3 Plants

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Table 3.1. *Methods described in this chapter and their applicability to different types of plant*

* usually applicable, + often applicable, ? sometimes applicable, and no symbol indicates that the method is never applicable to that plant type. The page number for each method is given.

Method	Trees	Shrubs	Herbs and grasses	Bryophytes	Fungi and lichens	Algae	Seeds	Page no.
Total counts	+	+	?					113
Visual estimates	*	*	*	*	*	+		113
Frame quadrats	+	+	*	*	*	+		115
Transects	*	*	*	?	?			117
Point quadrats			*	*				119
Harvesting	?	?	*	+	?	+		121
Plotless sampling	*	+	?					123
Seed-bank soil cores							*	124
Seed traps							*	128
Marking and mapping	*	*	*	?	?	?		130
Vegetation mapping	*	*	*					132
Phytoplankton						*		135
Benthic algae						*		137

Introduction

Most plant communities consist of individual plants arranged on a surface (e.g. soil or rock). These plants are sessile; i.e. they 'sit still and wait to be counted', as J. L. Harper put it. This makes some surveying jobs simple. For instance, it is very easy to wander through the vegetation and make a species list. However, species and individuals within species often vary enormously in size (i.e. biomass, photosynthetic biomass, height, horizontal spread etc.) causing problems in selecting the best measure of species abundance. The standard measure of abundance of animals is density, a count of individuals in a unit area. This can be used for plants but it has two drawbacks. It may be hard to distinguish individuals of clonal plants where the genetic individual (genet) may consist of connected ramets (e.g. tillers or shoots),

especially if the connections are buried rhizomes. It is sensible and usual in this case to estimate the density of ramets rather than genets and this has the added benefit that ramets will show less size variation. Variety in the size of plants (sometimes due to clonal growth) will mean that density measures lose a large amount of information about the community under study. For instance, there may be equal numbers of individuals of two species in your study area but the species with a larger average size will have a greater importance for the ecological processes in the area. Imagine comparing a herb and a tree species purely in terms of density. For these reasons measures have been devised which take into account both the size and density of plants.

Cover is a measure of the area covered by the above-ground parts of plants of a species when viewed from directly above. According to Grieg-Smith's (1983) commonly used description it is 'the proportion of ground occupied by a perpendicular projection onto it of the aerial parts of individuals of the species'. Because the vegetation may be layered the cover of all species often sums to more than 100%. Cover is a popular measure but as with other size-based measures it uses a particular definition of size, that of 'perpendicular projection'. Therefore, it favours species with spreading growth forms or larger leaves, and species which hold their leaves horizontally will have a higher cover than species with acute or obtuse leaf angles.

Biomass (usually the above-ground weight of the plants of a species) uses a more usual definition of size but also is biased, this time towards species with a greater tissue density or unit weight, such as woody species. The last common measure is frequency: the number of samples (usually frame quadrats, see p. 115) in which a species is found. This is extremely popular but is also a rather odd measure, being dependent on the cover of a species but also on the spatial pattern of the plants and on the size of the quadrats (see Frame quadrats, p. 115). Whichever measure is used, these descriptions and caveats should be kept in mind when you interpret your data.

The sessile nature of plants causes a clear and slow-changing spatial pattern in the distribution of species. Often patchiness in environmental variables, restricted dispersal of propagules and clonal growth all bring about a patchy distribution ('clumped', 'contagious' or 'overdispersed') of plants of a species. Your sampling strategy (see Chapter 2) must be designed to compensate for this patchiness and to give an accurate representation of the abundances of the species in the whole study area. If you keep this in mind, you will rarely have problems, although some measures (e.g. frequency and density by plotless sampling) will always be influenced by the form of the spatial distribution of species.

Species also show changes in relative abundance throughout the year particularly in response to seasons; what might be called temporal patchiness. This will be caused by temporal patterns in germination rates and/or in the growth form of plants. The latter is seen most spectacularly in plants which die back to, for instance, a bulb for part of the year. Species differ in their temporal behaviour. This should be accounted for in your sampling design or in your interpretation of the data.

Although plants are usually categorised into species, in surveys you could use a coarser system if you require a different type of information. This can be based on higher taxonomic

groupings such as genera, families or even orders. Loose classifications based on systematics can be used: e.g. mosses, grasses, herbs, trees etc. Another classification method based on the growth forms of plants was developed by Raunkiaer (see pp. 1–4 of Kershaw & Looney 1983). Algae can be classified by their morphologies (e.g. single cell, colony or filament) and cell type rather than by individual species. For the methods described in this chapter these classification systems can be used instead of species.

These methods can also be used on some other organisms: fungi, lichens and sessile animals such as corals or encrusting bryozoans. They are possible in many benthic aquatic plant communities, although in deep water specialised equipment such SCUBA may be needed. Phytoplankton and the propagules of adult sessile plants do not sit still and wait to be counted and so different measures must be used for them.

Total counts

Assessing density of large or obvious plants that are at low density

Method

This technique is so simple it might be overlooked. Every individual of a species or a number of species in the study area is counted.

Advantages and disadvantages

Because the study area is usually several orders of magnitude larger than the plants, this technique is often much too time-consuming (imagine counting every plant in a 1-ha grassland). However, you should be able to use this technique if a species has a low enough density and is easily spotted (e.g. trees in a prairie) and the whole of the study area can be covered.

Biases

This measures the true density rather than sampling it and therefore has no biases.

Visual estimates of cover

Cover of species in any vegetation

Method

Visual estimates are made of the cover of the species either in the whole study area or in sample plots, such as frame quadrats (see p. 115). Different measures can be used. The

Table 3.2. *The Domin and Braun-Blanquet scales for visual estimates of cover*

Value	Braun-Blanquet	Domin
+	<1% cover	1 individual, with no measurable cover
1	1–5% cover	<4% cover with few individuals
2	6–25% cover	<4% cover with several individuals
3	26–50% cover	<4% cover with many individuals
4	51–75% cover	4–10% cover
5	76–100% cover	11–25% cover
6		26–33% cover
7		34–50% cover
8		51–75% cover
9		76–90% cover
10		91–100% cover

simplest is the classification: dominant, abundant, frequent, occasional or rare (DAFOR). These classes have no strict definition and you must decide on your own interpretation. Percentage cover can be estimated by eye either by creating your own percentage classes, e.g. in 10% or 25% steps, or by using those given in the Domin or Braun-Blanquet scales (Table 3.2). Remember that because vegetation is often layered, percentage cover values can sum to more than 100%. You may find it useful to divide the vegetation into layers, e.g. a bryophyte layer, a herb layer and a shrub layer, and make cover estimates separately for each layer.

Advantages and disadvantages

Visual estimates of cover are made more easily where you can look down on the vegetation. Cover may be hard to estimate with any degree of accuracy in tall vegetation such as scrub or forest, although it is possible if you can look up at the canopy and estimate the cover of individual trees.

The great advantage of this technique compared to more complicated surveying is speed. However, it can be inaccurate because of the subjectivity of the estimates. This means that different people or one person at different times may make different estimates. DAFOR is vague, and if several people are surveying then the meaning of the classes should be carefully agreed before you start. You should also be careful that your own interpretation of the classes does not change as the survey progresses. These precautions are also necessary if you use estimates of percentage cover. Often it is much easier and quicker to make visual estimates using frame quadrats (see p. 115) rather than estimating from the whole study area. Cover estimates made in the small area of a quadrat will also be less prone to error. The information obtained is limited but may be all that is needed for a straightforward site description or for a preliminary site survey. Percentage cover estimates give more infomation than DAFOR. If percentage cover is estimated, the use of very small classes of, say, 5% steps may give the

impression of increased information but you may be fooling yourself that you can distinguish between these classes. Because these estimates are all scores you can only use non-parametric statistics on your data.

Biases

It has been suggested that more conspicuous species, such as those in flower or those forming clumps of individuals, may be given undeserved high cover estimates. This should not be a problem if you are careful.

Frame quadrats

Cover, density, biomass or frequency of species in any vegetation

Method

Often these are simply called quadrats. They are used to define sample areas within the study area and are usually four strips of wood, metal or rigid plastic which are tied, glued, welded or bolted together to form a square. It can be helpful to use bolts so that the quadrat can be dismantled for storage or transport. For aquatic macrophytes a wood or plastic frame will float and can be used to sample floating or emergent vegetation on the water surface. For large quadrats, over 4 m^2, a frame will be unwieldy and as an alternative you could measure out the quadrat using tape measures, folding rulers or string. Corners are marked by posts, and it is important to keep a constant quadrat shape, for example by using a set-square to measure out right-angles. Although a square is often used, the quadrat shape is unimportant as long as you know its area. For certain purposes the quadrat can be divided into a grid of equal-sized squares using regularly spaced lengths of string or wire.

There is a technique to determine the appropriate size of quadrat to give a representative sample for a study area (see Greig-Smith 1983) but the theory behind it is confused and it has little practical use. Experience has shown that different vegetation types requires different quadrat sizes. Vegetation with smaller plants, greater plant density or greater species diversity should require smaller quadrats. The sizes most often used are: 0.01 – 0.25 m^2 in bryophyte, lichen and algal communities (for instance, on rocks or tree bark), 0.25 – 16 m^2 for grassland, tall herb, short shrub or aquatic macrophyte communities etc., 25 – 100 m^2 for tall shrub communities and 400 – 2500 m^2 for trees in woods and forests. Different quadrat sizes can be used to survey different vegetation types within a study area, such as the understorey layer and canopy layer in a forest.

Quadrats are placed in the study area according to your sampling design and different measures can be used to survey the vegetation.

1. Density is measured by counting the number of individuals of the study species within the quadrat. Many plants will lie on the edge of the quadrat, and for this and other measures you must decide which to classify as inside the quadrat. Often only the plants rooted within the quadrat are counted.
2. Visual estimates of cover can be made within each quadrat rather than for the whole study area.
3. Frequency is a simple measure calculated as the percentage of the quadrats you have placed in which the species was present. For example, if 3 quadrats contained the species and 20 were placed then the species had a frequency of 15%. The number of plants in the quadrats is ignored. The 'shoot frequency' is measured if you define as present plants with any part inside the quadrat. If only rooted plants are counted this gives the 'rooted frequency'. A different measure is 'local frequency', which can be derived if the quadrat is subdivided into a grid and the percentage of grid squares containing the species is calculated. Local frequency is often used at points on a transect or where only a few quadrats can be used.
4. Biomass of species can be measured by harvesting (see p. 121).

Advantages and disadvantages

Frame quadrats are very easy to use and can be used in a wide range of studies. It can be difficult and time-consuming to measure out very large quadrats.

The different measures have different advantages and disadvantages. Those associated with the theoretical bases of density, cover and biomass measures are given in the Introduction. Counting individuals for density can be very time-consuming and difficult, unless the plants have a low density or you use very small quadrats, and is usually only used in studies of single species. Cover measures suffer from the problems of visual estimates (see p. 113). If, as is common, species have a non-random distribution over the study area, then estimates of cover, density or biomass from a *single* quadrat will be changed by the size of the quadrat. This is because larger quadrats will even out the patchiness in the vegetation more than smaller ones. Single quadrats are never used, and carefully planned sampling (Chapter 2) will eliminate these size effects.

Frequency is a very quick and easy method to use but the estimate of frequency will always be influenced by quadrat size. This is because frequency is a qualitative measure (of presence or absence in a quadrat) which is used to calculate a quantitative percentage and the quadrat, rather than being used to select a sample area, is used as the dimensionless unit of measurement. Therefore, larger quadrats will usually be more likely to find the study species and will give higher frequency estimates than smaller quadrats (Figure 3.1). Patchiness in species distribution will reduce the likelihood of a randomly placed quadrat finding the species and will therefore also reduce the frequency estimate. For these reasons you should take great care when interpreting frequency measures, especially when comparing different study areas. Local frequency has the same problems.

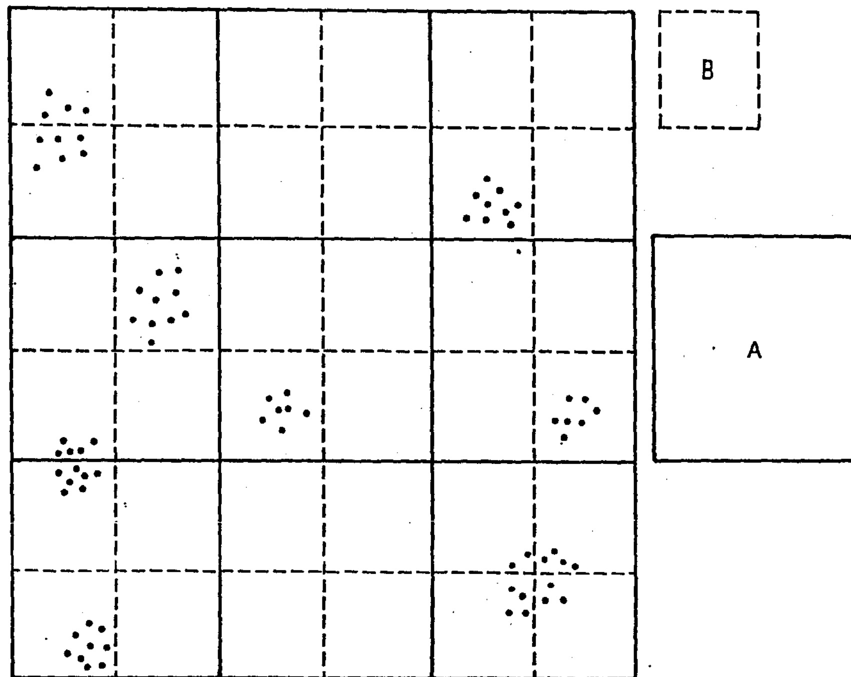

Figure 3.1 The effects of quadrat size on the measurements of biomass, cover, density and frequency. Quadrat A is four times the area of B. A single quadrat of size A will be more likely to hit an individual of a species (represented by a dot) than will a single B quadrat. If several quadrats are laid out (for simplicity the whole area is covered by quadrats in this example), the estimates of biomass, cover and density will be the same using A quadrats as if B quadrats are used. However, there will be more among-quadrat variation for the B quadrats. The different quadrat sizes give different estimates of frequency. This occurs for any distribution pattern of a species. In this example with a clumped distribution B quadrats give an estimate of $13/36 = 0.36$ and A quadrats give $7/9 = 0.78$.

Biases

Cover, density and biomass are discussed in the Introduction. Frequency can be biased against species with a more clumped distribution. Shoot frequency will be biased against smaller plants, but rooted frequency does not have this problem.

Transects

Variety of survey purposes in any vegetation

Method

Apart from the standard uses of transects (see Chapter 2) other transect-based methods can be used to survey vegetation. Transects are commonly used to survey changes in vegetation along an environmental gradient or through different habitats. This can be done using belt

transects or, for larger sample areas, gradsects. A second use is to estimate overall density or cover values of species in a single stand of vegetation by the line transect method. The length of the transect can be several centimetres or hundreds of kilometres, depending on the vegetation and the aim of the study.

1. The line transect or line intercept method involves using the actual transect line as a surveying implement. A simple measure is to count the number of plants of a species that touch the transect line to give a measure related to the density of plants. For longer transects you could count only touches at certain interval points along the transect, for instance at 10-mm, 10-cm, or 10-m intervals. Alternatively, percentage cover (see p. 112) can be estimated by measuring the length of transect line occupied by each species and using this to calculate the percentage of the length of the transect 'covered' by a species.
2. Belt transects consist of frame quadrats (see p. 115) of any size laid contiguously along the length of the transect. Cover or local frequency can be estimated for each quadrat, and the variation in the measure along the transect can be determined and correlated with the gradients in environmental factors.
3. Gradsects, or gradient-directed transects, are transects which are laid out to intentionally sample the full range of floristic variation over the study area. They are usually used to sample very large areas, sometimes being hundreds of kilometres long (e.g. Austin & Heyligers 1989). To accomplish this the transect is usually positioned to lie along a steep environmental gradient, for example due to altitude, land use or geology.

Advantages and disadvantages

In certain vegetation types it may be easier to use the line transect method than frame or point quadrats. It can allow more productive sampling in sparse vegetation and can be more practical in tall vegetation. In either case, sampling will be speeded up. If the vegetation is at all dense then counting touches will take a very long time. If plants are tussocky, form definite clumps or are large and distinct then the length of transect occupied by a species can be measured reliably and simply. Cover estimates will be very difficult in vegetation where plants are small and intermingled, and different methods should be used. Counting touches by individual plants is not only difficult in dense and intermingled vegetation where it is hard to distinguish individuals but also produces a measure with ambiguous meaning. It resembles a cover measure, being determined by the density and size of a species (bigger plants are more likely to touch the transect), but cannot be expressed as percentage cover. It is hard to see any use in this measure. Although they produce very detailed data, belt transects can be very time-consuming, and it is worthwhile to consider whether more sparsely spaced quadrats will fulfil your needs. Gradsects, if used to assess the range of vegetation types over a large area, will yield more information than randomly placed transects and are commonly used to survey little-studied areas. However, a knowledge of the environmental gradients is needed, and you run the risk of being guided by environmental factors which are relatively unimportant in structuring the vegetation.

Biases

The count of touches or estimate of cover will often depend on the height of the line transect in the vegetation, different species having a different vertical and horizontal structure. Gradsects for an estimation of the range of floristic variation will be biased by the particular environmental factor used to describe the gradient.

Point quadrats

Estimating cover of grasses, herbs, mosses, etc. in short vegetation

Method

A point quadrat is a thin rod with a sharpened tip and should usually be made of metal for rigidity and strength. Good materials are thick gauge wire, welding rod, knitting needles or even bicycle spokes. The point quadrat is lowered vertically through the vegetation and different recording methods can be used to get different types of data. There are a number of measures but many are confusing and difficult to interpret and I shall not discuss them. The most popular and acceptable measure is of the percentage cover (see p. 112) of each species in the vegetation. To sample this you should identify the species of each living plant part that the tip (and only the tip) of the point quadrat hits on the way down to the soil surface. The data recorded from that point quadrat are only the presence or absence of each species, i.e. whether or not the point quadrat hits a species; the number of hits on a species is unimportant. If all the hits on a species were counted this would give a measure of the 'total cover' of a species, a measure which reflects the size of plants of a species as well as their abundance in the vegetation.

The theory behind the use of point quadrats is fairly simple. Frame quadrats (see p. 115) could be used to estimate cover by this method, i.e. the percentage of quadrats covered by the species. In this case the quadrat can be wholly covered by a species, partially covered or not covered at all. If the frame is large then many quadrats will be only partially covered, introducing an ambiguity to the measure of cover. As the frame size is decreased and becomes smaller relative to the size of the plants, the frames become less likely to be only partially covered and the cover estimate becomes more accurate. Point quadrats are theoretically a frame quadrat of infinitesimally small area, i.e. a point. A plant can only be present or absent in an area of zero diameter and therefore there are no cases of partial cover. This gives a true value for cover. Of course, point quadrats do not give absolute points but are cylinders with measurable diameters and you are actually sampling a small circle: the diameter of the point quadrat. This fact has caused considerable excitement among some ecologists. Greig-Smith (1983) and Kershaw & Looney (1983) discuss the theory behind point quadrats extensively. The estimate of percentage cover for different species can vary a lot with the diameter of point quadrat used (Goodall 1952). You could get a zero diameter point by using optical cross

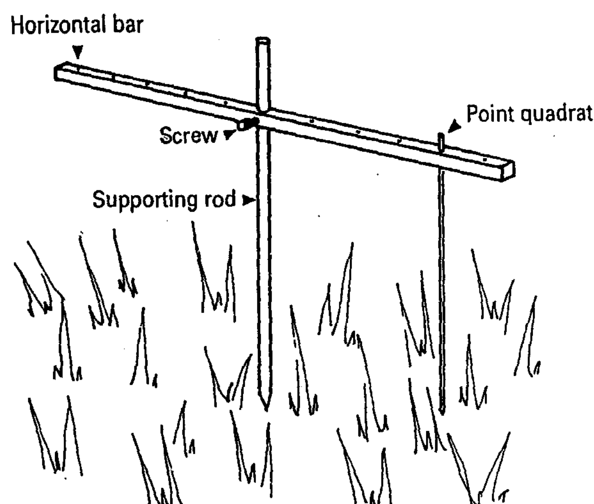

Figure 3.2 A point quadrat frame

wires, like those used in rifle sights, but these are well-nigh impossible to use in the field. The practical solution is to use point quadrats of as fine a diameter as possible. Steel wire of 1.5 – 2 mm diameter survives quite well in field use and a sharpened tip will narrow the point quadrat further. It is very important to use point quadrats of the same diameter in all your sampling, and if you want to compare your work with another study, find out what point quadrat diameter was used.

It is impossible to lower a point quadrat free-handed steadily and vertically through the vegetation while noting touches and identifying species. You should either stick the point quadrat into the soil and note touches along its length (it is important to have a very low diameter point quadrat for this) or use a 'point frame'. This consists of a sharpened metal supporting rod which supports a horizontal bar (Figure 3.2). The bar has holes along its length through which the point quadrat can be passed. The supporting rod is stuck vertically into the ground and readings are taken at the position of each hole in the bar. It is traditional, although not vital, for the frame to accommodate 10 point quadrats; the presence/absence readings from the 10 point quadrats are summed to give a score for the whole frame for each species (maximum = 10). You should never treat each of the 10 readings as independent measurements. Whatever method is used you should be careful not to disturb the vegetation when placing the point quadrat or taking readings; you could move plants onto or away from the point quadrat and cause errors in the sampling.

'Inclined point quadrats' are simply point quadrats which are lowered through the vegetation at an angle (usually 32.5°; see Warren-Wilson 1960) to the vertical. They are generally used in measurements of the canopy structure, which are particularly important in grazing studies. To measure canopy structure, not only are all the hits of the point quadrat noted, but also the height of each hit is recorded by marking a scale on the point quadrat.

Advantages and disadvantages

There is a sounder theoretical basis for using point quadrats to assess percentage cover than there is for visual estimates in frame quadrats, and canopy structure of short vegetation cannot be sampled any other way. Point quadrats are particularly useful in short vegetation, such as grasslands, and especially when it is difficult to distinguish individual plants. However, this technique can be very slow and fiddly especially in dense vegetation, and it involves crouching or lying on the ground for long periods. The vegetation should never overtop the point quadrat, and the point quadrats needed for some vegetation types such as hay meadows or tallgrass prairies will be too tall to be practical. Because a very small area is sampled very many samples may be needed to detect the rarest species.

Because leaves do not generally lie on the horizontal plane and inclined point quadrats enter the vegetation at an acute angle, this method will give more touches per point quadrat than will the vertical type. The inclined version will therefore give a more accurate sample of foliage area of each species, although this measure should not be confused with cover, which, being the proportion of ground covered by a species when viewed directly from above (see Introduction), can only be measured using vertical point quadrats. The measure of foliage area will also depend on the angle used. The practical problems of inclining the point quadrats at the same angle throughout the sampling programme and the ambiguity of the measure mean this approach is not usually worthwhile. Normal and inclined point quadrats can be used to measure the canopy structure, but because they give more touches, the latter seem to be preferred (e.g. Grant *et al.* 1985).

Biases

The biases involved in cover estimates are given in the Introduction.

Harvesting

Above-ground biomass of species in any vegetation

Method

The above-ground parts of the plants are cut at a certain height from the surface of the substratum, usually at or close to ground level. A knife, scissors, shears, saw or chainsaw may be used, depending on the vegetation type. Usually frame quadrats (see p. 115) should be used to define sample areas. The plant material should be taken to the laboratory, in bags or sacks when possible, and then sorted into species. Each species is either weighed as it is, giving a measure of 'fresh weight', or it is dried first, giving the 'dry weight'. The scales used will depend on the size of plants, but for any plants below the size of tall shrubs scales with an accuracy of at least 0.01 g should be used. Drying should be carried out in an oven at about 100°C for 1–2 days but if an oven is not available, natural drying for several days will work.

You must sort the species before drying or else the plants will be unidentifiable. It is important to remove or wash off any soil or detritus on the plant material before weighing.

Aquatic macrophytes may be harvested with a corer, which is effectively a quadrat frame that doubles as the harvesting tool. It is a sheet-metal cylinder with one rim sharpened and it is placed over the sample area and pushed into the substratum far enough to sever stems and roots. You could also use a grab to harvest an area of macrophytes.

Root harvesting (below-ground biomass) is too difficult and error-ridden to consider seriously.

Advantages and disadvantages

Harvesting has a large number of drawbacks and should be used only if you are certain that you need to measure the biomass of species. A particular instance of this is if vegetation is harvested to measure forage dry weight in grazing studies and you want to divide the biomass among the component species. This is known as 'destructive sampling', for obvious reasons, and should only be used in vegetation adapted to this sort of treatment (e.g. meadows), where the destruction is on such a small scale that it is unimportant, or where you and others do not mind the study area being destroyed. Large shrubs and trees are very difficult to harvest, transport and weigh. Very short vegetation, such as lawns, will be very difficult to harvest without massive error between samples caused by slight differences in cutting height. This method is really only appropriate for taller vegetation such as short shrub, aquatic macrophyte or meadow communities.

Cutting height is extremely important and you should usually cut at or close to ground level. However, the ground surface is rarely level and you may easily cut too high in some spots and too low (i.e. below the soil surface) in others, causing errors and contaminating the sample with soil. Corers for aquatic macrophytes cut below the substrate surface but there will still be variation in the depth of cutting. Grabs cut at very variable heights and also bring up a great deal of detritus and do not produce a reliable estimate of biomass at all.

Sorting of species can be very time-consuming and difficult, especially if the plants fall apart, leaving you with fragments to identify. Fresh weight is not a good measure. It varies with the moisture content of the plants and the moisture loss from cut plants means that fresh weight will be strongly affected by the time since harvest. Dry weight is a much better measure.

Biases

Low-lying species can easily be under-represented or even missed altogether if the cutting height is too high. If some species fall apart it is almost inevitable that you will lose and misidentify some plant parts. Fresh weight will bias the measure in favour of species with more effective moisture retention.

Plotless sampling

Estimating tree density in forests or woods

Method

A number of plotless sampling, or point-to-object, methods are possible. These are discussed more generally in Chapter 2, but I shall discuss only the two used commonly on trees. For either method a number of sample points are located at random in the study area. You can estimate the density either of all trees regardless of species or separately for each species. For the latter you can either lay out a different set of sample points for each species or use the same sample points and take separate distance measures for each species. The latter may be quicker. The minimum acceptable number of sample points will depend on the variation in the data, but as a rule of thumb you should use at least 50.

1. The nearest-individual method. The nearest tree to the sample point is located and the distance between it and the sample point is measured. The mean of the distances over all the samples is D_1 and the density of trees is worked out by the equation

   $$\text{density} = 1/(2D_1)^2$$

2. The point-centred quarter method. Two perpendicular straight lines which cross each other on the sample point are measured out. This creates four quadrants; in each quadrant measure the distance to the nearest tree, as for the nearest-individual method. The orientation of the lines should be fixed in advance. A useful method is to use compass points or, if a transect line is used to locate the sample points, this can be used as the first line. The distances of these four trees are averaged and the mean of these averages over all the samples is D_2.

   $$\text{density} = 1/(D_2)^2$$

Both methods involve the assumption that the distance is measured to the centre of the tree. You must estimate this distance. You must also decide whether or not to include saplings as trees in the survey. This is not usually done and you must decide at what size a sapling becomes a tree. This could be when it becomes part of the canopy layer.

Advantages and disadvantages

Neither of these methods gives a random sample of individual trees. Even with a random distribution of trees the method ensures that the more isolated trees are more likely to be sampled (see Chapter 2). If there is a patchy distribution the error is even greater. Some ecologists think that because of this non-randomness these plotless methods should not be used. The *T*-square sampling method can overcome this problem, and it is described in Chapter 2.

Plotless sampling is generally a much faster method for estimating plant density than

frame quadrats in woods and forests, where the quadrats must be large to give a reasonable sample of the vegetation. For this reason, the technique could also be used in other sparse vegetation where individuals are distinct such as semi-desert or maquis-type vegetation. Each sample takes a longer time for the point-centred quarter method than for the nearest-individual method, but the latter gives a more variable estimate and you must carry out more samples to overcome this. The choice of method will therefore depend on the particular circumstances, including your preference. If plants are at very low density it may take a long time to find which is the closest plant. This is a particular, and at times overwhelming, problem if you are surveying the densities of individual species separately, especially since the rarer species are, by definition, at low density. If you sample species separately you must decide before surveying which species you wish to study. For this reason, you might not survey all the species in the vegetation, especially not the rarest. The more species you survey, the more work you will have to do, with a new distance measurement carried out at each sample point of each species. Where the community is species-rich, having many species with medium or low density (such as many undisturbed tropical forests), this may be a great problem. The method is probably best for surveys of overall tree density, of certain species of interest, or in species-poor communities.

Biases

Because sampling is non-random and different species may have different spatial distributions, a biased measure of relative densities will be obtained.

Seed-bank soil cores

Estimating the density of seeds in the seed bank

Methods

Soil cores of known area and depth are taken at sample points throughout the study area. Seed banks show great variation in seed density and species composition over small areas owing to the patchiness of species distribution, which can be exacerbated by the fact that the seed bank may be the accumulation of several years of seeding. You must therefore take many samples to achieve an adequate estimate. There is often great variation in the seed bank over time, reflecting temporal changes in seed production and germination, for instance owing to the seasons. You should either take samples on at least two occasions to assesss this variation or choose the sampling time carefully, for instance after the late summer peak in seed production in cool temperate zones.

If large enough the core can be dug out, using a frame quadrat (see p. 115) to mark the area. Smaller cores can be taken using a sheet-metal cylinder which has one sharpened rim and is pushed into the soil to a certain depth (Figure 3.3). It should then hold the core when it is

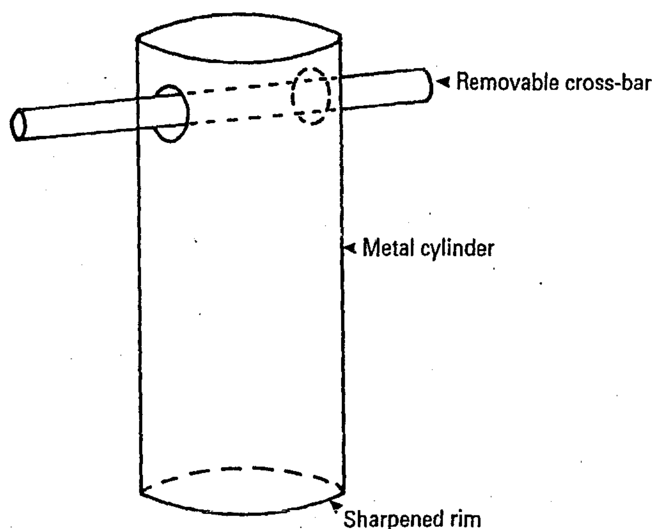

Figure 3.3 A soil corer

removed from the soil. A removable cross-bar is useful to aid the pushing of the cylinder into the soil and to push the core out of the corer.

There is no standard core diameter or depth but generally diameter is in the range 2–20 cm and depth between 5 and 20 cm. The diameter should reflect the density of the seed bank and the size of seeds. Studies of arable fields and cultivated grasslands have used core diameters of 2–5 cm. The smaller the cores you use the more samples you will have to take in order to sample a reasonable area. The core depth should be decided after considering the aim of the study. Viable seeds are strongly concentrated in the top 2–3 cm of the soil, and these are the seeds most likely to recruit naturally into the community. The deeper-buried seeds will give a more complete picture of the seed bank and will also show the seeds available to recruit following soil disturbance.

The soil cores are transported back to the laboratory in bags to avoid seed loss or contamination. Here the core can be separated into layers, if you wish, to find the vertical distribution of seeds in the soil. A common division is into layers 0–2 cm depth, 2–5 cm and > 5 cm. The top layer will contain litter and you could scrape this off and analyse the seeds in this separately. In some studies the seeds of the litter layer have been considered not to be part of the seedbank and have been discarded. There is no ecological reason for this idea.

An alternative to soil cores in aquatic communities, such as lake beds, is to use a dredge grab which is set to sample to a specific depth, again between 5 and 20 cm. You should be able to roughly estimate the area sampled by the grab.

The seed bank in the cores can be estimated by germination tests or by counting seeds. Methods involving flotation of seeds on a saline density gradient are inaccurate and difficult and I shall not discuss them.

1. Germination tests

The core or core section is air-dried to kill any vegetation and the soil is spread over a seed tray. The sample could be condensed by sieving (see below). Alternatively, you can mix in sterile soil (i.e. containing no viable seeds) as a spacer to decrease seed density. You could use either a seed compost or, to provide a more natural seed bed, soil removed from the study site. The seed trays are placed outside, in a glasshouse or in a growth room with simulated natural conditions (day length, diurnal temperature variation, humidity, etc.) and the soil is kept moist by watering or misting. If the trays are outside you should protect them from herbivores and contamination by airborne seeds. Germination tests for samples from submerged aquatic communities should usually be carried out in submersed seed trays, either in the original water body or in artificial conditions such as water tanks.

The trays should be monitored daily and any seedlings that emerge identified and removed. As the germination rate slows, monitoring can become more infrequent. The soil should be stirred occasionally to expose all the seeds. Seedling identification keys are available for some species, but in the absence of expert help it is best to repot unidentifiable seedlings and grow them on until the adult plants can be identified. It could be useful to maintain a reference collection of seedlings. Monitoring can stop when nothing has emerged for several weeks.

An extra procedure which may increase the germination rate of some plants of temperate regions is to chill the seeds. The soil samples are placed in a fridge at about 5 °C for 3–4 weeks and then germination is tested in seed trays as described above. The warming after chilling simulates the return of spring after the winter conditions of the fridge and this is often a trigger to break seed dormancy.

2. Counting seeds

The seeds are sorted from the soil core by wet sieving. The sample is passed through a graded series of sieves, starting with the largest mesh size. Seeds will be trapped in different sieves according to the seed size. The smallest mesh size should be fine enough to catch the smallest seeds but coarse enough to allow the soil particles to pass through. A typical size is 150–200 μm. It is usually quicker to do this under running water and to break up soil aggregates with your fingers. Sieving can be carried out with sophisticated hydropneumatic elutriation equipment (e.g. Gross 1990) or simply with a collection of sieves and a tap. This method serves to concentrate the seeds, and the material remaining in each sieve should be sorted to find these seeds. You should therefore use a range of sieve sizes to speed up this sorting, so that the majority of seeds are not mixed in with too many stones and large particles.

The seeds are sorted from the remaining detritus, maybe using a binocular microscope for the smaller seeds, and are identified. You may be able to use identification keys or find expert help, or you may have to germinate the seeds and create a reference collection, as described above. It is usual to count how many of the seeds are still viable since the seed bank is defined only by the *live* seeds. To save time this can be done on a random subsample of the seeds to establish the proportion of the seeds of each species that are viable. Viability can be tested by

germination tests in sterile soil or on dampened filter paper in a Petri dish or by tetrazolium and indigocarmine staining. Tetrazolium (triphenyltetrazolium chloride) stains living tissue red and remains colourless if the seed is dead. Indigocarmine remains blue if the seed is dead but goes colourless in live tissue. These tests are not always successful and so it is best to divide up the seed tissue and to carry out both tests using separate tissue samples. One positive result from the two tests is enough to indicate that the seed is viable. These stains are mildly poisonous so use them with care.

Tetrazolium

Fresh 1% 2,3,5-triphenyltetrazolium chloride is prepared in a phosphate buffer at pH 6–8. The seed is cut in half to expose its tissue and one half is incubated in the tetrazolium for 2 hours in complete darkness. Excess stain is washed away with distilled water. Living seed tissue will be stained a red colour.

Indigocarmine

0.05% indigocarmine is prepared in hot distilled water and the solution is filtered and allowed to cool. The seed tissue is submerged in the stain for 2 hours in the dark and then the stain is washed off with distilled water. If the tissue is colourless it is alive but a blue colour indicates dead tissue.

Advantages and disadvantages

Seed-bank sampling involves a lot of work no matter which method you use. Many samples and sampling on more than one occasion add to the effort. For aquatic samples, grabs are much quicker and easier than cores but there is much less accuracy in defining the area and depth of the sample.

Both methods for assessing seed numbers in the soil samples have drawbacks. It is virtually certain that not all the viable seeds will germinate in germination tests. While some species germinate readily others show seed dormancy which can only be broken by specific environmental factors. Chilling may break dormancy but other possibilities include after-ripening, scarification of the seed coat, high light intensity, widely fluctuating temperatures or even the severe heating of a fire. It is impossible to cover all the possibilities and most studies use just the standard conditions in the glasshouse or growth chamber. You must therefore remember that this method thus measures only the 'ready germinable fraction' of the seed bank. For these reasons it is also important to carry out germination tests in the same conditions for all samples.

By sieving the sample you will find most seeds, although the smallest seeds may be lost in the sieving and sorting processes. All stages in this technique are extremely labour intensive, and for this reason it is rarely used. The germination test for viability has the drawbacks described above. The chemical tests avoid these problems and provide the best method for fully sampling the seed bank, although they take a long time even if you use only a subsample.

Biases

The depth of soil core will probably affect the proportion of different species in your sample. Seeds are generally older further down the soil horizon and thus they represent both the longer-surviving species and the past composition of the plant community (which may be different to that at the present). The distribution of species in the soil can, however, be determined by separating the core into layers. The sample will also be biased towards those species that have produced seeds most recently. Sampling on different occasions will allow quantification of this bias.

The environmental conditions of the germination tests can strongly affect which species germinate. The sieving method may underestimate the very small-seeded species, such as the dust seeds of many of the Orchidaceae, since they may not be caught by the smallest mesh and may be too small to be seen in the sorting and sieving process.

Seed traps

Seed rain in terrestrial and non-submerged aquatic communities

Method

Seed traps are placed on the soil surface to estimate the density per unit time of seed arriving on that surface (the traps are sometimes placed vertically or at a slope but there is no benefit in doing this and it does not give a correct estimate of seed rain per unit area of the soil surface). A variety of trap types have been developed but sticky traps are by far the most popular. These prevent the loss of seeds from the traps. The basic premise of these is to fix a sticky surface to the ground which traps all seeds that come into contact with it. The sticky substance should be non-drying and not toxic to the seeds if they are to be germinated or tested for viability. There are a number of permanently sticky petroleum-based substances which can be used, for instance Tanglefoot, a bird repellant available in many countries and which can be smeared or aerosol-sprayed onto the trap surface. The sticky cards developed to sample flying insect pests can also be used. The surface used can be anything waterproof which can hold the sticky substance, for instance a pane of glass or polythene wrapped around wood or plastic. The insect cards could be used without modification. To be manageable the trap should have a diameter in the range 10–30 cm. Unless the trap is heavy you should fix it in place, for instance with long pins.

A popular alternative is to place a circle of paper (e.g. filter paper) smeared with the sticky substance in a Petri dish and use this as the trap. The dish can be either pinned to the soil surface or nailed onto a length of dowelling which you push into the soil to fix the trap in place. If you do the latter, the trap could be used to sample the seed rain onto the water surface in aquatic communities by fixing the dish above the water surface. You should make small holes in the dish to allow rain water to escape.

individuals then if you want to locate new recruits (e.g. seedlings or young plants), you must resample at each census.

Advantages and disadvantages

These methods will allow you to follow the fate or measure the performance (e.g. leaf, flower or seed production, change in basal area, clonal growth etc.) of individuals in a population; a technique that is central to much of population ecology, population genetics and community ecology. Marking, locating and identifying individuals can be very time-consuming and detailed work in a dense population, especially where the plants are small. Despite this you must always take great care to disturb the plants as little as possible because you may alter their survival and growth by the process of measuring them.

Permanent quadrats or their markers will move over time owing to soil movement (e.g. frost heave) and intentional or accidental interference from animals. Therefore, as time passes the position of a plant relative to the quadrat will change and mapped individuals may be lost or misidentified. This is only a real problem for dense populations. Certain types of markers may be lost through vandalism or interference from other animals. For instance, wire rings are lost very easily from grazed grass tillers. You should consider this problem and make the markers as permanent as possible.

If an individual is only mapped or if the marker is not fixed to the plant then if the plant dies and a new plant grows up in the same place between censuses, you might mistake this new individual for the old one. New individuals may grow through wire rings to create the same problem. You must decide on the likelihood of this happening in relation to the vegetation type (low in forests, high in fertile grasslands) and the frequency of censuses.

Biases

The loss of markers or poor mapping may underestimate the survival of individuals. Misidentification of new individuals for old, dead ones may lead to overestimation of survival.

Vegetation mapping

Estimating cover of vegetation types over a large area

Method

'Vegetation type' can have a variety of definitions for the purposes of vegetation mapping and provides a way of categorising areas of similar species groupings and/or plant growth form. Starting out with only a vague notion of what classification you will use will result in a waste of time and poor quality information. You can devise your own categories, for instance based on the dominant species or species combinations (e.g. *Calluna vulgaris*/*Erica cinerea*, *C. vulgaris*/*Ulex minor*, *Agrostis curtisii*/*E. cinerea* etc.) or coarser criteria (e.g. high

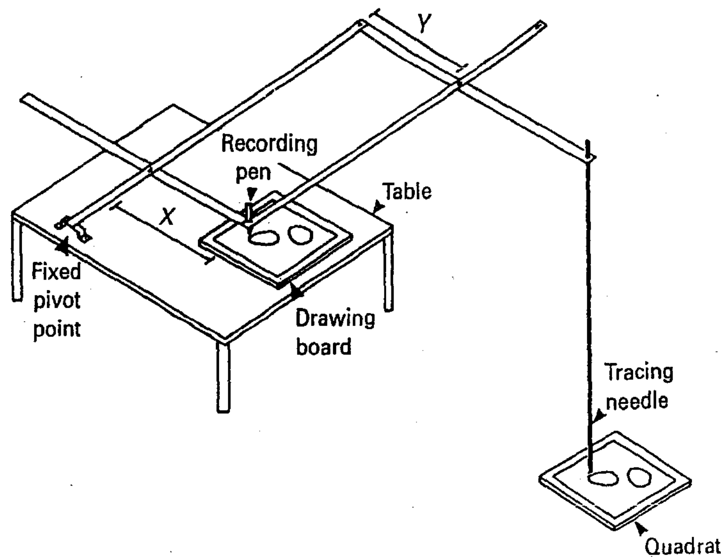

Figure 3.4 A pantograph. This can be either bought or made of wood, metal or plastic, although tubular aluminium with a plastic or plywood table are the best materials. You guide the tracing needle around the outlines of plants or point out the position of each plant. The recording pen inscribes these positions on a chart pinned out on the recording table. The resulting map can be scaled relative to the quadrat size, either larger or smaller depending on your needs. The scale depends on the ratio of the lengths X and Y. If $X > Y$ then the map is smaller than the quadrat.

number the plants but can distinguish the groups by the colours of the markers. The posts and tags described above can be coloured and you can also apply different coloured paints directly onto the plants. The paint should be non-toxic, fast-drying and hard-wearing, for instance artist's acrylic poster paint. With care this can be used even on very small plants such as grass tillers. Only small amounts of paint should be applied and only onto surfaces not important for light capture or gas exchange; the plant base is a good spot. The paint may need retouching at subsequent censuses.

On successive surveys each surviving individual is found again and you can make measures of performance on every plant. If individuals are numbered then you can simply note the performance measures against each number on your datasheets. If you have mapped plants without marking them with individual numbers, you must either write the measures on your map, or identify the individuals by their coordinates or by their position on the map (determined by overlaying consecutive maps) while you are in the field. Therefore, unless you are looking simply at survival between censuses, which can be determined by comparing consecutive maps in the laboratory, you should mark the individuals in the field. Mapping the outlines of individuals will allow you to calculate their basal areas. If you are censusing and marking or mapping all individuals in the study or sample area, you will locate new individuals at each census which you can then mark or map. If you are using a sample of

mangrove, mixed mangrove, salt marsh, savanna, pasture etc.). Usually this will supply sufficient information. Great detail can be obtained by classifying types by statistical ordination of species survey data (see pp. 276–319 in Kershaw & Looney 1983). Many standardised classification systems are available, the most famous being that of the Zurich–Montpellier school (see pp. 245–75 in Kent & Coker 1992 and pp. 156–64 in Kershaw & Looney 1983 for a full discussion of vegetation classification). The detail you can obtain will be limited by the mapping technique you use.

A number of techniques are available if you want to map and measure the cover (see p. 112) of different vegetation types over a large area, i.e. from a few to thousands of km^2. The vegetation types used (see Introduction) should depend on your needs and the scale of the survey.

1. Ground mapping

You can use a scaled-up version of visually estimating cover in quadrats with the quadrats being a convenient way of dividing up the survey area into manageable chunks. The size of these chunks should be determined by the total area to be surveyed and the detail you require. They can be measured out with reference to landmarks or as geographical entities (e.g. a hillside or the area between two streams) or you can use squares based on the grid of latitude and longitude. These can be located using a map and landmarks or by using a global positioning system (GPS) receiver, a hand-held device which uses satellite output to locate you precisely.

Either the cover of each vegetation type is estimated or their boundaries are sketched onto a map. The boundaries can also be positioned precisely using landmarks or a GPS. The areas on the map can be measured by hand or by using computer digitizers or image analysis systems.

2. Remote sensing by aerial photography or satellite images

This is highly technical (except for the most basic aerial photographs) and a full description is beyond the scope of this chapter. What is more, the technology is developing extremely rapidly, especially for satellite imaging. Curran (1985) is a good introductory text but you should consult experts. I shall merely give a background to allow you to decide whether you may find these techniques useful.

(a) You could simply take a normal photograph from a plane or helicopter directly over the vegetation. There are other more technical and more accurate procedures requiring specialised equipment. One popular alternative is to take aerial photographs as overlapping pairs, i.e. two parallel runs of photographs, one inclined from the left and the other inclined from the right. When these are viewed through a stereoscope a very clear three-dimensional image is seen. Aerial photographs at a range of scales are commercially available in many countries (see Curran 1985). The photographs may be infrared, multispectral (i.e. colour) or monochrome and are divided into patches of different tone, texture and pattern which correspond in some way to the vegetation. These cover types

are related to vegetation types by field surveys of representative areas. The boundaries of each cover type can then be transferred to maps and their areas measured using tracing paper, by projecting the image onto a map, using computer digitizers (for instance, those associated with geographical information systems), using image analysis systems or using specially designed transfer instruments such as Sketchmasters.

(b) Satellite imaging involves computer-driven interpretation of satellite images such as those from the Landsat Thematic Mapper. The resolution (i.e. the spatial detail) of these images is determined by the pixel size used. The pixel is the area of land from which a single spectral image is taken and can now be less than 100 m^2. The spectral image measures the intensity of light at a range of different wavelengths. Pixels or groups of pixels are classified into types based on these readings and these types are interpreted using field surveys. The vegetation types may be very coarse, e.g. forest, grassland etc.; but much greater detail may be achieved, e.g. differentiating conifer, deciduous and mixed woodland or wet heath, dry heath and bogs. These satellite maps can be transferred onto computer systems, including geographical information systems, and the areas measured. Useful references that give the background to satellite imaging techniques are Turner & Gardner (1990) and Haines-Young *et al.* (1993).

Advantages and disadvantages

To contradict my statement about the complexity of the remote-sensing techniques a simple method is to take a normal photograph from the air, identify the cover types by ground surveys, sketch or trace the boundaries onto a map and measure the areas by hand. The ground-mapping methods and the more complex remote-sensing techniques give more accurate measurements and you will probably have errors of perspective on the photograph owing to camera tilt or changes in ground relief, which may lead to miscalculation of areas. However, this is a quick, cheap and crude method for large-scale mapping of vegetation. At greater expense you could buy accurate aerial photographs and analyse them by the crude methods given above.

Ground mapping is cheap in terms of equipment, requires no technical expertise and can produce very accurate maps. However, it is very time-consuming compared to the other techniques and for this reason cannot be carried out over very large areas. You can cover large areas by remote sensing (satellites can cover thousands of kilometres) and you can survey areas which are impenetrable on land. Once a system is developed, analysis of remotely sensed images can be very rapid. Neither of the remote-sensing techniques has the resolution of ground mapping, and the cover types will not be as precise as can be achieved by a surveyor on the ground. These cover types may also be misidentified. These techniques can be expensive and time-consuming.

Biases

Different vegetation types may not be distinguished by remote sensing owing to similarities in appearance or in spectral image.

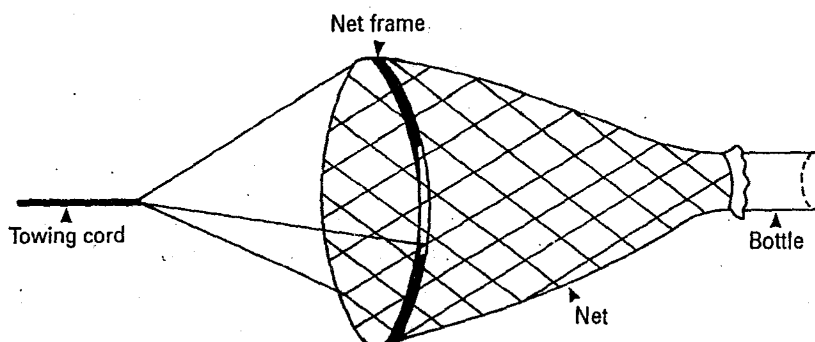

Figure 3.5 A plankton net. The towing cord can be replaced with a pole.

Phytoplankton

Density or volume of phytoplankton

Method

There are many methods for taking and analysing samples of freshwater and marine phytoplankton some of which involve specialised electronic equipment for *in situ* estimates. Analysis of samples can be by cell counts or indirect estimates of total cell biomass or volume (e.g. by chlorophyll *a* concentration). I shall describe only the simplest methods which use very little specialised equipment. I strongly recommend that you refer to the technical literature and consider other procedures (e.g. Bailey-Watts & Kirika 1981; HMSO 1983, 1984).

The water body can be sampled using a jar or container, although a plankton net can be used to cover a larger area and to concentrate the plankton sample. A plankton net (Figure 3.5) consists of a fine mesh net (e.g. 80 μm) ending with a bottle to hold the plankton. The net frame is usually square or circular, meaning that the sample area of the frame is easily calculated. The net should be small enough to be used easily and so the bottom of the water body is not disturbed (a problem especially in shallow streams). The net can be either fixed to a pole or to towing lines. For the latter the net can be towed either by you or by a boat. You can either hold the net against the current or sweep it through the water. The volume of water filtered depends on the net frame area, the speed of flow of the water, the speed you tow at and the time in the water. You should attempt to standardise this volume across all samples. Because of the fine mesh the net should be moved slowly through the water (> 1 knot). The phytoplankton will be stratified (i.e. species show a non-random vertical distribution in the water body) so the net should either be held at the same selected depth in all samples or be moved evenly through all depths.

The algal cells will decay rapidly and should be preserved immediately after collection if you want to count them. Various fixatives are used but the commonest is Lugol's iodine solution. A solution of 20 g potassium iodide in 200 ml distilled water is made and then saturated by adding 20 ml iodine. This is then acidified with 20 ml glacial acetic acid. The

solution should be kept in a dark bottle. 1–2 ml Lugol's iodine should be added to every 100 ml of the sample (remember to allow for this extra volume when calculating cell densities). Do not add fixative if you wish to test for chlorophyll *a*.

Densities of species or cell types (a less precise classification) can be determined by counting through a microscope. Cells can be counted but it may be better to count colonies or filaments of some species. Several pieces of apparatus are available (see HMSO 1990; Paert 1978) but the most commonly available is the haemacytometer, which is more usually used to count red blood cells. This holds a precise volume of liquid in which you can count the numbers of each species. Several counts should be made for each sample.

The concentration of chlorophyll *a* provides a relative measure of the volume of all phytoplankton in the sample. It does not distinguish species but can be useful for comparison of water bodies, for instance in studies of water quality. A number of methods are possible (see HMSO 1983; Riemann & Ernst 1982) but the simplest uses spectrophotometry. A defined volume of the sample is filtered (e.g. through a Whatman GF/C glass-fibre filter). You will need to carry out trial runs to determine the optimum volume to filter. The filter is then placed in 8–10 ml of 90% acetone and this is placed in a dark fridge for 20–24 hours to extract the chrorophyll *a*. The extract should be shaken two or three times during the incubation. Then the extract is removed from the fridge and allowed to warm up to room temperature in the dark. The extinction of the extract against that of 90% acetone should be measured in a spectrophotometer for the wavelengths 664 nm, 647 nm and 630 nm. Colloidal material in the extract will cause some turbidity and this can be corrected for by subtracting the extinction reading at 750 nm from these three readings. The relative concentration of chlorophyll *a* is calculated using the equation:

$$C_a = 11.85E_{664} - 1.54E_{647} - 0.08E_{630}$$

C_a is the concentration of chlorophyll *a* and E_i is the corrected extinction at wavelength *i*.

Advantages and disadvantages

These are the simplest and cheapest methods available. Netting phytoplankton will lose the smallest algae (nanoplankton) and other methods can be used to avoid this. Because algal cells vary widely in size (<1 to >200 μm), counting cells does not give a complete picture of the community. You can also estimate cell sizes (see Bailey-Watts & Kirika 1981). Chlorophyll *a* degrades during extraction and some of the products are not detected by this technique. Some algae are very difficult to extract (e.g. blue-green) and may extract incompletely. These will cause an inaccurate estimate of chlorophyll *a*.

Biases

Poor control of the sampling depth of the sampling effort at each depth will bias the counts of different species. The smallest species will not be sampled by netting.