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ESTIMATING OPTIMAL SAMPLE SIZE FOR TREE INVENTORIES IN PANAMANIAN RAINFORESTS.

Francisco Farnum[1]

Department of Botany, Centro Regional Universitario de Colón, Universidad de Panama,
Simón Bolívar Street, Panama City, Republic of Panamá, Phone (507) 66751782 email: frank0523@hotmail.com

ABSTRACT

Determination of an optimal sample size for documenting tree diversity in a rainforest setting would be a useful benchmark for comparing and validating information between researchers. We conducted a comprehensive inventory of Panamanian tropical rainforests by recording floristic descriptions, including all trees, at 14 sample sites. For each sampling site, the test area consisted of a rectangular transverse section measuring 1000 meters x 10 meters (one ha). Each test area was further subdivided into 10 rectangular consecutive sub-plots of 100 meters x 10 meters each. All shrubs and trees with 10 centimeters of diameter at breast height (DBH) or greater were taxonomically classified. Species richness, species abundance, life zones, forest type and soil chemical compositions were analyzed. Statistical calculations were performed for biodiversity indices, principal component analysis and cumulative species curves in order to derive the number of sub-plots that are needed to obtain a representative sample of these forests. Panamanian rainforests show high biodiversity values; tree inventories average 79 tree species per ha. Temperature and rainfall had no effect on tree diversity, whereas soil composition and altitude above sea level were found to have an impact on diversity in Panamanian rainforests. Our data suggests that the optimal sample size needed to obtain a representative flora sample of a Panamanian rainforest is 4.5 sub-plots measuring 100 meters x 10 meters each. Establishment of a standardized sampling plot will permit cross-study comparisons, facilitate efficient use of scarce resources, and promote conservation efforts by providing a more accurate description of local biodiversity.

KEY WORDS: biodiversity; cumulative species curves; deforestation; species richness.

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RESUMEN

El estudio se llevó a cabo en 14 sitios boscosos debidamente georreferenciados y clasificados como bosques húmedos tropicales de la república de Panamá. El objetivo del estudio es: determinar un tamaño óptimo de la parcela para obtener muestras representativas que sirva de patrón de comparación y validación para este tipo de bosques. En cada sitio de muestreo se ubicó una faja de 1000 metros de longitud por 10 metros de ancho (una hectárea). Las fajas se subdividieron en 10 subparcelas rectangulares consecutivas de 100 metros x 10 metros cada una. Los arbustos y árboles con 10 centímetros de diámetro a la altura del pecho, se numeraron e identificaron taxonómicamente. Las variables analizadas fueron riqueza de especies, abundancia de especies, zonas de vida, tipo de bosques, composición química de suelo. Se hicieron cálculos estadísticos para comparar índices de diversidad, análisis de componentes principales y curva de acumulativa de especies en función del área para determinar el número de réplicas (subparcelas de 1000m^2) que se requieren para lograr la representatividad. Se observó que los bosques de este estudio presentaron altos niveles de biodiversidad; registraron 79 especies arbóreas promedio por hectárea trabajada. La temperatura y la precipitación no afectan notablemente la diversidad; sin embargo, las condiciones del suelo y la altura condicionan los niveles de diversidad. Los datos obtenidos señalan que es posible obtener muestras representativas de la flora de los bosques húmedos de Panamá, a partir de 4.5 subparcelas de 100 metros por 10 metros cada una.

TROPICAL FORESTS ARE OF CRITICAL IMPORTANCE FOR THE CONSERVATION OF BIOLOGICAL DIVERSITY ON THE PLANET due to the very complex biological communities that they host (Condit *et al.* 1996, Ojo & Ola-Adams 1996, Ricklefs 2004). In terms of structure and diversity of species, tropical forests are home to 70 percent of all plant and animal species in the world (Ricklefs 1977, Wills *et al*. 2006). Even if only the trees are considered, tropical forests are extremely diverse and may contain over 200 tree species per ha (Richards 1957, Patiño 1997, Wright 2002, Taylor 2004).

Deforestation is the permanent loss of forest cover for other land use such as agriculture, pasture, human settlements, infrastructure and reservoirs. Tropical deforestation is recognized as one of the most important environmental problems facing the world today, with serious long-term economic and social consequences. This deforestation, which in large part had been ignored by developed countries and urban dwellers in developing countries until the 1980s, has received much attention in recent years. Most of

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the loss of forest area is a direct result of human intervention in the twentieth century (Fearnside & Laurance 1999, Laurance 1999, Dale *et al*. 2000, Achard *et al*. 2002, Lindsey 2004, Defries *et al*. 2005).

The original forest area of the planet is unknown. However, it is estimated that over the past 8000 years we have lost 40% of the original coverage, and that this loss represented approximately 6000 million ha (Laarman & Sedjo 1992). By the late twentieth century, it was estimated that forests covered an area close to 3500 million ha worldwide, representing nearly 27% of the Earth's surface. Of this total, 2000 million ha are in developing countries, mainly in tropical and subtropical regions (FAO 1997). Hence, in order to have an impact on preservation of remaining forests, it will be essential to develop research programs supporting the conservation of forests in these countries.

In order to facilitate the development of such research programs, policies should be established that encourage better research and thus an improved ability to compare data across different types of forests. This will facilitate establishment of conservation and management plans that could ensure continuity of sustainable ecosystems. One of the problems in the sustainable management of tropical forests is precisely the lack of a standardized size and shape for a sampling site, so that a given sample will allow us to study the characteristics of the site more efficiently and with less cost. As a critical element of the forest, tree populations can serve as indicators of the health of the entire forest. Considering that trees cannot usually be measured in total, it is important that samples are as representative as possible (SCBD 2002). In most cases, researchers prefer to use 0.1ha sampling unit, however, this is an arbitrary measure that, in practice, may not be representative of a forest and thus undermines the possibility of establishing comparable parameters in other similar locations (J. A. Mendieta, pers. comm.).

The intention of this study was to evaluate and establish an appropriate sampling size for the study of biodiversity of tropical rainforests. In doing that, biology researchers will: (1) obtain an index that can be used to compare across different forests; (2) target

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appropriate financial resources in the development of research; (3) maximize the chances of collecting the greatest number of individuals representative of the larger study area; (4) standardize information in order to solve any problems that may otherwise arise when comparing data from different countries that have used different methods to conduct inventories.

METHODS

STUDY AREA.—Panama is the southernmost of the Central American countries, with a total area of 75,517 km². The Isthmus of Panama is located between 7° and 10° N latitude and 77° and 83° W longitude. Panama has a tropical climate, with a prolonged rainy season (May to January) and a short dry season (January to May). Annual temperatures are uniformly high, averaging 27.5°C, as is the relative humidity (ETESA 2008).

AREA SETUP AND SAMPLING.—Fourteen sampling sites were chosen based on the aim of the study in terms of temperature, height above sea level, forest type, and relative humidity. Fig.1 shows the sites selected and their UTM coordinates. Study forests were located in the provinces of Panama, Chiriquí, Coclé and Colon. By evaluating the environmental variables temperature and precipitation, which are associated with forest type according to the classification of life zones of Holdridge (1978), all sample sites were categorized as tropical rainforests.

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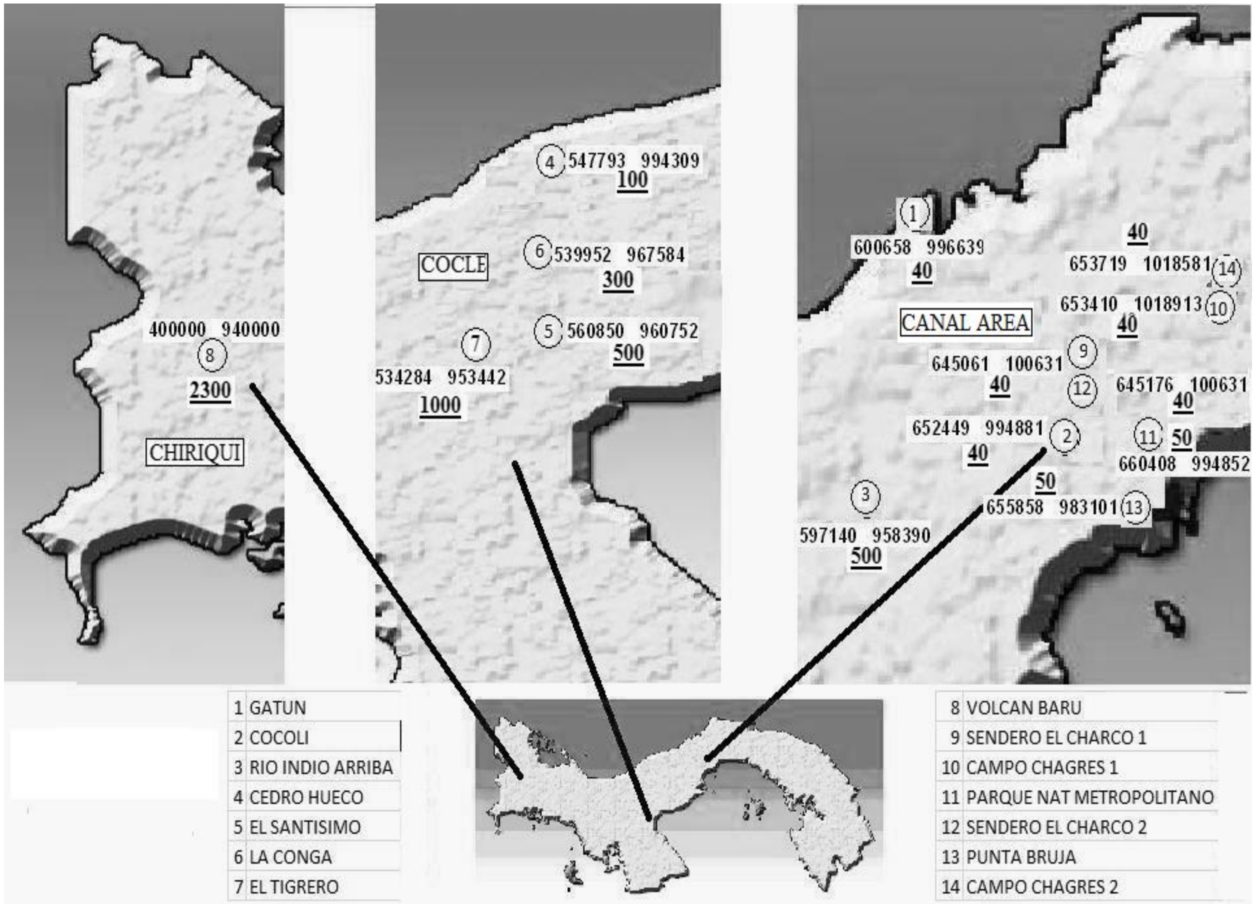

FIGURE 1. Sampling site locations. Forests were distributed throughout Panama towards the west, Chiriquí (1), the country's central province of Coclé (4), and in the Canal Area, the provinces of Panama and Colon (9). Sample site (circled numbers), height in meters above sea level (underlined numbers) and geo-data (plain black numbers) were recorded.

Based on preliminary information about the forests in the study area, sample sites were selected by a random procedure, by which transects were selected to include maximal local ecological variation, while sample plots were positioned along lines within sites. The test area consisted of a rectangular transverse section measuring 1000m x 10m (one ha). Each test area was further subdivided into 10 rectangular consecutive sub-plots of 100m x 10m (Fig.2).

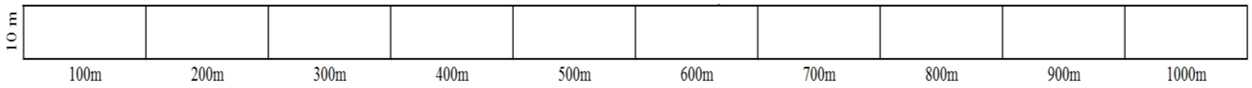

FIGURE 2. Sampling site plots**.** For each sampling site, the test area consisted of a rectangular transverse section measuring 1000m x 10m (one ha). Each test area was further subdivided into 10 rectangular consecutive plots of 100m x 10m each.

At establishment, reference points were located at the north east corner of the plot. The exact positioning and delineation of the sample plots were obtained using a global positioning system.

Random tree sampling was conducted within each plot. All shrubs and trees with 10cm DAP (diameter at chest height or 1.33m from the soil) or greater were taxonomically classified, numbered and entered into a database.

SOIL STUDY. —To determine soil characteristics we used information from the Instituto de Investigación Agropecuaria de Panamá (IDIAP) database that was developed from analysis of more than 100,000 soil samples from 1975 to 2005. In that study (IDIAP 2005), each sample was assessed using 13 different types of chemical analyses to determine its components. The parameters of comparison were taken from critical levels used by the Soil Laboratory of IDIAP for soils across the country. These critical levels are used to indicate the probability of growth from a crop after the application of a nutrient or fertilizer. When the level of a given component is low, the probability of observing a response to fertilizer is high and vice versa. Using the geo-data of that study, we extracted and compiled the data for soil characteristics of the sampling sites.

IDENTIFICATION OF SPECIES.—Fertile plant samples were collected and compared with those in the Herbarium of the University of Panama. Classifications were confirmed using the Catalog of Vascular Plants of Panama (Correa *et al.* 2004). An alphabetical list grouped into families, genera and species was produced.

BIODIVERSITY INDEX.—Given that it is more effective and scientifically accepted to use a combination of indices or measures to assess plant diversity (Caviedes 1999, Moreno 2001), the following multiple techniques were used:

1. Species richness was evaluated by considering Margalef's index of diversity (diversity = s-1/log N), where s is the number of species and N is the total number of individuals.
2. Simpson's index was used to evaluate different trends in plant diversity; this value is not logarithmic in nature and therefore is more sensitive to shifts in dominant plant species. In essence, equal value is given to the presence of any species, allowing the abundance of those species to increase the diversity value for a given plant community.

Simpson's index is calculated as follows:

$$\mathbf{SI} = \sum_{i=1}^{s} p_i^{2}$$

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where:

SI = Simpson's index of species diversity

S = number of species

pi = proportion of total sample belonging to the ith species.

3. The Shannon-Wiener index for a plant community is derived using the following equation:

$$\mathbf{H = -\sum_{\mathit{i=1}}^{\mathit{S}} (\mathit{p_i})(\ln \mathit{p_i})}$$

where:

H = index of species diversity

S = number of species

pi = proportion of total sample belonging to the *i*th species

ln = natural log

Due to its logarithmic nature, the Shannon-Wiener Index is sensitive to rare plant species and less sensitive to very common species. More value is given to the presence of each species than is given to the abundance of each species.

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4. Jaccard Similarity Index:

   We calculated the level of similarity using Jaccard's index **Sj** = c/(a + b - c), where (a) is the number of species in a region, (b) is the number of species in another region, and (c) is the number of species common to both. The values of **Sj** range between zero and one for complete dissimilarity to complete similarity, respectively.

MULTIVARIATE ANALYSIS.—A principal component analysis was carried out using the software Biodiversitypro26, version 2 (McAleece *et al.*1999) and the following sample site variables: Soil characteristics (pH, aluminum, texture, potassium, calcium, percent organic matter) and ecological conditions (life zone, forest type).

CUMULATIVE SPECIES CURVES.—For this analysis we used the program EstimateS (Colwell 2000a) and generated curves adjusted according to the modified Clench model (Soberón & Llorente 1993).

OPTIMAL SAMPLE SIZE.—Early sample analyses have shown that asymptotic curves assume a finite number of trappable species in a particular area, and when sufficient effort is applied this number of species will be caught (Thomson *et al.* 2003). For this reason, cumulative species curves for each of the 14 study sites were developed. The point at which the increase of new species should be less or equal to 10% was calculated for each curve (Oosting 1956). A newly designed computer program was developed for this study in order to calculate the optimal size for plot sampling. In this program the value obtained from the cumulative species curve was taken as reference, and the program proceeded to count the number of species present in each plot until it reached the reference value. This procedure was repeated for each site, and an average value was obtained for all 14 sites.

RESULTS

Physical and chemical properties of sample site soils were highly uniform among all 14 sites (Table 1). Soil type ranged from sandy soils to clay soils. For macronutrients such as Ca, K, P and Mg, values were low to medium, and aluminum concentration ranged from medium to low. Acid soils were present at all sites and organic matter content was low (0-2%) or medium (2-6%). According to these characteristics, most of the sample sites were suitable to support the development of forests with high diversity (Terborgh 1986, Gentry 1988).

TABLE 1. *Characteristics of sampling site soils.*

LOCATION	**CHARACTERISTICS**[a]									
	pH	**Al**[b]	**ST**[c]	**P**[b]	**K**[b]	**Ca**[b]	**OM**	**Cu**[b]	**Fe**[b]	**Zn**[b]
Cocoli, **Punta Bruja**	4–5	0–0.05	2	0–18	0–44	21-50	0–2	0–2	0–25	0–4
Bosque Gatun	4–5	0.06–1.0	4	0–18	0–44	0–20	2–6	0–2	25–75	0–4
El Santisimo, **R Indio Arriba,** **Cedro Hueco,** **La Conga**	4–5	0.06–1.0	4	0–18	45–150	0–20	2–6	0–2	25–75	0–4
P Metropolitano	6–7	0–0.05	2	0–18	0–44	21-50	0–2	0–2	0–25	0–4
El Charco 1, 2	6–7	0–0.05	2	0–18	0–44	21-50	0–2	0–2	0–25	0–4
Volcan Baru	6–7	0–0.05	3	18–54	45–150	0–20	2–6	2–6	0–25	4–14
Chagres 1, 2	6–7	0–0.05	4	0–18	0–44	21-50	0–2	0–2	0–25	0–4
El Tigrero	6–7	0–0.05	4	0–18	45–150	0–20	2–6	0–2	0–25	0–4

[a] Al=Aluminum, P= Phosphorus, K= Potassium, Ca= Calcium, OM = Percent Organic Matter, Cu= Copper, Fe= Iron and Zn= Zinc.
[b] mg/kg[c] ST= Soil Type. 1- Loamy, 2- Clay-Loamy, 3- Loamy-Sandy, 4- Clay-Loamy-Sandy.

Recibido: 17/04/14; Aceptado: 30/06/14

The total number of tree species identified at all sampling sites was 739 species, representing 106 families. The most common families recorded were Fabaceae, Lecythidaceae, Arecaceae, Rubiaceae, Myristicaceae, Anacardiaceae, Melastomataceae, Burseraceae, Clusiaceae, Myrtaceae, Euphorbiaceae and 312 genera including *Virola*, *Eschweilera*, *Socratea*, *Inga*, *Quercus*, *Miconia*, *Eugenia*, *Ocotea*, *Zanthoxylum*, and *Macrolobium*.

At all sampling sites, the values obtained for the indices of biodiversity (Margalef, Simpson and Shannon-Weiner) indicated a high level of tree diversity (Table 2).

TABLE 2. *Biodiversity indices***.** *Values for Margalef(richness), Simpson(dominant species) and Shannon-Weiner(uncommon species) diversity indices for the 14 sample forest sites using the software Biodiversitypro26, version 2 and EstimateS.*

PLACE	**Margalef**[a]	**Simpson**[b]	**Shannon-Weiner**[c]
Gatun	5.626	0.025	4.033
Cocoli	5.638	0.064	3.433
Rio Indio Arriba	5.551	0.046	3.543
Cedro Hueco	5.560	0.104	3.358
El Santisimo	5.552	0.029	3.942
La Conga	5.571	0.031	3.925
Cerro El Tigrero	5.407	0.023	4.458
Volcan Baru	5.631	0.213	2.650
Sendero El Charco 1	5.577	0.238	2.610
Campo Chagres 1	5.778	0.098	2.704
Parque Natural Metropolitano	5.726	0.102	2.838
Sendero El Charco 2	5.638	0.091	3.267
Punta Bruja	5.642	0.072	3.040
Campo Chagres 2	5.643	0.098	2.805
[a] Margalef's Index: values greater than 5 indicate high diversity.			
[b] Simpson' s Index: 0 represents infinite diversity and 1 designates no diversity.			
[c] Shannon-Weiner's Index: The greater the value is above zero, the greater the diversity; up to a maximum of 4.5.			

It should be noted that despite the high diversity that was observed, the distribution of species was not uniform because some dominant species were found at each study site.

In order to estimate similarity between all sites sampled, the abundance of species was assessed using the Jaccard Similarity Index. The results suggested that all sample sites were very similar in terms of species abundance. In general, higher similarity was observed between different forests in the same locality than between forests in different localities. Apparently, forest similarity was closed related to the environmental variables altitude, temperature and precipitation, which are all associated with life zones. This situation was confirmed by the dendrogram produced by cluster analysis (Fig. 3).

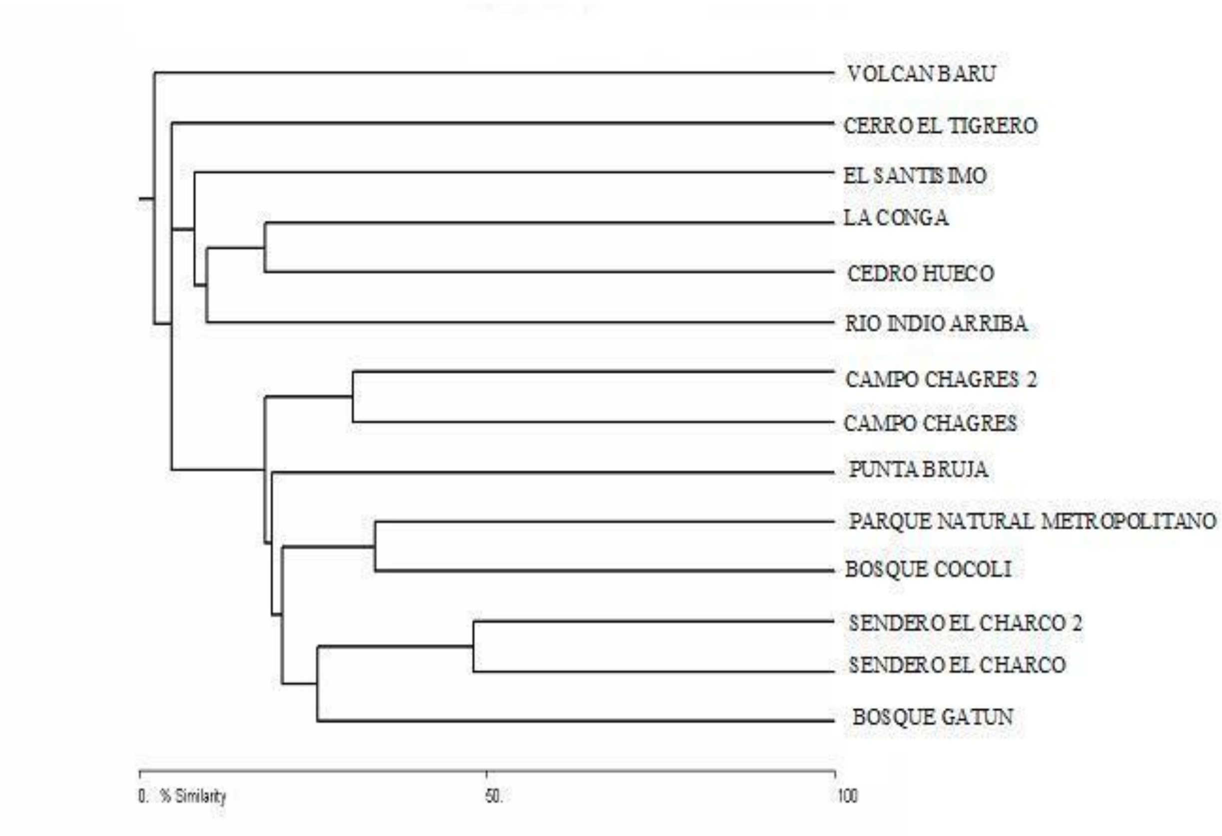

FIGURE 3. Cluster analysis dendogram considering Jaccard similarity coefficients for all sample sites, indicating biodiversity relationships between rainforests in Panamá.

Using the statistical approach of Principal Components Analysis, we calculated correlations across multiple environmental variables (Table 3a).

TABLE 3a. *PCA Correlation matrix. Soil variables were expressed according to soil characteristics. Environmental variables were expressed as life zone and forest type.*

Variables[a]	**pH**	**Al**	**Soil Type**	**K**	**Ca**	**O.M**	**LIFE ZONE**	**FOREST TYPE**
pH	1	-0.866	-0.135	-0.289	-0.149	-0.429	0.158	0.581
Al	-0.866	1	0.292	0.417	0.043	0.577	-0.068	-0.623
Soil Type	-0.135	0.292	1	0.564	-0.181	0.27	0.288	-0.202
K	-0.289	0.417	0.564	1	-0.258	0.577	0.73	0.048
Ca	-0.149	0.043	-0.181	-0.258	1	-0.447	-0.354	-0.186
O.M	-0.429	0.577	0.27	0.577	-0.447	1	0.474	0.083
LIFE ZONE	0.158	-0.068	0.288	0.73	-0.354	0.474	1	0.617
FOREST TYPE	0.581	-0.623	-0.202	0.048	-0.186	0.083	0.617	1

[a] *K= Potassium concentration, Ca=Calcium concentration, Al=Aluminum concentration, O.M.=Percent Organic Matter.*

We observed that percent organic matter content, and concentrations of aluminum and potassium could best explain the diversity observed in the forests studied (Table 3b and Table 3c).

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TABLE 3b. *Correlations of variables with principal components showed that the clusters of elements are positively associated to the first two eigenvectors*.

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalues	3.102	2.533	0.93	0.831	0.307	0.166	0.079	0.052
Percent Variance	38.777	31.664	11.628	10.384	3.833	2.074	0.988	0.651
Cumulative Percent	38.777	70.44	82.069	92.453	96.286	98.36	99.349	100

TABLE 3c. *The eight variables were reduced to two principal components, which explained 70% of the total variance*.

Components	Explanation Percentage	Explanation Variables
First	38.777	Percent Organic Matter, Aluminum Concentration, Potassium Concentration
Second	31.664	Forest Type, Life Zone and Soil pH
Total variance explained	70.44	

Finally, we performed cumulative species curves and observed that total diversity in these forests steadily increased, such that these curves do not reach an asymptote (Fig. 4).

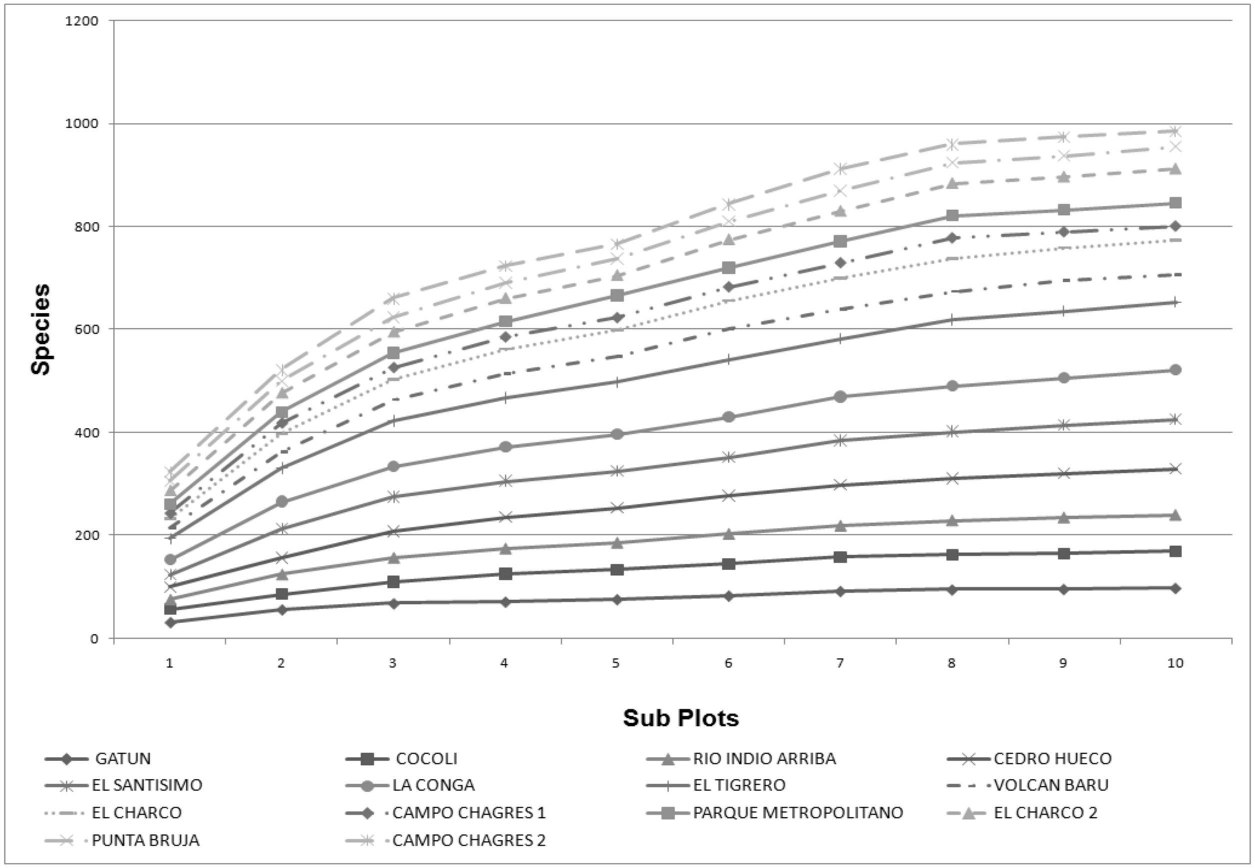

FIGURE 4. Cumulative species curve in 14 forest plots inventoried in Panamanian rainforest regions. All curves show that the number of species detected increased substantially until about 4.5 plots were sampled.

Nonetheless, this increase of new occurrences of species during the sampling did begin to decline after 67% of the species had been sampled. Critically, these calculations showed that representativeness in the samples is achieved with a sampling unit of not less than 4.5 sub-plots (Table 4).

TABLE 4. *Using Oosting`s break point, an estimate of the number of sub-plots needed in order to be representative of an area of 1 ha. of rainforest was calculated according to the number of species observed at each sampling site. Calculations were performed using a computer program specifically developed by the author of this paper.*

FOREST	OOSTING´S BREAK POINT	Number of Species Observed	% Species Observed	Plots needed to get Representativeness
1[jk1]	63	98	64.29	6
2	43	75	57.33	7
3	50	70	71.43	3
4	63	89	70.79	4
5	69	96	71.88	6
6	67	96	69.79	4
7	159	236	67.37	7
8	41	61	67.21	2
9	46	67	68.66	5
10	23	33	69.7	5
11	29	42	69.05	5
12	41	66	62.12	4
13	28	43	65.12	2
14	26	38	68.42	3
AVERAGE	53.43	79.29	67.39	4.5

DISCUSSION

Given their importance for the development of forest management strategies, tree inventories should be done according to established standards of accuracy, thus optimizing time and expense. In this context, determining the optimal dimensions of the plot and the number of sampling units required to achieve a certain degree of precision both stand out as important research tasks. This study has shown that it is possible to obtain representative samples of tropical rainforests from floristic inventories with a rectangular plot size of 4500 m^2. Whereas at present there is no standard measure of the size of sample plots for tree inventories in tropical rainforests, we recommend establishment of a standardized sampling plot to enable the ability to compare data and information between researchers.

The size of the sample plot for different forests has been a topic of much discussion among researchers, because it is a measure that is highly variable between studies (Howard 1982, Conde 1997). This particular feature may vary according to many factors, including legitimate concerns regarding the costs of conducting experiments, which can influence the plot sizes to be used. Ideally, when planning a study a researcher would select a suitable plot size that would minimize possible experimental error and thus be able to detect any significant differences that may exist between treatments. However, in most cases researchers choose to use a 0.1ha sampling unit, which has become widely accepted (J. A. Mendieta, pers. comm.). This is a somewhat arbitrary measure that may not be representative of the forest, and thus undermines the possibility of establishing comparisons to other similar studies.

In the tropical forestry literature, inventory sites are circular, rectangular or square in different dimensions, and no explanation is given for choosing these dimensions and shapes (Cochran 1977). In order to define the size and shape of the optimal site, the researcher should consider statistical power, and accuracy, as well as other practical measures, such as difficulty, time and cost. In this sense, we selected rectangular plots because they are always used in conjunction with survey lines, which simplifies their establishment in the field. They are most convenient if information on topography and forest composition is also required as part of the survey, and if dense undergrowth or difficult terrain necessitates spending a disproportionate amount of time on plot

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establishment. Conventionally, strips are run at right angles to the contours of the main topography of the area under survey because the fertility gradient usually runs in this direction (Schreuder *et al*. 1993).

The current study was performed for a very specific category of forest inventories, namely tropical rainforests. Although this type of analysis could serve as a guide for improving decision-making in the future about inventory and sampling methods for all flora of Panama, it can not necessarily be extended to decision-making and inventories regarding other forest types or in other geographic regions beyond Panama. It would be advisable to conduct similar studies in other types of forests of Panama and elsewhere in order to compare these results.

One of the environmental problems that has aroused great interest worldwide in this decade is the loss of biodiversity due to human activities, either directly (via overexploitation) or indirectly (via habitat alteration). Scientists have been able to educate government, private enterprise, and society in general to consider biodiversity a priority and thus to encourage greater efforts toward conservation programs. However, in addition to the traditional problems facing the study of biodiversity, such as species recognition and classification, conservation strategies, field and area specialists, and funds to develop conservation projects, are also those that arise from the lack of systematization of information and replication of results achieved. This is true in spite of the fact that the basis for an objective analysis of biodiversity and its change is its proper evaluation and monitoring (Chao *et al*. 2006, Moreno *op. cit.*).

Information relating to biodiversity is potentially infinite, due to the vast amount of variables involved and we lack effective technologies for large-scale processing and manipulation of basic biological data (Halffter *et al* 2001). Nonetheless, a better understanding of biodiversity, and the means of its preservation, will only come by systematically establishing accurate and universal standards to document, analyze and synthesize the vast amount of information involved.

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