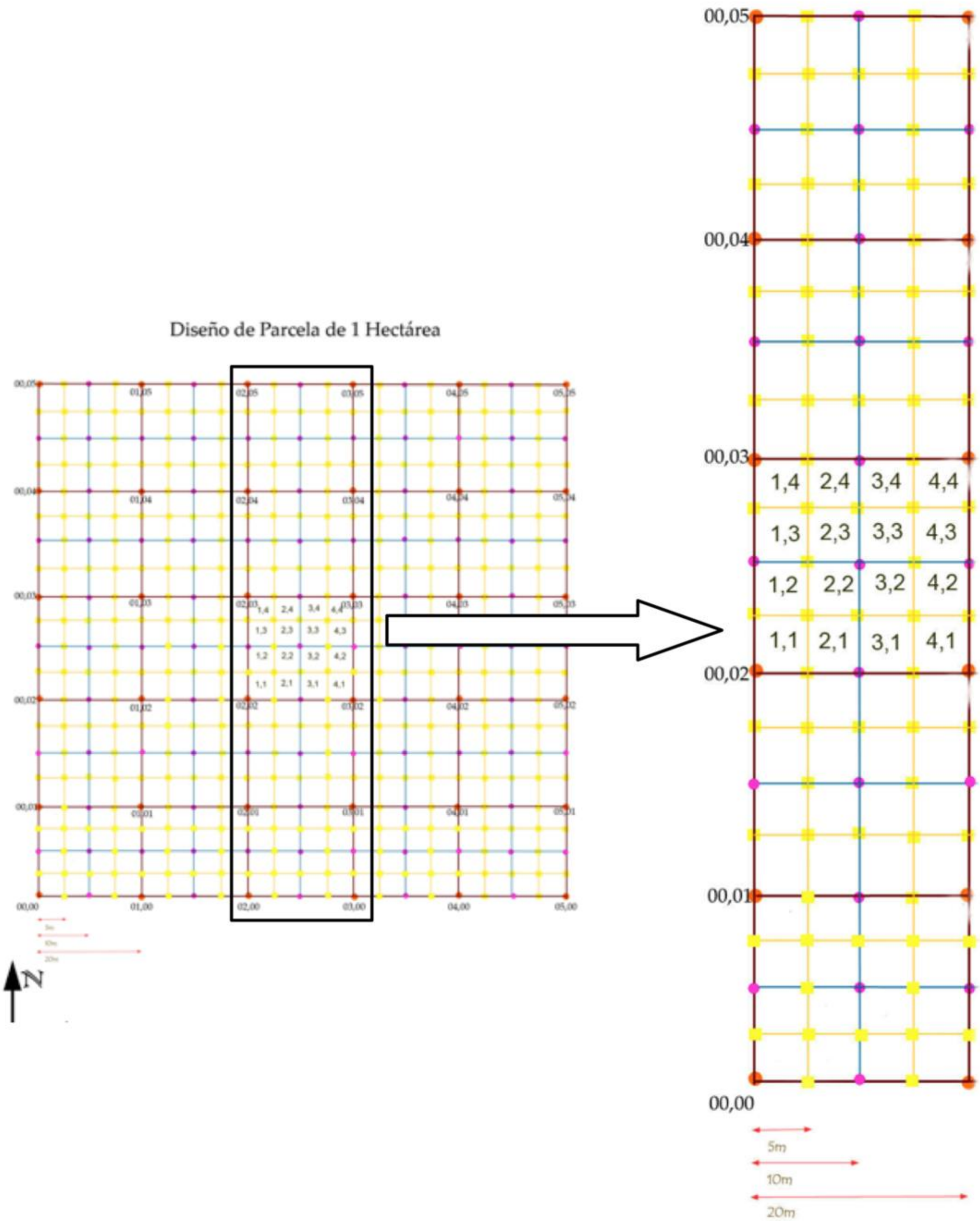


Figure 7.4. Quadrat numbering for a 100 x 100 m plot. The leftmost (ordinarily west) column is 00, and the bottom row is 00. Quadrats are designated with column-row. In the 20 x 100 m plot, quadrats should be numbered 0000 through 0500; note that this smaller plot is parallel to a stream and not oriented precisely north-south.

Quadrats and subquadrats

The 20 x 20 m quadrats into which the plot is surveyed become the unit of field labor; their accuracy is the basis for any future mapping of trees or other features. To be precise about tree-mapping, however, a 20x20 m quadrat is too big in dense forest, and a further subdivision into 5x5 m subquadrats is preferred. These markers, though, do not need to be permanent, and can be placed immediately before tree enumeration using tape measures (surveying them is not necessary). Methods for placing the subquadrats will be covered in the tree-mapping section.

Stake and quadrat numbering

The most convenient quadrat-numbering system for field work is to designate a quadrat by column and row, where rows are 20 m swaths numbered from south to north, and columns from west to east. Quadrat 0104 is column 01, row 04 (Fig. 7.4). The initial row and column are 00, thus quadrat 0000 is in the southwest corner, and 0005 is in the west-most column, row 05. We always use numbers with four digits in recording quadrats.

The base stake is at the lower-left, which is southwest for a plot on cardinal directions. The stake number matches the quadrat above and to the right. The lower-left corner of quadrat 0104 is stake 0104. The upper-right corner of the same quadrat is stake 0105 (Fig. 7.4). These numbers are marked on the stakes and on flags above them to allow navigating in the plot. To find any quadrat or a tree within, proceed first to the stake with the same number, then look into the quadrat to the northeast.

Summary of key issues for the topographic census

- Accurate measurement of the plot and placement of stakes is crucial to every subsequent measurement requiring plot area
- Stakes should be firmly placed and well-marked
- Two parallel lines from the base stake start the survey
- Regular side-checks ensure that two lines do not drift apart
- Keep records on side-checks since they can be used to assess accuracy of the topo map

Measuring and mapping trees

This is the core of the work and the reason for establishing the plot. All trees above the diameter limit of 100 mm, with all of their stems, must have their trunk diameter measured at a precise height. Measuring diameter is straightforward in the idyllic tree form, where the trunk is a straight cylinder; most of the details of the methods govern the non-idyllic tree: forked trunks, crooked or swollen trunks, buttresses, strangler figs, etc. Trees are numbered with tags, and their locations mapped relative to the plot stakes. Tagging and mapping are what allows trees to be relocated for future measurements. The species identity of every individual must also be recorded, and produces information on species composition, diversity, and rare or useful species.

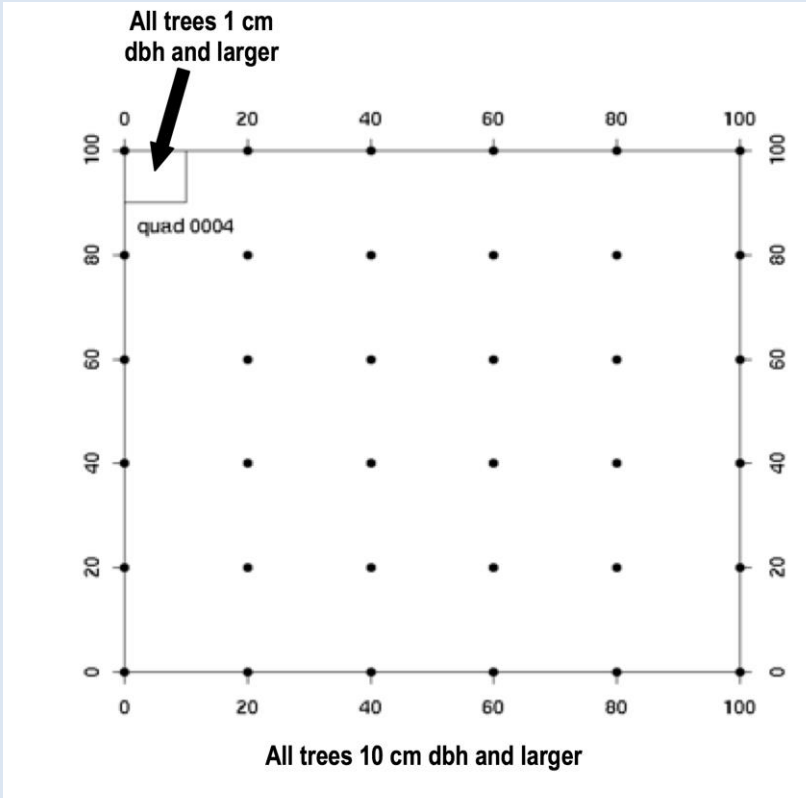


Figure 7.5. Location of the 10 x 10 m section where smaller trees are mapped. In the 20 x 100 m plot adjacent to a stream, the small section where saplings are included should be the upper-left 10 x 10 m subplot of quadrat 0000.

The methods that follow assume that the plot is already surveyed and gridded. Stakes are in the ground every 20 m, marked and numbered for easy navigation through the plot. The surveying does not need to be finished, though: the tree enumeration teams can start as soon as the initial quadrats are placed.

Plants to include

The purpose of a biomass and carbon inventory is to estimate how much wood is present in a forest, and this requires measuring all woody stems. In a tropical forest, this includes lianas, palms, strangler figs, and bamboo, not to mention trees with gigantic spreading buttresses. The need to include all simplifies the rule: if it is a woody stem larger than the minimum diameter limit at 1.3 m from the rooting point, it is included. The only stems that might be excluded are some herbaceous species with pseudo-woody stems (*Marantaceae, Araceae, Cyclanthaceae*). Some species in these families have soft green stems larger than 10 mm, but they are not included in the census.

The minimum diameter is 100 mm in most of the plot, but a subsection will include stems larger than 10 mm diameter (Fig. 7.5). The height of measure, 1.3 m, is traditionally called 'breast height', but for clarity, we use the acronym HOM, meaning the height-of-measure, as this is key in describing the rules for choosing where to measure a tree.

Demarcating subquadrats

Tree enumeration teams are responsible for filling in the 5 m grid markers, best done at the start of work in each 20x20 m quadrat. The 5 m points can be located with rope and tape measures alone. First, with a tape measure, locate the mid-point of all four sides of the quadrat and mark it with a stake. Note that the mid-point may not be 10 m from the ends, if the quadrat is inclined: if the two edge stakes are 21 m apart, the mid-point is at 10.5 m. Next, run two ropes straight across the diagonals of the quadrat, and place a stake directly below the intersection. (As long as the ropes are straight, this correctly locates the middle even if sloped.) This divides the 20x20 quadrat into four of 10x10. The identical procedure can be repeated to locate 5 m stakes.

Placing a stake every 5 m greatly facilitates the enumeration, making it less likely to miss trees and easier to map their locations. It is time-consuming, though, and the alternative of placing stakes only every 10 m is reasonable. In the details that follow, assume stakes are already placed every 5 m, but there are no great differences if stakes are placed every 10 m.

Ropes should be placed around the four edges of the 20x20 to show the boundaries clearly. This helps especially where two different teams are working at the same time in adjacent quadrats.

Work sequence

The 20x20 m quadrat, as marked during the survey, is the unit of work. Each mapping team works a single quadrat until it is finished. If there are two or three enumeration teams, each should work a single column of quadrats, starting with column 00 (Fig. 7.4). Thus, if there are three teams, they start in columns 00, 01, and 02, and the first to finish one column begins in 03. Work begins at the base of each column and follows the quadrat numbering. In a plot laid on a north-south axis, work proceeds northward up each column, and eastward from column to column.

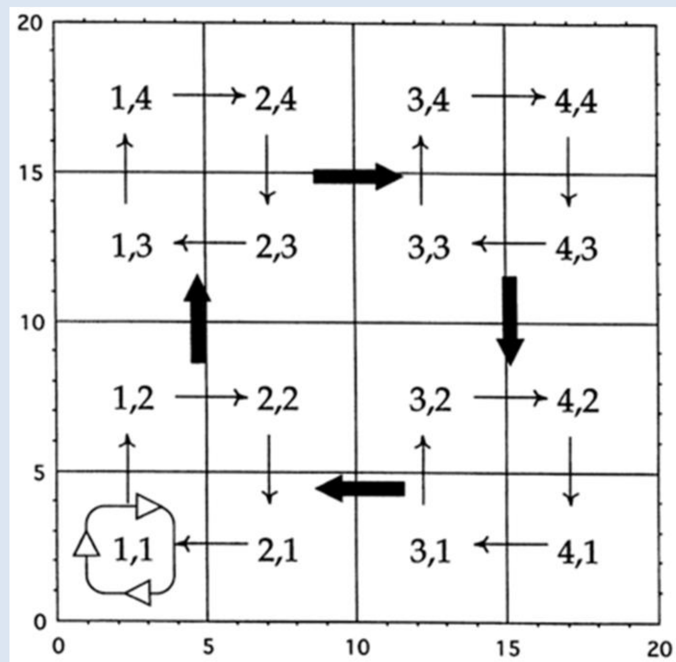


Figure 7.6. Recommended work sequence and subquadrat numbering within a 20 m x20 m quadrat. Each small square is 5x5 m. Black arrows indicate a work sequence between subquadrats; open arrows within a single subquadrat.

Within each quadrat, teams should follow a consistent sequence in working through the subquadrats of 5x5 meters. Subquadrats are numbered as in Fig. 7.6, one entire 5 x 5 m should always be completed before moving to the next. Solid arrows indicate a suggested work sequence from subquadrat to subquadrat, and open arrows a sequence within a subquadrat. Regardless of the order selected, following a consistent path is useful because the tag sequence is then predictable, and this makes later attempts to find a particular tree easier. Moreover, it helps assure that every stem is located.

Data Collection

i. Data sheets. Data from each 20x20 m quadrat go onto two data forms: the main datasheet plus a map. Each includes blank spaces at the top for recording the plot number, quadrat number, names of the field crew, and dates. Sample datasheets are provided at the end of this chapter. When data from one quadrat do not fill a sheet, data from a second quadrat should never be added; one sheet should only have data from a single quadrat. On the other hand, when a quadrat is not finished at the end of one day, data from the same quadrat on the next day should go on the same sheets.

ii. Mapping trees. Once a 5 m grid is marked, mapping the location of each tree is easy, indeed, mapping to a precision of 5 m is already accomplished. Trees are mapped entirely by eye by marking a spot on the field map. A sample map is provided at the end of the chapter.

Trees are mapped where they are rooted, so leaning or prostrate trunks require care: the crown and most of the trunk may not be in the rooting quadrat. If a prostrate tree has roots along a substantial length of the trunk, then the trunk base is mapped. Large trunks should be indicated with large circles, the center determining the tree's location.

Each tree's tag number is recorded next to its spot on the map. If tag numbers have more than four digits, and the initial digits do not change within a quadrat, then only the final four digits should be entered on the map, with full tag number of the first tag of the quadrat entered at the top of the map.

No coordinates are entered in the field. Rather, coordinates are captured later by digitizing the maps.

iii. Tagging trees. Tags are simply attached with loops of string around trunks < 150 mm in diameter. Loops must be large enough to allow for growth: 600 mm for stems < 60 mm diameter, 1 m for trees < 150 mm diameter. Larger trees (≥ 150 mm) are tagged with nails, with at least 50-60 mm of nail left above the bark. Nails should not be placed at the HOM (height of measure), where the diameter is taken.

Tags should be placed in a numerical sequence that matches the work sequence: clockwise within quadrats, northward up columns, etc. If several teams work at once, a sequence of numbers must be assigned to each column. Tag sequences should be prepared before going in the field, strung on a coat hanger or similar wire. A sufficient number of 600 -mm pieces of thread should be precut and carried so that single pieces can be easily pulled out. About 30 1 -m pieces should also be cut for every quadrat (for trees 60-150 mm diameter). Tagging of multiple stems within a single tree is described later.

iv. Trees to include. Within the entire 1 ha plot, all trees with a diameter ≥ 100 mm are mapped, tagged, and measured. This refers to the diameter taken following the rules below.

In a single 10x10 m section of the plot, all trees with diameter ≥ 10 mm should be censused following the same methods. This should be the northeast 10 m section of the 1 ha plot, within quadrat number 0004 (Fig. 7.4, Fig. 7.5); in the 20x100 m plot, it is the upper-left 10x10 section of quadrat 0000 (Fig. 7.4).

Figure 7.7. Measuring trunk diameter with calipers (left) and diameter tape (right).

v. Measuring trees. Here is the largest and most complicated section of all the methods, because good measures of stem diameter are critical for future measurements of stem growth. Recording the diameter is straightforward on regular, cylindrical stems: calipers are used for trees < 60 mm diameter, and a diameter tape for larger trees (Figure 7.7). In either case, the diameter must be measured exactly perpendicular to the trunk. Breast-height is found with a pole exactly 1.3 m in length, which is placed against the tree. Diameter should be recorded to 0.1 mm accuracy.

Unfortunately, stems are seldom perfect cylinders, and quite a variety of difficulties in measuring arise. Following are 10 rules of tree measurement designed to ensure replicability of the measurements.

- **Rule 1.** When using calipers, take the largest diameter. Most stems are in fact not circular in cross section, and calipers will record different diameters, depending on their orientation. With a diameter tape, this problem vanishes, but stems < 60 mm diameter cannot be measured accurately with tapes. Finding the largest diameter is easily accomplished by rotating the calipers while they are clamped lightly on the trunk. (This practices elevates the diameter of small trunks, and the amount of the bias should be evaluated carefully.)
- **Rule 2**. The HOM is always calculated 1.3 m above the ground on the uphill side. An HOM on the downhill side would be lower.
- **Rule 3.** Breast-height is taken along the lower side of a leaning tree, not the upper (Fig. 7.10).
- **Rule 4**. The HOM includes all stem above the ground, no matter the angle. Thus, a leaning stem may be measured only a few centimeters above the ground (Fig. 7.8), and on a sharply curved stem, 1.3 m must be measured around the curves with a tape measure. But in cases where a leaning stem has multiple rooting points so its origin is not clear, it should be treated specially, like a prostrate stem (Rule 8).
- **Rule 5**. Lianas, stranglers, and epiphyte roots should be pulled away from the tree trunk and the diameter tape slid underneath whenever possible. When the epiphytes cannot be moved, large calipers may be necessary. Any tree requiring large calipers should be marked as a big tree, to be measured later by the big-tree team (Rule 7).

The remaining five rules govern situations where something prevents measurement at the standard height of 1.3 m, usually an irregularity in the stem. In these situations, the diameter must be taken at a different height.

For all cases where the HOM is not 1.3 m (rules 6-10), it should be marked with paint so it can be precisely re-located. In all cases, the height from the ground where the measurement was taken should be recorded (see data sheets at the end of the chapter).

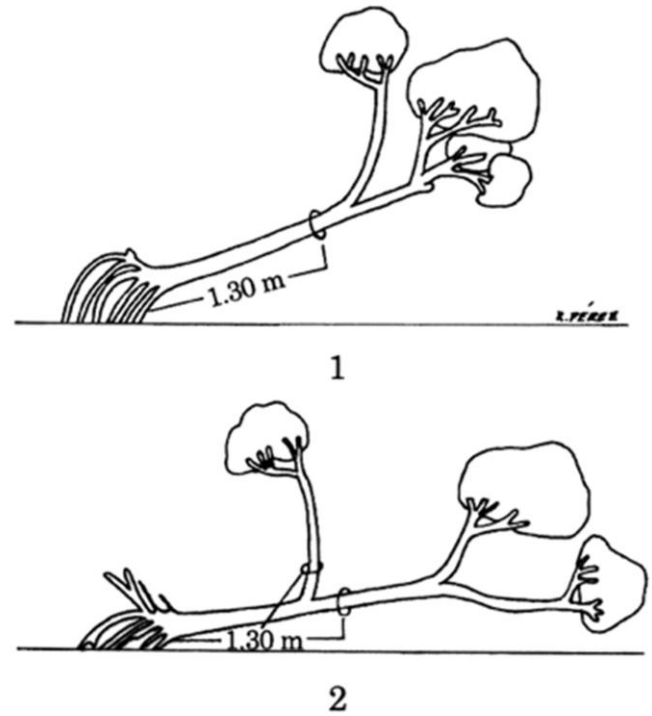


Figure 7.8. Measuring multiple and leaning stems. Each stem is measured 1.3 m from the rooting point, measured along the underside of the stem. Case 1 illustrated how a leaning or prostrate stem is still measured at 1.3 m along the stem.

- **Rule 6**. When an otherwise cylindrical stem has an obvious swelling or constriction at 1.3 m, the diameter is taken 20 mm below the lowest point of the irregularity. A thumb's width is used as an estimate of 20 mm. The trunk should be painted where it is measured.
- **Rule 7**. For trees with buttresses or stilts (Figures 7.9 and 7.10), the diameter must be measured at least 80 cm above the top end of the highest buttress (or stilt). If 1.3 m is sufficiently high, then the measurement is made as usual. But often it will be necessary to measure further up the trunk, and in some very large trees, a ladder will be needed. The HOM should always be painted. In most cases, I recommend these trees be marked as 'problems'; then all can be measured on the final day by a team of 3-4 people carrying the ladder throughout the plot (but in a one-hectare plot, there should seldom be more than one such big tree).

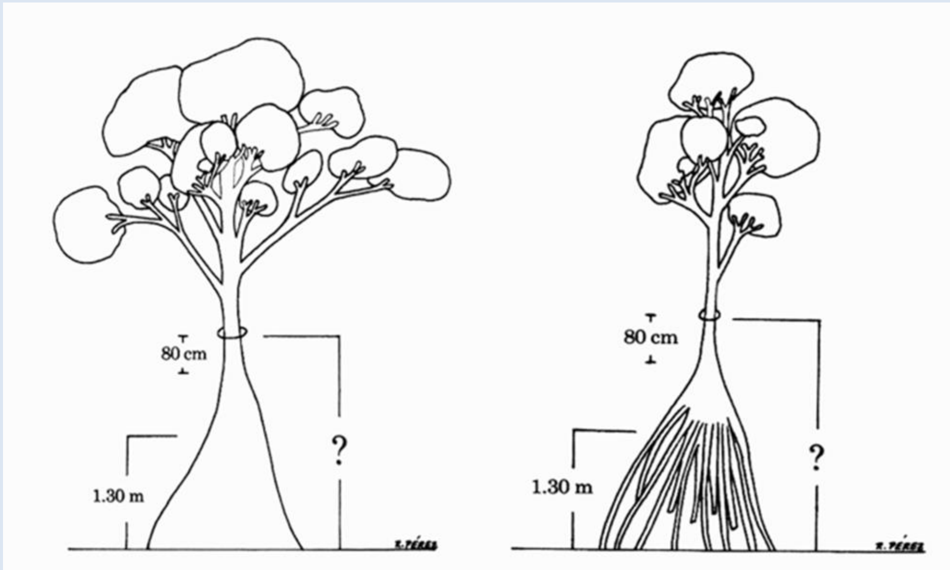


Figure 7.9. Trees with buttresses or stilts are always measured at least 80 cm above the point where the stem becomes approximately cylindrical.

Figure 7.10. Field crew measuring tree diameter above roots.

There are giant trees whose buttresses are so high that even a 5 or 7 m ladder is insufficient to reach above them. I have employed tree climbers with ropes and harnesses for a few trees in the Barro Colorado plot in Panama (all kapok trees, *Ceiba pentandra*). A crude alternative is to have one person climb to the top of a ladder and hold a 2 m measuring pole marked clearly in 0.1 m gradations. A second person can stand several meters from the tree and read an approximate trunk diameter (above the buttresses) off the pole; binoculars may make this easier. Although approximate, it is better than no measurement at all, and since these trees are so rare, it will not have much impact on final biomass estimates.

- **Rule 8.** Prostrate stems should always be paint-marked, since it is often not clear where 1.3 m along the trunk is. In general, they can be treated like leaning stems, with the diameter taken 1.3 m along the stem from its origin (rule 4), but multiple-rooting points and buried trunks can cause confusion. If so, a point above the ground should be selected and painted.
- **Rule 9**. When a trunk is extremely irregular at all heights, an HOM must be chosen as best as possible, then painted and recorded so the tree can be re-measured at the same spot in the future. For example, there are species with highly fluted trunks, and nothing can be done except to wrap the tape around the flutings. Also, there are species whose trunks taper sharply at all heights, and there is nothing to do but measure at the standard 1.3 m. A code indicating that the trunk was irregular at the HOM should be recorded so that future workers know.
- **Rule 10.** This is for trees where no rule works: there is no rule 10. There will inevitably be some trunks that simply defy measure, for instance when completely buried in a strangler fig. The best possible measurement must be taken -- at least some estimate is better than none -- and the location of the HOM painted and recorded. As long as these cases are rare, the best estimate of the team leader is acceptable. However, if there is a consistent problem unforeseen by rules 1-9, then a new technique must be developed and applied regularly.

vi. Multiple-stemmed individuals: we have left aside, so far, cases where a single plant branches or forks below 1.3 m, or has more than one stem connected underground. This situation requires special rules for mapping, tagging, and measuring (Fig.7.11), and it is particularly common among saplings, measured in the 10x10 m subsection of a plot.

Separate stems that are obviously connected to one another below 1.3 m, either above or below the ground, are considered part of the same individual. A single tag should be tied or nailed to the largest stem of the group. In addition, all stems, as defined below, should be given a separate type of tag following the same protocol for tagging as used on main stems (section 3 above). For example, in trees with two stems, the main trunk is given a primary tree tag (section 3), then each stem gets a stem tag, numbered 1 and 2; and so on for more stems. Stem tag 1 should always be the largest diameter stem (if several stems have the same diameter, then the tallest should be tag 1). Stem tags are typically not numbered in advance, but at the moment when they are applied, using thin aluminum tags with nails to etch numbers.

In cases where two or more stems arise from the ground at different locations, but are clearly part of the same tree, each should be indicated separately on the map. Each stem ≥ the minimum diameter cutoff is measured following the rules described above, and every one of the diameters is recorded on the main datasheet (Annex 7.2). If one stem is irregular or buttressed, its HOM may not be at breast height, but all other stems would still be measured at 1.3 m.

Any branch below 1.3 m is considered a second stem, even if it is horizontal, as long as it is above the minimum diameter cutoff at 1.3 m from the rooting point. The HOM is found by measuring from the ground, following any forks (Fig. 7.11). A branch above 1.3 m is not considered a stem. If there are extra stems but they are below the minimum diameter, the tree is not considered multiple.

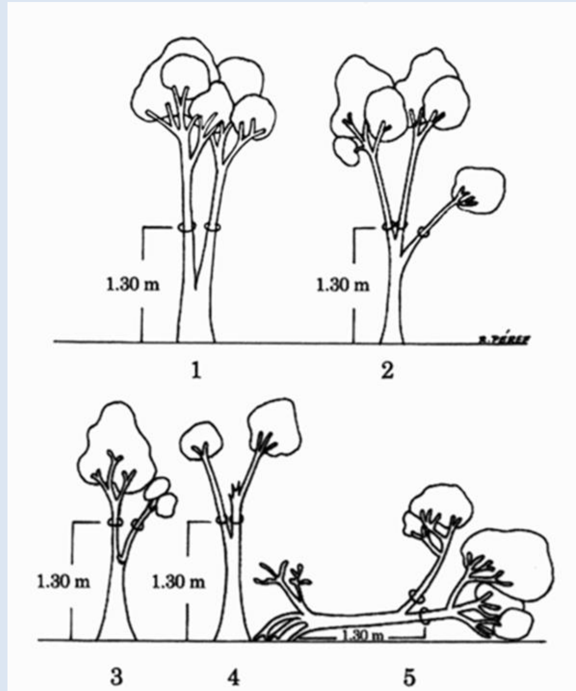


Figure 7.11. Measuring multiple stems. Each stems is measured 1.3 m from the rooting point, measured along the stem. Case 5 shows how a stem out of a horizontal trunk is measured.

Prostrate stems can have many vertical sprouts (Fig. 7.8, case 2). If the sprout is > 1.3 m from the rooting point, then it should not be considered a stem, following the rule that the HOM is measured along the stem. There are rare cases where prostrate stems grow more roots, and so have multiple rooting points. In this circumstance, all sprouts < 1.3 m of any rooting point should be tagged, and the main (prostrate) stem should be measured 1.3 m from what appears to be its original rooting point.

In some cases, it is not clear whether adjacent stems are connected underground. For species that do not normally grow as clones, a connection must be reasonably obvious to count two stems as one individual.

Multiple stems can create odd circumstances which create confusion. One case that is fairly common is where a trunk forks just below 1.3 m, and the fork causes a swelling. Rule 6 calls for this tree to be measured below the swelling, but this would require measuring below the stem fork, where there is only one trunk. Instead, the standard should be to measure the two stems above the fork, where they are regular, so that the tree is recorded with multiple stems. Another confusing case is where a buttressed tree requires a high HOM, for instance 3 m above the ground, but also has a trunk fork below 3 m. Because the fork is above 1.3 m, it should not be measured as a multiple stem, even though the main stem is measured higher. In all such cases, as for any unusual HOM, stems should be painted where measured.

vii. Palms. Most palms can be measured following the standard rules. A couple confusing features arise, though, special to palms. Some can be shorter than 1.3 m tall but have stems ≥ 100 mm diameter; this circumstance is rare in non-palms. Regardless, these are not included in the census. The following concerns only arise when a palm is approximately 1.3 m tall and has a large enough diameter stem to include. The top of the stem must be located exactly: it is defined as the base of the lowest living leaf sheath. A difficulty arises when dead leaf sheathes persist, hiding the base of the lowest living sheath, and then the location of the top of the stem can only be estimated. When measuring the diameter, dead sheathes are not removed, but are pressed as tightly as possible against the stem. Leaf petioles do not count as part of a stem even when they resemble a stem. Thus palms are often excluded even though their leaves are much taller than 1.3 m, and likewise for tree ferns.

viii. Stranglers. Stranglers are measured whenever they have a trunk above the minimum diameter at 1.3 m, whether it adheres to a living host or the host tree is dead and gone. Trunks are convoluted, and sometimes several trunks near the ground merge further up, so the HOM of stranglers is problematic (and thus should always be painted). In some cases, a single trunk is measured well above the merging point, as if they were stilts; but if there is no clear, single trunk at any height, separate trunks are measured at 1.3 m as if they were multiple stems.

ix. Lianas. Censuses of trees ≥ 100 mm generally include few lianas. When encountered, the sampling method matches that for trees: each stem should be tagged and measured 1.3 m from where it roots in the ground. What makes lianas unusual is that stems can cover great horizontal distances and also branch frequently, but these two characteristics do not require different methods. Rooting points must be located, and diameters measured following rules 1-6 above.

Most unusual with lianas is the tendency for stems to re-root far from their initial rooting point. In many cases, a single stem may rise high in the canopy then descend and root elsewhere, so it will not be evident that two different rooting spots belong to the same individual. The rule must be to measure 1.3 m from all rooting locations and to record these as two separate individuals, mapped at separate locations. A comment should be added that two roots belong to the same individual where this is detected.

x. Other data. Other than the diameter and information about the HOM, a few other pieces of information are important to record. Crowns or trunks broken or damaged above 1.3 m should be noted. Stems leaning by more than 45^o or prostrate should be indicated. Also, stems which show signs of an old break below breast height should be noted; this information will be useful during a later census. Each of these pieces of information with a single-letter code, to simplify recording and later data entry (Table 7.1). The sample data sheet at the end of the chapter includes a column for codes. Additional codes can be devised for additional topics that are relevant at a particular site. If one tree has multiple codes, they should be separated by semi-colons on the data form. There is no reason codes have to be single characters; this is just for brevity and convenience. Every enumeration team should have a copy of the codes table with them during the work.

Table 7.1. Codes for describing several important and routine circumstances regarding a stem or its measurement

Code	Description
L	Stem leaning by more than 45 degrees
F	Stem prostrate
Q	Stem broken above 1.3 m
I	Stem irregular where measured
B	Large buttresses
R	Signs that there was an old break below 1.3 m

The main data form also has a comments field where additional notes can be made.

xi. Problems. Any plant that requires future attention should be marked as a problem with a letter 'P' in the final column of the data form (see sample data forms in Annex 7.2). In addition, a brief comment can be recorded in the comments field to explain the problem. Problems include those situations where the rules seem not to apply and which prompt the supervisors to consult with each other or the chief scientists before deciding what to do. They also refer to simple things, such as lost tags, which cannot be corrected until the next day and cases where a big tree requires a ladder or perhaps other equipment to measure.

When the problem is resolved, the entry should be checked off to indicate clearly that no further attention is required. By marking all problems in one column on the data form, supervisors can quickly screen for them. Once a problem is resolved, the problem code can be erased; the comments should be left on the form, but marked to indicate the question was resolved.

Checking the Work

An advantage of working in teams, with field supervisors recording data, is that all work can be checked immediately. While recording data, each supervisor should also be monitoring the measurements and maps. Occasionally, especially in difficult cases, the supervisor should remeasure the diameter to check accuracy. This is especially important early in the census, when the field team is inexperienced.

In addition, each of the team members should be encouraged to check each other's work. Both the person drawing the map and the one tying the tags can look for trees and check whether any have been missed. Anyone in the group might also notice that a diameter called out seems way off, prompting a remeasure.

Finally, after returning to the field office at the end of each day, supervisors should double-check the datasheets for the current day. Are all sheets present? Does every record have a complete set of legible data? Are there duplicate tag numbers? Problems should be reviewed and resolved immediately, when possible.

If a quadrat is not finished, the datasheets should be set aside to be used the next day. When completed, the full set of data forms for the quadrat should be placed in a manila folder, with one manila folder per quadrat. The folders should be kept in a file cabinet at the field camp until the taxonomy teams need them.

3. Species Identification and Handling

Identifying tree species in Panama is the most challenging aspect of a tree census. Because the vast majority of individual trees in a forest do not have reproductive structures at any given moment, and most are juveniles, which will never reproduce, plants must be identified on the basis of sterile characteristics. Individuals must be recognized from leaves, stipules, branch form, odor, sap color, bark, etc. This requires experienced botanists, and every tree inventory requires one person with botanical training.

This section covers logistics of collecting and sorting specimens in an area where the flora is poorly known, as is the case in much of Panama. It is not a taxonomic key, as that would be well outside the current scope. As described in Chapter 2, a database of tree species known in Panama is now available online and should form the basis of any tree inventory. There are > 2300 species included, so it cannot be taken in the field nor memorized beforehand, but it should be consulted as plots are censused and identified. The Missouri Botanical Garden Tropico web site (see Chapter 2) should also be consulted to confirm species names in Panama. The site includes many specimen records in Panama, often with photos, and all tree names used should be checked against Tropicos, with spelling corrected as needed.

i. Botanical personnel. The tree census team should include two members whose sole focus is identification. They should work parallel to the members who are mapping and measuring, but separately if necessary (sometimes lagging behind due to difficult-to-identify specimens). One of the two must have botanical training, with experience in Panama. The second person should be prepared to help collect leaves, and skill with a sling-shot or pruning pole is important.

Species mnemonics

Species identified in the field should be recorded with an abbreviated mnemonic. These abbreviations have already been devised and appear in our online tree species list for Panama. They include six letters, comprised of the first four letters of the genus and the first two of the species. When two genera have the same initial letters, then the fourth position is a number, with the genus that is alphabetically first getting the lower number. For example, *Trichanthera* and *Trichilia* in Panama have genus codes TRI1 and TRI2. Likewise, two species names in the same genus with the same two letters at the outset must get one letter and a number.

Collection of specimens

The ideal botanical sample or specimen consists of a small branch with leaves, flowers and fruit; reproductive structures are necessary for proper identification. Not all plants will be encountered with flowers or fruit, however. For this reason, we suggest collecting additional specimens of plants that do have fruits and flowers outside of the boundaries of the plot. These specimens will make it possible to identify the trees more easily and enable comparisons with the sterile specimens (those without flowers or fruit). We recommend collecting at least 3 to 5 specimens per plant. The specimens should be stored in a plastic bag labeled with a field code. When dealing with shrubs or herbaceous species, snip the specimens with garden scissors close to the ground; in the case of trees, lianas or vines, use a tube trimmer with a guillotine accessory. Collection methods will vary depending on the botanical group (e.g., for *Poaceae* (*Gramineae*)) it is necessary to pull out the whole plant, including the root; for lianas, a transversal cut of the stem should prove sufficient). Some flowers and fruits might need to be placed in plastic vials with alcohol.

Treating the specimens

Once back from the collection site, the specimens should be placed inside folded newspaper sheets. This procedure is generally done in the field. Each botanical specimen will be placed inside folded newspaper sheets in such a fashion that the main features of the plant are easily observable. At least one of the leaves should be placed upside down to better demonstrate the veins. Thick fruit should be cut into longitudinal or transversal slices, but if this is not possible, it can be stored in paper bags labeled with the field code. Large leaves, such as *Arecaceae* and *Cyatheaceae* or compound leaves, such as *Fabaceae, Meliaceae, Anacardiaceae, Rutaceae or Sapindaceae*, must be cut into small enough segments to fit inside the newspaper sheets. The specimens must also be numbered with a pencil or a permanent ink pen and logged into a field book. For fieldtrips lasting longer than 10-15 days, specimens can be grouped into bales of 30 or 40 specimens individually placed into folded newspaper as above, and then tied up with string. Each bale should be placed vertically inside plastic bags and doused with one liter of a solution made with 50% alcohol and 50% water. The plastic bag should then be sealed and remain closed until it is time to dry and press the specimens. The solution will keep the specimens in good condition for up to a month.

Specimen drying and pressing

Leaf pressing requires a 50 cm long x 30-35 cm wide press, butcher paper sheets and aluminum sheets or corrugated cardboard. The process requires placing a sheet of aluminum or corrugated cardboard on one side of the press, then the botanical specimen inside of the folded newspaper sheet, a sheet of butcher paper, and another sheet of aluminum or cardboard. Each specimen must be prepared in the same fashion, folded in newspaper and separated from the rest of the specimens with cardboard. This procedure should be repeated for all specimens and duplicates. Aluminum and cardboard let air flow through the specimens, and the butcher paper absorbs humidity. This method will dry the leaves rapidly. Once all the specimens are ready, the press should be closed and tightly bound. Leaves should be dried as quickly as possible for two reasons: to free up the press for other specimens that require drying and for the best preservation of leaf color. The pressed specimens can also be dried inside an oven or dryer set at 46°C. The specimens should be ready in between 1- 8 days, depending on the type of dryer.

Mounting and filing of the specimens

The plant specimens should be mounted on exhibit board (approx. 29 cm x 41 cm) (Figure 7.12). A label with the information recorded in the field book will be affixed to the lower right portion of the board. The label should also include the name(s) of the collector(s) and their institution(s), as well as the scientific name of the specimen and the name of the botanist or specialist who identified it. The seal of the herbarium must be stamped on the lower right corner of the label; some herbariums add the number of the specimen (See Chapter 2 section on legislation in Panamá). The botanical specimen should then be pasted on the board, making sure it does not cover the herbarium's seal. Sometimes a small envelope is glued on the lower left side of the board for holding flowers, fruit, and seeds that have come detached from the mounted specimen.

Figure 7.12. Mounted specimen to be stored in the herbarium.

Identification of specimens

It is sometimes difficult to identify the specimens in the field, unless a group of expert botanists familiar with local flora accompany the expedition. Very often, plants that appear to be of the same species actually belong to different taxa upon closer inspection in the herbarium. For this reason, it is recommended to collect specimens from *all* of the plants that occur inside the plot. In order to identify a botanical specimen, it is necessary to relate it with the most common taxa and specific family, genus and epithet. Experts generally carry out identification, although botanists with experience in the flora of a region or country can also render accurate results. Taxonomic keys such as "The Flora of Panama", "Flora of the Neotropics", "Flora of Nicaragua", "Flora of Mesoamerica" (REFS), books and specialized magazine articles will aid in identification. Another useful resource is comparing specimens with those already filed in herbariums. The process of identification might take days, weeks or even years. Duplicates of the identified specimens must be forwarded to the Herbarium of the University of Panama, the Herbarium of the Smithsonian Institution of Tropical Research and to other herbariums of the world that have expertise on the taxonomic groups collected. It is recommended to keep one specimen for project files. Comparing the specimens with pictures or scanned images from reliable sources can also aid in identification. A good resource is http://ctfs.arnarb.harvard.edu/PanamaAtlas/.

The herbarium

A herbarium is a "plant library" of sorts, housing a collection of dried plants that have been prepared, identified, and preserved for future use. Herbarium staff collects, prepares, identifies, and preserves the specimens. The largest herbarium in Panama is the University of Panama's (internationally known as PMA), which maintains a reference collection of the flora of Panama and has more than 55,400 specimens of angiosperms and ferns and almost 10,000 bryophytes and lichens (http://www.up.ac.pa/herbariopanama/index.html). The National Environmental Authority of Panama (ANAM) requires that every research project collecting plants provide specimens to the National Herbarium (see Chapter 2, Laws and Permits).

Photographs. Once specimens are carefully organized, sorted into morphospecies and stored in cabinets, photographs should be taken of every individual species. For those species identified in the field and never collected because they are very well known, photos in the field should be taken. This provides a set of photographs covering every species found in the plots.

Consulting botanist. Once specimens are organized and photographed, an excellent option is to consult a botanical expert. There are several at the Smithsonian Tropical Research Institute who can offer assistance in Panama. Given well organized specimens, the expert can clear up outstanding questions and suggest which species might need additional collecting in only a few hours work in the laboratory.

4. Personnel and Training for the Tree Census

Accuracy hinges on the personnel, including those working in the field, entering data, and directing the work. Since some or most of the personnel may be new to the work, adequate training prior to the project is of course crucial.

Project director

An experienced scientist must oversee the entire project. As for the field team leaders, this person should be someone with a professional stake in local forest biology and conservation, and should have intimate knowledge of local forests and customs. Ideally, this would be a professor at a local university or a staff scientist in a local forestry department. If none of these options are possible, the funding behind the project may have to bring someone in from elsewhere, or provide the leader from their own staff. Depending on circumstances, the project may or may not have to provide salary support for this leader.

Moreover, the project requires an expert on local forests: where there are trails, who owns the land, and where and how to access. Most often, the project leader and the team leaders provide this knowledge. If not, the project leader needs to understand where to get this information, consulting local land owners or other forest biologists where needed.

The field crew

The ideal field team to work in the tree plots includes five people: a well-qualified field leader to organize the team and lead the work, two to work with the field leader in mapping and measuring trees, a taxonomic leader, and an assistant for taxonomic work.

The key element of the field team is the leader. This person is in the field every day checking on each of the teams: answering questions, solving problems. The leader should understand the project and thus be qualified to make decisions about unexpected circumstances, but of course reports to the overall project leader. A good leader needs to be demanding of the field workers while also being comfortable with them and understanding their needs.

The single most important trait that the leaders should have is dedication to the project: a deep personal desire to see it succeed. This is crucial because the field crew are always paid workers whose only bottom-line is employment; team leaders must feel ownership of the results. This may be best accomplished by involving students or other young scientists who have a stake in the project, perhaps as PhD theses or authorship on reports.

Camp assistants

Plot measurement work will require the field teams to be in one forest plot for 5-6 days. In remote locations, far from roads, this may be best accomplished by making camps in the forest. If so, assistants who carry gear, make camp, and prepare meals are most likely going to be involved. This needs to be decided by the project and team leaders.

Data entry

In addition, at least one but preferably two data entry clerks will be needed, however, these may be the same as the field technicians. Under such a plan, the field team works for three weeks setting up plots in the forest and then one week in the lab entering data. In addition, there should be a supervisor for the data entry clerks. An ideal option is to have a database manager who is a programming and data modeling expert. This person is responsible for working the data entry software, handling data during error-screening, and producing the final database. For a single project of about 25 forest plots, however, this is not a full-time job, so the data manager could be shared with other projects. Alternatively, the project leader could take this role, depending on background and interest.

Training

The project begins with the director and field leaders reviewing the methods to be sure they understand all steps in the field data collection. Training sessions should then be set up with the full field crews in easily accessible forest and non-forest areas, perhaps in an experimental forest at a university.

Field leaders and the project director should initially measure and mark about 20 test trees. These measurements should be deliberately chosen from easy to difficult. Supervisors then present methods to the field crews, and field workers measure the 20 test trees and compare with the supervisors' results. Discrepancies should be reviewed and discussed. Then the field workers should measure all trees in a test quadrat of 20x20 m, with supervisors nearby to check results and answer questions. These tests and reviews will help supervisors assign roles to all the field workers.

The clerks who enter data need to be shown the software for data entry and should spend a day entering test data with the plot director and data manager present to answer questions.

Summary on personnel and training

- The key to the project is the director and field leaders. They must understand the project, know the region and the workers, and -- most important -- feel a stake in the success of the work.
- Experts in local forests must be consulted.
- Field workers preferably are experienced in local forests and have some science background.
- Thorough training sessions should be arranged where measurement methods are practiced.
- Assuming two field teams working simultaneously, the project thus includes 1 director, 2 field leaders, 20 field workers, 2 data clerks, 1 database manager (half-time), 4 camp assistants (if needed), plus 1-2 consultants on local forests (if needed).

5. Estimated Budget and Labor for Tree Census

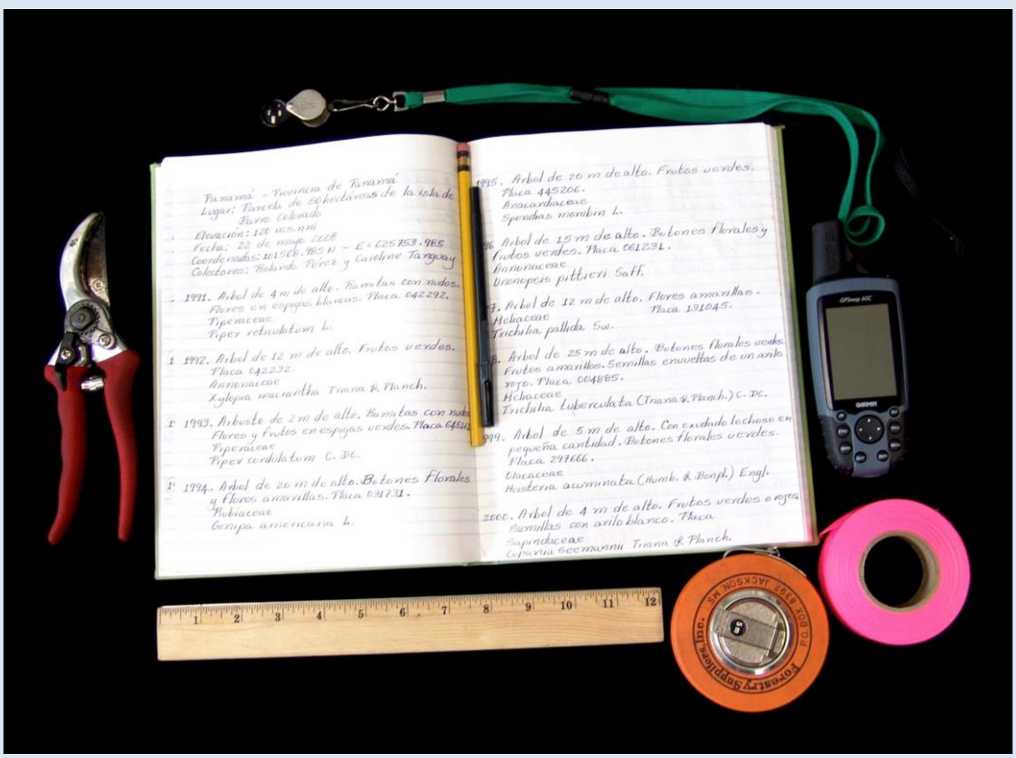


Figure 7.13. Required supplies for fieldwork

Equipment and supplies

Each enumeration team needs three diameter tapes (for two measurers plus the supervisor), two 25 m tape measures, two hand-held compasses, a hammer, pencils, two clipboards (for the two people recording data), and a small paint brush and paint (Figure 7.13 shows some of the required supplies). We recommend 1.6 m cloth diameter tape from Forestry Supplier (Forestry Suppliers Metric Fabric Diameter Tape, model 59571); one longer tape may also be needed for very big trees (model 59576). One of the enumeration teams also needs three tenth-millimeter accuracy metal dial calipers; these are only needed in a small portion of the plot, where small trees are measured (Forestry Supplier model 59911, for example; be sure it is metric). Other items are routinely available. Waterproof paper is useful for work in the rain forest, but not essential. If waterproof paper is not used, plastic sheets are necessary to protect the paper from rain. Each measurer also needs a straight pole exactly 1.3 m long, with flat ends, easily made from small trees near (not in!) the plot.

A large set of calipers may be needed for measuring the diameter of large trees in cases where a tape cannot be wrapped around the trunk.

The enumeration teams must also place the grid markers at 5 m intervals, and thus need 21 stakes for each 20 m x20 m quadrat along with ropes and tape measures for positioning them (as described in the previous chapter). One 40 m piece of light rope is necessary for demarcating each subquadrat.

Aluminum tags are used for marking trees. National Band and Tag Co. (Newport, Kentucky, USA) has 44x16x1.3 mm tags with rounded corners and numbers already engraved (see http://www.nationalband.com/ for many different options) (Figure 7.14). A less expensive alternative is to purchase sheets of thin aluminum tags that are soft enough to have numbers etched on with a nail. This latter approach should be used for replacement tags (when one tag number is lost) and tags for multiple stems.

Figure 7.14. An example of an aluminum tree tag located on a tree in the plot

For trees < 150 mm dbh, tags should be tied onto trunks with tough nylon or polyethylene thread. Aluminum nails, 100 mm long, can be used for attaching tags to larger trees. Waterproof paint that will last in wet forests is needed for marking trunks. Most oil-based exterior house paints or highway paints probably suffice. Each field team needs a paint can and brush.

An aluminum ladder extensible to 5 m is necessary for measuring large, buttressed trees. Only one ladder is needed, and it is only brought to the forest on one day. In some parts of the world, good tree climbers can be found, possibly obviating the need for a ladder.

Time and labor for placing the grid

Teams of 3-4 people can place 7-10 stakes per day. This means a one-hectare plot can be surveyed in four days by a team of four people. This does not include the time needed to fill in the 5 m grid; this is usually done during tree enumeration.

Time and labor for mapping trees

A general average for progress in tree enumeration is 50-80 trees per day per person in a field team, but this does not include the time needed to place 5 m stakes. The 1 ha plot described here will have 400-500 trees ≥ 100 mm diameter plus 40-50 ≥ 10 mm in typical forests of Panama; the smaller plot of 20x100 m will have 150 trees. A team of a supervisor plus two field assistants should be able to finish measuring two plots in three days. Assuming placing the 5 m stakes adds a day of work, and another day is needed for checking problems, measuring big trees, and remeasurements, five days is a reasonable estimate for finishing the tree enumeration. Taxonomists working in parallel will likewise be able to finish in five days.

This does not, however, include additional time that might be needed to sort and identify the plant specimens collected. It is difficult to anticipate this aspect, because it depends strongly on the location and how well the taxonomic team knows the species. In general, it is wise to assume that the taxonomists will need one extra week of work for every three weeks in the field.

Data entry

Entering the approximately 500 records into a computer system will take two days. Because data entry proceeds faster than the field work, a good plan is to have the supervisor and two technicians enter data after every three weeks of field work.

A budget for 25 forest sites

Calculations here assume that each site includes two forest plots, one of 100 x 100 m and a second of 20 x 100 m, and three weeks of work in the field followed by one week in the laboratory. Then it should be possible to finish three sites per month. Assume supervisors cost $1500/month and field technicians $1000/month (in 2013 $US). The price of computer equipment is not included, nor any costs for chief or consulting scientists.

Personnel	Quantity	Months per 25 plots	Total cost
Field supervisor	1	9	$13,500
Taxonomic leader	1	9	$13,500
Census assistant	2	9	$18,000
Taxonomic assistant	1	9	$9,000

Equipment	Quantity	Cost per item	Total cost
Tags	10000	$0.02	$100
Diameter tape	3	$100	$300
Calipers	3	$125	$375
Miscellaneous (office, nails, hammer)			$200
TOTAL			$54,950

6. Data Output

Data entry and management

The recommended methods presented here involve collection of data with pencil on sheets of paper in the field, and later transcribed by clerical personnel whose primary skill is reading and typing. Palm-pilots and even full-sized field laptops are now in use to replace pencil-and-paper, it seems inevitable that one day we will all switch to electronic recording. For now, though, penciled data remain the standard, and transcribing data off paper forms into computer systems is a step in the work that cannot be overlooked.

Specialized data-entry software

Assuming data must be transcribed from field data forms, great strides in efficiency and accuracy are possible by using specialized data entry systems, catered to the exact format of the data sheets. One necessary important feature is a data entry screen on the computer that precisely matches the data forms being transcribed: the same columns in the same order, and the same headers at the top. The other key feature is built-in data screening, preventing the entry of invalid records. Further refinements include key strokes that allow easy navigation between records, or even automatic filling in of some records. For example, the tree tag number might be automatically incremented by one when a new line is entered.

A tailored data-entry system can be programmed in HTML/PhP or Microsoft Access fairly easily, if some programming knowledge is available, and even a basic system is a substantial improvement over the use of spreadsheets such as Excel for data-entry. Moreover, the Smithsonian Tropical Research Institute has developed such software specifically for tree plots and will make it available to Panama's scientists. As part of a future edition of this document, we will include software matching the data forms provided.

Double data entry

If at all possible, transcribing all field sheets twice, by two different people, is a big advance in data quality. The two resulting files are then compared entry by entry, and discrepancies resolved by checking the entries against the original field sheets. This almost completely eliminates typographic errors, and it also helps overcome problems caused by poor handwriting (since hard-to-read entries may be typed differently). The Smithsonian Tropical Research Institute data management tool provides an automatic double-checking feature.

The final database

A good database format helps avoid many simple errors and clarifies the key results. The principle behind the outlined methods is to store data in separate tables, with each table covering one well-defined object, whether that object is trees, stems, measurements, plots, or people. This prevents unnecessary repetition of information. Below is a list of tables that we routinely use for storing plot data.

Plots	Each plot appears once, along with any plot information, which never changes, in particular, the GPS coordinates of the plot and the plot size
Personnel	The name of each person on the project appears on one row
Species	The name of every species encountered appears once (i.e. on one row), along with all species-level information, including taxonomic family, authority, and the level of identification
Trees	Every individual tree in all the censuses appears once in this table. It should include plot number, tag number, and information about the specific location (generally, a quadrat number plus precise x-y coordinates within the quadrat as estimated by digitizing the maps). This table does not include measurements, but it does includes fields to indicate status (tree, liana, dead).
Stems	This table is necessary for handling multiple stems on a single tree. It includes a single row for every stem, with tag number and stem number for identification.
Measurements	This is the core table for plot censuses. It includes a single record for every stem measurement, the tree tag and stem tag for identification, the date of measurement, plus the codes associated with the measurement. The reason measurements are not stored in the stem table is that a single stem can have more than one measurement.

All of these tables are stored together in a single database. They must be joined to produce useful reports. For example, to generate the diameter measurements from one plot, the species, tree, stem, and measurement tables must be joined, with only those records for the selected plot included. To include the location of that plot, the plot table would also have to be joined. Additional tables on geographic and remote-sensed information could also be included in the same database.

Examples of databases

Tables 7.2 – 7.4 provide some examples of tree plot databases.

Table 7.2. Example of database of the tree plot illustrating how the species data should be stored. The mnemonic corresponds to an abbreviated code used in the measuring table (Table 7.1). LevelID= is the degree of identification of each species; LevelID= “species” corresponds to complete identification while LevelID='genus' means that the genus is known, but not species name. Additional information about the species can be stored in this table, such as common name, lifeform, etc.

Mnemonic	LatinName	Family	LevelID
acac1	Acacia sp.1	Fabaceae	genus
acacme	Acacia melanoceras	Fabaceae	species
acacri	Acacia riparia	Fabaceae	species
acaldi	Acalypha diversifolia	Euphorbiaceae	species
acalma	Acalypha macrostachya	Euphorbiaceae	species
ade1tr	Adelia triloba	Euphorbiaceae	species
aegian	Aegiphila anomala	Lamiaceae	species
aegipa	Aegiphila panamensis	Lamiaceae	species
albiad	Albizia adinocephala	Fabaceae	species
albipr	Albizia procera	Fabaceae	species
alch3	Alchornea sp.3	Euphorbiaceae	genus
alch4	Alchornea sp.4	Euphorbiaceae	genus
alchco	Alchornea costaricensis	Euphorbiaceae	species
alchla	Alchornea latifolia	Euphorbiaceae	species

Table 7.3. Example of database of tree plots illustrating how the individual plot data should be stored. The most important part is the name of the plot and latitude/longitude. Additional data from each site can also be stored in this table, such as elevation, province, etc.

Plot	Shape of the site	Area	Latitude	Longitude
BCI	100x100	10000	-79,85530	9,15125
Plot 31	100x100	10000	-79,74810	9,35822
Achotines	100x100	10000	-80,16750	7,41381
Campo Chagres	100x100	10000	-79,59980	9,21146
Cerro Azul plot1	100x100	10000	-79,36220	9,20313
Cerro Azul plot2	100x5	500	-79,37130	9,20958
Cerro Minon	100x100	10000	-79,60970	8,93995
Cerro Patacon	100x100	10000	-79,59600	9,01382
Cerro Pelado	100x100	10000	-79,69730	9,12099
Chagrecito p1	100x100	10000	-79,32150	9,35590
Chagrecito p2	100x5	500	-79,32090	9,36099
Chagres - Chilibre	100x100	10000	-79,59790	9,21255

Table 7.4. Example of database of a plot illustrating how the diameter measurements should be stored. Each row corresponds to one diameter measurement. Label =number of label on the tree (label 532558 is a tree with multiple stems). Stem label =number of the stem, when the tree has multiple stems. Mnemonic=abbreviated code of the name of the species . DBH=diameter of the stem, with DAP=height above the ground where the diameter was measured. Codes=descriptive codes (B = big tree, measured above the buttresses; M=multiple stems).

Plot	Label	Stem label	Mnemonic	DBH	DAP	Date	Codes
P08	500852	NULL	socrex	106	2.3	30/08/96	NULL
P08	500853	NULL	socrex	109	2	30/08/96	NULL
P08	500854	NULL	socrex	124	1.9	30/08/96	NULL
P08	500855	NULL	tratas	382	1.3	30/08/96	NULL
P08	500856	NULL	welfre	201	1.3	30/08/96	NULL
P08	500857	NULL	tapigu	306	1.3	30/08/96	NULL
P08	500858	NULL	oxanlo	136	1.3	30/08/96	NULL
P08	500859	NULL	oenoma	108	1.3	30/08/96	NULL
P22	532555	NULL	swars1	109	1.3	29/01/97	NULL
P22	532556	NULL	faraoc	120	1.3	29/01/97	NULL
P22	532557	NULL	sch1zo	342	1.3	29/01/97	NULL
P22	532558	1	tet4jo	652	3.5	29/01/97	B;M
P22	532558	1	tet4jo	806	1.3	02/01/97	M
P22	532558	2	tet4jo	29	1.3	01/02/97	NULL
P22	532558	3	tet4jo	15	1.3	01/02/97	NULL
P22	532559	NULL	poutca	159	1.3	29/01/97	NULL

Annex 7.1 Ground Distances that Give 20 m or 10 m Horizontal Distances

Ground distances *d* that give 20 meter or 10 meter horizontal distances (see Fig. 7.2). The inclination □ is in degrees. For example, if the ground is inclined upward at 4.0°, the distance measured parallel to the ground should be 20.05 meters. The second column gives the correction for a 10 meter horizontal distance (always exactly half). For angles > 20 °, a table of corrections can be created using Equation V.1.

□	*d* (20 m)	*d* (10 m)
0.0	20.00	10.00
0.5	20.00	10.00
1.0	20.00	10.00
1.5	20.01	10.00
2.0	20.01	10.01
2.5	20.02	10.01
3.0	20.03	10.01
3.5	20.04	10.02
4.0	20.05	10.02
4.5	20.06	10.03
5.0	20.08	10.04
5.5	20.09	10.05
6.0	20.11	10.06
6.5	20.13	10.06
7.0	20.15	10.08
7.5	20.17	10.09
8.0	20.20	10.10
8.5	20.22	10.11
9.0	20.25	10.12
9.5	20.28	10.14
10.0	20.31	10.15

□	*d* (20 m)	*d* (10 m)
10.5	20.34	10.17
11.0	20.37	10.19
11.5	20.41	10.20
12.0	20.45	10.22
12.5	20.49	10.24
13.0	20.53	10.26
13.5	20.57	10.28
14.0	20.61	10.31
14.5	20.66	10.33
15.0	20.71	10.35
15.5	20.75	10.38
16.0	20.81	10.40
16.5	20.86	10.43
17.0	20.91	10.46
17,5	20.97	10.49
18.0	21.03	10.51
18.5	21.09	10.54
19.0	21.15	10.58
19.5	21.22	10.61
20.0	21.28	10.64
20.5	21.35	10.68

Annex 7.2 Sample Data Forms for the Tree Census and the Tree Map

Main tree inventory

Plot: ______ **Quadrat**: _______ **Start date**: _____________ **End date**: _____________

Field crew: ____

Supervisor: _________________________ Data entered by: _________________ Date: _________

GPS coordinates of 4 corners: __________ ___________, __________ ___________,

__________ ___________, __________ ___________

sub-quad	tag	stem tag[1]	status T/D/L[2]	diam (mm)	HOM[3] (m)	Codes	comments	prob
_,_	_________ _	___	_____	______	____	_______	______________ _________	___
_,_	_________ _	___	_____	______	____	_______	______________ _________	___
_,_	_________ _	___	_____	______	____	_______	______________ _________	___
_,_	_________ _	___	_____	______	____	_______	______________ _________	___

Main tree inventory: quadrat map

Plot: ______ **Quadrat**: _______ **Start date**: _____________ **End date**: _____________

____

1 Leave stem tag blank when there are no multiple stems
2 Tree, Liana, or Dead
3 Leave HOM blank when it is the standard 1.3 m

Field crew:

____

Supervisor: __________________________ Data entered by: _________________ Date: _________

Chapter 8. Data Presentation and Analysis

Richard S. Condit

Biodiversity inventory and monitoring projects are not finished until the information is reported in ways that are useful to scientists and resource managers. This chapter covers a series of data analyses aimed to produce the most important summary information about the flora and fauna of Panama. It starts with several basic presentations of the data in tabular format that allow quick calculations of crucial information: diversity, density, distribution maps, species abundances, species of concern, and forest carbon stocks. We also cover indicator species, both those invasive of non-forest areas and others restricted to mature forest. It is beyond the scope of a basic methods manual to include data analyses requiring advanced programming skills, such as models of habitat preference, spatial patterns, or detailed geographic information; and we set these aside for a manual dedicated to data manipulation and programming.

1. Basic Data Tables

Several tables presenting straightforward information about the plot and transect censuses are highly valuable to many users. The most important are tables of sampling sites and of species.

Sampling sites

A table providing information about each sampling site is of foremost importance. Chapter 2 provides detailed recommendations about the number and placement of sampling sites (Table 2.2). A single table with one sampling site per row should now be produced (Table 8.1). The information that should be given includes: site name, latitude, longitude, elevation, plus the number of plots and transects sampled at each for all taxa (ie, two tree plots, two bird transects, as described in chapters on individual taxa). The coordinates and elevation should be given for the initial site chosen, as described in Chapter 2.

Table 8.1. Sample rows for a table of sampling sites, one row per site. Data are hypothetical, for illustrative purposes only.

Site Name	Number of Sampling Units					Coordinates		
	Herp	Stream	Tree	Bird	Camera	Latitude	Longitude	Elevation
BCI Lower	2	2	2	2	65	9,1513	-79,8600	120
BCI Upper	2	2	2	2	85	9,2609	-79,8811	165
Soberania Road	2	2	2	2		9,1513	-79,8600	120
Soberania Upper	2	2	2	2		9,1513	-79,8600	220
etc.								

Species

A second more detailed table should then be created, foreshadowed by tables of sampling locations described in chapters on individual taxa (for example, Table 7.3). Those tables give information about individual plots (trees), transects (amphibians, birds), or stream collections (fish). A single summary table will have all plots and transects together, as illustrated with sample rows below (Table 8.2).

Table 8.2. Sample rows for a table of individual sampling units (plots, transects). The Site Name must match the names used in the table of Sites (Table 8.1). Data are hypothetical, for illustrative purposes only.

Site Name	Location	Taxa	Type	Size	Latitude	Longitude	Elevation
BCI Lower	Plot 1	Tree	Plot	1 ha	9,1513	79,8600	120
BCI Lower	Plot 2	Tree	Plot	1 ha	9,1700	79,8811	110
BCI Lower	Transect 1	Bird	Transect	500 m	9,1513	79,8600	120
BCI Lower	Transect 1	Bird	Transect	500 m	9,1513	79,8600	120
BCI Lower	Stream 1	Fish	Seine	40 m	9,1421	79,8781	105
etc.							

A map of all sites and individual units would then be a standard report for the project. It should be overlaid on topography, forest cover, roads, and towns, though details on the geographic software needed to create such a map are beyond the scope of this manual. Many analyses and presentation of the biodiversity data will include these site-summary tables, since they are necessary for linking the ecological results to geographic information on climate, soils, land use, or forest cover.

2. Basic Site-Specific Results

There are several routine analyses of the results per sampling location and unit that should be in an initial report. These include 1) number of individuals, 2) species richness, and 3) diversity indices, each calculated separately for the various taxonomic groups. These should be calculated for each location and for each individual sampling unit, so the rows of these tables match precisely the rows in Tables 8.1 and 8.2. For sites (Table 8.3), this means the pooled results from all individual units within the site. For example, at the hypothetical site called *BCI Lower*, there are two tree plots numbered 1 and 2. The pooled number of individuals is, of course, simply the sum of the number of individuals within the two plots. The species richness, however, is not simply the sum: it is the total number of species that appear at either, or both, plots. Likewise, diversity indices are never summed across units, but must be recalculated from the total list of species.

Species richness is simply the number of species observed. We recommend using Fisher's alpha as a diversity index, since it is the best way of comparing diversity at sites that differ in size. There are several programs available for calculating Fisher's alpha, given the number of species and individuals alone (both the *Vegan* packages and the *CTFS R Package* in the programming language R).

An additional calculation beyond these is the density of individuals, meaning the number of individuals divided by the area surveyed. For trees in particular, this is straightforward since the plot area is precisely demarcated. Tree density is usually given per hectare, so the following calculations are needed:

1) Density per ha of trees ≥ 10 cm dbh. In a single plot of 1 ha, this is simply the number of individuals counted. In multiple plots, simply divide the sum by the number of plots.

2) Density per ha of trees ≥ 1 but < 10 cm dbh. In the plot methods described in Chapter 7, these trees are only sampled in a subplot of 0.01 ha (10 m x10 m), so the density per ha is the number sampled divided by 0.01, equivalent to multiplying by 100. This can easily be extended to several plots by summing individuals, multiplying by 100, then dividing by the number of plots.

3) Subsequently, the total density ≥ 1 cm dbh is the sum of parts 1) and 2).

The number of fish per unit stream length is likewise straightforward to calculate using the distance over which seines were hauled. The assumption is that most fish are captured in the area seined. In the case of birds, amphibians, or reptiles counted along visual transects, the density can also be calculated simply by dividing the number observed by the length of transect. These methods allow counts to be compared even if the length of transects differ.

For mammals photographed with camera traps, the standardization procedure is to divide the number of animals photographed by the number of camera-days. That is, if 100 animals are observed in 70 traps after 30 days, the standardized number per camera per day would be $100/(70 \cdot 30) = 0.048$. Again, this is a straightforward way to compare results from two areas given different numbers of cameras and different time periods.

Table 8.3. Sample rows for a table of density and diversity for individual sampling sites of trees, one row per site. *N* = number of trees censused; *P* = density (*N/A*); *S* = number of species observed; *α* = Fisher's alpha. Data are hypothetical, for illustrative purposes only. Separate tables for fish, birds, mammals, frogs should be completed in the same format, though note that the density per ha is not so easily calculated for birds and mammals. Similar tables for individual sampling units (plots for trees) should be created, with one row per individual plot.

	Trees ≥ 10 cm dbh					**Trees ≥1 cm and < 10 cm dbh**					**All trees ≥ 1 cm dbh**			
	N	*A* (ha^{-1})	P (ha^{-1})	S	α	N	*A* (ha^{-1})	P (ha^{-1})	S	α	N	P (ha^{-1})	S	α
BCI Lower	805	2.0	402.5	102	30.9	65	0.02	3250	26	16.0	867	3652.5	111	33.8
BCI Upper	780	2.0	390.0	99	30.1	85	0.02	4250	32	18.7	865	4640.0	105	31.3
Soberania Road	990	2.0	495.0	131	40.4	78	0.02	3900	55	83.0	1121	4495.0	140	42.2
Soberania Upper	931	2.0	465.5	152	51.6	80	0.02	4000	44	40.2	1083	4465.0	162	52.8
etc.														

3. Forest Biomass

The carbon stored in forest is nearly all in the trees, or remnants of the trees buried in soil. The current emphasis on documenting and managing the earth's CO_2 has provided a common justification for forest plot inventories: measuring how much carbon is stored and fixed by trees (*fixed* means adding carbon via growth). The census methods described in Chapter 7 cover all that is necessary for often-used calculations of *above-ground-biomass* that are based on stem diameters and formulae derived sites where trees were felled and weighed piece-by-piece.

The formula appropriate for most of Panama's forests (excluding the dry arc of Cocle and high mountain forests) comes from Chave et al. (2005)[4] :

$$\log(B) = \rho + k_1 + k_2 \cdot \log D + k_3 \cdot (\log D)^2 + k_4 \cdot (\log D)^3 .$$

This is the formula for biomass *B* (in metric tons) of a single tree with diameter at breast height *D* (in centimeters), where the *k*s are constants (k_1 = -1.499, k_2 = 2.148 k_3 = 0.207, and k_4 = -0.0281). Carbon content can be found as 0.47*B*.

To find the biomass in one of the one-hectare plots, first find *B* for every individual tree. Call B_i the biomass of each tree >= 10 cm dbh, and B_j the biomass of those trees 1-10 cm in dbh, the latter censused in only 0.01 ha. The total biomass of the plot is

$$B_{total} = \Sigma B_i + 100 \cdot \Sigma B_j$$

where ∑ means the sum over all trees in the two categories.

4. Species List

Biodiversity inventories are about species diversity, and careful and accurate identification of all taxonomic groups has already been emphasized in chapters on field methods. The proper step for beginning analyses of species abundances and distributions is to assemble a table of all species encountered. This table resembles the species table described for individual taxonomic groups and can be started by simply combining tables of tree, bird, mammal, herp, and fish species.

Each row has information on a single species, starting with the genus, species, and taxonomic family. The authority who named the species must be included, and of course there needs to be an indication of the broad taxonomic group, for example *tree*, *bird*, *fish* or otherwise, matching the taxonomic category as it appears in other tables (8.1, 8.2). As was emphasized in previous chapters, this table should use names that match published lists of accepted taxa with accurate spelling. Use of out-date names and simple spelling errors greatly reduce the accuracy and reliability of the results, even if field data are otherwise excellent.

Every species found in one biodiversity project must be included, but additional species can be added. Indeed, a group of scientists working on several different inventories or monitoring projects may choose to assemble one comprehensive species table covering all the studies. The advantage of this is that it assures cohesive taxonomic information: consistent data

[4] Chave, J., Andalo, C., Brown, S., Cairns, M.A., Chambers, J.Q., Eamus, D., Fölster, H., Fromard, F., Higuchi, N., Kira, T., Lescure, JP., Nelson, B.W., Ogawa, H., Puig, H., Riéra, B., Yamakura, T. 2005. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. *Oecologia*, 145:87-99.

on every species across projects. Indeed, we envision a time soon when comprehensive species lists of all plants and vertebrates for Panama are readily available and can form the basis for all biodiversity research.

5. Species Abundances and Distribution

The most basic ecological information about species is abundance, and a straightforward yet crucial analysis is to assemble a table of abundances per sampling unit of every species. The table starts with the rows of the species table described in the previous section, with a column for every individual plot, transect, or camera during a single census. Each entry in the table is the number of individuals counted (Table 8.4). A second table of the same form, but showing abundance at each site, i.e. summing individual tree plots or multiple bird transects at a single site. The second table has as many columns as sites, the first table as many columns as individual units. These tables should be created separately for each taxonomic group.

Table 8.4. Hypothetical species abundance table. Each row gives number of individuals of a species in every individual sampling location. This can be immediately converted into an occurrence table by replacing all entries > 0 with a 1. Then every cell in the table has the value 1 if a species was observed at the site, and a 0 if it was absent. Both abundance and occurrence tables are useful in a variety of analyses.

Latin name (*Genus species*)	BCI Lower	BCI Upper	Soberania Upper	Soberania Road	etc. for all sites
Alseis blackiana	35	41	11	19	
Anacardium excelsum	2	0	0	0	
Buchenavia capitata	0	0	0	0	
Calophyllum longifolium	19	17	2	0	
etc. for all trees					
Black-striped woodcreeper	4	6	3	0	
Fasciated antshrike	91	77	32	19	
Southern nightingale-wren	0	0	3	1	
etc. for each taxonomic group					

The total abundance of each species is the sum of all entries on one row, and must be the same from both tables. The column sums are the site or unit abundances, identical to the abundances *N* (Table 8.3). These column and row sums can be inserted as a final column and a final row of each table.

An interesting and useful way of presenting these tables is to sort the rows by total abundance, descending from the most to the least abundant species. This shows the most important species in terms of abundance. The biomass per tree species, as calculated per site (Section 8.3) should be added to the tree table. Sorting tree species by highest biomass will generally show species quite distinct from those most abundant in terms of counts of individuals.

Tables of abundance are the data needed for three types of analyses of species distribution, given maps along with climatic and geographic information per site.

Distribution maps

A single row of the abundance table holds occurrences of a single species: positive abundance where it was found, zeroes where it was absent. In fact, converting the abundance table (8.4) to an occurrence table is a useful step: all zeroes remain unchanged, whereas all positive entries are changed to the number one.

Starting with a background map of the sampling sites, a species distribution map is created simply by overlaying points at those sites where the species occur: the columns with ones. Producing a booklet of maps for every species allows easy evaluation of distributions and makes an excellent report supporting the project description.

Abundance correlations

The abundance table (Table 8.4) provides results in a convenient format for assessing the correlation in abundance between two species across all sites. This is the correlation between the numbers in any two rows. Working with abundances, though, it is always necessary to log-transform the data, otherwise the few very abundant species control the results to the exclusion of hundreds of rare species. To log-transform, add one to every entry, then take the logarithm: $\log(N+1)$, where N is the numerical abundance of a species at a site (i.e. one entry to Table 8.4). Correlations are then carried out on these log abundances.

Positive correlations between species indicate that the two species tend to occur in the same areas. It is particularly interesting to consider the abundance of one animal species correlated with each of the tree species (one at a time). Positive correlations suggest that certain tree species are important to the abundance of that animal. Correlations among tree species show communities of trees. There are a variety of ecological clustering analyses aimed at defining groups of tree (or animal) species that tend to co-occur. Further details are beyond the current scope, but the abundance table is precisely the data needed.

Habitat models

The same occurrence table is the basis for understanding the environmental features regulating where species occur. Each of the columns has occurrences for a single site, and Table 8.1 – the site table – gives the elevation of each site. The second important feature that should be acquired for each site is rainfall. Combining elevation and rainfall records with occurrences of a species lead to an important model of habitat preference: how does occurrence change with either factor. This model requires *logistic regression*, a statistical tool used for correlation analyses where the response variable – in this case, the species occurrences – is always zero or one, whereas the predictor variables – in this case elevation and rainfall – are quantitative. Most statistical programming systems, including the language *R*, include logistic regression in their toolboxes. The results of the logistic regression for a single species show whether its range is influenced by rainfall or elevation, and in which direction (i.e. whether it is found most frequently at high or at low rainfall sites). Logistic regression also allows qualitative predictors, so for example, the presence of forest at a site (always yes or no) could be added to the model.

There is an enormous variety of habitat models, covering a wide range of predictors and many other modeling methods, and further description is beyond the scope of this manual. All methods, however, begin with the same data: occurrence records for a species at many sites, along with various measures of climate, soil, land use, etc., of the same sites.

6. Species of Concern

A major goal of biodiversity inventories is to gather information on species threatened with extinction. Two widely used sources of information for these rarest species are the IUCN Red List and a list of species endemic to Panama. An important component of the inventories is to assemble this information on threatened species, basing it on the master species list described in Section 4 of this chapter. The goal would be to create a table of every species for which the IUCN information is available, and include in it summary information on the geographic range of every species.

This table would then be matched with the abundance table (8.4) to find out how many threatened species were found across the entire study, and how many in each individual sampling site. Sites with the most threatened species should be targeted as 'hot-spots', particularly important for protecting the threatened species. Indeed, conservation priority should consider these tallies of the number of threatened species at each site.

A further analysis of great interest is to test for correlations between environmental variables, especially rainfall and elevation, on the number of threatened species found at a site. This gives insight into which habitats in Panama have the most species of concern. Further environmental variables, including land-use maps, could be added to the analysis.

7. Indicator Species

Based on prior work in forests of Panama, it is possible to identify species indicative of forest status vis-a-vis human perturbation. This works best for well known groups – trees, birds, and mammals – and especially for species that follow human disturbance. These are the weedy species and edge species that are uncommon-to-rare in old-growth forest but abundant where the forest has been opened by human intervention (Table 8.5). The first eight species in the Table are very common species in Panama, always associated with woodland, forest edge, or large forest clearings. If any one of those species has a density higher than 2-3 individuals in a one-hectare plot, it is likely the forest has been subjected to substantial clearing in the prior 30-50 years.

The other four species in Table 8.5 reveal past forest degradation by their size. At one extreme, *Anacardium excelsum, Ceiba pentandra, and Hura crepitans* can grow to immense size, and if individuals exceed 2 m (2000 mm) in trunk diameter, the trees are 500 years or older and the forest likely to be old-growth. On the other hand, *Enterolobium cyclocarpum, Ficus insipida, Pseudobombax septenatum, and Terminalia amazonica* can be used as indicators of mature secondary forest. There are many forests in Panama where those four species are the largest trees, reaching 500-1000 mm in trunk diameter, with no other trees near that size. Those are forests approximately 50-80 years recovering from substantial disturbance.

Table 8.5. Tree species indicative of forest status in Panama. Each is a common species in many parts of the country. Comments following each provide interpretations that can be made based on abundance or size. Dbh = stem diameter. Common names and further information can be found in Condit et al. (2011) *The Trees of Panama and Costa Rica* (Princeton).

		Indication		
Species	Size (dbh)	Young secondary	Mature secondary	Old-growth
Apeiba tibourbou (Malvaceae)	any	x		
Cecropia longipes (Urticaceae)	any	x		
Cecropia longipes (Urticaceae)	any	x		
Ficus inspida (Moraceae)	any	x		
Gustavia superba (Lecithydaceae)	any	x		
Luhea seemannii (Malvaceae)	any	x	x	
Ochroma pyrimidale (Malvaceae)	any	x	x	
Pseudobombax septenatum (Malvaceae)	any	x	x	
Enterolobium cyclocarpum (Fabaceae)	< 200 mm	x		
	> 500 mm		x	
Terminalia amazonica	< 200 mm	x		
	> 500 mm		x	
Anacardium excelsum (Anacardiaceae)	< 500 mm	x	x	
	> 2000 mm			x
Ceiba pentandra (Malvaceae)	> 2000 mm			x
Hura crepitans (Euphorbiaceae)	> 2000 mm			x

8. Species-Accumulation and Species-Area Analyses

A widely-used tool in ecology for judging the effectiveness of sampling species diversity is the species accumulation curve. This can be readily constructed from the table of occurrences, where species appear on rows and sites as columns; a 1 is entered everywhere a species was observed, a 0 otherwise (Table 8.4). The number of species at every site is then quickly found as the sums of the columns. A species-accumulation curve presents the number of species found in samples of increasing size. Given the occurrence table, this is calculated by finding the number of species found in any two sites combined, then in any three sites combined, and onward until all sites are combined. Given a combination of any two (or more) columns from the table, the total number of species observed includes all those seen at any one of the sites.

It should be obvious that the combined number of species is not found by adding together the species from the individual sites, because species may occur at more than one site. For example, if site A has 10 species and site B has exactly the same 10 species, then the combination of A and B also has 10 species. But if site C has 11 species, 6 of which are found at A but 5 are not, then the combination of A and C has 15 species. Once a large number of sites have been sampled, new sites may have very few additional species. This is when the accumulation curve approaches an asymptote: when nearly all species in a region have been found.

The precise way to calculate an accumulation curve requires the notion of random collections of sampling sites. In the occurrence table, this means combining randomly selected columns. All computer programming languages have a tool for drawing random numbers, and this must be done repeatedly. If there were 25 sampling sites, and thus 25 columns in the table, a random set of two columns is made by having the computer randomly choose two integers between 1 and 25. The number of species found in the combined collection of those two columns is then tallied; call this S_2, the number of species in two sites. Repeat this for random collections of 3, 4, 5 etc. sites, and then make a graph showing S_i as a function of *i*. The curve tends upward, with the final number, S_{25} giving the total number found in the entire sample. Doing this once, however, is not sufficient: the entire procedure should be repeated 100 times, each time having the computer select random subsets of the sites (columns). Because each selection is different, the values S_1, S_2, $S_3 \ldots S_{25}$ will be slightly different each time. The final curve is constructed by finding the mean $\hat{S}_2$ as the mean of all 100 replicate tallies S_2 and likewise for $\hat{S}_3$, $\hat{S}_4$, etc., and then graphing $\hat{S}_i$ vs. *i*. Each of the 100 replicates can also be used to calculate a standard deviation or confidence limits on each $\hat{S}_i$., providing an estimate of variability in species richness.

The species-accumulation curve is called a species-area curve if the horizontal axis shows the total surface area of a combined set of sites, rather than the number of sites *i*. That is, call A_1 the area of a single sampling unit, and $A_2 = 2A_1$ is the area of two combined samples, etc., then the species-area curve is the graph of $\hat{S}_i$ vs. A_i for all *i*. Species-area curves from ecological samples are nearly always linear when graphed on logarithmic scales, particularly after the first few sampling sizes.

Species-accumulation curves can be used for assessing species richness in wider areas. One straightforward technique is to examine how the number of species in *i* samples, $\hat{S}_i$, increases as *i* approaches the total number of samples. For example, if half the samples have already included all the species, meaning that adding the remaining samples adds no new species, then a reasonable conclusion is that the total inventory has been reasonably successful at find all the species.

Complete sampling might be approached in fairly low diversity groups, such as fish or frogs in Panama. However in diverse communities, such as trees in tropical forests, it will never happen in any practical inventory. New species will continue to be added even in very large samples. In this case, the log-log linear nature of the species-accumulation curve can be used as a quick, and approximate, way of extrapolating species richness. Define S_T as the total number of species found in the entire inventory, all sampling sites together, and $\hat{S}_{1/2}$ as the average number of species found in any half of the inventories. So if there were i = 20 inventories, then $S_T = S_{20}$, the number of species in all 20, and $\hat{S}_{1/2} = \hat{S}_{10}$, the mean number of species found in various combinations of 10 of the inventories. Find

$$z = (\log S_T - \log S_{1/2}) \div (\log 2),$$

where *log* means the natural logarithm. This is called the slope of the log-log species area curve, and usually falls in the range 0.10 to 0.25 across many ecological communities. To estimate species richness in an area that is k times larger than the current, use the formula

$$S_{larger} = S_T k^z .$$

For example, if $\hat{S}_{1/2}$ = 270 species were found in half the samples, and S_T = 300 species in all the samples, then

$$z = (\log 300 - \log 270) \div 0.693 = 0.152 .$$

The estimated number of species to be found after 10 times the sampling (180 more samples), is

$$300 \cdot 10^{0.152} = 426.$$

This should be considered only a rough estimate, and it should only be used after checking the species-accumulation curve visually on logarithmic axes to confirm that is reasonably close to linear.

9. Summary of Forest Status

One goal of biodiversity monitoring is to produce an overview on the conservation status of a forest in Panama, where status means the degree of human intervention and impact. At one end of the spectrum are forests largely undisturbed by humans whose original flora and fauna are largely intact, and at the other are forested sites where hunting or felling of trees has changed the community.

The best tools for an assessment of forest structure are biomass and indicator species. Old-growth and mature secondary forest (80 years since disturbance) average over 250 tons per hectare, while secondary and heavily perturbed forests average less than 200 tons. Species listed in Table 8.5 can support inferences suggested by biomass and together suggest whether a site has been cleared or had timber removed in recent decades.

Regarding degradation due to hunting, abundance of large mammals and birds is the clearest indicator. Any presence of deer, peccary, currasow, or guans suggests minimal hunting pressure. These species are typically present in large areas of mature to old-growth, only where hunting is absent. A good report on forest status is to prepare a table of the presence or absence of some of the key tree, mammal, and bird species, along with estimated mean tree biomass per hectare.

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