CHAPTER FOURTEEN

Parametric Analysis of Reptile Biodiversity Data

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Introduction

The analysis of biodiversity data is rarely straightforward, often *ad hoc,* and always challenging. In that respect, any chapter that describes techniques of analysis is necessarily incomplete. In this chapter we broadly outline the ideas and concepts that are important to consider when planning, initiating, and conducting studies designed to measure biodiversity. Beyond definition and description, we also include an extensive literature review as a starting point for interested investigators who wish to pursue a topic in greater detail. We begin with a discussion of biodiversity and its measurement, including richness indices, evenness indices, diversity indices, and rank-abundance models. We follow this with discussions of species-accumulation curves, rarefaction, and the measurement of taxonomic and phylogenetic diversities, and then conclude with a review of selected software packages potentially useful for the practitioner. Where appropriate, we provide opinions and caveats.

Biodiversity Measures

A review of the literature on the concept of biodiversity reveals a bewildering array of explanations and representations of the term, with little agreement on what should be included in the definition. Gaston (1996) began his response to the "What is Biodiversity?" question with the words "bandwagon, buzzword, growth industry, global resource, issue, and phenomenon." He went on to provide a selection of definitions found in the literature. We can, perhaps, agree that *biodiversity* is a measure of the "variety" of entities on earth. What goes into "variety," however, is left up to the individual researcher. Most biologists would likely agree that biodiversity should encompass the concept of total number of species and their respective frequencies of occurrence. One might also include the concept of multiple levels of diversity, for example, taxonomic, genetic, population, and ecosystem (Sandlund et al.1992). Given that biodiversity is a *construct,* or a collective representation of ideas, a suitable definition is not available in all circumstances. We can, however, conceptualize what we mean by "biodiversity" in the context of each particular research question.

Although biodiversity is difficult to define, it can be quantified if the researcher provides an *ad hoc* definition within the context of the given inventory, monitoring project, or research question. In some instances, for example, it may be represented simply by a species-frequency histogram or a list of species counts. In other circumstances, a mathematical expression combining species numbers and geographic distributions will be appropriate (e.g., estimation of density within a given geographical unit). Williams and Humphries (1996) suggested that when biodiversity is measured, an intrinsic, philosophical value of worth is placed on that measurement. Hence, prior to measurement and quantification of diversity, the important questions justifying a particular study should be examined. These questions are, necessarily, study specific.

Below we discuss a selection of potentially useful biodiversity measures, including richness indices, evenness indices, diversity indices, and abundance. We begin with a brief discussion of key concepts related to biodiversity and then revisit each concept to provide specific recommendations for its application, with examples from the literature. We refer readers interested in discussions more detailed than those presented here to the texts by Magurran (1988), Forey et al. (1994), Krebs (1989), and Southwood and Henderson (2000).

Reptile Biodiversity: Standard Methods for Inventory and Monitoring, edited by Roy W. McDiarmid, Mercedes S. Foster, Craig Guyer, J. Whitfield Gibbons, and Neil Chernoff.

TABLE 23

Mathematical Formulae for Traditional Diversity, Richness, and Evenness Indices

Index Name	*Analytical Formula*[a]	*Reference(s)*
Diversity Indices		
Simpson's	$\frac{1}{\sum_i \left(\frac{N_i}{N}\right)^2}$	Simpson 1949
Shannon-Weaver	$-\sum_i \frac{N_i}{N} \log \frac{N_i}{N}$	Shannon and Weaver 1949
McIntosh's	$\frac{N - \sum_i N_i^2}{N - \sqrt{N}}$	McIntosh 1967
Brillouin's	$\frac{1}{N} \log \frac{N_i!}{\prod_i N_i!}$	Brillouin 1962
Probability of interspecies encounter	$\frac{N}{N-1}\left(1 - \sum_i \left(\frac{N_i}{N}\right)^2\right)$	Hurlbert 1971
Berger-Parker	$\frac{N_{\max}}{N}$	Berger and Parker 1970
Richness Indices		
Margalef's	$\frac{S-1}{\log(N)}$	Clifford and Stephenson 1975
Menhinick's	$\frac{S}{\sqrt{N}}$	Whittaker 1972
Evenness Indices		
Simpson's I	$\frac{1}{S\sum_i \left(\frac{N_i}{N}\right)^2}$	Pielou 1969; Begon et al. 1990
Shannon-Weaver	$\frac{-\sum \frac{N_i}{N} \log \frac{N_i}{N}}{\log(S)}$	Pielou 1969; Begon et al. 1990
McIntosh's	$\frac{N - \sum_i N_i^2}{N - \frac{N}{\sqrt{S}}}$	Pielou 1969
Brillouin's	$\frac{\frac{1}{N} \log \frac{N!}{\prod_i N_i!}}{\frac{1}{N} \log \frac{N!}{\left(\left[\frac{N}{S}\right]!\right)^{S-\left(N-S\left[N/S\right]\right)}\left[\left(\frac{N}{S}+1\right)!\right]^{N-S\left[N/S\right]}}}$	Pielou 1969; Magurran 1988

a. N_i = number of individuals in the ith species ($i = 1, 2, \ldots, s$); N_{max} = number of individuals in the most abundant species recorded; N = total number of individuals recorded $(N = \sum_i N_i)$; S = total number of species recorded.

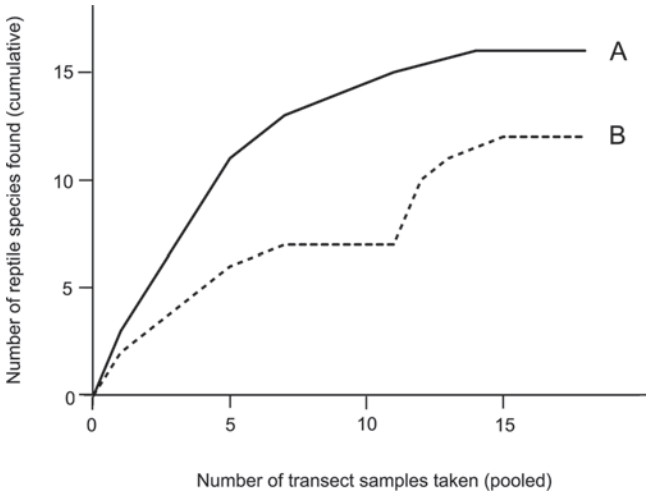

FIGURE 92 Sample species-accumulation curves for measuring reptile species richness at two field sites using transect samples. (A) Standard accumulation curve: increasing sampling effort by adding additional transects leads to an increase in the total number of species collected. The curve reaches an asymptote at approximately 15 species; 10 to12 transect samples would have been adequate to reach this asymptote. (B) Accumulation curve showing the influence of sample order. An initial asymptote is reached at approximately five transects; continued sampling shows that additional species are added with additional transects. This may be caused by the clumping of certain species, changing field personnel, seasonal behavior of certain species, or a variety of other factors. Under the assumption that the samples are fairly homogenous, a randomization procedure to generate plotted means might be a better choice for this site.

Species Richness

Species richness is simply the number of species present in a community (Begon et al. 1990). The utility of this measure is limited in that investigators rely on samples from communities, which they extrapolate to the entire community rather than censusing (actually identifying and counting every individual). Although samples are designed to represent communities as a whole, it is impossible to know how exhaustive or representative a data set really is. We can use simple *species-accumulation curves* (see "Species-Accumulation Curves," below) to obtain an idea of how the number of species being collected is related to the sampling effort and, therefore, to determine when it may be advisable to cease sampling because continued effort provides little additional return (Fig. 92). Often, however, efforts are not great enough to record all species in a given area or community, although complete species-level counts may be possible for some particular reptile groups (e.g., chelonians, crocodilians).

Investigators, conservationists, and politicians often wish to compare levels of biodiversity across communities. Using species richness as the criterion variable is sometimes inadvisable, because the underlying assumptions that sample areas, timing of sampling, and sampling efforts are roughly equivalent in the different communities have not been met. Investigators get around this problem by using methods such as rarefaction and diversity ordering (see "Species Evenness," below), but additional research on methodologies for comparing richness measures is needed. Some analytical methods are available for calculating richness indices (Table 23), and tests for statistical differences between or among communities based on these measures could be designed. Researchers must be sure, however, that tests do not violate the underlying assumptions of the statistical test (e.g., normality and homogeneous variability among communities).

Species Evenness

Species evenness (or *equitability*) is a measure that describes the uniformity with which individuals are distributed among species. Suppose, for example, that five lizard species are represented in a sample. Knowing that 10 individuals of each species are present leads to an interpretation of the community biodiversity different from the interpretation resulting from knowing that 46 individuals of one species are present, but only 1 individual each of the other species. We can express evenness mathematically by dividing a diversity index (see "Species Diversity," below) for a particular community by the maximum possible value it would have if all of the individuals were equally distributed among all species (Table 23).

Species Diversity

Species diversity is a measure that takes into account both species richness and species evenness. Whittaker (1972) suggested a useful classification of diversity, as follows: (1) *α-diversity*—local diversity, as within a habitat or community; (2) *β-diversity*—diversity (species composition) along an environmental gradient (and/or rate of change along that gradient), reflecting the presence of multiple communities along the gradient; and (3) *γ-diversity*—regional species diversity (a combination of α-diversity and *β*-diversity concepts). Southwood and Henderson (2000) discussed these concepts in detail and present methods for assessing and measuring them.

Hurlbert (1971) referred to species diversity as a "nonconcept." Although we do not fully agree with him, much can be said for this label because the literature contains multitudinous definitions and interpretations of diversity. Investigators must keep in mind that reporting a list of diversity indices from various communities is singularly uninformative unless certain assumptions about the sampling procedures have been met (see "Species Richness," above). Hypothesis testing involving diversity indices can be highly questionable because a number of confounding and extraneous variables associated with data collection make comparisons of two or more indices risky.

In Table 23 we list a variety of common diversity indices that have been used by ecologists. Although the indices may be useful in some studies, use of only a single number (the "index") derived from a set of raw data hides much of the story (much like reporting a mean without also reporting its associated variance and data distribution). Renyi (1961) discussed an entropy-based diversity ordering index, and Hayek (1994b) provided an extensive review of measures of association, both of which may be useful for comparing communities. We suggest that lists of species and numbers of individuals in each be reported rather than (or, at least, in addition to)

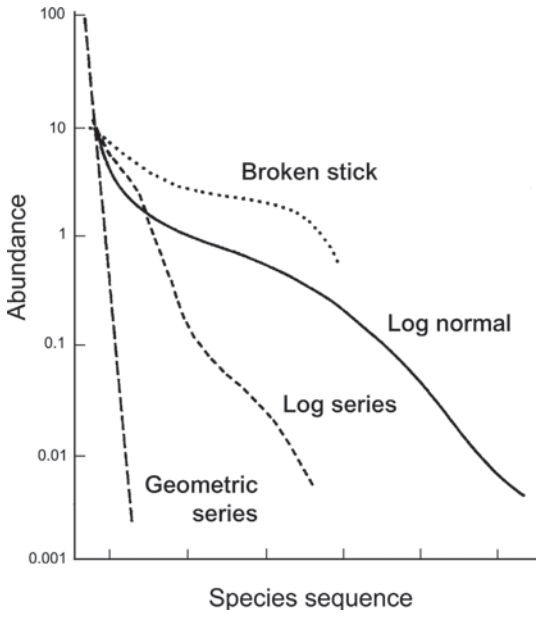

FIGURE 93 Plots showing the theoretical shapes of four common models used to describe rank-abundance patterns. In this diagram, abundance is log-scaled and is plotted against species rank (sequence). Descriptions and mathematical expressions for these models are given in the text. (From Magurran 1988; © A. E. Magurran, reprinted with permission.)

a single index. Having these data available will facilitate discussion, strengthen support for the ecological implications of the data, and reveal the limitations inherent in comparing communities with a single index.

Relative Taxonomic Diversity

For taxonomists, the most important type of diversity is generally α-diversity, that is, local, or within-habitat, diversity. Taxonomic diversity is often used somewhat synonymously with species richness (see "Taxonomic Diversity," below), with the unit of measure being the species and the data collected consisting of a list of those species. Such information is of primary importance for faunal surveys particularly if the following data are also available: (1) species composition and the associated distribution of each (e.g., for studies of biogeography; see Gaston and Williams 1996), (2) extent of current versus historic ranges (e.g., for studies of historical biogeography), (3) relationships between species compositions and environmental variables (e.g., for ecological biogeography), and/or (4) any information potentially useful for conservation and/or management programs.

For some faunistic and biogeographic studies, an index, or metric, of richness or diversity is sufficient for comparative purposes, although the investigator must acknowledge the potential short-comings of this simplified approach (see "Species Richness," above). Comparing species' lists is superior because it allows both common and rare species to be examined if samples have been taken in the same way and at the same scale across habitats or communities (Brown and Lomolino 1998). Surveyors employing appropriate experimental designs can obtain information for delineating biogeographic patterns (e.g., along elevational or latitudinal gradients) and revealing seasonal or community differences. Bannikov (1958), for example, examined the decrease in diversity of snakes in Eurasia along a west-to-east transect using species lists he accumulated, Scherbak (1986) analyzed herpetogeographic regionalization in the Palearctic region, and several authors have compared lizard communities (e.g., Cogger 1984; Kulikova et al. 1984; Orlova and Semenov 1986; Pianka 1986; Ananjeva 1997; Ananjeva et al. 1997).

Species Abundance

Species-abundance models (e.g., rank-abundance or species-abundance diagrams) are models used to describe the relationships between numbers of species and the abundance of each in a particular sampling area. They incorporate all of the information gathered from a community inventory. Consequently, they are more "complete" mathematically than a simple richness index (May 1975; Magurran 1988). Rank-abundance diagrams are constructed by plotting species rank (i.e., based on proportional abundance) on the abscissa and the respective proportional abundance (log-scaled) of each species, ranked from most to least abundant, on the ordinate. The proportion that each species contributes to the total is calculated by dividing the number of individuals in the *i*th species by the total number in all species combined (i.e., N_i / N). The species are then ranked by proportion and plotted as described above. Four models are used most commonly to describe the diversity of species in a rank-abundance plot of a sample (Magurran 1988): the geometric series, the log series, the log normal, and the broken stick (Fig. 93).

GEOMETRIC SERIES

In the *geometric-series model*, the most common species contributes the greatest fraction of the total number of individuals of all species combined, the second most common species a lesser proportion, and so on until all species in the sample have been included (Begon et al. 1990). This model is based on the "*niche-preemption hypothesis*" (*sensu* Magurran 1988) under the assumption that the most abundant species preempts the greatest proportion of resources in an area, the second most abundant species preempts the second greatest proportion of resources in an area, and so on. Hence, the rank abundances of species in a community, when ordered from the most abundant to the least abundant, produce a straight line when plotted (Fig. 93). The rank abundance of the *i*th ($i = 1, 2, \ldots, S$) species (N_i) as a function of niche preemption and total number of species is given by

$$N_i = \frac{Nk(1-k)^{i-1}}{1-(1-k)^s} \qquad (1)$$

where N = the total number of individuals; k = the niche-preemption parameter, which is generally estimated as the fraction of the total number of species over the total number of individuals, although newer methods for iteratively estimating k from sample data have been developed by Caruso and Migliorini (2006) and He and Tang (2008); and S = the number of species (May 1975). Field research by Whittaker (1977) suggested that this model is useful in species-poor areas or in early successional communities (see Magurran 1988) where

the niche-preemption hypothesis would suggest that the first species to arrive in an area will use the largest fraction of the available resources, the second species to arrive will use the *k*-fraction of the remainder, and so on.

LOG SERIES

The *log-series model* is closely related to the geometric series (May 1975) and may represent the logical progression of a generalized successional community through time (Fig. 93; Magurran 1988). Mathematically, the log series can be expressed using a constant "*a*" as a model constraint (defined in eq. 4 below), and an "*x*" that represents the number of species predicted to have one individual. The series then progresses such that $ax^2/2$ = the number of species expected to have two individuals . . . and ax^n/n = the number of species expected to have n individuals. We can obtain the total number of species (*S*) by'

$$S = a[-\log(1-x)] \tag{2}$$

where x is obtained from an iterative solution of

$$\frac{S}{N} = \frac{1-x}{x[-\log(1-x)]} \tag{3}$$

such that

$$a = \frac{N(1-x)}{x} \tag{4}$$

with *N* defined as the total number of individuals of all species present (Pielou 1969; Magurran 1988). In practice, the expected number of individuals can be calculated using the relations above, and these expected values can then be compared to the observed number using a likelihood ratio, chi-square, or related statistic (Magurran 1988; Sokal and Rohlf 1995).

LOGNORMAL SERIES

Many species' abundances in natural communities display a *lognormal distribution* (Fig. 93; May 1975; Sugihara 1980; Magurran 1988). This distribution is discussed in May (1975) and Gray (1987). The mathematical representation of the lognormal model is quite simple, with the distribution of the abundance classes represented by a histogram showing the numbers of species (*y*-axis) containing various numbers of individuals (*x*-axis; e.g., 4 species contain 3 individuals, 7 species contain 5 individuals, etc.). The equation can be written as

$$S(R) = S_o e^{(-2\sigma^2 R^2)} \tag{5}$$

(Pielou 1969) where S_o is the number of species in the modal class, σ^2 = variance of the lognormal distribution (i.e., a measure of the width of the distribution) calculated from the histogram, and *R* is the number of species in an individual class; therefore, *S*(*R*) = the number of species in a given class (*R*) to the left and right of the modal class (S_o). The observed distribution can be compared to a theoretical, expected distribution based on, for example, perfect normality around the modal class. Often, however, this distribution does not adequately represent species in the tails of the distribution because of sampling bias. Pielou (1975) showed how a truncated lognormal curve can be used to estimate the parameters of the equation when the relative abundance of very rare species produces a histogram with no left tail.

BROKEN-STICK SERIES

This model was first proposed by MacArthur (1957) to represent how individual species in a sample contribute to the total number of species assuming nonoverlapping niches with abundance proportional to the size of the niche (Pielou 1969; Magurran 1988). In the *broken-stick series* model, which has been referred to as a "biologically realistic expression of the uniform distribution" (Magurran 1988, p. 29), each species contributes a proportion to the whole such that the graph represents a stick that has been broken into random fragments and laid out by ranking the size of the fragments (Fig. 93; Begon et al. 1990). The mathematical representation for this model (May 1975) is

$$S(n) = \frac{S(S-1)}{N}\left(1-\frac{n}{N}\right)^{S-2} \tag{6}$$

where *S*(*n*) = the number of species in the abundance class containing *n* individuals, with *N* defined as the total number of individuals across all abundance classes, and *S* defined as the number of species observed. As before, expected and observed distributions can be compared to test the fit of this model. Magurran (1988) reviewed the literature concerning use of this model for many communities.

Species Density and Continuously Distributed Data

Types of Data

Variables that are measured in ecological studies can be classified in a variety of ways. It is important that variable type be kept in mind at every stage of an investigation (design, data collection, database management, analysis, and reporting) so that the appropriate type of data (e.g. continuous, categorical, etc.) will be collected to answer a specific research question. Often when designing studies, field biologists must balance resource (e.g., time, personnel, funds) availability against research needs (such as accuracy or precision) and must determine which data are needed to answer the questions at hand and how they should be gathered. Inasmuch as appropriate analyses require appropriate data, we briefly describe data types and general statistical considerations. Following the classification scheme of Sokal and Rohlf (1995), variables can be grouped into three broad categories: measurement variables, ranked variables, and categorical variables.

MEASUREMENT VARIABLES

A *measurement variable* is a variable based on a quantitative measurement. Investigators can determine whether differences between these variables (e.g., mean values for different

populations or the same population at different times), expressed as numbers, are significant statistically. Measurement variables fall into two broad data categories: continuous and discrete. *Continuous variables* are those in which a theoretically infinite number of values can fall between any two numbers, although in practice, instrumentation (the capability of a measuring device to measure smaller and smaller amounts) limits the realized continuity of any distribution (Hayek 1994b; Sokal and Rohlf 1995). *Discrete variables,* or *meristic variables,* have only fixed values. We may, for example, count ventral scales and record numbers such as 100 or 122, but not a number such as 100.115, which has no meaning. Measurement variables are quite useful in a statistical sense, as many established distributions (and tests derived from them) such as the normal, exponential, or gamma distributions for continuous variables, and the binomial, geometric, and Poisson distributions for discrete variables can be used to analyze these quantitative measurements.

RANKED VARIABLES

These variables, which are not necessarily based on measurements, represent an order or magnitude (Sokal and Rohlf 1995). Imagine that we record the order of emergence for five Copperhead (*Agkistrodon contortrix*) from their hibernaculum. We may record these as 1, 2, . . . , 5 (first, second, . . . , fifth), but unlike with discrete variables, we are not implying that the difference between adjacent variables (e.g., 1 and 2) is the same in magnitude as the difference between any other two adjacent variables (e.g., 2 and 3); snake 2 may have emerged 1 hour after snake 1, but 3 days before snake 3. Another way to consider ranked variables is in the context of nonparametric statistics. For example, after measuring the snout-vent lengths (SVL) of female *Thamnophis* from two populations, one may wish to compare their mean lengths. If preliminary testing reveals that we cannot meet the assumptions of normality and equality of variances for a two-sample *t*-test, the data may be ranked and then analyzed with a nonparametric Wilcoxon two-sample test (Sokal and Rohlf 1995). In this example, because the test is based on the absolute *ranks* of the data, we lose information about the absolute magnitude of difference between any two measurements.

CATEGORICAL (ATTRIBUTE) VARIABLES

Categorical variables are variables used to describe qualitative data. We might record *Varanus* females as "gravid" or "not gravid" or *Phrynosoma* females as "pre-reproductive," "reproductive," or "post-reproductive." Several statistical techniques have been designed especially for use with categorical variables (e.g., logistic regression and contingency table analysis); they are discussed in Agresti (1990) and Hosmer and Lemeshow (2000). Measurement variables are often grouped for convenience into categories (such as "docile" or "aggressive" or "very aggressive"), but that does not imply that the data are necessarily categorical in nature; rather, it may indicate that the researcher simply did not wish to divide the data any further (e.g. "somewhat aggressive" or "extremely aggressive").

Considering the way data are categorized is useful from a statistical design and analysis standpoint, because such categorizations dictate the type of analyses that can be done. Investigators often use a variety of univariate and multivariate statistical techniques. One of the most common violations of statistical assumptions seen in the literature is the failure to test for normality of the data (e.g., by comparing the empirical data that were collected to a theoretical distribution that is normal, using a Komolgorov-Smirnov or Shapiro-Wilk statistic), an underlying assumption of many well-known techniques. We strongly urge all scientists to seek the advice of a qualified statistician or biometrician prior to collecting data. These professionals can assist researchers in determining which variables to include in sampling and how to analyze them appropriately.

Species Density

DEFINITIONS

Species density is a common measure of biodiversity that refers either to the number of individuals of a single species or to the number of species present in an area during a given time or sampling effort (Hayek 1994b). *Population density* is a measure of the total number of individuals per unit area, whereas *relative density* is a measure of the densities of a number of populations relative to each other (Lancia et al. 1994). Another term common in the reptile literature is *linear density,* a measure of population density along a linear distance rather than an areal extent (King 1986; Parker and Plummer 1987; Cross 1998). Measures of linear density are particularly useful for aquatic and semiaquatic species (e.g., crocodilians), in which numbers per unit of linear shoreline may provide a more appropriate expression of the data.

ESTIMATES AND STATISTICAL CONSIDERATIONS

Estimating density using estimates of population size is not always straightforward, because the *effective area* (Lancia et al. 1994) and the area to which the density estimate is to be applied may not be equivalent, which leads to a biased estimate (White et al. 1982; Anderson et al. 1983). As an example, if one wishes to estimate the density of a species over 1 km^2, but does this with 10 subsamples of 10 m^2, then the effective area of the sample is the sum of the 10 subsamples (100 m^2) and not the desired 1 km^2. However, setting up a proper sampling design generally allows one to extrapolate over larger areas if the subsamples are, in fact, representative of the entire area of interest. If the area sampled is known (or assumed) to include the entire area of interest, then the estimate of population density, *D,* and its accompanying variance are given by

$$\hat{D} = \frac{\hat{N}}{A}$$
$$Var(\hat{D}) = \frac{Var(\hat{N})}{A^2} \qquad (7, 8)$$

(Lancia et al. 1994) where A = area, $\hat{N}$ = estimate of abundance, and $\mathrm{Var}(\hat{N})$ = estimated variance of the abundance estimate. If we assume that density is relatively constant over the length of a linear transect (e.g., shoreline), then we can calculate linear density by replacing length for area in the above equations. Because we can calculate both a point estimate (i.e., $\hat{D}$) and a variance, it is possible to test for a significant difference from a known (perhaps historic) density distribution under

the assumption of normality (Sokal and Rohlf 1995). Also, relative density distributions from two areas or two years can be compared using distributional statistics or nonparametric statistics. For a thorough review of field investigational techniques, including methods of determining density and abundance, see Skalski and Robson (1992).

Density can also be estimated using distance sampling. The primary sources of information on use of this technique are Burnham et al. (1980) and Buckland et al. (1993). *Line-transect sampling* (i.e., traversing a transect and measuring the distance and direction to a visible species of interest) and *point-transect sampling* (i.e., standing at a point and observing distance and direction to a visible species of interest) are commonly used to estimate bird and mammal densities (Buckland et al. 1993). The investigator establishes line or point transects through a study area and then estimates distances from a known point on the transect to any animals that are detected. These distances are then used to construct a detection function (i.e., the probability of detecting an organism that is present at a given distance) for adjusting density estimates (see "Transect Surveys, Including Line Distance," in Chapter 13). Area is estimated as twice the transect width (to account for left and right sides of the transect) multiplied by the transect length for line transects and by π times the square of the radius for point transects. Abundance is simply the number of animals encountered during the survey (assuming all animals were detected). Density is estimated by substituting these values into equations (7) and (8) for each point or line transect in the survey. Much has been published on the adjustment of estimates of animal detectability and errors in distance estimates (Burnham et al. 1980; Seber 1986; Buckland et al. 1993). These methods are not widely used by reptile biologists, perhaps because of the inherent difficulty of detecting some animals. Anderson et al. (2001) tested this methodology on *Gopherus agassizii* populations in the Mojave Desert. They discovered that even novice field personnel can adequately use transect sampling to estimate abundances, and they concluded that transect sampling should be considered for large-scale monitoring projects.

Species-Accumulation Curves

A *species-accumulation*, or *"collector's" curve*, is a relatively straightforward method for estimating local species richness (Fig. 92). The curve is constructed by plotting the cumulative number of species found as a function of the sampling effort (Soberón and Llorente 1993). Sampling effort is often represented by the number of samples collected, but many other measures, such as trap days, quadrat area, length of drift fence, and so forth, can also be used (Colwell and Coddington 1995). Species-area curves, wherein the total number of species is plotted against the size of the sampling area, are reviewed briefly in Hayek (1994b).

To optimize sampling effort, one can plot the number of species collected against the number of samples taken; when an "eye-ball" examination indicates that an asymptote has been reached, the investigator can stop sampling because the likelihood that additional species will be encountered is very small. However, if one wishes to compare estimates of species richness across sites or at one site over years, a more complex methodology must be used to find the maximum number of species expected—that is, the asymptotic maximum of the accumulation curve. Once a best-fit curve is fitted through the empirical data, then one can use iterative approaches to estimate the maximum (or supremum) of the curve; if known statistical distributions are used to plot the curve, then basic calculus can be used to find the maximum value the curve will attain. Many methods exist to find a best-fit curve; the program EstimateS is quite useful in this regard (see Appendix II).

The order in which samples are added to a survey can affect the shape of its accumulation curve; changes in the curve can be related to sampling error, amount of effort expended, or heterogeneity among sampling units (Fig. 92; Colwell and Coddington 1995). Randomizing the sampling order (e.g., of transects or sampling points; Colwell and Coddinton 1995) or using subsamples of the measure of effort (Holdridge et al. 1971) will help to control this problem when estimating average richness. The mean numbers of species calculated from the subsamples can then be plotted to construct a curve that is less affected by sample order; in addition, variances and confidence intervals can be constructed at each point along the curve.

Several models have been developed for fitting curves to accumulation plots in order to estimate an asymptotic maximum number of species. An early effort by de Caprariis et al. (1976) is equivalent to the *enzyme-kinetics model* of Michaelis-Menten in which the rate of substrate conversion (= rate of encountering additional species) is related to the concentration of the substrate (the number of species present). Much research on the statistical properties of this estimator can be found in the literature; we refer interested readers to reviews by Raaijmakers (1987) and Colwell and Coddington (1995). Another important model is the *negative-exponential model,* which estimates the probability that an additional individual will be a new species by assuming a linear dependence on the current species list (Miller and Wiegert 1989; Soberón and Llorente 1993). This probability approaches zero as the asymptote of the accumulation is reached, thus providing a common-sense interpretation of the model (Colwell and Coddington 1995). A third set of models, the *nonasymptotic models* (Colwell and Coddington 1995; reviewed in Palmer 1990), includes the *log-linear model* (accumulation increases with the log of effort) and the *log-log model* (equivalent to MacArthur and Wilson's 1967 island biogeographic species-area curve). These are only a few of the most common curve-fitting techniques. For a review of both parametric and non-parametric models for fitting curves to accumulation plots, see Colwell and Coddington (1995).

The most useful information provided by curve-fitting models is often an estimate of how richness is likely to change as a function of increased effort (Colwell and Coddington 1995). Also, one can compare species-accumulation curves (e.g., rate of increase) across time or space, or construct tests of point estimates (e.g., maximum richness) using a variety of goodness-of-fit statistics (Sokal and Rohlf 1995). Simply plotting maximum richness against time for a long-term study area may also be informative, as it can provide information about community stability and resilience, particularly if the community has been affected by a known event (e.g., fire, flood, introduction of an invasive species).

Rarefaction

A goal of many studies is to compare the species richness of different areas or of a single area at different times. Given that species richness generally increases with the number of

samples taken, up to a theoretical asymptote (Sanders 1968; Hurlbert 1971), the sample sizes of the entities being compared must be statistically equitable (Sanders 1968; Hurlbert 1971; Peet 1974; Heck et al. 1975).

Sanders (1968) devised a simple method to reduce samples to a common size before comparing them. He ranked the species in a sample relative to their representations in the sample, and calculated cumulative percentages. He then took random sub-samples of size n from the total pool of original samples taken (total of *N*), developing new species-accumulation plots, or *rarefaction plots*. The mean number of species found from these repeated subsamples was then plotted as a function of sub-sample size (Sanders 1968; Simberloff 1972; Hayek 1994b). An obvious drawback to this method is that it lacks a probabilistic framework (Hayek 1994b) and is strongly affected by sampling methodology (Fager 1972). Simberloff (1972, p. 417) suggested that "[n]ot only is the rarefaction method incorrect, the degree to which it is incorrect is markedly dependent on sample size." Sanders's (1968) original method consistently overestimated the expected number of species ($E(S_n)$) drawn randomly from a collection of *N* individuals and *S* species (Hurlbert 1971; Heck et al. 1975; Hayek 1994b), because the correct estimate of the number of species should be based on a hypergeometric probability distribution (Simberloff 1972; Heck et al. 1975) rather than on the *ad hoc* method he used. Based on the hypergeometric distribution Hurlbert (1971) gave the correct function for estimating $E(S_n)$:

$$E(S_n) = S - \sum_{i=1}^{S} \left[\frac{\binom{N-N_i}{n}}{\binom{N}{n}} \right] \tag{9}$$

where, $E(S_n)$ = expected number of species in the sample of n individuals that are randomly selected from a total of *N* individuals, *S* = the total number of species, and N_i = the number of individuals of the *i*th species in the unrarefied sample. Heck et al. (1975) provided a formula for calculating the variance associated with this estimate ($V(S_n)$); owing to the complexity of the expression, we refer interested readers to the original source.

If one samples with replacement instead of without replacement (i.e., the hypergeometric distribution), then a multinomial distribution can be used; Heck et al. (1975) provided the computational formula for $E(S_n)$:

$$E(S_n) = S - \sum_{i=1}^{S} \left(1 - \frac{N_i}{N} \right)^n \tag{10}$$

Again, the variance formula for $V(S_n)$ is quite complex and can be found in the original paper. The authors also provide a useful example of how to use $E(S_n)$ to estimate sufficient sample sizes for valid comparisons (i.e., simply solve for n in the equation and select a sample size which provides a desired proportion of the total number of species [*S*] to be taken).

When using a rarefaction technique, one must keep in mind the relationship between the population and the sample (i.e., the "parent population" and the "collections;" see Hayek 1994b). For the rarefaction methodology to be useful, samples must be collected at random. Given the habitat specificity of reptiles (and hence the sampling strategies used to collect them), sampling sites are often clustered in specific habitats rather than randomly distributed (Reinert 1993); therefore, rarefaction procedures can lead to biased results (Hayek 1994b). Nevertheless, rarefaction methods have been found to be particularly useful when sizes of species assemblages differ (Buzas 1979; Hayek 1994b), so long as sampling is random. This method can be particularly useful for comparing areas or time periods if samples were collected in a similar fashion (i.e., in the same season, same habitats, etc; Hayek 1994b).

Taxonomic and Phylogenetic Diversity

Taxonomic and phylogenetic diversity have become the vanguard of research in conservation, in which identification of areas with the greatest numbers of species available for protection is often a goal. In large part this reflects the many advances in techniques for elucidating phylogenetic relationships and species clustering patterns, and the development of diversity measures that take advantage of this new information. Interested readers should consult the excellent book by Forey et al. (1994) on this topic.

Taxonomic Diversity

Often, the only data that are available for groups of organisms is some measure of group membership based on taxonomic patterns. Consequently, investigators have developed measures of diversity based on the information inherent in classification branching points and nodes (Vane-Wright et al.1991, 1994; Williams and Humphreys 1996).

ROOT WEIGHT

In the *root-weight method* each species in a classification is weighted based on its distance from the root of the classification. Essentially, the number of branching points between the root and each of the species present in the classification is counted. Weights are then determined by dividing the total node count of the classification by the node count for each species; thus, species closest to the root node have the highest weights. These weights values are summed to provide a diversity score for a given biota (Vane-Wright et al. 1991).

HIGHER-TAXON RICHNESS

This method for calculating taxonomic diversity is an extension of the root-weighting procedure that gives greater weights to higher taxa (i.e., taxa farther from the initial branching node in the taxonomic tree) regardless of the number of species that each may include (Williams et al. 1991, 1993). For this measure, species are compared in pairwise fashion. In biotas with large numbers of higher taxa, the divergences of species tend to be closer to the root of the classification than in biotas with smaller numbers of higher taxa; thus, the latter biotas will have lower node counts. Again, investigators can convert taxonomic branches to ratio scores as above (counting the number of branching points between the root of the classification and each species and then dividing the total node count of the classification by the node count for each species); the sum of these weights for a given

classification is its diversity score (Williams and Humphreys 1994).

SPANNING-SUBTREE LENGTH

This methodology is based on a measure of the total amount of a classification that is represented in a given biota and, hence, is related to a common measure of phylogenetic diversity (Faith 1994; Williams and Humphreys 1994). In this case, the intervening nodes along a cladistic path are counted as a surrogate for path length, although the validity of this methodology has been strongly questioned (Faith 1994). These node counts are then converted to percentages of the total score for the entire classification. An extension of this method takes into account the actual cladistic divergence between species in a given biota to account for particularly evenly distributed species ("cladistic dispersion"; Williams and Humphreys 1994).

Phylogenetic Diversity

Measures of phylogenetic diversity were developed to introduce ideas and results from systematics into a conservation framework (e.g., see Engstrom et al. 2002 for work on endangered turtles). These measures are calculated using a mathematical function of the number of lineages implied by a phylogenetic tree, in order to discern the total amount of a classification that is represented by a given taxon or biota.

Faith (1994, p. 50) considered *phylogenetic diversity* to be a subset of species diversity and defined it as the "sum of the lengths of those branches from the estimated phylogeny that are spanned by the species subset." Say, for example, that one wishes to increase the phylogenetic diversity of a protected reserve. A subset of taxa on the preserve has a given phylogenetic diversity, and hence a logical question would then be "How much more diversity would be gained by introducing another taxon?" Species that are phylogenetically distinct should offer the largest contribution to the overall diversity of any biota. Hence, changes in branch length gained through adding an additional taxon provide a straight-forward approach for examining this question. Faith (1992a, 1992b, 1994) provided a variety of formulae for calculating these measures.

Molecular Advances

Recent advances in biochemical and molecular methods for determining phylogenetic relationships have provided new information that has changed our understanding of the phylogenies of several squamate groups, for example, the agamids and gekkonids of the arid Palearctic region (Macey et al. 1998, 1999, 2000) and the geckoes of Kazakhstan and northeastern China (Macey et al. 1997, 2000). A particularly useful method is *flow cytometry* in which cells (or chromosomes) suspended in a fluid medium are passed through a beam of light. The particles cause the light to scatter and may fluoresce. Detectors pick up and analyze the fluorescent emissions and the deflected and transmitted light, determining physical and chemical properties of the particles and sorting them. Several thousand particles per second can be analyzed and sorted with this technique. In addition, it does not require that specimens be sacrificed, making it especially useful for the study of rare or endangered species. It also reveals complexes of cryptic species. Data obtained with this technique can be presented as a phylogenetic tree or cladogram. Murphy et al. (1997) have outlined its use in biodiversity surveys. Also, see MacCulloch et al. (1996), who used this technique in their studies of *Anolis* lizards.

Reconstructive Biogeography

Reconstructive biogeography is a term used to describe the process of reconstructing taxonomies based on either morphological or genetic patterns (i.e., phylogeography) under the premise that contemporary geographic distributions of species are a function of historic processes or events related to population bottlenecks, immigration/emigration, and population expansion/contraction. Most often, both current molecular techniques and biogeographical histories are used simultaneously to construct species' origins and spread over time (for example, with poison frog species; Santos et al. 2009). Similar techniques are used to investigate fossil faunal assemblages (Smith 1994). In this instance, the number of taxa that could occupy the "morphospace" of a given size category is estimated based on the existing fossil record for a given geographic area. The occurrence of forms is recorded, and formal morphological character analysis is used to construct phylogenetic trees that permit the description of phylogenetic diversity in recent as well as fossil reptiles (Rasskin-Gutman and Buscalioni 2001; Ciampaglio et al. 2001; O'Keefe 2002).

Making Inferences Based on Monitoring Data

Making valid inferences from monitoring data depends on a number of factors, including the following:

1. *Project design*. Few things are more frustrating to a biometrician than being asked to consult on a project after the data have been collected only to find that the study was so poorly designed as to preclude any valid conclusions. All studies, particularly those that will involve monitoring over time, require specific statistical designs; otherwise, complex questions cannot be answered nor can practical management decisions be made.
2. *Scale*. The scale of a given habitat (e.g., microhabitat vs. macrohabitat) can be an exceedingly important issue to consider when designing a biodiversity study, particularly if target species are using available habitat resources at different scales seasonally or ontogenetically. Consulting the literature and other experts in the field is paramount to establishing the best sampling regime.
3. *Spatiotemporal considerations*. Monitoring data are inherently temporal, because a major goal of any monitoring project is to detect change (if any) over time. Thus, knowing when to sample so that the data collected are relevant to the research questions being asked is particularly important. If forecasting or time-series analyses (analyses of data, of which time is a dominant variable, taken at specified time intervals) are to be used, sampling periods must be equally spaced, a requirement that must be accommodated in the research plan (Bowerman and O'Connell 1993;

Fuller 1996). Relatively few ecologists consider the spatial nature (through space or time) of sampling, automatically assuming that all replicates are valid if they are carried out at different locations. The possibility of nonindependent samples (e.g., spatial correlations) cannot be ignored, however, and independence of different locations must be tested before data can be used as replicates in analyses. All ecologists involved in analyzing data from field studies should become familiar with the basics of spatial statistics theory (e.g., Isaaks and Srivastava 1989; Goovaerts 1997) and application (e.g., Fortin and Dale 2005).

4. *Statistical analyses and interpretations*. We highly recommend that investigators seek advice from a statistician familiar with ecological sampling designs and analyses before initiating a study. In addition, reports and publications must be written in such a way that even readers who are not well versed in statistics will understand that statistical significance does not necessarily mean that the results of a study contain useful biological information. Conversely, managers, journal editors, and others must recognize that the absence of statistical significance does not mean that a study and the data it encompasses are not important to the issue at hand. All researchers must be honest in their data gathering and reporting, even if a study does not warrant a peer-reviewed article. Additionally, researchers must be cautious when extrapolating beyond the limits of their data, which can be extraordinarily misleading and, in the worst case, provide impetus for short-sighted management decisions.
5. *Database management*. Heyer et al. (1994b), in their recommendations for amphibian-monitoring studies, suggested that monitoring data need to be stored, managed, and shared. We fully agree and strongly urge everyone involved in monitoring to store their data in a consistent way and to include metadata that describe the study, sampling techniques, and sampling locations in detail. This will allow future researchers to design comparative studies for use in potential meta-analyses. Without consistency over time and place, biodiversity monitoring may provide very little comparable information.

Analyzing Biodiversity Data

Biodiversity data should be analyzed using known, validated techniques. We do not mean to suggest that the development of new techniques is unimportant. Rather, adherence to methodologies that can be replicated and that are valid for answering specific research questions should be employed so that collected data are consistent and comparable over time and space.

Certain minimum data should be gathered and maintained as part of all monitoring studies. These include dates; exact location(s), preferably including corrected GPS coordinates; times of sampling; descriptions of techniques used to acquire data; and statistical sampling design. In many cases, it will be necessary to preserve voucher specimens, which must be adequately prepared, labeled, and stored so as to ensure their long term utility and availability for later verification of identification and research (see Chapter 6, "Voucher Specimens"). Noss (1990) has discussed the use of indicators for monitoring biodiversity.

We do not recommend any minimal set of statistical (or other) analyses that must be carried out on all data sets. The key is to make all data available in a usable format so that they can be easily retrieved and used over time. Individuals designing and carrying out monitoring (or other) studies will identify their own research questions and sets of pertinent analyses. Again, we urge the use of known, replicable techniques selected with the assistance (advice) of a statistician.

Computer Programs for Analyses of Biodiversity Data

Below we list selected computer packages that we have found to be useful for analyses of biodiversity data. The list is not exhaustive. We recommend these programs only because we have found them useful; we do not endorse their use or imply their superiority to other programs.

Biodiversity Analyses

BIOTA

Biota is an extremely flexible database management package (Colwell 2007). It is especially useful for managing collections as well as records and images.

ESTIMATES

Investigators can use this program (Colwell 2009) to compute randomized accumulation curves, a variety of diversity indices, and indices of similarity between samples, as well as to estimate richness.

RAMAS

This modeling program (Applied Biomathematics 2007) is useful at the landscape scale. It can be used to build predictive models of extinction, population growth, and other population parameters. It is quite useful for predicting trends through time based on vital rates.

WORLDMAP

This user-friendly software package (Williams 2001) is designed to explore geographic patterns in diversity at any spatial scale. It has been used extensively for assessing taxonomic diversity (e.g., Loiselle et al. 2003; Manne and Williams 2003).

Taxonomy and Phylogenetics

DELTA SYSTEM

Delta System is an integrated set of programs for formatting and managing taxonomic databases for use by other programs (Dallwitz 1980).