4 Collecting population data

> "*Crudely put, observers go afield to seek wildlife and return to tell the statistician how many they have found. It is then the statistician's task to determine how many animals they did NOT find.*"
>
> (Ram sey et al. 1988)

Definition of the population (the individuals of a species present in a defined area at a certain time) is central to most disease investigations and is also one of the most difficult aspects of any study of wild animals. Information about the abundance of animals is needed to assess the significance of disease, to decide on the need for management, and in most cases for assessing the effectiveness of management. There are a great variety of methods for describing a population but these usually involve elaboration of a few basic questions: (i) who is present? (ii) who is at risk? (iii) who is affected? and (iv) what effect is the disease having on the population? Answering these questions involves both a qualitative evaluation, e.g., which species are present, as well as determining the number of individuals in each group or class. This chapter will not provide a list of specific techniques for estimating populations of different species, as many references are available for that purpose. Lancia et al. (2005) provide a review and a conceptual framework for considering different methods. Emphasis here will be on the types of information that may be collected and on general principles related to data collection. It is necessary to state, at the outset, that there is no single perfect technique; all existing methods for assessing populations suffer from problems and have deficiencies, but different techniques are more useful in certain situations.

4.1 Basic features

The difference between a count and an estimate has been discussed earlier; a number of other terms used in describing population data require definition. An **absolute count** or census includes all the individuals present within an area or class and, as noted earlier, absolute counts of free-ranging wildlife are very seldom possible. **Relative counts** or estimates are used to detect changes in relation to some baseline and changes usually are reported in terms of a

proportion above or below the baseline. For example, if 400 deer were counted during a survey of an area prior to a disease outbreak, and 220 deer were seen during an identical survey after the outbreak, the relative change is a 45% decrease from the pre-disease baseline. In this example, neither the absolute number of deer on the area nor the number that disappeared is known, only a relative change has been observed.

Accuracy (or validity) is a measure of how closely the observed value corresponds to the actual state of affairs. If 200 ducks were released on a pond and 194 were counted during a survey done immediately after release, and before any population change had occurred, the survey method is correct to an accuracy of 6, or 3%. Because of the difficulty in determining absolute population numbers, the accuracy of most methods used in wildlife work is unknown. **Precision** (or reliability) is a measure of how closely a series of repeated measurements of the same thing match each other. For instance, if the same group of 200 ducks was surveyed by two different techniques, each of which was repeated five times, one might obtain the following counts:

	1	2	3	4	5	Average
Method A	180	156	227	197	210	194
Method B	196	192	189	199	194	194

The average estimate of the population size obtained with the two methods is the same, so the average accuracy of the two methods is equivalent but the precision is markedly different. The precision of an estimate is usually indicated by a measure of dispersion, such as the standard error of the mean, or one might calculate the 95% confidence limits (= ±1.96 X standard error if the estimator is distributed normally). Another way of comparing the amount of variation in the two samples is to calculate the coefficient of variation, which is the standard deviation expressed as a percentage of the mean (standard deviation X 100/mean). The 95% confidence interval for method A would include values from approximately 170 to 218, whereas that with method B would include values from approximately 191 to 197. Thus, the chances are that 19 of 20 estimates of this population made with method A will fall between 170 and 218 when the actual population is 200. The coefficient of variation of the two methods is 14.1 and 2.2%, respectively.

Estimates of population size should always contain an indication of the precision of the technique. In some circumstances, estimates may be highly precise but still be inaccurate, e.g., when a technique underestimates or overestimates the actual population by a fixed proportion. Such data, although inaccurate, still may be useful for detecting relative differences between areas or changes over time so long as the method is applied consistently. It is important to evaluate old data that may be available in planning new monitoring programs. Regular monitoring data can be combined with older sporadic data to estimate mortality rates and population growth, if the two types

of data are compatible. This was done to estimate the impact of morbillivirus outbreaks on harbour seals in England (Thompson et al. 2005).

4.2 Choosing a method

Techniques for collecting population information are chosen on the basis of the type of information required, how much information is needed, how much can be afforded, and if the results need to be comparable to those obtained by others or only to one's own data. The first choice that one must make is to decide if information is needed that describes the population as it exists at the instant (size, density, composition) or if information is required to understand changes in the population over time. If the latter is the case, it will be necessary to collect information on the four fundamental variables that result in changes in population size: natality, mortality, emigration, and immigration. Information of the first type may be sufficient during investigation of a short-term outbreak, while a more detailed study of the epizootiology of a disease will require the collection of both types of information. In many instances, it is more important to measure the **density** and the distribution of the population than to determine the total number of animals. Density is usually expressed as animals/unit of area but, in some circumstances, it may be more meaningful to express density in terms of some ecologic unit or resource, particularly if the unit is a limiting factor for the population. For instance, the number of deer using each waterhole in a xeric area may be more important for understanding disease transmission than is the number of deer/100 km^2. Measures of density are often used as indicators of population size in disease studies, e.g., Wandeler et al. (1974) used the number of foxes killed by hunting, accidents and disease/km^2 as an index of fox population size during studies of rabies. Measures of distribution and density will be considered later in this chapter.

Population estimates are usually used for comparison with other estimates. The ability to distinguish among groups and to recognize change is directly related to the precision of the method used. Precise methods are required to recognize small changes or differences. To illustrate this point, we can return to the example used earlier. Two weeks after placing 200 ducks on the pond, the number of birds present was estimated again using both methods A and B. The methods yielded identical estimates of 174 birds. With method A, this estimate is still comfortably within the 95% confidence interval (170–218) established when the actual population was 200. However, with the more precise method, B, the current estimate is well outside the confidence limits (191–197) and one should suspect that the population size had declined. The important point is that the more precise method allowed us to detect a probable population change, while any change that may have occurred was masked by the lack of precision in method A. By repeating the survey several times and calculating a mean and standard error, the estimates could be compared statistically

with the initial estimate. Unfortunately, estimates with high precision often are expensive to obtain because of the extra time and effort required to collect a large number of observations. Consequently, many of the techniques currently in use in wildlife studies have such low precision that only major changes in population can be detected.

4.3 Basic methods for determining animal numbers

Most techniques for estimating animal numbers consist of two steps: (i) **data collection**, which involves detection of the animals or some index to their abundance and (ii) **calculation** of population size. Lancia et al. (2005) identified two basic problems in any attempt to estimate animal abundance. The first relates to the probability of detecting animals that are present on the area. Most methods available do not detect all of the animals that are actually present, i.e., the probability of detection is <1. Calculation of population size usually involves some form of mathematical manipulation to account for the fact that only a proportion of animals in the population were detected and a major effort in developing population estimation methods has been in estimating the probability of detection under different circumstances. If all the individuals in a population can be counted directly, e.g., 27 cormorants on an island, the second step is then unnecessary. However, one should be aware of the problems inherent in making absolute counts, even of large birds on small islands (e.g., Haila and Kuusela 1982). The second basic problem relates to sampling. Because resources are usually limited, it often is impossible to survey the entire area occupied by a population and only a sample of the area can be examined. The dilemma lies in selecting samples that are representative and permit inference to the entire area. The choice of the appropriate method for data collection depends upon knowledge of the biology of the species and the particular situation, and many methods are available for data collection. In contrast, relatively few methods are available for calculation using the data. A critical point is that no statistical procedure or calculation will make poorly collected data into good data, nor will it allow data collected under differing conditions and circumstances to be comparable. The latter point is particularly important if data are to be compared with information collected by other investigators.

The value of replication in studies of population size can not be overemphasized. "*Unreplicated studies can lead to generalizations and unrestrained speculations; even one replication of a sample in a comparable habitat type should put some limitations on how the results are interpreted*" (Call 1986).

Methods for determining population size are based on two general assumptions: (i) that the population is stable during the period of data collection, i.e., that changes due to births, immigration, emigration and deaths are negligible, and (ii) that all members of the population have an equal probability of being

Table 4.1 Examples of the use of indices of animal abundance in studies related to disease

Species	Disease	Index
Brushtail possum	Bovine tuberculosis	Trap-catch index, fecal pellet counts[1]
House finch	*Mycoplasma* infection	Birds observed/hour[2]
Bank voles	Hantavirus infection	No. captured/100 trap nights[3]
European hare	Multiple factors	Annual hunter kill[4]
White-tailed deer	Tick infestation	Fecal pellet counts[5]
Harbour seal	Morbillivirus infection	Animals seen on haul out sites[6]

[1] Caley et al. (1999), Anonymous (2004)
[2] Based on North American Christmas Bird Count, Hochachka and Dhondt (2000)
[3] Olsson et al. (2003)
[4] Fickel et al. (2005)
[5] Rand et al. (2003)
[6] Thompson et al. (2005)

counted. Neither of these assumptions is likely to be completely valid during most studies. Problems related to the first can be minimized by keeping the data collection period as short as possible, and correction factors can be developed to correct for differences in countability among members or groups within the population.

Lancia et al. (2005) divided all techniques available into indices and population estimation methods. Indices do not actually estimate animal abundance, instead they measure some feature believed to be correlated with abundance. Examples of indices that have been used in the study of disease are shown in Table 4.1. An underlying assumption is that the relationship between the index and abundance remains constant under varying conditions, but this usually is untested. Lancia et al. (2005) caution against the use of indices, unless this assumption can be verified.

The second group of techniques is those designed to actually measure the abundance of animals. I have chosen to intermix indices and methods for estimating abundance in the following discussion.

The basic techniques for determining either an index to abundance or to measure population size consist of:

I. Counts:
  – of animals
    (a) total count
    (b) count of a sample
  – of some index of animal abundance
    (a) total count
    (b) count of a sample
II. Estimates based on removal or capture
III. Estimates based on mark and recapture

4.3.1 Population estimates based on counts

The simplest way to measure a population is to count the animals or to count some index to their abundance directly. For example, during a study of avian cholera among lesser snow geese on a lake, the entire lake could be photographed from the air and then the number of live and dead geese could be counted on the resulting photograph. Alternatively, some index such as tracks or feces, which is more easily counted than the animals, might also be used. The assumption with indices is that the abundance of the index object is directly proportional to that of the animal. Assume that we are interested in determining the population of muskrats in a marsh. It is difficult to count the animals directly due to their secretive habits; however, the number of muskrat houses might be counted from the air. It would then be necessary to determine the relationship between the number of houses and the number of muskrats. We might live-trap muskrats from a sample of houses and establish that, on average, muskrat houses in this marsh contain 2.6 muskrats with a standard error = 0.3. The estimated number of muskrats in the marsh could be calculated to be 2.6 times the number of houses, and the 95% confidence limits of the estimate would be that number $\pm 1.96 \times 0.3$. The relationship between abundance of the index and abundance of the animal must be determined for the specific area and circumstance under investigation. Extrapolation from other situations is very risky. For example, in a nearby marsh, the average muskrat house might contain 4.1 muskrats and the number of muskrats per house is likely to vary from season to season and year to year in a single marsh.

As noted earlier, a major problem with direct counts is that the proportion of animals or index objects present but not counted often is unknown. For instance, some of the snow geese in the population mentioned earlier may have been away from the lake, feeding in fields at the time of the photograph. Similarly, some muskrat houses may have been obscured by vegetation and missed during the aerial count. This type of problem can be reduced in some situations through replicate counts, e.g., by taking photographs of the geese at several times during the day and calculating the average population; or by having more than one observer count the animals on an aerial survey so that the probability of detection can be estimated (e.g., Potvin and Breton 2005). Correction factors can be developed to reduce this source of error. One could do an intensive ground search of a portion of the marsh and then compare the number of muskrat houses known to be present on the basis of the ground search to that observed from the air. The process of using ground searches to validate aerial observations is called 'ground-truthing'. The importance of ground-truthing is evident in a controversy about the use of aerial surveys for defining areas used by prairie dogs (Miller et al. 2005; White et al. 2005). Lancia et al. (2005) provide many references to methods to deal with detection probability.

If comparisons are to be made between areas, or between different time periods, the method of measurement must be consistent. Thus, a count of

snow geese made in late afternoon on a lake would not be comparable to a count made in early morning on the same or another lake, nor would counts of muskrat houses made from aircraft flying at different altitudes be comparable without some form of correction. As an example, Short and Hone (1988) found that approximately twice as many kangaroos were seen in an area during aerial surveys done at sunrise as were seen during surveys of the same area done 3 h later in the morning.

There has been very little effort directed toward assessing the efficacy and accuracy of methods used for collecting population information in relation to disease in wild animals. Even in outbreak situations, the only information that usually is available is an estimate of the number of animals found dead. Many such estimates are, in reality, only guesses. Direct body counts often have been used to calculate total mortality during the investigation of epizootics. However, the number of animals found dead, or of carcasses picked up during an outbreak, provides, at best, a *minimum* estimate of the actual number that died. The proportion of dead animals that were not found is usually unknown but may be very large. For instance, 'beach surveys' have resulted in the recovery of from 10 to 59% of marked dead seabirds of various species placed in the ocean to simulate losses during an oil spill (Beer 1968; Coulson et al. 1968; Hope Jones et al. 1970; Bibby and Lloyd 1977). Swenson (1979) estimated that a maximum of 27% of dead mule deer were found during a survey following an epizootic. Only 6% of duck carcasses placed 30 min earlier were found during a search for dead birds in a Texas marsh (Stutzenbaker et al. 1986) and we found that a line of searchers spaced 4 m apart detected only 62% of sparrow-sized models of dead birds in ungrazed pasture (Philibert et al. 1993). Fredrick et al. (1993) found that only 33–50% of dead heron chicks were detected during transects of a colony. The density of human observers in the area can have a marked effect on the probability of detection of sick or dead animals, e.g., Ward et al. (2006) placed marked crow decoys in different locations to simulate birds that might have died of West Nile virus infection. About twice as many of the birds were detected in an urban area compared to a rural area. Mark-recapture methods, described later in this chapter, are useful for estimating the proportion of dead animals that are found during surveys. Using a mark-recapture technique, we found that only about one-third of the duck carcasses present in a marsh during a botulism outbreak were collected during clean-up operations (Cliplef and Wobeser 1993). Madrigal et al. (1996) proposed a method for estimating bird mortality from pesticides. They used success in finding marked carcasses intentionally placed on the area to calculate a correction factor for birds not detected during searches.

A single count of sick or dead animals during an outbreak can only be used to calculate an estimate of the prevalence of the disease, i.e., the number affected at the time of the search. It cannot be used to estimate the total mortality. Table 4.2 shows the relationship between carcass disappearance and the number of dead animals that might be detected in searches on various

Table 4.2 Relationship between the number of carcasses detected during searches done on various days and the cumulative mortality during a disease outbreak. One hundred animals died on each of days 1 through 8, 50% of carcass disappeared /day, and 30% of the carcasses present were detected

		No. of carcasses present on each day									
Day	No. that died on day	1	2	3	4	5	6	7	8	9	10
1	100	100	50	25	13	6	3	1			
2	100		100	50	25	13	6	3	1		
3	100			100	50	25	13	6	3	1	
4	100				100	50	25	13	6	3	1
5	100					100	50	25	13	6	3
6	100						100	50	25	13	6
7	100							100	50	25	13
8	100								100	50	25
9	0									50	25
10											25
Cumulative mortality		100	200	300	400	500	600	700	800	800	800
No. of carcasses present		100	150	175	188	194	197	198	198	148	98
No. of carcasses found		30	45	53	56	58	59	59	59	44	29

days during a hypothetical outbreak. In this example, 100 animals died on each of the days 1 through 8, and mortality then ceased. It is assumed that carcasses disappeared at a constant rate of 50%/day, i.e., that the average half-life of a carcass was 1 day and that all carcass disappearance occurred overnight, and that 30% of the carcasses present were detected by the search method used. In real life, neither the rate of carcass disappearance nor the efficiency of searching is constant from day to day; however, the rate of disappearance used here is probably not unrealistic for passerine birds (Wobeser and Wobeser 1992) and the carcass recovery rate of 30% is similar to that which we have found in carcass cleanups during botulism outbreaks. It is evident from this hypothetical model that the number of carcasses recovered on any one occasion is a poor indicator of the total cumulative mortality. This becomes increasingly so as the outbreak continues over time (Fig. 4.1). In most outbreaks, the investigator does not know exactly when the outbreak began, so it is unclear where the disease is on the time scale shown in Fig. 4.1, which further complicates any attempt to extrapolate from a single carcass count to an estimate of total mortality.

Some years ago, we were interested in the extent of mortality of geese caused by avian cholera in a large area of western Saskatchewan. Our budget allowed

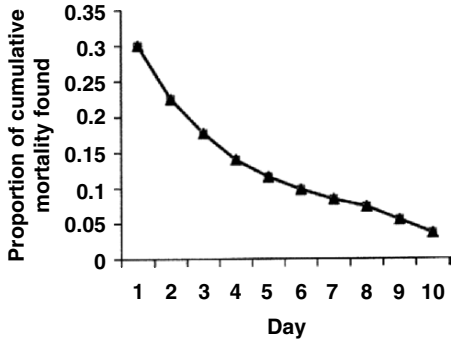

Fig. 4.1 Proportion of the cumulative mortality (all animals that died) that would be found on each day during a hypothetical die-off in which 100 animals died on each of days 1 through 8 and in which 50% of carcasses disappeared each day. The search method used detected 30% of carcasses present (data from Table 4.1)

one aerial survey of the area per week. Two questions arose in the planning stage of this work. Our first concern was related to the proportion of dead geese present in the area that would be detected from the air, i.e., how good was our search technique? The second related to the length of time that individual carcasses persisted in a recognizable form in the field. We needed an answer to the first question to understand the accuracy of the technique. This was determined by a process of ground-truthing in which we counted the number of carcasses present in marshes using ground searches and then compared these counts to counts made the same day from the air (this double sampling, using two different techniques, provided a measure of the probability of detection during the aerial surveys). We desired an answer to the second question to determine if there would be carry-over from one week to the next, i.e., were we likely to count the same carcasses on successive weeks and, hence, overestimate the incidence of disease. We marked recently dead goose carcasses with inconspicuous tags, left the birds in situ, and observed them daily until they disappeared. More than 50% of carcasses were gone within 4 days and all disappeared within 6 days. Combining the results of these two trials, we felt that our method provided a reasonably accurate count of the carcasses present and that we could be confident that few or no carcasses would persist from one weekly survey to the next. However, many birds that died between surveys would be missed because they had been removed, so that our estimate of the number of dead birds was conservative. This type of information might be used to derive a mathematical model that would allow estimation of total mortality on the basis of repeated surveys but, to my knowledge, this has not been done.

In most disease studies, a complete count of the population is impossible, and some form of sampling is necessary. A complete count might be done on a portion of the area, and then the population on the total area calculated

based on the assumption that the density of animals on the sampled area is representative of that in the total area: N (total population)/ A (total area) = n (number in sampled area)/ a (sampled area), in which case, N = An/a. Each individual count is a sample and must be supplemented by additional counts, (either repeated counts on the same area, or counts of several areas), so that an estimate of the population, together with confidence limits of the estimate, can be calculated. The type, size, shape, and number of sample plots that are used are based on knowledge of the biology of the species and methods available to the investigator. Often, sample plots are geographic areas but they may also be some ecologic unit, such as a tree or den-site. Sample plots may be of various shapes and each of circular, square and rectangular plots has particular advantages and limitations.

The line transect method is widely used for estimating density and abundance of wild animals (Buckland et al. 2001). It is appropriate for use during the study of disease but has received little attention. In a line transect, the observer moves along a randomly chosen straight line within the area, counting all the animals that are seen, and measuring either the perpendicular distance from the line to where the animal was seen or the distance to the animal and the sighting angle. It is assumed that not all animals are detected and that the probability of detection decreases with distance from the line. This probability can be calculated and this allows the density of animals to be estimated. We studied the line transect method for estimating the density of dead passerine birds in two habitat types and found it to be reasonably accurate, providing that the search line was sufficiently long so that at least 40 birds were located (Philibert et al. 1993). In a pasture with grass from 30 to 70 cm tall, search lines 1.6 to 4 km long were required to find sufficient birds when the known density was 50 birds/ha. Rivera-Milán et al. (2004) conducted field trials of line transect using chicken carcasses to establish the usefulness of this techniques for assessing pesticide-induced mortality of wild birds in Argentina. We used line transect to estimate density of nests and bird carcasses during a study of the role that Franklin's gulls play in waterfowl botulism (Soos and Wobeser 2006).

The location of sample plots or transect lines should be based on knowledge of the distribution of individuals within the area and it is a serious mistake to assume that the distribution of animals will be random or uniform. Dispersion may result from environmental factors, such as the availability of suitable habitat, or from behavioral factors, such as gregariousness or territoriality. Often a pilot study using random sampling on an area to determine the distribution of animals is necessary. Three general patterns of distribution are shown in Fig. 4.2. When the population is dispersed in a random or regular distribution, unrestricted or simple random sampling may be adequate. In this method, the area is divided into suitable sized plots by means of a grid and plots are selected randomly for sampling. This method meets the general requirement for random sampling, in that each plot has the same probability of being included as every other plot. When the population is found to be

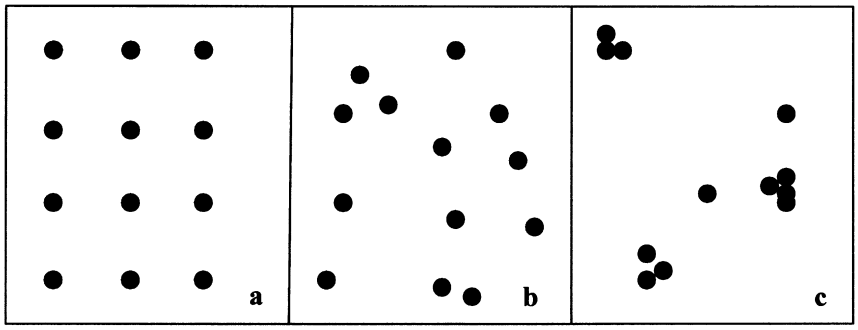

Fig. 4.2 Examples of three types of distribution of animals within an area: **a** Regular; **b** Random; **c** Aggregated

distributed in an aggregated or clumped manner, there may be advantages in using **stratified** random sampling. Many types of stratified sampling have been described, and the reader is referred to Davis (1982) for specific examples. The basic technique consists of dividing the area into sub-areas, often based on the density of animals in these **strata**, and then sampling within the strata in a random fashion. A major advantage of stratified sampling is one of efficiency, in that the sampling effort can be concentrated in the strata that contain most of the population. Whitlock and Eberhardt (1956) provide an early example of the use of stratified sampling for finding deer carcasses during a disease study.

The choice of the appropriate number of samples that should be collected in any survey is an important decision because collection of excessive samples is wasteful and an inadequate sample size may limit confidence in the estimate. The appropriate sample size is determined by the size of the difference one wishes to detect. As noted earlier, greater precision (and a larger number of samples) are required to detect small as compared to large changes in the population. Methods for determining minimal sample size under various conditions will be discussed in Chap. 7. Davis (1982) includes several examples of the use of various techniques for determining sample size in studies of population size. The choice of an appropriate sample size is not an easy matter, and assistance should be sought from a knowledgeable biometrician whenever possible.

4.3.2 Population estimates obtained by removal or capture of animals

These methods have not been used extensively in disease studies but may be appropriate in certain circumstances, particularly for evaluating the effectiveness of some types of disease management. The simplest method of this type is to calculate an index of animal abundance by measuring the number of animals captured relative to catch effort. This system has been used in many studies of

small mammals. For example, the number of meadow voles captured/1,000 trap nights, (a trap night is one trap set for one night) provides an index to the number of voles present, and this can be used to compare the relative abundance of animals in an area at different times or to compare the density in different areas, providing that the same trapping method is used in all instances and that the probability of detection remains constant at different levels of population. Rosatte et al. (1986b) measured the effectiveness of skunk population reduction for control of rabies in Alberta by comparing the number of skunks caught per unit of catch effort at various stages of the program. A standardized trap-catch index is used to assess the effect of brushtail possum control in New Zealand (Anonymous 2004; Coleman et al. 2006).

When animals are removed from a population and the removal operation is repeated again and again, the number of animals caught during each successive trapping period should decrease. The progressive decrease in the number caught can be used in a variety of ways to estimate the original population. The assumptions for these methods are that each animal in the population is equally likely to be caught, that the probability of capture does not change during the removal process, that the population is closed (no increase or loss except through capture), and that the number caught is proportional to the number on the area. Two simple graphical methods for using this type of data are shown in Fig. 4.3. The graphs might depict the number of skunks captured each week during a hypothetical trapping campaign to control rabies in an area. Obviously, home range and activity of the animals, length of the removal period, and immigration into the area, will have a great effect on this technique. The assumptions listed above are seldom completely valid in real life. For more details of this type of procedure and the related mathematical methods for calculation, see Lancia et al. (2005). As an alternative to actually removing animals from the area, captured animals may be marked and released. Marked individuals are then treated as though they were not present (although they make traps unavailable to capture new animals, so that the number of trap-nights must be reduced for calculations). An advantage of this method over removal is that habitat is not left empty on the study area, reducing the likelihood of immigration of new animals from outside the area (Bracher et al. 1986).

Another method uses the change in ratio of occurrence of some feature or index of the population, as a result of removal of animals, to estimate population size. Swenson (1979) used the change in the proportion of bucks in a deer population, as a result of the hunting season, to estimate the population in an area before an epizootic. Prior to the hunting season, 18% of the deer observed on the area were males, while after the hunting season males comprised only 9% of the deer seen. About 44 bucks were known to have been killed on the area by hunters. The change in proportion of males from 18% (S_1) to 9% (S_2) was assumed to be the result of removal of these 44 (n) animals. If N is the population of males on the area prior to the hunting season, then: $S_1 - S_2/n = S_1/N$ or 18–9/ 44 = 18/ N and N = 88. If there were 88 males on the area prior to the hunting season, the total population = $88/18 \times 100 = 488$.

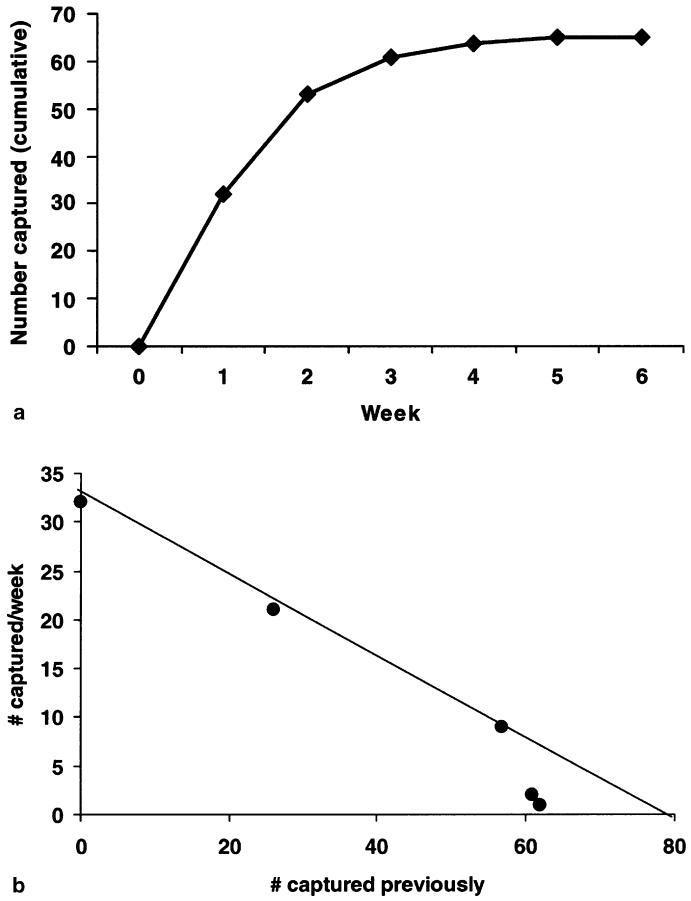

Fig. 4.3 Two simple graphical methods for using trapping data to estimate population size. In both cases, the animals captured were removed from the population. In **a**, the cumulative number of animals captured is plotted and the total population size is estimated by the asymptotic point on the resulting line. In **b**, the number captured in each time interval is plotted against the number captured previously with the total population being estimated by the intercept of the resulting line with the x-axis

Similar calculations can be done using track counts or other indices measured before and after a period of depopulation, such as in the skunk control program shown in Fig. 4.3.

A basic and serious problem with this method is that each of the values used in the calculations (e.g., the proportion of bucks in the population) is an estimate with an error component. When such estimates are used in calculations that involve division or subtraction, the compound error increases dramatically. The error component of the final estimate (population size in this example) might easily be ± 100% of the estimate. Lancia et al. (2005) should be consulted for other assumptions required for this technique.

4.3.3 Estimation of population based on mark-recapture

Estimation of population size based on recapture of marked individuals is one of the most widely used techniques in wildlife work. An array of methods are available (Manley et al. 2004) but most are derived from tests (Peterson method, Lincoln index) based on the assumption that the ratio of marked to unmarked animals in a sample collected from the population is representative of the same ratio in the population: N (population size)/ M(number marked and released) = n (number in sample)/ m (marked animals in sample). Assume that 100 animals were captured, marked, and released in an area. A few days later, ten marked animals were recaptured among a sample of 40 trapped animals, then: N/100 = 40/10, and the estimated population N = 400. Mark-recapture techniques may be useful in any situation in which animals or objects can be marked and recaptured later. For example, Swenson (1979) used this technique to determine the efficiency of a search for carcasses during an epizootic in deer. We used a mark-recapture method to test the effectiveness of carcass collection during a botulism outbreak in ducks (Cliplef and Wobeser 1993). Dead ducks were marked with inconspicuous tags and replaced where they had been found in the marsh just prior to the start of a clean-up operation by other individuals. All carcasses collected were then examined for tags prior to disposal. In one trial, 103 dead ducks were marked. Of the 85 carcasses collected during cleanup of the area, 20 had been tagged. The carcass collection was only about 19.4% effective (20 of 103 marked carcasses were collected). Using the formula N/M = n/m and solving for N, the estimated number of carcasses present in the area was 438. The actual number of carcasses actually present was likely even greater, since some dead birds were undoubtedly missed during both the initial search when we marked carcasses, and the carcass collection.

If conspicuous marks are used, or if the animals have distinctive natural marks, the animals may be observed visually or by other means without being captured, e.g., Bartmann et al. (1987) used radio-collars to relocate deer during a study of the accuracy of aerial surveys. Mowat and Strobeck (2000) used mark-recapture analysis based on DNA recovered from hair samples collected in "*hair-catchers*" to estimate abundance in a population of grizzly bears. Many elaborate methods for dealing with mark-recapture information are available (see Manley et al. 2004), with the Jolly-Seber model (Jolly 1965; Seber 1965) being most important. The same basic assumptions are required in all these methods: (i) the marks are not lost during the study period, (ii) there is no addition to the population during the study, (iii) the marking process does not affect subsequent survival of the animal (i.e., mortality is the same for marked and unmarked animals), and (iv) marked and unmarked animals have the same probability of being captured.

Methods have been developed for testing how well data fulfill some of these assumptions (Davis and Winstead 1980) and techniques are available to deal with variable probability of capture (Rexstad and Burnham 1991); however,

mark-recapture techniques often have been used without regard to these assumptions, or to the limitations of the methods. Investigators should be particularly concerned that the capturing and marking process does not, in itself, act as a morbidity or mortality factor. If marked animals develop capture myopathy or suffer other injury during capture and handling, estimates of population size, and survival rate based on the recapture of these individuals will be biased and not representative of the actual population (Höfle et al. 2004; Abbot et al. 2005). In general, a large proportion of a small population must be marked to obtain reliable estimates and this may nullify the advantages of these techniques (Bartmann et al. 1987). Manley et al. (2004) and Lancia et al. (2005) should be consulted for further discussion of mark-recapture methods.

4.4 Population distribution

The distribution of animals within an area is a fundamental feature of a population but "*it is a feature that is extremely difficult to describe in precise and meaningful terms*" (Clark and Evans 1954). As noted earlier, it is foolhardy to assume that any population of animals is distributed randomly across the landscape. Some species maintain and defend territories, while others share or have extensive overlap among adjacent home ranges. The spread of infectious diseases geographically is influenced by the degree of overlap and interaction between neighbors. Aggregated or clumped distributions create special problems for measuring animal abundance and often are extremely important in understanding the ecology of both infectious and non-infectious diseases. For example, Wright and Gompper (2005) describe the effect of a clumped distribution on parasites of raccoons. One technique for quantifying spatial relationships is by use of nearest neighbor analysis (Clark and Evans 1954). In this analysis, the expected average distance from an individual to its nearest neighbor in a randomly distributed population can be calculated based on the number of animals and the size of the area. This then serves as a basis for comparison with the average measured distance between individuals and their nearest neighbor in the population under consideration. Nearest neighbor analysis also can be used to compare groups, such as infected and uninfected individuals, as in studies of tuberculosis in badgers and cattle in Ireland (Olea-Popelka et al. 2005) and the United Kingdom (Woodroffe et al. 2005). In both of these studies, spatial clustering of animals with tuberculosis was detected. Woodroffe et al. (2005) found that infection with *Mycobacterium bovis* was clustered spatially at a scale of 1–2 km in both badgers and cattle, which has obvious implications for management.

The distribution of animals may change at different times of the year and this may be important in disease transmission if, for example, the rate of contact is higher between infectious and susceptible individuals when they are aggregated. Animals also may be aggregated artificially, enhancing disease

transmission, as is thought to be important in transmission of tuberculosis among white-tailed deer aggregated by artificial feeding in Michigan (Miller et al. 2003) and in transmission of brucellosis among elk concentrated on feeding grounds (Thorne et al. 1982).

A feature of animal distribution that is important for understanding disease is dispersal of animals, since this may explain in part how disease moves across the landscape. Dispersal has been defined as the movement an animal makes from its point of origin to the place where it reproduces (Caughley 1977). Dispersal is difficult to detect or measure. The traditional method has been to use mark-recapture, and particularly radiotelemetry, to follow individual animals. For instance, in studies of tuberculosis in wild elk near Riding Mountain National Park, Manitoba, most marked individuals stayed close to the original site at which they were marked but a few individuals dispersed across many kilometers of open farm land to another area of suitable elk habitat, so that sampling for tuberculosis had to be extended into this area. Buechner (1987) defined dispersal in terms of the number of territories, home ranges, or units of area capable of supporting a resident animal that are crossed by a dispersing individual. This is a useful concept for considering dispersal in terms of disease because it provides an image of the number of resident animals with which a dispersing animal is likely to have contact. In a study of tuberculosis in ferrets in New Zealand, Caley and Morriss (2001) found that very few juveniles dispersed, i.e., left the home range where they were born. The distance that animals move may be influenced by habitat conditions, such as vegetation conditions, and animals living in areas of poor or patchy habitat may move greater distances and contact more conspecifics than animal in uniformly good habitat (Root et al. 1999). Disease may also alter the distance that animals move or disperse, e.g., the average distance moved by non-rabid raccoons during a study in New Jersey was 1.5 ± 0.5 km, while rabid raccoons moved an average of 8.4 ± 4.3 km (Roscoe et al. 1998).

Another method for estimating dispersal (and immigration) in a population is through use of molecular techniques to identify the population structure by examining the genetic profile of individual animals (Waser and Strobeck 1998). Through the use of assignment tests, the natal population of individuals can be identified and the proportion of immigrants can be estimated more rapidly and with less fieldwork than is required for mark-recapture studies (Berry et al. 2004). This was used to characterize feral pig populations in Australia and allowed assessment of the efficacy of population control, identification of groups that acted as source for reinvasion after population control, and delineation of reinvasion corridors along river courses (Hampton et al. 2004). Immigration may confound interpretation of a local disease event. Baker et al. (2001) found increased genetic diversity in bank voles from contaminated sites at Chernobyl but could not determine if this resulted from increased mutation because of radiation or from increased immigration into the contaminated site because of higher mortality there.

4.5 Vital statistics

Changes in population size and density occur because of variations in the rate of entry of animals into the population through birth or immigration and in the rate of loss of animals through death or emigration. The methods discussed to this point have been concerned only with the abundance of animals and do not provide information on vital statistics, such as sex and age ratio, natality, recruitment, survival, and mortality, which may be as important as the number of animals for understanding a disease. For instance, Mills et al. (1999) found that the apparent prevalence of antibody to hantavirus in a population of wild rodents was not proportional to population density. This seems counter-intuitive but could be explained when sex and age composition of the population over time was known. When the population was increasing, the prevalence of animals with antibodies to hantavirus was low, because the population was being diluted continuously by the addition of young animals that had not yet become infected. When environmental conditions were less favourable, reproduction declined and the population decreased, and the population consisted largely of older animals that had been infected and had antibodies. In some animals, it may be very difficult to detect an effect of disease on the population without considering the sex/age structure. For example, seabird populations are made up of many overlapping generations and the population contains a pool of non-breeding birds. Losses, such as might occur from an oil spill in which an entire age cohort dies, may not be obvious because of recruitment from the pool of non-breeders, as well as other forms of compensation (Burger and Gochfeld 2002). Vital statistics related to the population are calculated by observation of samples of living animals, or examination of samples of dead animals that have been collected, harvested, or found dead. Dinsmore and Johnson (2005) provide a very thorough review of methods for collection and analysis of this type of population data.

The samples used must be representative of the population and, for this to be true, each animal in the population must have an equal opportunity to be identified and sampled. Most samples of wild animals are biased in some way and, as a general rule, one should treat all samples as biased until proven otherwise. It is better to assume a biased sample and to search for causes of bias (so that they can be measured and reduced early in the study) than to assume that sampling is free of bias, only to discover later that the data are flawed. Samples collected by observing free-ranging animals may be biased by differential behavior, activity, distribution or visibility of the various sex and age groups. This variation may change diurnally or seasonally, e.g., brightly hued, singing male songbirds are much more conspicuous than are their mates during the breeding season, but this bias may be less severe at other times of year. Connolly (1981) felt that counts of mule deer conducted during the summer underestimated the number of males in the population because males moved less than females at this time of year. Counts in late autumn were thought to reflect the population composition more accurately than those done in the summer.

It is difficult (or impossible) to distinguish the sex and age of many species at a distance and some type of trapping or capture may be necessary. Samples collected by trapping or other means of capture are usually biased. Juvenile animals may be unusually susceptible to capture because of naivety, males may have an increased likelihood of encountering a trap because of larger home range size, and social dominance may determine which animals enter the trap first (Garrott and White 1982). Even mass-capture techniques such as cannon-netting or drive-trapping of waterfowl may not produce random samples from the population (Raveling 1966; Sulzbach and Cooke 1978; but see Morez et al. 2000).

Animals killed by hunters are a common source of samples for disease studies. Such samples may be biased not only by differences in vulnerability of animals to hunting but also by conscious or subconscious selection by the hunter (Coe et al. 1980; McCracken et al. 2000). Animals dead of other causes, for instance road-kills, may also be used, but disease investigators (if anyone!) should be aware that most mortality factors affect each sex and age group at a different rate and that such samples are often not representative of the population. During carcass collections, conspicuous species are likely to be found at a proportionately greater rate than are cryptic species (Linz et al. 1991; Philibert et al. 1993; Cliplef and Wobeser 1993).

There is no single method for avoiding bias and obtaining representative samples. Techniques should be chosen on the basis of a thorough knowledge of the biology and behavior of the species being studied, and of the local area. The advice of experienced field biologists is particularly valuable in this regard. It is a sound principle to examine and compare samples collected in more than one way from the population, whenever it is possible to do so. For example, assume that we are studying the impact of a disease on a deer herd. We find that there is a small proportion of fawns among a sample of deer killed by hunters. This might be the result of a low proportion of fawns in the population, perhaps because of disease, or it might be because of some other factor such as active selection against fawns by hunters. Evidence of the age composition of deer harvested in the same area in earlier years, and in the same year in adjacent deer herds would be helpful for interpretation, if such data are available. One could also be more confident that the proportion of fawns in the population was actually reduced if few fawns were seen during an aerial survey of the area and if there was also a paucity of fawns among a sample of road-killed deer. In this instance, all the data sources would be corroborative. Connor et al. (2000) described a method for detecting bias in data from hunter-killed animals. Bias may have little effect if the same technique is used repeatedly to measure relative changes over time or between areas, so long as all samples are biased in a similar manner.

4.5.1 Sex ratio

Knowledge of the gender composition of the population is needed for the calculation of other vital statistics, many of which differ between the sexes, and it is necessary for understanding the reproductive potential of the population. Sex is

an important intrinsic determinant of disease and many diseases are distinctly sex-oriented. These include diseases that are: (i) related to structures or functions that occur only in one sex, such as mastitis and uterine infections in the female, and reduction of lipid soluble PCBs and other chlorinated hydrocarbon residues in females as a result of lactation (Addison and Brodie 1977), (ii) related to sexually oriented activities, such as the occurrence of brain abscesses in male deer as a result of injuries suffered during the rut, (iii) transmitted venereally, as well as diseases such as brucellosis in which the major impact is on the reproductive organs. As an example, young male bison are particularly prone to contract brucellosis because they are particularly interested in materials associated with the birth process that are the major route of transmission (Rhyan 2000). Many other diseases occur more commonly in one sex than the other, although the reasons for this are unclear. For example, many male white-tailed deer have some degree of degenerative joint disease by the time they reach 5 years of age, while this condition is uncommon in females of any age (Wobeser and Runge 1975a). Males of some species of game birds are better able to withstand cold and starvation than are females, while the reverse is true in other species (Latham 1947). A striking example of a sex-associated disease is the synchronous mortality of the entire male segment of the population that occurs annually in the dasyurid marsupial *Antechinus stuartii* (Barker et al. 1978).

During a disease outbreak, it often is possible to determine sex-specific numerators by counting and determining the sex of affected and dead individuals. However, such counts may be biased by differences in visibility between the sexes, e.g., male birds usually are more conspicuous than females, or because of differential expression of the disease in the two sexes. It is more difficult to obtain suitable sex-specific population denominators needed to calculate rates. This is particularly true for inconspicuous species that lack obvious sexual dimorphism. It is important to remember that an unequal sex distribution is normal within some animal populations.

The proportion of each sex in the entire population is the general sex ratio; age-specific sex ratios also may be calculated. The sex ratio traditionally is expressed as the number of males per 100 females (e.g., 114 males:100 females) but there may be advantages in expressing it as a proportion (males = 0.53, females = 0.47) if the ratio is to be used in other calculations.

4.5.2 Age composition

Information on the age distribution within a population is needed to describe a disease, for calculating other ratios, and also may provide important information on the history of the population and its response to disease. Age is an important determinant of disease and many diseases are distinctly age-associated. Some diseases occur only in the very young, e.g., myiasis (infection by fly larvae) caused by the fly *Wohlfahrtia vigil* is limited to nestlings (Craine and Boonstra 1986). This parasite, and the mortality it causes, would be completely overlooked unless this age group is examined. Many infectious

diseases occur at the greatest prevalence among young animals, in some cases because older animals in the population have protective immunity acquired as a result of infection when they were younger. Other diseases, such as rabbit hemorrhagic disease, are found predominantly in older animals. This may be because of transient protective immunity acquired from the young from the dam, susceptibility associated with the aging process (many degenerative diseases and neoplasia appear to be of this type), cumulative exposure (certain long-lived parasites and many cumulative toxins), or because the disease is slow to develop and only becomes evident in older individuals. As an example of the latter situation, macroscopic cysts of the protozoan parasite *Sarcocystis rileyi* are not found in hatch-year ducks during fall migration because the parasite requires at least 5 months development in the duck before cysts are visible to the naked eye (Cawthorn et al. 1981). Anderson and May (1985) present evidence that in many diseases of humans there also may be age-related changes in the rate of infection of susceptible individuals. It is probable that similar phenomena exist among wild animals.

The ease with which observers can differentiate among age groups varies among species. In birds, it often is only possible to distinguish between hatch-year and adults although, in some species, sub-adults that have not bred but are more than 1 year old also may be distinguishable. The actual age of many mammals can be determined by examining the replacement of deciduous teeth in young animals and by the presence of cementum annuli in permanent teeth of adults. However, cementum annuli may be unreliable in some situations (McCullough 1996). In the field, differentiation between young-of-the-year and adults may be all that is possible. Depending on the method used for counting, there may be serious bias because of differences in visibility of one age or sex group. For example, aerial surveys gave a good estimate of the total number of adult bison in a group, but the number of calves was underestimated markedly (Wolfe and Kimball 1989).

The young/adult ratio is important in most disease studies because it is a measure of reproductive and rearing success. In the investigation of certain diseases, it may be necessary to measure this ratio at several times during the year because different disease mechanisms act at different stages of life. Consider a moose population in which the calf/cow ratio has been noted to be very low during surveys done in the autumn of successive years. Further sampling at several times of year might reveal a variety of different scenarios, each of which suggests mechanisms that should be investigated:

Calf/Cow ratio

Mid-gestation	Post-partum	Autumn	Potential mechanisms
Low	Low	Low	– poor breeding success, low conception rate, early fetal death
High	Low	Low	– late abortion, stillbirth, high perinatal mortality
High	High	Low	– high mortality of calves after perinatal period

The last of these scenarios was found in a moose population in Saskatchewan in which the loss of calves was attributed to predation. It also was found to be the situation in certain bighorn sheep bands in Colorado where lambs were dying as a result of transplacentally transmitted lungworm infections that caused severe pneumonia in mid-summer when the nematodes matured (Woodard et al. 1974; Schmidt et al. 1979).

When suitable information is available, it may be useful to construct an **age pyramid** (Fig. 4.4). Such information must be interpreted with care, but it may provide evidence of the past history of the population, particularly if pyramids for a succession of years can be compared. In Fig. 4.4, population A has a high reproductive rate, indicated by the large number of young, a relatively high rate of mortality of animals in their first year (assuming that the yearling population was similar to that of the current young), and then a lower rate of mortality among older age groups. This general pattern is thought to be normal for many wild animal populations. Population C appears to have had an extremely low reproduction or survival of young for the past 3 years and, based on the sample, it appears that recruitment into the herd has been very low. This was the type of pattern seen in bighorn sheep herds that suffered successive years of high mortality of lambs from mid-summer pneumonia. Population B appears to have experienced 1 year of poor reproduction and/or survival so that one age class or cohort is almost absent from the population. This is the type of pattern observed in arctic-nesting birds as a result of a year with unfavorable nesting conditions.

Information on the average age at which individuals become infected, age-specific prevalence of infection and immunity, as well as the population age structure and average life expectancy, is critical for understanding the population biology of any disease. The most common method for collecting this type of data is through cross-sectional surveys, and serologic surveys in particular, in which the occurrence of various factors can be related to age. Figure 4.5 illustrates the proportion of animals of various ages that have experienced a disease, based on the prevalence of antibodies to the agent. The average age at which infection occurs in the population can be estimated from this type of data, and this statistic can be used to estimate other values, such as R_0 the basic reproductive rate of the disease (this subject will be discussed in Chaps. 10 and 13). Studies by Van Rensburg et al. (1987) and Harris and Smith (1987) provide excellent examples of the use of age-related information of this type in the study of the impact of a disease, and of a control program, respectively, on the demography of wild populations.

4.5.3 Measures of reproduction

Knowledge of the reproductive ability and success of a population is essential for any understanding of the population ecology of a disease. This information is needed to define the effects of disease on the population, for predicting

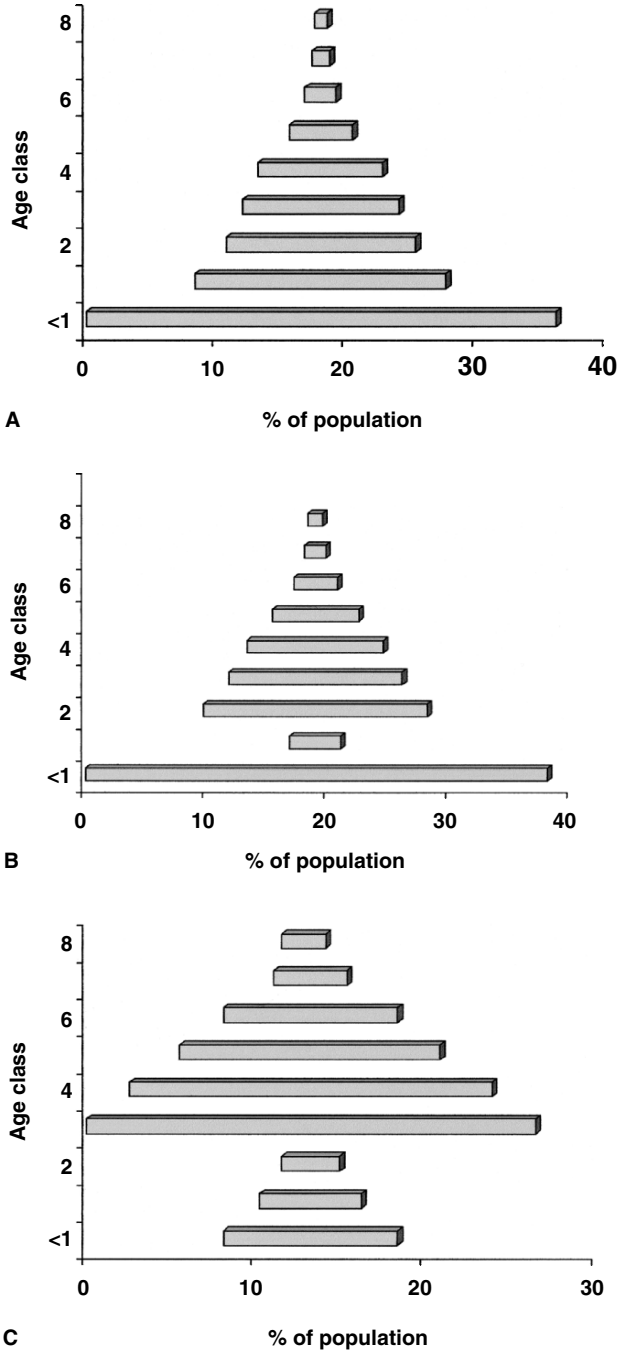

Fig. 4.4 Age-pyramids for three hypothetical populations of wild animals. The differences in pattern among the populations are discussed in the text

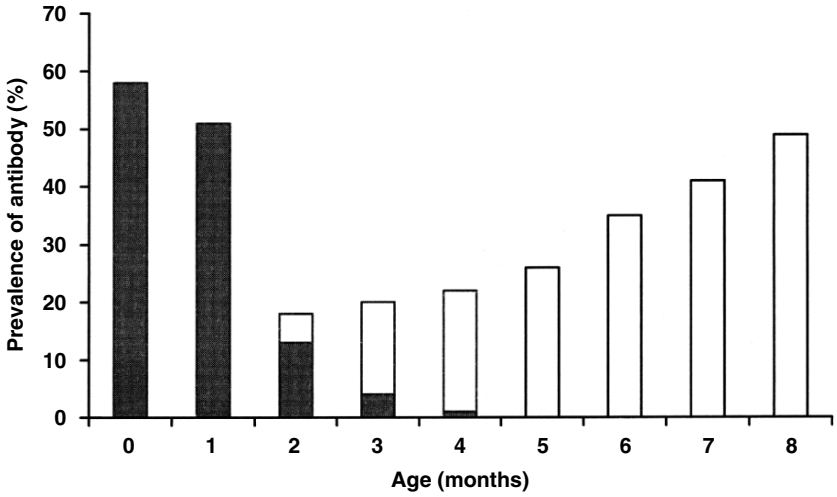

Fig. 4.5 Age-specific prevalence of antibody to disease agent X in a population. Many neonatal animals had antibody acquired passively from the dam (indicated by the *filled portion of the bars*). The prevalence of this type of antibody waned rapidly during the first 3 months and the subsequent increase in antibody prevalence was the result of exposure to agent X. This actively acquired antibody is indicated by the *open portion of the bars*

the response of the population to a disease, and for designing and assessing the effect of a management program. Studies by Wandeler et al. (1974) and Bogel et al. (1974) provide an insight to the importance of the reproductive biology of a host (the fox) in the epizootiology of a disease (rabies), and the impact of a high reproductive rate on the success of attempted control procedures.

Fecundity is the term used to describe the potential reproductive output of a species. Fertility is the actual reproductive performance of the population and is usually expressed as a rate. A number of terms have been used for this rate, including reproductive rate, birth rate, and natality rate. Each of these is a ratio of the number of live offspring produced during some period to some measure of the population during that period. Unfortunately, the term 'offspring' is interpreted arbitrarily, depending on the stage of development that is measured. It might mean the number of fertilized zygotes, the number of implanted embryos or eggs laid, the number of young born alive, or the number of young that hatch. Each of these is a valid measurement and each may have some particular significance for an individual disease but the researcher must take care to define the rate used. The most commonly used numerator in natality rates is the number of young born or hatched alive. In human populations the annual birth rate is expressed in relation to a denominator of the average number of persons alive in the population during the year. For reasons

discussed earlier, this statistic is seldom used for wild animals, and most natality rates are expressed in relation to the adult female (fetuses/pregnant female, fawns/adult doe, ducklings/adult hen). If the number and proportion of adult females in the population are known, more general rates can be calculated.

Measurement of reproductive rates is done by sampling the population and is subject to all the biases discussed earlier. Calculations are done in the same manner as for other population variables and, because the sample size usually is small, the precision of the resulting estimates is often poor. Until recently, measurement of reproductive success prior to parturition (during pregnancy) involved post-mortem examination of the reproductive tract. Application of techniques, developed for use in domestic animals, such as field laparoscopy (Zwank 1981), ultra-sound examination (Smith and Lindzey 1982), analysis of blood hormone (Seal and Plotka 1983) and pregnancy-specific protein B (Noyes et al. 1997; Russell et al. 1998), rectal palpation in large species (Follis and Spillet 1974) and measurement of fecal steroid metabolites (Schoenecker et al. 2004) allow the researcher to follow individual pregnancies and to measure in utero reproductive loss. However, there must always be concern that capture and handling, necessary to examine the animals, may affect their reproductive performance adversely. Studies such as that by DelGiudice et al. (1986) to determine the impact of immobilization on pregnant deer are needed to validate data resulting from these techniques. In some species, examination of the uterus for placental scars, the ovaries for corpora lutea, the mammary glands for milk, or the plumage for the presence of a brood patch may allow retrospective assessment of the recent reproductive history of an individual female. The number of young seen with adults or the age ratio in samples of harvested animals can be used as an index of fertility. Another number, the recruitment rate, i.e., the number of young, particularly of females, that reach reproductive age and, hence, are recruited into the productive segment of the population is often very important in understanding the impact of disease at the population level.

As noted in Chap. 2, a difference between investigating disease in wild animals and investigating disease in humans and domestic animals, is the need to consider the impact of disease on life-time reproductive success or fitness. This is extremely difficult, except in small populations that can be followed intimately over many years, as has been done with red-billed choughs (Reid et al. 2003), or through the use of extensive radio-marking as has been done with caribou (Adams and Dale 1998).

4.5.4 Mortality and survival

Although mortality is a stock-in-trade of the disease investigator, the term is seldom used in its population sense in papers dealing with disease in wild animals. In contrast, wildlife managers use the concept regularly. The **mortality rate** is a measure of the probability of death occurring during a prescribed

interval of time, and is defined by the equation: mortality rate = number of deaths during period/number alive at beginning of period. It is important to note that the mortality rate applies only to those individuals alive at the beginning of the period. This is in contrast to the **death rate**, which appears in the literature occasionally and may be confused with mortality rate. The death rate equals the number of deaths during period/average number in population during period. Death and mortality rates are equal if the time period under consideration is instantaneous, or if additions to the populations match the number of deaths exactly but, in most instances, the rates are different. Death rate will not be considered further here.

A third rate, **survival,** is used widely and is the reciprocal of mortality, i.e., survival = 1 – mortality, and is defined by the formula: survival rate = number alive at end of period/number alive at beginning of period. As with mortality, the survival rate refers only to the individuals alive at the beginning of the period.

Information on the death of deer during winter taken from Potvin et al. (1981) illustrates these rates. During the winter of 1974, an estimated 100 deer died from a population of about 480. The mortality rate over the winter was 100/480=0.21 and the survival rate was 380/480 = 0.79.

Survival rates for consecutive periods may be multiplied to calculate a cumulative survival rate. If the survival rate for a group of birds in April, May, and June was 0.89, 0.92, and 0.89, respectively, the overall survival rate during the 3-month spring period is the product of these, or 0.73. If any two of the population at the beginning of a period, the population at the end of a period, or the number of deaths are known, mortality and survival rates can be calculated.

What is measured in most studies is the apparent survival rate rather than the true survival rate, because fidelity to the area is usually not measured. If animals leave the area permanently (emigrate), the apparent survival will be biased low relative to the true survival. Return rate to the nesting colony in the following year has been used to measure the effect of parasite treatment (Hannsen et al. 2003) and immunization (Hannsen et al. 2004) on annual survival of female common eiders. It was believed that apparent survival was very similar to true survival in these situations because fidelity to the colony was known to be strong.

Studies of survival/mortality usually involve marking and releasing animals. The assumption is that the capture and marking process has no effect on survival. That this is not a safe assumption is illustrated by the examples in Table 4.2; however, other studies have not detected an effect of the system used for marking on survival (Swenson et al. 1999; Esler et al. 2000; Conway and Garcia 2005; DelGiudice et al. 2005; Powell et al. 2005). Whenever possible, a marking system should only be used when its potential effect on the results has been assessed. Radiotelemetry has been used extensively for direct measurement of mortality rates in wild animals and is particularly useful for studying neonatal or cryptic animals that are hard to find. This technique has the advantage that animals can be located for necropsy shortly after death if motion-sensitive transmitters (mortality switches) are used. The results obtained

from radio-marked individuals may be very different from those animals found by other means. For instance, in a study of mortality among reintroduced Eurasian lynx, 72 dead lynx were examined of which 15 were found because they were radio-marked. In the entire group, 18% died of infectious disease, while 40% of the radio-marked individuals died of infections (Schmidt-Posthaus et al. 2002). The survival rate of offspring has been measured by placing a radio on the mother, so that the group can be located for observation (Duncan 1986; Eberhardt et al. 1989). Evelsizer (2002) used radiotelemetry to compare the survival of ducks during botulism outbreaks on wetlands where carcasses were collected to that of ducks on wetlands with no carcass cleanup. Even if animals can only be relocated occasionally, the data collected may be useful, e.g., Ringelman and Longcore (1983) used a technique for estimating average survival time of ducks that were located infrequently.

A number of techniques have been developed for calculating mortality rates mathematically. Many of these were derived from methods developed in entomology or fisheries and only simple examples will be presented here.

Catch:effort: It often is easier to measure some index to the population than to determine population numbers, as indicated earlier. Changes in catch:effort can be used to calculate mortality, provided that all the assumptions mentioned previously in this chapter are valid. For example, during a study of long-tailed weasels, an average of 8.7 animals was captured/1,000 trap nights in the autumn, while only 4.3 were trapped/1,000 trap nights in the spring. The estimated mortality rate over the winter (during which no additions occurred as a result of births) = 8.7–4.3/8.7 = 0.51, and the survival rate = 4.3/8.7 = 0.49 (one assumption in this example is that weasels are equally susceptible to capture in autumn and spring, which may or may not be true).

Mark-recapture: A number of techniques are available for estimating mortality or survival using mark-recapture information. If animals are marked at one time and then recaptured on two occasions subsequently, a modification of the catch:effort method can be used to measure mortality in the interval between the two captures. If animals can be recaptured repeatedly, the survival rate can be estimated by plotting the proportion of the marked animals known to be alive against time (Getz 1970). The hypothetical data set in Table 4.3 illustrates information from a population of 12 marked animals in which recapture was attempted at monthly intervals. Paradis et al. (1993) used capture/recapture information in a model to estimate sex and age-related survival in a small rodent population. Newman et al. (2002) used mark-recapture to compare the survival of foxes affected by sarcoptic mange to that of uninfected foxes. Infected foxes survived only about one-fifth as long as uninfected foxes.

Another method called the "*triple catch trellis*" by Ricker (1958) requires two mark-and-release operations with different marks applied at each time, and one recapture. If 120 muskrats (M_1) were captured, marked and released in autumn and an additional 60 (M_2) were captured and marked and released early the following spring, the proportion of each, ($R_1 = 30$, $R_2 = 25$), captured during a later trapping period could be used to estimate over-winter survival: survival = $R_1M_2/(R_2 + 1)(M_1) = 30 \times 60/(25 + 1)(120) = 0.58$ (Table 4.4).

Table 4.3 Examples of studies that have detected negative effects of capture/marking on subsequent survival of animals

Species	Handling or marking procedure	Effect
Canada goose	Neck bands	Reduced survival[1]
Mallard	Radio transmitter	Reduced survival[2]
Wild turkey	Radio transmitter	Negative effect on wing growth[3]
Grey partridge	Radio transmitter	Adverse effect on survival, reproduction and body mass in some years[4]
Northern pintail	Radio transmitter	Reduced body mass[5]
Cassin's auklets	Radio transmitter	Reduced growth of chicks from radio-marked adults[6]
Emperor goose	Neck collar, radio transmitter	Reduced survival, breeding, clutch size[7]
Blue-winged teal	Radio transmitter	Altered behavior[8]

[1]Castelli and Trost (1996), [2]Paquette et al. (1997), [3]Hubbard et al. (1998), [4]Bro et al. (1999), [5]Fleskes (2003), [6]Ackerman et al. (2004), [7]Schmutz and Morse (2000), [8]Garretson et al. (2000)

Table 4.4 Example of using capture-recapture information to estimate the survival rate of a group of animals by using the proportion known to be alive at various times during the study. The animals were marked in December and an attempt was made to recapture each animal at monthly intervals

Animal	January	February	March	April	May	June
1	R[a]					
2						
3	R					
4						
5	A[b]	R				
6	A	A	A	R		
7						
8	A	A	R			
9	R					
10	A	A	A	R	A	R
11	R	R				
12	A	A	R	A	R	
Proportion alive	.75	.50	.33	.25	.17	.08

[a] R – recaptured
[b] A – assumed to be alive because recaptured later

For derivation of this formula and variance calculation, see Ricker (1958) and Seber (1973). Bird-banding analyses are derived from this general principle but have become very sophisticated (see Brownie et al. 1985) but a huge number of birds need to be banded to estimate survival with precision, because of the low rate of recovery (Sheaffer and Malecki 1995).

Change-in-ratio: Changes in the proportion of some ratio, usually sex or age, during a period of mortality can be used to estimate mortality. This technique is used extensively to estimate mortality as a result of hunting and deserves consideration for use in disease outbreaks. The general requirements are that the population contains two groups that can be readily distinguished, e.g., males-females, young-adults, or two species and that, during the period of mortality, one of the groups is removed at a higher rate than the other. The proportion removed from the entire population (i.e., the overall mortality rate) is defined by the formula: mortality rate =P–R/R–K, where P is the proportion of one group within the population prior to the removal, R is the proportion of the same group in the population after removal, and K is the proportion of the group among those removed. A hypothetical avian cholera epizootic will be used to demonstrate how this method might be used. Prior to the outbreak, the ratio of snow geese:white-fronted geese in the area was 30:70 (P=.30). The ratio among a large sample of dead birds collected during the outbreak was 50:50 (K=.50), and the observed ratio following the outbreak was 10:90 (R=.10). Assuming that all losses were due to the disease and that no birds moved into or out of the area during the period, the proportion of the total population that died (the general mortality rate) = .30 – .10/.10 – .50 = 0.50. The species-specific mortality rate can be calculated by multiplying the general mortality rate by the appropriate K/P value: thus, the mortality rate for snow geese = 0.50 × .50/.30 = 0.83 and for white-fronted geese = 0.50 × .50/.70 = 0.36.

The technique obviously works best in situations in which the groups can be distinguished at a distance in the field. The ratios observed must be representative of the true situation and, if the ratios are similar, small biases or errors in any ratio will affect the estimated mortality greatly (Davis and Winstead 1980). Dinsmore and Johnson (2005) suggest that because the assumptions required for this method are stringent, these should be considered carefully before the method is used.

Life tables (mortality-survival tables): The methods described above have been concerned with general mortality and survival rates of the population. In some circumstances it may be necessary to know the age-specific mortality or survival rate. The concept of a life table has been developed for the study of age-specific mortality and longevity in human populations. A life table presents the history of a group of individuals or cohort born simultaneously (usually in 1 year) by tabulating the number surviving at the end of each interval (often a year) until the last individual is dead. Construction of such a table for a human population requires relatively few assumptions because records are kept of all deaths and the total population is measured at regular

intervals by census. In contrast, those working with wild populations usually have incomplete population data and must make many assumptions and inferences in the construction of a life table (Davis and Winstead 1980). The techniques may be useful in long-term studies where information is available over a period of years but attempts to estimate age-specific rates from a single census or sample of a population taken at one time require that the population have a stationary age distribution, and such estimates are plagued by problems of sampling variability (Polacheck 1985). Those interested in these techniques should consult Caughley (1966, 1977) as well as Lancia et al. (2005).

4.5.5 Cause-specific rates and special ratios

Much of the information in this chapter has dealt with general rates (mortality, death, and survival). The disease investigator usually is interested in cause-specific rates, i.e., as a result of a single disease. The same general principles and techniques are used for collecting such information; however, care must be taken to ensure that both the numerators and denominators used are appropriate. A common mistake during the investigation of outbreaks of disease in wild animals is to assume that all of the individuals found dead succumbed to a single factor. It should be obvious that animals are dying continuously of a number of conditions and that these non-specific deaths continue to occur, even in the midst of a catastrophic epizootic. Whenever possible, a large sample of individuals should be examined in a diagnostic laboratory to determine the proportional mortality rate for each cause of death, i.e. the number of deaths attributable to each cause/total number of deaths. This rate can then be used to adjust the numerator.

The appropriate denominator for general rates is the total population but some individuals within the population may not be at risk of developing a particular disease because of age, sex, prior exposure, or other factors. Cause-specific rates should be calculated using only the segment of the population that is at risk as a denominator. This may require additional sampling to determine the proportion of the population that has identifiable resistance. A hypothetical outbreak of canine distemper in raccoons may illustrate these points. The number of raccoons that died in a county was estimated to be 300, and the total population in the area prior to the outbreak was estimated to be 800. A sample of 40 raccoons found dead was submitted to a diagnostic laboratory and, of these, 28 (70%) were found to have died of canine distemper, while the other 12 died of a variety of other causes. Serum collected from a sample of raccoons captured in the area shortly before the outbreak was available in a serum bank. Of these animals, 65% had antibody to canine distemper at a titre considered to be protective. Thus, only about 35% of the population was actually at risk of developing canine distemper. The general mortality rate during the epizootic was $300/800 = 0.38$, while the cause-specific rate for canine distemper among the animals at risk was: $(300 \times .70)/800 \times .35 = 0.75$.

4.6 Summary

- Wild animals seldom can be counted directly and most population parameters must be estimated.
- Accuracy is a measure of how closely an estimated value corresponds to the actual value. Most estimates of wild populations are of unknown accuracy.
- Precision is a measure of the extent to which repeated measurements of a single population agree with their mean. Population estimates should include an indication of their precision.
- Methods for determining animal numbers consist of two steps: (i) detecting the animals (or some index to their abundance) for counting, and (ii) using the number detected to estimate population size. The second step involves mathematical manipulations to account for the proportion of the population that is not detected. Measurement of the probability of detection should be a part of all studies.
- Most methods for measuring animal abundance assume that the population is stable during the data collection period and that all members of the population have an equal probability of being detected. Neither of these assumptions is totally valid in most measures of wild populations.
- Animal abundance may be estimated by: (i) using counts of animals or of some index to their abundance, (ii) measuring changes that occur when a known number of animals are removed, or (iii) measuring the proportion of previously marked animals that can be recovered or observed.
- Additional methods are required to collect life history information, such as sex and age ratios, reproductive performance, mortality and survival rates, needed to understand the population effects of disease.
- Most samples of wild animals are biased in some way. The effects of techniques, such as animal capture and marking, on the factors being measured should always be assessed.
- There is no perfect technique for collecting information on animal abundance; various techniques have advantages under some circumstances.
- Calculation of cause-specific information is necessary to separate the relative effects of different disease factors.