Quantitative Analysis of Marine Biological Communities
Field Biology and Environment

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Preface

There have been some outstanding books and computer programs published on quantitative methods. Among these are old and new editions of Cochran (1977 and earlier editions), Southwood (1966, 1978), Southwood and Henderson (2000), Krebs (1989, 1999, 2000 and earlier editions), Davis (1973,1986), Sokal and Rohlf (1981,1995), Manly (1997), Zar (1999), Mead (1988), Legendre and Legendre (1998), Jongman et al. (1987), Buckland et al. (2001), Fowler et al. (1998), Borchers et al. (2002) McCune et al. (2002), Thompson (2002) and others. Selected information was condensed and simplified then included in this book. The Internet was very useful in locating information and in obtaining photos of marine science equipment. The many reviewers have been exceptionally helpful in improving the text.

This book is an attempt to combine ordinary quantitative techniques with relatively new advances in quantitative methodology. These quantitative methods are frequently used in many disciplines outside of biology. The idea is to present one or two specific examples (e.g., equations) for each quantitative topic, hopefully the best techniques. The book is an introduction with few exceptions (e.g., environmental impact assessments are discussed in considerable detail). Some topics receive greater emphasis than others because of the popularity of the topics and the interests and knowledge of the author. Emphasis is placed on shoreline and nearshore habitats, especially intertidal (littoral) and scuba-depth regions. Both tropical and nontropical examples are given. Chapter 8 offers information on equipment used offshore and in deeper waters.

This book is designed for advanced undergraduate and graduate students interested in marine biology and field biology, although much of the information can also be used in terrestrial biology. For this reason, limited terrestrial examples are given. Terrestrial examples are also offered to make marine biologists aware of some techniques in ecology that may be of use to them. The book will also be useful as a general introduction for professionals, such as marine biologists in consulting firms, fish and game or fish and wildlife workers, and pollution specialists. **The emphasis is on marine biology and community ecology**, classical population ecology receiving scant coverage. It is suggested that Google or other search engines be used to locate topics. This exposes the reader to many sources of information on the same topic. Most of the chapters are rather straightforward (Chapter 2 – Biometrics) and some complex (Chapter 4 – Community Analyses; Chapter 5 – Multivariate Techniques). Chapter 3 (Quantitative Methods in Field Ecology) is an eclectic mixture of various topics that have been of interest and of help to the author. Many of them are intended to introduce the student to a discipline rather than offering detailed coverage of the topic. Many references are cited for further information. Some

reviewers have legitimately stated that I should have offered my personal opinion on certain subjects. Numerous topics are covered in this book. I can claim to have firsthand field experience with perhaps a couple dozen of them and may be considered a specialist in but a few. Consequently, I have relied on the expertise of many others, citing their opinions frequently, especially when they are conflicting.

My biological career began with studies of terrestrial plants, birds and mammals as an undergraduate student. This was followed by population studies on a stream bird (Dipper) in Montana (Bakus, 1959ab). Several years were then devoted to the taxonomy and development of marine sponges in Washington (Bakus, 1966). This led to 30 years of research on the chemical ecology of coral reefs (e.g., Bakus, 1986), all originally due to the fact that I could not initially locate sponges for a NSF-supported field study at Fanning Island, an atoll in the central Pacific (Bakus, 1964). Later, I discovered that the very few exposed sponges present were toxic to fishes, leading to studies in chemical ecology.

I became interested in quantitative techniques in biology because of co-teaching twice with a visiting professor of marine biology from Australia. My first book on quantitative methods (printed in India where I was involved in training programs, but distributed in Europe and elsewhere by Balkema, Rotterdam – see Bakus, 1990) was dedicated to Prof. William Stephenson, Department of Zoology, University of Queensland, Brisbane, Australia, and to my graduate students. Prof. Stephenson frequently referred to his area of research as "the numbers game". Prof. Stephenson died since then. It is through efforts of fine people such as these that we continue on. My dedication here is to the many people interested in field biology, natural history, and quantitative methods.

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Chapter 1

Biological Sampling Design and Related Topics

1.1 PROFILING METHODS AND UNDERWATER TECHNIQUES

1.1.1 Introduction

Because so many marine studies are conducted in the intertidal or littoral zone, **a review of methods of profiling beaches is now given**.

1.1.2 Profiling a Beach

Profiling a beach involves measurements of changes in elevation from the top of the beach to the water. These changes are then plotted as a figure, appearing as if you were looking at the slope of the beach from the side. This enables one to then **record the zonation of species above mean lower low water so that you know at what tidal level a density study of a species occurred**. There are often two high tides and two low tides each 24 h on the Pacific coast of North America. Thus there is a high low and a low low tide each day. The yearly average of the low low tides is the mean lower low reference point.

There are several methods of obtaining profiles on a beach. Some of these are easier than others; some are more accurate. The method chosen will depend on the availability of equipment and time. The Sight method: Stand at point A (facing the ocean) and ask someone to stand at point B (perpendicular to the shoreline and in line with point A), *X* m downslope from point A (Fig. 1-1). The distance between points A and B will depend on the slope. The steeper the slope, the shorter the distance. The individual at point B holds a calibrated rod (2–3 m long) in a vertical position with the lower end of the rod resting on the average basal level of the substratum (e.g., between rocks on a rocky shore). The individual at point A then sights the horizon at point B and reads the intercepted height value on the rod. The distance

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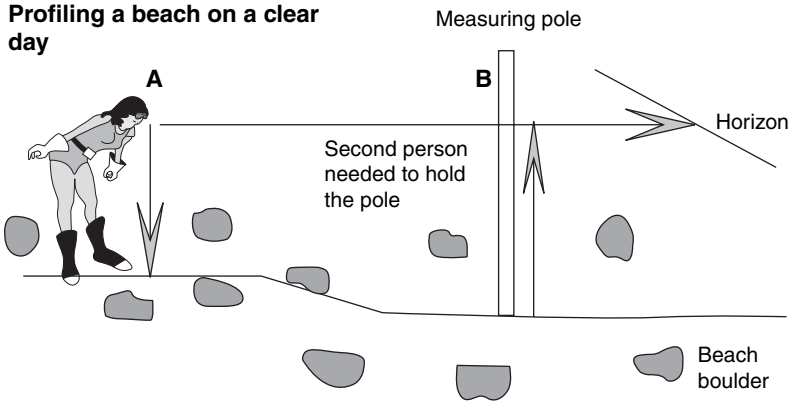

Figure 1-1. Profiling the beach. Sight from the upper part of the beach to a spot lower down the beach. Measure (1) the height from the eyes to the average beach floor, (2) the height from the average beach floor to the height at the pole where the visual sighting intercepts the horizon, and (3) the distance from the eyes to the pole. Subtract the two height measurements. This is the change in slope over the distance measured. Continue doing this down the beach to the water's edge. Combine the measurements and draw a simple profile figure of the beach. Now that you have a beach profile, you need to determine the tidal level of the profile. Record the position of the water's edge with respect to your profile and the time. Go to a tide table (source: fish tackle store or library). Look for the high and low tidal levels for the day you were profiling the beach (unfortunately not given in metric measurements). Interpolate the tidal level (between high and low tide), based on the time you recorded at the beach, and the times of high and low tides. This gives a reference point tidal level. You can then plot your final beach profile as tidal height (*y*-axis) vs. distance (*x*-axis), converting English units (i.e. feet) to metric units (meters) if you wish.

from the average basal level at point A to the individual's eye line is measured and this value is subtracted from the horizon height at point B, giving the change in elevation over a selected distance. A string or twine placed between points A and B (Fig. 1-1) and leveled with a carpenter's level can be used as a substitute for the horizon in foggy weather.

Other methods include using a hand level (with internal bubble for leveling), a Brunton compass (Fig. 1-2), plastic tubing with water, a self-leveling Surveyor's instrument, and a Geographic Positioning System (GPS) or an altimeter with a high degree of accuracy for elevations.

1.1.3 Underwater Profiles

The angle of slopes underwater can be measured with a homemade inclinometer. View the slope sideways, estimating the angle (Fig. 1-3). [An inclinometer can also be used to measure slopes as well as the height of trees on land.]

For oceanographic studies, underwater seafloor profiles are obtained with a precision depth recorder or with sidescan sonar (Fig. 1-4) coupled with the GPS. Sidescan sonar can cover vast areas of the seafloor with a single sweep (the system GLORIA has a two-mile swath).

Figure 1-2. The Brunton compass is used extensively by field geologists. It is tricky to operate as you must peer through two metal holes to the waterline and simultaneously look into the mirror and rotate a knob on the back until the bubble is level, then read off the angle or grade. The advantages are that you can easily measure slopes over long distances with only one person. The distance (d in m) between where you are standing (point A) and the site (Point B or waterline) is measured. Trigonometric functions are applied. $h = d \sin a$ where: h = change in elevation (m), d = distance measured (m) between two points, a = angle measured (degrees). Once h is calculated, the distance from your eye level to the average substratum needs to be subtracted from h. The Brunton compass is accurate to about 1/2° for elevations. Example: $d = 50\,\text{m}$ $a = 20°$ ($\sin a = 0.0159$) therefore $50 \times 0.0159 = 0.8\,m$ change in the level of the substratum.

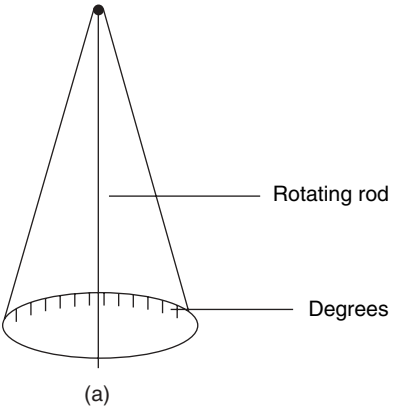

Figure 1-3a. The plastic clinometer is held up sideways underwater on a reef and the angle of the slope estimated by moving the rotating rod until it follows the average slope line.

1.1.4 Underwater Techniques

Coyer and Whitman (1990) present a comprehensive book on **underwater techniques for temperate and colder waters**. A book by Kingsford and Battershill (2000) is recommended for **techniques of studying temperate marine**

(b)

Figure 1-3b. The metal clinometer reads % slope or angle and is used on land to measure the height of trees. Stand above the base of the tree and measure the % slope to the base of the tree. Measure the % slope to the top of the tree. Measure the distance from the clinometer to the tree. Add the percentages and multiply by the distance measured. For example, the % slope to the base = 30%, the % slope to the treetop = 60%, and the distance = 40 m. Then 30% + 60% = 90% × 40 m = 36 m (the height of the tree).

Figure 1-4. Sidescan sonar. This sonar system (fish) is lowered aft of the ship and towed underwater. It sends out radar and records the topography of the seafloor back on deck. We used it successfully to locate a ship anchor and chain lost offshore from the Port of Los Angeles after two days of operation.
Source: http://www.woodshole.er.usgs.gov/stmapping/images/dataacq/towvehicles/sisi000.jpg

environments by habitat type. An excellent book on **sampling techniques in the tropics** is by English et al. (1997). Hallacher (2004) presents an interesting overview of underwater sampling techniques on coral reefs. See also Fager et al. (1966), UNESCO (1984), and especially Munro (2005). Divers can use a clipboard and waterproof paper (polypaper). The sheets are held down with two large rubber bands (Fig. 1-5). A pencil is tied to the clipboard and the clipboard attached to a brass link on the diver's belt. Alternatively, small polypaper notebooks are available. A very useful tool for measuring distances [e.g., using the Point-Center Quarter (PCQ)

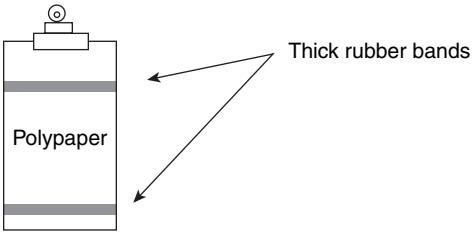

Figure 1-5. A diver's clipboard with polypaper (waterproof paper) and two stout rubber bands to hold the paper down. The clipboard is attached with twine to the diver's belt clip. This mode of operation was designed by Tim Stebbins as a graduate student.

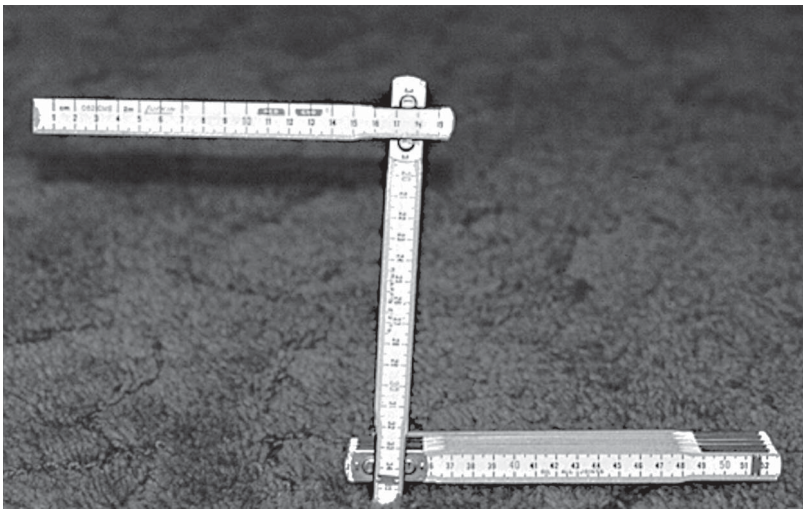

Figure 1-6. Carpenter's collapsible rule. A handy underwater tool for measuring distances. When configured into a square, it forms a 0.25 m^2 quadrat.

method] is the collapsible rule. This rule can also easily substitute as a 0.25 m^2 quadrat frame (Fig. 1-6). Underwater recording systems are available for divers but they are expensive. WetPC and SeaSlate are recently developed underwater recording systems (see p. 310 in Chapter 8)

1.2 SAMPLING POPULATIONS

1.2.1 Introduction

The procedure by which the sample of units is selected from a population is called the sampling design. Adequate sampling design requires that the correct questions are asked and the study is carried out in a logical, systematic manner. The activities or stages in the study should flow as follows: **purpose → question → hypothesis → sampling design → data collection → statistical analysis → test of hypothesis → interpretation and presentation of the results**. Reasons for sampling populations often involve the need for estimates of densities of organisms and their distribution

patterns (e.g., random, clumped, even). These data can then be used to compare community structure or to conduct population studies.

Sampling populations can be accomplished by survey designs (e.g., quadrats, line intercepts) or by model-based inference (Buckland et al., 2001). In a design-based approach to survey sampling, the values of a variable of interest of the population are viewed as fixed quantities. In the model-based approach, the values of the variables of interest in the population are viewed as random variables (Thompson, 2002). Model–based methods use a statistical model of the distribution of organisms based on likelihood methods (e.g., maximum likelihood estimation, Bayes estimation). One area of sampling in which the model-based approach has received considerable attention is with ratio and regression estimation (Thompson, 2002). It has been prevalent in sampling for mining and geological studies. Here we emphasize the use of survey designs. The classical text on sample design is Cochran (1977). An informative book on sampling is Thompson (2002). Murray et al. (2006) recently authored a book on monitoring rocky shores, a valuable source of information on sampling techniques with marine algae and macroinvertebrates.

Krebs (1999), in a leading text on ecological methodology, and Green (1979), in an excellent review of sampling design and statistical methods, **each** present 10 commandments for the field biologist. **They are combined here**. Italic or boldface fonts are explanations, additions, or emphases by the present author.

(1) Find a problem and state concisely what question you are asking.

(2) Not everything that can be measured should be. Use ecological insight to determine what are the important parameters to measure.

(3) Conduct a preliminary survey to evaluate sampling design and statistical analysis options. **Preliminary surveys are critical for well-designed studies**.

(4) Collect data that will achieve your objectives and make a statistician happy. Take replicate samples for each condition (time, space, etc.). See Hessler and Jumars (1974).

(5) **Take an equal number of random replicate samples (at least two) for each condition**. Replicate samples often have 50–90% similarity. **Equal numbers of samples are required for many statistical tests**.

(6) Verify that your sampling device or method is sampling the population with equal and adequate frequency over the entire range of sampling conditions to be encountered.

(7) If the sampling area is large-scale, break it up into relatively homogenous subareas and sample them independently. Allocate samples proportional to the size of the subarea. If an estimate of total abundance is desired, allocate samples proportional to the number of animals in the subarea. *Optimal allotment is to allocate on the basis of within stratum variances* (*Stuart Hurlbert, pers. comm.*).

(8) Adjust the sample unit size (i.e., number of samples needed) relative to sizes, densities, and spatial distribution of organisms to be sampled. Choose the optimal quadrat size (*see Southwood, 1978 and p. 17 in this chapter*). *Estimate the number of replicates needed to obtain the precision you want*

(*Gonor and Kemp, 1978, Krebs, 1999, and see p. 10 in this chapter*). *Fractal methods* (*Chapter 3, p. 168*), *analysis of variance* (*Chapter 2, p. 88*), *and power analysis* (*Chapter 2, p. 100*) *can also be used to determine the required sample size.*

(9) Test the data to determine whether the error variation is homogenous, normally distributed (i.e., has a bell-shaped curve), and independent of the mean. If not, as in most field data, (a) transform the data (Chapter 2, p. 66), (b) use nonparametric analysis (Chapter 2, p. 102), (c) use sequential sampling design (*see p.* 27 *in this chapter and Krebs, 1999*), or (d) test against simulated H_0 (null hypothesis) data (*Connor and Simberloff, 1986 and Chapter 3, p.* 141).

(10) Stick with the result. Do not hunt for a better one.

(11) Some ecological questions are impossible to answer at the present time. For example, historical events that have helped establish future ecological patterns (e.g., *asteroid impacts, rats*).

(12) Decide on the number of significant figures needed in continuous data before an experiment is started.

(13) Never report an ecological estimate without some measure of its possible error.

(14) Always include controls (*in experimental studies*).

(15) Be skeptical about the results of statistical tests of significance. Cut-off points such as $P = 0.05$ (95% confidence level in your statistical answer) should be considered as shades of gray instead of absolute boundaries.

(16) Never confuse statistical significance with biological significance. Biological characteristics are often much more important than results from a statistical test.

(17) Code all your ecological data and enter it on a computer.

(18) Garbage in, garbage out. Poor data give poor results, no matter what kind of data analysis is used.

Two worthwhile books on terrestrial statistical ecology are those of Ludwig and Reynolds (1988) and Young and Young (1998). Dale (1999) and Fortin and Dale (2005) discuss spatial analysis. Sutherland (1996) discusses basic ecological census techniques then covers specific taxa (plants, invertebrates, fishes, amphibians, reptiles, birds, mammals) and environmental variables. For standard methods in freshwater biology see p. 353 in Chapter 8. See also Elliott (1977) and Gonor and Kemp (1978).

The most important thing one can do when planning a field study is to make a preliminary survey of the study site. This will indicate whether the organisms are present and provide some information on their density, distribution, and possibly their role in community structure. This preliminary step automatically biases the sampling procedure since further sampling will often take place where the organisms are relatively abundant, but it saves considerable time, effort, and costs for the definitive study.

Four major methods of obtaining population estimates include (1) sampling a unit of habitat and counting organisms in that unit, (2) distance or nearest neighbor techniques, (3) mark-recapture, and (4) removal trapping (Southwood and Henderson, 2000). Removal methods have poor precision and the potential for a large degree of bias (Buckland et al., 2001), thus will not be considered here. Frontier (1983) discusses sampling strategies in ecology.

1.2.2 Sampling Design

Sampling design varies considerably with habitat type and specific taxonomic groups. Kingsford and Battershill (1998) present sampling designs and data analysis based on specific marine habitats. Design analysis in benthic surveys is discussed by Underwood and Chapman (2005). Sampling design begins with a clear statement of the question(s) being asked. This may be the most difficult part of the procedure because the quality of the results is dependent on the nature of the original design. A preliminary survey of the proposed study area is essential as spatial and temporal patterns of selected species can be assessed. If the sampling is for densities of organisms then at least five replicate samples per sampling site are needed because many statistical tests require that minimal number. Better yet, consider 20 replicates per sampling site and in some cases 50 or more. If sample replicates are less than five then bootstrapping techniques can be used to analyze the data (see Chapter 2, p. 113). Some type of random sampling should be attempted (e.g., stratified random sampling) or a line intercept method used to estimate densities (e.g., Strong Method). Measurements of important physical–chemical variables should be made (e.g., temperature, salinity, sediment grain size, etc. – see Chapter 8). Field experiments need to be carried out with carefully designed controls (see Chapter 2, p. 97). The correct spatial scale needs to be considered when planning experiments (Stiling, 2002). Environmental impact assessments ideally attempt to compare before and after studies. For example, a coastline destined to have a new sewage outfall constructed could be studied in detail prior to its initial operation. This study then could be repeated two years after the outfall system begins operation. Because before and after studies are often not feasible, an alternative is to compare impacted areas with nearby unaffected (control) areas.

Peterson et al. (2001) analyzed four major sampling designs in shoreline studies of the impacts of the Exxon Valdez oil spill in the Gulf of Alaska. Two studies employed stratified random sampling techniques and two had fixed (nonrandom) sites. For an explanation of these methods, see pp. 20 and 23 in this chapter. There were differences in sampling sites, sampling dates, effort, replication, taxonomic categories, and recovery data. That the studies came to different conclusions is no surprise (for a similar example of differing interpretations but with the same ecological data see Ferson et al., 1986). The results emphasize how important is sampling design. Gotelli and Ellison (2004) and Odum and Barrett (2005) have informative chapters on sampling design. Diserud and Aagaard (2002) present a method that tests for changes in community structure based on repeated sampling. This may be

especially useful in pollution studies and studies on natural catastrophes. See also Cuff and Coleman (1979), Bernstein and Zalinski (1983), Frontier (1983), Andrew and Mapstone (1987), Gilbert (1987), Eberhardt and Thomas (1991), Fairweather (1991), Thompson (1992), Stewart-Oaten and Bence (2001), Peterson et al. (2002), and Lindsey (2003).

1.2.3 Physical–Chemical Factors

Physical and chemical measurements (temperature, salinity, etc.) are frequently carried out when sampling organisms. Techniques for collecting physical–chemical data are discussed in Chapter 8 for marine biology and oceanography. Multivariate analysis of physical–chemical–biological data is discussed in Chapter 5.

1.2.4 Timing of Sampling

The timing of sampling varies with season, age, tides, sex, and other factors. For example, many nocturnal fishes are inactive during the day and seldom observed at that time (Bakus, 1969), thus sampling needs to be done at dawn, dusk, or during nighttime hours for these fishes. Some abundant tropical holothurians move from cryptic habitats and subtidal depths into shallower waters as they mature (Bakus, 1973). There are numerous others changes that occur among species over space and time. These behaviors need to be considered to optimize field studies.

1.2.5 Size of the Sampling Area

The size of the sampling area is highly variable. One must compromise between the overall size of the habitat and the distribution, size, and habits of the organisms, and the statistical measures to be employed before all data have been collected.

1.2.6 Scale

The effects of scale on the interpretation of data have become a very important issue in ecology. The scales commonly encountered in ecology include the individual, patch of individuals, community, and ecosystem (Stiling, 2002). Data based on different spatial scales can yield answers to different questions or even result in different conclusions. One of the earliest discussions on the effects of scale on the interpretation of data from the marine environment is that of Hatcher et al. (1987). For more recent developments see Podani et al. (1993), Schneider (1994), Peterson and Parker (1998), Scott et al. (2002), and Seuront and Strutton (2003). See Fig. 3-1 on p. 124 for examples of how changes in scale can result in different interpretations of the same data. Also see Mann and Lazier (2005).

1.2.7 Modus Operandi

The following sections describe quantitative techniques that give numbers of samples required or densities of organisms. Many of these techniques originated in terrestrial studies and were later employed in aquatic habitats. The examples described herein often center around shorelines or terrestrial sites because most people are familiar with these habitats. Moreover, relatively few students have had shipboard experience to relate to. Nevertheless, these quantitative techniques are often modified and used in seafloor and water column studies as well. For example, plankton sampling can be performed haphazardly, by systematic sampling, or by following a transect line. Infaunal sampling can be carried out with simple random sampling and coordinate lines, stratified random sampling, or line transects. A submersible can perform systematic sampling, belt or strip transects, line intercepts, and so forth. For information on benthic and water column sampling devices see Chapter 8. For information on seafloor sampling techniques see Holme and McIntyre (1984), Mudroch and MacKnight (1994), and Eleftheriou and McIntyre (2005). For information on water column sampling techniques see Hardy (1958), Strickland (1966), Harris et al. (2000), and Paul (2001).

Many of the sampling designs are relatively simple but some (e.g., sequential sampling, mark or tag and recover) can be complex and involve pages of equations and calculations. For those cases, the author refers the reader to references that provide details. A number of special sampling techniques (e.g., coral reef surveys, large scale sampling, etc.) are presented after the discussion of common plot and plotless methods. Collected data can be stored on Microsoft Excel spreadsheets for analysis.

1.2.8 Sample Size or Number of Sample Units Required

Density is the number of individuals per unit area or unit volume. The number of sample units required for a density study is dependent on the variation in population density and the degree of precision required. There are numerous methods for estimating the sample size (i.e., number of samples) needed in any study. The traditional methods have emphasized the variance to mean ratio, such as in the following example for a **normal distribution** (Cochran, 1977):

$$n = \frac{t^2\,\mathrm{SD}^2}{(E\,\bar{X})^2}$$

where

n = number of sample units

t = t value

SD = standard deviation

E = allowable error (e.g., 10% = 0.1)

$\bar{X}$ = mean

First conduct a preliminary sampling then calculate the sample mean and the sample variance (σ^2 – see Chapter 2, pp. 76 and 77). Look up the critical t value at $P = 0.05$ and the degrees of freedom (number of samples – 1). Enter the table t value in the equation and the allowable error, say 10% (use 0.1).

Example: The density of brown giant kelp (*Macrocystis pyrifera*) or trees per 100 m^2 is: 17, 7, 8, 5, 3, 5.

The t value for 5 degrees of freedom at $P = 0.05$ is 2.6.
The mean = 7.5 and the variance = 24.7. With an allowable error of 0.1 (10% error):

$$\text{No. of samples units needed} = \frac{(2.6)^2\,(24.7)}{(0.1\times 7.5)^2} = 223$$

This large number is based on limited preliminary sampling. Taking more sample units during preliminary sampling could further reduce the number of sample units (decrease the variance) required for the definitive study. A preliminary survey is essential in obtaining precursory density estimates in order to use a preferred method to estimate how many sample units will be needed for a final or definitive study. If this is not possible then a survey of the literature of similar studies is essential.

For population studies, the approximate **number of sample units needed with a Poisson (random) Distribution** is estimated by Krebs (1999:244) as follows:

$$n \cong \left(\frac{200}{r}\right)^2 \frac{1}{\bar{\bar{X}}}$$

where

n = sample units required (e.g., number of quadrats or plots)

$\cong$ approximately equal to

r = allowable error (%)

$\bar{X}$ = mean

Example

For a mean of 10, a 10% allowable error, and $\alpha = 0.05$ (95% confidence level – see Chapter 2, p. 81):

$$n \cong \left(\frac{200}{10}\right)^2 \left(\frac{1}{10}\right)$$

$$n \cong (400)\,(0.1)$$

$n \cong 40$ samples (e.g., quadrats).

Krebs (2000a) has a computer program for this – listed under "quadrat sampling." See the Appendix.

The approximate **number of sample units needed with a negative binomial (aggregated) distribution** is estimated by Krebs (1999:245) as follows:

$$n \cong \frac{(100\,t_\alpha)^2}{r^2}\left(\frac{1}{X}+\frac{1}{k}\right)$$

where

n = sample units required (e.g., number of quadrats)

$\cong$ = approximately equal to

t_α = t value for $n-1$ degrees of freedom (= 2 for 95% confidence level)

$\overline{X}$ = mean

k = estimated negative binomial exponent

r = allowable error (%).

$$\text{Approximate estimation of } k = \frac{(\overline{X})^2}{(S)^2 - \overline{X}}$$

where

$\overline{X}$ = mean

S = standard deviation.

Krebs (2000a) has a maximum likelihood estimation computer program for this – listed under "quadrat sampling." This produces a more precise estimate of k.

Example

For a mean of 4, error of 10%, and negative binomial exponent of 3.

$$n = \frac{(200)^2}{(10)^2}\left(\frac{1}{4}+\frac{1}{3}\right)$$

$$n = 400\ (0.25 + 0.33)$$

$$n = 232 \text{ samples (e.g., quadrats).}$$

The major problem with many of these equations is that the precision level (i.e., 10% allowable error, an arbitrary value) results in too many sample units being required (i.e., often several hundred in the intertidal zone). Hayek and Buzas (1997) state that a precision level of 25–50% is all that is reasonably attainable in many field studies. The 10% sample error may often be met by terrestrial plant ecologists. They contend neither with the tides nor with slow underwater operations. I call this the 1:5:10 rule of thumb, that is, intertidal density studies may take about five times longer, and subtidal studies 10 times longer to obtain the same amount of density data (using plot sampling) as that of many terrestrial studies (e.g., tree densities). When temporal or spatial variation in a population is large, a small number of sample units provides imprecise estimates of population values, so that models derived from such data may be quite distorted (Houston, 1985).

The best sample unit number is the largest sample unit number (Green, 1979). It is better to sample the same total area or volume by taking many small sample units rather than few large ones, according to Green (1979) and Southwood and Henderson (2000). However, this does not consider edge effects, cost considerations, and so forth. Population density (and variance) is always fluctuating thus too much emphasis should not be placed on a precise determination of the optimum size of the sampling unit (Southwood and Henderson, 2000). See Krebs (1999) and Southwood and Henderson (2000) for a discussion of this topic and Krebs (2000a) for a computer program. If one wishes to sample community structure, another method of determining sample size is to use a species area curve (see Chapter 3, p. 145). A newer method of estimating required sample unit number is **power analysis**, discussed in Chapter 2, p. 100, regarding experimental methods. See also Green (1989).

Bakanov (1984) published a nomogram for estimating the number of sample units needed with an aggregated distribution. Manly (1992) discusses bootstrapping techniques for determining sample unit sizes in biological studies. Keltunen (1992) estimates the number of test replicates required using ANOVA.

A correction factor (fpc or finite population correction factor) is employed when sample unit sizes represent more than about 5% of the population. This can be used to reduce the sampling error or the sample unit size required. The equation is:

$$\text{fpc} = \sqrt{\frac{N-n}{N-1}}$$

where

fpc = finite population correction

N = size of the population

n = size of the sample

Assume $N = 2000$ and $n = 200$

$$\text{fpc} = 0.901$$

For example, if the estimated number of sample units needed is 162 and the fpc = 0.901, then the corrected number of sample units needed is:

$$162 \times 0.901 = 146 \text{ samples}$$

In sampling small populations, the fpc factor may have an appreciable effect in reducing the variance of the estimator (Thompson, 2002). For further information see the Internet for numerous examples.

For pollution studies, if you want to know **how many sample units to take** in order to determine if pollution standards have been exceeded, the following equation has been used:

$$N = Y \frac{Zs^2}{D^2 \bar{X}^2}$$

where

N = no. of sample units required

Y = expected level of change (% expressed as a decimal)

s = standard deviation

D = allowable error (10% or 0.1)

$\overline{X}$ = mean

Z = a function of the distance from the mean in standard deviation units.

2-tailed test: $Z\ (p = 0.05) = 1.96$ (=95% confidence level)

$Z\ (p = 0.01) = 2.58$ (=99% confidence level)

Example

Assume a Z of 1.96 (95% confidence level), 20% change, allowable error of 10%, mean of 10, and standard deviation of 4.

$$Y = 0.2\frac{(1.96)(4)^2}{(0.01)(10)}$$

$$Y = 63 \text{ samples}$$

1.3 QUANTITATIVE SAMPLING METHODS

1.3.1 Introduction

Major methods of sampling marine benthic organisms for abundance can be conveniently categorized as plot and plotless. This section will give only a brief introduction as to how these sampling programs are carried out. The reader is referred to Southwood (1978), Seber (1982), Hayek and Buzas (1997), Krebs (1999), and Thompson (2002) for detailed information. Eleftheriou and McIntyre (2005) discuss methods for the study of marine benthos. The seasonal timing of sampling is determined by the life cycle (Southwood and Henderson, 2000). **Plot methods incorporate the use of rigid boundaries**, that is, squares (quadrats), rectangles, or circles (circlets, unfortunately also called quadrats by some investigators), and circumscribe a given area in which organisms are counted or collected. **They are used to save time, instead of conducting total counts or a census of organisms, and to remove bias in sampling**. Bias is a systematic, directional error (McCune et al., 2002).

Some traditionally plotless sampling techniques become plot techniques when boundaries are added for convenience (e.g., PCQ – see below), and coordinate lines in simple random sampling create sample points rather than fixed boundaries or plots. Establishing transect lines or cluster sampling can be followed by either plot or plotless sampling techniques. Thus plot and plotless are somewhat flexible terms yet are convenient to use.

The plot method of sampling generally consists of three major types: (1) simple random or random sampling without replacement, (2) stratified random, and (3) systematic (Cochran, 1977). Simple random sampling with replacement is inherently less efficient than simple random sampling without replacement (Thompson, 2002). It is important not to have to determine whether any unit in the data is included more than once. Simple random sampling consists of using a grid or a series of coordinate lines (transects) and a table of random numbers to select several plots (quadrats), the size depending on the dimensions and densities of the organisms present (Fig. 1-7 and see p. 19). The advantage of using these standardized sizes is that comparisons can be easily made between the densities of species in different regions and with data collected from the past. Some divers have used circular frames (e.g., using 3 lb. metal coffee cans [approximately 8 inches (20 cm) high by 6 inches (15 cm) in diameter] to core surface sediments in the shallow waters of the coastal Arctic Ocean because this is a convenient way to collect infauna in that region).

The basal area of trees or forest stands has more functional significance than most descriptors of forest structure. Density measurements are of relatively little value with plants unless applied to restricted size classes (McCune et al., 2002).

See Arvantis and Portier (2005) for information on natural resource sampling methodology.

1.3.2 Table of Random Numbers

In the past, few texts had tables of random numbers in columns of two digits, which gave numbers from 1 to 99, convenient for ecologists. The tables were typically columns of four digits. A random number generator starts with an initial number then uses a deterministic algorithm to create pseudorandom numbers (Michael Arbib, pers. comm.). A table of random numbers is shown in Table 1-1. Tables of random numbers are used to take samples randomly. Samples are taken randomly to remove bias.

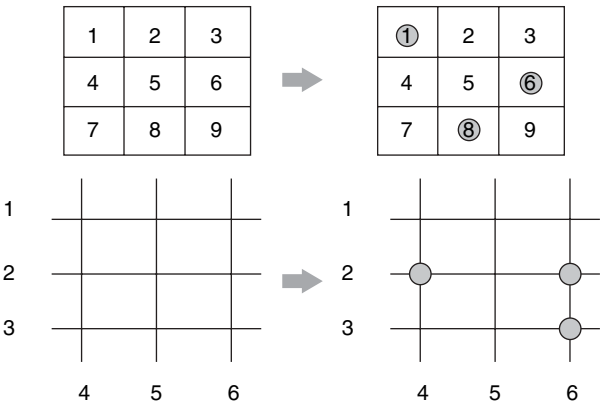

Figure 1-7. Simple random sampling. Random numbers from a table of random numbers give 1,6,8 for the squares and 2-4, 2-6, 3-6 for the coordinate lines, indicating the areas or points to be sampled (e.g., to count animals).

Table 1-1. A table of random numbers.

20 17	42 28	23 17	59 66	38 61	02 10	86 10	51 55	92 52	44 25
74 49	04 19	03 04	10 33	53 70	11 54	48 63	94 60	94 49	57 38
94 70	49 31	38 67	23 42	29 65	40 88	78 71	37 18	48 64	06 57
22 15	78 15	69 84	32 52	32 54	15 12	54 02	01 37	38 37	12 93
93 29	12 18	27 30	30 55	91 87	50 57	58 51	49 36	12 53	96 40
45 04	77 97	36 14	99 45	52 95	69 85	03 83	51 87	85 56	22 37
44 91	99 49	89 39	94 60	48 49	06 77	64 72	59 26	08 51	25 57
16 23	91 02	19 96	47 59	89 65	27 84	30 92	63 37	26 24	23 66
04 50	65 04	65 65	82 42	70 51	55 04	61 47	88 83	99 34	82 37
32 70	17 72	03 61	66 26	24 71	22 77	88 33	17 78	08 92	73 49
03 64	59 07	42 95	81 39	06 41	20 81	92 34	51 90	39 08	21 42
62 49	00 90	67 86	93 48	31 83	19 07	67 68	49 03	27 47	52 03
61 00	95 86	98 36	14 03	48 88	51 07	33 40	06 86	33 76	68 57
89 03	90 49	28 74	21 04	09 96	60 45	22 03	52 80	01 79	33 81
01 72	33 85	52 40	60 07	06 71	89 27	14 29	55 24	85 79	31 96
27 56	49 79	34 34	32 22	60 53	91 17	33 26	44 70	93 14	99 70
49 05	74 48	10 55	35 25	24 28	20 22	35 66	66 34	26 35	91 23
49 74	37 25	97 26	33 94	42 23	01 28	59 58	92 69	03 66	73 82
20 26	22 43	88 08	19 85	08 12	47 65	65 63	56 07	97 85	56 79
48 87	77 96	43 49	76 93	08 79	22 18	54 55	93 75	97 26	90 77
08 72	87 46	75 73	00 11	27 07	05 20	30 85	22 21	04 67	19 13
95 97	98 62	17 27	31 42	64 71	46 22	32 75	19 32	20 99	94 85
37 99	57 31	70 40	46 55	46 12	24 32	36 74	69 20	72 10	95 93
05 79	58 37	85 33	75 18	88 71	23 44	54 28	00 48	96 23	66 45
55 85	63 42	00 79	91 22	29 01	41 39	51 40	36 65	26 11	78 32

The numbers are arranged into columns of two digits, ideal for the field biologist. Other tables of random numbers may have columns of three or four digits. The digits in a two-column random numbers table range from 01 to 99 usable numbers, in a three-column random numbers table from 01 to 999, and in a four-column random numbers table from 01 to 9999. To use the table, one can proceed from top to bottom (e.g., 20 to 55). Begin with the first column and proceed to the bottom then go to the top of the second column and proceed to the bottom, and so forth. You can also start from a haphazard location in the table (Thompson, 2002). Note that some of these numbers are very close to one another (e.g., 32, 35, 33) by chance. This can skew the results of your survey if you are sampling by the simple random sampling method (see Figure 1-9). This is why ecologists use some type of stratified random sampling in plot techniques. If you need more numbers go to the computer and generate more.

1.3.3 Quadrat Shape

Ecologists have used squares, rectangles, and circles (e.g., 3 lb. coffee cans to core sediments by hand; a 1 m long piece of twine tied to a stake and rotated in a circle as one counts benthic organisms; in songbird surveys). The most common shape for sampling benthic marine organisms is a square (67%), followed by circles (19%), and rectangles (14%) (Pringle, 1984). Rectangular frames with a size ratio of 2:1

tend to give slightly better results with population estimates than do square frames in terrestrial studies (Krebs, 1999). Thompson (2002) compared nine types of plots and compared their detectability functions. **Long, thin rectangular plots are more efficient than square or round plots**. Various line transects, variable circular plots (radial transects), and plots with holes in them (i.e., torus or doughnuts) gave intermediate results. However, **if there is a clinal gradient of some type, a rectangular quadrat can be aligned parallel or perpendicular to the cline and the variance in the density can be very different**. Long quadrats cover more patches, whereas narrow rectangles (size ratios higher than 4:1) can create a severe edge effect, in which too many organisms may cross the boundary of the quadrat, resulting in more frequent counting errors. **Typically, animals intercepting the top and left-hand boundaries are counted (Southwood and Henderson, 2000). Edge effects often produce a positive bias or a number greater than the true density** (Krebs, 1999). Edge effects, in theory, are least with circles, intermediate with hexagons, and greatest with squares and rectangles because bias introduced by edge effects are proportional to the ratio between the boundary length and the area within the boundary (Southwood and Henderson, 2000). Circles are the poorest shape for estimation from aggregated distributions, resulting in high variances (McCune et al., 2002). Squares are also poor and rectangles better for aggregated distributions, especially narrow rectangles, but narrow rectangles may exhibit severe edge effects.

1.3.4 Optimal Quadrat Size

The optimal size for a quadrat depends on many factors. Changes in quadrat size (i.e., scale) can result in differences in the interpretation of field data, such as abundance, associations between species, and the degree of aggregation within a species (Fig. 3-1 on p. 124). One rule of thumb is to select a size of quadrat that will not give frequent yields of zero counts of individuals. **Use the smallest quadrat that is practical or easiest to use but will also sample organisms adequately**. The larger the species the larger the quadrat size. The optimal size for aggregated species is the smallest size relative to the size of the species (Green, 1979). For example, when counting small, numerous barnacles, you may use a $0.1\,m^2$ quadrat frame, but then subdivide the frame into 50 or 100 small squares. A smaller size often results in increased precision of estimates with aggregated distributions because the boundary is small, thus one would be less likely to either double-count or undercount individuals. Moreover, smaller sizes often result in a smaller variance around the mean but scaling factors may alter this (Greig-Smith, 1964). Pringle (1984) found that the $0.25\,m^2$ quadrat was the most efficient size for sampling benthic marine macrophytes. Dethier et al. (1993) concluded that 10×10 cm quadrats were effective for visual estimates of the abundance of sessile benthic marine organisms. A compromise in frame size must be made when more than one species is being studied and counted within the same quadrats. Interactions between adjacent organisms (e.g., production of allelochemicals) may result in the species growing only a certain distance from each other. These interactions should also be considered when determining quadrat size, especially on coral reefs (Wilfredo Licuanan, pers. comm.).

Techniques have been developed to determine the most appropriate group frame size (Southwood, 1978) but field experience seems to be the most efficient and effective determinant of frame size. Southwood (1978) suggests that the relative net precision of a unit of a given size is as follows:

$$\mathrm{RNP} = \frac{1}{\mathrm{Cu}\,S^2\,\mathrm{u}}$$

where

RNP = relative net precision

Cu = relative cost of taking a sample (usually time)

S^2u = variance among unit totals.

Example

Cost (Cu) = 4 h

Variance = 25

$$\mathrm{RNP} = \frac{1}{4 \times 25} = \frac{1}{100} = 0.01$$

The highest value of RNP is the best unit. For multiple species, sum the relative net precision values for each quadrat size over all species of interest and choose the unit with the highest sum. If certain species were more important than others (i.e., ecologically as numerical dominants or as keystone species), weighting of their relative precision values would be appropriate. Krebs (1999) recommends the Wiegart method (Wiegart, 1962) in which quadrat size (*x*-axis) is plotted against relative cost (i.e., time, *y*-axis) (Fig. 1-8). The size of quadrat with the lowest "cost" is preferred. Krebs (2000a) provides computer programs for determining optimal quadrat size. See the Appendix.

In practice, ecologists often use a range in the size of quadrats from 0.1 to 1.0 m^2 (but also 0.01 m^2 for small organisms such as barnacles and 100 m^2 when sampling the distribution and abundance of trees) to cover all of the possibilities in

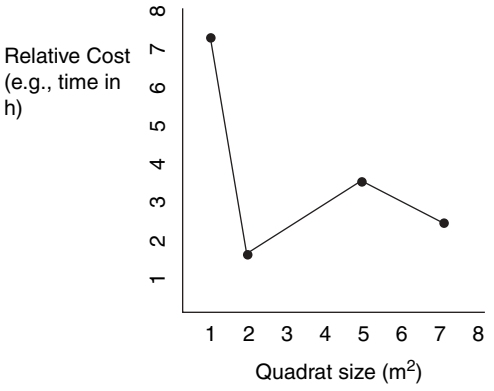

Figure 1-8. The Weigert method for determining the best quadrat size. It is 2 m^2 in this example. Source: modified from Krebs (1999).

a standardized fashion (e.g., number of organisms per 1, 10, or 100 m^2). However, one cannot always accurately extrapolate species richness or density in a small area (e.g., 0.1 m^2) to species richness or density in a larger area (e.g., 1 m^2) because the relationship between the two areal sizes is often nonlinear. Such extrapolations are done frequently for convenience, but must be interpreted carefully. See West (1985) for an interesting discussion on nonlinearity.

When counting organisms in a quadrat, one should examine each quadrat in a similar manner. For example, in looking down on a quadrat from above, you may wish to exclude animals in cracks and crevices (because including cracks and crevices creates numerous complications such as differences in crevice size, shape, depth, etc.). This standardizes the procedure and greatly simplifies the sampling process.

1.3.5 Simple Random Sampling

Simple random sampling consists of using a grid or a series of coordinate lines (transects) and a table of random numbers to select plots (e.g., squares, quadrats). The bottom right side of Fig. 1-9 shows **the main pitfall of the simple random sampling technique, that is, that the random numbers may occur in such a fashion as to concentrate sampling effort mostly in one part of the study area, missing important parts of the study area**. The other major criticism is that the simple random sampling method is unfeasible for large areas (for example, Marsden squares in the ocean or dense forests) since too much time is wasted in moving from one place to a distant site. Marsden squares represent areas on a Mercator chart of the world, each square measuring 10 degrees of latitude by 10 degrees of longitude.

1.3.6 Haphazard (Convenience, Accidental, Arbitrary) Sampling

Haphazard sampling is often carried out in the field to substitute for random sampling. It is sampling without the use of a classical sampling design. Bias is always a problem in haphazard sampling. A diving project in the Maldive Islands required random sampling. Random sampling would have taken an inordinate amount of time and time was limited, thus haphazard sampling was employed. A biologist had

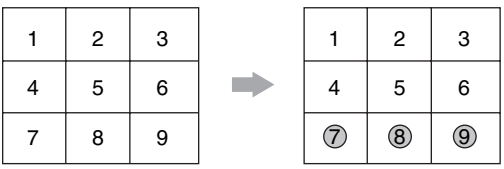

Figure 1-9. Problems with simple random sampling. Three numbers were chosen randomly from a set of number ranging between 1 and 9. By chance they all fell in the lower part of the sampling area. If this were an intertidal site, the study would give an incomplete picture of community structure as it would leave out the middle and upper intertidal zones.

initially and casually swum through the potential site to finally select it as a suitable study area (i.e., it had living hard coral growth rather than continuous sand). He then swam across a flat coral reef area, dropping weights haphazardly every 30 sec, without looking at the seafloor. These weights then became corners of quadrats to be sampled. Some bias was thus removed without random sampling and the effort was highly time efficient. McCune et al. (2002:17) refer to this technique as "arbitrary but without preconceived bias."

1.3.7 Stratified Random Sampling

The sampling design is called stratified random sampling if the design within each stratum (e.g., habitat or elevation) is simple random sampling (Cochran, 1977; Thompson, 2002). In some cases it may be desirable to classify the units of a sample into strata and to use a stratified estimate, even though the sample was selected by simple random sampling, rather than stratified random sampling. Stratified random sampling involves choosing subsamples with a table of random numbers from each of the major plots or quadrats which are arranged in strata in the study area (Fig. 1-10). **This method is frequently used since the sampling is conducted throughout the study area. The advantage of using either simple random or stratified random sampling techniques is that standard statistical procedures can be applied**. Stratified random sampling uses a table of random numbers and is often considered to be the most precise method of estimating population densities other than a direct total count or census, for two reasons. It covers the entire study area and samples randomly from each subdivision of the study area (Southwood and Henderson, 2000). Nevertheless, contrary to assumption, **stratified random sampling is not necessarily the most accurate method of sampling the environment** (because too few samples may be taken and because it may not be as accurate as some line intercept methods with highly aggregated organisms – see p. 43) and **it is often labor intensive for divers and for surveys in dense forests when compared to some plotless methods**.

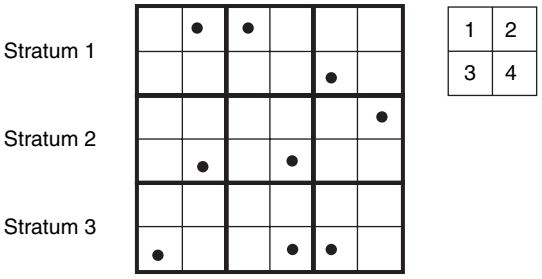

Figure 1-10. Stratified random sampling. The study area is divided into nine large squares (in this example) and each large square into four smaller squares. A table of random numbers is used to select a number (i.e., the dots) between 1 and 4 in each of the larger squares. Thus all strata (3 from top to bottom) are sampled and each large square is sampled randomly.

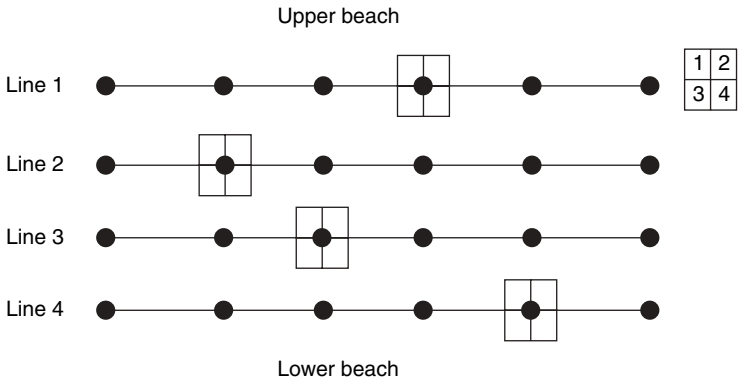

Figure 1-11. Stratified random sampling. A series of transect lines (metric tapes) are lain across the beach. Clothespins are placed at 5 m intervals. A table of random numbers is consulted and one number from 1 to 6 is selected for each transect line. A 0.1 m^2 quadrat frame is placed in four positions at those random spots and numbered 1–4. A table of random numbers is used to select one number between 1 and 4 for each box on each transect line. The organisms in the selected subunits are then identified and counted, the clothespins removed when the counting is completed. A total of four counts are made in this example.

Stratified random sampling can be carried out in various ways. A grid can be constructed and subdivided into strata, each stratum being subdivided into smaller plots. A table of random numbers is then used to select one of the smaller plots from each of the larger subunits of the stratum (Fig. 1-10). Another method of accomplishing the same goal is to arrange transect lines or coordinates across a study area then mark off every 5 m along each line. A table of random numbers (Table 1-1) is used to select some of the designated points along each line for sampling (Fig. 1-11). A better alternative to this is to mark off the line at each 5 m interval then set up a grid at each point, selecting, for example, one subunit of each set of four subunits per grid using a table of random numbers. (Fig. 1-12). This method covers the entire study area and is sampled randomly.

1.3.8 Systematic Sampling

Systematic sampling is used when a uniform coverage of the area is desired. It can be safely used for convenience when the ordering of the population is essentially random (Cochran, 1977). It is often used in marine studies where the primary interest is to map distributions or monitor sites with respect to environmental gradients or suspected sources of pollution (Southwood and Henderson, 2000; McDonald, 2004). Systematic sampling involves choosing a constant sampling pattern (for example, every other quadrat or every third quadrat, see Fig. 1-13). Note that the systematic pattern may conform with an environmental pattern (e.g., quadrats 3-5-7 in Fig. 1-13) and this biases the overall results. For example, the systematic pattern could follow a ridgeline of serpentine soils or an intrusive ribbon of intertidal rock of a different characteristic

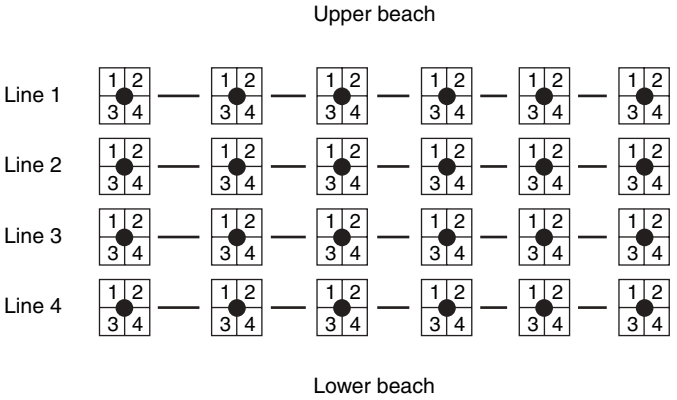

Figure 1-12. Stratified random sampling. A series of transect lines (metric tapes) are lain across the beach. Clothespins are placed at 5 m intervals. A 0.1 m^2 quadrat frame is placed in four positions at each spot and numbered 1–4. A table of random numbers is used to select one number between 1 and 4 for each box on each transect line. The organisms in the selected numbered box are then identified and counted, the clothespins removed when the counting in finished. A total of 24 counts are made.

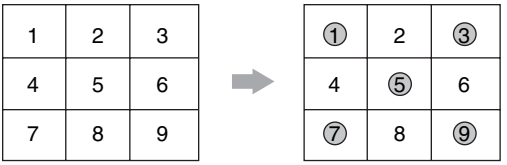

Figure 1-13. Systematic sampling. Begin with quadrat 1 and select every other quadrat that remains (or every third, fourth, etc.). Note that this has created an artificial diagonal or X pattern. If quadrat Nos. 1, 5, and 9 follow a specific sediment type (e.g., marine clays) then the plants or animals living there may be different than those in other areas and they would be emphasized in the collection data.

than the surroundings (Fig. 1-14). The sampler would thus collect more endemic plants that grow on serpentine soils or a different assemblage of marine invertebrates, thus biasing the overall picture. **Because there is no element of random sampling in this method, standard statistical tests cannot be used** (Southwood and Henderson, 2000). When statistical tests are applied to data from systematic studies, the probability (*p*) values are not accurate (McCune et al., 2002). One major advantage of the systematic method is that it often simplifies logistics involved in sampling and is useful in fields such as forestry (mensuration) or deep-sea sampling. It may also increase the probability of collecting uncommon species in species-rich areas. A higher density of clams was detected in Prince William Sound, Alaska, in systematically located sites than in preferred clam habitat (McDonald, 2004). One can combine methods, such as using systematic sampling to cover large areas with stratified random sampling within each of the systematic sampling plots. See Buckland et al. (2001), Hayek and Buzas (1996), and Thompson (2002) for general sampling techniques and Keith (1991) and Mueller et al. (1991) for environmental sampling.