Chapter 3

Experimental Design

"An experiment is a question which science poses to Nature, and a measurement is the recording of Nature's answer." - Max Planck

In many ways, there is no more difficult aspect to science than the design and execution of a well-conceived experiment. Experimentation lies at the very heart of scientific inquiry; so much so, it would almost seem a rather mundane task. But nothing could be farther from the truth—the design of our experiment, as it turns out, requires us to be both philosopher and mathematician; stalwart logician and inspired visionary. Not an easy role to pull off.

As we have already discussed, the sole purpose of any scientific experiment is to provide us with the necessary data, so that we may test our original contemplations in order to establish some pattern of correlation or causation. Although the scientific method provides us with a stepwise guide to do just that, it is up to us to structure our experiments so that they provide us with objective data of sufficient precision and accuracy that we require to perform our analyses.

The question is, how? Our universe is filled with an infinite number of complex curiosities, any number of which we might seek to describe. But if that be the case, how can we possibly hope to arrive on the proper course of action, perfectly designed to reveal those details? Well, let's begin with the basics first.

Key Concepts

- All scientific experiments can be loosely categorized as either a method comparison, single-element assessment, multi-element correlation, or synthetic analysis.
- The single most helpful guide to experimental design is a thorough review of relevant scientific literature.
- The usefulness of inferential statistics relies heavily on the establishment of sufficient sample sizes and appropriate sampling methods.
- Several standard field methods exist that can be duplicated (or modified) to fit the needs of the researcher, either within predefined boundaries (as a plot method) or without any spatial limits (as a plotless method).

Starting with the Basics of Experimental Design

Of course, the first order of business for any field research program is to define the overall goals of the project; to use our hypothesis to envision what the outcomes of our research should be. It is of great value to consider, in very concrete terms, what the research products shall be, as a way to "reverse engineer" the most appropriate field program specifically designed to deliver the goods.

Although the natural world, in all its complexity, presents us with a limitless smorgasbord of research opportunities, we need not be paralyzed with the enormity of the infinite possibilities before us. To move forward in the planning phase of our experiment(s), it is necessary that we focus our attention on defining just a few, very specific objectives that our research is intended to accomplish. After all, what is it that you intend to demonstrate with your research? This seems like a simple question, but it requires that you think very seriously about what you are most interested to explore in your research, and what you can (and cannot) realistically hope to complete in the course of that research. In essence, the research **objective** is an explicit definition of the critical goal(s) you intend to accomplish using a scientific approach.

When defining our specific objectives, it is critical that we structure them in such a way that we can then create hypotheses to test different aspects of the larger research goal. For each hypothesis we wish to test, we will also have to determine which measures will be necessary (that is, what data we must collect) in order to conduct those tests. Those data, the equipment used to collect them, and the method by which they are collected are all dependent on your chosen experimental method. Thus, it is impossible to design an appropriate experiment without first defining the specific research objective (and the attendant hypotheses) that will ultimately allow the researcher to make sense of the data.

In concept, the preliminary steps in experimental design are very straightforward:

1. Define the larger goal(s) of your research as an objective (or list of objectives).
2. Devise a hypothesis to test a specific assertion, relevant to your research objective(s).
3. Design an experiment that will allow you to test that specific hypothesis.

In reality, this is more difficult to do because you must wade through all the interesting possibilities to explore and settle on just a few, very specific research objectives you intend to accomplish. Then you must logically construct a number of testable hypotheses that are required to provide you with sufficient evidence in support of (or in conflict with) your assertions. And for each hypothesis, you must create an experiment designed for the sole purpose of testing that specific hypothesis.

As an example of how we might first approach the daunting task of experimental design, let us consider shoal grass (*Halodule wrightii*; see Figure 1.5), a commonly occurring species of seagrass that we used as earlier examples in Chapters 1 and 2. Let's say we were interested in studying shoal grass, but weren't quite sure what kind of research project we'd like to perform. Our first step would be to define the objective of our research; that is, to define what we are specifically interested in.

In a very general sense, research objectives usually fall into one of these themes:

- Method comparison
- Single-element monitoring and assessment
- Multiple-element correlation or causation
- Complex synthetic analysis

While a field research program may contain more than one of these themes within the research objective, it is really only necessary (and more common) to have one. Although there are always exceptions, method comparisons and single-element assessments are generally considered to be less challenging (and are therefore more appropriate for early-career researchers). Since we had earlier decided to use shoal grass as the general subject of our field research, let us continue that example to see what sort of research objectives we might be able to define, using these themes as guidance.

Method Comparison

These are projects designed to test the accuracy (or veracity) of existing research methods. The simplest form of method comparison is to merely repeat a preexisting experimental protocol to confirm earlier results as a type of method replication. And although method replication may be lacking in the "sizzle" department, it is the foundational method of science—it provides the confirmation of consistent results and is the best vehicle for reducing any bias and/or error in earlier works.

Of course, the history of science is rife with examples of how new and improved methodologies, either borne from inspired researchers or from ingenious inventors of new scientific equipment and technology, led to a complete revision of what was once held as scientific dogma. Hence, method comparisons that focus on the augmentation and improvement of historic methodologies are always of great value to the scientific community. Occasionally, method comparisons that expand the number of pertinent variables, or the temporal/spatial scope of those methodologies, employed by earlier researchers can also provide valuable insight.

Let's say we were interested in performing a method comparison as the general theme of our objective, and we wanted to use shoal grass. In Chapter 2, we used shoal grass to demonstrate the 30–300 rule, as it applied to the measurement of leaf-blade length and width using a metric ruler with 1 mm resolution (see Figure 2.3). In that example, we were able to show that 1 mm resolution was sufficient for measuring leaf-blade lengths, but was insufficient for measuring leaf-blade widths (according to the 30–300 rule). However, if you wanted to avoid using microscopic analysis of leaf-blade widths, you might be motivated to demonstrate that the use of a metric ruler is a perfectly sound method for measuring leaf dimensions of shoal grass, regardless of the 30–300 rule.

If that is your objective, you would essentially be advocating a comparison between the use of a metric ruler (1 mm resolution) and the use of microscopy (0.1 mm resolution), to see if there were significant differences in the leaf width (*lw*) measurements collected using these two different methodologies. So, now that you have a clear objective, you could formulate at least one testable hypothesis designed to meet that objective:

$$H_o : \overline{X}_{lw-ruler} = \overline{X}_{lw-scope}$$
$$H_a : \overline{X}_{lw-ruler} \neq \overline{X}_{lw-scope}$$

In this example, the mean leaf widths measured with a ruler ($\overline{X}_{lw-ruler}$) could be tested against the mean leaf widths measured microscopically ($\overline{X}_{lw-scope}$) to see if any significant differences exist. Of course, we won't know the answer to that question until we perform the experiment and gather the data. But regardless of the outcome of the statistical test, the answer will fulfill our research objective.

Now that we have our hypothesis clearly stated, our next step would be to design an experimental method that will provide us with the data we need to test that hypothesis. In this example, we would need access to shoal grass specimens, the equipment necessary to perform these measurements, and a clear understanding of what variables we were measuring (in this case, the leaf-blade widths). As you can see from this example, it is absolutely essential to first define the research objective, and then the hypotheses, before we can think about the design of our actual experiments. The same thought process (objective → hypothesis → method) is required for the following themes, but more sophisticated research objectives will naturally require more hypotheses to test, which in turn require more sophisticated methods for data acquisition and analysis.

Single-Element Monitoring and Assessment

These are projects that seek only to quantify (or categorize) the measurement of a single variable over the spatial and/or temporal scale of the investigation, without really engaging in any correlation or causation analyses of those measurements (a scientific endeavor sometimes referred to as "monitoring"). Of course, all monitoring projects require that a sufficient methodology exists for measuring the variable of interest, with appropriate accuracy and precision.

Although most monitoring projects typically measure more than one variable at a given place or time, if these variables are not analyzed with any relational context, the data are simply collected and cataloged as single variables, disconnected from each other and constrained to their place and time of collection. That is not to say that monitoring and assessment projects are not a critical part of field research. On the contrary, they provide the bulk of the raw field data that other investigators may use to provide analyses of complex interactions within the natural system from which the data were initially collected. But the mere collection of data, without much thought to the analysis of those data, serves a very shallow research objective.

Multiple-Element Correlation or Causation

These projects build on the foundations laid by proven methodologies and the acquisition of pertinent field data, all in an effort to connect at least two measured variables (and oftentimes more) and provide the context of their connectivity. The "connectivity" of field data is usually viewed through the prism of **correlation** or **causation**.

Correlation does not require that the researcher fully understand how the variables are connected, merely that the quantity (or quality) of one variable has a measurable effect on another (that is, one variable is dependent on another, by some unknown mechanism). If we had measured two different variables, A and B, and discovered that B was dependent on A, we could state the relationship logically as

$$A \rightarrow B, \quad \text{or} \quad \text{if } A \text{ then } B$$

If we were interested only in establishing the correlation as our research objective, we would not engage ourselves in determining why B is affected

by *A*—it would be enough to simply demonstrate that the two variables are related to each other in a very predictable manner. By contrast, determining causation is a much more challenging task, because our research objective would then require us to determine the pertinent mechanism(s) at work in the natural system, affecting *B* as a function of *A*. Naturally, the hypotheses needed to test correlation or causation are quite different from each other, so our methodologies will be quite different as well (but more on that later).

Complex Synthetic Analysis

A **synthetic analysis** requires that we consider each of the described, complex relationships (preferably as causative) and combine them into a grand unifying concept of precisely how complex systems function, and how each of the variables within that system is connected. Obviously, this is a tremendously challenging task and often requires years (if not decades) of foundational research—comparing and perfecting several different methodologies of measurement, acquiring huge volumes of field data over multiple spatial and temporal scales, and describing the most critical correlations and causative relationships within the overall system.

So daunting is this task that it typically cannot be accomplished using traditional field studies. Instead, we must rely on the construction of numerical models (Chapter 10) to mathematically describe and test these complex interrelationships using high-performance computing resources to "simulate" natural systems in their entirety.

Proper Experimental Design Requires Significant Preparation

At this point in the process of designing our field program, we should have a general idea of the phenomenon we wish to investigate and a preliminary hypothesis we will ultimately test, using the results of our experiment(s). To that end, we should also have made some basic decisions regarding just how involved our research program will be. Do we wish to compare different methodologies, or merely repeat them? Do we want to engage in a monitoring project to gather raw data for separate variables, or will we investigate the connectivity of those variables, using a variety of correlation and causation analyses?

Before we get too far down the road of designing the perfect field program, it is important for us to take the time for an honest assessment of our operational constraints. This will require that we exercise due diligence in our planning and design of the best plan for the limitations we are forced to accommodate. This due diligence will require us to make some early decisions, informed by our thoughtful consideration of

- Literature review
- Data requirements
- Research strategy
- Scope of investigation
- Equipment and facilities needs
- Preliminary site survey(s)
- Sampling effort

Literature Review

Because the process of science relies so heavily on earlier works, it is absolutely critical that any new scientific endeavor include a thorough review of the published literature, particularly as it relates to the specific research objectives you shall pursue in your proposed field program. Just as there is

no sense in re-inventing the wheel, there is no sense in performing research on a particular topic that has already been thoroughly investigated (unless you wish to challenge those earlier findings and/or conclusions).

A competent literature review has the additional benefit of providing deeper context to the topics you wish to explore, and establishes to the scientific community that you have "done your homework" and are fully informed as to the most current research in your particular field of interest. In the planning process, this can be a tremendous boon, as there may be other investigators in your field who can provide advice and inspiration with regard to your choices of the most appropriate objectives, methodologies, and scope of your proposed research.

Data Requirements

These are critical decisions that must be settled very early in the process of designing your experiment(s), as the data collected will determine the fundamental value of your research. As we discussed earlier in Chapter 2, the type of data you collect will determine what sort of numerical analyses shall be possible, especially in the context of statistics. You must also be prepared to make the critical decisions of precisely what variables you wish to measure, taking care to limit your investigation to only those variables that are germane to the objectives of your research. That is a task much more easily said than done, which is yet another reason to review the current body of knowledge on such matters in the scientific literature.

Research Strategy

On a very basic level, the research strategy employed in field studies can be reduced to a simple dichotomy: Shall the proposed research rely on the acquisition of data through passive observation of the natural system, or does the investigator intend to become an active driver of the observed phenomenon by perturbing the natural system, through true experimentation? Typically, observational studies are far easier to perform, simply because it is so challenging to establish the appropriate experimental controls in a perturbed natural system. There may also be some serious legal impediments to manipulating natural systems, as most regional and national regulatory agencies are reluctant to allow "unnatural" changes in an otherwise natural system. However, there are often very specific questions that can only be answered through active perturbation of the system, and monitoring its response thereafter.

Scope of Investigation

It is also critical to recognize that all field research programs are limited by geographic and temporal constraints that shall define the overall scope of the investigation. Every research program must have its beginning and its end, but drawing those boundaries can be a challenging task in itself. Again, you must focus on the geographic and temporal scales that are most relevant to your research objectives and limit your investigation according to those boundaries; otherwise, your research workload will become unmanageable.

It is also important to note that these geographic and temporal constraints not only define the boundaries of the overall project, but also define the boundaries of the actual method(s) employed in the field for data acquisition. In the field, very rarely are we afforded the luxury of **synoptic** data; that is, data gathered instantaneously across a broad geographic range. With the exception of remotely sensed data from aircraft or satellite, field data are more typically gathered from several field stations, located quite distant from each other geographically. Add to this the expiration of time as we transit from one research station to another, and we soon recognize that the scope of our investigation can introduce

significant spatiotemporal bias—the more grandiose the scope, the more significant the bias.

Equipment and Facilities Needs

Accomplishing any objective requires the expenditure of some combination of time, money, and effort; scientific research is no different. Resources that are critical to the research effort must be assessed honestly, and if the budget cannot support the proposed research, there are only three possible solutions: either reduce the cost/scope of the investigation, seek additional funding from alternate sources, or abandon the proposed research. And although the investments required in human capital, equipment, facilities, travel costs, and consumable supplies to conduct the field research are the most straightforward expenses to consider, do not underestimate the costs associated with the analysis phase of the research (which should include any publishing costs as well).

Preliminary Site Survey(s)

If at all feasible, a preliminary site survey is always indicated once the fundamental experimental design has been sketched out. Not only does this allow the investigator to perform a "dry run" of the proposed research in the field as practice, but there are invariably unforeseen complications in the field that will allow for some last-minute revisions to the method, station location(s), or sampling frequency. Preliminary surveys are also critical in defining the sampling effort that shall be required to meet the research objectives.

Sampling Effort

One of the most difficult aspects of any scientific endeavor, but particularly in field research, is determining the minimum required effort for the maximum benefit—deciding when enough data have been collected for the task at hand. In the context of statistics, sample sizes of 30 or more will afford a sufficient number of observations to allow statistical analyses, as a general rule of thumb. However, there is no guarantee that a sample size of 30 is an appropriate subset of the larger population from which it was taken, so any predictions made from small sample sizes are always dubious.

It is far more important to consider whether our subset of data is truly representative of the larger population we are trying to describe in our research. If our data are normally distributed, we can estimate how many samples are "enough" by using the Cochran method, which uses Equation 3.1 to calculate the variance-to-mean ratio

$$n_o = \frac{d_\alpha^2 \cdot s^2}{E^2 \cdot \overline{X}^2} \tag{3.1}$$

as an estimate of the minimum number of required samples (n_o). As we can see from the example given in **Example Box 3.1**, the sample size (n_o) is determined as a function of the mean ($\overline{X}$) and standard deviation (s) of the population being investigated, based on the chosen significance level ($d_\alpha = 1.96$ when $\alpha = 0.05$, from Equation 2.13) and the relative predicted error (E).

Keep in mind that the Cochran method assumes that our data are normally distributed and that the preliminary survey was conducted using random sampling. If our data more closely resemble a Poisson (or any other non-Gaussian) distribution pattern, we can instead use the Krebs method (see Equation 3.2), which defines the minimum number of required samples as

$$n_o = \left(\frac{d_\alpha}{E}\right)^2 \cdot \left(\frac{1}{\overline{X}}\right) \tag{3.2}$$

EXAMPLE BOX 3.1

Determining Sample Size Using the Cochran Method

A preliminary site survey involved 30 measurements of soluble lead (Pb^{2+}) in the estuarine waters near a textiles manufacturing plant. Based on this small random sampling effort, our estimate of the mean concentration of Pb^{2+} was 1.30 nM, with a standard deviation s of 0.89 nM. If our chosen significance level α is 0.05 ($d_\alpha = 1.96$) and we want to make sure our estimate of the Pb^{2+} mean concentration is within 10% ($E = 0.10$) of the true mean,

$$n_o = \frac{(1.96)^2 (0.89)^2}{(0.10)^2 (1.30)^2} \approx 180$$

the Cochran method would indicate that at least 180 random samples are needed for an adequate study of lead contamination in this particular estuary.

If you wish to reduce the number of samples indicated by the Cochran method, it may be necessary to loosen your restrictions on the relative predicted error by increasing the value of E. Reducing the variance s^2, perhaps through the use of better-performing analytical equipment or by improving the collection method, will also reduce the number of samples needed. Of course, additional observations (beyond the initial 30) may also reduce the variance (and therefore reduce the number of samples indicated).

If the Krebs method is used to determine the minimum sample size, we need not worry about whether our data are normally distributed. Although that may at first seem like somewhat of an advantage, it is that same "lack of normality" that makes it more difficult to predict how much data is sufficient with regard to our sampling effort. As a result, the Krebs method usually indicates that a greater sampling effort is needed to offset the inherent uncertainty in data that are not normally distributed (as demonstrated in **Example Box 3.2**).

A much less technical approach is to simply plot the accumulation of new information against the total effort, such as the number of samples taken,

EXAMPLE BOX 3.2

Determining Sample Size Using the Krebs Method

Recall from our earlier example that the mean concentration of Pb^{2+} at our study site was 1.3 nM. If we determined the minimum number of required samples using Equation 3.2,

$$n_o = \left(\frac{1.96}{0.10}\right)^2 \cdot \left(\frac{1}{1.3}\right) \approx 296$$

The Krebs method indicates that 296 measurements of Pb^{2+} are required, instead of the 180 suggested by the Cochran method. This is because the Krebs method assumes that the data are not normally distributed, so it is harder to predict the distribution of nonnormal data (hence the higher indicated sample size).

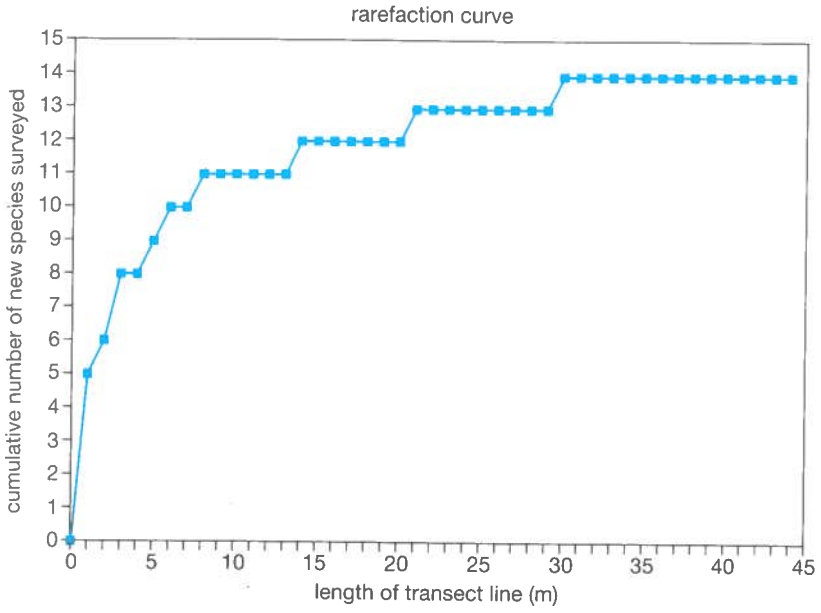

Figure 3.1 A typical rarefaction curve, indicating the accumulation of new information on the *y*-axis, relative to the expenditure of effort on the *x*-axis. In this example, the new information gained is the cumulative number of new species surveyed, and sampling effort is represented by the length of the transect used in the survey. If these data were collected from a preliminary site survey to assess species richness, the investigator could reasonably conclude from the rarefaction curve that there is no benefit to extending the transect lines beyond 30 m. (Adapted from the US Department of the Interior, National Park Service [2001] Virgin Islands National Park Coral Reef Monitoring Manual.)

number of replicates performed, and so on. (**Figure 3.1**). This is occasionally referred to as a **rarefaction curve**, especially in the context of ecological studies. During initial sampling, the slope of the resultant line will be positive and quite steep. In subsequent samples, the gain of new information decreases and the slope of the resultant line will trend asymptotically toward zero. When the slope of the line reaches zero, there is no further gain of information regardless of how many more samples are taken, thus establishing the number of samples needed to sufficiently describe the population under investigation.

Sample Size Determination is the Key to Success in Every Research Endeavor

Determining what is the "appropriate" sample size (n_o) for laboratory and field studies is not a task to be taken lightly. Regardless of whether n_o is determined using the Cochran or Krebs (or any other rarefaction) method, there are four critical factors that must be known to provide the best estimate for n_o:

1. Difference threshold (the magnitude of the difference you wish to test between groups)
2. Standard deviation of the population (for continuous variables only)
3. Relative error ($1 - E$, usually 0.9 by convention)
4. Significance level (α, usually 0.05 by convention)

Dichotomous Variables Can be Used to Determine n_o

Dichotomous variables are those expressed as rates of occurrence or the proportion of a specific outcome. Generally speaking, these are variables that possess only one of two possibilities as a binary condition that are mutually exclusive of each other (like present vs. absent, dead vs. alive, male vs. female). In practice, these can most easily be illustrated by comparing a specific occurrence in an experimental group compared to the control. For example, if we wished to test for the occurrence of death in the control versus a treatment (experimental) group, we could determine n_o by using the proportion of dead specimens in the control (p_c) relative

to the proportion of dead specimens in the experimental group (p_e). Thus, 10% death in the control versus 50% death in the experiment would yield $p_c = 0.10$ and $p_e = 0.50$. According to the Fleiss function (see Equation 3.3), we can determine n_o as

$$n_o = C\frac{p_c q_c + p_e q_e}{d^2} + \frac{2}{d} + 2 \tag{3.3}$$

where $q_c = 1 - p_c$, $q_e = 1 - p_e$, and $d = |p_c - p_e|$. The value C depends on the relative error E and significance level α chosen by the investigator, according to the following table.

$1 - E$	$\alpha = 0.05$	$\alpha = 0.01$
0.8	7.85	11.68
0.9	10.51	14.88

For a relative error of 10% ($1 - E = 0.9$) and a significance level α of 0.05,

$$n_o = 10.51 \times \frac{(0.10)(0.90) + (0.50)(0.50)}{(0.40)^2} + \frac{2}{0.40} + 2 \approx 29.33$$

Thus, a minimum sample size of 30 is indicated for each of the control and experimental groups.

Continuous Variables Can Also Be Used to Determine n_o

Continuous variables are those expressed as values that vary along some continuous gradient or range (like the concentration of Pb^{2+}, or the leaf width of a blade of shoal grass). The most common statistical tests utilizing continuous variables are those that compare group means (although any measure of central tendency would suffice). If comparisons are made between independent groups, sample size can be calculated by the Snedecor and Cochran function (see Equation 3.4) as

$$n_o = 1 + 2C\left(\frac{s}{d}\right)^2 \tag{3.4}$$

where s represents the population's estimated standard deviation and d represents the magnitude of the difference the investigator wishes to test between the two groups (and C is determined in the same manner described by Equation 3.3).

As an example of the Snedecor and Cochran function, let's say we wanted to test the differences in leaf-blade lengths of shoal grass (*Halodule wrightii*) growing in two different regions: one considered to be a pristine coastal region, while the other is influenced by runoff from a nearby river. The sediment load from the nearby river does seem to affect water clarity, so perhaps we'd like to test whether the turbid waters affect seagrass growth when compared to the seagrass at the pristine site (using leaf-blade lengths as our test variable). So how many seagrass blades should we measure?

Recall from our earlier example in Chapter 2 that we have already performed a preliminary survey of shoal grass leaf lengths ($N = 29$) and found $\overline{X} = 131$ mm and $s = 7$ mm (see Example Box 2.6). To detect a 5% difference in the mean leaf lengths between the pristine and affected sites, we employ

Equation 3.4 and use d = (131 mm)(0.05) = 6.55 mm, and C = 10.51 to limit our relative error E to 10%, so

$$n_o = 1 + 2(10.51)\left(\frac{7\,\text{mm}}{6.55\,\text{mm}}\right)^2 \approx 25$$

Hence, we would need to conduct a minimum of 25 observations at each site. Since we've already collected 29 observations from the pristine site, our work there is done; all we need to do now is collect a minimum of 25 observations from the affected site. Since it is always best to use the same number of observations when making statistical comparisons, you would be wise to go ahead and collect 29 observations at the affected site.

Note that if the groups being tested are paired (that is, dependent on each other, such as pre- and post-treatment comparisons), sample size is instead computed as

$$n_o = 2 + C\left(\frac{s}{d}\right)^2 \tag{3.5}$$

An Introduction to Quantitative Sampling Methods

Once we have reached some early decisions regarding our project goals, we must turn our attention to the actual method(s) we shall employ in the field to acquire our data. Although there are many different quantitative sampling methodologies in the literature that we can utilize, a vast majority of them are designed for application in terrestrial systems. Aquatic systems, quite literally, add a new dimension of complexity to any quantitative sampling method, as we must account for **heterogeneity** in both the horizontal and the vertical dimensions (in Super-3D, so to speak).

In the aquatic environment, studies limited to the **benthos** will require very few changes to the traditional terrestrial methodologies that exist, as they are both defined predominantly by horizontal, rather than vertical, variability. However, if a fully three-dimensional sampling regime is necessary to resolve three-dimensional variability, there are relatively simple ways to expand traditional two-dimensional sampling methodologies to include the vertical dimension (which we shall discuss shortly). For now, let us review the classic terrestrial methods to provide the context and conceptual foundation for any modifications we may wish to make to our chosen method.

In addition to the basic question of whether we must consider a two- or three-dimensional sampling regime, it is also important to consider whether we shall employ a plot or a plotless method. **Plot** methods are those whereby the data are acquired from areas (or volumes) bounded by strict geographic boundaries. The main benefit to plot methods is that the variables being measured are inherently relatable in terms of their spatial distribution (such as coverage per acre, or counts per m^3). Although plot methods typically yield data that are most representative of the population from which they were sampled, they can also be quite labor and time intensive to collect.

In contrast, **plotless** methods are those that do not constrain data to arbitrary areas or volumes; rather, data are gathered from discrete points in space or along **transect** lines and are most commonly related in terms of frequency, proximity, or point-to-point distances. Because plotless

methods are not constrained by boundaries, they are often easier to conduct in the field, especially in dynamic regions where establishing semipermanent boundaries may be impractical. However, plotless methods are generally considered to be inferior to plot methods since plotless methods make it impossible to return to the exact same location to conduct repeated measures over time.

Basic Plot Methods

Since the scope of any investigation is limited by geographical constraints to some extent, it shall be necessary for us to define the geographic boundaries of the project as a whole. Unless we seek to perform a complete census of all pertinent measures within the confines of that boundary, we will rely instead on our ability to take multiple subsamples that are representative of the larger population from which they came. By choosing to employ a plot method, we are simply choosing to place spatial boundaries on those subsamples.

For us to be able to relate our data to the true dynamics of the system under investigation, it is absolutely critical that our subsamples are representative of the larger population from which they were gathered. When choosing an appropriate plot method, we must take care to consider all of the following:

- Sampling effort
- Plot shape
- Plot size
- Random sampling

Sampling Effort

Whether or not we have expended sufficient effort in our sampling is largely determined by the number of samples taken (n) for a given measure, for each group or at each site. As we have already discussed, there are a multitude of ways to determine the most appropriate number of samples that are required for a given study (see Equations 3.1–3.5).

Plot Shape

Since data collected from all plot methods are inherently related to the unit area (or unit volume) within the boundary of the plot, it is usually easiest to construct the plot as a regular quadrilateral (that is, a square). Traditionally, most plots are designed as **quadrats**, either square or rectangular in shape, primarily for their ease of use in the field. The regular geometry of the quadrilateral makes it a very simple device to scale by subdividing the quadrat into a number of smaller, constituent quadrats (**Figure 3.2**). Similarly, any quadrat can be used to define a fixed-area grid, containing any number of equidistant points depending on the desired scale of measurement.

However, as an interesting consequence of geometry, it is best to maximize the "area-to-perimeter" ratio of the quadrat, so that irregularly shaped elements are more likely to be contained within the area of the quadrat (rather than falling along the edge of the quadrat). Deciding whether an element should be counted as "in" or "out" of the quadrat can occasionally lead to a positive **bias** in the data, sometimes called the **edge effect**. We can generally minimize this bias by choosing quadrat dimensions that maximize the quadrat area and minimize the quadrat perimeter (edge). This is done simply by using Equation 3.6 to analyze the area-to-perimeter (A:P) ratio:

$$\text{Quadrat } A{:}P = \frac{L \cdot W}{2L + 2W} \tag{3.6}$$

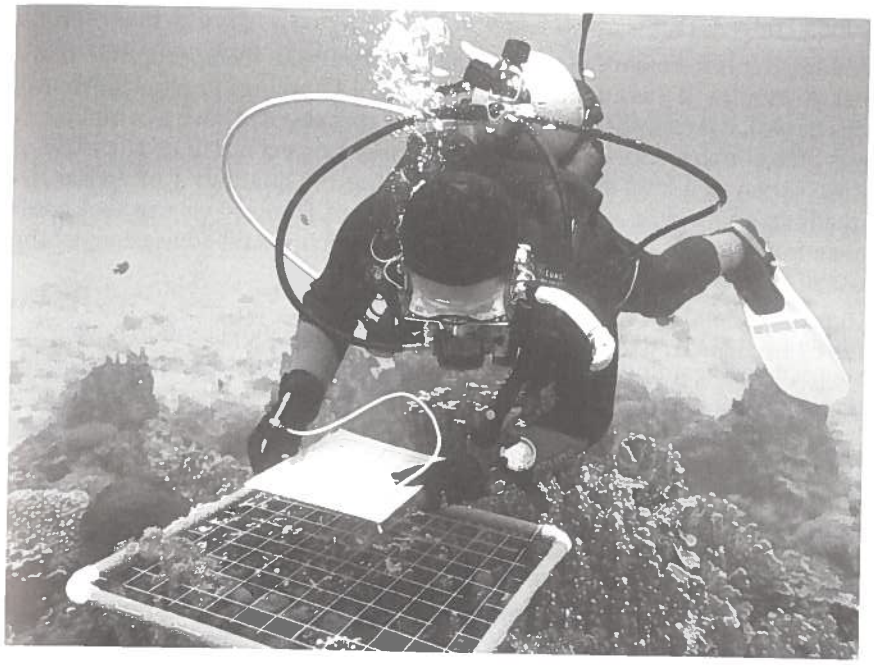

Figure 3.2 A nested quadrat, when placed over the substrate, serves as a versatile instrument for conducting a plc census. This device is especially versatile in the field, as various plot methods can be employed using either the entire quadrat area or a specified number of randomly selected sub-quadrats. Note that the same device can also be used to gather gridded, point-intercept data at each of the "cross-hairs." (Courtesy of the Griffith School of Environment & Australian Rivers Institute, —Coast & Estuaries. Brisbane Australia.)

Using this simple calculation, it is easy to demonstrate that not all quadrats are created equal. As an example, let us assume we wish to employ a quadrat of fixed area, such as 4 m^2. Let us now compare the A:P ratios of a 2 × 2 quadrat compared to a 1 × 4 quadrat:

$$2 \times 2 \text{ Quadrat } A{:}P = \frac{2 \cdot 2}{2(2) + 2(2)} = \frac{4}{8} = 0.5$$

$$1 \times 4 \text{ Quadrat } A{:}P = \frac{1 \cdot 4}{2(1) + 2(4)} = \frac{4}{10} = 0.4$$

Thus, it is always preferable to use quadrat dimensions that yield the highest possible value of A:P (to minimize the edge effect).

For any circle, Equation 3.6 can be rewritten as

$$\text{Circle } A{:}P = \frac{\pi r^2}{2\pi r} = \frac{r}{2} \qquad (3.7)$$

where r is the unit radius of the circle. If we continue with our analysis of the best possible quadrat, we can show that a circle of 4 m^2 area must possess a radius of approximately 1.128 m:

$$A = \pi r^2 \quad \therefore \quad r = \sqrt{\frac{A}{\pi}} = \sqrt{\frac{4\,\text{m}^2}{\pi}} \approx 1.128\,\text{m}$$

If we use Equation 3.7 to analyze our circular "quadrat," we will find

$$\text{Circle } A{:}P = \frac{r}{2} = \frac{1.128}{2} \approx 0.56$$

Because of this interesting geometric relationship, we can demonstrate that it is impossible to improve on the circular quadrat in terms of minimizing its edge effect (thus making the circle the best shape for any size quadrat).

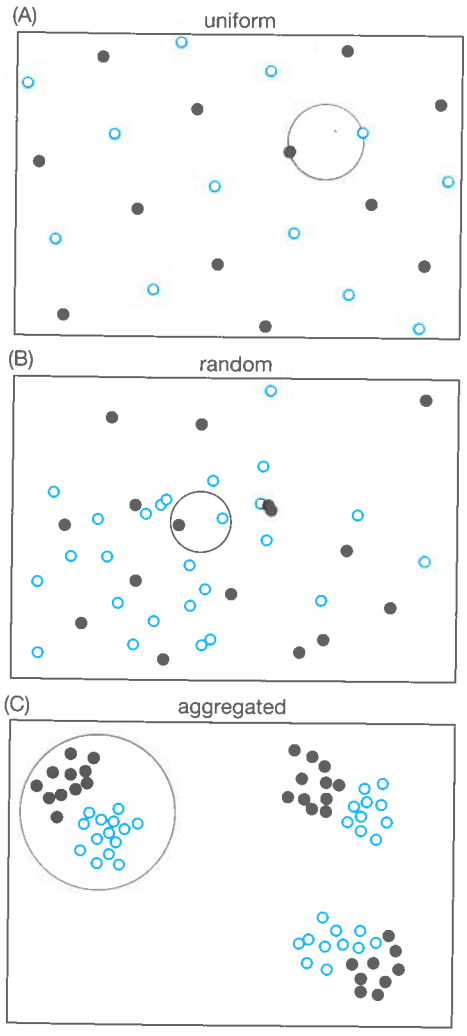

Figure 3.3 Idealized spatial distribution patterns of two different populations, typical of (A) uniform, (B) random, or (C) aggregated distributions. Note that both the uniform and aggregated distribution patterns indicate a spatial relationship between members of population ϕ (open blue circles) and those of population θ (closed black circles), which is somehow associative.

Plot Size

Although it is necessary to independently determine the appropriate number of samples (n_o) as an indicator of sufficient sampling effort (as previously discussed), it is easy for us to recognize that plot size will have a complimentary effect on that effort. For example, we might expect our field data to differ if we surveyed a total area of 100 m^2 by using 100 quadrats 1 m^2 in size, 50 quadrats 2 m^2 in size, or 25 quadrats 4 m^2 in size. Here too we can seek guidance from the 30-300 rule (see Chapter 2), which would advise against the use of 25 quadrats 4 m^2 in size.

Although readers are encouraged to perform a thorough literature review for suggestions regarding the appropriate plot size for their specific study, quadrat sizes of 0.1 m^2 to 1 m^2 are typical for most ecological applications (although it may be necessary to scale the quadrat up or down, depending on the size of the elements being surveyed). If it is possible for the investigator to perform a preliminary site survey, an appropriate plot size can be quickly and easily estimated by using the "3 & 3 rule" (not to be confused with the 30–300 rule).

The 3 & 3 rule simply requires each of three different quadrat sizes to be chosen (as the investigator's "best guess") and used in triplicate to randomly survey the same location. The survey data from each quadrat size are then analyzed by calculating the simple **variance-to-mean ratio** (***VMR***) using Equation 3.8, and the quadrat size that yields the lowest *VMR* is the size that should be selected for all further surveys:

$$VMR = \frac{s^2}{\bar{X}} \tag{3.8}$$

Although the 3 & 3 rule is usually sufficient for most general survey methods, it may not provide sufficient resolution for those studies that seek to establish density-dependent, spatial relationships (such as geographical distribution patterns as a result of competitive exclusion).

To detect these sorts of associative patterns, Sanjerehei presents the simplest general method to determine plot size. According to his work in ecological modelling, Sanjerehei was able to show that the most appropriate circular quadrat diameter ($\bar{d}$) for detecting an association between members of two different populations (ϕ and θ) is dependent on the spatial distribution of ϕ and θ, and can be determined simply as

$$\bar{d} = \sqrt{1/m_{\phi\theta}}, \quad \text{and} \tag{3.9}$$

$$m_{\phi\theta} = (m_\phi + m_\theta) \tag{3.10}$$

where m_ϕ and m_θ represent the population densities of ϕ and θ, respectively, per unit area.

As written, the Sanjerehei method is applicable regardless of whether the members of ϕ and θ are uniformly or randomly distributed (**Figure 3.3A, B**). If members of ϕ and θ instead follow an aggregated pattern (**Figure 3.3C**), certain adjustments must be made when calculating m_ϕ and m_θ. For example, if duplicate instances of ϕ are in contact with or overlap each other, they must be treated as a singlet when determining m'_ϕ:

$$m'_\phi = m_\phi - \left(\frac{\%\,\phi \text{ as duplicates}}{2} \times m_\phi \right) \tag{3.11}$$

If ϕ is present as a significant aggregation, each aggregation must be treated as a singlet when determining m''_ϕ:

$$m''_\phi = \left(\frac{m_\phi}{\text{mean } \phi \text{ per aggregation}} \right) \tag{3.12}$$

Of course, the same analyses (see Equations 3.11–3.12) must also be done to determine m'_θ and m''_θ for the members of population θ as well.

To demonstrate how plot size ($\bar{d}$) is determined using the Sanjerehei method, let us assume that the areas depicted in Figure 3.3 each represent 24 km^2 (4 km $\times$ 6 km). In the example of a uniform distribution (see Figure 3.3A), we have 12 members of population ϕ (blue) and 12 members of population θ (black); thus, m_ϕ = 0.5 per km^2 and m_θ = 0.5 per km^2, respectively. Using Equation 3.10, $m_{\phi\theta}$ is simply 1.0 per km^2. Using Equation 3.9,

$$\bar{d} = \sqrt{1/1.0 \text{ km}^{-2}} = 1.0 \text{ km}.$$

In the example of a random distribution (see Figure 3.3B), the distribution of ϕ and θ is a little more complicated. Although there don't seem to be any significant aggregations, there are at least two instances where individuals among population ϕ overlap each other, and one instance of overlapping among population θ. Let us first calculate m_ϕ (28 per 24 km^2 $\approx$ 1.167 per km^2) and m_θ (14 per 24 km^2 $\approx$ 0.583 per km^2). Since we had only two instances of duplication (overlap) among the 28 members of ϕ (7.14%) and only one instance of duplication (overlap) among the 14 members of θ (7.14%), we must utilize Equation 3.11 to calculate m'_ϕ and m'_θ as

$$m'_\phi = \frac{1.167}{\text{km}^2} - \left(\frac{0.0714}{2} \cdot \frac{1.167}{\text{km}^2} \right) = \frac{1.125}{\text{km}^2}$$

$$m'_\theta = \frac{0.583}{\text{km}^2} - \left(\frac{0.0714}{2} \cdot \frac{0.583}{\text{km}^2} \right) = \frac{0.562}{\text{km}^2}$$

Using Equation 3.10, $m_{\phi\theta}$ is $m'_\phi + m'_\theta$, or 1.687 per km^2. Using Equation 3.9,

$$\bar{d} = \sqrt{1/1.687 \text{ km}^{-2}} = 0.77 \text{ km}.$$

For the aggregated distribution (see Figure 3.3C), we first calculate m_ϕ (35 per 24 km^2 $\approx$ 1.458 per km^2) and m_θ (27 per 24 km^2 $\approx$ 1.125 per km^2). Since we have significant aggregation, we must then calculate the mean number of ϕ and θ per aggregation (11.67 and 10.33, respectively) and use Equation 3.12 to calculate m''_ϕ and m''_θ as

$$m''_\phi = \frac{1.458/\text{km}^2}{11.67} = \frac{0.125}{\text{km}^2}$$

$$m''_\theta = \frac{1.125/\text{km}^2}{10.33} = \frac{0.109}{\text{km}^2}$$

Using Equation 3.10, $m_{\phi\theta}$ is $m''_\phi + m''_\theta$, or 0.234 per km^2. Using Equation 3.9,

$$\bar{d} = \sqrt{1/0.234 \text{ km}^{-2}} = 2.07 \text{ km}.$$

Random Sampling

Unless the field survey is specifically designed to locate rare or concealed objects (or events), most field methods will be heavily dependent on the

Figure 3.4 Gaming dice are an excellent, low-tech solution for generating random numbers in the field, so long as you use the results from single rolls. Two 10-sided dice are a perfect way to generate random percentages or even probabilities from 1 to 100.

premise that the subpopulation being surveyed is representative of the larger population. In order to best assure ourselves that the data captured in our quadrats are truly reflective of the larger system from which they were taken, our quadrats must be placed randomly throughout the system.

As silly as it sounds, "purposeful randomness" is one of the most effective ways of minimizing bias from any field survey. Unfortunately, it is also one of the more difficult aspects to put into practice while in the field. For any investigation, we are compelled to select the most appropriate method and the most appropriate site to test our hypotheses; it is only natural that we would seek to maintain that sense of control, whether it be on the placement of our quadrats or determining where our "starting points" will be. However, once the spatial limits of our investigation are defined, we must relinquish control over some of the decision-making and rely instead on some sort of system that can aid us in determining the random placement of our plots within the boundaries we have defined.

In most applications, it is easiest to define the larger study area according to a gridded map, where each subregion is assigned a unique numerical value. Random number generators (or random number tables) are then used to generate a series of random numbers that indicate which subregions should be surveyed. Depending on the remoteness of the study site, a low-tech solution may be a better option: just ask an old pen-and-paper gamer for their gaming dice (**Figure 3.4**). Now there's a simple random number generator for you to use.

Simple Random Sampling is the Foundation of Most Field Methods

The easiest plot method to employ in the field is simple random sampling, where the entire study area is divided according to a regularly spaced grid pattern, without taking into consideration any of the ecological gradients that may be present in the system. That is, the placement of each plot is determined purely by chance, and it is assumed that any inherent differences between plots shall be captured in the data so long as enough random quadrats are analyzed.

A common practice in most simple random sampling methods is to use a series of nested plots, where the overall study area is first divided into a regular, large-scale grid so that the number (and location) of each quadrat can be randomly determined and surveyed. Then each of the randomly selected quadrats may be divided into even smaller quadrats, to determine the number (and location) of each minor quadrat to be surveyed (**Figure 3.5**). This method can be further adapted to determine "point contact" data by measuring only those objects located at the vertices of four contiguous minor quadrats. This variant method can also be used to yield simple estimates of percent cover. For example, the large quadrat depicted in Figure 3.5 contains a total of nine vertices. If four of nine vertices were in contact with the same object or species, it would be a simple task to estimate the percent cover as 4/9, or ~44.4%.

Although this method is quite simple to execute in the field and is usually sufficient for comparisons between different study areas, its very design makes it difficult to perform comparisons within a single study area. For that, we would need to design a stratified plot method.

Stratified Random Sampling Can Be Used to Organize the Study Area into Horizontal or Vertical Strata

The stratified random sampling method simply seeks to divide the study area according to inherent ecological gradients, or **strata**, so that a simple random

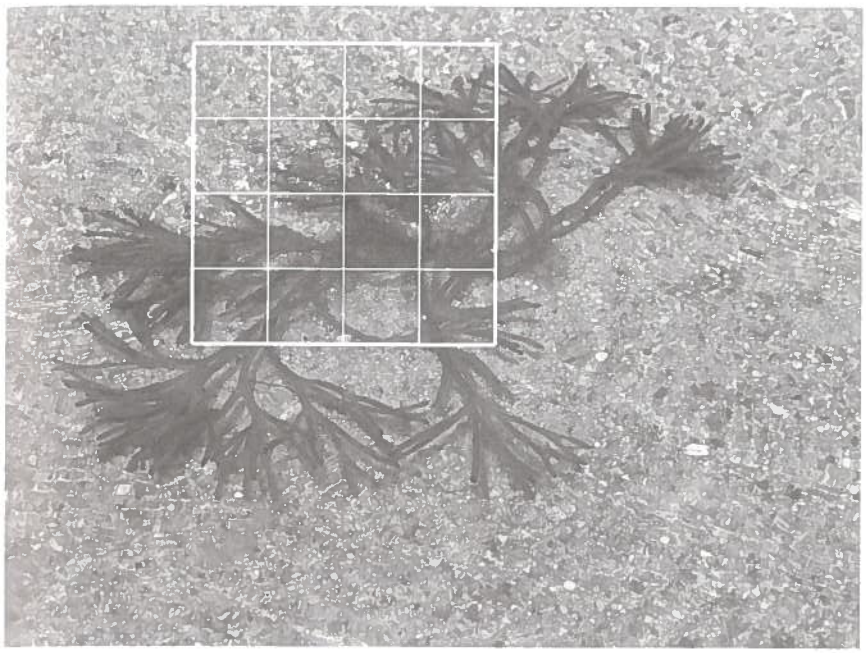

Figure 3.5 A simple random sampling method might require that the entire area first be gridded out on a map, and the location of where the larger quadrats should be placed can then be determined randomly. Once the quadrat has been placed, the investigator may choose to survey either the entire quadrat, any number of randomly determined minor quadrats, or simply at the "cross-hairs" formed by four contiguous minor quadrats (the point-contact method).

sampling method can be employed in each of the defined strata, separate from each other. In essence, this method simply divides the study area into two or more regions that are exclusive of each other according to some discriminating feature (**Figure 3.6**). In most aquatic systems, the ecological gradients present in the horizontal dimension can be quite different from those in the vertical. In practice, it is usually easier to treat the horizontal and vertical strata separately from each other (although it is certainly possible to define complex three-dimensional strata, if that is the investigator's desire).

Because survey data are organized according to the strata from which they were gathered, it is possible to perform comparisons between strata. With the use of statistics, this sampling method is particularly powerful and versatile, as comparisons can be made between different strata in the same geographic area, or between similar strata in different geographic areas.

Transect Interval Sampling Is a Variation of the Belt/Line Transect Method

Unlike the simple random sampling method, where the overall study area is divided into equally sized subdivisions, investigators utilizing transect

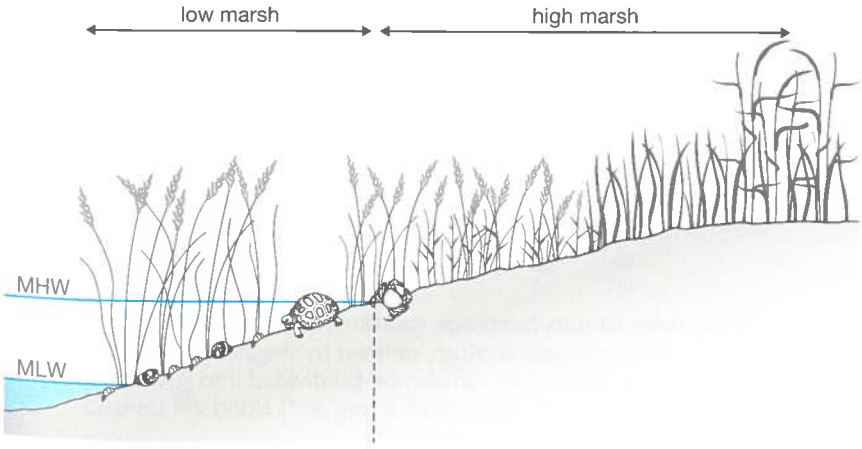

Figure 3.6 A coastal marsh can be defined by those regions found above mean high water (MHW) and mean low water (MLW), according to the local tidal cycle. As a result of differential tidal inundation, a natural ecological gradient is established. This relationship can be used to define different marsh strata, where sampling conducted in the "high marsh" area (above MHW) can be conducted separately from the "low marsh" area (below MHW). (From Laresen C et al. The Blackwater NWR Inundation Model. US Geological Survey, Open File Report 04-1302.)

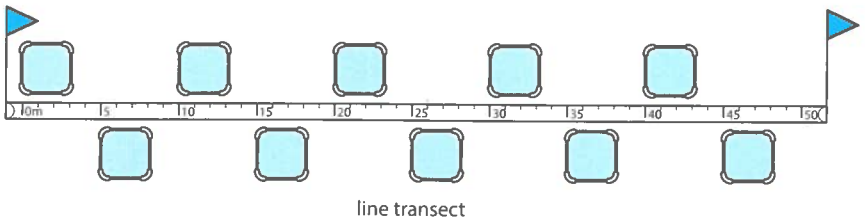

Figure 3.7 Transect interval sampling requires the random placement of a transect line, where a number of quadrats are placed along the transect line, according to either a random or predefined interval.

interval sampling simply lay out a transect line with regular divisions (such as a tape measure, as depicted in **Figure 3.7**) and place their quadrat(s) according to some predetermined rule. If the location and orientation of the transect is determined randomly, it is permissible to define a regular interval of measurement (such as one quadrat every 5 meters) without endangering the "randomness" of the survey. However, if the investigator is forced to orient the transect in a nonrandom manner (for example, according to some set ecological pattern or strata), the points of quadrat deployment along the transect line should be randomized instead (for example, one quadrat every 1 to 10 meters along the transect line, determined randomly each time).

For most benthic surveys, it is relatively simple to use a physical transect line resting on the **substrate**. However, three-dimensional surveys of aquatic systems typically require the use of a "virtual" transect line, usually defined by using standard map coordinates (such as latitude and longitude). Using this method, surface measurements are treated as though the transect line had been "placed" on the surface of the water. However, because of the strong vertical gradients present in all aquatic systems, it will be necessary to divide the vertical dimension into different strata if vertical profiles of the transect line are needed (**Figure 3.8**). It is also important to note that the measurements taken along a transect line must possess some areal or volumetric frame of reference (such as abundance per km^2, or concentration per m^3) in order for this to be considered a true plot method.

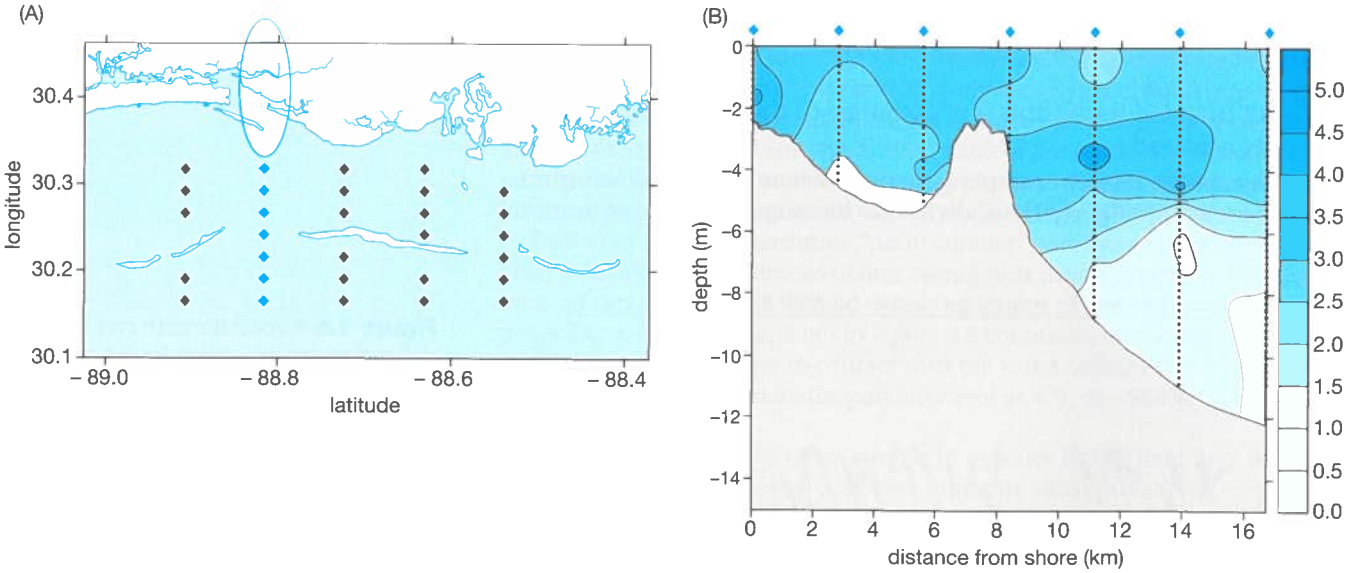

Figure 3.8 A series of regularly spaced station locations (using fixed latitute–longitude coordinates) can be easily oriented in a grid pattern, so each station location can serve as a point within the horizontal stratum, defined by the along-shelf and cross-shelf transects that compose the grid (A). At each station, the vertical dimension can also be subdivided into strata, typically by sampling at specific depth intervals. In this figure, measurements of *in situ* chlorophyll *a* (mg m^{-3}) along the transect highlighted in blue were pooled according to the vertical strata, at 0.25 m depth intervals (B).

Adaptive Sampling Is an Excellent Method for Detecting Rare Events

Unlike most plot methods, adaptive sampling is only quasi-random, since its primary purpose is the directed (nonrandom) survey of rare or elusive features. A typical method is to start with a simple random sampling method (nested quadrat or point contact), with a subsampling rule designed to concentrate effort only on those quadrats or grid points that successfully detect the feature under investigation (**Figure 3.9**). The subsampling rule (or "search pattern") can be just about anything and is limited only by the investigator's imagination. However, once the adaptive sampling rules are chosen, they should remain consistent throughout the duration of the survey.

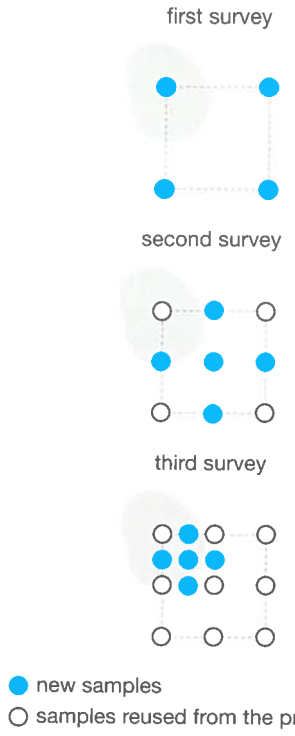

Figure 3.9 For adaptive sampling methods, a random point-contact grid is first established, with an adaptive sampling rule to halve the distance between contiguous grid points for every successful "hit." In the first survey, the rare event (shaded in grey) is detected in only one of four locations. In each successive survey, the investigation becomes increasingly focused on those regions where previous attempts were successful in detecting the event.

Basic Plotless Methods

Although plot methods are generally considered to yield data of greater accuracy than plotless methods, plot surveys are usually more labor intensive and time consuming. If the features under investigation are assumed to be stationary and spatially distributed in a random manner (as depicted in Figure 3.3B), it may be preferable to use a plotless density estimator for ease of use in the field, or in those locations where it would otherwise be impractical to use a plot method. Even nonrandom distributions can be surveyed with a plotless method, provided that an appropriate sample size and methodology is chosen.

Plotless Density Estimators (PDEs) Do Not Require Exhaustive Surveys of an Area

There are a variety of methods used to estimate object densities by measuring the relative distances between objects rather than using counts per unit area. The simplest of these **plotless density estimators** (or PDEs) requires only a tape measure and a number of random starting points within the area of study. Although there are many different PDE formulations, they all require the investigator to begin from some random location in the study area and continue in a regular search pattern until the object of interest is encountered. By calculating the relative distances between a series of random starting points and each encountered object (**Figure 3.10**), the density of those objects in the study area can be estimated.

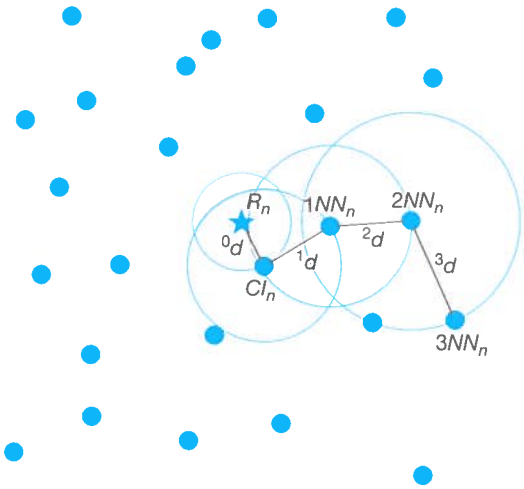

Figure 3.10 Schematic representation of how the simplest PDEs are implemented in the field. R_n is the nth random starting point; CI_n is the closest individual to R_n; $1NN_n$ is the first nearest neighbor to CI_n; $2NN_n$ is the second nearest neighbor to CI_n; $3NN_n$ is the third nearest neighbor to CI_n; ^0d is the shortest distance between R_n and CI_n; ^1d is the shortest distance between CI_n and $1NN_n$; and ^2d is the shortest distance between $1NN_n$ and $2NN_n$.

Basic Distance Estimators (BDEs)

The simplest of all common PDEs, the closest individual method (*CIM*) simply uses the mean distance ${}^{0}d_n$ between each random starting point R_n and the closest individual CI_n to R_n for N number of samples to estimate the object density:

$$D_{CIM} = \frac{1}{\left[4\left(\frac{\sum {}^{0}d_n^2}{N}\right)\right]} \tag{3.13}$$

Nearest neighbor methods are a variation of the *CIM*, and comparative studies have shown that distance measurements ${}^{1}d_n$ between the closest individual CI_n and its first nearest neighbor $1NN_n$ generally yield more accurate object density estimates:

$$D_{1NN} = \frac{1}{2.778 \cdot \left(\frac{\sum_1 d_n^2}{N}\right)} \tag{3.14}$$

Often, density estimates can be further improved by using the distance measurement to the second nearest neighbor $2NN_n$ instead of $1NN_n$:

$$D_{2NN} = \frac{1}{2.778 \cdot \left(\frac{\sum_2 d_n^2}{N}\right)} \tag{3.15}$$

In a comprehensive review of PDE performance, an average of the results from each of the *CIM*, *1NN*, and *2NN* methods ($\bar{D}$) yielded the best general performance compared to all other PDEs, provided a minimum sample size N of 10:

$$\bar{D} = \frac{D_{CIM} + D_{1NN} + D_{2NN}}{3} \tag{3.16}$$

Ordered Distance Estimators (ODEs)

This method is very similar to the closest individual method (see Equation 3.13), but uses the distance between a randomly determined starting point R_n and the ith closest individual (${}^{i}d_n$), which differs significantly from the nearest neighbor method of distance measurement (**Figure 3.11**). Although the second- and third-closest individual are most commonly used, researchers have found that for uniformly distributed objects, the formulation utilizing the third-closest individual ($i = 3$) performed best for sample sizes N in excess of 25:

$$D_{OD} = \frac{(3N - 1)}{\pi \cdot \sum {}^{3}d_n^2} \tag{3.17}$$

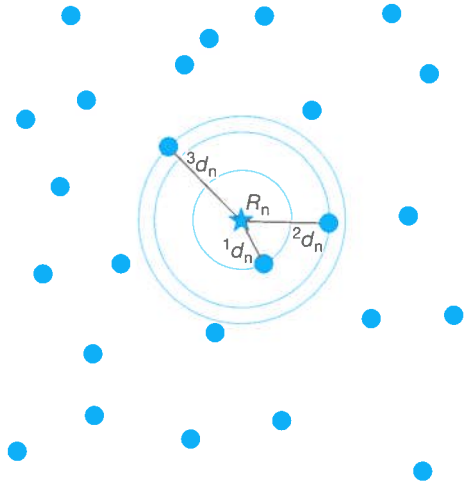

Figure 3.11 Schematic representation of the ordered distance estimator method, where the distance from the third-closest individual (${}^{3}d_n$) to some random starting point (R_n, blue star) can be used as the best PDE for uniform distributions.

Angle-Order Estimators (AOEs)

This method is a variant of the ordered distance estimation method, except that the study area in proximity to a randomly determined starting point R_n is divided into four quadrants, and the distance to the ith closest individual in each of the

EXAMPLE BOX 3.1

Calculating the Basic Distance Estimators (BDEs)

Let us assume that Figure 3.10 represents an area of 1 m^2 (10,000 cm^2), and that the "true" density is 25 individuals per m^2. If we used 10 random starting points R_n in this area, and measured the distances to the closest individual (^0d_n), the first nearest neighbor (^1d_n), and the second nearest neighbor (^2d_n) for each R_n, our data might resemble Table 1.

Table 1 Measured distances (cm) to the closest individual (^0d_n), the first nearest neighbor (^1d_n), and the second nearest neighbor (^2d_n) using a variety of plotless methods

n	^0d_n	$^0d_n^2$	^1d_n	$^1d_n^2$	^2d_n	$^2d_n^2$
1	8.7	75.69	14.1	198.81	14.7	216.09
2	6.5	42.25	11.3	127.69	9.9	98.01
3	9.2	84.64	10.1	102.01	12.3	151.29
4	7.8	60.84	9.6	92.16	18.7	349.69
5	8.1	65.61	6.9	47.61	16.3	265.69
6	11.3	127.69	7.8	60.84	10.0	100.00
7	9.2	84.64	13.6	184.96	7.7	59.29
8	10.7	114.49	9.5	90.25	11.6	134.56
9	8.9	79.21	12.3	151.29	8.9	79.21
10	13.4	179.56	18.9	357.21	6.7	44.89
$N = 10$		$\Sigma = 914.6$		$\Sigma = 1412.83$		$\Sigma = 1498.72$

These data are then used to calculate the density estimators following the closest individual (D_{CIM}, Equation 3.13), first nearest neighbor (D_{1NN}, Equation 3.14), and second nearest neighbor (D_{2NN}, Equation 3.15) methods:

$$D_{CIM} = \left(\frac{1 \text{ individual}}{4 \cdot \left(\frac{914.62 \text{ cm}^2}{10} \right)} \right) \approx 27.3 \text{ ind m}^{-2} \qquad D_{1NN} = \left(\frac{1 \text{ individual}}{2.778 \cdot \left(\frac{1412.83 \text{ cm}^2}{10} \right)} \right) \approx 25.5 \text{ ind m}^{-2}$$

$$D_{2NN} = \left(\frac{1 \text{ individual}}{2.778 \cdot \left(\frac{1498.72 \text{ cm}^2}{10} \right)} \right) \approx 24.0 \text{ ind m}^{-2} \qquad \overline{D} = \left(\frac{27.3 + 25.5 + 24.0}{3} \right) \approx 25.6 \text{ ind m}^{-2}$$

Although the *1NN* analysis provided the most accurate estimate of the true population density in this particular example, the average of these three methods ($\overline{D}$), calculated using Equation 3.16, is generally the best choice among the basic distance estimators.

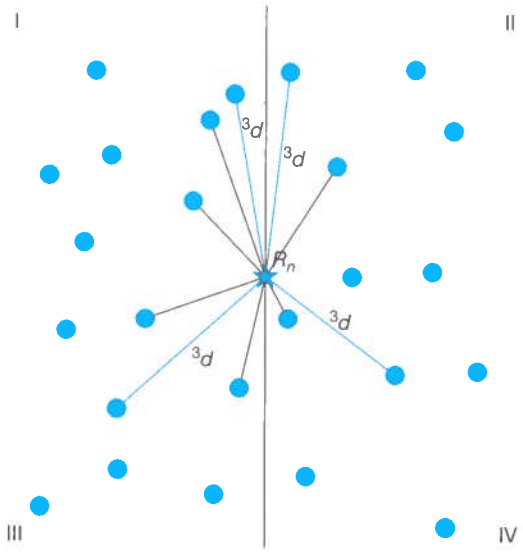

Figure 3.12 Schematic representation of the angle-order estimator method, where the study area is subdivided into four quadrants and the distance from the third-closest individual (^3d_n) is determined for each quadrant.

four quadrats (^id_n) is determined (**Figure 3.12**). Although this method is somewhat time consuming, it performs quite well for objects with an aggregated distribution pattern, especially when using the formulation that utilizes distance measurements to the third-closest individual ($i = 3$) for each quadrant:

$$D_{AO} = \frac{44N}{\pi \cdot \sum\left(1/{}^3d_n^2\right)} \tag{3.18}$$

Line Transect Density Estimators (LTDEs) Are the Best PDE for "Line-of-Sight" Search Patterns

As we have discussed, many PDEs require that the investigator follow a radial search pattern to determine the shortest distance between the survey starting point and the *i*th closest individual (or its *n*th nearest neighbor). However, the use of line transects may be preferable when the terrain would otherwise make it difficult to utilize a radial search pattern. Typically, **line transect density estimator** (or **LTDE**) methods simply measure the distance between the starting point of the transect and some encounter with the object or event along the transect line.

Variable Area Transect (VAT)

This method is not a true line transect method, as it uses a fixed-width, variable-length belt transect with a randomly selected starting point, and is extended in a random, straight direction until the object of study is encountered. Although this method can be used for "first" encounters, some researchers have found that the *VAT* method performed best when the distance l to the third encounter ($i = 3$) was used (**Figure 3.13**). Using a belt transect of uniform width w, the formulation is simply

$$D_{VAT} = \frac{(3N - 1)}{w \cdot \sum l} \tag{3.19}$$

Generally, the *VAT* is very easy to perform in the field and performs moderately well in most cases; however, the *VAT* performs best when sample sizes are relatively large ($N > 50$) and for those objects or events that exhibit a Poisson distribution.

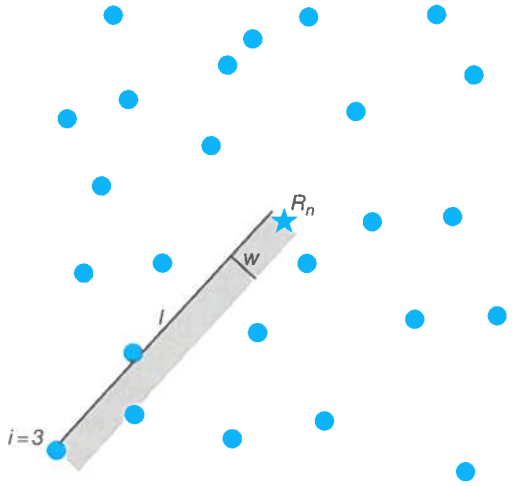

Figure 3.13 Schematic representation o the variable area transect (*VAT*) method, where a belt transect of fixed width *w* and variable length *l* is extended out from a random starting point R_n until the *i*th closest individual comes in contact with the belt transect. In most cases, the *VAT* method provides the most accurate results when the belt transect is extendec out to the third encounter.

The Strong Method (SM)

The Strong method utilizes a true line transect and estimates object densities as a combined function of their overall abundance per unit area, as well as the objects' physical size and orientation (as these will also affect the probability of randomly encountering the object within the study area). For any transect of a fixed length *l*, every object encountered along the length of that transect is described instead by its maximum orthogonal width w_n (**Figure 3.14**), so the density can be estimated as

$$D_{SM} = \frac{A}{l} \times \sum \frac{1}{w_n} \tag{3.20}$$

where *A* simply represents the unit area. Note that Equation 3.20 requires that *A*, *l*, and w_n all share consistent units.

Plotless Methods for Mobile Subjects Require a Strategy Different than Most PDEs

As we have seen from all the plotless methods discussed thus far, they each assume that the object or event being surveyed is somewhat fixed in place. In fact, it is that presumption of semipermanence that allows the investigator to measure distances between some theoretical starting point and the object of interest (either as the *i*th closest individual or as its *n*th nearest neighbor). But what are we to do if we wish to perform a plotless survey of objects that move freely (and often) throughout the study area? Unfortunately, most PDEs are insufficient for estimating population densities of mobile species; for them, you'll need a different approach.

Roaming Surveys

Fishes are notoriously difficult to assess using standard plotless methods, and are virtually impossible to assess using a plot method (unless the plot is inescapable, such as a net or cage). For rapidly or haphazardly swimming species, it is often advisable to estimate the fish density D_{RS} using the Southwood method (see Equation 3.21) for rapid visual assessments:

$$D_{RS} = \frac{Z}{2000rV} \tag{3.21}$$

where *Z* is the number of encounters per hour, *r* is the effective viewing radius of the surveyor (in meters), and *V* is the average swimming speed of the organism (in kilometers per hour).

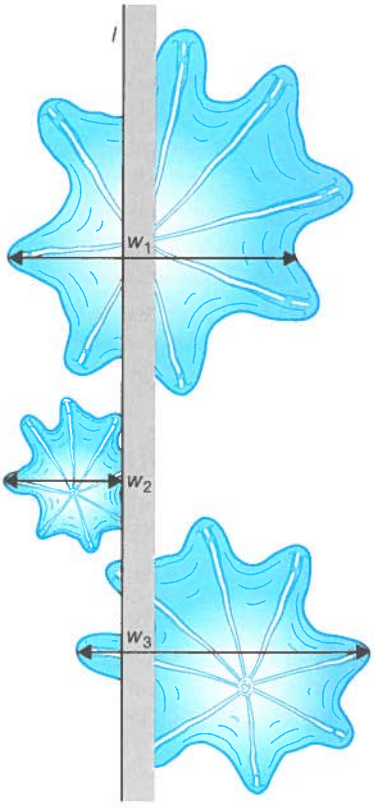

Figure 3.14 Schematic representation of the Strong method, which uses the maximum orthogonal width w_n for each object encountered along a line transect of fixed length *l*.

Mark and Recapture

These methods simply involve the capture and marking (or tagging) of individuals, which are subsequently released. At a later time, the investigator returns to capture more individuals and simply count those that were already marked from a previous capture. Of course, capture (and recapture) efficiencies are usually quite low, so these methods require a long-term commitment to the mark and recapture regime. However, such data can be extremely useful not only in establishing the population densities, but also in estimating birth, death, and emigration rates. These methods are discussed more thoroughly in Chapter 6.

Catch per Unit Effort (CPUE)

One of the simplest methods to perform in the field, measurements of CPUE can be used in virtually any biological context to estimate densities as a function of the collection device. For example, crabs harvested from a number of baited traps can be measured according to any number of relevant biometrics (size, quantity, biomass, etc.). As long as the collection device is used consistently, those biometrics can be related to the sampling effort. Although the CPUE method may not be the best choice for estimating instantaneous densities, CPUE data can be easily used for trend analyses in continuous, long-term data sets. These methods are discussed more thoroughly in Chapter 7.

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