Chapter 6

Census Methods for Benthic Organisms

"Consider what each soil will bear, and what each refuses." – Virgil

Unlike hydrologic data, the information we seek to gather from living organisms is far more ephemeral. After all, we expect organisms to exhibit variable features, even among members of the same species. Virtually every conceivable measure of an organism's features—its body shape, its coloration, its biomass, or even its gender—will be different from its conspecific fellows. And while variety may be the spice of life, it certainly makes it more difficult for us to assess what is a "typical" biometric for that species.

Fortunately for us, a wide variety of census techniques are available for the assessment of highly variable biological communities. And among the biotic habits of organisms found in the sea, those that make their home on or in the bottom are the easiest to measure, largely because we can ignore the vast volumes of the ocean and restrict our attention to the two-dimensional world of the bottom-dwellers. That is good news, because it is always easier to analyze complexity in two dimensions rather than three. So, as long as our study subjects stay on the bottom we can use the classic terrestrial census techniques to assess population density, frequency of occurrence, percent occupation of the habitat, or virtually any other biometric we may wish to quantify.

Key Concepts

- The census methods used for benthic aquatic organisms are similar to those used in classic terrestrial ecology assessments.
- The biotic habit of the target species will define which census method is most appropriate for use.
- If a particular plot or plotless method is indicated for sessile benthic species, the same method can be used to assess motile benthic species as well; however, the corollary may not be true.
- Benthic species that can freely migrate into or out from a study area are said to be from "open" populations and may require highly specialized census techniques to estimate their true population densities.

Choosing the Right Method for Each Biotic Habit

When deciding on the most appropriate census method for a particular biological community, it is critical that you first consider the **biotic habit** of the marine organism(s) you wish to study (**Figure 6.1**). Since a biotic habit is essentially an organism's preferred lifestyle, it only makes sense that we would have better success studying an organism "where it lives."

If an organism in the ocean is powerless against the currents and will float wherever the ocean takes it, it is considered to be **plankton**. Although plankton can be found floating and/or drifting anywhere in the water column, those plankton that can only be found at the surface belong to a special class of organisms called **neuston**. If an organism is powerful enough to swim against the currents of the ocean, it is classified as **nekton**. Because the plankton and nekton can be found anywhere (and everywhere) within the water column, we are usually forced to adopt more complex, three-dimensional census methods when surveying these kinds of **pelagic** organisms (which we shall discuss in the following chapter).

Of course, there are many marine creatures that neither swim nor float. Those organisms that are associated with the bottom are considered to be **benthic** in their biotic habit; that is, they are part of the **benthos**. Within the larger context of the benthos, the **epifauna** are those animals that make their living upon the substrate, while the **infauna** are those that live within the substrate itself.

Although all of the pelagic organisms can be assumed to be **motile** (either carried by the currents or swimming on their own), this is not necessarily the case among benthic organisms. Certainly, there are many benthic organisms that are also motile (that is, free to move about and within the substrate). However, one of the most significant consequences of the benthic habit is that it allows certain species to remain stationery, either attached to or buried within the substrate. Hence, those creatures that move extremely slowly (or not at all) are considered instead to be nonmotile, or **sessile**.

In general, benthic organisms are much more easily surveyed because we know exactly where they can be found: somewhere on (or in) the bottom. That means we can utilize the more traditional, two-dimensional census

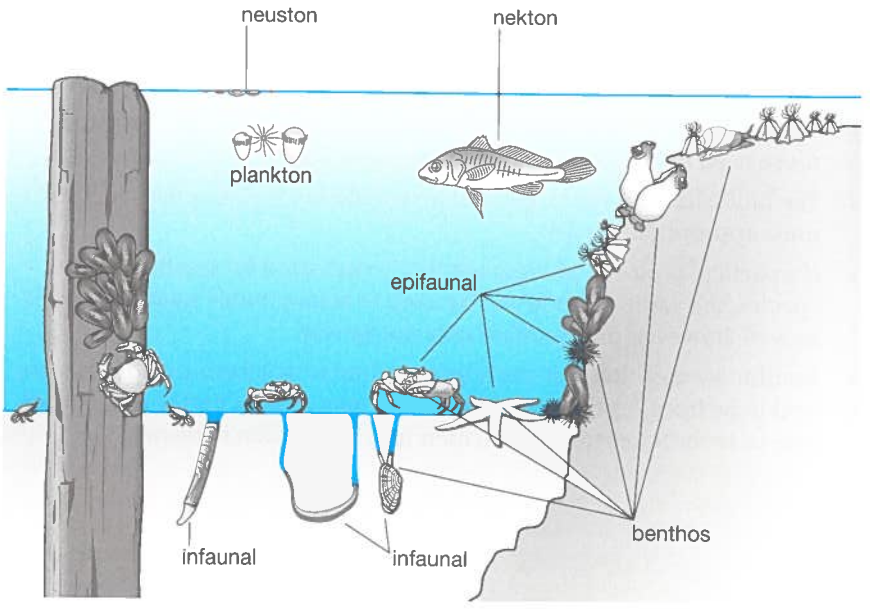

Figure 6.1 The classic paradigm of benthic and pelagic habits is depicted here. Within the pelagic environment, organisms that are powerful enough to swim against the currents are categorized as nekton, while those powerless to resist water movement are categorized as plankton (with the neuston constrained to the surface). Within the benthic environment, animals that live upon the substrate are categorized as the epifauna, while those that live buried (or encapsulated) within the substrate are called the infauna.

methods used in terrestrial ecology—adapted for use in an aquatic environment. But unlike the pelagic organisms (which are all motile), you must take great care in choosing the most appropriate census method for benthic organisms, particularly if the community you wish to investigate has a complicated mixture of both motile and sessile forms.

Starting Simple Is the Key to Every Biological Census

As a field researcher, you should never feel limited by what others have done in the past, or by what is considered to be a "typical" census method. By the same token, it is often useful to consider such things and to seek guidance from other investigations as inspiration for your own. In most cases, biological surveys seek to accomplish one or more of the following assessments in an ecological context:

1. Population density (number of individuals per unit area)
2. Frequency of occurrence (relative abundance)
3. Live coverage (area occupied per unit area of available habitat)
4. Living biomass (or other relevant biometric, such as body length)

Although these sorts of data may seem a bit routine or perhaps simplistic, it would not be possible to perform any of the higher-order assessments of community dynamics without them. So for now, let us focus on the fundamentals—we will have ample time to explore how these data can be used in the analyses of single- and multivariate dynamics in later chapters.

Take Note Whether the Census Includes Open or Closed Populations

Because benthic populations can have a mixture of motile and sessile forms, it is often a challenge to account for population gains and losses over the period of investigation. Not surprisingly, an organism's capability for reproduction may cause the population size within the area of study to increase specifically as a function of **natality** and/or larval **recruitment**. Conversely, the population size may decrease as a result of **mortality**. Each of these represent an inherent change to the overall population size, in the sense that reproductive additions to the population size (or growth additions to the population biomass) are determined by an organism's intrinsic life cycle and can only be "undone" by the organism's death.

However, there can also be changes to the population size (or biomass) that are caused by transient migration events. Any organism migrating into the study area from another location will of course represent an increase to the population size due to **immigration**; likewise, any organism migrating out of the study area will cause the population size to decrease as a result of **emigration**. In either case, the true population size is not affected at all, but there will be an apparent change to the population size within the area of study depending on the migration rates into or out of the confines of your census area (**Figure 6.2**).

Although one might expect a natural population to always fluctuate as a result of reproduction, growth, and mortality, field researchers must consider whether motile species are free to immigrate into (or emigrate from) the study site—a truly "open" population. Sessile species will also be affected by reproduction, growth, and mortality, but they can usually be considered to belong to a "closed" population because their numbers are largely unaffected by migration. That being said, it is important to keep in mind that there are very few populations that are truly closed, because most aquatic species spend the early phases of their life cycle

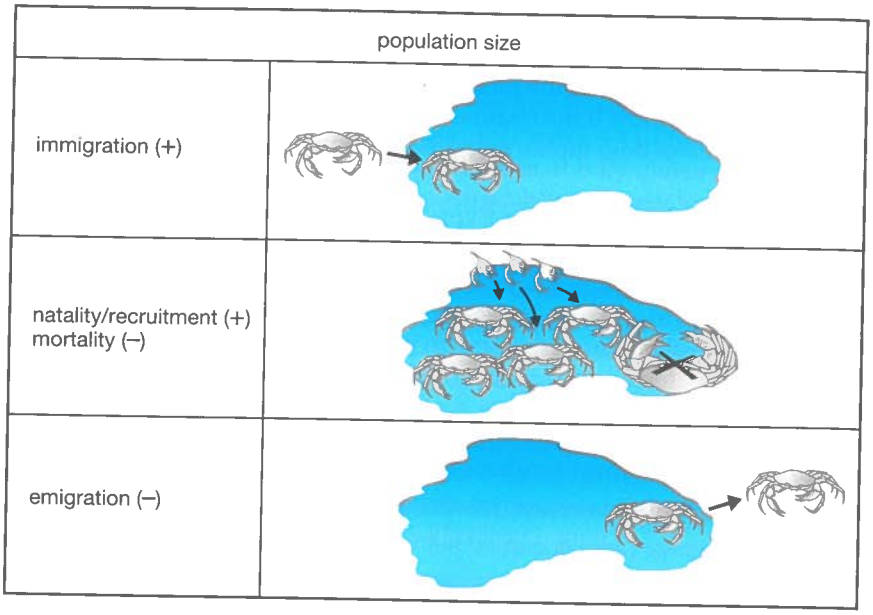

Figure 6.2 Changes in population size will typically increase as a result of reproductive gains (as natality or recruitment) or immigration into a fixed study area. Decreases in population size will result from mortality losses and/or emigration out of the study area. Although all organisms are affected by natality and mortality, only the motile species are affected by migration.

as planktonic larvae (so there is always some capacity for drifting larvae to migrate).

The duration of the field study can also have an influence on whether the population(s) are considered to be open or closed. If a field survey is conducted over a long enough time period that the organisms under investigation become subject to changing climactic and/or breeding cycles, the population must be assumed to be open. Similarly, slow-moving motile species can be considered to be closed populations, so long as the field study is conducted over such a brief period of time that migration effects are eliminated. Although there are ways to compensate for these effects, it is nonetheless important for the field researcher to consider whether the population is open or closed when deciding what census technique to use.

Benthic Organisms Require a Two-Dimensional Census Method

Because benthic organisms must be located on (or in) the substrate, it is almost always appropriate to think in terms of population density, live coverage, or biomass relative to some unit area rather than by volume. In a very practical sense, it is far easier to conduct the general plot and plotless methods discussed earlier when you're dealing with two spatial dimensions rather than three.

Although the bottom is never truly "flat," in areas where the bottom has very limited topographic relief, it is perfectly acceptable to assume planar geometries when surveying the epifauna, which can easily be accomplished with a plot method utilizing rigid quadrats. However, for those epifauna living upon highly irregular surfaces, it is more appropriate to use a survey method utilizing a line or belt transect that can be draped over an irregular bottom topography. This method has the added benefit of providing an objective measure of substrate **rugosity**, whereby biological data obtained from the plot method can be related to (1) the true surface area of the substrate (A^0), (2) the idealized surface area of the substrate (A'), and/or (3) the overall rugosity of the substrate (**Figure 6.3**). Of course, one might opt to abandon the plot methods altogether in favor of a plotless one.

For assessments of infauna, it may be more appropriate to utilize a stratified sampling technique, where infauna can be assessed at discrete depth

intervals in the substrate, which are treated as successive, two-dimensional layers extending down into the substrate. For example, let us presume that the organisms depicted in **Figure 6.4** are each found in a different "depth zone" in the sediment, but were each collected with a box core with a cross-sectional area of 0.50 m^2, 25 cm deep into the sediment. If Layer 4 is explicitly defined as inclusive of sediment depths 7–12 cm, all of the organisms collected within Layer 4 (9 cm mean depth) can ultimately be cited relative to the 0.50 m^2 sampling area of the box core.

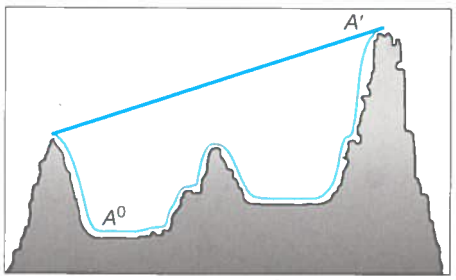

Figure 6.3 Rugosity as a measure of small-scale variations in height across an irregular surface height. Mathematically, it is simply defined as a ratio of the actual (true) length or surface area A^0 in relation to the idealized (geometric) length or surface area A'.

Census Methods for the Sessile Benthos

Among the most versatile of all census methods in marine science are the "nearest neighbor" plotless methods and the "line transect" and "quadrat" plot methods discussed earlier. With very little modification, these census methods are ideal for use in surveys of sessile benthic organisms. However, there are several other options that may have more specific utility, depending on the habit of the organisms that are at the center of your field investigation.

Rare Species Are Best Surveyed Using the T-Square Plotless Method

For those sessile organisms that occur very rarely, or are sparsely distributed, it is very likely that most of the investigator's time in the field will be wasted conducting multiple plot assessments in which the species of interest is simply not recorded. In such cases, it is usually advisable to conduct a plotless "T-square" survey instead. Similar to the nearest neighbor method (*1NN*, see Equation 3.14), the T-square method employs a series of random starting points (P_i) within the study area, as illustrated in **Figure 6.5**. For each randomly determined starting point P_i in the study area, the nearest individual of the target species (O_i) is sought out and its shortest distance to P_i is measured as x_i. A conceptual line is then drawn perpendicular to the

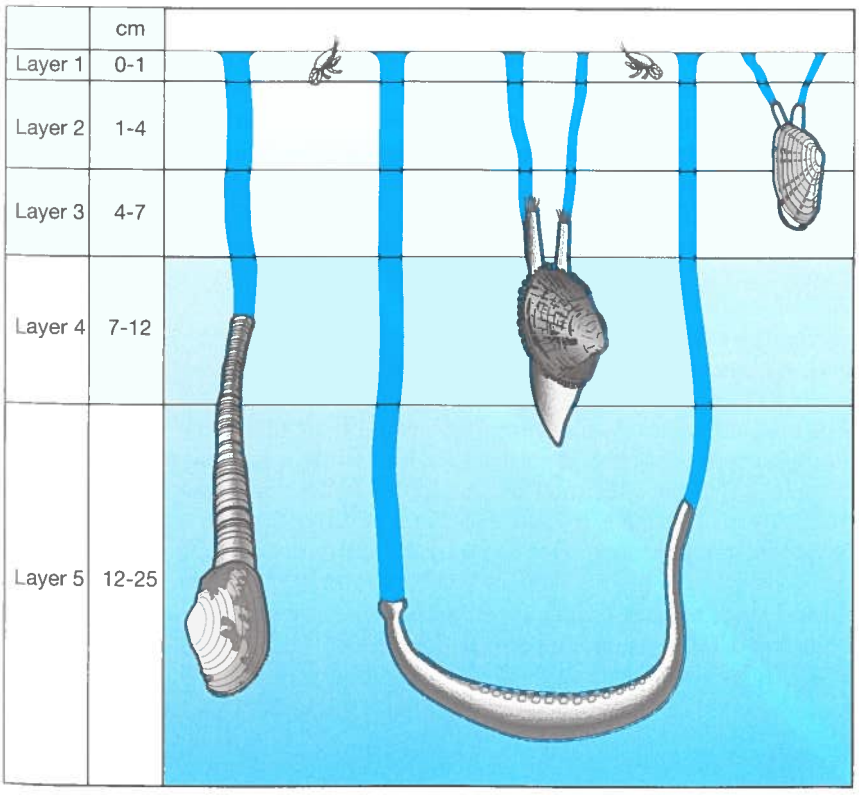

Figure 6.4 Despite the vertical differences of infauna diversity and biomass, if several discrete vertical layers are chosen and each is surveyed as a single "section," the data collected from each section may be reported as biomass per unit volume (for example, mg cm^{-3}), performed for each layer).

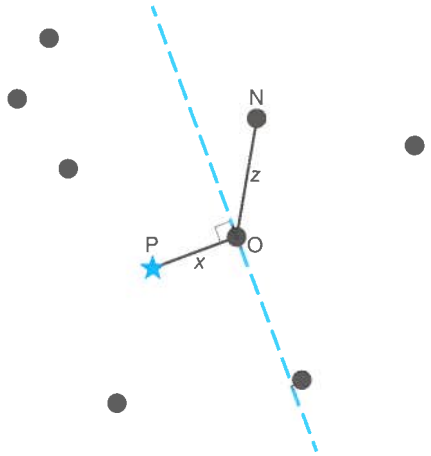

Figure 6.5 The T-square method. The black circles represent individual sessile organisms belonging to the target species. O is the nearest individual to the random starting point P. N is O's first nearest neighbor on the opposite side of the line perpendicular to OP.

line between P_i and O_i, and the distance to the first nearest neighbor (N_i) to O_i is then measured as z_i (as long as N_i is located on the opposite side of the line, relative to P_i).

The population density D (the number of individuals per unit area) can then be estimated using Equation 6.1:

$$D = \frac{\left(\sum i\right)^2}{2.828\left(\sum x_i \cdot \sum z_i\right)} \tag{6.1}$$

where i is simply the number of random starting points used in the assessment. In order to test whether the organisms are distributed randomly throughout the study area, you may use Equation 6.2 to calculate the associated test statistic t':

$$t' = \left(\sum i\left[\frac{x_i^2}{\left\langle\left(x_i^2 + z_i^2\right)/2\right\rangle}\right] - \frac{i}{2}\right) \cdot \sqrt{\frac{12}{i}} \tag{6.2}$$

Determining the distribution pattern of a particular species can often yield information about whether the individuals in that population are associative and therefore exhibit some kind of density-dependent relationship. In virtually all cases, the distribution patterns can be categorized either as uniform, random, or aggregated (see Figure 3.3). Using the test statistic calculated in Equation 6.2, the distribution pattern can be determined as

$t' > +2$	Uniform
$-2 < t' < +2$	Random
$t' < -2$	Aggregated

The Distance Sampling Plot Method Is Best for Closed Populations

Distance sampling can be used for assessments of either sessile or motile species, but it is particularly useful for closed populations that are assumed to have relatively constant species densities throughout the study area. Distance sampling is so named because it assumes that the investigator's ability to detect members of the target species decreases as a function of increasing distance from the transect line. This decline in "detectability" can thus be used to estimate the number of specimens that were not recorded during the execution of the survey. As with all survey methods, it is often necessary to conduct several replicate surveys to minimize errors.

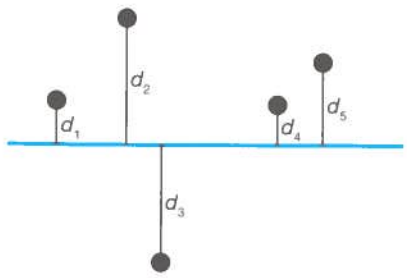

Figure 6.6 The distance sampling method. The black circles represent individual sessile organisms belonging to the target species; their shortest distances from the line transect are measured as d_i.

This method is remarkably simple, as it simply involves an observer moving along a predetermined route through the study area (usually along a line transect). When a specimen of the target species is spotted at any distance from the survey route, its shortest distance from the line transect is determined and logged as d_i (**Figure 6.6**). When the observer has traveled the entire length L of the line transect, the total number of specimens detected (i) and their respective distances d_i from the line transect can be used in Equation 6.3 to estimate the population density D:

$$D = \frac{i\sqrt{\left(2i/\sum\left\langle d_i^2\right\rangle\right)}}{2L} \tag{6.3}$$

The Line Transect Plot Method Is Among the Simplest to Use

The line transect method simply involves a line (or chain) of a specified length randomly laid out within a study site. The observer then transits the line transect, recording biometric data for the organisms of interest that are encountered (touching the line) at various points along the length of the transect (**Figure 6.7**). If the observer wishes to conduct a complete count, everything in contact with the transect line is surveyed. However, if the data volume generated from a complete count is too burdensome, the investigator may instead survey only those organisms encountered at regular distance intervals along the line (such as every 1 m along a 100-m line). Once interval data are collected, they can be used to provide an estimate of species densities, based on the encounter rate of each species and the relative distances between encounters (**Example Box 6.1**).

In low-energy environments, the use of a tethered nylon rope or tape measure is an excellent choice. For interval sampling along the line, knots can be tied in the line (or marked with permanent ink) according to the chosen sampling interval. Of course, the use of a nylon tape measure is the most straightforward and has the added benefit of allowing the investigator to monitor "along-transect" distances at a glance. In higher energy environments, it may be necessary to instead use a heavy brass chain to prevent the line transect from becoming dislodged from its original position. Individual links in the chain can easily be marked (or tagged) according to the chosen sampling interval.

Line transects have the added benefit of deformability; that is, either the line can be stretched taut and surveyed as a straight geometric line, or it can be draped over the substrate or along horizontal contours. Typically, line transects are deployed either parallel to shore to investigate "along-shelf" gradients, perpendicular to the shore to investigate "across-shelf" gradients, or along depth contours for **isobathyal** surveys. Their ease of placement makes them an ideal choice as a plot survey method on structurally complex substrates.

Recall that when plot methods were first introduced in Chapter 3, it was apparent that any plot method must be chosen with careful consideration when determining (1) sufficient sampling effort, (2) the shape and size of the plot method, and (3) the randomness of plot placement. Although line

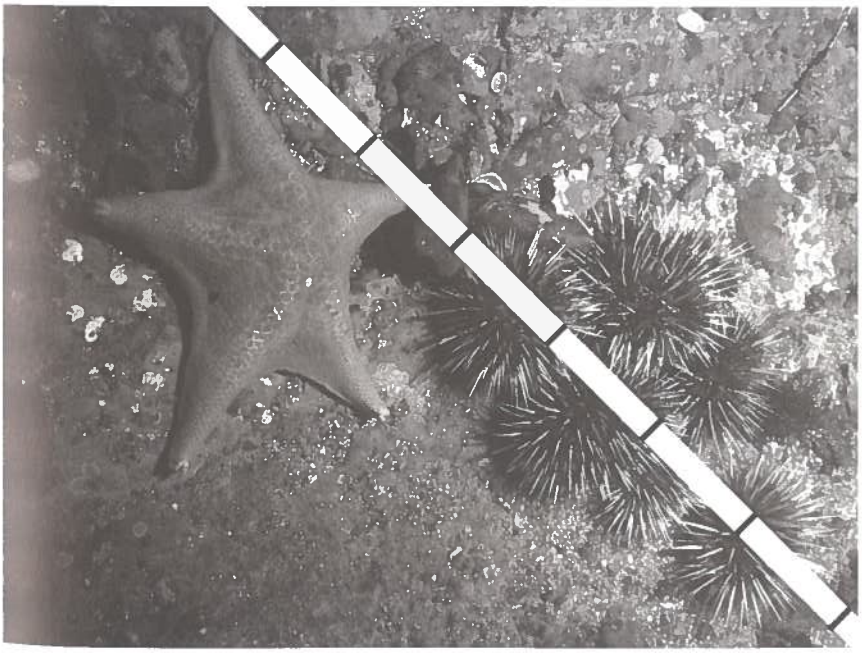

Figure 6.7 A line transect draped over the substrate to be surveyed. For complete counts, everything touching the white transect line is surveyed. For interval sampling, only those species touching the line at predetermined intervals (black hash marks) would be surveyed.

EXAMPLE BOX 6.1

Estimating Population Densities from Line Transect Data

Because line transects are inherently one-dimensional, it is not possible to directly determine population densities from line transect data. However, since species are detected along the line transect as a distance relationship, we can easily determine the mean distances between members of the same species:

Distance interval	Coral species
0.00 m	PPOR
0.25 m	AAGA
0.50 m	MANN
0.75 m	MCAV
1.00 m	SSID
1.25 m	PPOR
1.50 m	SSID
1.75 m	MANN
2.00 m	SSID
2.25 m	MANN
2.50 m	PPOR
2.75 m	MCAV
3.00 m	SSID

According to these line transect data, the coral species *Montastraea annularis* (MANN) was encountered at 0.5 m, 1.75 m, and 2.25 m. The distance between $MANN_1$ and $MANN_2$ was measured as 1.25 m, while the distance between $MANN_2$ and $MANN_3$ was 0.50 m; thus, the mean distance between MANN colonies is calculated as 0.87 m. This means we can expect to encounter 1 MANN colony in each 0.87 m × 0.87 m (0.76 m^2) area. Thus, we can estimate our population density as 1 MANN per 0.76 m^2 (or 1.32 MANN m^{-2}). Although this example demonstrates how easy it is use line transect data to estimate population densities for each species encountered, we would obviously want to collect much more data than are presented here.

transects are easy to deploy in just about any environment, they are a simple linear survey with limited power to resolve the spatial heterogeneity of the study site. The addition of several replicate line transects can ameliorate this, but the quadrat method is generally considered to be superior to the line transect method for most ecological assessments.

True Area Surveys Are Accomplished Using Quadrat Plot Methods

The term "quadrat" generally refers to a square frame of a predetermined size used as a measuring unit by placing the frame over (or upon) the substrate and subsequently documenting the data for all objects or organisms contained within the boundary of the quadrat. As we first discussed in Chapter 3, the use of a circular "quadrat" is preferable to a square frame in order to reduce the sampling bias at the edges of the quadrat boundary. However, the use of a

square frame quadrat is perfectly acceptable, so long as the size of the quadrat is appropriate for the size of organisms (or objects) that are the focus of the research.

An appropriate quadrat size can be determined analytically by selecting the quadrat size with the lowest variance-to-mean ratio (VMR) according to the "3 & 3 rule" (see Chapter 3). However, densely populated areas and/or small organisms are usually sampled using 0.25 m^2 quadrats; more sparsely populated areas and larger organisms are sampled using either 0.5 m^2 or 1.0 m^2 quadrats. The use of quadrats larger than 1.0 m^2 may certainly be indicated for large field investigations; however, this usually requires that a plot of land be staked off and defined using a line or light chain stretched taut around the perimeter of the study area.

Gridded quadrats offer the greatest flexibility in the field (**Figure 6.8**), as the observer can use the same device to either (1) perform complete counts over the entire quadrat, (2) randomly determine some number of nested subquadrats in which to perform complete counts, or (3) perform counts only at the intersection points within the gridded quadrat (which is sometimes called a "point-intercept" method). Quadrats can also be used to improve the line transect method by performing the quadrat surveys at predetermined intervals along the line transect.

Because all of the objects surveyed within a quadrat are automatically assessed as a function of spatial coverage, the use of quadrats makes it incredibly easy to determine population densities, frequencies, and areas of live coverage in relation to the area contained within the quadrat. For example, if you conducted a complete count and found 20 individuals within your 1.0 m^2 quadrat, your final count represents your estimate for the actual population density per unit area (that is, 20 individuals m^{-2}). Frequencies f are calculated in Equation 6.4 as

$$f = \frac{n}{N} \tag{6.4}$$

where n is the total number of quadrats in which the species was observed and N is the total number of quadrats performed in the entire survey.

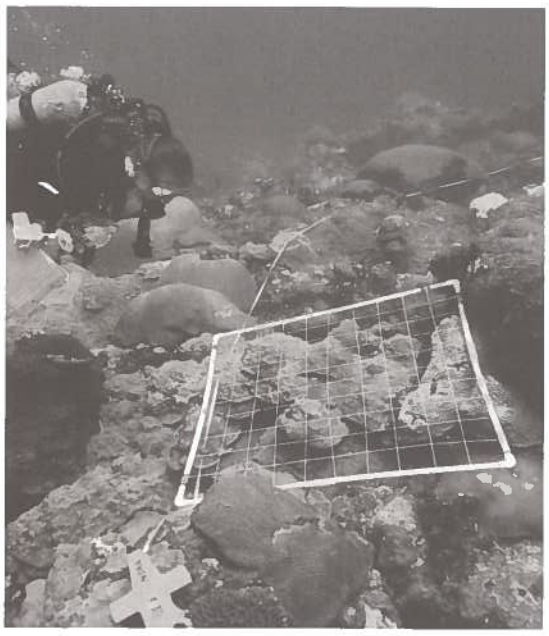

Figure 6.8 A gridded quadrat placed over the substrate to be surveyed. For complete counts, everything contained within the main body of the quadrat is surveyed. Otherwise, a set number of smaller sub-quadrats can be selected randomly for complete counts. The simplest analysis requires the observer to count only those organisms found beneath each of the intersection points of the grid. (Courtesy of the Flower Garden Banks National Marine Sanctuary; Hickerson/FGBNMS.)

Calculations of percent live coverage are typically used to assess distribution patterns of benthic species (like corals, algae, seagrass, or barnacles), but can also be used to determine changes in the availability of habitable space on the substrate. Since measures of percent live coverage are unitless, we can use data either from line transects or from quadrats. For point-intercept data from line transects or gridded quadrats, percent live cover (%) is calculated in Equation 6.5 as

$$\% = \frac{n_P}{N_P} \times 100 \tag{6.5}$$

where n_p is the number of points along the line transect (or within the gridded quadrat) where the living species was encountered and N_p is the total number of points along the line transect (or within the gridded quadrat).

For gridded quadrats, another easy method for assessing percent live cover is to simply determine the number of sub-quadrats where the species in question occupies more than 50% of that sub-quadrat (n_{50}). When related to the total number of sub-quadrats (N_T) within the "parent" quadrat, percent live cover (%) can be imprecisely estimated by Equation 6.6:

$$\% = \frac{n_{50}}{N_T} \times 100 \tag{6.6}$$

Of course, the most precise estimates of percent live cover require that the observer define the physical dimensions of the living organism in relation to the total area within the quadrat. In the field, this may be too time consuming for practical application. However, if it is possible to map all of the quadrats within the survey (usually done by taking digital photographs), the contents of each quadrat can be analyzed at a later date when more time can be dedicated to the analysis.

The use of "photo-quadrats" is a practice well used in oceanography, because digital images can be analyzed in several different color spectra and at several different spatial resolutions. As long as each photo-quadrat depicts a ruler (or some other size standard), the observer can later convert the real dimensions of the photo-quadrat into digital pixels and thereby "measure" the complex geometries of irregularly shaped organisms by tracing their shapes and then calculating the total number of pixels contained within that tracing (**Figure 6.9**). These measurements are then back-calculated to meaningful dimensions, from which precise calculations of percent live cover (or any other spatial assessments) can be conducted.

Long-Term Studies May Require a Fixed Quadrat Plot Method

For long-term monitoring programs, it is sometimes advisable to establish a series of semipermanent (fixed) quadrats that are repeatedly surveyed according to a predetermined time interval. These are especially useful in monitoring the larval settlement, growth, and mortality of certain species over time. This method is also extremely useful when conducting traditional before-after-control-impact (BACI) studies, where several semipermanent quadrats are established in an area before an ecological perturbation, and then surveyed again after the impacts of the perturbation are realized. So long as several quadrats are located in similar but unaffected (controlled) habitats, the observer can use data from the BACI investigation to monitor ecosystem resilience and recovery.

Fundamentally, there is no difference between the standard quadrat plot method and the fixed quadrat method, save that the fixed quadrats are not

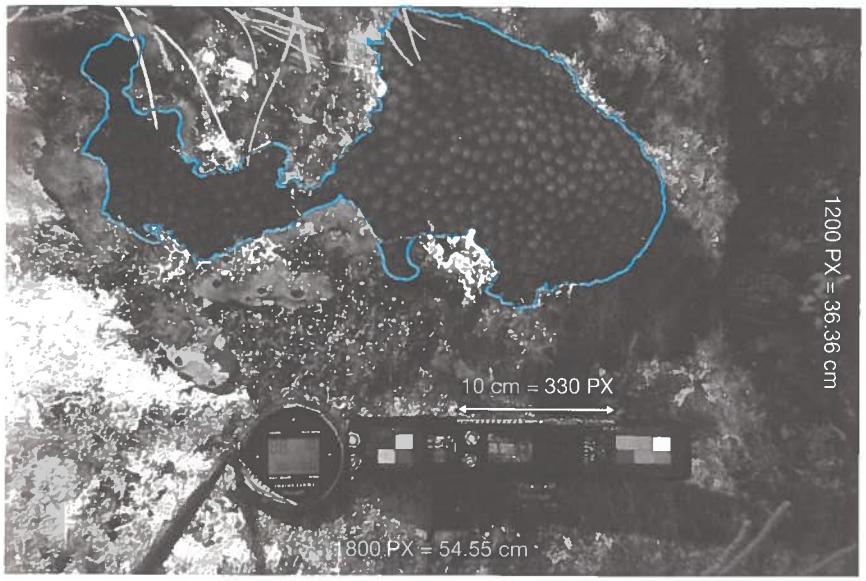

Figure 6.9 An example of a photo-quadrat used in a coral reef survey. Note that the device depicted in the photograph contains a 10.0 cm strip of silver duct tape to serve as a size standard. Using planigraphic software, the physical dimensions of each coral colony can be traced as a complex polygon, and the number of pixels contained within that polygon can be easily determined. By simply measuring the number of pixels along the 10.0 cm size standard, pixel information can be used to determine the total area surveyed within the photo-quadrat (for example, 54.55 cm × 36.36 cm = 1,983 cm^2). Then each coral colony polygon can be expressed in terms of percent cover (total pixels of polygon ÷ total pixels of quadrat) or by its estimated area (in cm^2) of live coverage.

moved once they are initially placed in the area of interest, and they are repeat-sampled. It is important that the investigator consider the appropriate periodicity of repeat sampling within fixed quadrats, as many species exhibit different sensitivities to diurnal, tidal, breeding, and seasonal cycles (which can all significantly impact the results from repeat-sampled study areas).

Census Methods for the Motile Benthos

All the census methods discussed earlier for the sessile benthos are perfectly suited for motile species as well. This section is instead devoted to the few, specialized census techniques that are uniquely suited for bottom-dwelling organisms that are capable of migration—a special circumstance that we have ignored until now.

Because motile species are capable of migration into or out of the study area, we can use these behaviors as an integral part of the census methods used to assess their numbers. This is typically done in one of two general ways: either we can physically collect the migrators and assess their number as a function of trapping success, or we can devise some clever labeling system to mark the organisms we have already surveyed and assess trapping success by keeping track of how many individuals are captured in successive collections (using "mark-and-recapture" methods).

Trapping and Removal Methods Are the Most Common Census Strategy for Motile Benthic Specimens

All trapping methods essentially estimate population densities as a function of catch per unit effort (CPUE), a concept first discussed in Chapter 3. Among the benefits of the various trapping methods, the most significant is the fact that the organisms collected within the traps may be analyzed for virtually any biometric of interest to the investigator. Species identification and enumeration are the most basic data collected from traps, but with a little additional effort, the field researcher can also assess size and biomass data from each collected specimen. If specimens are collected alive, they can be easily transported to the laboratory for behavioral experiments or sacrificed for dissection, tissue analysis, or preservation and long-term archival.

It should be noted that trapping efficiency will never be consistent across study sites and among species. As a result, there are significant biases in

data collected from traps. Although the investigator can compensate for this inherent bias by increasing the quantity of traps used in a particular study, the use of passive traps will always carry the risk that the specimens captured are not truly representative of the larger population from where they came. Despite these imperfections, comparisons can still be made between CPUE and trapping data that follow the same methodology, for the same species and areas of interest. So long as we maintain consistency in our chosen sampling method, the biases inherent to those methods can be constrained.

It is also important to recognize that a haphazard network of traps serves as a plotless census method. As a result, it is usually quite difficult to assess population densities because there is no way to preserve the "spatial history" of each collected organism. After all, how would you know if two specimens collected in a trap were originally located within 5 cm of each other, or within 0.5 km of each other, prior to their journey to the same trap? In an effort to minimize this fundamental weakness among trapping methods, it is useful to adopt a geometric pattern of trap placement, either as a rectilinear trapping grid or as a radial trapping web.

Trapping Grids/Webs: Trapping grids are used when it is assumed that a motile species can be readily trapped, and that the density (and mobility) of the organisms is the same throughout the study area. The simple geometry of a symmetrical, rectilinear grid (**Figure 6.10A**) can be used in Equation 6.7 to estimate the population density D as

$$D = \frac{N_{MR}}{\left(L^2 + 4LW + \pi W^2\right)} \tag{6.7}$$

where the total number of organisms estimated to be present in the study area (N_{MR}) must be determined by a mark-and-recapture method (discussed later in this chapter). Although the overall length of the grid (L) is easily defined, the width of the outermost strip of habitat (W) from which organisms may migrate into the main grid and become trapped is unknown. However, if multiple trapping grids of different sizes (ΔL) are used, W can be estimated using Equation 6.7 as long as we assume N_{MR} and D do not vary.

Trapping webs simply utilize a uniform number of traps arranged in concentric circles (**Figure 6.10B**). Such webs are also used to introduce a gradient of trapping efficiency, whereby organisms located near the center of the web are more likely to encounter a trap and become ensnared compared to those located near the outermost ring of traps.

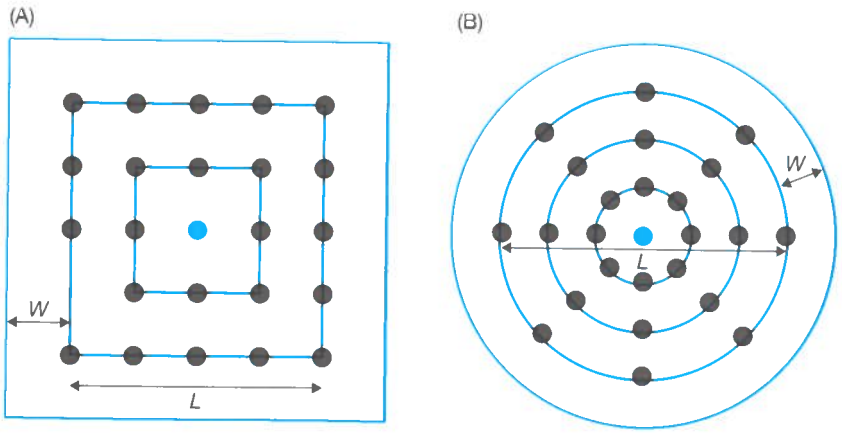

Figure 6.10 Typical geometric patterns used to define (A) a rectilinear trapping grid and (B) a radial trapping web. Trapping grids can be used to establish population densities as a function of some unknown width (W) from whence "outside" specimens will immigrate into the grid, relative to the overall size of the trapping grid (L). Trapping webs are typically used with an attractant (or deterrent) located in the center of the grid or web.

Because of this gradient in trapping efficiency, successive surveys can be used to determine migration patterns into (or out of) the center of the study area. Since it is assumed that organisms in the center of the study area are more likely to be caught in the innermost ring of traps, capture rates in the interior of the web should decline over time. By comparison, capture rates in the outermost rings will more than likely be due to "new" organisms entering the study area. The placement of an attractant (or deterrent) at the center of the trapping web can also be used to investigate the differences in random versus induced migration.

Beyond the investigator's choice of trapping pattern, it is also important to choose the appropriate style of trap for the organism you wish to capture. Although there are a wide variety of traps available, there are four general types: (1) pitfall, (2) attractant, (3) emergence, and (4) enclosure and exclosure traps. Take care that you consider the motility and migration behavior of the species you wish to capture when deciding which kind of trap to use.

Pitfall Traps Are Best for Capturing Small, Motile Species Above Sea Level

Pitfall traps can be constructed from simple plastic cups, buried just beneath the surface of the substrate, and protected by an overhanging platform to prevent unwanted debris from falling into the trap (**Figure 6.11**). The space between the substrate and the overhanging platform can be modified to exclude larger organisms by simply not giving them enough space to crawl under the platform. To prevent trapped organisms from crawling out, it is best to use tall, sheer-walled containers (hard plastic or glass). Of course, the traps can also be baited in order to increase capture rates; just be sure to use a bait consistent with your target organism's diet.

In most cases, you should check your traps daily, especially if you wish to collect live specimens. If your traps are located in a coastal area that will not be inundated with water, you may wish to place a small amount of formalin in the bottom of your traps to ensure that any captured organisms are killed and preserved before they have a chance to escape. Of course, it is not advisable to place any toxic fixatives or anaesthetizing agents in submerged traps, as these chemicals may be "flushed out" by tides or currents and pollute nearby habitats.

Attractant Traps Are Excellent for Capturing Forager and Scavenger Species

Attractant traps are those that are placed in plain view on the substrate and are baited with some kind of attractant. A classic type of attractant trap is the crab trap, usually with one or two entry points, oriented horizontally to

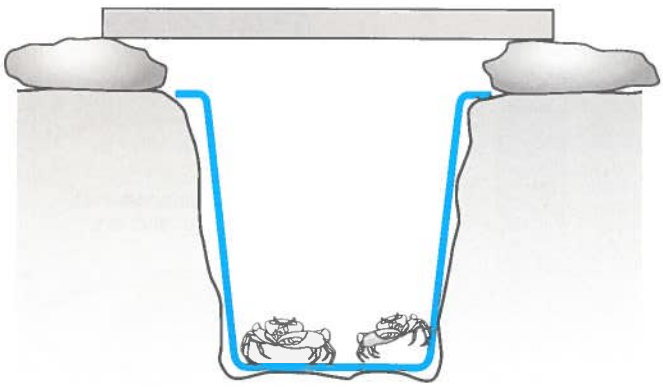

Figure 6.11 A pitfall trap, buried in the substrate so that the lip of the trap is just below grade. The use of an overhanging platform will help prevent unwanted debris from cluttering your trap. If the trap is baited, a cover will also keep unwanted scavengers from stealing the bait.

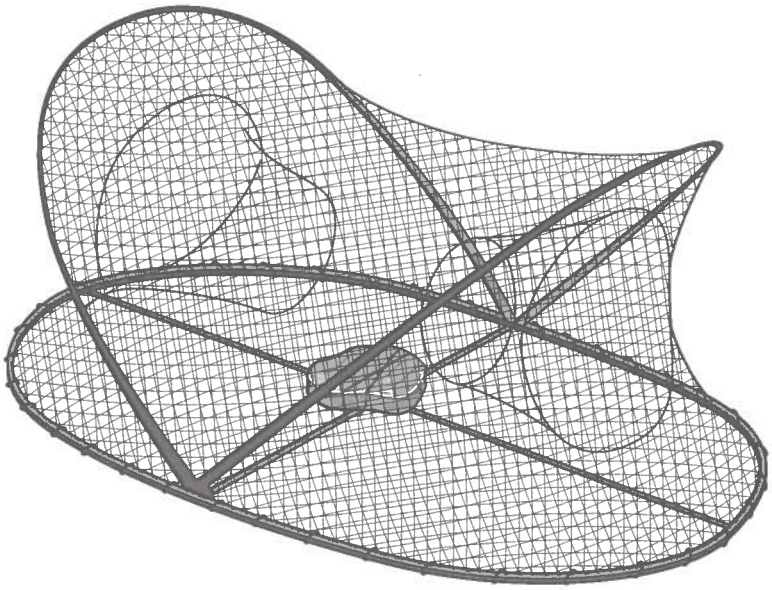

Figure 6.12 A wire-mesh crab trap, baited and used as a classic attractant trap.

the substrate, that functions as one-way "valves" to allow entry and prevent escape. Many trap varieties simply use an "inverted funnel" design rather than a physical barrier to prevent escape, but either type is suitable (**Figure 6.12**).

Most attractant traps will open easily to allow the investigator to change bait and/or collect the captured animals. Traps can either be tethered to fixed objects (such as rocks or wharf pilings) or be anchored in soft sediment and marked with a floating buoy. Many sizes and varieties of these traps are easily available, but take care that you select a size most appropriate for your species of interest. It is not uncommon for your larger prisoners to feast on the smaller ones, so it is best to limit the size of the trap openings to discourage larger carnivores from entering your traps, and be sure to check your traps often. To catch very small organisms, you may wish to use glass mason jars fitted with an inverted funnel before screwing on the collar. Otherwise, the smallest organisms can easily sneak out of the mesh traps.

Emergence Traps Are Best Used in the Capture of Cryptic Species

Emergence traps are very similar to attractant traps, but they are typically oriented so that the funnel mouths of the traps are perpendicular to the substrate in order to capture motile species emerging from the substrate (**Figure 6.13**). These traps are particularly effective at trapping cryptic

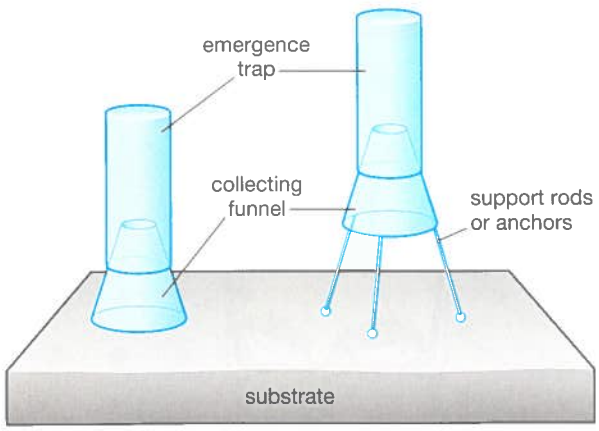

Figure 6.13 Emergence traps can be placed directly upon the substrate (*left*) or anchored and allowed to float a set distance above the substrate (*right*), with the collecting funnel oriented with the broad opening facing downward in order to trap organisms emerging from the substrate and swimming/floating upwards.

and infaunal species, which spend their daylight hours hidden within the substrate. After dark, when there is no danger of them being seen by predators, they will leave the relative safety of their hiding places and move or swim upwards. Like attractant traps, emergence traps can also be baited.

Enclosure and Exclosure Traps Are Best Suited for Mesocosm Studies

Traps that are constructed to enclose (or exclude) a specific organism are not designed to "collect" specimens; they are more commonly used to perform controlled ecological experiments in a natural environment, whereby the subjects of the study are prevented from leaving (an enclosure system), or the subjects of the study are protected from marauding outsiders (an exclosure system). The most common type of enclosure/exclosure trap is a wire cage with a uniform mesh size that is smaller than the shortest body dimension of the organism of interest. Wire cages have the benefit of allowing free exchange of water into and out of the trap while preventing the migration of the target species. However, if you wish to specifically disallow water exchange in the trap to create a truly closed aquatic **mesocosm**, it may be preferable to use a large, solid-walled tube or box that can be firmly fixed to the substrate (**Figure 6.14**).

Mark-and-Recapture Methods Are a "Trapless" Solution to Census Motile Species

Once a suitable trap design is chosen, the investigator may choose an appropriate "mark-and-recapture" method in order to make sense of the data collected from those traps without sacrificing the caught specimens. Although the methods we have discussed earlier require that specimens be removed from consideration once collected, other methods require that the collected specimens be tagged and allowed to return to the population unharmed, where they may be subsequently collected again (and again).

For mark-and-recapture methods to be useful, we must assume that, in a well-mixed population, the proportion of marked specimens in any collection will be the same as the proportion of marked specimens in the population from which they were collected. If n_1 represents the number of animals marked and released in the first outing, then n_2 can be used to represent the total number of specimens collected in the second outing. Among the n_2 organisms collected, the number that bear the markings placed on them from our first outing can be represented as m_2. Thus, we should be able to

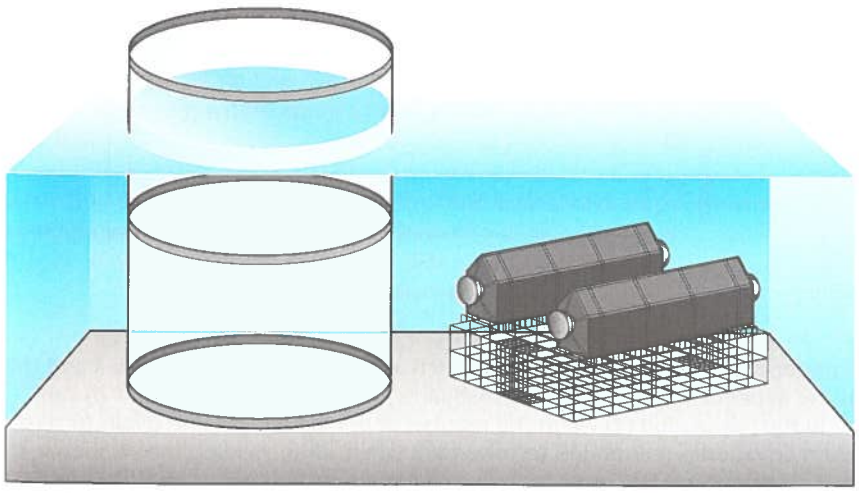

Figure 6.14 Enclosure and exclosure traps are typically fashioned as nets or wire-mesh cages designed to function as a flow-through system (*right*) or as solid-walled traps to literally trap the water along with the organisms enclosed therein (*left*). By their nature, these traps are generally not used for migration studies; instead, they are more commonly used to study closed-system dynamics.

use Equation 6.8 to estimate the total population size N_0 according to the mathematical relationship

$$N_0 = \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} - 1 \qquad (6.8)$$

Although this represents the simplest of all mark-and-recapture relationships (that is, a two-sample method), it is the mathematical foundation on which all mark-and-recapture methods are built.

The Pseudo-removal Method Can be Used to Estimate the Size of a Closed Population by Simply Marking Specimens

In a closed population, it is easy to see that as animals are continually trapped, marked, and released, the number of unmarked animals captured in successive collections should decline. At some point, every member of the population will be captured and marked, and at that point, the total number of individuals in the population will be known.

Of course, it is not practical to mark and recapture specimens until every member of the population is collected. However, by plotting the number of unmarked animals collected (on the y-axis) against the cumulative number of marked animals from all previous collections (on the x-axis), a least-squares regression of the resultant line ($y = mx + b$) can be used to estimate the total population size x' when $y = 0$ (**Figure 6.15**). If animals are simply removed when trapped, this same method can be applied to the results by plotting the number of organisms caught and removed (on the y-axis) against the cumulative catch (on the x-axis). As more and more members of the closed population are caught and removed, $y \rightarrow 0$.

The Wileyto Removal Method for Closed Populations Uses a Combination of Marked and Removed Specimens to Assess Population Size

Another simple method for closed populations is the Wileyto method, which relies on two different sorts of traps deployed simultaneously. The first is a simple trap that catches and permanently removes animals from the population. The second trap is identical to the first, except that animals entering it are marked and immediately set free. Since the capture trap will collect

collection series	no. unmarked specimens per collection series (y)	cumulative catch (x)
1	47	0
2	52	47
3	25	99
4	33	123
5	11	156
6	8	167
7	8	175
8	10	184

x = 223 total individuals when y = 0

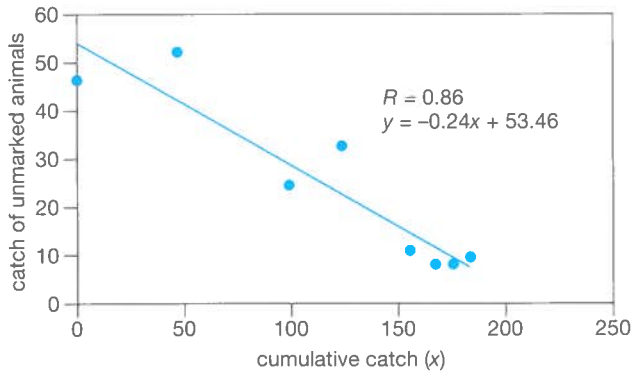

Figure 6.15 An example of the pseudo-removal method. By using the cumulative catch as the independent variable x and the number of unmarked specimens per collection as the dependent variable y, a least-squares linear regression ($y = mx + b$) can be used to estimate the total number of members in the population (x) when the number of unmarked specimens falls to zero (that is, when $y = 0$). In this example, the regression equation is rearranged to solve for x when $y = 0$, yielding the solution $x = 233$ as the estimated size of the total population.

both marked and unmarked specimens, their proportions can be used in Equation 6.9 to provide an estimate of the total population size (N_0) according to the formula

$$N_0 = \frac{(U + M)^2}{2(M + 1)} \tag{6.9}$$

where U is the number of unmarked animals caught in the "permanent removal" trap and M is the number of marked animals caught in the same trap. From these simple results, a 95% confidence interval can be estimated using Equation 6.10:

$$N_0 \pm \frac{2\left(\sqrt{N_0\langle U(U + M) + M(U - M)\rangle}\right)}{M} \tag{6.10}$$

The Multiple Mark-and-Recapture (Schnabel) Method for Closed Populations is among the Most Rigorous Estimates of Population Size

Also known as the Schnabel method, this particular method requires that specimens are marked only when they are first captured; successive captures do not merit successive markings. Assuming that the collected organisms do not vary in their "trapability" over time, estimates of population size can be calculated from the proportion of marked and unmarked specimens captured in successive collections. Although the Schnabel method is similar to the pseudo-removal method discussed earlier, it utilizes more information when estimating population size. Generally speaking, this makes the Schnabel method the preferred method, but it also requires a more rigorous mathematical treatment of the data.

The fundamental variables for use in the Schnabel method are defined as follows:

i = Number of collections
n_i = Number of total specimens in the ith collection
m_i = Number of marked specimens in the ith collection
u_i = Number of unmarked specimens in the ith collection
M_i = Cumulative number of specimens marked prior to the ith collection

From these data, Equation 6.11 is used to define the following relationships:

$$A = \sum_i n_i M_i^2; \quad B = \sum_i m_i M_i \tag{6.11}$$

As an example, let us use the data in **Table 6.1** for a mark-and-recapture study of lightning whelk snails (**Figure 6.16**) collected from a South Texas mud flat.

According to the Schnabel method, the final estimate of population size (N_0) is calculated in Equation 6.12 simply as

$$N_0 = \frac{A}{B} \tag{6.12}$$

Using the data from Table 6.1, the quantity (A/B) is calculated to be 64.7627; rounded to the nearest whole number, the Schnabel method estimates that the total population size of lightning whelk snails in our example study area is 65 individuals.

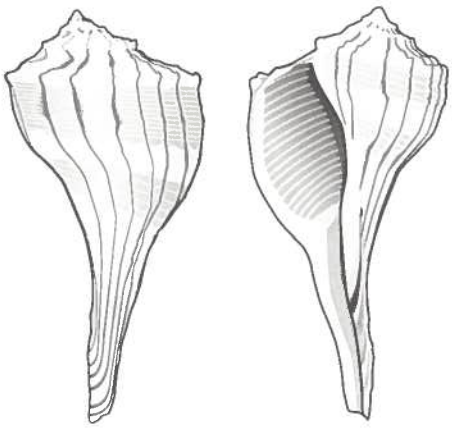

Figure 6.16 The lightning whelk (*Busycon contrarium*) is a large, predatory snail commonly found on sandy and muddy substrates in the Gulf of Mexico and the Caribbean.

Table 6.1 Data for Lightning Whelk (*Busycon contrarium*)

i	n_i	m_i	u_i	M_i	$n_iM_i^2$	m_iM_i
1	23	0	23	0	0	0
2	39	13	26	23	20631	299
3	27	23	4	49	64827	1127
4	44	34	10	53	123596	1802
SUM					$A = 209054$	$B = 3228$

Specimens collected using the Schnabel method, indicating the collection number (i), the total number of specimens in each collection (n_i), the number of marked (m_i) and unmarked (u_i) specimens in each collection, and the cumulative number of specimens collected in previous collections (M_i).

Open Populations are More Challenging to Assess and Require Complex Multiple Mark-and-Recapture Methods

For populations that are subject to immigration and emigration of specimens, the method used to estimate N_0 requires that the investigator conduct a minimum of three mark-and-recapture collections and requires that the specimens are marked with unique symbols or colors each time they are collected (thereby preserving their "capture histories").

The method most commonly used for multiple-recapture data from open populations is the Jolly–Seber method. Although this method is an excellent choice for estimating the total number of individuals within an open population, it is also quite rigorous in its execution and in the mathematical computations required for the manipulation of collected data. For further information on the Jolly-Seber method, please refer to the Further Reading section.

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