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SOUTHWOOD'S

ECOLOGICAL METHODS

FIFTH EDITION

PETER A. HENDERSON

Southwood's Ecological Methods

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Fifth edition

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Preface to the Fifth Edition

The title of this book honours the memory of Sir Richard Southwood, the original author of *Ecological Methods*, and I am grateful to his family for their permission to use his name in the title. The book is the fifth edition of *Ecological Methods*, which was originally published in 1965. The objective remains to provide a handbook of methods pertinent for the study of animals. In this edition there is no longer an emphasis on insects, although the extraordinary abundance and species richness of insects in terrestrial environments justifies the space given to insect sampling methodologies. The focus is on the methods which are peculiar to ecologists, both in the field and in the laboratory. As in previous editions, I do not cover the measurement of abiotic environmental variables; while certainly an important aspect of the ecologists' work, the subject is too wide to be usefully summarized here and the methods are the domain of the physical scientist. Similarly, microbial ecology—a field which is producing exciting work on species diversity particularly since the development of DNA sequencing techniques—is not covered, as the field has specialist methodologies.

The growth in the use of R software packages has increased dramatically since the previous edition, and there is now an R package for almost every analytical task undertaken by ecologists. The range of statistical and ecological packages available is amazing, and one result is that the package I have chosen to feature in *Ecological Methods* is in some cases rather arbitrary. Often, I have used a package which offers a range of ecological analyzes so that the researcher can reduce the number of packages with which they need to become familiar. The organization of the data for input can vary greatly between packages, so there are advantages in using the minimum number possible. It has become normal for new methodologies to be supported by an R package, which allows ecologists to quickly try new techniques, many of which would be arduous to program. Given that R is an open-source programming language available to all, it has become a one-stop shop for many ecologists. It is, however, not cost-free, as time needs to be invested in learning how to use it. This is rapidly becoming an essential skill for the ecologist, and once learnt offers huge potential. The edition offers in grey boxes R code listings for all the major analytical methodologies presented.

Ecology is gradually acquiring the large temporal and spatial scale datasets required to study ecosystem structure and stability and to understand the ways in which we are altering the natural world via habitat destruction, over-hunting, and climate change. This is, in part, driven by automated systems such as camera traps, fish counters, electronic tags, drone-based survey systems, and environmental DNA sequencing systems. The traditional pursuits of the biological recording schemes are also producing greatly enlarged datasets, and citizen science projects which use the internet to harness the efforts of huge numbers of people are producing appreciable insights. The analysis of large datasets and statistical modelling has grown in importance in line with these developments, and now features more extensively.

Almost every chapter in this book assumes the availability of a computer and the internet to download and run R packages and obtain supporting information. It is normal for ecologists to spend long periods working with a personal computer, manipulating and analyzing data. I would urge all to remember that ecology remains an observational science, and every ecologist benefits by spending

Dick Southwood, in the yellow raincoat, setting olive fly emergence traps in a Greek olive grove in the 1970s.

time observing and sampling the natural world. Our datasets span only a brief period—often fifty years or less, and only occasionally extending to 200 years or more. They are also concentrated in a few spatial clusters on the surface of our planet. We have only recorded a small fraction of the range and type of abiotic variability, population dynamical behaviour, species losses, pathogen outbreaks, and changes in local diversity which most of the species alive today have experienced over the last 500,000 years. We need more observational data in a wider range of habitats.

By undertaking sampling, one gains insight into the accuracy and reliability of a method under different conditions. It is common to read powerful claims of major changes in the natural world based

on relative sampling methods which can change greatly in efficiency with environmental conditions. Many such claims are, at best, unproven and in some cases highly misleading. *Ecological Methods* extensively covers the issues that can influence the reliability and accuracy of absolute and relative sampling methods. Ecologists need to be aware that their claims may be uncritically taken up by the general media. If *Ecological Methods* helps us to critically appraise the wilder claims, it will have proved useful.

One of the decisions made with each passing edition concerns which references to retain. Compared with most scientific texts, *Ecological Methods* refers to a large number of papers published between 1920 and 1980—a period which covers the development of ecological science. References to methods that are no longer useful have been culled. However, early references to sampling techniques in use today are retained. Often these papers provide far more detail about construction and deployment than do recent publications, and it is a source of pleasure that this edition honours so many fine scientists by referencing their work. My aim is to make *Ecological Methods* a book presenting the latest techniques within their historical context.

The completion of this book would not have been possible without the kind help of fellow ecologists, and in partcular I would like to acknowledge Dr Richard Seaby, Professor A. E. Magurran, and Mr C. Hambler. As I grow older and, inevitably, a little more set in my ways, their help was invaluable in pointing me to exciting new techniques and viewpoints.

Peter Henderson
Lymington, July 2020

Preface to the Fourth Edition

My co-author for the third edition, and the original author of *Ecological Methods*, Sir Richard Southwood FRS, known to his many colleagues and friends as Dick, died on 26 October 2005. For those interested in reading about his long and highly distinguished career, his Wikipedia page, http://en.wikipedia.org/wiki/Richard_Southwood, provides links to obituaries and his Royal Society biographical memoir. Dick was a wonderful man to work with and a fine head of department. While achieving senior academic positions at an unusually young age, he retained an open and pleasant manner and a love of natural history, and he remained accessible to the most junior members of staff, all of whose names he would invariably know. When he was head of Department of Zoology at Imperial College and I was a first-year undergraduate, I was astonished that he knew the name of every undergraduate in his department. I was keen to revise *Ecological Methods*, in part to honour Dick's memory, but also because, I feel, the book still serves a useful purpose in acting as an ecologist's handbook of methods and sources of information. While the Web now provides ecologists, even in isolated spots, with access to a huge amount of information, it can be difficult to glean the full range of possibilities for experimental approaches, sources of information, and sampling gears. The old problem of how to design a successful sampling scheme and build samplers remains with us.

The trends in ecological research noted in the preface to the third edition have continued at an accelerating pace. Computation and data handling have advanced greatly, and the present edition includes many references to R, the computing language and environment for statistical analysis and graphics. The dramatic growth in R packages for ecologists, all of which are offered free of charge, is one of the most important developments since the publication of the third edition, and I have included many examples of R code in the present edition. Electronic developments in radar, sonar, remote sensing satellites, miniature tags, geographical positioning, movement detectors, lights, digital cameras, mobile phones, and batteries have all greatly increased the opportunities for data acquisition. These advances, combined with novel biochemical techniques such as species detection from amplified DNA fragments, is creating tremendous opportunities for ecological research. We now have tools and resources that would have seemed incredible to ecologists in the 1950s and 1960s. And yet many of the techniques we use are still based on the ideas developed and refined between 1930 and 1980. Indeed, some of our sampling methods would have been familiar to our hunter–gatherer ancestors. One of my aims has been to maintain continuity with this great body of earlier knowledge. In part, this is because earlier papers are able to describe techniques and equipment in far more detail than is normal today. But it is also the case that our predecessors often had great insight, and in many cases we can reapply their ideas by using our superior electronics and data handling to good effect. It is heartening to note that as journals have fully digitized their back numbers, many earlier papers are being cited regularly,

Early ecologists suffered from a lack of long-term time series. With each passing decade, datasets are becoming larger and the opportunities for more detailed analysis of temporal dynamics increases. In addition, remote sensing and large-scale observation, as undertaken in particular by bird and

butterfly watchers, have greatly extended the opportunity for spatial analysis. Recent concerns about species loss, habitat destruction, and fragmentation, and the effects of climate change, are dependent on the collection and analysis of temporally and spatially extensive datasets. The collection and handling of these data and the computation of indices of change, species richness, and diversity are important fields which continue to develop.

Dick Southwood is still included as an author of this fourth edition of *Ecological Methods*, because there are still many parts of the book which were originally authored by him and have been little changed. However, I answer for the inadequacies of the present edition.

Peter Henderson
Lymington, February 2015

Preface to the Third Edition

We have been encouraged to prepare this third edition by the continuing use of the earlier editions. In doing so we have been struck by contrast between the advances in some areas, especially data handling, and the enduring value of various other techniques. Ecology has continued its advance into the popular and political domain, though far from everything that has gained the 'eco-' prefix falls within our purview. The underlying paradigm of ecology has however shifted. In particular the concept of the metapopulation is now recognized as central to the understanding of the distribution and abundance of animals and its exploration aided by the accessibility of numerous datasets, often with large temporal or spatial scales. The availability of molecular techniques has encouraged the consideration of genetic and phylogenetic aspects and has permitted the growth of quantitative comparative analyses described by P. H. Harvey and M. D. Pagel (1991), *The Comparative Method in Evolutionary Biology* (Oxford).

The extent to which we have felt that revision was necessary has varied greatly in different parts of the book. Where there has been little change in the method, we have retained the early references. We have done this on the bases that journal editors formerly permitted more detailed description of methods, that these papers will not be located by computer searches, and that these pioneers continue to deserve credit. Other portions have required considerable modification; we have deleted one chapter (12) and added a new one (15), as well as reorganizing the structure in some places. Although the primary focus remains on insects, which are in terms of species the dominant animals, we have taken the opportunity to explicitly expand the coverage to all major macroscopic groups.

The widespread availability of high-capacity PCs, with software packages and access to the internet, has totally changed the speed and ease of handling (and sometimes accessing) data. We have therefore given references to some relevant software packages and websites, whilst eliminating many descriptions of time-consuming graphical methods. However, we believe that the advice given in the preface to the second edition is even more applicable today. The researcher, who relies entirely on the output of a computer, is in danger of drawing false conclusions and overlooking possible insights. It is essential to understand the features of the data (are there any outliers?), the assumptions of the methods, the biological basis of the analysis, and to acquire a feel for the capabilities and responses of the species under study.

The interpretation of 'ecological methods' remains as described in the preface to the first edition, namely those methods peculiar to ecologists, either in their origin or in modification. Just as the measurement of physical factors, using the methods of the physical sciences, has always been outside the book's scope, so are the methods of molecular biology. These are described in a number of works, such as 'Molecular markers for population ecology' (1998), *Ecology* 79, 359–425, edited by A. A. Snow and P. G. Parker.

We are most grateful to many ecologists who sent comments on the second edition. In particular, generous help has been given by Drs C. Henderson, D. J. Rogers, A. E. Magurran, W. D. Hamilton, G. R. W. Wint, and Mr C. Hambler.

T. R. E. Southwood
P. A. Henderson
Oxford, October 1998

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CHAPTER 1

Introduction to the Study of Animals

Information about animal populations is sought for a variety of purposes; *the object* of a study will largely determine the methods used, and thus this must be clearly defined at the outset. Very broadly, studies may be divided into *extensive* and *intensive* (Morris, 1960). Extensive studies are carried out over larger areas or longer time periods than intensive studies, and are frequently used to provide information on distribution and abundance for conservation or management programmes (e.g. monitoring the status of mammal populations; Macdonald et al., 1998, or assessing the extent of an insect pest outbreak, Fierke et al., 2005). Developments in remote sensing capability and geographical information system software have given great impetus to extensive studies in recent years (see Chapter 16). For pests or parasites, they provide assessments of incidence or damage, and may also guide the application of control measures. Aphids, for example, have been monitored in the UK since 1964 by the Rothamsted Insect Survey (RIS) suction trap network, (http://www.rothamsted.ac.uk/insect-survey/STAphidBulletin.html; Harrington and Woiwod, 2007). In extensive surveys, an area will often be sampled once or at the most a few times per study period. The timing of sampling in relation to the life cycle of the animal is obviously of critical importance, and for many species only limited stages in the life cycle can be sampled. Extensive studies produce information about the spatial pattern of populations, and it is often possible to relate the level of the population to edaphic, oceanographic, or climatic factors. Recently, long-term data have proved invaluable to quantify the effects of climate change on distribution and phenology.

Intensive studies involve the *repeated* observation of the population of an animal. Usually, information is acquired on the sizes of the populations of successive developmental stages so that a life-table or budget may be constructed. Then, using this table an attempt is made at determining the factors that influence population size and those that govern or regulate it (Varley and Gradwell, 1963; Sibley and Smith, 1998). It is important to consider at the start the type of analysis (see Chapter 11) that will be applied and so ensure that the necessary data are collected in the best manner. Intensive studies may have even more limited objectives, such as the determination of the level of parasitism, the amount of dispersal, or the overall rate of population change. The census of populations and the stages at which mortality factors operate are necessary first stages in the estimation of the productivity (see Chapter 14) of ecosystems.

When resources allow it can be advantageous to combine intensive and extensive components within a single study. For example, Fierke et al. (2005) in a study of red oak borer, *Enaphalodes rufulus*, in the Ozark Mountains, USA, used intensive data collected by felling and examining entire tree boles to check the reliability of a less resource-hungry subsampling protocol which was subsequently used to estimate borer densities in an extensive survey of the region.

1.1 Population estimates

Population estimates can be classified into a number of different types; the most convenient classification is that adopted by Morris (1955), although he used the terms somewhat differently in a later study (Morris, 1960).

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1.1.1 Absolute and related estimates

For large animals that are easily observed and have small, countable, populations such as rhinos, elephants, tigers, whales or some birds, it may be possible to express the global or metapopulation size as a total number of individuals. However, for most animals, numbers will be expressed as a density per unit area or volume or per unit of the habitat. Such estimates are given by distance sampling and related techniques (Chapter 9), marking and recapture (Chapter 3), by sampling a known fraction of the habitat (Chapters 4–6) and by removal sampling and random walk techniques (Chapter 7).

1.1.1.1 Absolute population

This is defined as the number of animals per unit area. For planktonic animals, the number per unit volume can be more appropriate. It is almost impossible to construct a budget or to study mortality factors without the conversion of population estimates to absolute figures. This is because other measures of habitat such as leaf area are variable. For example, the amount of plant available to an insect is always changing; further, insects often move from the plant to the soil at different developmental stages. *The importance of obtaining absolute estimates cannot be overemphasized*.

1.1.1.2 Population intensity

This is the number of animals per unit of habitat, for example per leaf, per shoot, per plant, per host. Such a measure is often, from the nature of the sampling, the type first obtained (see also p. 119). When the level of the animal population is being related to habitat availability or plant or host damage, it is more meaningful than an estimate in absolute terms. It is also valuable when comparing the densities of natural enemies and their prey. However, the number of habitat units per area should be assessed (Chapter 4), for differences in plant density can easily lead to the most intense population being the least dense in absolute terms (Pimentel, 1961). When dealing with different varieties of plants, differences in leaf area may account for apparently denser populations, in absolute terms, on certain varieties (Bradley, 1952). Thus, the choice of the leaf or of the plant as the unit for expressing population intensity can affect the relative population estimate obtained (Broadbent, 1948) (Figure 1.1). Similarly, with litter fauna—owing to the effects of seasonal leaf fall—the intensity measure (on animals/weight of litter) will give a different seasonal picture from an absolute estimate per square metre (Gabbutt, 1958). These examples also underline the importance of absolute estimates when interest lies primarily in the animal population.

1.1.1.3 Basic population

In some habitats, especially forests and orchards, it is often convenient to have an intermediate unit between that used for measuring intensity and absolute measures of ground area, for example 1 m^2 of branch surface (Morris, 1955) or branches of apple trees (Lord, 1968).

1.1.2 Relative estimates

Relative methods are important in applied areas such as fisheries or game management, where most of the information available may be derived from fishing or hunting returns. In fisheries research, catch per unit effort is often difficult to calculate from landing statistics because of changes in catch efficiency as fishing technology and the economy changes. The behaviour of fishermen frequently changes with the abundance and monitory value of the target species.

1.1.3 Population indices

These are generated when the animals themselves are not counted, but their products (e.g. frass, webs, exuviae, tubes, nests) or effects (especially plant damage) are recorded.

Both population indices and relative estimates of population can sometimes be related to absolute population (if this is measured at the same time) by regression analysis. If such a study has been based on sufficient data, subsequent estimates from relative methods or indices can be converted to absolute terms using various correction factors; such an approach is common in fisheries research (e.g. Beverton and Holt, 1957).

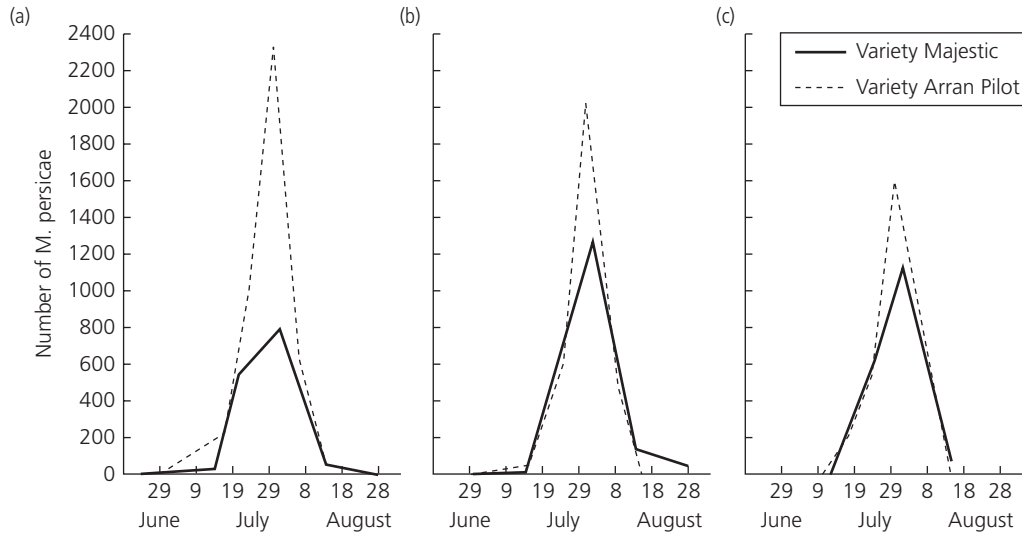

Figure 1.1 The influence of habitat unit on relative population levels when these are measured in terms of population intensity: the populations of Myzus persicae on different varieties of potato; (a) per 100 leaves; (b) per plant; (c) per plant, corrected for proportions of upper, middle, and lower leaves. (Adapted from Broadbent, 1948.)

1.2 Errors and confidence

The statistical errors of various estimates can usually be calculated, and the upper and lower boundaries of an estimate are referred to as the confidence or fiducial limits (the estimate (x) being expressed as $x \pm y$, where y is the fiducial limits). For ecologists, the distinction between confidence and fiducial is, in practice, unimportant. The confidence limits are calculated for a given probability level, normally 0.05, which means that there are only five chances in 100 that the range given by the limits does not include the true value (hence the expressions 5% probability level and 95% fiducial or confidence limits). If more samples are taken the limits will be narrower, but the estimate may not move closer to the actual value. Biologists are often worried that assumptions about sampling efficiency may be incorrect (e.g. does the 'knockdown' method really collect all the weevils on a tree?). Intuitively, most believe—quite correctly—that this estimate should be compared with another method that has different assumptions. If the estimates are of the same order of magnitude, then the investigator can have much greater confidence that the result of the study is not misleading. It is therefore sound practice to contemporaneously estimate the population or other variables by more than one method.

When two estimates have been obtained from different sampling procedures, and provided they are internally consistent (e.g. a t-test shows that the means are not significantly different), they may be combined to give a weighted mean, weighting each estimate inversely as its variance (Cochran, 1954). Under some circumstances Bayes' theorem could be used (see p. 62, equation 3.22) to give the combined estimate. Laughlin (1976) has suggested that the ecologist may be satisfied with a higher probability level (say 0.2) and thus narrower confidence limits for estimates based on more than one method, because such estimates have a qualitative, biological assurance, additional to that from the consistency of the data, that the true mean lies close to the estimate.

Most population studies are based on sampling, and the values obtained are considered to have a generality that scales with the area from which the samples were drawn. All estimates have fiducial limits, and the level of accuracy that should be aimed at is difficult to determine. Morris (1960) has

aptly said that '*…we are not likely to learn what precision is required by pessimistic contemplation of individual fiducial limits*.' Excessive concerns about accuracy can always be used by those who favour the warmth of the hearth to being in the field. As the amount of time and labour that can be put into a problem is limited, it should always be borne in mind that the law of diminishing returns applies to the reduction of the statistical errors of sampling. In the long run, more knowledge of the ecology of the animal may be gained by studying other areas, by making other estimates, or by taking further samples than by straining for a very high level of accuracy in each operation. Against this must be set the fact that when animals are being extracted from samples, the errors all lie below the true value as animals will occasionally be missed. A number of very carefully conducted control samples may allow a correction factor to be applied, but the percentage of animals missed may vary with density; sometimes, more are overlooked at the lowest densities (Morris, 1955).

An alternative to sampling is the continuous, or regularly repeated, study of a restricted cohort, such as the population of an aphid on a particular leaf or leaf-miners on a bough. These studies have a very high level of accuracy but they sacrifice generality. A combination of some cohort studies with larger-scale sampling often provides valuable insights.

1.2.1 Calculating confidence limits about the mean using R

Numerous R libraries will calculate confidence limits about the mean. Box 1.1 uses three different R packages to calculate the mean and confidence limits for the oak borer data of Fierke et al. (2005). The t.test function in the standard stats package can be used. Alternatively, the CI or MeanCI functions in the Rmisc and DescTools give identical results. To avoid making assumptions about the underlying distribution the MeanCI function can make bootstrap estimates for the confidence intervals (see Box 1.1). As these are based on random resampling the estimates will vary with each run.

1.2.2 Jackknife and bootstrap estimation of confidence limits

It is frequently difficult to estimate the accuracy of ecological indices because no equation exists for the variance of the statistic and the sampling distribution is unknown. Jackknife and bootstrap methods, which offer a non-parametric means of estimating parameters, are now widely used by ecologists to estimate the accuracy of population parameter estimates and indices for diversity or similarity. Both techniques are based on the repeated estimation of

Box 1.1 Example R code to calculate confidence intervals using a variety of methods. Oak borer data of Fierke et al. (2005).

```
# Confidence interval calculations. (Data from Fierke et al 2005.)
Input =("
Red_oak_no Borer_density
 1          274.1
 2           41.2
 3          181.6
 4          328.9
 5          112
 6          320.5
 7          142.5
 8          378.2
 9           63.9
10          112.5
11           61.1
12            9.6
13           58.7
")
Data = read.table(textConnection(Input),header=TRUE)
#Three packages all give the same result
t.test(Data$ Borer_density,conf.level=0.95) #t test for 0.95 confid int.
library(Rmisc) # Using CI in Rmisc package for confid. Int.
CI(Data$ Borer_density,ci=0.95)
library(DescTools) # Using MeanCI in DescTools package confid int.
MeanCI(Data$ Borer_density,conf.level=0.95)
#Bootstrap confidence interval calculation
MeanCI(Data$ Borer_density, method="boot", type="basic", R=10000)
```

Box 1.2 An example bootstrap calculation of the confidence intervals for the coefficient of variation of a set of observations held in the vector obs.

```
#Bootstrap confidence intervals for coefficient of variation
obs <-c(2, 3, 5, 2, 4, 2, 1, 3, 3,5,3,5,6,3,2) # Enter data
#generate a vector to hold 1000 bootstrap values
bootest <-numeric(1000)
#Define function to calculate coefficient of variation
CV <- function(obs) sqrt(var(obs))/mean(obs)
CV(obs) #Calculate CV
#Bootstrap 1000 CV estimates
for (i in 1:1000) bootest[i] <- CV(sample(obs,replace=T))
mean(bootest) #calculate the mean of the bootstrap estimates
var(bootest) #calculate the variance
quantile(bootest,0.975) #calculate value at upper 97.5%
quantile(bootest,0.025) #calculate value at lower 2.5%
#Calculate bias, difference between original and mean
 bootstrap CV
bias <- mean(bootest) – CV(obs)
# corrected estimate of the CV is the original estimate
 minus bias
CV(obs) – bias
# If normal, the approximate 95% confidence interval is
 given by
CV(obs) – bias – 1.96*sqrt(var(bootest))
CV(obs) – bias + 1.96*sqrt(var(bootest))
# Efron's confident limit
quantile(bootest,0.975)
quantile(bootest,0.025)
#Hall's confidence limits
2*CV(obs) – quantile(bootest,0.025)
2*CV(obs) – quantile(bootest,0.975)
```

the parameters of interest on a subsample of the data for which a computer is essential. Perhaps their most important application is for the estimation of error limits and confidence intervals for estimated parameters. There are computer programs for their calculation, and examples using R are given in Boxes 1.1 and 1.2.

The jackknife method uses less computational effort than a bootstrap analysis and is thus often quicker and easier to undertake. However, it cannot be used to estimate confidence intervals. The general scheme is as follows. Consider a situation where a parameter is to be estimated from n samples, e.g. a species diversity index from 10 kick net samples from a stream.

1. Use all n samples to calculate the parameter of interest, E, the diversity index.
2. Now remove one of the samples at a time and recalculate the parameter of interest, E_i.
3. Calculate the pseudovalues for the parameter of interest: $\varphi_i = nE - (n-1)E_i$, where ϕ is the pseudovalue for jackknife estimate i, n is the total number of replicates, E is the estimate for all n samples, and E_i is the estimate with sample i removed.
4. Estimate the mean and standard error of the parameter of interest from the n pseudovalues. The jackknife estimate of bias is E—the mean of the pseudovalues, and the estimate of the standard error of the sample is simply the standard error of the pseudovalues:

where ϕ is the mean of the pseudovalues. $se = \sqrt{\dfrac{\sum(\phi_i - \bar{\phi})^2}{n(n-1)}}$

The bootstrap method differs from the jackknife in the means of sampling the original dataset. The subsets of observations used to repeatedly calculate the parameter of interest are each selected at random with replacement. This allows a given observation to be included in a subset more than once. This procedure is repeated, often hundreds of times, so that many combinations of the original observations are generated. For example, if a sample comprised ten species and 100 individuals, then each subset of data would be a randomly selected 100 individuals taken from a population that represented all the species according to their original frequency of observation. Each of these samples is then used to generate estimates of the parameter of interest (e.g. a diversity index) as for the jackknife method. The bootstrap estimate of the bias is simply

found by subtracting the observed parameter value from the mean parameter obtained from the bootstrap replicates. The estimate of the standard error of the sample is simply the standard error of the replicate parameter estimates:

$$se = \sqrt{\frac{\Sigma(\phi_i - \bar{\phi})^2}{b-1}}$$

where ϕ is the mean of the replicates and b is the number of bootstrap samples taken.

A number of methods can be used to estimate confidence intervals. The percentile bootstrap is the simplest and most commonly used. The 2.5 and 97.5 percentiles of the bootstrap distribution of the parameter are used as the 95% confidence intervals. For example, if 1000 bootstrap replicates have been taken, then these are arranged in order of magnitude, and the 2.5th percentile calculated as the average of the 25th and 26th smallest values, and the 97.5 percentile as the average of the 975th and 976th smallest values.

Jackknife and bootstrap calculations are easily undertaken in R. Box 1.1 calculates bootstrap confidence intervals using the DescTools package. Box 1.2 calculates bootstrap confidence intervals for the coefficient of variation of a series of observations held in the vector obs.

Bootstrap and jackknife estimates are used to estimate species richness (Chapter 13), population size (Chapter 3), and population growth rates (Chapter 11). Introductions to bootstrap and jackknife methods are given in Efron and Tibshirani (1986), Scheiner and Gurevitch (1993) and Potvin and Roff (1993).

1.3 Studies of communities

In survey and conservation work, the species make-up of the community and temporal changes in its diversity (p. 481) associated with human activities are most frequently the features it is desired to measure. The measurement of biodiversity requires careful planning, as the type and intensity of sampling can have a marked influence on the conclusions drawn. The estimation of species richness invariably requires repeated sampling of the habitat, with special methods of analysis needed to estimate the total species number (see Chapter 13). Species richness studies almost always need to be intensive as they require repeated sampling within a restricted local. It is frequently useful to identify core (resident) and transient (tourist) members of a community (p. 402), and this necessarily requires repeated sampling. The most direct approach is to sample regularly over an extended number of seasons to identify transient species as those not constantly present (e.g. Magurran and Henderson, 2003, for estuarine fish; Ulrich and Ollik, 2004, for forest hymenoptera, Coyle et al., 2013, for birds, Snell et al., 2018, for a general review of transient species occurrence). With large teams of observers as occurs with citizen science (Chapter 16), geographically extensive studies reach the sampling intensity required to estimate species number and recognize core and transient species across wide geographical zones. The proportion of the community classified as transient varies with the spatial scale and environmental heterogeneity as shown by Jenkins et al. (2018) using the geographically extensive North American Breeding Bird Survey.

The relative abundance of species (Chapter 13) is a key attribute which may give insight into the functioning and health of a community, though difficulties usually arise because of the impossibility of recording the abundance of all the species living within a habitat with equal efficiency. When considering ecosystem function it can be useful to remove transient species from the analysis.

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CHAPTER 2

The Sampling Programme and the Measurement and Description of Dispersion

2.1 Preliminary sampling

2.1.1 Planning and fieldwork

At the outset you must be quite clear as to the problem you are proposing to investigate. As it is normally impossible to count and identify all the animals in a habitat, it is necessary to estimate the population by sampling. Naturally, these estimates should have the highest accuracy commensurate with the effort expended. This requires a plan that includes a sampling programme laying down the number of samples, their distribution, and their size. While the statistical principles for sampling are given in texts (e.g. Elliott, 1977; Cochran, 1977; Green, 1979; Seber, 1982; Mead, 1990; Scheiner and Gurevitch, 1993; Underwood, 1997; Thompson, 2012; Lohr, 2019), there is no universal sampling method. Knowledge on the biology of the focal species will influence the methodology. *The importance of careful formulation of the hypothesis for test cannot be overstressed*.

Detailed and now classical accounts of the development of sampling programmes for various insects are given by Morris (1955, 1960), LeRoux and Reimer (1959), Harcourt (1961, 1964), Lyons (1964) and Coulson et al. (1975, 1976), amongst others. Sampling insects for integrated pest management is discussed by Binns and Nyrop (1992). Terrestrial sampling programmes for vertebrates, small mammals, and birds are described by Caughley (1977), Smith et al. (1975), and Blondel and Frochet (1987), respectively. For aquatic systems, estuarine and coastal sampling programmes are described for many groups by Baker and Wolff (1987), sampling marine benthos by Eleftheriou (2013), marine rocky substrates by Bianchi et al. (2003), and for zooplankton by Omori and Ikeda (1984).

In community studies, preliminary work should consider species richness and potential problems with species identification. Taxonomic difficulties should be identified and addressed. The appropriate degree of taxonomic discrimination must be decided, as it is important to maintain a consistent taxonomy. Sample sorting and species identification are often the most labour-intensive parts of a study, and it may be useful to process a trial sample to assess the effort required. Saila et al.(1976) found that a single marine grab sample cost US$25 to collect and US$300 to sort. By 2019, the cost of picking and identifying to species the contents of a single marine benthic grab sample can cost £650 (US$840).

The planning of the timing of sampling requires knowledge of life cycles. Preliminary work will be necessary to gain some knowledge of the distribution of the animals. From these observations, an estimated cost per unit sample in terms of time, resources, and money can be made.

2.1.1.1 Deciding upon the scale of sampling

The first decision concerns the scale of the universe to be sampled. Whether this is to be a single habitat (e.g. field, woodland or pond) or representatives of the habitat type from a wide geographical area will depend on whether an intensive or an extensive

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study is planned. The correct definition of the target population or community is essential; if too small, it may not produce results representative of the structure as a whole; if too large, it will waste resources and probably fail to be completed.

Both spatial and temporal scale need to be considered. The scale chosen will influence your conclusions. In an extensive study two species may occur sympatrically and appear as potential competitors. However, small-scale studies may show they occupy different parts within a single plant, are active at different times of the day or at different seasons and thus rarely interact.

Small spatial scale favours control, replication, and precision. The possibilities for experimental manipulation, replication, and the selection of control sites all becomes increasingly difficult as the area of interest increases in magnitude. Ecological experimental manipulations cannot study the spatial scales required to capture large-scale phenomena (Kareiva and Anderson, 1989). Increased focus on large-scale issues has been accompanied by the need to develop and test large-scale hypotheses in ways that move beyond classical experiments without compromising scientific rigour. Citizen science (p. 492) is one approach now regularly used to resource large-scale studies. Determining the appropriate spatial scale requires knowledge about the biology of the focal species. Sale (1998) argued that the appropriate scale when sampling reef fish is determined by the extent of movement of the life stage under study, and the same approach can be usefully applied to terrestrial organisms. A good description of this decision process for plankton is given by Omori and Ikeda (1984).

Temporally short-term studies overemphasize temporal variability as they cannot show long-term stability. Figure 2.1 A and B demonstrates the dramatic differences in conclusion that may arise with variation in duration of a study. Over the full thirty-eight years of study in the Bristol Channel, Henderson (2019) observed no significant trend in the abundance of whiting, *Merlangius merlangus*, indicating long-term stability. However, over the first seven years there was a significant fit to an exponential curve which, at the time, suggested unconstrained population growth. Some research objectives may require demandingly long time series. The statistical detection of density-dependence by Bulmer's method (see p. 477) can require time series >100 generations in extent. In an examination of Hudson Bay fur-trapping data Bulmer (1975) noted that a series of sixty-two annual observations for a species is only likely to reveal the existence of strong density-dependence.

Wiens (1977) argued that short-term studies will produce erroneous conclusions because they are likely to miss unusual or extreme events. However, Weatherhead (1986) concluded that short-term studies actually record too many unusual events. The reason given for this counter intuitive result is that we tend to exaggerate the unusual nature of our more extreme observations in short-term studies because we lack the perspective afforded from long experience. Long-term studies will inevitably be required to study (1) slow processes (e.g. long-lived tree or vertebrate population dynamics), (2) rare events, (3) subtle processes, when the signal is of small magnitude compared to the noise, and (4) complex phenomena involving a combination of multiple parameters (Strayer et al., 1986).

It is inevitable that financial resources and the duration of doctoral grants often determine the scale of a study, or what is appropriate to study. Weatherhead (1986) in a survey of 308 studies found a mean duration to be 2.5 years, and Tilman (1989) found only 1.7% of field studies published in *Ecology* had a duration >5 years. The spatial extent of manipulative field studies is similarly limited; Kareiva and Anderson (1989) report that 44% of studies examined had a physical scale <1 m and only 25% >10 m.

2.1.1.2 Accuracy and precision

The second decision must be to define the accuracy or precision of the population estimates required. Accuracy measures how close an estimate is to the real value, while precision measures the reproducibility of the estimate (Figure 2.2). If a systematic error is intrinsic to a sampling method then increased sampling may improve the precision, but it will not increase the accuracy of the estimate. The decision on accuracy is taken by considering both the objectives of the study and the variability of the system under study. For example, many species of insect pest exhibit ten- or even hundredfold

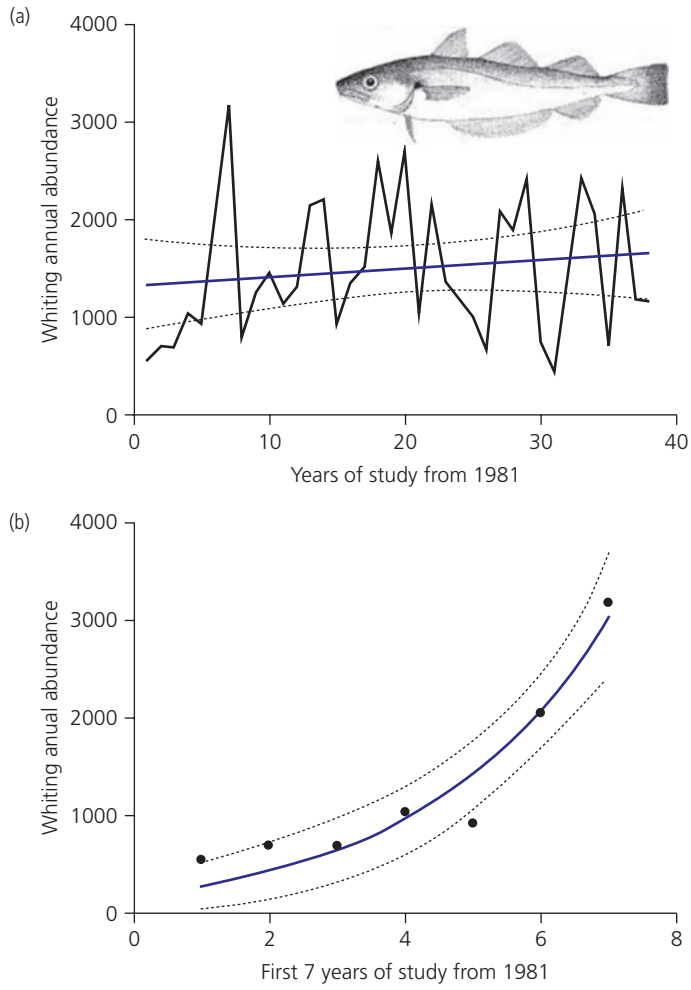

Figure 2.1 Temporal trends in the abundance of the whiting, *Merlangius merlangus*, in the Bristol Channel, UK (after Henderson, 2019). A. Annual abundance derived from monthly sampling between 1981 and 2018 inclusive with a linear trend fitted by regression ($R^2 = 0.017$). There is no significant long-term trend. B. Annual abundance of the same time series for the first seven years with an exponential curve fitted by regression ($R^2 = 0.93$). 95% confidence intervals plotted as dotted lines.

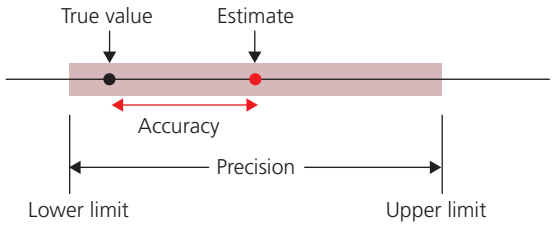

Figure 2.2 An illustration of the difference between accuracy and precision.

population change within a single season. For such variable species an estimate of population density with a standard error of about 25% of the mean, enabling the detection of a doubling or halving of the population, is sufficiently accurate for damage control (Church and Strickland, 1954). Similar levels of accuracy are often appropriate for fisheries studies; fish recruitment can vary tenfold between years (Cushing, 1992). For life-table studies, more especially on natural populations, a higher level of accuracy—frequently set at 10%—will be necessary.

2.1.1.3 The quality of species inventories

Conservationists often seek to build species inventories. Here, completeness will replace accuracy as a measure of quality. Whereas, for large mammals or birds the aim might be to record all resident species, for high-diversity groups such as beetles the objective may be set at only 5–10%. Community studies often aim to generate summarizing statistics such as measures of diversity or species richness (Chapter 13), which can be used to compare localities or changes through time. The accuracy and precision of these estimates must be carefully considered if changes are to be detected at the desired resolution. The detectability of animals changes with the climate, so it can be difficult to maintain constant sampling effort.

2.1.1.4 The focal life-stage and the timing of sampling

In extensive work, the amount of sampling in a particular locality will be limited and therefore a further decision concerning both the age-group to be studied and the timing of sampling will need to be made. For insects, the best stage for sampling may be that most closely correlated with the amount of damage (Burrage and Gyrisco, 1954). Alternatively, if the purpose of the survey is to assess the necessity for control, the timing should be such that it will give advanced information of an outbreak (Gonzalez, 1970). Direct sampling methods such as sweep-netting or counts of individuals per leaf are often most effective for less mobile, immature, stages (Martini et al., 2012).

For marine organisms, timing is often determined by the reproductive cycle. For example, in temperate waters, marine benthic surveys carried out in autumn will show a population dominated by recent recruits. The same survey carried out in the spring will show the resident community which has survived both competition for space and the rigours of the winter (Bamber, 1993). In freshwater habitats, samples collected in spring and autumn can differ markedly. Carlson et al. (2013), in a study of Swedish streams, found chironomids to be most abundant in autumn, which they related to the timing of emergence. Other seasonal changes were linked to the harsh conditions during the spring ice melt when water discharge is high and temperatures low. Similarly, large seasonal changes occur in tropical waters such as the Amazon linked to the flood cycle. Henderson et al. (1998) reported order-of-magnitude changes in floodplain fish density between high- and low-water seasons as typical.

While the life history stage to be sampled will depend on the objectives, the stage must not be one whose numbers change greatly with time and it must be present for a period sufficient to allow the survey to be completed. Further, the easier the stage is to sample and count the better—birds, butterflies, and some mammals may be most easily counted during courtship and mating. Extensive surveys can result in different areas holding populations that differ in their position in the reproductive cycle. The reliability of samples in an extensive survey may be particularly sensitive to current weather conditions (Harris et al., 1972). The influence of latitudinal gradients on the timing of sampling can be important. In extensive surveys of marine plankton, the timing of blooms or larval production can vary by one month over 1 degree of latitude.

Although the preliminary sampling and analysis of the assembled data will provide a measure of many of the variables, the actual decisions must still, in many cases, be a matter of judgement. Furthermore, as the density changes, so too will many of the statistical parameters, and a method that is suitable at a higher density may be found inadequate if the population level drops. Shaw (1955) found that Thomas and Jacob's (1949) recommendation for sampling potato aphids—one upper, middle, and lower leaf from each of fifty plants—was unsatisfactory in Scotland, in certain years, because of the lower densities.

2.1.1.5 Subdividing the study area

The next step is to subdivide for sampling purposes. These subdivisions may be orientated with respect to an environmental gradient. If none is apparent, it is often convenient to divide the area into regions of regular shape, but this may be neither possible nor desirable. Ideally, the within-subdivision variability should be much less than the between-subdivision variability in habitat. The habitat must be considered from the biological angle and a decision made as to whether it might need further division. If it is woodland, for example,

the various levels of the tree—upper, middle, and lower canopy and probably the tips and bases of the branches—would on *a priori* grounds be considered as potentially different divisions. The aspect of the tree might also be important. In herbage or grassland, if leaves or another small sampling unit is being taken, the upper and lower parts of the plants should be treated separately. Similarly, aquatic samples may be taken from different depths, position with respect to flow, and from different plants or substrates.

It is often of value to take at least two different sized sampling units (Waters and Henson, 1959). In benthic surveys a 0.1 m² grab sample suitable for sampling macroinvertebrates can itself be core sampled for the meiofauna prior to processing (McIntyre and Warwick, 1984). In an insect study, the aim should be to sample towards the smallest possible limit, for example a leaf blade or half-leaf. As a general principle, a higher level of reproducibility is obtained (for the same cost) by taking more smaller units than by taking fewer large ones.[1] Small sampling units may also enable precision to be increased by distinguishing between favourable and unfavourable microhabitats; Condrashoff (1964) found with a leaf miner that the upper and lower leaf surfaces should be considered as separate units. Two examples of any one size-sampling unit should be taken within each sampling plot or subsection. For example, for a field crop a preliminary plan could be as in Figure 2.3, which gives a total of 160 samples.

In community studies where it is essential to gain some idea of species diversity and the species–area relationship, a number of sampling methods may be

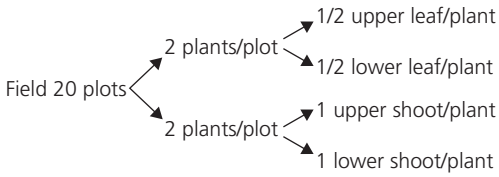

Figure 2.3 Example of a simple sampling plan.

[1] The only disadvantage of sampling by small units is the number of zeros that may result at low densities; this truncation may make analysis difficult and has led to the suggestion that larger-sized samples should be taken (Pradham and Menon, 1945; Spiller, 1952). The decision must be related to the density of the animal, although moderate truncation can be overcome by suitable transformation; in other cases, it may be necessary to increase the size of the unit.

needed. When benthic sampling for example, a pilot study might use dredges to obtain a general idea of animal presence and distribution and a limited number of grab samples to estimate densities.

During a pilot study a record should be kept of the cost of each part of the sampling routine, normally expressed in man-hours.

2.1.2 Data control and statistical aspects

Before the data gathered in the preliminary samples can be analyzed, some aspects of data organization and statistics need to be considered.

2.1.2.1 Data storage, software, and statistical analysis considerations

A sampling plan needs to consider the processes involved in: (1) data acquisition; (2) organization and storage; (3) analysis; and (4) presentation. Smooth and rapid progress along this chain almost always uses computers, but problems arise if data cannot be easily transferred. If different software products and operating systems are used, then compatibility must be considered. Computational power is now so great that even small, portable, machines can handle 1,000 × 1,000 arrays of data, which is quite sufficient for most researchers' needs, as few of us will ever count the abundance of 1,000 taxa in 1,000 samples. However, computational demands soon start to rise to demanding levels when automated forms of data acquisition are used. For example, the analysis of sonar data for fish counting or recordings of bat echolocation calls can rapidly generate huge amounts of digital information that needs to be analyzed. Particular care is needed when automated recording is to be undertaken to ensure the data can be analyzed within the required time-scale. Both software and hardware capability need to be considered for each stage of the study. During data acquisition it is important to assess the data storage and processing requirements. Data have only been acquired when it has been processed into a usable form. Data acquisition and processing rates are often different. A common problem is the analysis of video recordings; for example, the study of salmon movement through a fish pass. In one such study over a ten-week period about 1680 hours of video was recorded of the water in the fish pass. Because of variable light, turbulence,

and debris in the water the images were of poor quality. It was originally planned to use an image analysis program to identify and to count the fish on the video. However, at the end of the fieldwork it was found that the image analysis software worked at less than one-tenth of real time, and required over 16,800 hours of computer time to analyze the images. In practice, this meant that image analysis would only just be complete by the beginning of the next salmon migratory season. The only way to acquire the fish count data within the required time was to employ three people for six weeks to view the video and manually input observations. A possible solution to such problems may be the citizen science approach (p. 492).

Many software products are available for initial data organization. Those most frequently used by ecologists are spreadsheets such as Excel, which has become the industry standard. When choosing the product to use, it is important to consider carefully its capability to hold, manipulate, and convert the data into other formats. A general data file format that can be imported and used by many programs is a csv file. These can be easily read into R programs using read.csv (see p. 465 for an example). Much historical data from the 1970s and 1980s has become difficult to access because it was recorded on tape or disc formats that are no longer used. This should be less of a problem in the future as datasets can be uploaded to web-based data storage. Consideration should be given to long-term data archiving and maintenance. It may be a condition of publication that the data are made available on an open access digital archiving service. Knowing where your data will be archived and the format required should be identified at an early stage.

Ecological datasets of animal abundance can often be organized within a three-dimensional array of species (or other taxa) by station (locality) by time. This is the data structure that spreadsheets are designed to handle. If a spreadsheet has the capability to hold the datasets then they are often the preferred software for data organization and elementary analysis for they allow the rapid production of summary statistics, tables and graphs. Specialist database applications such as Microsoft Access, ADABAS, MySQL, and IBM DB2 can also be used to hold and organize data. These programs are better than spreadsheets if there is a need to store and relate together different types of data, but they are usually inferior to spreadsheets in their ability to *easily* undertake calculations and plot graphs. If you will be gathering a number of different types of data, e.g. physical data and biological, you might require a relational database. This can be thought of as a collection of data tables linked together so that different combinations of data can be extracted.

You will also need to consider where you wish to enter the data and if this will be undertaken by a number of people. Web-enabled databases allow the data to be accessed remotely and interactively; they may be useful if you require access while in the field. Cloud-based systems have the advantage that your data will be archived and protected by a third party. Database options and their strengths and weaknesses can be summarized as follows.

Desktop database

- Runs on single computer, simple to organize.
- Data availability, storage, and functionality limited by computing power.
- Not scalable, e.g. cannot adapt to multiple user inputs or remote access.
- Inexpensive, but the user does all management.

Web-enabled database

- Has a permanent link to the internet, a web server, and a firewall.
- Utilized online, and all data can be accessed remotely and interactively.
- Moderately scalable.
- More expensive to run than a desktop database.

Server database software

- Only sensible if massive data storage anticipated.
- Multiple users can simultaneously access, modify, and update data.
- Scalable and powerful.
- Expensive and likely to require specialist support.

The ease and ability to move data into other programs should be considered before committing to a particular database or spreadsheet. The most common requirement is to export data to a statistical, graphical or word-processing package. Large statistical software packages often offer their own spreadsheet capability, as do graphics packages such as SigmaPlot. When using R it is useful to have the data in csv format.

A wide range of powerful statistical and data manipulation packages, such as R, SAS, SPSS, Stata, Systat or Genstat, are available. Many ecologists now use R, the open source statistical analysis environment available from the Comprehensive R Archive Network (CRAN) at http://cran.r-project.org, because it is not only free but also offers a huge range of statistical tests and procedures, including more advanced methods such as general and generalized linear models, time series analysis, multivariate analysis, cluster methods, and a wide range of graphical displays such as heat maps (Figure 13.6). It also offers extensive graph-plotting procedures. It is, however, a far from intuitive environment within which to work, and those with little programming experience can find it difficult to use and will usually prefer commercial statistical packages.

A common problem is backward compatibility. While most new programs will be able to handle older file formats, it is often impossible to import new formats into older software packages. Care must be taken if an old but well-tested and valued program is to be used to ensure that it can run with current data formats. Care must also be taken if data are to be moved between machines using differing operating systems, for example Windows and Apple PCs.

Particular care is needed to define date and number formats, as Europe and the USA differ in day and month order. The real number represented as 3,14 in continental Europe is 3.14 in Britain or the USA. There are also many formats used to represent missing values.

2.1.3 The normal distribution and transformations

The most important and commonly used of the theoretical distributions is the normal or Gaussian distribution. This has a probability curve that is a symmetrical bell-shape. The probability density of a normal variable $P(x)$ is:

$$P(x) = \frac{1}{\sqrt{2\sigma}} e^{\left[-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)\right]} \qquad (2.1)$$

where μ is the mean and σ the standard deviation. This distribution is symmetrical about the mean, and the shape is determined by the standard deviation (Figure 2.4 A and B).

The normal distribution has a key role in statistics because the mean of many random variables independently drawn from the same distribution is distributed approximately normal, irrespective of the form of the original distribution. This is termed the 'central limit theorem'. Thus, if an ecologist calculates a series of means from samples obtained from a population which conforms to a non-normal distribution, such as a negative binomial, the distribution of the means will be normal. While many distributions obtained from observation (e.g. the heights of humans) are approximately normal, the spatial distribution of individuals is seldom, if ever, normal. The importance of the normal distribution to ecologists arises solely from the fact that many statistical methods, such as linear regression, assume that the errors in measurement are normally distributed. General linear models, which include analysis of variance, assume homogeneity of variance and normality of error. These assumptions need to be tested to ensure that statistical significance is correctly determined. The *glm()* and related functions in R offer a range of diagnostics to ensure the assumptions are met. Box 2.2 and Figure 2.6 show the use of a Q-Q diagnostic plot to check for normality. While it is viewed that analysis of variance is quite robust to deviations from normality—and in some respects more so than the χ^2 test (Reimer, 1959; Abrahamsen and Strand, 1970)—data whose frequency distribution is considerably skewed and with the variance closely related to the mean cannot be analyzed without the risk of errors.

Ecological data are usually skewed. The skew can be negative or positive, and the terminology is confusing as a negative skew is biased towards the right (see Figure 2.5). The negative skew shown in Figure 2.5 was generating using the R code in Box 2.1.

Such distributions are usually transformed by taking logarithms or square-roots. For example, if the square-root transformation were applied to 9, 16, and 64 they would become 3, 4, and 8, and it will be observed that this reduces the spread of the larger values. The interval between the second and third observations (16 and 64) is on the first scale nearly seven times that between the first and second observations; when transformed, the interval

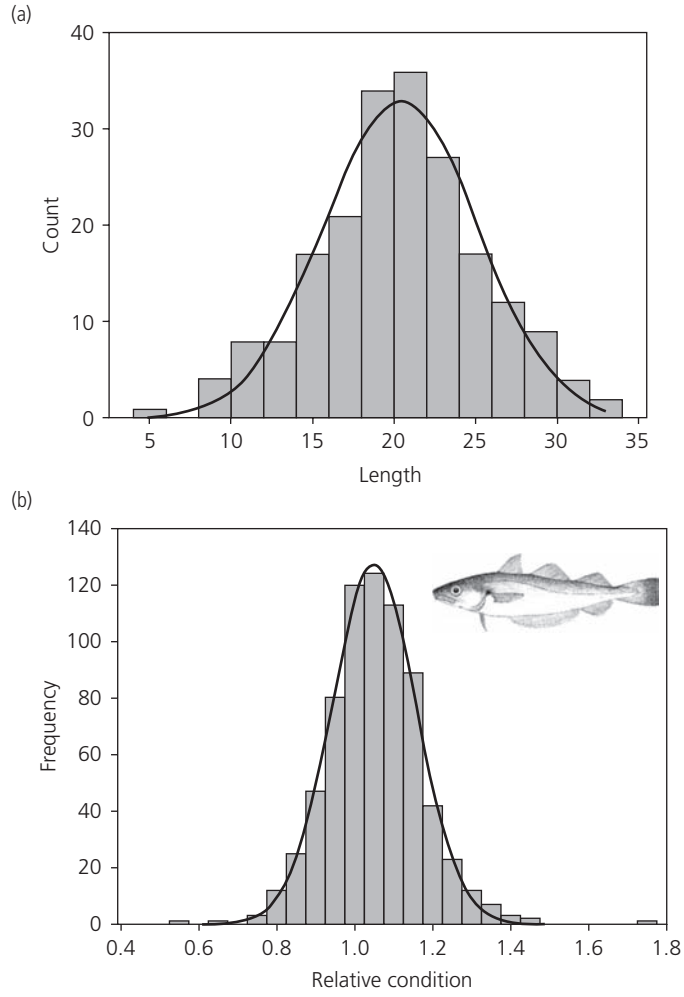

Figure 2.4 The normal distribution. A, randomly generated normal distribution of 200 observations and a fitted normal curve. The values were generated in Excel using the formula = NORMINV(RAND(),20,5). B, An actual example of an approximately normal distribution. The frequency distribution of relative condition of whiting in November. The greater the condition, the greater the stored fat (From Henderson, 2019).

Box 2.1 R code to generate a skewed distribution. The sn package is used to generate a skew-normal distribution.

```
library(sn)
r <- rsn(1000, 5, 2, -10)
hist(r, breaks=30, density=20)
```

between the second and third observations is only four times that between the first and second. A transformation of this type would tend to 'push' the long tail of a skew distribution in, so that the curve becomes more symmetrically bell-shaped. If all the observations (x) are positive and no *a priori* reason exists to choose between the many types of transformation possible, the Box–Cox empirical method can be used (Box and Cox, 1964). The general transformation function from x to x' is:

$$x' = \frac{\left(x^{\lambda} - 1\right)}{\lambda} \; when \; \lambda \neq 0, and$$
$$x' = ln \; x \; when \; \lambda = 0 \qquad (2.2)$$

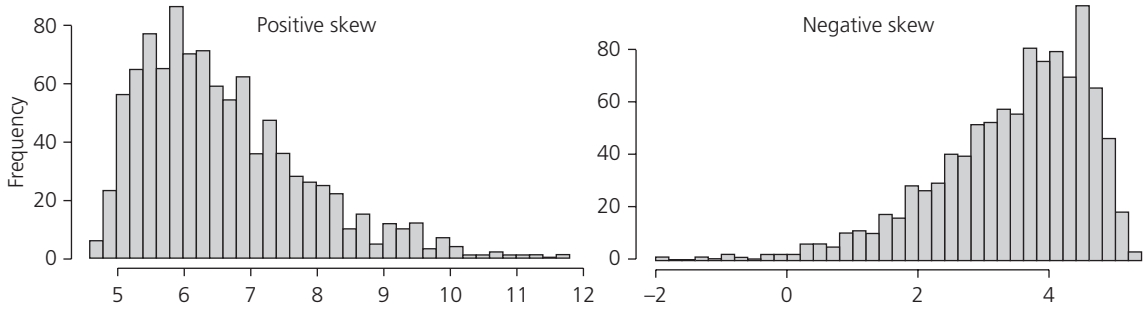

Figure 2.5 Examples of positive and negative skew. These distributions were generated in R using the sn package; see Box 2.1.

Table 2.1 Values of γ obtained by fitting either the Box–Cox transformation function and their corresponding transformations.

λ	Transformation
1	linear $y = ax + b$
0.5	Square-root $y = \text{SQRT}(x)$
0	logarithmic
–0.5	reciprocal square-root
–1	reciprocal $y = 1/x$

The best transformation to normality is the value of exponent, λ, usually estimated using a maximum likelihood method. A suitable value of λ can be identified using the boxcox function in the MASS package in R. The R code presented in Box 2.2 gives an example R code used to transform a series of values for the average density of the mysid *Schistomysis spiritis*. The data are the average density per 100 m^3 for a sequence of generations within the Bristol Channel. As shown in Figure 2.6 A and B they are clearly non-normal. The transformed data gave a reasonable fit to a normal distribution (Figure 2.6 C and D).

Examination of the general transformation function (Equation 2.2) will show that certain values of λ correspond to commonly used transformations; these are listed in Table 2.1. If the calculated value for λ falls close to that corresponding to a common transformation, then this transformation should be used. For example, the mysid data in Box 2.2 give $\lambda = 0.06$, so a value of $\lambda = 0$ would be appropriate and a logarithmic transformation used.

Box 2.2 R code to test for normality and select an appropriate transformation to normalize the distribution.

```
# Input average mysid density per generation
Mysid_density = c(16.25,294.75,390.25,47.13,1084.5,
    117.13,52.63,489.5, 1.25,53.63,203.88,10,28.5,132.5,
    1.38,60.33,123.63,37,23,449.25,3.38,42.88,39.88,
    17,2.63,271.13,31.5,19.25,37.38,9.38,6.38,155.88,4.88,
    5.75,46.75,3.75,34.75,120.25,12.25,142.63,218)
#Examine data for normality
library(rcompanion) # Open rcompanion
plotNormalHistogram(Mysid_density) # Data shows positive
    (right) skew
#Normal Q-Q plot shows deviation from normality
qqnorm(Mysid_density,ylab="Sample Quantiles for Mysid
    density")
qqline(Mysid_density,col="red")
# Open MASS package to use Box-Cox procedure
library(MASS)
# Transform Mysid)density.Try values -6 to 6 by steps of 0.05
Box <- boxcox(Mysid_density ~ 1,lambda = seq(-2,2,0.05))
Cox <- data.frame(Box$x, Box$y) # Data frame holds results
Cox2 <- Cox[with(Cox, order(-Cox$Box.y)),] # sort by y
    the data frame
# Display the lambda with maximum log likelihood
Cox2[1, "Box.x"]
lambda <- Cox2[1, "Box.x"] # Extract that lambda
Trans_mysid <- (Mysid_density ^ lambda - 1)/lambda #
    Transform the original data
# Plot transformed data to check normality
plotNormalHistogram(Trans_mysid)
qqnorm(Trans_mysid,ylab="Sample Quantiles for trans-
    formed mysid density")
qqline(Trans_mysid,col="red")
```

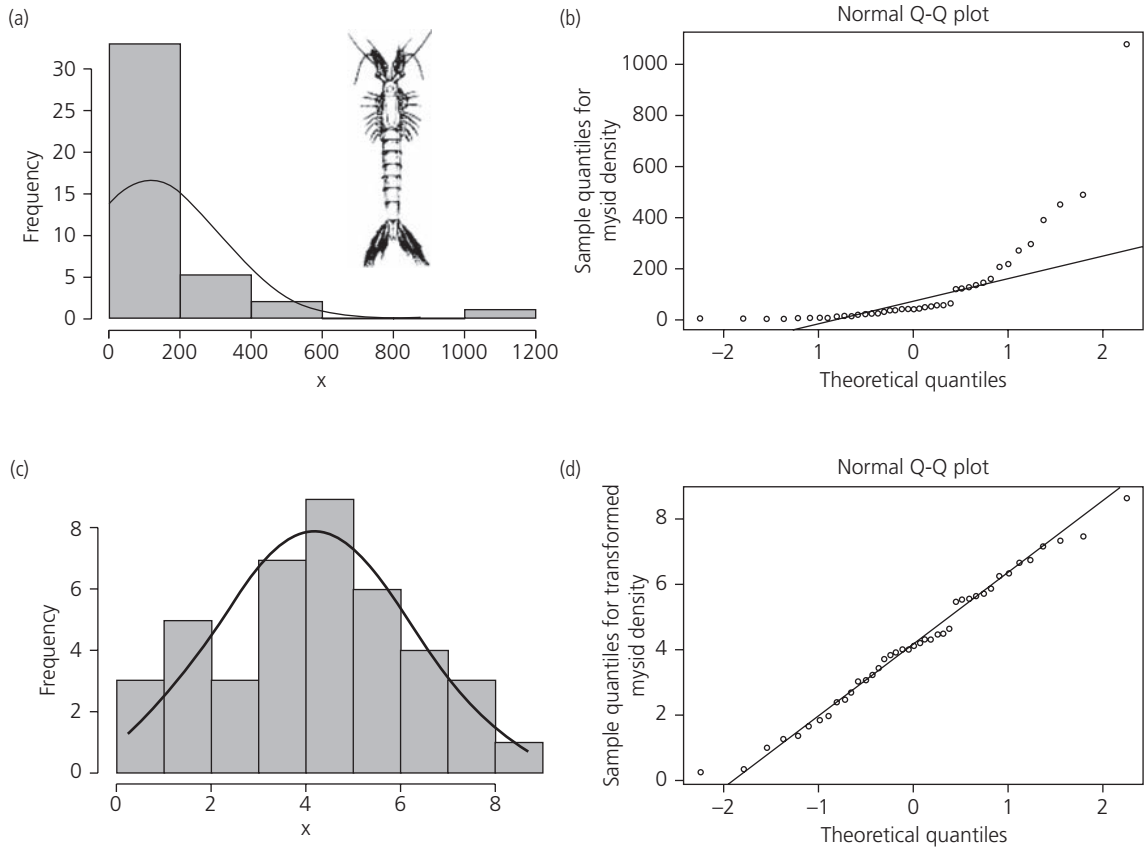

Figure 2.6 The effects of transformation on the mysid density data. The original data and the R code used to generate these graphs is shown in Box 2.2. A The frequency distribution of the original data showing a right skew. B A normal Q-Q plot showing a poor fit to a linear relationship. C The frequency distribution of the transformed data. D The normal Q-Q plot for the transformed data showing a good linear relationship.

Taylor's power paw (see p. 34), which relates the variance to the mean, has been used to identify suitable transformations. If the goal is to minimize the squared errors in predicting the mean, then better methods are sometimes available. For example, if replicate counts are taken from a Poisson-distributed population, but sampling error allows the mean to vary from count to count, it can be shown that the variance–mean relationship is:

$$\sigma^2 = \mu + c^2\mu^2 \qquad (2.3)$$

where c is the coefficient of variation of the Poisson means.

It must be stressed that transformation does lead to difficulties, particularly in the consideration of the mean and other estimates (see below). It should not be undertaken routinely, but only when the conditions for statistical tests are grossly violated (LeRoux and Reimer, 1959; Finney, 1973). In many applications, Generalized Linear Models (GLMs) should be used rather than seeking a suitable transformation.

Following the development of Generalized Linear Models (GLMs) (McCullugh and Nelder, 1989) in which all additive effects and the relationship between mean and variance are specified separately, the need for variance-stabilizing transformations prior to statistical analysis has been made unnecessary. When dealing with count data, when there cannot be negative counts, the basic GLM is a Poisson model with a log link (e.g. Roslin et al., 2006, in a study of the leaf miner, *Tischeria ekebladella*, on

oak, see p. 20). The glm() function in R will meet most ecologists' GLM needs.

In order to overcome difficulties with zero counts in log transformations, a constant (normally 1) is generally added to the original count (x); this is expressed as 'log (x + 1)'. Anscombe (1948) has suggested that a better transformation would be obtained by taking log ($x + k$ /2), where k is the dispersion parameter of the negative binomial (see below). As k is frequently in the region of 2, in many cases this refinement would have little effect. Andersen (1965) has shown that if the mean and k are very small (less than 3, and approaching zero, respectively) then the variance will not be stabilized by $k/2$ or any of the common transformations. However, if independent samples are pooled, or the size of the sampling unit is increased, the data may be satisfactorily transformed. When you have zero values, consider using a square root transformation.

It has been customary to transform percentage or proportion data using an arc-sine transformation, calculated in R using *asin(sqrt(x/100))*, where x is a percentage. Generally, this can be avoided by the choice of a GLM binomial model with a logit link function. One field where Crawley (2005) still recommends the use of the arc-sine transformation is for percentage cover data as collected by botanists. After transformation, such data can be analyzed using traditional linear methods.

The use of transformations can lead to problems and, as stressed above, transformation should not be routinely undertaken. If the fiducial limits are calculated from the transformed mean this may be erroneous (Abrahamsen and Strand, 1970). The biological interpretation of estimates based on transformed data is often difficult. There is indeed much to commend the use of the arithmetic mean (that based on the untransformed data) in population studies (van Emden et al., 1961; Lyons, 1964), and if the distribution of the animal is random (see below) the fiducial limits are available in tables (Pearson and Hartley, 1958). If data have been transformed, the means of the untransformed and transformed values should be provided; back transforms of, say, geometric means from logarithmic transformations are more difficult to interpret and contain biases unless the variances are small (Finney, 1973). Beauchamp and Olson (1973) note that values calculated by regression using log transformed variables cannot be simply untransformed by taking the antilogarithm without introducing bias; they show that the bias will be corrected by using properties of the log normal distribution for detransforming.

2.1.3.1 Checking the adequacy of the transformation

An adequate transformation should eliminate or considerably reduce two attributes of the data that are easily tested for. These are listed below.

1. Excess skewness of the frequency curve causing large deviations from normality, detected either from a probit plot or by statistical test (Snedecor and Cochran, 1989). Using R, a Q-Q plot using the function qqnorm() produces a plot in which departures from a straight line suggest violations of normality (Box 2.2 and Figure 2.6), alternatively the shapiro.test() function performs a Shapiro–Wilk normality test. The nortest package in R offers five omnibus tests for normality.
2. The dependence of the variance on the mean (instability or non-homogeneity of the variance) which may be shown graphically (Figure 2.7). In R, the bartlett.test( ) function provides parametric and the fligner.test( ) function non-parametric tests for homogeneity of variances. Box 2.3 shows these tests applied to the plant growth dataset supplied with R, the Bartlett test output p-value is 0.2371, indicating no significant difference in the variances.

Box 2.3 R code to test for homogeneity of variances.

```
# Tests of homogeneity of variances
# As an example, we use the plant growth dataset in R
# Comprises control and 2 treatments
PlantGrowth # Print data
bartlett.test(weight~group, PlantGrowth) #Barlett's test
fligner.test(weight~group, PlantGrowth) #Fligner-Killeen
    test
```

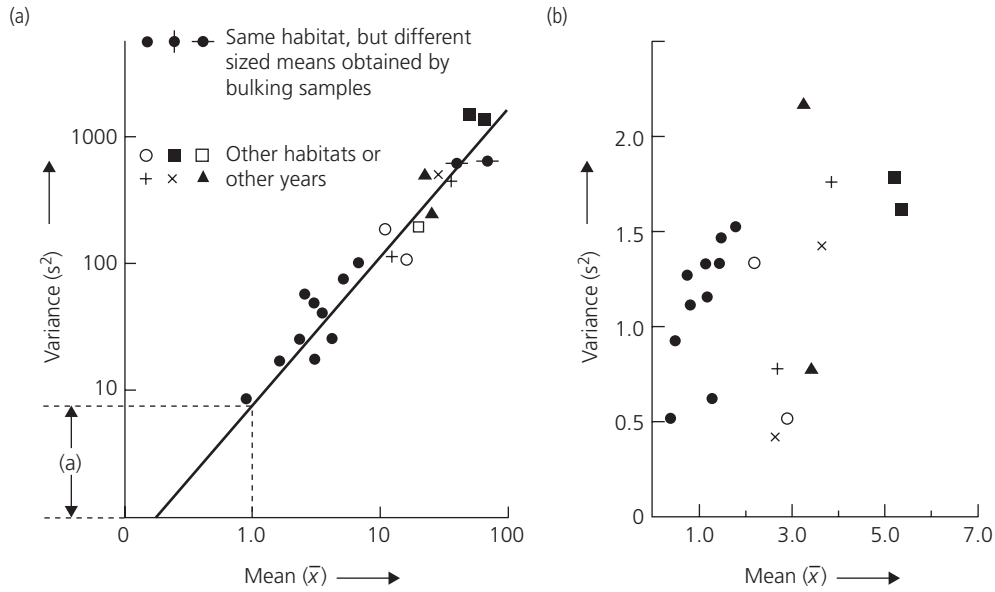

Figure 2.7 (a) The plot of variance against the mean on a log/log scale to obtain the constant a of Taylor's power law, data from samples of olive scales, Parlatoria oleae, per twig; (b) The same data transformed to $x^{0.4}$, showing the relative independence of the variance from the mean.

Generally, as Hayman and Lowe (1961) have pointed out, '…as non-normality must be extreme to invalidate the analysis of variance it is better to concentrate on stabilizing the variance of the samples.' A correct transformation for this property will also ensure a third property necessary for the analysis of variance, the additivity of the variance (Bliss and Owen, 1958). Indeed, all three properties are related, and for practical purposes the distinction between transformation for normality and that for stabilizing the variance need not be emphasized.

The adequacy of a transformation in stabilizing the variance may be tested for graphically (Figure 2.7) or by using the hovPlot() function in the HH package in R.

2.2 The sampling programme

2.2.1 The number of samples per habitat unit (e.g. plant, host, or puddle)

There are two aspects: first, whether different regions of the unit need to be sampled separately; and second, the number of samples within each unit or subunit (if these are necessary) that should be taken for maximum efficiency. Although the habitat unit could, for example, be the fleece of a sheep, a bag of grain, a rock in a stream, or an area of seabed, for convenience the word 'plant' will, in general, be used in its place in the discussion below.

2.2.1.1 Subdivision of the habitat

If the distribution of the population throughout the habitat is biased towards certain subdivisions, but the samples are taken randomly, then what LeRoux and Reimer (1959) aptly term *systematic errors* will arise. This can be overcome either by sampling so that the differential number of samples from each subdivision reproduces in the samples the gradient in the habitat, or by regarding each part separately and correcting at the end. The question of the estimation of the area or volume of the plant is discussed in Chapter 4.

2.2.1.2 Subdivision of plants

The amount of subdivision of the plant that various workers have found necessary varies greatly. Variability within a single tree is an important factor for herbivores (Suomela and Ayres, 1994). On apple, the eggs, larvae, and pupae of the tortricid moth, *Archips argyrospilus*, were found for most of

the year to be randomly distributed over the tree so that only one level (the lower for ease) needed to be sampled (Paradis and LeRoux, 1962). In contrast, on the same trees and in the same years, the immature stages of two other moths showed marked differences between levels at all seasons (LeRoux and Reimer, 1959). With the spruce budworm, *Choristoneura fumiferana*, Morris (1955) found that there were '…substantial and significant differences from one crown level to another', and that there was a tendency for eggs and larvae to be more abundant at the top levels, but there was no significant difference associated with different sides of the same tree. A similar variation with height was found with the eggs of the larch sawfly, *Pristiphora erichsonii*, although here it was concluded that in view of the cost and mechanical difficulties of stratified sampling at different heights a reasonable index of the population would be obtained by sampling the mid-crown only (Ives, 1955). When studying all of the organisms on aspen, *Populus tremuloides*, Henson (1954) found it is necessary to sample at three different levels of the crown. A strategy of simply counting the thrip, *Frankliniella schultzei*, on the apical leaf of each branch of water melon plants was used by Pereira et al. (2016). This methodology was considered representative of the total number of insects on the plant, gave a variance lower than 25% and was fast and cheap.

Roslin et al.(2006) report on the performance of the lepidopteron leaf miner, *Tischeria ekebladella*, on oak, *Quercus robur*. They sampled the moth larvae at five hierarchical scales: (1) across individual oak stands; (2) among trees within stands; (3) among branches within trees; (4) among shoots within branches; and (5) among leaves or samples of several leaves within branches. When modelling leaf miner count data, a Generalized Linear Mixed Model with a log link function and Poisson-distributed errors was used, and it was concluded that the greatest variation in leaf mine abundance was between shoots within branches. This was greater than the variation between branches, within a tree, or between trees within a single stand. Thus, it was concluded that a single oak is '…a mosaic of heterogeneous resources'.

Even with field crops, height often needs to be considered. Broadbent (1948) recommended that potato aphids be estimated by picking three leaves—lower, middle, and upper—from each plant, and when estimating the population of the European corn borer, *Ostrinia nubilalis*, Hudson and LeRoux (1961) showed that the lower and upper halves of the maize stem needed to be considered separately as the former contained the majority of the larvae. The age of the plant may also affect dispersion (Hirata, 1962; Peng and Brewer, 1994). In contrast, Teulon and Cameron (1995) found few significant differences in the distribution of thrips' eggs and adults at different heights in sugar maple trees.

2.2.1.3 Aspect and exposure

Aspect is sometimes important; for example, in Nova Scotia in the early part of the season the codling moth lays mostly on the south-east of the apple trees, but later this bias disappears (MacLellan, 1962). Aspect has also been found to influence the distribution on citrus of the long-tailed mealy bug, *Pseudococcus adonidum* (Browning, 1959), the eggs of the oak leafroller moth, *Archips* (Ellenberger and Cameron, 1977), and of three species of mite, each of which was most prevalent on a different side (Dean, 1959), but not that of the pine beetle, *Dendroctonus* (Dudley, 1971). Variations in the spatial distribution of similar species in the same habitat, which complicates a sampling programme designed to record both, has also been recorded for two potato aphids by Helson (1958). Some insects are distributed without bias on either side of the mid-vein of leaves, so that they may be conveniently subsampled. Similarly, Nelson et al.(1957) recorded that when estimating populations of sheep keds, *Melophagus ovinus*, the fleece of only one side need be sampled.

Occasionally, sampling may be restricted to one part of a plant upon which a large and constant proportion of the population of the animal lives. Wilson (1959) showed that in Minnesota, 84% of the eggs of the spruce budworm, *Choristoneura fumiferana*, are laid on the tips of the branches, and if sampling is confined to these, rather than entire branches, the sampling time may be reduced by up to 40%.

Exposure to water and flooding can also influence the distribution and abundance of terrestrial insects. For example, Julião et al.(2018) in a study of Amazonian flooded forest found the abundance of galling insects in trees was influenced by the water depth reached during the flood season.

Aspect can also be important in aquatic systems. For example, in the northern hemisphere north-facing

rocks in the intertidal zone offer the coolest and most shady habitat available, and thus tend to favour red algae and its associated fauna (Baker and Crothers, 1987). Conversely, in Caithness, at the northern limit of its range, the inter-tidal barnacle, *Chthamalus*, is more abundant on south-facing slopes. In aquatic habitats, orientation with respect to flow and the degree of exposure are important (Baker and Crothers, 1987). Intertidal communities were classified in terms of their exposure to wave action by Ballentine (1961).

2.2.1.4 Hierarchical design and nested analysis of variance

A hierarchical design is one in which, for example, a number of plants are sampled from each of a number of plots from within a number of fields. If a certain number of samples are collected randomly within each strata this is often termed *nested random sampling*, and is analyzed by a *nested analysis of variance*. There may be two or three hierarchical levels, rarely more. Examples of such sampling programs are Bancroft and Brindley (1958), Harcourt (1961), Buntin and Pedigo (1981) and Steffey et al. (1982). The R function *aov* can handle nested and hierarchical models.

2.2.1.5 The number of samples per subdivision

To determine the optimum number of samples per plant (or part of it) (n), the variance of within-plant samples (s_s^2) must be compared with the variance of the between-plant samples (s_p^2) and set against the cost of sampling within the same plant (c_s) or of moving to another plant and sampling within it (c_p):

$$n_s = \sqrt{\frac{s_s^2}{s_p^2} \cdot \frac{c_p}{c_s}} \qquad (2.4)$$

If the interplant variance, s_p^2, is the major source of variance, and unless the cost of moving from plant to plant is very high, n_s will be of the order of one or less (which means one in practice). Interplant variance has been found to be much greater than within-plant variance in many insect populations, such as the spruce sawfly, *Diprion hercyniae* (Prebble, 1943), the lodgepole needle miner, *Recurvaria starki* (Stark, 1952), the cabbage aphid, *Brevicoryne brassicae* (Church and Strickland, 1954), the spruce budworm, *Choristoneura fumiferana* (Morris, 1955), the diamondback moth, *Plutella maculipennis* (Harcourt, 1961a), the cabbage butterfly, *Pieris rapae* (Harcourt, 1962), the Western pine beetle, *Dendroctonus brevicomis* (Dudley, 1971), the pine chermid, *Pineus pinifoliae* (Ford and Dimond, 1973), and the spider mite, *Panonychus ulmi* (Herbert and Butler, 1973). In most of these examples the within-plant variance was small so that only one sample was taken per plant or per stratum of that plant, although of course when this is done the within- and between-tree variances cannot be separated. However, with *Eoreuma loftini* on sugar cane the within-plant variance, from stalk to stalk, was found to be high, as was field-to-field variance, the lowest value being within a field (Meagher et al., 1996). On apples the within-tree variance of insect numbers may also be high, particularly at certain seasons, when as many as seven samples may be taken from a single tree (LeRoux and Reimer, 1959; LeRoux, 1961; Paradis and LeRoux,1962).

Often, a considerable saving in cost without loss of accuracy in the estimation of the population, but with loss of information on the sampling error, may be obtained by taking at random a number of subsamples which are bulked before sorting and counting. This is especially true where the extraction process is complex, as with soil samples; Jepson and Southwood (1958) bulked four random, 76.25 mm (3 inch) row samples of young oat plants and soil to make a single 305 mm (1 ft) row sample that was then washed and the eggs of the frit fly, *Oscinella frit*, extracted. Such a process gave a mean as accurate as that obtained by washing all the 76.25 mm samples separately at greater cost. Paradis and LeRoux (1962) sampled the eggs of a tortricid moth, *Archips argyrospilus*, on apple by bulking twenty-five cluster samples.

2.2.2 The sampling unit: its selection, size, and shape

The criteria for the sample unit are broadly those of Morris (1955):

1. It must be such that all units of the universe have an equal chance of selection.
2. It must have stability (or if not, its changes should be easily and continuously measured—as with the number of shoots in a cereal crop).

3. The *proportion* of the population using the sample unit as a habitat must remain constant.
4. The sampling unit must lend itself to conversion to unit areas.
5. The sampling unit must be easily delineated in the field.
6. The sampling unit should be of such a size as to provide a reasonable balance between the variance and the cost.
7. The sampling unit must not be too small in relation to the animal's size as this will increase edge effect errors.
8. The sampling unit for mobile animals should approximate to the average ambit of an individual. This 'condition' is particularly significant in studies on dispersion involving contiguous sampling units, Lloyd (1967) has suggested that a test of the appropriate size would be provided by several series of counts of animals in contiguous quadrats conforming to a Poisson series.

A sampling unit defined in relation to the animal's ambit or territory (e.g. the gallery of a bark-beetle) will give different information from one defined in terms of the habitat (e.g. Cole, 1970). *This re-emphasizes the need to be completely clear as to objectives and hypothesis before commencing a sampling programme*.

To compare various sampling units in respect to variance and cost it is generally convenient to keep one or other constant. The same method of sampling must, of course, be used throughout. From preliminary sampling the variances of each of the different units (s_u^2) can be calculated; these should then be computed to a common basis, which is often conveniently the size of the smallest unit. For example, if the smallest unit is 1 m of row, then the variance of 2 m row unit will be divided by 2 and those of 4 m by 4. The costs will similarly be reduced to a common basis (C_u). The relative net cost for the same precision for each unit will then be proportional to:

$$C_u s_u^2 \qquad (2.5)$$

where C_u is the cost per unit on a common basis, and s_u^2 is the variance per unit on a common basis. Alternatively, the relative net precision of each will be proportional to the reciprocal of Equation (2.5). The higher this reciprocal, the greater the precision for the same cost.

A full treatment of the methods of selecting the optimum size sampling unit is given in Cochran (1977) and other textbooks, but as population density—and hence variance—is always fluctuating, too much stress should not be placed on a precise determination of optimum size of the sampling unit. An example of the determination of optimal sample size is Zehnder's (1990) study of larval and adult Colorado potato beetle *Leptinotarsa decemlineata*. Zehnder found that while a five-stem sampling unit was most efficient, a three-stem unit would reduce costs with only a small reduction in sampling precision. Pieters (1977) compared the estimates of mean density and precision of arthropod samples collected from cotton using a D-Vac® suction sampler, and found that mean estimates increased with reduced sample size while precision remained unchanged; this led to the conclusion that small sample sizes are better. Similarly, when sampling for benthic macrofauna no advantage has been generally shown for 0.2 m^2 over 0.1 m^2 grab samplers (McIntryre et al., 1984). With insects on plants, the nature of the plant usually restricts the possible sizes to, for example, half-leaf, single leaf, or shoot (see p. 120). Because the sample size range initially chosen for examination is often arbitrary, it may not include the optimal sample size.

The shape of the sampling unit when this is of the quadrat type, rather than a biological unit, is theoretically of importance because of the bias introduced by edge effects. These are minimal with circles, maximal with squares and rectangles, and intermediate with hexagons (Seber, 1982), because they are proportional to the ratio of sample unit boundary length to sampling unit area. If the total habitat is to be divided into numbered sampling units (for random number selection), then circular units are impractical because of the gaps, and it is doubtful if the reduction of error from the use of hexagons normally justifies the difficulties of lay-out. Clearly, the larger the sampling unit, proportionally smaller is the boundary edge effect. The size of the organism will also influence this effect: the larger it is in relation to the sample size, the greater the chance of an individual lying across a boundary. This problem has been investigated for subcortical insects where

the damage to the edge individuals by the punch, and the curved nature of the sampled substrate pose special problems (Safranyik and Graham, 1971). In general, edge effects can be minimized by a convention (e.g. of the animals crossing the boundaries only those on the top and left-hand boundaries are counted).

2.2.3 The number of samples

Precision (see p. 9) is measured by the scatter about the mean of the results obtained and is sometimes expressed as the coefficient of variation of the samples. This is different from the accuracy of a method, which measures how close the estimate is to the actual population. The total number of samples depends on the degree of precision required. This may be expressed either in terms of achieving a standard error of the mean within a predetermined magnitude, or as a probability that the estimated mean is within a selected value of the mean (Karandinos, 1976; Ruesink, 1980). For many purposes, a standard error of 5% of the mean is satisfactory. Within a homogeneous habitat the number of samples (n) required is given by:

$$n = \left(\frac{s}{E\bar{x}}\right)^2 \tag{2.6}$$

where s is the standard deviation, $\bar{x}$ is the mean, and E is the the predetermined standard error as a decimal of the mean (i.e. for a standard error of ±5%, then $E = 0.05$). This expression compares the standard deviation (s) of the observations with the standard error (E) acceptable for the contrasts we need to make; it will be noted from this equation that in any given situation the value of the standard error will change with the square root of the number of samples: thus, a large increase in n is necessary to bring about a small improvement in s.

Where sampling is necessary at two levels, for example a number of clusters per tree, the number of units (n) that need to be sampled at the higher level, such as trees (LeRoux and Reimer, 1959; Harcourt, 1961b), is given by:

$$n_t = \frac{\left(\frac{s_s^2}{n_s}\right) + S_p^2}{\left(\bar{x} + E\right)^2} \tag{2.7}$$

where n_t is the number of samples within the habitat unit (calculated as above), s_s^2 is the variance within the habitat unit, S_p^2 is the variance between the habitat unit (interplant variance), and $\bar{x}$ is the mean per sample (calculated from the transformed data and given in this form and E as above).

If the dispersion of the population is well described by either the Poisson or negative binomial distribution, the desired number of samples is given by:

$$n_t = \frac{\left(\frac{s_s^2}{n_s}\right) + S_p^2}{\left(\bar{x} + E\right)^2} \tag{2.8}$$

and

$$n = \frac{\frac{1}{\bar{x}} + \frac{1}{k}}{E^2} \tag{2.9}$$

where k is the dispersion parameter of the negative binomial (see p. 28).

The second approach uses confidence limits so that the estimate is within a selected distance from the mean with a given probability. The general formula then becomes:

$$n = \left(\frac{ts}{D\bar{x}}\right)^2 \tag{2.10}$$

where t is the 'Student's t' of standard statistical tables and depends on the number of samples and approximates to 2 for more than ten samples at the 5%, level, and D is the predetermined half-width of the confidence limits for the estimation of the mean expressed as a decimal (e.g. ± 10% = 0.1). It will be seen that normally this gives a similar estimate to Equation (2.6), provided the values of E and D are adjusted to accord with their meanings. The procedure is, perforce, somewhat approximate, depending on the preliminary estimates of the mean and standard deviation, and the inclusion of t does perhaps give it a slightly bogus air of precision! Additionally, there is normally a biological aspect to consider: as population characteristics change with time, so will the optimal number of samples (e.g. Bryant, 1976; Kapatos et al., 1977).

If the distribution of the animal can be described by a Poisson or negative binomial distribution, or if

Taylor's power law applies, then s^2 can be substituted by other expressions (Buntin, 1994). For example, the formula when Taylor's power law applies is:

$$n = t^2 a\bar{x}^{b-2} D^{-2} \qquad 2.11$$

where parameters a and b are those in Equation (2.24). Ward et al. (1986) in a study of the cereal aphid, *Sitobion avenae*, on winter wheat, demonstrated that the mean and variance conformed to Taylor's power law with the same equation throughout the season and were able to use this relationship to apply Equation (2.11) throughout the season.

Equation (2.11) should be used with caution. Because, as discussed in Section 2.4.2, Taylor's power law may give a poor description of the variance–mean relationship at low densities, Riddle (1989) showed Equation (2.11) to substantially underestimate the required sample size for low-density benthic animals. Shelton and Trumble (1991) concluded that Equation (2.11) should only be used for $b < 2.0$.

Another type of sampling programme concerns the measurement of the frequency of occurrence of a particular organism or event; examples are the frequency of occurrence of galls on a leaf or of a certain genotype in the population (Oakland, 1950; Cornfield, 1951; Henson, 1954; Cochran, 1977). Before an estimate can be made of the total number of samples required, an approximate value of the probability of occurrence must be obtained. For example, if it is found in a preliminary survey that 25% of the leaves of oak trees bear galls, the probability is 0.25. The number of samples (N) is given by:

$$N = \frac{t^2 \rho(1-\rho)}{D^2} \qquad (2.12)$$

where p is the probability of occurrence (i.e. 0.25 in the above example), and D is as defined for Equation (2.10).

If it is found that the leaves (or other units) are distributed differently in the different parts of the habitat, they should be sampled in proportion to the variances. For example, Henson (1954) found from an analysis of variance of the distribution of the leaf-bunches of aspen that the level of the crown from which the leaves had been drawn caused a significant variation, and when this variance was portioned into levels, the values were lower 112,993, middle 68,012, upper 39,436. Therefore, leaf-bunches were sampled in the ratio of 3:2:1 from these three levels of the crown.

Worked examples of the calculation of sample number for a variety of sampling programs are given by Greenwood (1996).

When choosing a sampling method it is important to choose a method with a low and stable level of bias, as this cannot be reduced by increasing sample number (Dahlsten et al., 1990).

2.2.4 The pattern of sampling

Again, it is important to consider the object of the programme carefully. If the aim is to obtain estimates of the mean density for use in, for example, life-tables, then it is desirable to minimize variance. But if the dispersion (distribution, pattern) of the animal is of prime interest, then there is no virtue in a small variance.

In order to obtain an unbiased estimate of the population, the sampling data should be collected at *random*—that is, so that every sampling unit in the universe has an equal chance of selection. In the simplest form—the *unrestricted random sample*—the samples are selected by the use of random numbers from the whole area (universe) being studied (random number tables are available in many statistical works, or may be generated using a computer or phone app). The position of the sample site is selected on the basis of two random numbers giving the distances along two coordinates; the point of intersection is taken as the centre or a specified corner of the sample. If the size of the sample is large compared with the total area, then the area should be divided in plots which will be numbered and selected using a single random number (e.g. Lloyd, 1967). Such a method eliminates any personal choice by the worker whose bias in selecting sampling sites may lead to large errors (Handford, 1956).

However, a random choice method is not an efficient way to minimize the variance, since the majority of the samples may turn out to come from one area of the field. The method of *stratified random sampling* is therefore to be preferred for most ecological work (Yates and Finney, 1942; Abrahamsen, 1969), where the area is divided into a number of

equal-sized subdivisions or strata and one sample is randomly selected from each strata. Alternatively, if the strata are unequal in size, the number of units taken in each part is proportional to the size of the part; this is referred to as self-weighting (Wadley, 1952). Such an approach maximizes the accuracy of the estimate of the population, but an exact estimate of sampling error can only be obtained if additional samples are taken from one (or two) strata (Yates and Finney, 1942). The taking of one sample randomly and the other a fixed distance from it has been recommended by Hughes (1962) as a method of mapping aggregations. The fixed distance must be less than the diameter of the aggregations that are assumed circular; the standard error cannot be calculated. However, the method has been found useful for soil and benthic faunas (Gardefors and Orrage, 1968).

When it is apparent that there are systematic variations in the density of the study animal across the study area, stratified sampling should be used. In general, the strata are chosen to minimize within-stratum variance. Individual strata need not form continuous patches within the study area. When the habitat is stratified, biological knowledge can often be used to eliminate strata in which few animals would be found. Such a restricted universe will give a greater level of precision for the calculation of a mean than an unrestricted and completely random sample with a wide variance. Prebble (1943), with a pine sawfly, found that satisfactory estimates of the pupae were only obtained if sampling was limited to the areas around the bases of the trees. The variance of completely random sampling throughout the whole forest was too great, as many areas were included that were unsuitable pupation sites (see also Stark and Dahlsten, 1961).

The other approach is the *systematic sample*, taken at a fixed interval in space (or time). In general, such spatial data cannot be analyzed statistically, but Milne (1959) has shown that if the *centric systematic area-sample* is analyzed as if it were a random sample, the resulting statistics are '…at least as good, if not rather better' than those obtained from random sampling. The centric systematic sample is the one drawn from the exact centre of each area or stratum, and its theoretical weakness is that it might coincide with some unsuspected systematic distribution pattern. As Milne points out, the biologist should, and probably would, always watch for any systematic pattern, either disclosing itself as the samples are recorded on the sampling plan or apparent from other knowledge. Such a sampling programme may be carried out more quickly than the random method, and so has a distinct advantage from the aspect of cost (see also p. 22). Systematic sampling is often used in marine studies where the primary aim is to map distribution with respect to environmental gradients or suspected sources of pollution. In the absence of environmental gradients, marine benthic or plankton sampling is often undertaken at regularly distributed stations (McIntryre et al., 1984; Omori and Ikeda, 1984). This allows easier contour mapping of animal density, particularly if computers are used.

An example of an unbiased systematic method is given by Anscombe (1948). All the units (e.g. leaves) are counted systematically (e.g. from top to bottom and each stem in turn). Subsequently, every time a certain number (say 50) is reached, that unit is sampled and the numbering is commenced again from 1; only one allocation of a random number is needed and that is the number (say somewhere between 1 and 20) allotted to the first unit.

Biologists often use methods for random sampling that are less precise than the use of random numbers, such as throwing a stick or quadrant or the haphazard selection of sites. Such methods are not strictly random; their most serious objection is that they allow the intrusion of a personal bias, quite frequently marginal areas tend to be undersampled (nobody wants their quadrat to disappear into a bed of nettles or a small pond!).

It may be worthwhile doing an extensive trial comparing a simple haphazard method with a fully randomized or systematic one, especially if the cost of the latter is high when compared with the former. Spiller (1952) found that scale insects on citrus leaves could be satisfactorily sampled by walking round the tree, clockwise and then anticlockwise, with the eyes shut and picking leaves haphazardly. For assessing the level of red bollworm eggs, *Diparopsis castanea*, to determine the application of control measures, Tunstall and Matthews (1961) recommended two diagonal traverses across the field, counting the eggs at regular intervals.

Bias may intrude due to causes other than personal selection by the worker. Grains of wheat that contain the older larvae or pupae of the grain weevil, *Sitophilus granarius*, are lighter than uninfested grains. Hence, the most widespread method of sampling is to spread the grains over the bottom of a glass dish and then scoop up samples of a certain volume; as the lighter infected grains tend to be at the top, this method can easily overestimate the population of these stages (Howe, 1963). In contrast, for the earlier larval instars, before they have appreciably altered the weight of the grain, such a simple method gives reliable results (Howe, 1963). It was undoubtedly this difference that led to Krause and Pedersen (1960) stressing the need for samples to contain a relatively high proportion of the same stage if good replication was to be obtained. The distribution of parasitized animals may be such that they are more vulnerable to capture. For example, roach infested with *Ligula intestinalis* swim close to the surface (Loot et al., 2002).

2.2.5 The timing of sampling

In the absence of sampling constraints, the seasonal timing of sampling will be determined by the life cycle (e.g. Morris, 1955). In extensive work when only a single stage is being sampled, it is obviously most important that this operation should coincide with peak numbers (e.g. Edwards, 1962). This can sometimes be determined by phenological considerations (Unterstenhofer, 1957), but the possibility of a control population in an outdoor cage (Harcourt, 1961a) to act as an indicator should be borne in mind. The faster the development rate, the more critical the timing. With intensive studies that are designed to provide a life-table, regular sampling will be needed throughout the season. It is not always realized that the time of day at which the samples are taken may also have a considerable effect. The diurnal rhythms of the insects may cause them to move from one part of the habitat to another, as Dempster (1957) found with the Moroccan locust, *Dociostaurus maroccanus* (Figure 2.8).

Many grassland insects move up and down the vegetation not only in response to weather changes but also at certain times of the day or night (p. 220); during the day a quite large proportion of active insects may be airborne (cf. the observations of Southwood et al.(1961) on the numbers of adults of the frit fly, *Oscinella frit*, on oats). There is a marked periodicity of host-seeking behaviour in many blood-sucking invertebrates (e.g. Camin et al., 1971; Corbet and Smith, 1974). Diurnal and tidal changes in activity and distribution are common in aquatic organisms. Plankton may become concentrated in

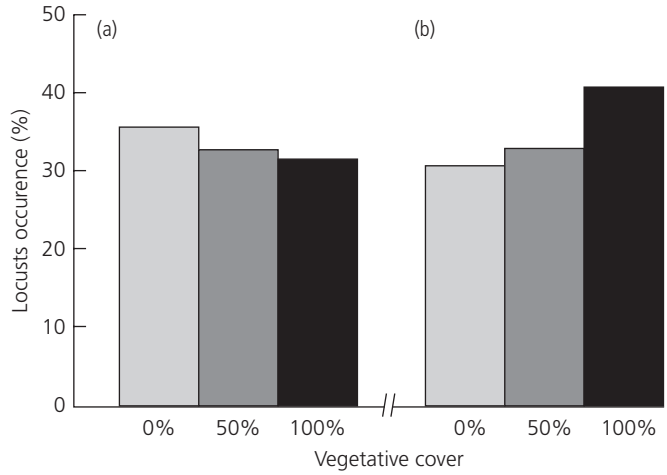

Figure 2.8 The variation in the distribution of adults of the Moroccan locust, *Dociostaurus maroccanus*, at different times of day. (a) Early morning before 09:30 h; (b) morning, after 09:00 h and before noon. The histograms show the relative numbers on bare ground and areas with moderate vegetation. (Data from Dempster, 1957.)

surface waters at night, and fish that remain hidden by day become active and vulnerable to trapping at night or dawn and dusk. Sampling methods that depend on the activity of the organism, such as gill nets for fish (p. 228) or pitfall traps for spiders, insects and small mammals (p. 228), will vary in efficiency as activity levels change. Aquatic insects may emerge at a particular time of day or phase of the lunar cycle (Corbet, 1964). The ecologist may find that some sampling problems can be overcome, or at least additional information gained, if they work at night, dusk or dawn, rather than during conventional working hours.

2.3 Dispersion

The dispersion of a population, the description of the pattern of the distribution or disposition of the animals in space, is of considerable ecological significance. Not only does it affect the sampling programme and the method of analysis of the data, but it may also be used to give a measure of population size (nearest-neighbour and related techniques) and, in its own right, is a description of the condition of the population. Changes in the dispersion pattern should be considered alongside changes in size when interpreting population dynamics. For example, if a mortality factor reduces the clumping of a sessile organism this is an indication that it acts most severely on the highest densities, or if the dispersion of a population becomes more regular then intensification of competition should be suspected (Iwao, 1970a). An understanding of dispersion is vital in the analysis of predator–prey and host–parasite relationships (Crofton, 1971; Murdie and Hassel, 1973; Hassel and May, 1974; Anderson, 1974). Hilker et al. (2009) discuss the Allee effect (population disadvantaged at low densities) in parasite dynamics, and Start and Gilbert (2018) give an example Allee effect in a gall-forming aphid, *Melaphis rhois*.

There is a vast literature on the spatial analysis of plants and animals; Perry et al.(2002) reviewed the statistical methods for quantifying spatial pattern, and Fortin and Dale (2005) provided a good ecological introduction to the huge field of spatial analysis. Two other important recent works on species distribution modelling are those of Franklin (2010) and Peterson et al.(2011). In R, species distribution modelling methods are available in the *dismo* and *raster* packages. Spatial Analysis by Distance Indices (SADIE) is available in the *epiphany* package (see p. 42).

2.3.1 Mathematical distributions that serve as models

It is necessary to outline some of the mathematical models that have been proposed to describe the distribution of organisms in space; for a fuller treatment, reference can be made to early works such as Anscombe (1950), Wadley (1950), Cassie (1962) and Katti (1966), and to textbooks; for example, Bliss and Calhoun (1954) and Patil and Joshi's (1968) dictionary of distributions. In R, the *fitdistr()* function in the MASS package and the *fitdistrplus* function in the fitdistrplus package (see p. 29 for an example application) undertake maximum likelihood fitting of the following univariate distributions; beta, Cauchy, chi-squared, exponential, *f*, gamma, geometric, log-normal, lognormal, logistic, negative binomial, normal, Poisson, *t*, and Weibull.

2.3.1.1 Binomial family

In the binomial distribution the variance is less than the mean, for the Poisson it is equal to the mean and for the negative binomial it is greater than the mean. A full discussion of variance and its calculation will be found in most statistical textbooks (e.g. Bailey, 1959; Zar, 1984), but the estimated variance (s^2) of a distribution may be calculated using:

$$s^2 = \frac{\sum x^2 - \left[\frac{(\sum x)^2}{N}\right]}{N-1} \qquad (2.13)$$

where $\sum$ is the sum of x = various values of the number of animal/sample, and N is the number of samples.

A random distribution: the Poisson series

The central place in this family is occupied by the *Poisson series* which describes a *random distribution* (see Figure 2.9). It is important to realize that this does not mean an even, regular or uniform distribution (Figure 2.9), but that there is an equal probability of

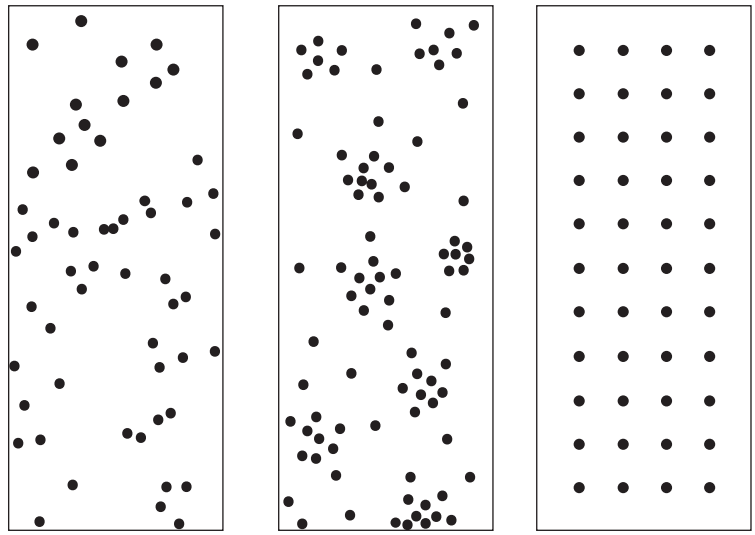

Figure 2.9 Three different types of spatial distribution. A Poisson distribution describes a random distribution and a negative binomial distribution describes a clumped pattern.

an organism occupying any point in space and that the presence of one individual does not influence the distribution of another. Because, for the Poisson distribution, the variance (S^2) is equal to the mean ($\bar{x}$) the probability of finding a certain number ($\bar{x}$) of animals is described by one parameter as follows:

$$p_x = e^{-\bar{x}} \frac{\bar{x}^x}{x!} \tag{2.14}$$

where e is the base of natural (Napierian) logarithms.

The goodness of fit of a set of data to the Poisson distribution may be tested using a χ^2 test on the observed and expected values.

A regular distribution

Occasionally, it may be found that the variance is less than the mean; this implies a more regular (or uniform or even) distribution than is described by a Poisson series (Figure 2.9).

A clumped or contagious distribution: the negative binomial

Most commonly in ecological studies the variance will be found to be larger than the mean, that is, the distribution is contagious[2] (Figure 2.9)—the population is clumped or aggregated. Many contagious populations that have been studied can adequately be expressed by the negative binomial distribution. Examples of its use in insect and marine benthic populations have been provided by Bliss and Owen (1958), Lyons (1964) and Harcourt (1965). This distribution is described by two parameters, the mean and the exponent k, which is a measure of the amount of clumping and is often referred to as the dispersion parameter. The mean and variance of the negative binomial are given in terms of c and k by:

$$\mu = \frac{k}{c} \tag{2.15}$$

[2] The term 'contagious' is a mathematical one coined in connection with work on epidemiology, and has certain implications that to some extent make its use in ecology inappropriate (Waters and Henson, 1959). An alternative is the term 'over-dispersion', first introduced into ecology by Romell (1930), with its opposite—for more uniform spacing—'under-dispersion'; unfortunately, however, the use of these terms has been reversed by some ecologists, therefore the terms used here are contagious and regular, which are also those commonly used in plant ecology (Greig-Smith, 1978).

and:

$$\sigma^2 = \frac{k}{c} + \frac{k}{c^2} \tag{2.16}$$

Defining for convenience $p = c/(c+1)$ and $q = 1 - p$, the probabilities of 0,1,2,3……events (animals per sample) is given by the terms of the expansion of $p^k (1-q)^{-k}$, for which the first few terms are:

$$p^k \left\{1,\ kp,\ \frac{k(k+1)}{2!}q^2,\ k(k+1)\frac{(k+2)}{3!}q^3,\ldots\ldots\ldots\ldots\right\} \tag{2.17}$$

Generally, for natural populations, values of k are in the region of 2; as $k \to \infty$ the distribution tends to the Poisson, whilst as $k \to 0$ the distribution tends to the logarithmic series. The value of k is not a constant for a population, but often increases with the mean (Anscombe, 1949; Bliss and Owen, 1958; Waters and Henson, 1959) (see p. 36). The truncated Poisson may be of value where the distribution is non-random, but the data are too limited to allow the fitting of the negative binomial (Finney and Varley, 1955).

Calculating k *of the negative binomial*

This is now easily accomplished using standard statistical software. In R, the *fitdistr()* function in the MASS package can be used. A package with enhanced features to handle both censored and non-censored data is *fitdistrplus* (Delignette-Muller et al., 2013). A simple example application using data on the annual captures of the conger eel, *Conger conger*, at Hinkley Point, UK, is given in Box 2.4. The parameter estimates are termed mu, which is the mean, *l* which is the dispersal parameter (*k*) of the negative binomial distribution respectively. The summary function gives the goodness-of-fit statistics Loglikelihood, AIC and BIC. The default plot of the observed and predicted distributions are shown in Figure 2.10.

Calculating a common k

Samples may be taken from various fields or other units and each will have a separate *k*. The comparison of these and the calculation of a common *k* (if there is one) will be of value in transforming the data for the analysis of variance and for sequential sampling (p. 46). The simplest method is the moment or regression method (Bliss and Owen, 1958; Bliss, 1958). Two statistics are calculated for each unit:

Box 2.4 Testing the fit of annual abundance data to a negative binomial distribution using the R package fitdistrplus.

```
library(fitdistrplus) #load package
# Input annual abundance of conger eel
Conger_number = c(11,13,4,12,8,6,5,5,8,7,7,9,8,6,13,18,
   13, 6,4,8,7,53,15,7,2,10,7,7,5,3,8,2,1,4,16,10,10,23)
#Fit of a negative binomial distribution to data
fitnb <- fitdist(Conger_number,"nbinom")
summary(fitnb) # Summary of result
#Plot the fit
plot(fitnb)
```

$$x^1 = \bar{x}^2 - \left(\frac{S^2}{N}\right) \tag{2.18}$$

$$y^1 = s^2 - \bar{x} \tag{2.19}$$

where $\bar{x}$ is the mean, S^2 is the variance, and N is the number of individual counts on which $\bar{x}$ is based. When y^1 is plotted against x^1 (Figure 2.11) (including occasional negative or zero values of y^1) the regression line of y^1 on x^1 passes through the origin and has the slope 1.

An approximate estimate of the common k (k_c) is given by:

$$\frac{1}{k_c} = \frac{\sum y^1}{\sum x^1} \tag{2.20}$$

It may be apparent from the plotting of y^1 on x^1 that a few points lie completely outside the main trend, and therefore although their exclusion will mean that the resultant k is not common to the whole series of samples, it is doubtful if the k derived by including them would really be meaningful.

A further graphical test of the homogeneity of the samples is obtained by plotting $(1/k) = (y^1/x^1)$ against the mean, $\bar{x}$, for each subarea or group of samples. If there is neither trend nor clustering (Figure 2.12), we may regard the fitting of a common k as justified.

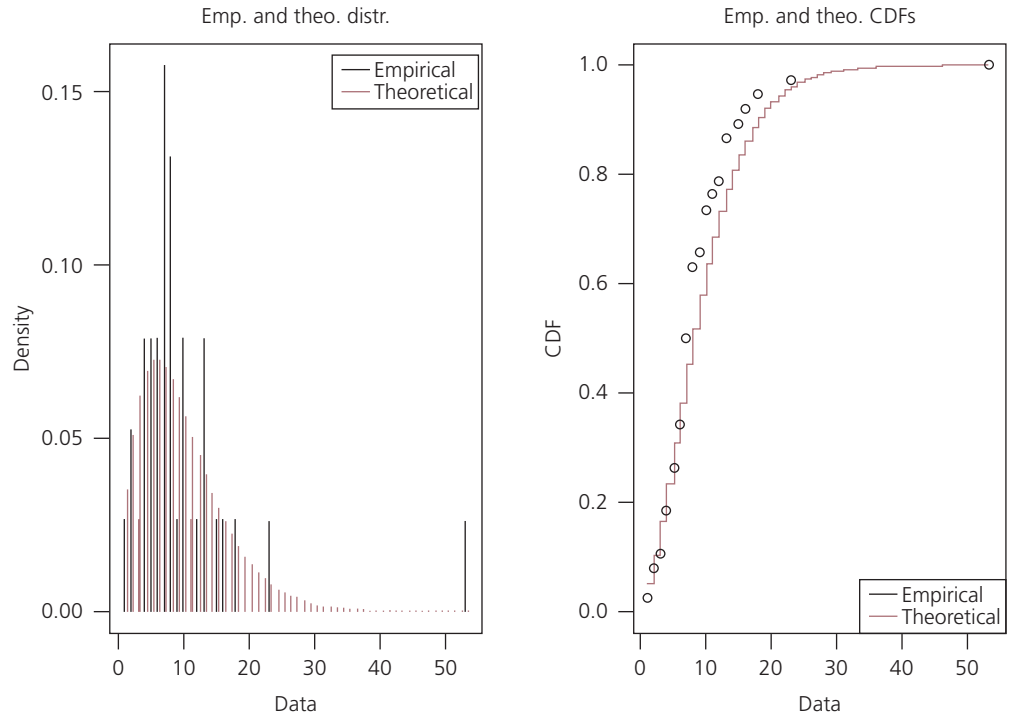

Figure 2.10 Plots of the observed and fitted negative binomial distribution generated by the fitdistrplus package in R. The data are the annual captures of conger eel given in Box 2.4.

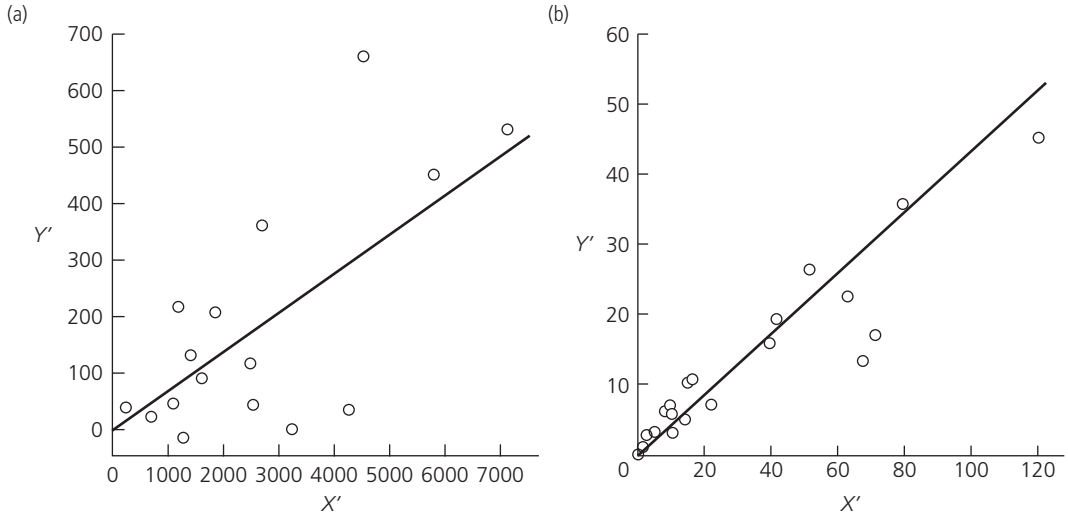

Figure 2.11 Regression estimate of a common *k* for: (a) Colorado beetle, *Leptinotarsa*, in eight plots within each of sixteen blocks; (b) wireworms (Coleoptera, Elateridae) in 175 sampling units in each of twenty-four irrigated fields. (Data from Bliss and Owen, 1958.)

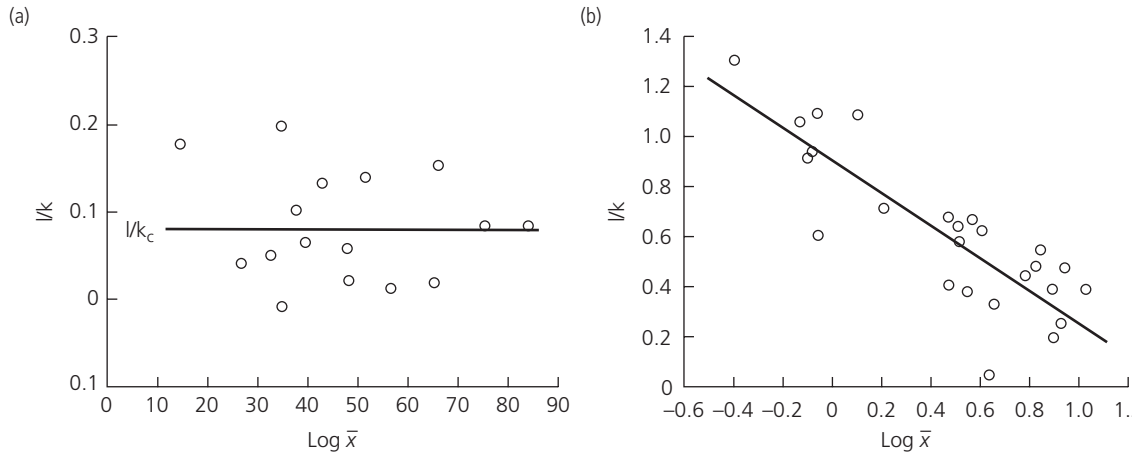

Figure 2.12 The relationship of 1/k to the mean for: (a) Colorado beetle; (b) wireworms, based on the same data as in Figure 2.8. (Data from Bliss and Owen, 1958.)

Fairly rough estimates of k_c are usually adequate, but in critical cases a weighted estimate of k_c should be obtained, and whether or not this lies within the sampling error can be tested by the computation of χ^2. The method is given by Bliss (1958) and Bliss and Owen (1958). The latter authors describe another method of calculating a common k that is especially suitable for field experiments arranged in restricted designs (e.g. randomized blocks).

2.3.1.2 Logarithmic and other contagious models

A number of other mathematical models have been developed to describe various non-random distributions, and several of these may have more than one mode. Anscombe (1950) and Evans (1953) reviewed these, and they include the Thomas, Neyman's types A, B, and C, and the Polya-Aeppli (also termed the geometric Poisson distribution). The Thomas (1949) type is based on the assumption of randomly distributed colonies whose individual populations are values plus 1, from a Poisson series. Neyman (1939) distributions are similar and are intended to describe conditions found soon after insect larvae hatched from egg batches; the modes are equally spaced. Skellam (1958) has shown that Neyman's type A is particularly applicable where the organisms occur in compact clusters, but that it can be used as an approximation in certain conditions when the clustering is less compact. The Polya–Aeppli distribution describes the situation when an initial wave of simultaneous invaders has settled and produced clusters of offspring, the number within each of which follows a geometric distribution; it may have one or two modes. Bimodal distributions are observed in marine benthic communities following settlement.

As mentioned above, the logarithmic model describes situations for which the negative binomial would give a very small value of k. The logarithmic series (Fisher et al., 1943), which is derived from the negative binomial with k tending to zero and with the zero readings neglected, and the *discrete and truncated* (and censored) *log normal* distributions (Preston, 1948; Grundy, 1952) have been found of most value in the description of the relationship between numbers of species and numbers of individuals and are discussed later (p. 396). Also, they have been found to give a reasonable description of the distribution of the individuals of some insects, including the citrus scale insect, *Aonidiella ornatum* (Spiller, 1952), and the eggs of the larch sawfly, *Pristiphora erichsonii* (Ives, 1955).

Working with plankton, with large mean values, Cassie (1962) suggested that the action of a series of environmental factors led to the population being distributed in a succession of Poisson series, the means of the series being themselves distributed

according to the log normal model. He called this the *Poisson log normal*, and it differs from the negative binomial mainly in the left-hand flank, as ordinarily plotted, where it allows for fewer zero values.

2.3.1.3 Biological implications of the distribution model

Neyman's distributions are based on precise models, and as Upholt and Craig (1940) found, if the biological assumptions underlying the distribution are not fulfilled it will not adequately describe the dispersion of the population. It is perhaps for this reason that Neyman's distributions have been used relatively little by ecologists, most preferring the other distributions that can be derived from a number of different hypotheses. Maldonado et al. (2015) in a study of the spatial distribution of the banana root borer, *Cosmopolites sordidus*, found a negative binomial gave the best fit in 93% of surveys, followed by the Neyman type A which gave a good fit 90% of the time. Frequently, it is found that the negative binomial offers an adequate description of a moderately clumped population.

The negative binomial can arise in at least five different ways (Anscombe, 1950; Waters and Henson, 1959):

1. **Inverse binomial sampling**. If a proportion of individuals in a population possess a certain character, the number of samples, in excess of k, that have to be taken to obtain k individuals with this character will have a negative binomial distribution with exponent k.
2. **Heterogeneous Poisson sampling**. If the mean of a Poisson distribution varies randomly between samples, under certain conditions, a negative binomial results (Pielou, 1969). A biological example of this is the observation of Ito et al.(1962) that a series of counts of a gall-wasp on chestnut trees were distributed as a Poisson for each single tree, but when the counts from all trees were combined they were described by a negative binomial. The distribution of oribatid mites in the soil (Berthet and Gerard, 1965) and of a tapeworm in fish (Anderson, 1974) also appear to arise according to this model.
3. **Compounding of Poisson and logarithmic distributions**. If a number of colonies are distributed as a Poisson but the number of individuals per colony follows a logarithmic distribution, the resulting distribution per unit area (i.e. independent of colonies) will be a negative binomial. Counts of bacteria (Quenouille, 1949) and the dispersion of eggs of the cabbage butterfly, *Pieris rapae* (Kobayashi, 1966) have been shown to satisfy this model.
4. **Constant birth–death–immigration rates**. The former two expressed per individual and the immigration rate per unit of time will lead to a population whose size will form a negative binomial series.
5. **True contagion**. Where the presence of one individual in a unit increases the chance that another will occur there also.

The logarithmic series (Fisher et al., 1943) can also be derived from several modes of population growth (Kendall, 1948; Shinozaki and Urata, 1953).

It is clear, therefore, that from mathematical considerations alone it is unsound to attempt to analyze the details of the biological processes involved in generating a distribution from the mathematical model it can be shown to fit or, more often, approximately fit (Waters and Henson, 1959). There is value in expressing the various possible mechanisms in biological terms: this has been done for parasite–host interactions by Crofton (1971) and Anderson (1974). These then provide alternative hypotheses that may be tested by other observations.

2.3.1.4 Changes in the degree of clumping

The extent of clumping and the changes in it provide important evidence about the population. The uses and interpretation of the parameters will be discussed later (see p. 34), but here changes in the actual type of distribution will be discussed. The main distinction lies between regular, random, and contagious distributions (see Figure 2.9), with the respective implications that the animals compete (or at least tend to keep apart), have no effect on each other, or are aggregated or clumped. One must be careful to define the spatial scale over which the dispersion is described. The behavioural significance of the spacing pattern within the colony area (see below) is different from that between colonies.