

CHAPTER 30

POPULATION ESTIMATES

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INTRODUCTION

The size of a population is of fundamental importance in determining the conservation status of a species. Understanding a population usually involves a survey of the number of individuals in it.

Concerns regarding wildlife conservation increased dramatically during the twentieth century due to the extinction or quasi-extinction of several species caused both by human related (*e.g.* hunting and fishing, habitat degradation) or natural (*e.g.* catastrophic events, disease outbreaks) sources. These revealed the need for the establishment of effective conservation and management actions in order to preserve populations or promote their recovery. These actions usually rely on some knowledge of the status or trends of the population of interest. Therefore estimates of population size are fundamental for wildlife conservation. In addition, knowledge of population abundance also has other applications such as ecosystem modeling research.

This chapter will describe the basic techniques used in population surveys that apply to cervids. The language used will be appropriate to the public interested in this subject, but will not go into great mathematical or statistical detail.

Census and Sampling

The size of a population can be determined by two main techniques: census or sampling. A census is a total enumeration of all the individuals in the population and is usually not feasible or, if feasible, is often prohibitively expensive for most wildlife populations (*e.g.* Borchers et al. 2002). Therefore, statistical sampling methods must be used in most cases. All sampling methods assume that total population size can be estimated when only a fraction of the population is detected by one or more observers. When this is the case, it is important to assess the accuracy of the estimate. ‘Accuracy’ means how close the estimate is to the real number, and should not be confused with ‘precision’, which means how close an estimate is to its repetitions.

Population Estimates

The goal of a population estimate is to determine the number of individuals, or estimate the total population in an area. When assessing populations that

occur over extensive areas, the only way to do a survey is by using samples. The population of the entire area will be estimated by extrapolation of the sampling results. A common mistake is to only visit places considered the best environment for finding the species. This should not be done unless it is absolutely certain that the species occurs exclusively in that area. It is impossible to estimate the total population without covering all the possible environments in which the species might occur. The best estimates come from random samples. Ideally, the study area should be divided into plots. The greater the number of visits to each plot, the greater is the accuracy of the results. If there are different environments within a plot, they must be separated into strata. Each stratum represents one kind of environment, and the results found in each one can be compared to the results found in the same kind of stratum in different plots. This provides an estimate of the number of individuals in each environment, and finally, the total population size.

Bias

An aspect that is worth mentioning is something called a sampling bias. This phenomenon occurs as a result of uncontrolled variables. It means the sampling technique was poorly applied. In a total enumeration, if the observer fails to count all the animals, the result will be biased. Another example of this is when individuals are counted only where they are most common. There are ways to later determine if the method that was used has probable sources of bias. Corrections can be made if bias identification and effect were registered carefully and in detail. Idealized statistical models that do not adequately reflect reality are also a source of bias. Capture-recapture models, for example, usually assume that capture probability is the same for each individual; the distance sampling method assumes that the spatial distribution of individuals is random. These presumptions are frequently incorrect and create a source of bias. When a source of bias is identified, it is best to change the method. When that is not possible, a population rate can be used instead of the total number. Wrong conclusions can be drawn from poor estimates, and result in equally wrong decisions that can cost the environment dearly.

Concept of Population

A population has been defined as a group of organisms of the same species that inhabits a certain place and has certain characteristics that the individuals do not, such as: density, birth rate, etc. (Odum 1972). It has also been defined as a group of organisms of the same species that occupy a given place at a given moment, because the boundary of a population (in time as well as space) is vague, being arbitrarily established by the observer (Krebs 1972). Another definition is that a population is an arbitrarily defined group in time and space (Margalef 1974). From the descriptions above, we can see that it is a concept that is not clearly defined. In general, a population can be considered as a group of individuals arbitrarily delimited in time and space.

The techniques for census or estimates found here will be linked to this definition of population, which we accept. When we delimit a population, we should use common sense. We will have to decide if our definition of population suits the method we will use. It is important for the researcher to always be aware of the relationship between the population as indicated by his methods and the real population, whose parameters he intends to delimit (Caughley 1977).

Understanding the Animal

Understanding the basic biological aspects of the species of concern is important when deciding which method to use. It is very important to have information on the home range, as well as the behavior of the species. The environment plays the main role in determining which techniques can be used. Direct counting is easier with animals that live in open areas, such as the pampas deer (*Ozotoceros bezoarticus*) that lives in grasslands, or the marsh deer (*Blastocerus dichotomus*) that lives in wetlands (Fig. 1). Behavior such as migration, shifts, and activity hours determine the possibility of detecting the individuals in different environments. Nocturnal species (e.g. *Mazama bororo*) that live in forests require special attention when choosing a method, since there is very little chance that they will be sighted directly. In this case, we can use traps or indirect counting techniques, which use traces left behind by the animals. Several other aspects related to the population and species of concern deserve attention and will be crucial in determining the most appropriate method to use.



Figure 1 - An aerial perspective of a marsh deer habitat at Paraná River Basin, Brazil.

Spatial Distribution

The spatial distribution of a population can, in general, be classified into three basic types (Fig. 2):

Uniform Distribution: The number of individuals per surface unit is constant. It is rare to find this kind of distribution in nature.

Random Distribution: There is the same probability of finding each individual in every place of its environment, which means the presence of an individual is independent of the other individuals of the population. This kind is also not commonly found in nature.

Aggregated Distribution: Individuals tend to group into clusters, as a consequence of their mutual attraction or of differences in environmental conditions. This kind of distribution is the most common in nature. However, it is the least convenient for estimates in terms of being representative, since it causes high variance.

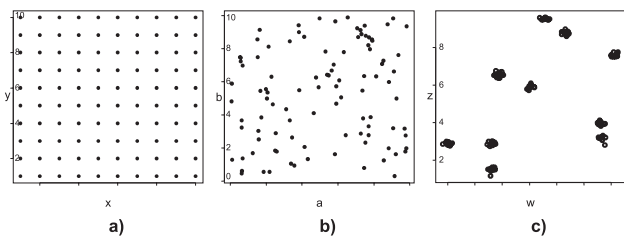


Figure 2 - Possible spatial distribution of a population. a) Regular Distribution, b) Random Distribution, c) Aggregated Distribution.

Distribution is linked to the different kinds of environments where the species occur. In the real world, the environment has discontinuous characteristics due to different climates, soil, etc. So it is natural to presume that animals too do not occupy areas homogeneously. Since a species chooses an area suitable to its biological characteristics and to its needs, there are individuals of the same population that prefer certain places. These are called *home range* or *vital area* (Burt 1943). There are also other factors that are important and that influence distribution. Social factors such as *gregariousness* and *territoriality* influence the way a population uses the environment. Living in social groups, being gregarious, affects the behavior of each individual in different ways, according to species, age and sex (Krebs and Davies 1981). Territorial defense and dominance are characteristics of territoriality and determine spatial relations. All of the factors mentioned above deserve a good deal of attention and should be carefully considered while planning a population estimate.

Distribution in Time

Through time the environment changes and also affects distribution, abundance and the probability of finding the animals. These changes must be taken into consideration in all estimation methods. It will be mentioned in the section about monitoring that the rainy season affects the behavior of the marsh deer. The succession of days and nights, seasons, the phases of the moon, are all relevant cyclical processes. In general, living beings too, have endogenous cycles that determine cyclic

patterns of physiological and behavioral changes. These changes are called biological rhythms and allow individuals to be prepared for environmental variations. Environmental variables synchronize endogenous cycles. Birth rate and mortality, emigration and immigration all vary throughout the year, so it is important to know their patterns because they affect the number of animals in the study area. We will describe later the study on marsh deer in which the sighting rate declined during the rainy season. This adaptation strategy affects the distribution of individuals and their abundance in certain areas as well. Behavior that varies throughout the day (Andriolo et al. 2003) can affect the probability of detection if the animals hide during certain periods of the day (Fig. 3 and 4). These variations affect not only direct counting techniques, but also techniques that use traps.

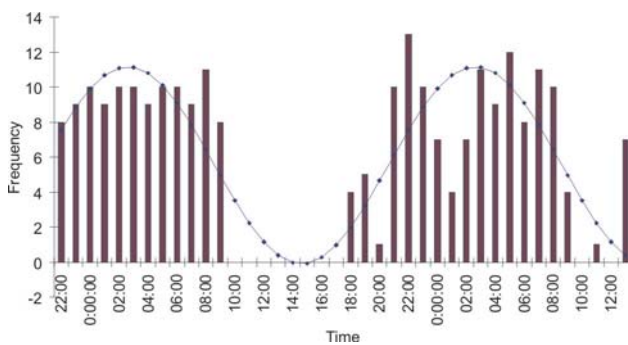


Figure 3 - An example of daily variation of activity period of mammalian species that can affect the probability of detection if the animals hide during certain periods of the day.



Figure 4 - A marsh deer resting behind bushes. This behavior potentially affects the probability of detecting the animal.

Cervids belonging to the genus *Mazama* frequently use latrines, which are used by one or more individuals to deposit feces and urine (Black-Decima 2000; Black-Decima and Santana 2002; MacNamara and Eldridge 1987; Vogliotti 2003). Even though the shape of the feces of each species is relatively homogenous, amount, size and consistency of the pellets can vary, depending on the diet of the animal. Because of this, other evidence such as traces, tracks or photos (taken with camera traps) should also be used, whenever possible, to confirm that they really belong to the group of concern.

With time, feces also tend to decompose and break up, especially in wet environments where there are dung beetles. We should keep this fact in mind, since abundance estimates based on counting feces depend on correctly identifying new fecal matter deposited in a certain area during a certain period of time.

Population estimates can be obtained by counting feces along transects, or in randomly or systematically determined plots within the study area (Sutherland 2000; Wemmer et al. 1996). The feces that are found can be marked or removed from the study area so that during the next sampling, only the feces that were deposited during that period would be considered (Sutherland 2000). After setting a defecation rate for the animal being studied it will be possible to estimate the number of animals per plot, which will be converted into the number of animals per area unit (i.e. number of animals / km²).

ABUNDANCE ESTIMATES

In the section below, methods used or potentially useful to estimate deer abundance are described and, when applicable, software used for estimation is presented.

Total counts

Total counts involve the count of all individuals in a geographically defined population of a species. The methodology is simple, as no calculations are done beyond adding all individuals. Total counts were the standard technique before the 1960s (Caughley and Sinclair 1994). However, we will rarely be able to count all individuals of a species in a determined study area (Wilson et al. 1996). This technique has some flaws and it is difficult to determine how many individuals were missed. Given the cost and the inaccuracies, this technique has been generally abandoned. The only condition where this technique is still used, and is actually useful, is when the size of the area is relatively small and the species to be counted is very conspicuous. Some conspicuous species such as hippopotami (*Hippopotamus amphibious*) and African elephants (*Loxodonta africana*) can be accurately counted, especially from the air.

Forest species and species that conceal themselves despite living in open areas are almost impossible to include under this technique. Thus, most deer species from Latin America cannot be the subject of a total direct count, as some individuals will be counted twice and others will be missed. To be as accurate as possible, large crews are needed, and the area considered has to be scanned with care. This technique is not recommended for species with high mobility such as deer species. However, a tame population would allow the presence of people without much disturbance.

This technique, despite missed individuals, was used for *Odocoileus* species in the United States up to 1950, and the number obtained was considered as the minimum population size in the area, or the known-to-be-alive estimate (Caughley and Sinclair 1994). This resulted in an underestimate of the population but that is better than an overestimate, which may lead to wrong conservation conclusions and/or inappropriate management. When attempting a total count for deer species, the main

assumption, that all individuals are observable, is not met and a proportion of the population is not counted. The effort has to be too large and too expensive to obtain an accurate count that will fit within determined confidence limits. Thus, despite the fact that the total count is hard to achieve, the technique can be used and may be the most appropriate if we clearly understand that only a proportion of the population will be counted, and that the number obtained will mean at least the population is that large. For conservation and management purposes, it is safe to use the counted number.

Population Indices

All species leave signs of their presence in a variety of ways (Wemmer et al. 1996). There are different signal categories – visual, olfactory or auditory – and the capability to recognize them depends on a series of attributes, such as biological knowledge of the species and field experience.

Some of those signs can be used as indices of population size. An index of population size for a species is a manifestation or sign that will change with a change in population size. That is, if the population decreases or increases in size, the index will change in a predictable way that will indicate that decrease or increase. The calculation of population indices is useful and inexpensive.

Although indices are not as accurate as other counting methods, they may be the most reliable methods for estimating the populations of some species. The main reason for using an index instead of counting is usually the difficulty in observing, counting or capturing the individuals, because of their habits or size (Wilson et al. 1996). Another reason is that sometimes only a measurement of relative abundance is needed. The relationship between the index and the population size is usually positive. However, it may vary depending on weather or habitat. For example an index based on sightings will absolutely vary depending on weather conditions (Wilson et al. 1996).

Presence Absence Index: The most simple of all indices, is an index of presence or the lack of it, which will suggest absence. To use a presence / absence index, we should find a clear indicator that will imply that there is evidence for the presence of the species in a determined area. Once the evidence is found, the presence of the species is confirmed. However, the absence of evidence should not be taken as evidence of absence of the species in a determined area. This index is very useful and it is required for endangered species whose presence in a determined area is in doubt. For deer species, finding the remains of individuals is the most reliable evidence and the only one that will not lead to uncertainties in the situation in which more than one species occurs in an area. A cast antler is an example of hard evidence of the presence of a deer species in a determined area.

The presence / absence index will only provide evidence of the presence of a species in a determined area. However, the evidence used to determine the presence of a species in an area could be used numerically to estimate the population in a specific area. For this purpose, we need a previous study on the relationship

between the number of pieces of evidences and the total population size. The numerical relationship between the index of presence and the actual abundance should be positive. The association might not be direct, that is, a straight line, and sometimes will be graphed as a curve. A direct relationship will indicate that doubling the pieces of evidence means doubling the total population in an area.

Tracks: the presence of tracks positively indicates the presence of a specific species in an area. Numerically, some relationships have been calculated between the number of tracks and the number of individuals, but those relationships are species specific, locality specific and season specific. We need to know certain traits from the species and habitat to be studied to determine any relationship. For example, if the area A has the same number of tracks as the area B, it does not mean that it has the same population size, as the number of tracks in area A may be related to food scarcity and the need to walk for longer periods. Also, the permanence of a track is related to soil material and to weather conditions. However, track counts have proven accurate as an index of abundance in some circumstances, but the authors admitted that the relationship was not constant over time or areas (Wilson et al. 1996).

In the case of deer species, tracks are very similar among species of the same size and are, therefore, hard to use in areas where more than one species coexists. The best way to use tracks as an index may be related to the counting of deer paths crossing a surveyed trail. It will be best to relate number of paths in a determined length of surveyed transect or trail to population density or size. To achieve an accurate relationship, several surveys will be needed prior to the estimation of how many paths mean how many individuals. Therefore, if a direct and positive relationship between number of tracks and abundance is achieved, track counts are best used to infer relative differences in abundance between successive counts through time. For example, if the goal is to determine population trends, track counts can be used to determine sequential indices, inferring relative differences in abundances.

Pellet groups: the presence of pellet groups also positively indicates the presence of a specific species in an area. As with tracks, some numerical relationships have been calculated between the number of pellet groups and the number of individuals. Those relationships are also species specific, locality specific and season specific. The duration of a specific pellet group depends on weather conditions and the speed of coprophagous insects (Wiles 1980). In dry areas pellets will endure longer than in wet areas, and the same applies for seasons. However, the individual defecation rate is more constant than the distance traveled per day per individual, and can be more accurately calculated for specific seasons and areas. The procedures to estimate population sizes and determine population changes are described in White and Eberhardt (1980).

For deer species, some indices have been determined in the past, e.g. Eberhardt and van Etten (1956) for white-tailed deer. However, most research and

relationships have been done for deer in temperate environments. Any research on defecation rates of South American deer species will provide a baseline for the estimation of population sizes in specific areas. As a general rule the pellet group count is reliable as an index for population size given that the defecation rate is known.

The cervids that belong to the gender *Mazama* usually defecate in latrines accumulating faecal pellets and urine of one or more individuals (Black-Decima 2000; Black-Decima and Santana 2002; MacNamara and Eldridge 1987; Vogliotti 2003). Although the cervid fecal shape is relatively homogeneous for each species, this can vary in quantity, size and consistence, depending on the animals' diet. So, to confirm the species identification it is necessary to have other evidence such as marks, footprints and pictures using camera traps. To make abundance estimates it is necessary to consider that the dung degrades over time, and control the new pat in the area.

To perform a population estimate dung can be counted in the transects or in plots randomly distributed in the area (Sutherland 2000; Wemmer et al. 1996). The dung found can be marked or removed to be distinguished from the new ones in the next survey (Sutherland 2000). Assuming a daily rate of defecation for a specific species, it is possible to estimate the number of animals in the plot, which will be converted in animals per unit of area.

Prudence should be applied if a study on habitat use is based on pellet-group counts, as the species may be spending more time in a different habitat type than the one used for defecation. Also, detection of pellet groups may differ among habitat types (Wilson et al. 1996). Before any conclusion is reached the connection between defecation rate and habitat type has to be determined (Wilson et al. 1996).

Local people accounts: oral communications from local people can be used as an index of presence / absence, and as an index of relative abundance. Some people living in rural areas close to the distribution range of a species spend the whole year in areas where researchers only spend limited time. Therefore, local people have usually more data on a species researchers are beginning to study. However, we should be cautious with the behavioral accounts, and even on the presence of a determined species. Local people sometimes separate males and females as species or consider antlered individuals as two different species depending on the stage of the antler, whether it is in velvet or hard antler.

When listening to local accounts to determine the presence of a species in an area, we should never instigate or press local people to say what we want to hear. It is best to start talking about animals in general and see where we arrive. Frequently, they will describe the species we are looking for, and eventually they may describe behaviors or specific patterns unknown to us.

Capture-Recapture Methods

Capture-recapture (or mark-recapture, tag-recapture, sight-resight, capture-mark-recapture) methods, hereafter referred to as CR, have been widely used to estimate population size. General reviews of models and

assumptions have been presented. The reader is referred to Otis et al. (1978); Seber (1982); White et al. (1982) and Borchers et al. (2002) for more details on assumptions, statistical modeling and examples.

CR methods rely on sampling a proportion of the population of interest, marking and releasing this sample back into the range of the population, allowing a time interval for marked and unmarked (not sampled) individuals to mix, and then sampling the population again. The new sample reveals the proportion of animals marked, which is then used to estimate population size. CR methods assume that the number of individuals marked in the population is known. The loss of marks (or death of individuals in the case of closed models – see below) violates this assumption and causes bias in the estimates. They also assume that the marked and unmarked individuals have the same likelihood of being observed or captured.

CR techniques can be broadly divided into two major categories: closed and open models. The former assume that, during the survey period, animals do not move in or out of the study area (geographic closure), and that there is no mortality or recruitment in the population of interest (demographic closure). The most simple mark-recapture model is the two capture occasion Lincoln-Petersen estimator (Chapman 1951; Seber 1982; White et al. 1982). Models with multiple (>2) capture occasions and different parameterizations are preferable to test assumptions of varying capture probabilities (e.g. Burnham and Overton 1978; Darroch 1958; Schnabel 1938).

Specific CR abundance estimators are often needed for radio collared (tagged) animals, when sightings of animals are used to generate the recapture for population size estimates. White and Garrot (1990, Chapter 10) provide a series of population size and variance estimators and discuss the preferred estimator for experiments conducted under these circumstances.

Sampling periods (occasions) for closed population models must be short-term relative to the life span of the species of interest to ensure that the assumptions of demographic and geographic closure hold. An increase in the length of the sampling intervals increases the probability that birth, death, emigration and immigration will affect (bias) the population size estimates. Thus open population models are more appropriate in this case. Open population models do not require demographic closure and allow for the estimation of immigration, emigration, mortality and birth rates (e.g. Pollock et al. 1990; Seber 1982). The most traditional open population CR model is the Jolly-Seber (Jolly 1965; Seber 1965). Unlike closed models, where only one estimate of population size is obtained, the Jolly-Seber model allows for one estimate per sampling occasion because the estimates will vary due to natural processes (e.g. birth and immigration), the capture probability and the data. Pollock et al. (1990) observed that heterogeneity in capture probabilities could cause important negative bias in population size estimates with the Jolly-Seber model, particularly when capture probabilities are low.

A relatively new field in CR studies is the use of combined closed and open population models. Combined

models offer the benefit that data from separate designs are combined to improve precision of parameter estimation and that new parameters may be identified (Lindberg and Rexstad 2002). One example of a combined model is the Robust Design (Kendall et al. 1995; Pollock 1982), which was formulated with open, primary sampling occasions and closed, secondary occasions. Abundance is estimated in the closed component of the model and survival and immigration/emigration rates between the primary occasions.

Software: Software is available for estimating population size with CR approaches. The program CAPTURE (Rexstad and Burnham 1992) calculates population size estimates for eleven different models for closed populations and computes tests for model selection following the methods described by Otis et al. (1978).

A relatively new, more sophisticated, Windows based software is MARK (White and Burnham, 1999). MARK incorporates CAPTURE and therefore calculates population size from closed models. It also implements open population models for estimating abundance (e.g. Jolly-Seber) and different variations of the Robust-Design. MARK presents a variety of other open population models to estimate survival, birth, movement rates and other parameters. MARK allows for modeling of covariates to improve parameter estimation. The model selection is based on the Akaike Information Criterion and model averaging procedures are available.

Program NOREMARK provides four different estimators to calculate population size with a known number of marked animals and one or multiple resighting occasions (White, 1996). It also contains simulation procedures for determining estimator performance and for calculating sample sizes.

Line transect

Developed in the late 70's (Burnham et al. 1980; Buckland et al. 2001), the line transect methodology (LTS) has been widely used to estimate animal abundance in the past 30 years. LTS is a variation of Distance Sampling, which also includes point transect sampling. The reader is referred to Buckland et al. (2001) for the standard uses of Distance Sampling. Advanced techniques are presented in Buckland et al. (2004).

Software DISTANCE (Thomas et al. 2009) is available for estimating abundance with line transect sampling.

In LTS, one or more observers travel along a line and record (count) the objects of interest that are detected (seen) (Fig. 5). Different platforms can be used (e.g. on foot, by car, motorcycle, aircraft, etc.) to estimate deer abundance. These platforms will produce different possible effects on the count and, consequently, in the results. The perpendicular distance (or radial distance and angle) from the object to the line (or to the observer) is measured or estimated. Perpendicular distance data is used to estimate the probability of detecting an object given its distance from the line, which can be converted into the effective strip half-width (ESW). ESW corresponds to the distance from the survey line where all objectives are effectively detected on each side of the line. Density is then estimated by dividing the number of objects counted

by the observer(s) by the area effectively surveyed (2 times ESW times the length of the survey line). If objects occur in clusters (groups), density is then multiplied by the average cluster size. Population size is then obtained by multiplying density by the total area surveyed. An example is given by the use of LTS to evaluate the impact of a flooding dam on marsh deer population (Andriolo et al. 2005).

Three assumptions are necessary for reliably estimating populations size with LTS methods (Buckland et al. 2001):

- (1) All objects present on the survey line are detected with certainty (that is they are detected with probability equal to 1 or ' $g(0) = 1$ ');
- (2) There is no responsive movement from the object to the observer prior to detection (that is, animals don't move away or closer before being seen);
- (3) Distance (and angles, where relevant) are measured accurately.

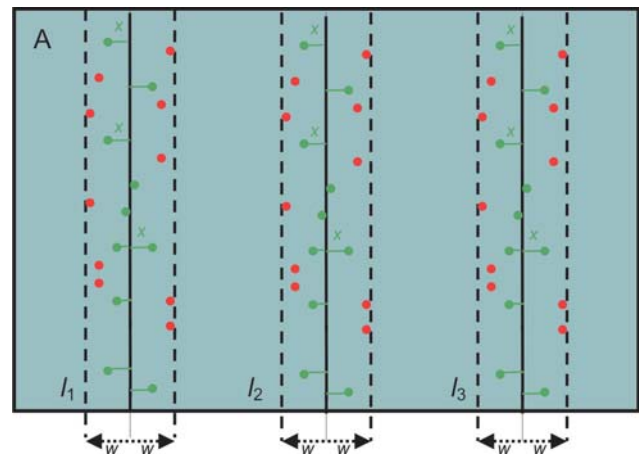


Figure 5 - An observer travels along the line (l_1, l_2, l_3) and records (counts) the objects of interest that are detected (green dots). The red dots are missed objects. The perpendicular distance from the object to the line (X) is measured or estimated. Strip half-width corresponds to the distance from the survey line where all objectives are effectively detected on each side of the line (W).

Failure to meet those assumptions will cause different degrees of bias in the estimates of population size. Detecting animals on the survey line with probability equal to one is rarely achieved in most wildlife surveys. Often animals are available to be detected but are missed by the observer (perception bias) or are hidden under the water, vegetation or under the ground and therefore not available for the observer (availability bias). The second assumption depends on the animals' responses to the observer, which may also depend on the ability of the observer to remain undetected, but is considered more difficult to control (Ward et al. 2004) (Fig. 6). Missed animals cause, often substantial, negative bias in the estimates. Methods to estimate the proportion of objects that are missed must be developed to avoid or minimize biases. Ward et al. (2004) evaluated the responses of roe deer (*Capreolus capreolus*) to the observer during distance sampling surveys in order to test compliance with the second assumption and discussed the implications of common

survey practices for density estimation. Such methods and analytical procedures are thoroughly discussed in Laake and Borchers (2004).



Figure 6 - Evasive behavior performed by a marsh deer due to the approach of an observer.

An implicit assumption of distance sampling is that the probability of detection of objects depends only on their distance from the line. In practice, however, several variables may cause detection probability to vary. For example, animals in large aggregations (groups) are easier to detect than solitary individuals. Therefore there is a greater probability of detecting larger groups. Buckland et al. (2001) presented two ways to reduce heterogeneity in detection probability caused by these variables. One is to stratify the data according to one or more covariates (e.g. group size categories) and then estimate detection probability separately for each stratum. Stratification can be done *a priori* if information is available about possible factors that affect probability of detecting an object, or *a posteriori* using information collected during sampling. The most recently developed approach is to directly incorporate covariates in the detection probability estimation procedure via a multi-variate function (e.g. Marques and Buckland 2003; Ramsey et al. 1987). This procedure is currently known as Multiple Covariate Distance Sampling (MCDS) and allows for the inclusion of covariates in the estimation of detection probability. The advantage of this method is that it prevents sample size reduction due to stratification and therefore minimizes bias and loss of precision. It also allows for several covariates to be incorporated into the estimation procedure (Marques and Buckland 2003).

NONINVASIVE GENETIC SAMPLING (more details in chapter 36)

The amazing biotechnological advances of recent years enabled the development of methodologies for population estimates based on noninvasive DNA sampling. In order to do this, hair, feathers and feces have been used as samples, from which the individuals in an area can be genetically identified and the population size estimated. Even though this kind of technology is very recent, its potential grows steadily as new studies use it to solve technical problems in collecting and analyzing data. Until now, only one study (not yet published) has used this new methodology with neotropical cervids. So the

reason this item was included in the chapter on population estimates is to call special attention to the possibilities this new approach offers, while shedding light on some key points that should be given attention by those who want to use it in their studies.

Genotyping Methods and Genotyping Error Avoidance

Although several molecular markers are available and can be used for individualization, microsatellite markers are the ones most commonly used for this purpose at the moment. Microsatellite markers are based on the amplification of repetitive regions using PCR. They are highly polymorphous and are easy to use with species for which they have already been developed. Despite the advantages, several authors have called attention to genotyping errors that occur while obtaining these markers (Bonin et al. 2004; Hoffman and Amos 2005). In addition to the general errors associated with manipulating samples and the data they generate, genotyping based on noninvasive samples can generate errors caused by the amplification and typing of very small quantities of DNA (Broquet and Petit 2004; Taberlet et al. 1996) and/or of low-quality DNA (Gerloff et al. 1995; Hoffman and Amos 2005). Genotyping errors can be defined as differences between two or more molecular genotypes obtained independently from the same sample. These happen mainly when an allele fails to amplify (allelic dropout) (Gagneux et al. 1997; Navidi et al. 1992; Taberlet et al. 1996; Walsh et al. 1992), or because false alleles are amplified due to errors that happen during PCR (Bradley and Vigilant 2002; Taberlet et al. 1996). These problems can lead to errors in the identification of individuals (Paetkau 2003; Taberlet and Luikart 1999) and distortions in population size estimates (Creel et al. 2003; McKelvey and Schwartz 2004). Several protocols have been developed in an attempt to avoid these problems, such as: repeated independent amplifications of DNA samples (Navidi et al. 1992); comparison of genotypes obtained from noninvasive samples with those obtained from blood or tissue (Fernando et al. 2003; Parsons 2001; Sloane et al. 2000); and the reamplification of loci where these errors potentially occur (Morin et al. 2001), among others. Even though it is technically difficult, we should strive to assess these errors during the investigation and report them later in scientific publications. By doing this, we will be forming a set of data that will make it possible in the future to better assess the effects of these errors on population estimates and improve methodologies that minimize the consequences of these errors. Besides, it is a way of making the results we obtain more precise and of validating them. For more information on ways of detecting and quantifying genotyping errors, see Bonin et al. (2004) and Broquet and Petit (2004).

Estimating Population Size Using Noninvasive Genetic Sampling

Several estimates and indexes are used to make population estimates from genetic data. Though there is a great variety of available methods, two are most

used: the capture-recapture method (Seber 1982), and rarefaction analysis (Eggert et al. 2003; Kohn et al. 1999).

In order to use the capture-recapture method, identical genotypes must be grouped into a report of capturing and recapturing (sampling periods), which should be determined by the idealized experimental design. For example, if sampling lasted one month, we can consider each week a sampling period. This way, the feces of an animal that is captured two or more times during the same period is counted as only one capture, and if it is captured during the following periods it is counted as recapturing. Data organized in this way is then analyzed as any other conventional type of data normally used in estimates based on the capture-recapture method, and can be analyzed with computer programs like MARK (White and Burnham 1999), which allows the effect of heterogeneity, temporal variation and difference between sexes to be incorporated into the probability of detecting the individuals.

In rarefaction analysis, population size is calculated by an asymptotic curve formed by the relation between the cumulative number of unique genotypes found and the number of samples analyzed in a certain area. Kohn et al. (1999) describes this curve with the equation $y = (ax)/(b + x)$, where a is the asymptote, x is the number of fecal samples, and b is the decline rate of the curve. Eggert et al. (2003) developed a similar estimate with the following equation: $y = a(1 - e^{-bx})$.

Papers that compare different methodologies are still practically nonexistent. In a recent paper that compared the methods we cited above, Bellemain et al. (2005) concluded that capture-recapture methodologies produced more reliable estimates. As new studies are done, differences between different methodologies and between noninvasive methods and those that are conventionally used to obtain population estimates will slowly be determined, producing a new group of procedures that will certainly be of great use to those who study wildlife populations.

Population Estimates of Neotropical Cervids by DNA Analysis of Feces – Case Study

Until now, only one study has used DNA analysis of feces to obtain population estimates of neotropical cervids. In this preliminary study (see summary in Duarte et al. 2005), fecal samples from cervids of the genus *Mazama* were collected from a 9.86 km² area in Intervalas State Park (in the state of São Paulo, Brazil) and typed by mitochondrial DNA analysis, in order to identify the species they belonged to. Three loci containing microsatellite regions were used to genotype and individualize the samples identified as belonging to the species *Mazama bororo*. The data this generated was used in a capture-recapture model to calculate the density of animals in the study area and to estimate the population size. The authors estimated a 1.44 animal / km² density in the sampled area. Extrapolating this number to the total of protected areas in the region where the species is presumed to be distributed, the estimate is that the total population of *Mazama bororo* is made up of around 5,500 individuals. Even though the study was a very simple

preliminary analysis, lacking larger and more careful sampling, the results show the potential of this technique, as it provided valuable information about a species that was practically unknown until now.

COMPARISON OF METHODOLOGIES AND THEIR APPLICATION TO DEER SPECIES

All methodologies are useful for deer species under determined circumstances but their applicability will be especially influenced by what we need to know. Also, the reason for using a specific method depends largely on the species and habitat. Here we summarize the application of various methodologies for population estimation to different Neotropical deer species.

The use of indices should be limited to studies where there is no need for a precise calculation of density or abundance. Indices are the best choice when assessing the presence of the species in a particular area and when assessing population trends. Another limiting factor on when to use indices is the economic one; if resources are limited, the best choice will be to use an inexpensive index to approximate the population size in a given area. However, the use of indices is not appropriate when a more precise number is needed (Conroy 1996). For small and secretive species such as *Pudu mephistophiles*, *P. puda*, *Mazama chunyi*, *M. rufina*, *M. bricenii*, *M. nana* and dwarf mountain subspecies of *M. americana*, the use of indices are the best choice to determine their presence and relative abundance in an area. Indices, then, could be useful for calculating population trends and even for approximating population sizes if the index can be related numerically to the density or total population.

Small deer living in dense humid forests along mountain chains would need a huge sampling effort to estimate their abundances by transect sampling or by indices based on direct observations. However, their populations could be accurately calculated by the mark-recapture methodology given a large enough sample size and a capture spot where several individuals converge. We should take into account that most of these species are probably territorial, as was suggested for all *Mazama* and *Pudu* species (Geist 1998), but with territory or home range size largely unknown for most of the species. The best way would be to deploy a large number of traps, probably close to a hundred and arrange them separately, covering a large area.

As was explained in the section with that name, the direct total count methodology will be expensive if we need accuracy. This methodology should be only applied to small areas and in open environments. Direct total counts were the rule for *Odocoileus* species fifty years ago, but only to determine the minimum population size (Rasmussen and Doman 1943). More recently, this method has been used for species living in open areas such as *Ozotoceros bezoarticus* and *Hippocamelus antisensis* but even then the count was restricted to small areas (Barrio 1999; Cosse 2002).

The method most recommended for calculating densities and populations for most deer species is transect sampling. The methodology is best applied to deer living in open landscapes including open forests. Among the

deer species that would be best sampled using this methodology we find *Odocoileus virginianus* and *O. hemionus*, *Ozotoceros bezoarticus*, *Hippocamelus antisensis*, *H. bisulcus*, *Mazama gouazoubira* and *Blastocercus dichotomus*. Transect sampling is also useful for medium sized species living in lowland rainforests, but where densities are high or the sampling effort is large. In some areas the method can be used for forest deer such as *Mazama americana*, *M. pandora*, *M. temama* and *M. nemorivaga*.

Comparing 3 methods (track count, pellet group, and direct count from transects, Mandujano and Gallina (1995) found the transect method to be more precise in the wet season, whereas the track and pellet counts produced more precise results in the dry season. Using the double-count procedure Mandujano (2005) generated 2 models in which track counts were calibrated to estimate the density of white-tailed deer in dry forest on the Mexican Pacific Coast. With the double sampling procedure, 2 equations were obtained that resulted from calibrating tracks counts on the basis of the density obtained with the line-transect sampling. He concluded that both models derived in this study are useful for monitoring the population. Special attention should be directed to the location of the transects, as they must be randomly distributed.

ASSESSMENT OF IMPACT

Aerial estimates have been widely used for doing population surveys with cervids all around the world. Linear transects are useful when studying the marsh deer, since they tend to live in flat open areas in wetlands and are widely distributed throughout the environment. Aerial surveys of marsh deer have been done since 1976 (Andriolo et al. 2005; Mauro et al. 1995; Schaller and Vasconcelos 1978). Even though they used different techniques, Mourão and Campos (1995) and Pinder (1996) arrived at similar estimates for marsh deer in the area influenced by the Porto Primavera dam, in the state of São Paulo, Brazil, by flying over the study area in helicopters. The results they obtained show that animals are denser in the remaining areas, which is no surprise, given the size of the population and the abrupt habitat loss that happened when the lake formed by the dam filled. However, in general, the total population in the northern region is smaller. This result also indicates the impact the lake, when it fills, has on the population, which is decreasing due to flooding, habitat loss, and an increase in hunting, etc (Andriolo et al. 2001).

MONITORING THE POPULATION

Population dynamics are very interesting phenomena to follow. Identifying the dynamics involved in the growth, decline or stability of the number of individuals in a population can answer important questions about how the population relates to the environment. For example, prey/predator relationships can be observed by monitoring both populations. The growth of one, and the consequent growth of the other, or vice-versa, can explain the dependence between them.

Many studies try to document the degree of change occurring in a population. When the issue is conservation, this kind of information is extremely relevant. Monitoring is done by repeated estimates of the same population. It is fundamental that the same technique be used in each stage of the study. If changing the technique becomes necessary, then there should be a period when both techniques are used at the same time, so that it will be possible to calculate the relative efficiency of each technique, and then the data can be calibrated.

Monitoring environmental variables in the study area is important so that the results of the estimates can be correlated with the changes in the population. Changes in environmental variables might be affecting the estimation technique directly. Andriolo et al. (2001) found that the probability of detecting marsh deer (*Blastocercus dichotomus*) was compromised when using aerial monitoring during the rainy season, since the animals sought higher areas (Fig. 7). These higher areas are small woods spread on the wetlands, and this hindered the sighting of the animals. After a forest fire burned Emas National Park (Brazil), Rodrigues (2003) took advantage of the fact that it was easy to observe the animals and used the line-transect methodology with one of the roads in the park to do a population estimate of the pampas deer.



Figure 7 - During the rainy season marsh deer sought higher areas changing detection probability.

The regularity with which estimates need to be done will depend on the question whose answer is being sought, as well as on the general characteristics of the population being studied. Some species have fast rates of growth and decline, so estimates would need to be done more frequently in order to appropriately follow their variations. For populations that change more slowly, less frequent monitoring would not compromise the study, and would also save time and work.

REFERENCES

- ANDRIOLO, A., U. PIOVEZAN, M. J. R. PARANHOS DA COSTA, J. L. LAAKE, AND J. M. B. DUARTE. (2001) Estimação aérea de abundância e distribuição do cervo-do-pantanal (*Blastocercus dichotomus*) na bacia do Rio Paraná, entre as barragens de Porto Primavera e Jupia, em avaliações pré e

- pós enchimento da primeira cota da UHE Sérgio Mota. in O cervo-do pantanal de Porto Primavera: Resultado de dois anos de pesquisas. (Duarte J.M.B. ed). CD-Rom, Fundação de Estudos e Pesquisas em Agronomia, Medicina Veterinária e Zootecnia, Jaboticabal, São Paulo, Brazil.
- ANDRIOLO, A., M. J. R. PARANHOS DA COSTA, U. PIOVEZAN, H. A. TORRES, AND J. M. B. DUARTE. 2003. Activity period of marsh deer (*Blastocerus dichotomus*) monitored by telemetry. *Revista de Etologia* 5:141-141.
- ANDRIOLO, A., U. PIOVEZAN, M. J. R. PARANHOS DA COSTA, J. L. LAAKE, AND J. M. B. DUARTE. 2005. Aerial line transect survey to estimate abundance of marsh deer (*Blastocerus dichotomus*) (Illiger, 1815). *Brazilian Archives of Biology and Technology* 48(5):807-814.
- BARRIO, J. 1999. Población y hábitat de la taruka en la Zona Reservada Aymara-Lupaca, Perú. Pp. 453-460 in Manejo y Conservación de Fauna Silvestre en América Latina (T. Fang, O. Montenegro and R. Bodmer eds.). La Paz, Bolivia.
- BELLEMAIN, E., J. E. SWENSON, D. TALLMON, S. BRUNBERG, AND P. TABERLET. 2005. Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. *Conservation Biology* 19:150-161.
- BLACK-DECIMA, P. 2000. Home range, social structure, and scent marking behavior in brown brocket deer (*Mazama gouazoubira*) in a large enclosure. *Mastozoologia Neotropical* 7:5-14.
- BLACK-DECIMA, P., AND M. SANTANA. 2002. Brocket deer identify and scent mark foreign dung in dung piles. *Animal Behavior*, Available in: <<http://www.animalbehavior.org/ABS/Media/AbstractStatus.html>> Access in: 30 April 2005.
- BONIN, A., E. BELLEMAIN, P. B. EIDENSEN, F. POMPANON, C. BROCHMANN, AND P. TABERLET. 2004. How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* 13:3261-3273.
- BORCHERS, D., S. T. BUCKLAND, AND W. ZUCCHINI. 2002. Estimating Animal Abundance. Closed Populations. Springer-Verlag, London, U.K.
- BRADLEY, B. J., AND L. VIGILANT. 2002. False alleles derived from microbial DNA pose a potential source of error in microsatellite genotyping of DNA from faeces. *Molecular Ecology Notes* 2:602-605.
- BROQUET, T., AND E. PETIT. 2004. Quantifying genotyping errors in noninvasive population genetics. *Molecular Ecology* 13:3601-3608.
- BUCKLAND, S. T., D. R. ANDERSON, K. P. BURNHAM, J. L. LAAKE, D. L. BORCHERS, AND L. THOMAS. 2001. Introduction to Distance Sampling. Estimating abundance of biological populations. Oxford University Press, New York.
- BUCKLAND, S. T., D. R. ANDERSON, K. P. BURNHAM, J. L. LAAKE, D. L. BORCHERS, AND L. THOMAS. 2004. Advanced Distance Sampling. Estimating abundance of biological populations. Oxford University Press, New York.
- BURNHAM, K. P., AND W. S. OVERTON. 1978. Estimation of the size of a closed population when capture probabilities vary among animals. *Biometrika* 65:625-634.
- BURNHAM, K. P., D. R. ANDERSON, AND J. L. LAAKE. 1980. Estimation of density from line transect sampling of biological populations. *Wildlife Monographs* 72:1-202.
- BURT, W. H. 1943. Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy* 24:346-352.
- CAUGHLEY, G. 1977. Analysis of vertebrate populations. Wiley & Sons, London, U.K.
- CAUGHLEY, G., AND A. SINCLAIR. 1994. Wildlife Ecology and Management. Blackwell Science, Cambridge, Massachusetts, USA.
- CHAPMAN, D. G. 1951. Some properties of the hypergeometric distribution with applications to zoological censuses. University of California Publications in Statistics 1:131-60.
- CONROY, M. 1996. Abundance Indices. Pp. 179-192 in Measuring and monitoring biological diversity. Standard Methods for Mammals (D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran and M. Foster, eds.). Smithsonian Institution Press, Washington, D. C.
- COSSE, M. 2002. Dieta y solapamiento de la población de venado de campo "Los Ajos", (*Ozotoceros bezoarticus* L, 1758) (Artiodactyla: Cervidae). Master thesis, Universidad de la República, Montevideo, Uruguay.
- CREEL, S., et al. 2003. Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. *Molecular Ecology* 12:2003-2009.
- DARROCH, J. N. 1958. The multiple-recapture census: I. Estimation of a closed population. *Biometrika* 45: 343-359.
- DUARTE, J. M. B., et al. 2005. Distribution and abundance of the small red brocket deer (*Mazama bororo* Duarte, 1996): Is it an endangered species? p.62 in Annals of 19th Annual Meeting of the Society for Conservation Biology.
- EBERHARDT, L., AND R. VAN ETTEN. 1956. Evaluation of the pellet group count as a deer census method. *Journal of Wildlife Management* 20:70-74.
- EGGERT, L. S., J. A. EGGERT, AND D. S. WOODRUFF. 2003. Estimating population sizes for elusive animals: forest elephants of Kalum National Park, Guana. *Molecular Ecology* 12:1389-1402.
- FERNANDO, P., T. N. C. VIDYA, C. RAJAPAKSE, A. DANGOLLA, AND D. J. MELNICK. 2003. Reliable noninvasive genotyping: fantasy or reality? *Journal of Heredity* 94:115-123.
- GAGNEUX, P., C. BOESCH, AND D. S. WOODRUFF. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Molecular Ecology* 6:861-868.
- GEIST, V. 1998. Deer of the World: Their Evolution, Behavior, and Ecology. Stackpole Books, Mechanicsburg, Pennsylvania.
- GERLOFF, U., C. SCHLOTTERE, K. RASSMAN, et al. 1995. Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). *Molecular Ecology* 4:515-518.
- HOFFMAN, J. I., AND W. AMOS. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology* 14:599-612.
- JOLLY, G. M. 1965. Explicit estimates from capture-recapture data with both death and immigration stochastic model. *Biometrika* 52:225-247.
- KENDALL, W. L., K. H. POLLOCK, AND C. BROWNIE. 1995. A likelihood-based approach to capture-recapture estimation of demographic parameters under the robust design. *Biometrics* 51:293-308.
- KOHN, M. H., E. C. YORK, D. A. KAMRADT, G. HAUGHT, R. M. SAUVAJOT, AND R. K. WAYNE. 1999. Estimating population size by genotyping feces. *Proceedings of the Royal Society of London B* 266:657-663.
- KREBS, C. J. 1972. Ecology: the experimental analysis of distribution and abundance. Harper & Row Publishing, New York .
- KREBS J. R., AND N. B. DAVIES .1981. An introduction to behavioural ecology. Blackwell Scientific Publications, Oxford, United Kingdom.
- LAAKE, J. L., AND D. L. BORCHERS. 2004. Methods for incomplete detection at distance zero. Pp.108-189 in Advanced

- Distance Sampling (S.T. Buckland, D.R. Anderson, K.P. Burnham, J.L. Laake, D.L. Borchers and L. Thomas, eds.). Oxford University Press, New York.
- LINDBERG, M., AND E. REXSTAD. 2002. Capture-recapture sampling designs. Pp.251-262 in Encyclopedia of Environmetrics (A.H. El-Shaarawi and W.W. Piegorisch, eds.). Wiley, Chichester, U.K.
- MACNAMARA, M., AND W. ELDRIDGE. 1987. Behaviour and reproduction in captive pudu (*Pudu pudu*) and red brocket (*Mazama americana*), a descriptive and comparative analysis. Pp.371-387 in Biology and management of the Cervidae. (C.M. Wemmer, ed.). Smithsonian Institution Press, Washington, D.C.
- MANDUJANO, S., AND S. GALLINA. 1995. Comparison of deer censusing methods in tropical dry Forest. Wildlife Society Bulletin 23(2):180-186.
- MANDUJANO, S. 2005. Track count calibration to estimate density of White-tailed deer (*Odocoileus virginianus*) in Mexican dry Tropical Forest. The Southwestern Naturalist 50(2):223-229.
- MARGALEF, R. 1974. Ecologia. Omega. Barcelona, Spain.
- MARQUES, F. F. C., AND S. T. BUCKLAND. 2003. Incorporating covariates into standard line transect analyses. Biometrics 59:924-935.
- MAURO, R.A., G.M. MOURÃO, M. PEREIRA DA SILVA, M.E. COUTINHO, W.M. TOMAS AND W.E. MAGNUSON 1995. Influência do habitat na densidade e distribuição de cervo (*Blastocerus dichotomus*) durante a estação de seca no pantanal Mato-Grossense. Revista Brasileira de Biologia, 55(4):745-751.
- MCKELVEY, K. S., AND M. K. SCHWARTZ. 2004. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. Journal of Wildlife Management 68:439-448.
- MORIN, P. A., K. E. CHAMBERS, C. BOESCH, AND L. VIGILANT. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). Molecular Ecology 10:1835-1844.
- MOURÃO, G. AND Z. CAMPOS 1995. Survey of broad-snouted caimam *Caiman latirostris*, marsh deer *Blastocerus dichotomus* and capybara *Hydrochoerus hydrochaeris* in the area to be inundated by Porto Primavera Dam, Brasil. Biological Conservation, 73: 27-31.
- NAVIDI, W., N. ARNHEIM, AND M. S. WATERMAN. 1992. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. American Journal of Human Genetics 50:347-359.
- ODUM, E.P. 1972. Ecologia. Interamericana. Mexico.
- OTIS, D. L., K. P. BURNHAM, G. C. WHITE, AND D. R. ANDERSON. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62:1-135.
- PAETKAU, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12:1375-1387.
- PARSONS, K. M. 2001. Reliable microsatellite genotyping of dolphin DNA from faeces. Molecular Ecology 1:341-344.
- PINDER, L. 1996 Marsh Deer *Blastocerus dichotomus* population estimate in the Paraná River, Brasil. Biological Conservation, 75, 87-91.
- POLLOCK, K. H. 1982. A capture-recapture design robust to unequal probability of capture. Journal of Wildlife Management 46:752-757.
- POLLOCK, K. H., J. D. NICHOLS, C. BROWNIE, AND J. E. HINES. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs 107:1-97.
- RAMSEY, F. L., V. W. WILDMAN, AND J. ENGBRING. 1987. Covariate adjustments to effective area in variable-area wildlife surveys. Biometrics 43:1-11.
- RASMUSSEN, D. I., AND E. R. DOMAN. 1943. Census methods and their application in the management of mule deer. Pp. 369-380 in Transactions of the 8th North American Wildlife Conference. American Wildlife Institute, Washington, D.C.
- RODRIGUES, F. H. G. 2003. Estimating Pampas Deer Population in Emas National Park, Brazil. Deer Specialist Group News 18:10-12.
- SCHALLER, G.B. AND J.M. VASCONCELOS 1978. A marsh deer census in Brazil. *Oryx*, 14:345-351.
- SCHNABEL, Z. E. 1938. The estimation of a total fish population in a lake. American Mathematical Monthly 45: 342-358.
- SEBER, G. A. F. 1965. A note on the multiple recapture census. Biometrika 52:249-259.
- SEBER, G. A. F. 1982. The Estimation of Animal Abundance and Related Parameters. 2nd ed. Edward Arnold, London, United Kingdom.
- SLOANE, M. A., P. SUNNUCKS, D. ALPERS, B. BEHEREGARAY, AND A. C. TAYLOR. 2000. Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. Molecular Ecology 9:1233-1240.
- SUTHERLAND, W. J. 2000. The conservation handbook: research, management and policy. Blackwell Science, Oxford, United Kingdom.
- TABERLET, P., et al. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189-3194.
- TABERLET, P., AND G. LUIKART. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68:41-55.
- VOGLIOTTI, A. 2003. História Natural de *Mazama bororo* (Artiodactyla; Cervidae) através da etnozootologia, monitoramento fotográfico e radiotelemetria. Master thesis, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, São Paulo, Brazil.
- WALSH, P. S., H. A. ERLICH, AND R. HIGUCHI. 1992. Preferential amplification of alleles: mechanisms and solutions. PCR Methods Applications 1:241-250.
- WARD, A., P. C. L. WHITE, AND C. H. CRITCHLEY. 2004. Roe deer *Capreolus capreolus* behaviour affects density estimates from distance sampling surveys. Mammal Review 34(4): 315-319.
- WEMMER, C., T. KUNZ, G. LUNDIE-JENKINS, AND W. MCSHEA. 1996. Mammalian Sign. Pp. 157-176 in Measuring and monitoring biological diversity. Standard methods for mammals (D.E. Wilson, F.R. Cole, J.D. Nichols, R. Rudran and M. Foster, eds.). Smithsonian Institution Press, Washington, DC.
- WHITE, G. C., AND L. E. EBERHARDT. 1980. Statistical analysis of deer and elk pellet-group data. Journal of Wildlife Management 44:121-131.
- WHITE, G. C., D. R. ANDERSON, K. R. BURNHAM, AND D. L. OTIS. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory Rep. LA-8787-NERP, Los Alamos, New Mexico, USA.
- WHITE, G. C., AND R. A. GARROTT. 1990. Analysis of wildlife radio-tracking data. Academic Press, San Diego, CA.
- WHITE, G. C. 1996. NOREMARK: population estimation from mark-resighting surveys. Wildlife Society Bulletin 24:50-52.
- WHITE, G. C., AND K. P. BURNHAM. 1999. Program MARK: Survival estimation from populations of marked individuals. Bird Study Supplement 46:120-138.

WILES, G. 1980. Faeces deterioration rates of four wild ungulates in Thailand. *Natural History Bulletin of the Siam Society* 28:121-134.

WILSON, D. E., F. R. COLE, J. D. NICHOLS, R. RUDRAN, AND M. FOSTER, eds. 1996. *Measuring and monitoring biological diversity. Standard methods for mammals.* Smithsonian Institution Press, Washington D.C., U.S.A., and London, U.K.

SOFTWARE

Program CAPTURE (REXSTAD AND BURNHAM, 1992), MARK (WHITE AND BURNHAM, 1999), NOREMARK (WHITE, 1996) and their respective instruction manuals and relevant documentation can be freely downloaded from the Fish and Wildlife Biology Department at the Colorado State University, USA. The website is: <http://www.cnr.colostate.edu/~gwhite/software.html>.

POPAN implements the Jolly-Seber models and provide estimates of population size. Free downloads are available from the University of Manitoba, Canada, at <http://www.cs.umanitoba.ca/~popan/>.

DISTANCE (THOMAS, L., J. L. LAAKE, E. REXSTAD, S. STRINDBERG, F. F. C. MARQUES, S. T. BUCKLAND, D. L. BORCHERS, D. R. ANDERSON, K. P. BURNHAM, M. L. BURT, S. L. HEDLEY, J. H. POLLARD, J. R. B. and T. A. MARQUES, 2009) is currently the standard software used to estimate abundance with line transect sampling. The software and manual are available for free download from the Research Unit for Wildlife Population Assessment at the University of St. Andrews, Scotland, at the following website: <http://www.ruwpa.st-and.ac.uk/distance/>.