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Proper survey methods for research of aquatic plant ecology and management

JOHN D. MADSEN AND RYAN M. WERSAL*

INTRODUCTION

Although there are well-established methods used by foresters, land managers, and plant ecologists to collect data on diverse stands of plants in the terrestrial environment, the same standardization has been largely lacking for aquatic plant communities. Commonly-accepted methods not only lead to repeatability, but also assist in defining what is being measured when communicating results across the country. Standardized methods are of great utility in surveying and monitoring for operational aquatic plant management programs, as well as for research on the effects of aquatic system manipulation and management.

Many management programs are satisfied with qualitative surveys and simple mapping, which provide some basic information but do not allow for statistical analysis of intra- and interannual trends, or assessment of treatment effects. The purpose of this paper is to present an overview of quantitative survey techniques for the distribution and abundance of aquatic plant communities for the purpose of management and research.

Why quantitative surveys? Most monitoring projects and a number of research projects utilize subjective assessments to assess plant communities and plant management operations. These subjective surveys might be a site- or plot-wide ranking, grading, or score without any replication. Even a visual percent cover or percent control estimate without any replication makes it impossible to perform statistical assessments. However, these subjective methods introduce a level of bias into the results, even among the most careful researchers. Additionally, subjective methods are difficult to repeat and results can vary from researcher to researcher; for instance, 90% control to one researcher might only be 60% to someone else. Therefore, four reasons to utilize quantitative assessments are: 1) quantitative data are objective measurements rather than opinion; 2) quantitative data can be subjected to rigorous statistical analysis that provides an authoritative answer to whether there is a difference in treatment, changes over time, or significant changes in a plant community; 3) quantitative data can be repeatable although collected by different observers; and 4) quantitative data can be utilized by individuals other than the observer for statistical comparison and analysis (revised from Madsen and Bloomfield 1993).

*First Author: Research Biologist, U.S. Department of Agriculture, Agricultural Research Service, Exotic and Invasive Weeds Research Unit (USDA ARS EIWRU), University of California–Davis, Department of Plant Sciences, Davis, CA 95616; Second author: Aquatic Plant Scientist, Lonza Water Treatment, 1200 Bluegrass Lakes Parkway, Alpharetta, GA 30004. Corresponding author's E-mail: jmadsen@ucdavis.edu.

Scientific method and hypothesis testing. Most resource managers, and many scientists for that matter, have neglected the basic elements of the scientific method. Many are content with simply making an observation, thinking that an observation is an authoritative statement by an expert. An observation, however, is only the beginning. All scientists should have a healthy skepticism of observations, whether their own or by others. The observation merely serves as a starting point to developing a testable hypothesis, followed by designing and conducting an experiment that generates quantitative data, and an analysis of data in which statistical tests are used to decide whether the hypothesis is accepted or rejected. Each of these steps is critical to the scientific method (Figure 1). Therefore, generating objective quantitative data from the experiment for statistical analysis is critical.

Sampling design and statistical analysis. Sampling design is a critical aspect of surveying or data collection that should be considered carefully because it serves as the basis for the remainder of the project, and will profoundly affect the outcome. Sampling designs can be categorized as completely random, stratified random, random–systematic, and systematic designs (Figure 2). Completely random sampling can be employed in areas with a homogeneous environment to avoid sampling bias. Stratified-random sampling is most amenable when there is a known environmental gradient (or strata) that is known to be significant. For instance, if the hypothesis is that depth is a significant factor in distribution, then equal numbers of sample locations could be randomly located within known depth strata (e.g., 0 to 1 m, 1 to 2 m, 2 to 3 m). Random-systematic methods ensure that the selected point is randomly located, but avoids clustering points unevenly. A systematic method, such as a regular grid of points, might be the easiest to set up and follow, and can be used when there are no underlying patterns of environment as yet known. Current Geographic Information Systems (GIS) can allow designing and deploying any of these sampling designs, when used in conjunction with modern Global Positioning System (GPS) receivers.

Two extremes should be avoided in designing sampling regimes: undersampling or oversampling. Undersampling results in having inadequate data to statistically separate treatments. Oversampling results in more data than is necessary, at the cost of labor and effort in the field. The best way to avoid these extremes is to take a preliminary sample and conduct a power analysis to ensure that an adequate number of samples are collected (Madsen 1993, Madsen and Wersal 2017). For instance, highly variable biomass or extremely rare plants will require more sampling to resolve than more homogeneous plant growth with

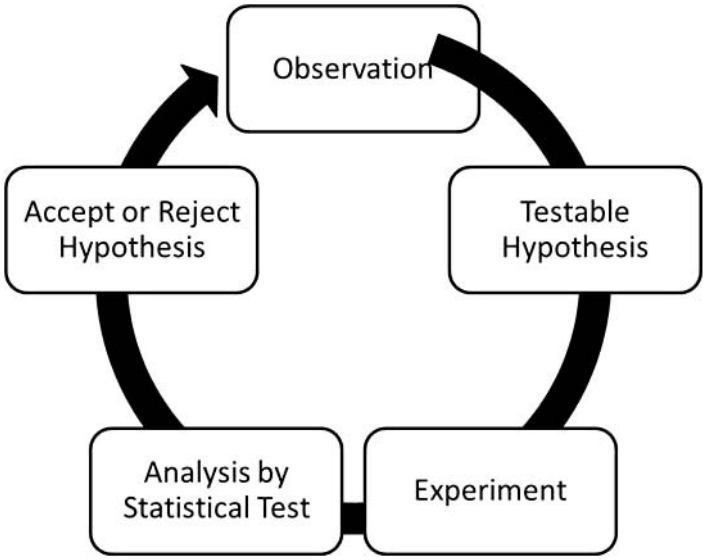

Figure 1. Scientific method progresses by making an observation, developing a testable hypothesis, designing an experiment to test the hypothesis, conducting the experiment, and analyzing the data statistically to accept or reject the hypothesis. Accepting (more correctly, not rejecting) or rejecting the hypothesis often leads to a new hypothesis.

common plant species. Sampling requirements are discussed further for biomass (Madsen 1993) and point-intercept sampling (Madsen and Wersal 2017) elsewhere.

RESEARCH-GRADE METHODS

Basic and applied ecology search for patterns of the distribution and abundance of organisms related to the environment, seeking to explain these patterns by interspecific interactions or environmental drivers. Techniques for collecting data on distribution and abundance are substantially different, in part because classic abundance is measured by either biomass (collecting all of the living material per unit area) or density (counting all of the individuals per unit area), and these techniques are tedious and difficult to do across an extensive area, especially in an aquatic environment. In contrast, distribution data is collected at many more sites by techniques that are much less intensive. Both approaches provide an important insight into plant communities, both in managed and unmanaged sites.

Distribution methods

As stated above, methods to measure distribution and species composition are typically much less labor intensive, and can be employed over a larger area than the typical abundance measures. The most common of these are subjective surveys, semiquantitative surveys, quadrat methods, line intercept, and point intercept (Table 1).

Subjective surveys. In subjective surveys, the observer selects a plot or site and wanders over the site, recording species observed. This is useful mostly for getting a list of species and in searching for rare, threatened, and endangered species. Some individuals add a notation of how widely

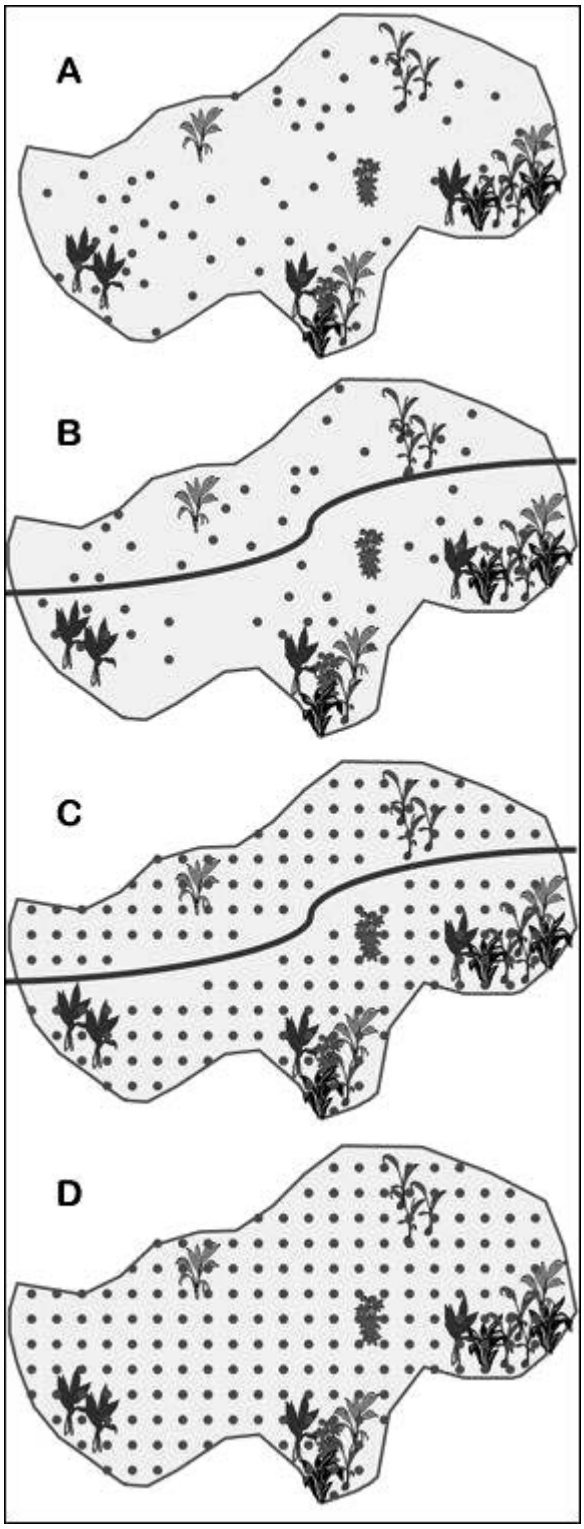

Figure 2. Sampling designs illustrated in a simulated lake, with points indicating sample locations. A. completely random, B. stratified random, C. random–systematic, D. systematic. Each sampling design has strengths and weaknesses, which make them amenable to specific experimental designs or situations (adapted from Madsen and Wersal 2017)

Table 1. Field survey and assessment methods for quantitative assessment of aquatic plant communities, with notes on applications and limitations.[1]

Technique	Measures	Sample Unit	Type of Plants	Application	Limitations	Reference
Distribution Methods						
Subjective estimates	Species composition	Plot or area	S, F, E	Observation, species list, RTE	No statistical evaluation	Poore 1955, Daubenmire 1968, Mueller-Dombois and Ellenberg 1974
Semiquantitative	Species composition and relative distribution	Plot or area	S, F, E	Observation, species list, RTE	No statistical evaluation, interobserver variation	Daubenmire 1968
Quadrat percent cover	Percent cover distribution	Quadrat unit area	S, F, E	Occurrence, percent cover	Cover estimates are subjective	Grieg-Smith 1983, Madsen et al. 1996
Line intercept	Percent cover distribution	Transect interval	S, F, E	Occurrence, percent cover	Quantitative cover	Grieg-Smith 1983, Madsen 1999
Point intercept	Percent cover distribution	Point	S, F, E	Occurrence, percent cover	Quantitative cover	Madsen 1999, Wersal et al. 2010
Abundance Methods						
Quadrat biomass	Plant dry weight per unit area	Quadrat	S, F, E	Abundance	Labor-intensive	Madsen 1993
Rake	Plant weight per unit rake pull	Hard-tine rake	S	Abundance	Moderately labor-intensive	Johnson and Newman 2011
Box corer	Plant dry weight per unit area	Large dredge	S	Abundance	Labor-intensive	Madsen 1993, Madsen et al. 2004
Dredge (Eckman, Ponar)	Plant dry weight per unit area	Small dredge	S	Abundance	Labor-intensive	Madsen 1993
Corer	Plant dry weight per unit area	PVC or metal coring device	S, F, E	Abundance	Labor-intensive	Madsen 1993, Madsen et al. 2007
Density	Number of individuals per unit area	Quadrat, dredge, or core	S, F, E	Density	Labor-intensive, difficulty in identifying an "individual"	Center and Spencer 1981
Propagule density	Number of propagules per unit area	Quadrat, dredge, or core	S, F, E	Density	Labor-intensive	Woolf and Madsen 2003, Madsen et al. 2016
Nondestructive Methods						
Abundance class	Classification of abundance	Rake	S	Abundance	Semiquantitative; not consistent between observers	Yin and Kreiling 2011
Plant height	Height or other allometric measure	Ruler	S, F, E	Plant dimensions	Must be compared to biomass subset	Maceina et al. 1984, Spencer et al. 2006
Hydroacoustic	Biovolume	Hydroacoustic	S	Abundance	Statistical evaluation protocol pending	Valley et al. 2006, Sabol et al. 2009, Radomski and Holbrook 2015
Remote sensing	Light reflection and absorbance	Light or energy sensor	F, E	Distribution and/or abundance	Well-used for floating and emergent plants, use for submersed plants limited	Everitt et al. 1999, 2003

[1]Abbreviations: E, emergent; F, floating; PVC, polyvinyl chloride; RET, rare, threatened, or endangered; S, submersed.

distributed each species is within the site (e.g., rare, common, abundant). This technique is not amenable to any statistical analysis. Although not commonly used by research scientists in the United States, this type of survey is employed by scientists studying vegetation of communities in Europe and forest inventories of the western United States (Daubenmire 1968, Mueller-Dombois and Ellenberg 1974). In these studies, the plots are often referred to as relevés, and vary in size depending on the size of plant species or forest layer studied (Poore 1955).

Semiquantitative surveys. Semiquantitative surveys add an element to the simple species surveys above, in which the observer attempts to estimate the percent cover or cover class of the entire plot or area. Although no statistical analysis is possible, some individuals still see the value, particularly if they are comparing plant species distribution before and after management activity. Observers can estimate percent cover visually, or employ cover class groups such as those developed by Daubenmire (1968). Although this presents a numerical estimate, no statistical comparisons can be made, and the values can vary between observers.

Quadrat methods. Quadrat methods have been employed in both terrestrial and aquatic vegetation assessment for many

years. Traditionally, observers used a quadrat or frame of known and constant size and either simply recorded the species present in the quadrat, or attempted to estimate the percent cover of plant species visually within the quadrat (Grieg-Smith 1983). The presence/absence of species within a quadrat is not subjective, so occurrence data can be analyzed with significant confidence. Percent cover estimates, however, are subjective and vary widely between observers or sampling intervals. For instance, Richardson and others (2001) compared digital imaging to line intercept methods and visual percent cover estimates in turf, and found that visual estimates were the most variable. A comparison of data from six divers performing percent cover estimates on permanent quadrats in Lake George determined that cover estimates varied widely between observers, although individuals were consistent in their own cover estimates. The identification of species present in quadrats did not vary between observers (Madsen et al. 1989).

The size and shape of the quadrat can vary, with common shapes being rectangular, square, and round, and size varying from 0.1 to 100 m^2. The quadrat should be easily transportable, and sized to fit the type of vegetation being sampled. Quadrat data is amenable to statistical analysis, whether it is occurrence data or percent cover estimates (Madsen et al. 1991; Madsen et al. 1996). Quadrats have traditionally been deployed in contiguous blocks, along line transects, or at randomized locations (Titus 1993, Getsinger et al. 1997).

Line-intercept methods. What differentiates a line intercept method from deploying quadrats along a transect is that the line intercept method does not use any quadrat. There are two approaches to using a line intercept method: 1) the beginning and end of contiguous cover is recorded along a tape or other measured device, and thus only one or a few species are studied (Grieg-Smith 1983); and 2) the transect is subdivided into segments of equal length, and all the species in each segment are recorded (Madsen 1999). In either instance, the percent of occurrence is equivalent to percent cover.

Point-intercept methods. Point intercept methods have been used in terrestrial plant ecology for many decades, usually distributed by a random walk type method (Grieg-Smith 1983). The application to aquatic plant quantification did not occur until the availability of GIS and handheld GPS allowed researchers to first set predetermined points, and navigate to those points (Madsen 1999). Although points can be distributed in any fashion (as discussed under sampling design), generally a regular distribution of points is easiest for navigation. Point intercept can be used for whole-lake or basin surveys (Madsen et al. 2002) or plot studies (Wersal et al. 2010, Madsen et al. 2015). In general, point intervals of less than 10 to 20 m are difficult to sample, so in those instances line transects or point frames might be preferable. For plot studies, a minimum of 30 points per plot is recommended for adequate statistical power. For whole-lake or basin studies, a minimum of 100 points in the littoral zone is recommended (Madsen and Wersal 2017). Although many researchers have suggested using a semiquantitative estimate of abundance or distribution with point intercept surveys (Hauxwell et al. 2010), the additional time required can slow survey efficiency and complicate statistical analysis. If only plant species presence/absence data are collected, along with water depth at each point, an investigator should be able to collect more than 100 points in a single day, and statistical analysis is not complicated (although if the same survey is repeated multiple times a repeated measures test needs to be used).

Estimating plant abundance

Plant abundance is important for studies of plant management, because the nuisance is typically caused by shoot biomass, and the best method then for estimating effectiveness is to measure the change in abundance (Madsen 1993). Despite the obvious desirability of measuring abundance, relatively few individuals use these methods because they are very labor intensive. Collecting plant samples to sort, dry, and weigh is tedious and produces large amounts of samples to handle. In addition, biomass samples are notoriously variable, so statistical resolution of differences could require a large number of samples.

The size of the sampling device is critically important in biomass studies. Fewer large sampling devices are required for statistical resolution (Madsen 1993), but a study by Downing and Anderson (1985) found that smaller sampling devices actually require a shorter period of time to collect and less labor to achieve statistical relevance than large sampling devices (although more sampling devices need to be collected). Samples should be sorted by species or taxa, and taxa dried separately. Although it might be desirable to separate plant parts into different components, this expands the number of samples to weigh. Samples should be handled consistently, with the same plant components collected for each sample. After sorting and washing, plants should be dried to constant weight, which is usually achieved in 48 h if a forced-air oven is set between 50 and 110 C. If no tissue analyses are to be performed, a higher drying temperature can be used. If tissues are to be analyzed for carbohydrates or other constituents, they should be dried between 50 and 70 C. Four general techniques are used in collecting plant biomass: quadrats, box corer, dredge, and coring devices.

The quadrat is the most commonly used device for sampling aquatic plant biomass, and is almost universally used for collecting emergent and floating plant biomass. A quadrat is a square, rectangle, or circle made of polyvinyl chloride (PVC) or metal that is rigid and maintains a set area. As discussed above, the area is critical to the number of samples collected. Although many researchers use quadrats of 1 m^2, a quadrat this size leads to unmanageably large samples. Generally, a quadrat of 0.1 m^2 is most appropriate for most aquatic plants. A sample of this size typically requires 10 to 20 samples for statistical significance. For emergent and floating plants, a quadrat can be deployed by wading or from a boat. For sampling submersed plants, quadrat sampling requires snorkeling or scuba diving. Although this was common in the 20th century, the issues of liability and long-term health hazards have greatly restricted the use of the self-contained underwater

breathing apparatus (SCUBA) for biomass sampling (Madsen 1993, Madsen and Wersal 2017).

Since the decline in the use of SCUBA, many researchers have looked to various samplers for collecting biomass. A box corer is a very large box-type dredge that must be deployed from a large boat, because it requires a davit arm and winch to position, sample, and retrieve (Madsen et al. 2004). The samples are typically large, and require significant time and labor to sort. The box corer is good at collecting both shoot and root-zone biomass, including tubers or rhizomes. A variety of handheld samplers are also used, including Eckman dredges and the Ponar dredge. These are not as effective at collecting underground biomass, but are consistent in collecting stem material. Although these are deployed from a boat, additional equipment is not necessary for their use.

A variety of coring devices have been used to collect biomass, including commercially-available 5- and 7.5-cm diam sediment corers. A coring device made from 15-cm-diam PVC pipe produces more consistent results for both shoot and underground biomass. These are particularly useful when a significant amount of the biomass is below ground (Madsen et al. 2007). Although the corer has been used across a range of sediment types, the coring samplers are less useful in either flocculent organic sediments or in cobble. With a 15-cm sampler, anywhere from 30 to 100 samples should be collected to find a statistically-significant value.

Density

Density is the measure of the number of individuals per unit area. Density is commonly measured with animal populations, because an "individual" has a readily understood value. For aquatic plants, the concept of an "individual" can be problematic. For some species, a ramet is easily defined—a growing unit that might be genetically identical to others, but maintains a clearly identified plant unit. For instance, the rosettes of waterhyacinth or water chestnut, a culm of flowering rush, or lemma of duckweed are readily identified ramets. On the other hand, many submersed plants have no readily identified ramet (such as Eurasian watermilfoil, egeria, or coontail) in which the stems readily branch and growth is indeterminate. Density as a measure of abundance rarely holds much meaning for these plants, because the size of the individual of a given age can vary greatly. Studies of distinct ramet-forming species provide insight into the population dynamics of vegetative reproduction (Madsen 1991). For instance, ramet-based sampling determined that waterhyacinth responds initially in the spring by increasing ramet density, then allocates more resources to leaf biomass (Center and Spencer 1981).

Demographic studies of propagules can be crucial to understanding the reproduction and spread of species that form distinct propagules, whether those are stem fragments (Madsen et al. 1988), tubers (Nawrocki et al. 2016), turions (Woolf and Madsen 2003), or rhizome buds (Madsen et al. 2016). In these instances, a distinct unit is identifiable that can be counted, and the size of this unit is fairly predictable and measurable. Progressive sampling and tracking of propagule formation can indicate the timing of spread (Madsen et al. 1988), or demonstrate the potential for long-term control (Madsen et al. 2016). Individual samples are collected as with biomass, but the number of propagules per sample are counted.

Nondestructive methods

Given that collecting biomass samples is tedious and labor-intensive, not to mention costly, many investigators have turned to a variety of nondestructive sampling techniques. These techniques can be parameterized with a traditional destructive biomass technique.

Abundance class. The widespread application of an abundance class approach has largely been used for estimating abundance of submersed aquatic plants. The most common approach is to assess the fullness of a rake collection based on the number of tines or depth of the tines covered (Yin and Kreiling 2011). This type of technique has been compared to quadrat-based biomass samples; a strong correlation exists between abundance class and biomass, but the regression line differs by species, time of year, and location. Given this level of variability and lack of comparability to other systems, this method is not recommended because it is too subjective.

Plant height. The most common nondestructive method used for all aquatic plant species is to regress plant or leaf height to biomass, and thereafter measure only plant height (Spencer et al. 2006). A second allometric technique is to measure leaf area, but this technique is much more labor intensive. Plant height can be measured either directly using a pole or ruler, or indirectly by using hydroacoustic readings for submersed plants (Maceina et al. 1984). Plant height is a useful measure when studying species that cannot be damaged, such as rare, threatened, and endangered species, or species of concern.

Hydroacoustic technology. With the advent of sensitive hydroacoustic gear and advanced algorithms, the use of hydroacoustic technology to map submersed plant abundance has become widespread (Valley et al. 2006, Sabol et al. 2009). The most commonly used gear prior to 2010 was a BioSonics hydrophone and implementation of the submerged aquatic vegetation early warning system (SAVEWS) algorithm and program (Sabol and Johnston 2001, Sabol et al. 2009). More recently, much less expensive electronics of a Lowrance unit can be utilized with the data processed through a publicly available SAVEWS Jr. platform (Sabol et al. 2014), or the proprietary BioSonics platform (Radomski and Holbrook 2015). Hydroacoustic identification of species is preliminary (Farrell et al. 2013), so generally biovolume is calculated as total plant abundance, with some speciation identified from rake sampling. Although the integration of hydroacoustic sampling to hypothesis-testing science has been slow, the use of hydroacoustics for operational programs managing submersed aquatic plants has become widespread. While hydroacoustics have great potential for research and operational assessments, what has been lacking to this point is the use of explicit statistical testing to show that there are in fact differences in data sets.

Remote sensing. Remote sensing uses fixed-wing or rotary aircraft, or satellite-borne sensors, to detect differences in

the reflectance of light or light wavelengths. With development of plant absorption spectral signatures, the presence and abundance of vegetation and specific species can be identified. Although remote sensing is widely used for emergent and floating vegetation, confident use of remote sensing for submersed plant applications is still limited due to the problems with light absorption in water. Unless submersed plants are at or near the water surface, their presence or abundance will not be consistently represented.

Data analysis and interpretation

Once distribution or abundance data are collected, a wide variety of analytical approaches can be utilized. The larger the dataset, the more tempting is the prospect of data fishing to "find a relationship." However, the scientific process is to develop a hypothesis, design an experiment, and evaluate collected data to either reject or not reject the hypothesis. Analyses, as much as possible, should be designed into the experiment *a priori*, and not devolve to *a posteriori* comparisons. Two specific analyses to consider as part of the experimental design process are species diversity measures and statistical analyses.

Species richness and diversity. Species richness is simply the number of species in a given sample or site. Species diversity is a much more complex suite of expressions that include the distribution of species, relative abundance, and evenness. A wide variety of measures have been derived for the estimation and expression of species richness and diversity. These measures have been discussed in depth by Peet (1974) and Whitaker (1972). Unfortunately, most of the indices that have been developed are not amenable to statistical comparison. Therefore, the simplest measure of diversity that can be statistically tested is to use the species richness or number of species per sample, which can be analyzed using a parametric statistic such as a *t*-test or analysis of variation (ANOVA) (Madsen 1999).

Statistical analysis. The entire purpose of collecting quantitative data in research is to lead to an appropriate statistical test. The statistical test should be selected as part of the experimental design. Normally distributed parametric or quantitative data, such as biomass, should be tested using a parametric statistic, such as a *t*-test or ANOVA (Madsen 1993). Occurrence data follow a binomial or Poisson distribution, and should be analyzed using an appropriate test, such as a chi-square test (Madsen 1999). However, occurrence data that have been collected using the same sites over multiple surveys cannot be analyzed using a simple chi-square test. These data can no longer be considered independent because the same points or sites have been sampled multiple times. A number of options are available for both types of data for more elegant statistical analysis or mathematical modeling (Cox et al. 2014, Madsen et al. 2015).

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