VIII PLANNING A SAMPLING PROGRAMME

The statistical principles of sampling are the subject of several books (e.g. Yates 1960, Stuart 1962, Cochran 1963), and only some aspects are discussed in this section. It is first necessary to define clearly the objects of the study and the area to be sampled. The frequency of sampling will depend upon the objects of the study. Samples may be taken at weekly intervals in detailed studies of life histories, or only once a year in some general surveys. Most investigations are either extensive faunal surveys or intensive quantitative studies. These two broad categories are now discussed separately.

8.1 Faunal Surveys

A survey of a large sampling area (lake, river, etc.) usually precedes a quantitative investigation, but may be an end in itself. The whole sampling area is usually divided into sections of equal area, and one or more stations are selected in each section. Whenever possible, the stations should be selected at random. Alternatively the sampling area is divided into different biotopes (e.g. stony substratum, mud, moss, etc.) and stations are selected at random in each biotope.

The chief objects of a faunal survey are to discover which species are present, and to estimate the relative abundance of each species at each station. As it is important to catch the rare species at each station, each sample should cover a large area of bottom. A simple method is to collect over a large sampling unit with a pond net. This technique can be used in flowing water and still water. Collections in rivers are obtained by disturbing the bottom, either by hand-turning of stones or by kicking with the feet, and allowing the current to carry the dislodged material into a collecting net. The collector slowly moves upstream over a fixed distance and thus covers a large area of bottom. A similar technique can be used on the stony substratum of lake shores. Stones are lifted and cleaned in the mouth of the net which is quickly swept underneath the stones. The collector slowly moves along the lake shore over a fixed distance. More than one collection may be necessary when a station covers a large area of bottom. It is sometimes suggested that collections should be taken over a fixed time, e.g. 2 min, rather than a fixed distance. As the distance covered in a fixed time will vary considerably with the operator, the type of habitat and the flow conditions, this procedure cannot be recommended.

The advantages of these simple techniques are: (1) they do not require elaborate apparatus; (2) they usually catch a high proportion of the total species present at each station; and (3) they often provide fairly comparable figures, especially when the biotopes and collector are the same for all samples; it is also possible to compare the percentage composition of the benthos at each station.

The disadvantages are: (1) they cannot be used in deep water; (2) the samples do not provide estimates of numbers per unit area, and comparisons between samples are usually limited to relative abundance. As the expression of the numbers of each species as a percentage of total numbers is strongly influenced by numbers in the rest of the samples, comparisons of percentage composition are of limited value.

If a quantitative technique is preferred to the above methods, there is a choice of numerous samplers (Welch 1948, Macan 1958). Although the size of the sampling unit is known with these techniques, it usually covers a small area of bottom. Therefore the major problem is to decide how many sampling units are necessary to ensure that the sample includes most of the species present. If the sampling units are taken in a long transect line which runs parallel to some obvious environmental gradient, there is a high probability that most species will be taken at least once. When species can be identified in the field, the sampling units are taken at random and the number of new species is noted in each successive sampling unit. No more sampling units are taken when three successive units have failed to add any species to the total list. The number of sampling units required at each station will depend upon the diversity and dispersion of the bottom fauna. Gauin, Harris & Walter (1956) give a formula for calculating the average number of new species contributed by the Kth successive sample (K = 1 or more), and Harris (1957) proposes a formula for computing the standard errors of such estimates.

8.2 Quantitative Studies

The methods described in sections 4-7 are all used in quantitative studies and require a knowledge of numbers per unit area. Some problems of quantitative sampling were mentioned in previous sections, and the major considerations are (1) the dimensions of the sampling unit (quadrat size), (2) the number of sampling units in each sample, and (3) the location of sampling units in the sampling area. It is often impossible to make a complete and accurate estimate of the numbers of all species in a large area of bottom. Therefore most quantitative investigations are restricted to a study of a small number of species in a large area, or a larger number of species in a small area.
It is important to define the area in which the investigation takes place; thus if only a section of a lake or river is sampled, then the area of this section must be clearly defined.

8.2.1 The dimensions of the sampling unit (quadrat size)

The defined area is divided up into sampling units of equal size, and the whole aggregate of sampling units forms the population (statistical meaning, see section 2.1). Therefore the total number of available sampling units in the population depends upon the relationship between the area of the population (= total sampling area) and the area of the sampling unit (= quadrat size).

A small quadrat size is most suitable for a study of the dispersion of a Population (ecological meaning, see section 2.1), and the problems of detecting non-randomness were fully discussed in section 5.4. If the dispersion of a Population is truly random, all quadrat sizes are equally efficient in the estimation of population parameters. Efficiency is defined in terms of the relative amounts of sampling required to give estimates of equal precision. Several workers (e.g. Beale 1939, Finney 1946, Taylor 1953) have investigated the effects of the size of the sampling unit on the efficiency of sampling, and they conclude that a small unit is more efficient than a larger one when the dispersion of a Population is contagious. The advantages of a small sampling unit over a larger unit are: (1) more small units can be taken for the same amount of labour in dealing with the catch; (2) as a sample of many small units has more degrees of freedom than a sample of a few large units, the statistical error is reduced; and (3) since many small units cover a wider range of the habitat than a few large units, the catch of the small units is more representative. In general, the smaller the sampling units employed, the more accurate and representative will be the results.

Although the ideal solution is to use the smallest possible sampling unit, many practical factors will set a lower limit to the dimensions of the sampling unit, e.g. stone size will be a limiting factor on a stony substratum. It must also be remembered that with a small sampling unit, the sampling error at the edge of the unit is proportionally greater. Therefore the choice of the final quadrat size is always a compromise between statistical and practical requirements.

8.2.2 The number of sampling units in each sample

As the dispersion of many species is frequently contagious, a large variation is encountered in sampling natural populations and small samples are statistically inaccurate. Therefore published quantitative data are often unreliable because the samples are too small. The simplest solution to this problem is to always take very large samples, i.e. with over 50 sampling units in each sample (n > 50). Unfortunately, it is usually impossible to sort and count all the different species in a very large sample, especially when samples are taken at frequent intervals. Therefore a compromise must be made between statistical accuracy and labour.

The following simple method can be used to find a suitable number of sampling units. Take 5 sampling units at random and calculate the arithmetic mean. Next take 5 more units at random and calculate the mean for 10 units. Continue to increase sample size by 5-unit steps, and plot means for 5, 10, 15, etc. units against sample size. When the mean value ceases to fluctuate, a suitable sample size has been reached and this sample size can be used for that particular station. As it is often impossible to calculate means at the time of sampling, this simple method is of limited application.

Sample size can be calculated for a specified degree of precision. First decide how large an error can be tolerated in the estimate of the population mean. The percentage error can be expressed as either the standard error of the mean (section 6.1), or confidence limits of the mean (section 6.2). For a given standard deviation (or variance $s^2$), the standard error is a function of the number ($n$) of sampling units in each random sample. The ratio of standard error to arithmetic mean is an index of precision ($D$). For example, if we can tolerate a standard error equal to 20% of the mean (a reasonable error in most bottom samples), then $D$ is given by the general formula:

$$D = 0.2 = \frac{\text{standard error}}{\text{arithmetic mean}} = \frac{1}{\bar{x}} \sqrt{\frac{s^2}{n}}$$

Therefore the number of sampling units in a random sample is given by:

$$n = \frac{s^2}{D^2 \bar{x}^2} = \frac{s^2}{0.2^2 \bar{x}^2} = \frac{25s^2}{\bar{x}^2} \text{ for a 20% error}$$

If the Poisson series is known to be a suitable model for the samples, then $D$ is given by:

$$D = \frac{1}{\bar{x}} \sqrt{\frac{n}{\bar{x}}} = \frac{1}{\sqrt{n\bar{x}}}$$

Note that $n\bar{x} = \Sigma x = \text{total count for the sample}$. Therefore the precision of the estimated population mean depends upon the total
number \( (\bar{n}) \) of animals in the sample, rather than sample size. For example, for a tolerable error of 20%:

\[
D = 0.2 = \frac{1}{\sqrt{n\bar{x}}}
\]

Therefore

\[
\frac{1}{D} = \frac{1}{0.2} = 5
\]

Therefore the product \( n\bar{x} \) (\( = \Sigma x \)) must be always 25 for an error of 20%. The number of sampling units in a random sample is given by:

\[
n = \frac{\bar{x}}{D^2 \sigma^2} = \frac{1}{D^2 \bar{x}} = 25 \quad \text{for a 20% error}
\]

Therefore optimum sample sizes (to nearest integer) for a 20% error and various values of \( \bar{x} \) are:

<table>
<thead>
<tr>
<th>( \bar{x} )</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>25</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

If the negative binomial is known to be a suitable model for the samples, then \( D \) is given by:

\[
D = \frac{1}{\bar{x}} \sqrt{\frac{\bar{x}}{n} + \frac{\bar{x}^2}{nk}} = \frac{1}{\bar{x}} \left( \frac{1}{n} + \frac{1}{nk} \right)
\]

and

\[
n = \frac{1}{D^2} \left( \frac{1}{x} + \frac{1}{k} \right) = 25 \left( \frac{1}{\bar{x}} + \frac{1}{k} \right) \quad \text{for a 20% error}
\]

When \( D \) is the relative error in terms of percentage confidence limits of the mean, the above formulae must be multiplied by \( t_i \), where \( t \) is found in Student’s \( t \)-distribution (\( t = 2 \) for 95% probability level of \( D \)). For example, if we can tolerate 95% confidence limits of ±40% of the mean (equivalent to standard error of about 20% of the mean), then \( D = 0.4 \). Therefore the sample size needed to obtain an estimate of the population mean within ±40% of the true value is given by the general formula:

\[
n = \frac{t_i^2 \sigma^2}{D^2 \bar{x}^2} = \frac{2^2 \sigma^2}{0.4^2 \bar{x}^2} = \frac{25 \sigma^2}{\bar{x}^2}
\]

If the Poisson series is a suitable model for the samples, then:

\[
n = \frac{t_i^2}{D^2 \bar{x}} = \frac{2^2}{0.4^2 \bar{x}} = 25 \frac{1}{\bar{x}}
\]

If the negative binomial is a suitable model for the samples, then:

\[
n = \frac{t_i^2}{D^2} \left( \frac{1}{\bar{x}} + \frac{1}{k} \right) = \frac{2^2}{0.4^2} \left( \frac{1}{\bar{x}} + \frac{1}{k} \right) = 25 \left( \frac{1}{\bar{x}} + \frac{1}{k} \right)
\]

* If \( n<10 \), repeat calculation using value of \( t \) for \( n-1 \) degrees of freedom. This process can be continued until the equation is balanced.

8.2.3 The location of sampling units in the sampling area

The terms random sample and sampling units taken at random are frequently used in this account. As many statistical methods require a random sample, it is important to understand the principles of random sampling.

The defined area for the investigation is the total available sampling area, and the number of available sampling units in this area is given by:

\[
N = \frac{A}{a}
\]

where \( A \) is the total sampling area and \( a \) is the area of the sampling unit (= quadrat size). Therefore \( N \) sampling units form the population from which a sample of \( n \) sampling units is selected. As \( n \) is usually much smaller than \( N \), we must decide how to select the small sample of \( n \) units from the large population of \( N \) units. The sample must be representative of the whole population, and therefore the sampling units must be selected without bias: These conditions are fulfilled when the sampling units are selected at random from the population.
In simple random sampling, every sampling unit in the population has an equal chance of selection. True random selection is often difficult to achieve and the most reliable method is to use a table of random numbers. Suitable tables are included in many statistical textbooks and a large table is given in Fisher & Yates (1963, Table 33). To use these tables, simply select the first $n$ numbers with values less than $N$. If all the available units in the population are listed from 1 to $N$, the $n$ units in each sample are easily located. This method is often laborious in a large population. Therefore it is easier to use a large two-dimensional grid with each square equal to the area of a sampling unit. Some squares of the grid are redundant when the sampling area is irregular. The squares on two adjacent sides of the grid are numbered, and each sampling unit is thus located by a pair of co-ordinates. Random numbers are then drawn in pairs and these are used as co-ordinates to locate each unit in the sample. This is simply done by laying down two graduated lines at right angles, or by pacing out the required co-ordinates when the sampling area is large.

As nearly all the randomly-selected units in a sample may fall in one part of the sampling area, simple random sampling is not very efficient, especially when $n$ is much smaller than $N$. Therefore the more efficient method of stratified random sampling is always preferable to simple random sampling. The purpose of stratified sampling is to increase sampling efficiency by dividing the population into several sub-populations or strata. These strata should be more homogeneous than the whole population, and should be well defined areas of known size. Stratification also increases the accuracy of population estimates and ensures that subdivisions of the population are adequately represented. The data from the different strata can be compared by a one-way analysis of variance (sources of variation: between strata, within strata).

In the simplest form of stratification, the whole sampling area is divided into areas of equal size (= strata). All the units in the sample are divided equally between strata and these units are located at random in each stratum. If the strata are unequal in area, the units in the sample are divided unequally between strata and the number of units allocated to each stratum is proportional to the area of the stratum, i.e. the total number of available units in the stratum. With proportional allocation of the units in a sample, the sampling fraction in each stratum is the same, i.e.

$$\frac{n_1}{N_1} = \frac{n_2}{N_2} = \cdots = \frac{n_k}{N_k} = \frac{n}{N}$$

where we select random sub-samples of $n_1, n_2, \ldots, n_k$ units from $k$ strata containing $N_1, N_2, \ldots, N_k$ units. The total numbers of sampling units in the sample ($n$) and in the population ($N$) are given by:

$$n = n_1 + n_2 + \cdots + n_k$$

$$N = N_1 + N_2 + \cdots + N_k$$

Note that:

$$\frac{N_1}{N} = \frac{n_1}{n}, \frac{N_2}{N} = \frac{n_2}{n}, \ldots, \frac{N_k}{N} = \frac{n_k}{n},$$

where $N_1/N, N_2/N, \ldots, N_k/N$ are the relative weights attached to each stratum. With proportional stratified sampling, the sample is self-weighting and the arithmetic mean of the whole sample is the best estimate of the population mean. This is calculated in the usual way, or from the means of the $k$ strata:

$$\bar{x} = \frac{\sum x_i}{n} = \frac{n_1 \bar{x}_1 + n_2 \bar{x}_2 + \cdots + n_k \bar{x}_k}{n}$$

where $\bar{x}_1, \bar{x}_2, \ldots, \bar{x}_k$ are the arithmetic means of the different strata.

Standard error of mean = $(1/n) \sqrt{n_1 \sigma_1^2 + n_2 \sigma_2^2 + \cdots + n_k \sigma_k^2}$ where $\sigma_1^2, \sigma_2^2, \ldots, \sigma_k^2$ are the variances of the different strata. If the sampling fraction exceeds $10\% (n/N > 0.1)$, then:

Standard error = $(1/n) \sqrt{(n_1 \sigma_1^2 + n_2 \sigma_2^2 + \cdots + n_k \sigma_k^2)(1-n/N)}$

where $(1-n/N)$ is the finite population correction.

Although proportional allocation is most frequently used in stratified sampling, the theoretical optimum allocation of the units in the sample is the one that minimizes the standard error of the estimated mean for a given total cost of taking the sample. This is achieved when the sampling fraction for each stratum is proportional to the standard deviation for the stratum. As the standard deviations for the strata are rarely known before sampling, the method of optimum allocation is rarely used. With optimum allocation, the sample is no longer self-weighting, and therefore a weighted mean and standard error must be calculated (Snedecor & Cochran 1967).

Stratified sampling is of greatest value when the sampling area contains a diversity of biotopes. For example, we wish to sample a section of river with a total sampling area of 200 m², and the area of the sampling unit is 0.05 m². Therefore 4000 sampling units form the population ($N = 400$) from which a random sample of 40 units must be selected ($n = 40$). The following five strata ($k = 5$) were recognised (units in each stratum given in parentheses): Large stones on gravel ($N_1 = 1000$), Gravel ($N_2 = 500$), Plant A on gravel ($N_3 = 1500$),
Plant B on gravel \( (N_4 = 800) \), Mud \( (N_5 = 200) \). As the standard deviations for the strata are not known, optimum allocation of units cannot be used. Therefore the units in the random sample \( (n = 40) \) are allocated in proportion to the areas of the strata thus:

\[
\begin{align*}
n_1 &= 10 & n_2 &= 5 & n_3 &= 15 & n_4 &= 8 & n_5 &= 2
\end{align*}
\]

These units are selected at random from the available sampling units in each stratum. Note that the sampling fraction is the same for each stratum \( (n/N = 0.01) \).

Other methods for selecting a sample from a population have a more limited application than random sampling. If the object of the investigation is to determine the mean and variance of the population, random sampling is essential. If the object is to determine numbers in relation to position within the ecosystem, systematic sampling may be preferable. In systematic sampling, the first unit in the sample is selected at random from the population and then the next units are selected at fixed intervals, e.g., every 10th unit in the population is taken until the required sample size is obtained. The advantages of systematic sampling are:

1. It is easy to draw a sample, since only one random number is required;
2. The units in the sample are distributed evenly throughout the population.

The disadvantages are:

1. The sample may be very biased when the interval between units in the sample coincides with a periodic variation in the population;
2. There is no reliable method for estimating the standard error of the sample mean.

A variation of systematic sampling is the centric systematic area-sample which can be treated as a random sample (Milne 1959). In this method, each sampling unit is taken from the exact centre of each stratum.

Another method is to use a grid of contiguous quadrats (Greig-Smith 1964). This method is used in plant ecology and facilitates the detection of mosaic patterns in non-random distributions of species. The method is limited to small areas of bottom and to biotopes which are suitable for a contiguous arrangement of quadrats. Another method of mapping aggregations is to take sampling units in pairs with one unit located at random and the second unit located at a fixed distance from the first (Hughes 1962).

### 8.3 Sub-sampling in the Laboratory with Large Catches

Although it is usually possible to count all the invertebrates in a sampling unit, the task is often laborious when the catch per sampling unit is very large. This problem can be solved by reducing the size of the sampling unit, but this is often impossible. An alternative solution is sub-sampling (also called two-stage sampling). First, a sample of \( n_1 \) primary sampling units is selected in the usual way from the population in the field (see section 8.2.3). In the second stage, a sub-sample of \( n_2 \) sub-units (or second-stage units) is taken from the total catch for each primary unit. The large catch for each primary unit is concentrated in a known volume of water (or preservative), and is well agitated in a container before sub-sampling. If the invertebrates are distributed randomly in the container before sub-sampling and only a small proportion of the total catch is removed in each sub-unit, then the counts of the sub-sample should be distributed according to a Poisson series. This hypothesis should be checked by the \( \chi^2 \) test (variance to mean ratio, section 4.1.2) on a sub-sample of at least five sub-units. If agreement with a Poisson series is accepted, then it can be assumed that the invertebrates are distributed randomly before sub-sampling, and subsequently a sub-sample of only one sub-unit need be taken. Thus a single count can be used to estimate the total numbers for each primary sampling unit, and the accuracy of this estimate depends upon the size of the count (section 6.2.2).

Sub-sampling is used extensively in the quantitative study of plankton populations and several papers provide a detailed account of statistical methods (e.g., Ricker 1937, Holmes & Widrig 1956, Kutkuhn 1958, Lund, Kipling & Le Cren 1958). The following imaginary example outlines the chief statistical methods.

Large numbers of chironomid larvae were taken in a random sample of 10 (primary) sampling units \( (n_1 = 10) \). The other less-numerous invertebrates were removed from the total catch for each primary unit, and then chironomid larvae plus detritus were transferred to 4 litres of preservative in a flask. This process was repeated until there were 10 flasks, each containing the total catch of chironomid larvae for one primary unit. After thorough agitation of a flask, a sub-unit of 50 ml was removed with a pipette. This process was repeated until a sub-sample of 5 sub-units had been taken from one flask. Chironomid larvae in each sub-unit were counted, and the following counts were obtained for the first sub-sample:

\[
\begin{align*}
20, & \ 25, & \ 25, & \ 30, & \ 40; & \ \mu = 28, & \ \sigma^2 = 57.5, & \ n_2 = 5
\end{align*}
\]

The \( \chi^2 \) test (variance to mean ratio, section 4.1.2) gave a value of \( \chi^2 = 8.2 \) with 4 degrees of freedom. As this \( \chi^2 \) value clearly lies between the 5% significance levels in Fig. 8, agreement with a Poisson series is accepted and it is
assumed that the chironomid larvae were distributed randomly in the flask before sub-sampling. Therefore we can now estimate the total numbers of larvae in the first flask, i.e. total numbers for the first primary sampling unit. The accuracy of this estimate increases as the size of the count increases. Therefore the total count for the sub-sample (i.e. 140) is used and 95% confidence limits for this count are 117 to 163 (from Crow & Gardner 1959). As the total count is for a volume of 250 ml (5 sub-units of 50 ml each) or \(\frac{1}{5}\) of the total volume in the flask (4 litres), the count and its confidence limits are multiplied by 16 to give an estimate of the total numbers in the first flask. Therefore the estimated number of chironomid larvae for the first primary unit is 2240 with 95% confidence limits of 1872 and 2608 (or 2240 ± 368).

These calculations are now repeated for each flask and hence for each primary sampling unit. Sufficient sub-units should be taken to ensure that the total count for each sub-sample is at least 100 (for 95% confidence limits of ±20%; see section 6.2.2). As the estimation of total numbers and confidence limits by the above methods requires agreement with a Poisson series, it is important to ensure that the invertebrates are distributed randomly before sub-sampling. Therefore various methods of mixing should be tried on preliminary samples until a satisfactory technique is found.

If the counts of a sub-sample do not follow a Poisson series and the variance of the sub-sample is significantly greater than the mean, then the invertebrates were distributed contiguously before sub-sampling. It is now more difficult to estimate total numbers and confidence limits for each primary sampling unit. The simplest method is to transform the counts of a sub-sample to logarithms, and then calculate the geometric mean with its 95% confidence limits (see section 6.2.4, example 22).

8.4 SUMMARY GUIDE

Define the objects of the study and the area in which the study takes place. Most studies fall into two broad categories:

(A) Faunial surveys (section 8.1): Chief objects are to discover which species are present, and to estimate the relative abundance of each species at different stations in the sampling area. Therefore the sample at each station should cover a large area of bottom.

(B) Quantitative studies (section 8.2): Chief object is to estimate numbers per unit area for each species, and therefore quantitative comparisons can be made. Major considerations are:

(1) The dimensions of the sampling unit (quadrat size). The smallest possible sampling unit should be used, but the chosen quadrat size is always a compromise between statistical and practical requirements (section 8.2.1).

(2) The number of sampling units in each sample. Large samples \((n > 50)\) are preferable, but the size of small samples can be calculated for a specified degree of precision:

\[
 n = \frac{s^2}{D^2 \bar{x}^2}
\]

where \(D\) is an index of precision, i.e. the required standard error as a proportion of the mean. This formula is multiplied by \(t^2\) \((t = 2)\) when \(D\) is the relative error in terms of percentage confidence limits of the mean. Approximate values of \(\bar{x}\) and \(s^2\) are guessed from previous samples or a preliminary sample. There are special formulae for a Poisson series and negative binomial when either of these distributions is a suitable model for the samples (section 8.2.2).

(3) The location of sampling units in the sampling area. The units in the sample are usually located at random in the sampling area and all the available units in the population must have an equal chance of selection for the sample. A table of random numbers is used to select the units for the sample. The sample must be representative of the whole population, and therefore stratified random sampling is preferable to simple random sampling. The sampling area (= population) is divided up into several strata (= sub-populations). These strata can be unequal in area, e.g. when the strata are different biotopes. The units in the sample are allocated to each stratum in proportion to the area of the stratum. The arithmetic mean of the whole sample is the best estimate of the population mean.

Other methods for selecting a sample from a population are briefly reviewed (section 8.2.3), and the method of sub-sampling in the laboratory is described (section 8.3).
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