CHAPTER 2

An overview of sampling issues in species diversity and abundance surveys

Scott A. Bonar, Jeffrey S. Fehmi, and Norman Mercado-Silva

2.1 Introduction

Homer S. Swingle was a pioneer in pond management and professor of fisheries at Auburn University. Results from his scientific research on the principles of fish interactions in lakes and ponds, and methods to improve fish production are used worldwide, and he is credited with improving lives of countless people by increasing the amount of food available to those in developing countries and providing recreational opportunities to a multitude of anglers. His advice was sought by US presidents, prime ministers, the United Nations and countries across the globe (Byrd 1973).

His biological work was groundbreaking, yet there is little evidence that Homer Swingle used sampling procedures when conducting his work. His major works contain no reports of sampling error with his estimates (Swingle 1950, 1952) and his contemporaries have pointed out that he actively avoided subsampling in favor of census. His methods for enumerating a population were simple. At the conclusion of his pond experiments, he would census every fish involved, either by draining or treating the pond with piscicide (fish poison). Being a student on Swingle's piscicide crew meant counting *every* dead fish as it floated to the water's surface in the hot Alabama sun over a 3-day period. Those involved in the third-day pickup were the unluckiest, and often the least senior members of the crew—a smelly task indeed!

While a complete census of all experimental or survey subjects provides the most complete information, few of us have the luxury of counting every subject in a population to estimate population characteristics. It simply costs too much, takes too much time, or is infeasible in some other way. Therefore we have to sample a portion of the population to estimate diversity or population parameters such as abundance. For example, a marine biologist estimating the number of clams in the Gulf of California cannot hope to count every one—he will expand an estimate calculated from a sample of plots. A plant biologist measuring the biomass of grasses in a field will not weigh every grass blade in the field—she will estimate the biomass through a sample of plots containing grass. A wildlife biologist will not count all mice in a national park to determine relative density of different species. Density will be determined by a subset of captures from different areas.

To ensure the most useful information, a biologist must be familiar with basic sampling issues. Here we discuss some issues that all biologists should know when sampling diversity, abundance, or other parameters. Consideration of these issues allows the biologist to sample in a manner that, while not a perfect reflection of the population, will provide the best representation of the true population as possible.

2.2 State of the field

Successful sampling of plant and animal communities address similar basic issues. In the following sections we discuss considerations that every sampler should understand before beginning a survey.

2.2.1 Setting objectives

Critical to any good survey are clear objectives. These objectives should answer the fundamental questions for the survey. What is needed from the survey? How will the survey data be used? If the sampling design or methodology becomes confusing and complicated, returning to the objectives of the survey can help clarify what needs to be done. Deviating from the objectives of the survey may make the information obtained from sampling much less valuable for answering the original questions.

Explicit objectives can improve the study design. A poorly worded objective for a survey might be: 'Estimate the effect of outflow from the Brandon Chemical Plant on freshwater clams.' More precise would be: 'Estimate the effect of outflow from the Brandon Chemical Plant on abundance, growth, and species diversity of freshwater clams.' Wording objectives as specifically as possible will allow biologists to design a survey that will meet those objectives.

Objectives that drive plant and animal sampling efforts include estimating conservation status, the potential effect of human action or inaction on communities, or, perhaps the most common, comparing a trend in a site over time to adapt management.

There are several other considerations when sampling diversity or abundance which help to define study objectives more clearly.

2.2.2 An important partner: the statistician

Just as a business executive needs the services of a good lawyer and a good accountant, a biologist needs a good statistician. Go to any academic library and scores of books on sampling techniques are available. Which methods should be used to design a survey? Any biologist should have some background of sampling techniques, which may suffice for simple surveys. However, consultation with a good statistician can dramatically improve the sampling design. Finding a good statistician is like finding a good mechanic. The best ones are incredibly busy, and the biologist will probably need persistence to encourage their involvement in a study. However, they can make the difference between a failed study and a study that can provide the most information for the lowest price. Which are the best statisticians for the biologist? They tend to be those who have on-the-ground, practical experience with biological studies in addition to a solid statistical background. One talented statistician at a Pacific Northwestern University in the USA had a solid knowledge of his subject, and had also been a commercial salmon fisherman and an active participant in field projects. The biologist could be assured that this individual would help to design a study that met the statistical requirements, but was also practical and logistically possible.

2.2.3 What species to sample

When sampling plant or animal diversity or abundance, a decision must be made as to which species to sample. At a recent meeting of the Ecological Society of America, Pulitzer Prize winning ecologist E.O. Wilson stated that a person could conceivably spend their entire life studying the species found associated with one tree stump. There are literally thousands of species, from bacteria to mammals, that make their home there—a staggering level of diversity. Practically, there are many species in any given area, so which do biologists sample?

Study objectives can help to identify the species that should be studied and the methods required to study them. Typically there is more interest in some species than others. For example, in plants, sampling may be structured around plants associated with the abundance of food and habitat of an important wildlife species, invasive plants that impact desired plants, or rare plant species of special conservation concern. Plants with neutral or little impact on the survey objectives tend to be discounted. For animals, species of special conservation concern can also be overall drivers of the study, such as those on threatened and endangered species lists. Other species might be indicators of ecological conditions such as the presence of Plecoptera (stonefly) or Chironomidae (chironomid) larvae in streams of varying water-quality conditions. Still others might be important species that drive a community, such as the most abundant picivorous (fish-eating) and insectivorous (insect-eating) fish of a lake community that make up most of the biomass.

Not only species must be considered when sampling, but life stages and size as well. For plants, larger perennial plants are much more likely to be detected than smaller ephemeral plants. (Plant scale differences can be dealt with by changing the plot size as discussed later) (see also Chapter 3). For animals, one must cover all the areas where the different life stages of a given animal (in the case of abundance estimations) or all species (in the case of the estimation of diversity) might be. This may require the use of different sampling methodologies for each stage or require that sampling efforts be carried out in different seasons or at different times of the day. For example, to detect the presence of *Salvelinus confluentus* (bull trout) in Pacific Northwest streams, juveniles are most often sampled near the substrate at night because this is when they are most commonly seen (Bonar et al. 1997). The sampling design and methods for animals with large habitats (or ranges) will necessarily be different from those of sessile animals or animals with very low motility. The size of the species of interest can affect the sampling. It will take a much larger plot to sample a grove of *Sequoiadendron giganteum* (sequoias) than *Bromus tectorum* (cheatgrass).

2.2.4 Where to sample

Where should a biologist sample if he or she wants to know something about an underlying community or population? At first glance, this sounds like a simple question, but it can be complex. Below are tips to help the biologist select from where the sample should be taken.

The *target population* is the population or ecological resource of interest. For example, the target population might be all sea urchins in the waters off San Juan Island, Washington, mule deer on the Kaibab Plateau, or plant communities of the northeast Siberian coastal tundra. The *sampling frame* is the physical representation of the target population. It represents what or where the samples are taken from to characterize the target population. For the above examples the sampling frame might be the littoral zones of the San Juan Island from the shore to 20 m deep at high tide, the Kaibab Plateau, or the montane and lowland tundra distributed between the Laptev and Chukote Seas, respectively.

For best results, the sampling frame should mirror or represent the target population as closely as possible. For example, say the biologist's objective was to estimate the proportion that each aquatic macrophyte (vascular plant) species contributed to the total macrophyte biomass in Lake Taupo, New Zealand. Because most macrophytes will be found in depths of less than 15 m, a sampling frame consisting of the littoral zone up to a depth of 15 m should adequately bracket the community. However, if the sampling frame consisted only of sampling sites less than 1 m deep, it would not adequately encompass the target population of macrophytes. Sampling in waters less than 1 m deep would potentially overestimate the proportion of shallow water emergent plant species biomass in the entire community and would not meet the research objective.

Sometimes the sampling frame is obvious. The area bounds are established along jurisdictional or management boundaries as specified by the project funding (e.g. Saguaro National Park, Posey County, Indiana, the island of Barbados, or the site of the new subdivision). In some situations defining the sampling frame can be challenging. Perhaps an estimate of *Cancer magister* (Dungeness crab) density is needed for the Pacific Ocean off Washington State, USA. Three boundaries of the sample frame are easy to determine: the frame is bounded on the south at the Columbia River at the Oregon border, at the north at the middle of the Strait of Juan de Fuca on the Canadian boundary, and at the east by the waterline of the Washington coast. However, defining the western limit of the sampling frame, how far offshore to sample, is less clear. *Cancer magister* are not found in the deepest abyss, but become less dense in progressively deeper water. It would make no more sense to sample *C. magister* in a deep ocean trench than selecting sites containing no people (such as a wilderness area) when surveying human population characteristics. Using the biological characteristics of the *C. magister* (it is rarely found in water deeper than 180 m) one can set an outer depth limit to the sampling frame.

The sampling frame may be restricted to where sampling gear can be used effectively, even though

the organism may be found outside the frame. For example, a common method to sample freshwater fish in lakes is electrofishing, but electrofishing cannot generally be used to capture fish in water deeper than about 3 m. The sampling frame for electrofishing sites in lakes would include segments along shorelines up to 3 m deep, not transects in deep offshore areas where there would be little chance of catching fish by this method. Often the samples taken from such a frame may not reflect the entire community diversity or abundance, but can provide an important index for trends or similar work.

Because the sampling frame can vary among studies, the frame must be defined and reported in any report or publication. This is especially important if other biologists are not familiar with the type of survey and cannot assume its sampling frame.

2.2.5 Bias, sampling error, and precision

A biologist known by one of the authors wasted 2 years of his working life and 4 years of a study, costing his project sponsors thousands of dollars, because he did not know the fundamentals of sampling procedure. The biologist was tasked with studying how littoral (shallow water) fish populations changed following reduction in aquatic plant communities. He was to record the abundance of various fish species in the littoral zone for 2 years before the plants were removed and 2 years following. The 2 years of samples prior to plant removal had already been collected by others using springtime electrofishing. The biologist had other tasks in the spring, so he decided to collect his electrofishing samples in the autumn. Unfortunately, unknown to the biologist, this was a fatal flaw for his study. Different species and sizes of fish use the littoral zones of lakes in the spring and in the autumn. The sampling conducted pre treatment in the spring could not be compared to the sampling conducted by the biologist post treatment in the autumn because the effects of plant control could not be separated from the effects of seasonal differences in fish use of the littoral zone. The biologist failed to consider bias. Bias, and also the concepts of precision, sampling error, and accuracy should be known by all who collect samples.

The terms sampling bias, precision, sampling error and accuracy are all related to the fundamental question in survey design: 'How well does the sample estimate parameters of the target population?' *Accuracy* is the closeness of agreement between an observed value and an accepted reference value (Locke 1994) and is a function of how much *bias* and *sampling error* is in the sample. Bias and sampling error are often confused, but they are different.

A sample is biased when, to some degree, it does not represent the population from which it was taken. Bias can be subdivided into measurement bias and sampling bias. *Measurement bias* occurs when measurements are taken incorrectly. Perhaps the biologist was sampling lizards and did not set traps correctly, so the abundance of lizards was underestimated. Perhaps a poorly trained crew recorded birds seen in only a portion of the area of interest, biasing the study towards those birds found only in that portion. Perhaps a fish-measuring board started at 5 mm instead of 0 mm, so all fish lengths were overestimated. These are examples of measurement bias, which can be reduced by careful crew training and ensuring sampling equipment is properly calibrated and working correctly. *Sampling bias* occurs when the sample does not include all groups of interest in the population. Stated in another way, this means that a sampling method does not capture all organisms equally. Every sampling method has inherent sampling biases that can affect estimations of animal diversity and abundance (Willis & Murphy 1996; Krebs 1999; Southwood & Henderson 2000)

Sampling or method-specific biases need to be understood to adequately estimate the accuracy of animal diversity and abundance values. Sampling can be biased by organism size. A net used in estimations of fish diversity or abundance, for example, can have a mesh size that very small fishes can escape through, therefore the net is said to be biased towards larger fish, that is it captures larger fish in a disproportionately greater amount than they occur in the population as a whole, so the mean fish length of the sample will be greater than that of the population. Other methods or a smaller mesh size may be effective for capturing smaller

fish, but they will have their own limitations (Lyons 1986; Mercado-Silva & Escandon-Sandoval 2008; Rabeni et al. 2009). Sampling can also be biased towards particular species. A trawl might capture smelt effectively because of their schooling behaviour in the water column, but *Micropterus salmoides* (largemouth bass), which are associated with the lake bottom and structure, may be harder to capture, therefore the gear is biased for smelt. Depending on the species, the differing amounts of bias present can be difficult or impossible to measure, and present particular problems when evaluating diversity using species proportions (e.g. Shannon or Simpson's index). Those species which are easier to capture can be overrepresented in the indices (see further discussion of this point in Chapter 3).

Size and species bias are also present in plant sampling, as each sampling method is typically better at detecting some groups of plants than others. In most instances, larger and more persistent plants are much more likely to be detected than smaller, more ephemeral, or juvenile plants. Juvenile plants of species judged important can be especially problematic in that they can be difficult to detect compared to adults because all plants are small early in their lifecycle. Plant scale differences can be dealt with by changing the plot size, as will be discussed later.

Bias can make comparisons with the true population difficult, but there are some ways to account for bias in a study. Ensuring crews are properly trained and biologists are familiar with the amount of bias that may occur using a particular sampling gear aids in the evaluation of the importance of bias in surveys.

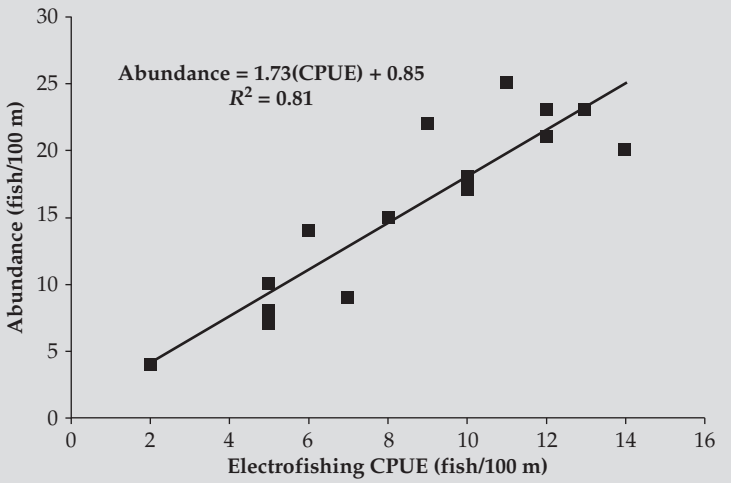
If bias is the same over time (trend surveys) or space (for status surveys) the sample data can be used for comparison or to follow trends. A trawl net could be used to monitor fish population trends in a lake over time, under similar conditions, with the understanding that fewer small fish would be captured than larger fish in each sample, but abundance of all might increase or decrease over time. However, if a biologist uses a gill net one month to catch fish and the next month uses electrofishing for trend monitoring, the bias would be different for the two gear types and the samples could not be compared. The biologist would not know if trends in the data were due to the actual changes in the population or just differing gear bias. Similarly, if trawling was conducted in very different habitats, or in different seasons of the year when fish behaviour varied, the biologist would not know if differences in capture rates were due to how well the trawl fished in the different habitats or time periods, or in the actual numbers of fish present. This is the error the biologist made in the example above. The bias of the electrofishing surveys was not the same in the spring as in the autumn in the lake in question.

If the objective is to capture a diverse sample, a combination of methods should be used to cancel out the bias of the different sampling gears as much as possible. This should be tried to collect a wide range of (1) species with different habitat use, (2) species with different behaviour, and (3) different life stages of a given species with a specific size and ethology. The sampling methods selected should cover all possible niches that exist in an ecosystem. Lethal methods such as the application of poison or explosives in fish studies, although they are destructive, may in some cases be the only alternatives to fully sample a community. Still, even these may fail at capturing certain elements of the community (e.g. fish eggs resistant to poison effects, individual fish that die but cannot be captured).

Perhaps the best methods to correct for sampling bias involve double sampling while surveying or conducting preliminary studies that calibrate gear, that is evaluating sampling gear *efficiency* for the studied species under a wide variety of conditions (Peterson & Paukert 2009; Chapter 3). In double sampling a randomly selected subset of samples from the overall survey are sampled twice: once using the sampling method and then using an unbiased population estimator, such capture–recapture for species richness or abundance (Williams et al. 2002) or an occupancy estimator for species detection (MacKenzie et al. 2002). The 'true' population estimates are then regressed against the samples and correction factors are developed for samples in the survey that were not double-sampled (Box 2.1). Gear efficiency studies are more labour intensive than double sampling as they use abundance

Box 2.1 An example of correcting for bias and estimating precision

Micropterus dolomieu (smallmouth bass) are collected using backpack electrofishing at 50 block-netted sites to estimate their mean abundance in a stream. To correct for sampling bias, a randomly selected subset of 15 sites is selected for double sampling. In each of the double-sample sites, *M. dolomieu* catch per unit effort (CPUE) and an unbiased population estimator (capture–recapture estimate) of abundance are determined. Next the abundance estimate is regressed against CPUE to calculate a correction for all samples.



All 50 samples are then converted from CPUE to estimated abundance using the regression equation developed above. How precise is the mean abundance of *M. dolomieu* in this stream? The mean abundance and associated standard deviation of the 50 converted samples can be calculated using formulas from any standard statistics text or computer software. Notice that there is still variability that was not captured during the conversion of CPUE to estimated abundance. More variability could have been accounted for by developing a more labour-intensive gear calibration model, accounting for the effects of habitat complexity and other factors.

estimates obtained through capture–recapture or removal methods (which can have bias associated with them as well) to calculate the efficiency of the gear in a separate study before the survey. Gear efficiency depends on species-specific attributes such as capture avoidance, size, patterns of aggregation, and habitat complexity. Gear efficiency studies help to determine the true rarity of a given species, as some sampling methodologies, being relatively inefficient at capturing a certain species, may give the false idea that a species is less abundant than it actually is (Lyons 1986; Bayley & Herendeen 2000; Longino et al. 2002; Chapter 3). If gear efficiency is not calculated, sometimes a lower threshold of gear efficiency can be used, which is based on pilot surveys or the literature. Bonar et al. (1997) assumed a lower threshold of 25% snorkelling efficiency for encountering the *Salvelinus confluentus* (bull trout) that were present in Pacific Northwest streams. This was used in the calculation of sample sizes needed to detect *S. confluentus* if they occurred in actual densities of less than 0.15 fish/100 m of stream. Even if no correction is used for bias, it is important to understand the limits of sampling methods being used and create correction factors between sampling methods. These could be used to calculate method bias that should be accounted for in data interpretation.

Sampling error is the inverse of the amount of *precision* in an estimate. Unlike sampling bias, sampling error is easily measured, quantified, and reported as *variability*, *standard deviation*, *standard error* or some other measure of dispersion. For example, an estimate is needed of fish density in a lake. A trawl net that samples a specific volume of water is used to catch the fish. Fish are spread evenly throughout the lake, and on six sequential trawls 5, 6, 4, 5, 6, and 3 fish are captured, respectively. The average or mean number of fish per trawl sample is 4.8. Here the biologist may have a lot of confidence that the true mean of the fish population available to trawling (excluding any bias) is close to 4.8. Sampling error is low (0.48) and the sample is precise. If the fish are distributed in clumped groups throughout the lake, the six sequential trawls may capture 2, 11, 0, 14, 0, and 2 individuals, respectively. There is more 'spread' or experimental error in the later estimate (with an error of 2.48) and the average (4.8 fish/trawl) calculated from this estimate is less precise. The biologist is less confident that this average represents the actual population mean, again assuming there is no bias.

One way to illustrate precision and bias is by reference to targets (Fig. 2.1). The bull's eye of the target is the true population parameter. For the first target on the left, a tight cluster of samples to the right of the bull's eye is precise (the samples are tightly clustered) and biased (the samples are not centred on the target but are off to the side). A loose cluster of samples to the right (second target) is imprecise and biased. A loose cluster of samples in the middle is imprecise and unbiased (third target). A tight cluster of samples in the centre of the target is precise and unbiased (fourth target).

Now we know our objectives, what species we will sample, where we will sample, and some of the factors that can affect sampling surveys, such as bias and precision, we will discuss how to sample and how much to sample.

2.2.6 How to sample

Nets, plots, traps, bird-calling surveys, dredge samples: what can be used to quantify the animal or plant population? Below we discuss important considerations about selecting gear types or plots when sampling. Often previous studies or reference books of sampling techniques (e.g. Bonar et al. 2009b) can be used to choose an appropriate method for sampling the species of interest.

The minimum size unit in sampling is called a *sampling unit*. For animal surveys, the sampling unit could be a location for a pit-fall trap set, a transect for sighting birds, or a shoreline length where all frogs are captured. For plants, the sampling unit is often a plot. A subset of sampling units is drawn from the sampling frame in a way to ensure the subset represents the area of interest. Units to be sampled within a sampling frame are usually chosen *randomly*, which means that every sampling unit has an equal chance of being selected. They can also be assigned *systematically*, which means using a random starting point and taking a sample at every *n*th unit. Some type of random allocation of sampling units is almost always preferred, but systematic sampling can be equivalent to random sampling if the ordering of the individuals is independent of the attribute being measured. Descriptions of various commonly used sampling designs, such as simple random sampling, stratified random sampling, systematic sampling, adaptive sampling, and cluster sampling, are provided in sampling texts (Cochran 1977; Williams et al. 2002; Thompson 2004; Scheaffer et al. 2006; Bonar et al. 2009b).

Researchers must be careful to adopt sampling methodologies that will minimize the effects on individuals, populations, and habitat. The humorist Don Novello wrote a comic letter to NASA, which was testing for life on Mars in the mid-1970s by burning a small sample of Martian soil and testing for carbon residue. Novello wrote 'That doesn't mean there *is* life on Mars—that means there *was* life on Mars—You killed it!' In the past, it was common to sample animal populations using destructive techniques. For example, toxicants were widely used to sample fish communities in a variety of ecosystems. Today these methodologies are often discouraged, although they are still in use for a variety of management purposes (Bettoli & Maceina 1996). Techniques that destroy habitats (e.g. bottom trawling for fishes or marcoinvertebrates [Freese et al. 1999]), change animal behaviour, or hurt

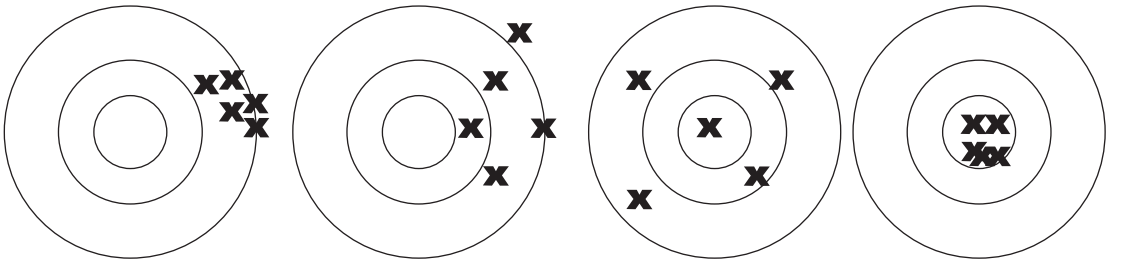


Figure 2.1 Depiction of bias and precision, with centre of target representing true population value. Far left figure shows sample that is biased and precise, sample on second target is biased and imprecise; sample on third target is unbiased and imprecise; sample of far right target is unbiased and precise.

animals (i.e. gill nets [Murphy & Willis 1996]) should also be carefully considered before using. When possible, observation and other sampling techniques that do not involve the taking of individuals may be good options to estimate the diversity and abundance of certain species, especially those easy to identify. However, sometimes it is necessary to take a small number of individuals to provide data to protect the population as a whole.

Pilot surveys are small studies carried out before the initial survey, and are an indispensable component of most surveys. They can be used to help identify the species present, the scale at which organisms occur, and the heterogeneity of populations for sample variance estimates in sample size calculations. For example, in *S. confluentus* surveys, Bonar et al. (1997) recommend that an informal snorkelling survey is used to document presence before extensive time and resources are spent designing an expensive statistically intense study for the reach. Bonar et al. (1993) used pilot surveys to calculate the sample size needed to adequately estimate biomass of aquatic macrophytes.

For animals, motility is perhaps the most important factor that determines which sampling method to use. Sessile or low-mobility animals are usually detectable by a variety of active sampling methodologies and cannot easily evade detection. Although it is clearly possible to capture mobile animals using active methodologies, animals with moderate to high mobility can, and often will, attempt to evade being captured by active methods. Passive methods (e.g. attractants, traps), observation (e.g. direct observation, distance sampling), or techniques based on animal signs (e.g. food remains, tracks, scats) are often better suited to mobile animals. See Bonar et al. (2009b), Murphy and Willis (1996), and Krausman (2002) for various techniques to sample fish and wildlife populations. Other references such as Hauer and Resh (2006), and Sørensen et al. (2002) discuss sampling invertebrate populations.

For plants, plots are usually the unit sampled, and the biologists must choose a plot shape and plot size. Square, rectangular, and circular plots, and transects (a one-dimensional rectangle (Pueyo et al. 2006)) have all been used to sample diversity. Squares and rectangles can be the easiest to delineate in the field because strings or tapes can be stretched between the corner points. Narrow rectangular plots are often recommended because they can capture more patchiness within each plot, although this will depend on the elongation of the plot and the homogeneity of the site. Circular plots for surveying plants can be difficult to set up in areas with trees and shrubs because plots are usually marked by attaching a string to the plot centre and moving it around in a circle. The movement of the string radius is obstructed by plants above the surveyor's height. Larger plots capture more different landscape elements and plant associations, but lose fine detail. This can be corrected by measuring small and large plants at different scales using nested plot designs (Fig. 2.2), including the Whittaker plot (Shimida 1984) or the modified Whittaker plot (Stohlgren 2007). More methods on determining the appropriate size of the plot will be discussed in the section on how many samples to collect below. See Stohlgren (2007), Bonham (1989),

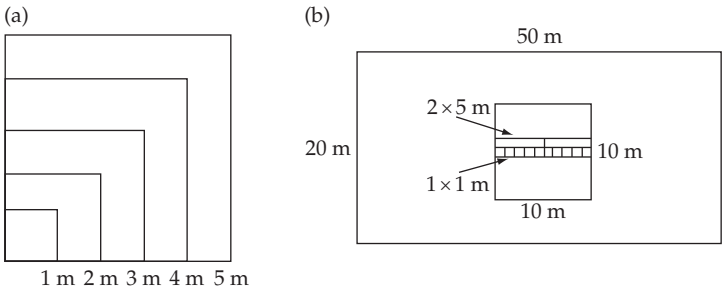


Figure 2.2 Two common nested plot designs. (a) A simple geometric progression of plot sizes typical of a pilot study. (b) A Whittaker plot (Shimida 1984), which is commonly used as well as numerous published modifications (Stohlgren 2007).

and Elzinga et al. (1998) for various techniques used to sample plants.

Detecting rare or elusive species sometimes requires specialized sampling designs (Thompson 2004). For plants that are rare because they are concentrated in a small part of the area of interest (a clump or cluster), use two-stage sampling (e.g. Elzinga et al. 1998). In two-stage sampling, the plot is searched by walking, driving, or remote sensing until the clump of rare plants is discovered, and then the clump is subsampled. Adaptive sampling for plants and animals is similar (e.g. Smith et al. 2004). Here biologists search for a species of interest at predetermined locations and if the species is found, they sample nearby. To search for rare plants, Poon and Margules (2004) recommend stratifying a region of interest using environmental variables and then noting in which strata populations of rare species are known. New searches are then concentrated in similar environments in the same general location or in other geographical areas.

2.2.7 Quantifying the sample

When a sample is taken, what will the biologist measure to quantify abundance and diversity? A measure of abundance is important because diversity is not the same as species richness and depends on the relative commonness and rarity of the species present. There are many options, for example the number of organisms, their weight, the amount of area they occupy, or their presence. Which of these factors are measured depends on the objectives of the study.

Abundance measures tend to be more problematic with plants than with animals because plant growth can exhibit considerable plasticity under different environmental conditions and plants commonly have both asexual and sexual reproduction strategies. With animals, abundance measures are made difficult because of movement, different life stages and sizes, and ontogenic changes. Common choices for measuring abundance include density, biomass, and cover (usually for plants). These data allow calculation of the many diversity indices currently available. Commonly used ones include Simpson and Shannon (Stohlgren 2007).

Density is the number of individuals by species per unit area. The number of individuals is simply counted within each plot. For animals, direct counts are often not possible and a method such as catch per unit effort, mark–recapture, removal technique or distance sampling is used to estimate the animal density in an area (Murphy & Willis 1996; Buckland et al. 2001; Krausman 2002; Bonar et al. 2009b). Sometimes counts of animals in an area are so great that subsampling for density (e.g. zooplankton (Karjalainen et al. 1996)) or using biomass measures are easier (e.g. zooplankton, marine fish (Ware & Thomson 2005)). Some plant types do not fit well into counting density (sod-forming grasses, multi-stemmed shrubs, clonal trees, etc.) because determining which part represents an individual is a daunting and time-prohibitive exercise. Without digging up roots to look for a connection between individuals, it can be impossible to assess observationally which plants are individuals. This problem expands as the total area covered by these plants

increases within the area of interest. While each situation is somewhat unique, a rule stating that individuals with known clonal propagation must be greater than a fixed distance apart to be considered individuals (e.g. for bunch grasses a separation of more than 10 cm) will allow data collection despite the uncertainty.

An enticing but less useful alternative is gather frequency data (presence or absence) for each plot and then attempt to convert it to density. While this has had some success in the field (see Bonham (1989) for plants), it remains overly dependent on the assumption that the underlying distribution of organisms is random. However, for plants, as the plot size reaches or exceeds the size of a plant of interest, the density data collected are essentially frequency data, which can add complexity to the analysis. Determining if the plant is in the plot or not can sometimes be difficult because of the plant's plastic growth form. A typical rule is that if an individual plant's basal area is more than half in the plot and rooted in the plot it counts as being in that plot.

Measuring areal coverage is usually conducted for plants and sessile animals such as sponges (e.g. Lauer & Spacie 2004) and coral (e.g. Gardner et al. 2003). Again, because of the growth plasticity of plants, areal coverage measurements can sometimes be problematic. Using cover mitigates some of the difficulties with collecting density as well as being more or less independent of the scale of the plant in terms of the time needed to collect data for a plot—individuals do not need to be enumerated. Cover has been successfully used in many studies of diversity but it can make comparison between studies difficult because small differences in procedure can produce large differences in the data (see the section below). In addition to procedural differences, many vegetation protocols only advise measuring the surface layer of the cover, which discounts or dismisses those species that occur beneath the canopy. This gives disproportionate weight to plants dominant at the time of observation. In some systems, the aerially dominant plant is relatively fixed throughout the season, as mentioned above, yet in other systems it varies from week to week throughout the growing season, which would change the diversity measure based simply on the time of sampling rather than from more substantive differences. A simple fix for this difficulty with measuring cover is to observe the cover of all species independently of one another, but this takes more time and cannot be done with remote sensing or photography because it requires evaluation of each layer of cover. Remote sensing data can give information valuable to diversity studies in that the vegetation alliance can often be identified, but species other than some large plants and invaders with unique phenology cannot be usually identified (Gillespie et al. 2008). Remote sensing can also help with plot stratification and offer support for inferences about the extent and grain of the field sampling.

Directly measuring the biomass of animals is usually a straightforward procedure and can vary from simple to time-consuming depending on the study. Individuals are weighed (either wet or dry weight depending on the study) and summed for the sampling unit; a subsample from the unit is weighed and a mean obtained that is multiplied by the number of individuals in the unit, or the group is weighed in mass. For plants the biomass option can be very time-consuming because rather than observationally collecting these data, plants are typically harvested, sorted, bagged, oven dried, and weighed.

Conversion between the different measures of abundance between animals can often be conducted through regression or other types of analyses when animals of the same species have similar morphology. The plastic growth of plants (where individuals can mature in a range of sizes spanning orders of magnitude) makes conversion between different measures of abundance problematic. However, there are limited situations where the individual plants are similar enough to make cover density and biomass well correlated. Only evaluation of the actual conditions at the time of data collection can help to determine if a conversion between measures of abundance is well advised.

2.2.8 When to sample

Determination of diversity and abundance in animal communities is heavily influenced by seasonality, time of day in which a sample is taken, and the reproductive stage of a given taxon. Sampling efforts should occur during a season which allows for most taxa to be susceptible to being

captured. This includes considerations of migratory behaviour. Similarly, species' diurnal/nocturnal movement cycles should be taken into consideration when planning sampling procedures. Often, entire assemblages are most detectible at a given time of day. It is at these times when samples should be taken to maximize species richness.

Timing is also a very important consideration for plant studies. Each growing season, the range of plants, their density, their distribution, and even their size can be different. Species can colonize or be extirpated from the area of interest in short periods of time. Even within a growing season, the presence of perennial plants waxes and wanes, while this pattern defines the lifecycle of many annuals and ruderal perennials. Within the herbaceous zone, switches in dominance are not uncommon as the season progresses, despite relatively stable plant populations. Some plants mature early and others later. A similar change can occur in woody communities, where dominance changes can seem sudden between seasons as later succession species in the community overtop those that arrived earlier. In deserts, plants can remain dormant in the seed bank for years until suitable growing conditions occur. An overarching statement about the appropriate time period in which to observe diversity is not possible but instead depends on the within- and between-year site variabilities and how they relate to the larger question.

2.2.9 How many samples to collect

The size of sampling error depends on (1) the heterogenity among sampling units in the sample frame and (2) the number of sampling units observed/collected (sample size) (Groves 1989); therefore, the more heterogeneity among samples, the more samples must be taken to estimate the parameter with a given degree of confidence. In addition, the more samples taken the better the chance of collecting rare species. Because of these principles, collecting more small samples is usually better than collecting fewer large samples.

The most precise and accurate estimations of animal diversity and abundance come from intense sampling on a system for a long time period. These studies reveal temporal variation of a species' appearance in collections, and have experienced specialists who can identify the collected species and know which species from adjacent areas might appear in their collections (Longino et al. 2002). More commonly, diversity or abundance studies are of limited duration or geographical scope, and need to employ efficient sampling methodologies that can provide accurate estimates with small sample sizes.

Sufficient sample sizes are obtained in different ways, depending on study objectives. Sometimes sample sizes are previously set based on previous studies. For example, in plant studies, no matter how large the plot size, at least five independent sampling units are recommended to allow enough plots to capture the variability (Stohlgren (2007) recommends seven). In other studies sample sizes are calculated based on the accepted level of error in the estimate and the variability of the parameter estimated through a pilot study, previous study, or, sometimes, an educated guess. The cost of sampling can be a factor in decisions. If developing 99% confidence intervals for an estimate requires 100 000 samples, a biologist might have to accept 95, 90 or even 80% confidence intervals for the estimate if it only requires 30–100 samples and does not substantially affect survey objectives. Surveying references provide overviews of how to assign samples and calculate sample sizes for means, totals, and proportions using common sampling designs, including simple random, stratified random, cluster, and systematic sampling (Bonham 1989; Elzinga et al. 1998; Magurran 2004; Bonar et al. 2009b).

Advances in computer science and mathematics have resulted in substantial progress in the development of monitoring designs for trends that allow the biologist to maximize power by allocating samples over space and time based on the variance structure of initial samples (e.g. King et al. (1981), Gibbs et al. 1998, Urquhart et al. (1998), Urquhart & Kincaid (1999), and Larsen et al., (2001, 2004)). Software programs such as MONITOR (Gibbs 1995) and TRENDS (Gerrodette 1993) are available to help the biologist allocate samples and maximize power in order to detect trends.

Species accumulation curves to estimate the amount of sampling needed for diversity sampling have recently seen increased use. Here, plot size or the number of samples taken is recorded on

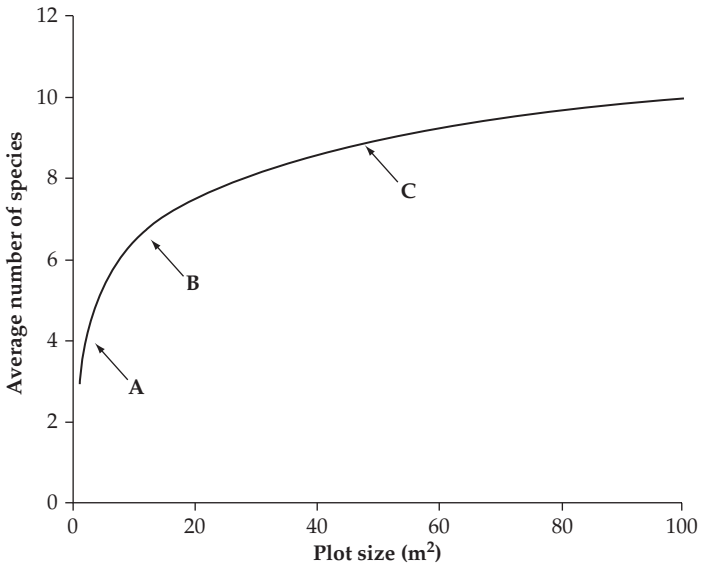


Figure 2.3 A example of a species area curve from nested square plots sampling perennial plants in the Sonoran desert, Arizona in 2007. If the plot area increases from 1 to 10 m^2 (point A), the plot captures twice as many species. Once the plot size increases to 20 m^2 (point B), the plot has doubled in area but only captured one additional species. As the area of the plot continues to increase (point C), the number of new species increases only very gradually as rare species are encountered. An efficient plot area in this example might be 20 m^2.

the *x*-axis and the number of species collected is recorded on the *y*-axis (Fig. 2.3). Increasing the number of samples or plot size initially has a dramatic effect on the number of species encountered (A). However, as more samples are taken or plot size is increased, the curve flattens where more sampling does not result in many more species collected (B). It is at the point where the curve levels that the number of samples to be collected or the plot size is optimized (C). Species accumulation curves

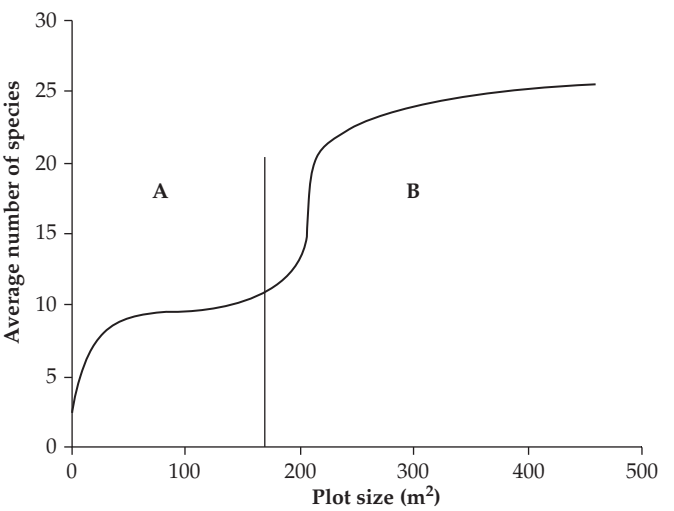


Figure 2.4 A theoretical species area curve for a nested plot as it increases in area. The segment marked A is the traditional species area curve with the line flattening as all common species have been captured and occasional rare species are encountered. As the line enters segment B, the line shows the plot expanding out of the current vegetation association into a different type. For example, in the Sonoran Desert, Arizona, this could be moving from an upland site into riparian vegetation or onto a slope. If a large enough plot could be censused, this stair-stepped pattern would be repeated as the area increases to encompass all of the vegetation associations in the area of interest. In practice, the part of the site represented in segment A would be stratified and sampled separately from that in segment B.

for nested plots occur at different scales and can be more complex (Fig. 2.4).

Given the difficulty of detecting rare species in assemblages, and the limitations that exist in carrying out field surveys to estimate biodiversity, it is often necessary to use statistical techniques that can help in determining the amount of effort that would be necessary to capture the entire diversity. Three methods for estimating species diversity are (1) fitting a statistical distribution to rank abundance data, (2) extrapolating a species accumulation curve to its asymptote, and (3) estimating the asymptotic number of species with non-parametric estimators (Longino et al. 2002). For individual-based (abundance) data, the area under a fitted, lognormal abundance distribution has been used to estimate the total number of species, including undetected rare species (Chao et al. 2009). Other species abundance models such as the log-series, geometric, negative binomial, Zipf–Mandelbrot, and the broken-stick (Magurran 2004) can also be fit to abundance data to estimate asymptotic species richness. Curve-fitting methods, which can be applied to both abundance data and incidence data, extrapolate a fitted function such as the Michaelis–Menten equation or a mixture model out to the asymptote of the species accumulation graph (Soberon & Llorente 1993; Colwell et al. 2004). Other parametric and non-parametric methods are useful in the estimation of biodiversity in a species assemblage (Chao et al. 2006; Chao et al. 2009).

2.2.10 Comparing information from different surveys

The objective of studies on the diversity or abundance of animals is often to compare these metrics through time or from one area to another. Conversion techniques have been developed for comparing data collected using different techniques (e.g. Scheiner et al. 2000; Peterson & Paukert 2009), but these are often labour intensive and can introduce additional error. Methods used at different times or in different areas can also be standardized (Bonar & Hubert 2002; Bonar et al. 2009a,b). By using the same methodology in each sampling effort, it is possible to eliminate the variability introduced by modification of sampling methods, although validation still needs to occur to relate the standardized sample to the true population. Standardization not only refers to the equipment used or how it is used, but also to other aspects of sampling such as timing of sampling, the habitats that are sampled, and effort. Care should be exercised to make data from independent collections as comparable to one another as possible. Standardization is useful even in cases when large-scale time or space comparisons are not the focus of a given study. Today, many databases exist that compile information from a variety of independent studies that were carried out using standard techniques. These often serve as a basis for large-scale studies. Standard methods for animals such as fish (see Bonar et al. 2009b) have been developed, but standard methods of sampling plant diversity have been difficult to develop (Stohlgren 2007) and general methods must be modified for each unique set of questions, abiotic-, biotic-, and budgetary-realities.

2.2.11 Preparing for the field

While objectives are being set, and the survey design developed, there are additional tasks to consider. Often a permit from a natural resource management agency will be required to carry out sample. Always plan enough time to obtain the permit. For example, obtaining federal or state permits to sample endangered species in some areas of the USA can take up to a year, and because diversity or abundance surveys are often used to quantify a species in peril, long wait times can be the norm. After permits have been obtained, the appropriate landowners and natural resource agency personnel should be notified to inform them of specific dates when sampling will take place. If notification does not occur, law enforcement personnel are often called out to the site to investigate the 'suspicious activity' occurring. Field sampling is frequently conducted in remote locations using specialized equipment. Project logistics and the salaries of surveyors are usually the most expensive parts of any project. Checklists should be used to ensure that all field equipment is ready and loaded before the survey, and contingency plans (such as having a spare set of equipment available) for when equipment failure occurs should be developed to

avoid unnecessary expense. When surveying a field site, it is important to move quickly and accurately, minimizing unnecessary breaks and ignoring minor discomfort to complete the site on schedule. Above all, the emphasis should be on safety. Surveys should not be continued if dangerous weather conditions develop, sampling equipment poses a risk, or other conditions associated with the sampling are compromised.

2.3 Prospectus

Proper planning of surveys and consideration of fundamental issues such as bias, accuracy, and precision can help ensure a useful, valid study (Box 2.2). Biologists who initiate surveys without considering the basics of survey design risk embarrassment and the outlay of considerable funds with few results. Those who carefully plan their surveys from the start will provide information that can advance science, influence politics, or shape laws and policies.

Box 2.2 Guidelines for sampling

- Develop clear, detailed objectives for the survey.
- Define the sampling frame—select what, where, and how to sample.
- Seek advice from statisticians when designing surveys.
- Consider how bias and sampling error will affect estimates. Correct for bias (calibrate sampling) if possible and maximize numbers of samples collected and allocate samples in time and space to reduce sampling error.
- Incorporate some form of randomization when selecting samples.
- Chose methods to sample that minimize impact to the organisms studied.
- When possible, sample using standard techniques so results among studies can be compared.
- Incorporate time and planning for obtaining sampling permits and conducting safe surveys, and develop contingency plans for when things go wrong in the field.
- When in doubt about survey design or logistics, refer back to survey objectives.

Comparison across large regions and communication among diverse researchers are becoming increasingly important, therefore the adoption and development of standard sampling techniques will play even a greater role in the future, similar to the standard methods already developed for climate science, water chemistry, geology, and medicine.

With the advent of technology, analysis procedures, and automation, many sampling procedures will become easier over time. However, the basic considerations will remain the same. A fundamental knowledge of basic sampling issues will help those surveying for biological diversity, no matter what tools are available in the future.

2.4 Key points

1. Set clear objectives for surveys, with a carefully defined sampling frame.
2. Design the survey, seeking help from a statistician when needed. Define when, where, and how to survey, using methods that minimize mortality to surveyed organisms and incorporate some form of randomness.
3. Precision and bias affect the utility of sampling estimates. Account for the effects of bias by using the same techniques over time to monitor trends, using a variety of gears with different bias to cancel species-related bias for point estimates, or, the best way, by validating sampling techniques with true population parameters. Maximize precision by increasing sample sizes and careful consideration of sample allocation over space and time.
4. Pilot surveys can help define the sampling effort required and identify the logistical challenges the main survey will face.
5. Sample using established standard sampling methods when possible to maximize the comparability of the data among studies. Ensure safe surveys and use field etiquette, including obtaining sampling permits in advance, to maximize survey efficiency.