1. COURSE NAME & CREDIT LOAD
COURSE CODE: WRM 503
COURSE TITLE: Wildlife Management Techniques
NUMBER OF CREDITS: 2 Credits
COURSE DURATION: Three hours per week for 12 weeks (36 hours)
As taught in (2009/2010) session (2 hrs for Lectures and 1 hr for Practicals).

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3. COURSE DETAILS
3.1 Course Synopses:
Observation and records, capturing and marking wild animals, necropsy in birds and mammals, physiological indices of reproduction, sex and age structure, estimating population, habitat study, improvement and evaluation, elementary telemetry, human factors in wildlife management.

3.2 Course note:

WRM 503

Field Work in Mammalogy

Field investigations conducted by mammalogists have been fundamental to the accumulation of our current knowledge on the biology of mammals. Field studies enhance our understanding of the complexities of mammalian relationships in time, space,within and among species, and with other components of the biotic and abiotic environment. Knowledge gained from field studies of mammals provides a basis for prudent decisions regarding the welfare and survival of all mammals, including ourselves.

In its simplest form, field work consists of direct observation of free-ranging mammals under natural conditions.However, most species of mammals are secretive, nocturnal, or both and, thus, are not suited for study by direct observation. Furthermore, most kinds of information and data used in mammalogical research cannot be obtained by simple observation. Therefore, the objectives of most studies mandate that individual mammals be captured one or more

times. Hence, these guidelines for the capture, handling, and care of mammals apply to virtually all field research involving mammals.

Why Mammalogists Collect Specimens

Research and teaching in mammalogy typically involve both the judicious collection and live capture of mammals in the field. Information obtained not only permits accurate identification of species, but also contributes to our understanding ofsystematic and evolutionary relationships among species, various genetic phenomena, population dynamics, community structureand dynamics,comparative anatomy and physiology, behavior,parasites and diseases, economic importance, geographic and microhabitat distributions, ecology of mammals in their natural or managed environments, and other scientifically important phenomena. Advances in the science of mammalogy foster the growth of other disciplines, and vice versa, and help to formulate management policies for game and nongame species, endangered species, economically important species, conservation of habitats,ecosystem analysis, control measures for pest and disease-bearing species, management of predators, and domestication of species.

Many mammals (or parts thereof) that are collected in the field eventually are deposited in natural history museums or biological banks. Museums are managed repositories for whole specimens and their parts, whereas biological banks are collections of histologically or cryogenically preserved organs,sera, tissues (including live cultures), cells (including gametes), or embryos. Both kinds of repositories permit qualified researchers to study specimens in these collections(Yates, 1996), and many are linked electronically. Such collections are invaluable as sources of research materials for use in current and future scientific investigations. Voucher specimens should be retained from those field investigations in which animals are killed or salvaged. These specimens (including any tissues, parasites, etc.) should be deposited in museum collections that meet standards established by the American Society of Mammalogists (Committee on Systematic Collections,1978) for curation of such collections so that they will be available for use by future investigators.

What is an Adequate Sample?

Researchers in mammalogy need to obtain samples of sufficient size to permit them to answer questions and test hypotheses. An adequate sample, therefore, may be defined as the number of specimens or other data needed to ensure empirical and statistical validity. The sample size required for a study will depend on the nature of the research and the extent of variation in the organisms and parameters being studied. In general, field studies require larger samples than laboratory studies because field investigators have less control over

conditions (both biotic and abiotic) that produce variation. Furthermore, natural populations exhibit considerably greater individual variation than highly inbred and genetically more uniform laboratory stocks. For certain anatomical studies and cladistic analyses, one or two specimens, or parts thereof, may comprise an adequate sample; however, much larger samples generally are necessary for research involving population and community phenomena and for environmental monitoring. Computer modeling, simulation, and appropriate statistical methods sometimes can reduce the number of individuals required for an adequate sample, as can use of specimens preserved in museum collections. However, objectives of research may require that additional specimens be collected. For this purpose, the investigator should collect no more specimens than needed and should be prepared to explain or justify why a particular sample size is required. Nevertheless, care should be taken to ensure that sample sizes are large enough to address any questions being asked with a high degree of statistical rigor.

Sampling in Threatened Habitats

In many parts of the world where mammals are poorly known, natural habitats are experiencing rapid and widespread destruction and many species of mammals only remain in small patches of habitat. Efforts to protect indigenous species often are dependent on our ability to learn which species are present and to gather basic information about their habitat requirements, systematics, distribution, ecology, anatomy, physiology, and reproduction. Such basic information can lead to action that promotes the survival of these and other ecologically associated species, some of which may be unknown to science. However, scientists studying mammals in threatened habitats must proceed with sensitivity and careful judgment so that populations under study will not be affected adversely by the studies that are intended to help protect them. Concern for the welfare of the species being studied should be foremost. This issue is especially important in many areas of the tropics where data on the natural history of resident species may be extremely limited. In such cases, initial studies involving removal trapping often are necessary. The investigator must design sampling procedures that minimize the likelihood that populations will suffer any significant damage. To achieve this, we recommend that: 1) no more than a small percentage of the habitat be trapped; 2) sampling sites be well-separated from one another so that recolonization can take place easily from surrounding populations; and 3) under circumstances where animals are collected for preservation as museum specimens, all reasonable efforts should be made to collect as much information as possible from each animal. In all cases, the investigator must be prepared to cease the relevant portion of the sampling if there is evidence that populations of a given species are being adversely affected. Once basic information on

relative abundance and habitat use is obtained, sampling procedures should be refined to answer specific questions and to avoid causing stress to vulnerable populations.

COMPLIANCE WITH LAWS AND REGULATIONS

Although the focus of this section is on federal and state regulations in the United States, researchers in mammalogy, regardless of nationality or location of their research, should be aware that wherever they are working, there may be local, state/provincial, federal/national, or international laws or regulations that pertain to scientific collecting, transport, and possession of specimens or parts thereof, or other activities involving native species of mammals. Therefore, each mammalogist must have knowledge of, and comply with, all relevant laws and regulations pertaining to the field collection of mammals. Ignorance of the law or even inadvertent violation of regulations may result in prosecution (Choate and Genoways, 1975).Federal regulations in the United States pertaining to collection, import, export, and transport of scientific specimens of mammals previously were reviewed by Genoways and Choate (1976). Researchers based in or conducting research in the United States must obtain permits issued by various federal agencies for the following purposes: 1) to import or export specimens of non-endangered species through a non-designated port of entry; 2) to import or export endangered wildlife through any port; 3) to import injurious wildlife; 4) to import, export, ship interstate, take, or possess endangered species or parts thereof for research or propagation; 5) to take, harass, possess, or transport marine mammals; 6) to import or transfer etiological agents or vectors of human disease and living non-human primates; 7) to collect scientific specimens on national wildlife refuges; 8) to import ruminants and swine, including parts, products, and by-products; and 9) to import organisms or vectors, tissue cultures, cell lines, blood, and serum. When moving specimens into or out of the United States, researchers should always request and file United States Fish and Wildlife Service (FWS) form 3-177 and any necessary permits from the Convention on International Trade in Endangered Species (CITES), if specimens are listed by CITES or the United States FWS. Mammalogists working outside the United States should expect similar regulations in other countries and should take steps to ensure that they comply with all applicable regulations dealing with species of special concern.

Mammalogists must ascertain whether additional permits are needed when they review the state/provincial and federal/national laws and regulations that relate to their planned field investigations. Investigators must be familiar with the current list of mammalian species deemed threatened or endangered and must comply with all rules and regulations pertaining to capture of these and all other categories of

mammals. A list of threatened or endangered species/subspecies under the United States Endangered Species Act is available from the Office of Endangered Species, United States Department of the Interior, Fish and Wildlife Service, Washington, DC 20240. Regulations relating to these taxa are published in the Code of Federal Regulations, Title 50, Chapter 1. The Federal Register publishes amendments to regulations under Title 50. Most states and provinces now require scientific collecting permits, and mammalogists must comply with this requirement and other regulations imposed by agencies in the states or provinces in which they do field work. Lists of all mammals (as well as other animals and plants) that are regarded as threatened or endangered or are controlled by wildlife regulations in each of the 50 states and the United States Virgin Islands are published periodically (Berger and Neuner, 1981), together with the addresses and telephone numbers of conservation personnel who can respond to questions regarding regulations and permits.

Some cities, counties, agencies, and other organizations in the United States and most foreign countries have regulations regarding scientific uses of wildlife on lands under their jurisdiction. Compliance with these regulations is essential. Finally, permission of the owner, operator, or manager of privately owned land always should be obtained before commencing field work thereon.

Many institutions, as well as state, provincial and federal governments, have regulations or recommendations concerning the handling and sampling of rodents that may be carriers of serious human diseases. Investigators must ensure their own safety and that of their employees or students by understanding the disease carrying potential of the mammals they study, by taking appropriate safety precautions, and by complying with appropriate regulations (see HEALTH PRECAUTIONS).

METHODS FOR COLLECTING SPECIMENS

Live Capture

Researchers seeking to capture, mark, and release mammals have a special responsibility to both the integrity of their research and the animals they handle to be certain that their capture methods are humane and that animals are released in the best possible condition. Methods of live capture, primarily by trapping and netting, must be designed to keep captive animals alive, uninjured, well provisioned, and in comfortable microclimatic conditions while awaiting subsequent processing and release. Live traps of various sizes, shapes, designs, and materials are available from numerous commercial outlets (e.g., Sherman, Havahart, Longworth, Little Critter, National, and Tomahawk), or they can be custom-made. Live capture methods have the advantage of allowing non-target species or individuals (e.g., lactating females) to be released unharmed.

For non-fossorial mammals, live traps should enclose a volume adequate for movement therein of the target species; for fossorial mammals, trap diameter typically approximates that of the burrow (e.g., Baker and Williams, 1972). The trap mechanism should not inflict injury and should be effective in containing the captive so that it does not became stuck or partially held in the trap door. In certain circumstances, padded leghold traps may be appropriate for live-trapping larger mammals. Live traps must be checked frequently to prevent mortality and to maintain captive mammals in prime condition. Therefore, the number of traps set should be based on the number and energy of persons available to check them, the conditions of the study area, weather, and species of mammal being studied. The time interval between trap checks will depend on the type of live trap, type and activity of the mammals to be trapped, configuration of the traps, climate, and season. Typically, live traps for nocturnal species should be set before dusk and checked as soon as possible after dawn. They should be closed during the day after the morning check to prevent accidental capture of diurnal species. However, live traps for shrews should be checked ca. every 1.5 h to minimize mortality (Hawes, 1977; Michielsen, 1966), although Churchfield (1990) suggests that four visits per 24 h (e.g., dawn, midday, late afternoon, and evening) are sufficient. In general, live-trapping of Insectivora requires more frequent checks of traps due to the higher metabolism of these species. Special care also is required to maintain these species in captivity, even for short periods of time. During warm weather, live traps for diurnal species should be shaded or positioned so as to avoid full exposure to the sun and should be checked every few hours to prevent heat stress of captured mammals. During cold weather, energy demands of thermoregulation require that an adequate supply of food and nesting material be placed in live traps. Where disturbance of traps by raccoons or other animals is a significant problem, trap enclosures (Getz and Batzli, 1974; Layne, 1987) may be required. The field researcher is obligated to find and inspect every live trap each time the trapline or grid is checked, and to remove all traps from the field or lock traps open at the end of the sampling period. If live traps are not set in a systematic fashion (i.e., in a grid or transect), they should be numbered and set sequentially, or trap sites should be tagged or flagged and numbered sequentially to ensure that all traps are found each time traps are checked, and that no traps are left in the field upon completion of sampling. Pitfalls, which are an appropriate type of live trap for some mammals, also must be checked frequently and should contain nesting material and adequate food to last until the next time traps are checked. As for other kinds of live traps set for shrews and other small mammals with high metabolic rates, pitfalls may have to be checked as often as every few hours to prevent starvation. Pitfalls may need securely fastened raised

covers to keep out predators such as raccoons, as well as rain and direct sunlight.

Some species of mammals can be captured by hand. When done with care, this is an effective and humane capture technique; however, precautions should be taken to avoid being bitten or contaminated with body fluids or ectoparasites.

Corral traps are designed to enable herding of large mammals along fences or runways into a corral. This technique commonly is used by wildlife personnel in research or management procedures involving large ungulates and kangaroos. As with cannon nets, another technique of choice in the wildlife profession, care should be taken to avoid injury to captured mammals. When corral traps or nets are used, all animals captured must be attended to as quickly as possible to prevent panic or injury.

Mist nets, harp traps, and similar devices are effective and humane methods of capturing live bats (Kunz and Kurta, 1988). These devices are best set immediately before sunset and dismantled or rendered inoperative before sunrise and between capture efforts. Mist nets should be tended continuously, and all captured animals should be removed immediately to avoid injury from undue entanglement or from predators. Mist nets should not be deployed at sites where large numbers of bats may be captured (for example, at entrances to cave or mines); in such circumstances, harp traps are recommended. Harp traps should be monitored regularly, but do not require constant vigilance as do mist nets.

Particular attention should be given to the time of year when bats are collected from communal roosting sites. Maternity colonies generally should be avoided during the period when young are born and during the entire time females are nursing to reduce disturbance-related mortality. Repeated disturbance and arousal of hibernating bats will cause depletion of critical fat stores, which can lead to high mortality.

Use of "CAP-CHUR" guns or darts to shoot a sedative into the shoulder or hip of a large mammal requires knowledge of proper dosage and adequate logistical support to track a darted mammal until the sedative takes effect. Unless the investigator has considerable experience in the use of this capture method, we recommend that the advice of a wildlife veterinarian be obtained.

Location, habitat, and time required for sedation should be considered to avoid injury or drowning of sedated mammals. In cases where treed mammals (e.g., mountain lions and bears) are shot with tranquilizer guns, precautions must be taken to ensure that the animal is not injured if it should fall from the tree, e.g., by positioning a net or pad under the animal. Sedated mammals should be monitored closely and should not be released until they recover normal locomotor capabilities. Exceptions would be large, dangerous species that would pose a risk of injury or death to the investigator. Such species

should be placed in secure sites where they will not be subject to physical harm or extremes of temperature, and can be monitored from a safe distance.

We recommend that captured small and medium-sized mammals be handled by methods that restrain the body and appendages, yet permit easy breathing. Covering the eyes may help, because many mammals will not struggle to escape if their eyes are covered. Restraint by means of a mesh or cloth bag permits marking, measuring, biopsying, or otherwise sampling of the mammal through the mesh or partially opened end of the bag. A captured mammal also may be manipulated safely by confining it in a heavy-duty clear plastic bag for brief periods. Such bags also are useful when anesthesia must be induced (e.g., small mustelids). An anesthetic (e.g., halothane, methoxyflurane) can be introduced into the bag by dripping it onto cotton or gauze in a jar with perforated lid or in a tea strainer, thus precluding direct contact between the captive animal and the anesthetic. Larger mammals may require mild sedation before they are removed from traps for examination.

Rodents that are reservoirs for serious human diseases may be anesthetized before handling to reduce the chance of infection via bite or contact with potentially infectious excretions, feces, or secretions. Depending upon type of anesthesia, ectoparasites that carry diseases transmissible to humans may be rendered inactive, thereby reducing chances for the spread of disease.

Kill-trapping and Shooting

Some types of research in mammalogy require the killing of individuals, either by use of traps or firearms. Investigators must endeavor to ensure that such collecting does not adversely affect the populations being sampled. In such collecting, it is essential to employ methods of trapping or shooting that will ensure that death occurs as quickly and painlessly as possible without damage to any body parts needed for research. Some species may be taken effectively only by use of specialized traps such as snap or break-back traps (e.g., Victor or McGill traps for rat-sized mammals and Museum Special traps for smaller species); pitfalls for shrews or other small terrestrial mammals; Macabee and comparable traps for pocket gophers; harpoon traps and similar devices for moles; Conibear or similar body-grip traps for medium-sized mammals. These latter traps are preferable to leg-hold traps where appropriate. Kill traps must be positioned with care so as to ensure the highest probability of capture of "target" species and the lowest probability of capture of other animals. Traps must be secured well and marked conspicuously to prevent loss. Traps must be checked at least once each day to remove captured mammals. If a captured animal is not already dead, it should be killed immediately and humanely.

Snap traps set strictly for nocturnal species should be removed or sprung during the day to avoid accidental capture of diurnal species. Pitfalls may be used as kill traps only when no other effective method of killtrapping is available. The use of formalin or ethylene glycol in pitfalls is not approved. Mammalogists are encouraged to use the least traumatic kind of trap that will serve the purpose. If only leg-hold traps will do, it is recommended that modern types that minimize the incidence of injury to captured mammals be used (Kuehn et al., 1986) and that such traps be checked frequently, at least twice each day, preferably more often. Shooting is the most effective way, and in some cases the only way, to collect certain species. This is particularly true for tree-dwelling species that seldom if ever come to the ground where they would be subject to capture in traps. Investigators who employ this technique should be experienced in the safe and proper use of firearms and must comply with laws and regulations governing their possession and use. Humane use of firearms necessitates that mammals be killed outright. Therefore, the firearms used should be appropriate for the species to be collected. Mammals the size of chipmunk or smaller mammals can be shot with a .22 caliber pistol or rifle loaded with number 12 or dust shot. A .22 caliber rifle loaded with conventional bullets or a 12, 16, 20, or .410 gauge shotgun with appropriate loads is better suited for medium-sized mammals (as large as a raccoon). The shooting of large mammals may require use of a high-powered rifle with appropriate ammunition. Shooting nocturnal species with the aid of a spotlight (when legal) demands extra safety precautions and skill because of limited visibility.

METHODS FOR SAMPLING TISSUE FROM LIVE MAMMALS

Both non-invasive and invasive techniques used in sampling tissues from live mammals require humane procedures and astute professional judgment aimed at obtaining maximal scientific data from a minimum of individuals or samples. The advice of a veterinarian may be helpful in planning such procedures. Only trained, experienced personnel should take tissue samples from live animals. Judgment about the use or non-use of local anesthetics when sampling peripheral body tissue and tissue fluids, such as blood, lymph, sperm, and tissue samples from body openings, should be based on a conscious effort to avoid or minimize pain to the mammal. If pain is slight or momentary, it may be judicious not to use anesthesia so that the mammal can be released immediately.

Generally, however, any procedure that causes pain or significant distress requires the use of an appropriate anesthetic. Selection of anesthetics and analgesics for specific animals should be based on evaluation by a specialist such as a veterinarian. If physiological measurements are to be made, this may affect the choice of agent(s) used. Tranquilizers used to immobilize large mammals are not

acceptable substitutes for anesthesia when subsequent treatment produces more than slight or momentary pain. If a mammal is destined to endure prolonged pain or discomfort resulting from the effects of capture or treatment, euthanasia is warranted. Although aseptic techniques are difficult in the field, cleanliness in all surgical or puncture techniques is essential to minimize the potential for infection and to provide reliable biological samples. Researchers and educators performing invasive procedures (e.g., implanting abdominal transmitters) in the field should utilize acceptable surgical procedures (e.g., gloves, facemasks, and sterilized instruments) to minimize the risk of infection. They also should administer antibiotic drugs when there is a risk of infection following surgery or other invasive procedures in the field. Use of antibiotics should only be done following consultation with a veterinarian. An affected mammal must be maintained under close observation and not released until it has recovered from treatment. Small amounts of blood can be obtained from small terrestrial mammals by an incision at the tip of the tail. Blood can be obtained from bats by venipuncture in the tail membrane or along the leading edge of the wing. If larger volumes of blood are needed, venipuncture of the femoral or jugular vein, the orbital sinus, or any of several venous plexuses can be performed on most mammals without significant risk of mortality. The use of anesthesia for blood sampling will depend upon the procedure and species. Because some species are highly sensitive to anesthesia, the use of anesthesia should be weighed against the risk of mortality from the anesthesia. Cardiac puncture under anesthesia may yield moderate amounts of blood with low risk of mortality. In instances where a large amount of blood is needed from a small mammal, appropriate methods would include terminal thoracotomy under anesthesia followed by exsanguination, decapitation and collection of trunk blood, and exsanguination by cardiac puncture.

External tissue samples, such as skin clips, require aseptic conditions and anesthesia. Internal tissue samples, obtained by large-bore needle biopsy, generally require immobilization and anesthesia, but can be performed in the field if care and sterile instruments are used. For certain large, terrestrial, and marine mammals, tissue may be taken from free-ranging individuals with biopsy darts (Karesh et al., 1989; Barrett-Lennard et al., 1996). Consultation with a veterinarian is essential in such field procedures.

In processing karyotypic preparations, it often is necessary to increase the mitotic index with a mitogen. Methods that are acceptable include the yeast-stress method (Lee and Elder, 1980) and the use of recognized mitogens, such as phytohemagglutanin, that cause minimal discomfort to the specimen.

United States National Institutes of Health (NIH) guidelines recommend that surgery in the field or laboratory be done under aseptic conditions. Mammalogists working in the United States should be familiar with regulations promulgated by the United States Secretary of Agriculture (CFR, Title 9, Subchapter A, Parts, 1, 2, 3, and 4) with respect to the care, handling, and treatment of vertebrate animals held or used for research, teaching, or other activities supported by federal grant awards and the United States Animal Welfare Act (P.L. 89-544, 1966), as amended (P.L. 91-579 and P.L. 94-279). Moreover, mammalogists working in the United States or its territories and receiving financial support for their research from the U.S. National Science Foundation (NSF), NIH, or other federal agency are expected to follow guidelines described in the "Guide for the Care and Use of Laboratory Animals," (National Academy of Sciences, 1996; previously NIH Publication 85-23), and to comply with the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training" (included as an appendix to the National Academy of Sciences Guide).

Finally, mammalogists should be familiar with the American Society of Mammalogists' "Guidelines for the Use of Mammals in Research" (ad hoc Committee for Animal Care Guidelines, 1985).

Specific safety guidelines have been published for handling and sampling small mammals that are known reservoirs for agentsthat can cause severe human diseases such as hemorrhagic fever or hantavirus pulmonary syndrome (Mills et al., 1995a, 1995b). It is important that mammalogists who handle such reservoir species in the field in endemic areas, or who process tissue or blood samples from these species in the laboratory, adhere to these safety guidelines (see HEALTH PRECAUTIONS).

SOCIAL INTERACTIONS

Some species of mammals are members of groups with complex social interactions (e.g., ground squirrels, prairie dogs, certain primates). When studying such species, investigators should endeavor to minimize the impact that holding or removing individuals will have on the welfare and social interactions of both the individual and group. In live-trapping studies of social species, simply minimizing the length of confinement can materially reduce the adverse impact of such procedures.

METHODS OF EUTHANASIA

When live-caught animals are retained as voucher specimens or when specimens are injured or distressed and cannot be released, they must be euthanized humanely. Field methods used to euthanize mammals should be quick, as painless as possible, and compatible with both the design of the investigation, and

the size and behavior of the species of mammal under investigation.

Also, in the United States, researchers receiving federal support must comply with relevant provisions of the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions. Acceptable methods of euthanasia vary among species (American Veterinary Medical Association, 1993), but typically are related to size of the animal. Use of inhalants such as carbon dioxide, halothane, methoxyflurane, ether (carcinogen, flammable and potentially explosive), or other gases (except chloroform, which is not recommended by United States Public Health Service guidelines because of hazards to the investigator) for euthanasia is acceptable (American Veterinary Medical Association, 1993), but sometimes is impractical under field conditions. Under open-air field conditions, chloroform may be appropriate due to the fact that it also kills ectoparasites, that may pose greater risks to the researcher through transmission of diseases such as plague and typhus. If chloroform is used, it always should be outside in well-ventilated areas and by experienced personnel. For euthanizing small mammals, cervical dislocation and thoracic compression are commonly used methods because they are quick and impart little pain, thus meeting the criteria for euthanasia methods of the United States Department of Agriculture's Animal and Plant Health Service (APHIS). Euthanasia by shooting or other traumatic means also is humane and effective if the result is instantaneous death, but should not be employed except by experienced investigators. Other methods of euthanasia have been reviewed by the American Veterinary Medical Association's Panel on Euthanasia (American Veterinary Medical Association, 1993). Regardless of method used, death of the animal should beconfirmed.

METHODS FOR MARKING AND TRACKING

The objective of marking a mammal is to permit its reidentification, either upon recapture or from a distance. Marking may be temporary or permanent. The method of marking employed should be as painless as possible and should not restrict the normal activity or affect the well-being of the mammal. The selection of a method of marking should involve both assessment of the objectives of the study and the characteristics of the species being studied. For example, toe-clipping should be avoided for arboreal, scansorial, semi-fossorial, and fossorial species. Although ear-tagging may be preferable in some cases, frequent loss of tags may render this method less reliable than others, including toe-clipping. Also, ear tags may limit the ability of small mammals to groom their ears effectively. In Peromyscus leucopus this results in higher infestations of ticks (Ixodes scapularis), which are vectors responsible for the transmission of Lyme disease (Ostfeld et al.,1996). The small, cryptic ears of some species such as

Shrews (Soricidae) preclude ear-tagging as a viable method of marking.

To ensure the comfort of the marked mammal and easy reidentification, marking methods should be appropriate for the size, future growth, body form, and habits of the species. Metal or plastic tags should be applied properly and should not burden the mammal or make it vulnerable to injury or predation. Sequentially numbered or color-coded markers can be inserted into the ear, around the neck or leg, or into loose body skin (using topical anesthesia if necessary).

Bats are best marked with wing bands or bead-chain necklaces(Barclay and Bell, 1988). Generally, wing bands should be applied loosely so they slide freely along the forearm. If young bats are to be banded, the bands should be large enough to allow for growth to adult size. The wing membrane of some species may need to be slit to accommodate the band properly (Barclay and Bell, 1988). Because, in the tropics, wing bands often lead to infection, bead-chain necklaces are the better option. If bead-chain necklaces are used, extreme care should be taken to ensure proper fit (Barclay and Bell, 1988).
When no other marking methods are feasible, ear-punching and toe-clipping are quick, long-term marking methods that cause only brief and minor discomfort to small mammals (shrew to rat-sized).

A poultry punch is an effective marking instrument for the ear margins of small mammals. All clipping methods should be performed with sharp instruments. No more than one toe per foot should be clipped. These methods should not be used on bats because of the important roles of the pinna in echolocation and the toes in roosting.

Radiotelemetry is an especially useful method of locating and tracking medium-sized and large mammals whose wanderings are difficult or impossible to monitor by frequent live-trapping or direct observation. This method is appropriate for use on mammals that can carry the transmitter and antenna without encumbrance. The transmitter normally is incorporated into a collar or harness that, like any other tagging device, should be secured without restricting or abrading the body parts. Collars placed on young, growing mammals should be of an expandable or break-away type if there is a low probability of recapturing the mammal to remove the collar before it becomes too tight. For terrestrial mammals, the radiotransmitter normally should not exceed 5% of body mass. This is especially important in the case of small bats (body mass < 70 g). For bats, transmitters are most successfully attached to the mid-dorsal region using surgical adhesive (Barclay and Bell, 1988). In studies on some mammals, the transmitter may be implanted surgically.

Investigators are obliged to monitor the condition of marked mammals and, if practical, remove transmitters at the completion of a study.

Passive integrated transponders (PIT tags) provide a new method of permanently marking mammals. PIT tags are injected under the skin with large-bore hypodermic syringes. Care should be taken to avoid contamination of PIT tags prior to implantation. Once implanted, these can be "read" with a scanner that activates the tags; however, with few exceptions (e.g.,Harper and Batzli, 1996) specimens must be recaptured and handheld for the scanner to function. PIT tags are expensive in terms of both the tags and the readers; however, they are more reliable than ear tags in terms of frequency of loss by marked animals (Harper and Batzli, 1996; Williams et al., 1997). Temporary marking with non-toxic dyes or dry fluorescent pigments, by spot-shaving, or by injection of low dosages of short half-life radioisotopes should be employed when practical, if the study is short-term or seasonal. More permanent marking methods, such as tagging, collaring, banding, PIT tags, earpunching,toe-clipping, tattooing, and freeze branding are more suitable for long-term studies.

Other acceptable tagging methods involve use of low-level radioactive tags, light-emitting diodes (LEDs), Beta lights, and chemical light tags. Radioactive tags are especially valuable for studies of fossorial species for which radiotelemetric methods may be impractical. All relevant federal, state, local, and institutional regulations must be followed if this method is used. When the study is completed, marked animals should be recaptured so the radioactive material can be removed, and all contaminated materials should be disposed of according to established safety standards.

HOLDING AND TRANSPORTING CAPTIVE MAMMALS

Captured mammals to be retained for brief periods (no more than a few hours) or transported to a laboratory must be placed in appropriate holding cages, which can include live traps if those traps are provided with adequate ventilation, food, and a source of moisture, and if they encompass sufficient space with appropriate padding and bedding to ensure the comfort of captive mammals. Live traps also should be positioned to permit drainage of urine produced by captive animals. Acceptable holding devices for bats were described by Kunz and Kurta (1988).

Mammals are endotherms and homeotherms, and as a consequence have high food and water requirements. While being transported, mammals should be provided with adequate food, sources of moisture (e.g., moist fruits, if water is not a practical option), and an appropriate environment for thermoregulation.

Mammals in transport should never be subjected to thermal environments that exceed their limits of tolerance. Cages for transporting mammals should be kept out of the sun, wind, and precipitation and at a comfortable temperature. Captives should

be checked frequently. Most field vehicles are not mobile laboratories and conditions in a vehicle cannot be maintained as they are in a laboratory facility. Rather, the precautions used for the humane transport of household pets should be applied when transporting research animals. Care also should be taken to minimize psychological stress on certain species by shielding cages from excessive light, noise, and human activities.

On occasion, wild-caught mammals are brought into a laboratory where they are kept for a period of time before being processed. While in captivity, these mammals must be maintained under conditions that meet their needs and tolerances for food, moisture, nesting, space, and microclimate. Researchers receiving federal support must ensure that conditions in the laboratory comply with guidelines described in Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996; previously NIH Publication 85-23), and any local regulations that may apply. Those guidelines typically also must be met if a permanent or long-term colony is maintained.

However, guidelines for maintenance of animal colonies do not apply to the design of research intended to simulate naturalconditions in the laboratory, in experimental studies using enclosures or environmental manipulations in the field, or when wild mammals kept in captivity require conditions other than those prescribed by the NIH guidelines; obviously, in such instances, professional judgement must prevail. In this regard, methods for the special care and housing of bats in the laboratory were summarized by Wilson (1988).

Special precautions are necessary when holding, transporting, or initiating laboratory colonies of species that are known reservoirs for serious human diseases, most especially those transmissible by aerosol. These precautions are outlined in Mills et al. (1995a, 1995b).

MAINTENANCE OF WILD-CAUGHT INDIVIDUALS IN CAPTIVITY

Cages or enclosures to hold wild-caught mammals and their offspring should be designed to accommodate salient features of their ecology, morphology, physiology, and behavior. To house certain species (e.g., desert granivores, shrews, and fossorial species such as moles) under conditions prescribed for laboratory rodents is not in the best interest of such species and may amount to inhumane treatment. Desert granivores need fine sand for dust-bathing and caching of seeds. Burrowing species require soil or other suitable substrate in which to construct tunnels. Methods useful for maintaining mammals that have been bred in captivity for many generations may not be appropriate for wild-caught mammals. For example, allowance should be made for less-frequent cage cleaning and inclusion of more objects (e.g.,materials for nest construction and play) in many wild species.

Although basic cleanliness and hygiene remain a high priority, wild mammals should be disturbed less often and allowed to accumulate familiar odors, which are important to species that are olfactorily oriented. Furthermore, mammals that are hibernating require different caging and housing than the same individuals when not hibernating. Particularly important is the need to maintain sufficiently high humidity levels and to keep temperatures at optimal levels to minimize energy expenditures. In some cases, this may involve keeping ambient temperatures within only a few degrees of freezing, depending on the thermal optimum during hibernation for each species.

Experienced field researchers often are more knowledgeable about the care and welfare of wild-caught mammals than individuals whose expertise is limited to laboratory animals. In such situations, researchers should be permitted to care for captive mammals using procedures that best meet the needs of the animals based on the known ecology, physiology, and behavior of the species in question, even if these are outside guidelines established for the care of laboratory mammals. Mammalogists sometimes study natural populations of mammals inside field enclosures in order to manipulate population size, group membership, or movements. For many small mammals, such as mice and voles, enclosures as small as 0.10 ha are sufficient for maintaining normal population processes (e.g., natality and mortality rates) and home range sizes (i.e., area typically covered by individuals during routine activities). Thus, animal care procedures necessary under laboratory conditions, such as provisioning of food and water and changing bedding, are not necessary in such experimental enclosures within natural habitat.

RELEASING PREVIOUSLY CAPTURED LIVE MAMMALS

There are few exceptions (for example, reestablishment of previously extirpated populations) to the rule that field-caught mammals must be released only at the sites where they were captured. To do otherwise potentially would upset natural populations and reduce the chances for survival of released animals. Translocated mammals also have been implicated in the rapid dissemination of disease agents, such as rabies, that pose a threat to humans and other mammals (Nettles et al., 1979).

Moreover, mammals should be released as soon as possible after capture to minimize behavioral or physiological stresses resulting from the conditions of captivity, or immigration of replacement individuals. Finally, consideration should be given to releasing mammals at times coincident with their normal daily and seasonal activity patterns.

HEALTH PRECAUTIONS

All wild mammals are potentially dangerous to researchers either from traumatic injury due to direct contact or from

infectious diseases that are carried by mammals or their parasites. Therefore, researchers dealing with wild-caught mammals in the field or laboratory should work under the assumption that the animals they are handling pose some risk to their health and safety, as well as that of their students and staff. The risk can be substantially reduced by common sense and good personal hygiene (e.g., wash hands often with soap and water). Researchers should endeavor to minimize the chances of being bitten or scratched (e.g., wear leather or fabric gloves) and should avoid use latex gloves to unnecessary exposure to blood or other body fluids and feces, which may contain parasites or pathogens that affect humans. In high-risk areas, care should be taken to immobilize or kill ectoparasites before handling specimens. Special care also should be taken to avoiding needle punctures when using syringes and similar devices.

Moreover, investigators who work with carnivores or bats should be especially careful to avoid being bitten and should be immunized against rabies (Constantine, 1988). All field workers should maintain up-to-date tetanus immunizations. In studies on bats, care also should be taken to avoid breathing potentially lethal gases (present in some caves and mines), to minimize exposure to anticoagulants that have been used in buildings to kill bats, and to avoid being infected by Histoplasma capsulatum (a fungus which causes histoplasmosis). A number of infectious diseases that are transmitted by arthropod vectors may be acquired without direct contact with mammals. Arthropod-borne diseases such as Lyme disease, ehrlichioses, Rocky Mountain spotted fever, and the equine encephalitides in North America, and dengue fever and malaria throughout tropical regions are examples of these agents. Mammalogists should be aware that these diseases represent a risk of conducting field studies in specific geographic areas. Reduction of that risk requires knowing what agents occur in a region and taking appropriate precautions to minimize exposure. In addition, mammalogists should recognize the risks of contracting diseases that are associated with direct contact with mammals or their parasites. For example, bubonic plague is caused by a bacterium that can be transmitted to humans by fleas that occur on certain rodents, especially sciurids (squirrels), or indirectly by close contact with certain carnivores (e.g., domestic cats). Such risks must be considered when selecting a method of euthanasia for mammals. Preference should be given to agents that kill ectoparasites as well as mammals. Tularemia is a bacterial disease, primarily of lagomorphs (hares and rabbits), that can be transmitted to humans by arthropods or by handling or eating infected animals. Mammals may also serve as reservoirs for numerous other agents such as relapsing fever, murine typhus, salmonellosis, histoplamosis, toxoplasmosis, leptospirosis, and pasteurella. The list of pathogens that humans can acquire directly or

indirectly from mammals continues to grow, principally because new technologies have become available to detect them. Recently, mammalogists have become aware of the potential for acquiring hantavirus pulmonary syndrome following exposure to several species of sigmodontine rodents that serve as reservoirs for hantaviruses. Guidelines established by institutional safety committees and the United States Centers for Disease Control and Prevention should be consulted when working with known reservoir species (Mills et al., 1995a, 1995b).

In mammalogy, as in other fields of science, decisions must be based on cost-benefit analysis. An attempt to avoid or reduce the risk of one health problem may result in increased probability of another health problem. For example, exposure to arthropod-vectored diseases may be increased by not using a method of euthanasia that kills ectoparasites, but such euthanasia agents pose some risks to humans. Reasonable approaches must be considered when dealing with such agents as hantavirus, which requires considerable efforts to ensure absolute safety. Protocols such as properly equipping one individual to handle high-risk mammals in a class situation could alleviate the unreasonable case of equipping every student with respirators and level-4 viral training. Finally, it should be remembered that perhaps the greatest risk in most field studies in mammalogy involves travel to and from the study site.

Investigators or students who become ill following field work involving mammals should inform physicians immediately of their possible exposure to agents carried by mammals or their parasites, and the geographic regions in which their field work was performed. Physicians rely heavily on exposure histories in deciding the courses of diagnosis and treatment. Informing physicians of possible exposures may be critical to receiving prompt and appropriate testing and treatment.

A key component of safety in the field is common-sense personal hygiene. Investigators should wash their hands frequently and should wash their field clothes and any other materials that come in contact with mammals or their blood or body fluids. They also should take precautions to prevent contamination of food and living areas with droppings and urine. The history of mammalogy suggests that common sense, coupled with prudent hygiene, can serve to reduce the risk of disease from mammal-borne pathogens to acceptable levels.

ESTIMATING WILDLIFE POPULATIONS

To establish and to appraise management practices, wildlife managers must estimate the sizes of wildlife populations. For game species, such inventories are ideally taken 3 times a year: during the breeding season, after the young are born or hatched and before the start of the hunting or trapping season, and after the hunting or trapping season. In practice, population estimates are usually done only once a year, at best, because of manpower and funding

shortages.

Wildlife managers use 4 general approaches to estimate population sizes of wildlife: total counts, incomplete counts, indirect counts, and mark-recapture methods. We shall examine each of these methods and detail some of their advantages and disadvantages.

COMPLETE COUNTS OR TOTAL COUNTS

A complete count, or total count, counts every member of a population. Where populations of large species occur in open areas, such as waterfowl on lakes, seals on breeding beaches, or pronghorns on shortgrass prairie, aerial counts of most individuals are possible, especially with the aid of photography. Sometimes, wildlife managers can count deer in enclosed populations using a drive approach: a large group of people crosses the enclosure in a line, counting all deer that pass in each direction. Distances between the members of the drive crew are critical for success because *all* deer must be counted, even those hiding. Nonetheless, wildlife managers seldom use this approach because lack of funds or personnel usually make censussing an entire population impractical or impossible and, in addition, such an undertaking disturbs, and can even destroy, the population or its habitat. Even when used, this approach is usually expensive.

INCOMPLETE COUNTS

An incomplete count involves counting part of a population and then extrapolating to the entire population. Quadrats may be established in a sample area and an attempt made to count all the individuals in each quadrat. A "deer drive" census, using large sized quadrats, can be an effective way to estimate deer populations on wooded areas. Stationary observers stand along 3 sides of a quadrat and count all deer leaving and entering the area in front of a drive crew walking across the quadrat from the 4^{th} side. The total number of animals is then calculated as the sum of the animals leaving the area ahead of the drive crews plus the animals passing back through the drive line minus the animals entering the quadrat through one of the sides or through the drive line. As with complete counts, distances between observers and between members of the drive crew are critical for success.

Strip censuses, roadside counts, flushing counts and booming or drumming ground counts are all incomplete count methods. A strip census can be used to estimate grouse population sizes. An observer walks a transect through a representative section of habitat and records the distances at which birds flush to either side. The population size, *P*, is estimated to be

$$P = \frac{AZ}{XY}$$

where *A* is the area of the habitat censussed, *Z* is the total number of grouse flushed, *X* is the total distance walked and *Y* is twice the average distance from the observer to the bird when flushed. The fundamental assumptions of this method are 1) birds vary randomly in distances at which they flush, 2) birds are scattered randomly across the study area and 3) the average flushing distance is a good estimate of the "true" average. Which of these assumptions are likely to be met? What if some birds will not flush? A Wildlife Monograph has dealt extensively with these types of population size estimates (Burnham et al. 1980).

INDIRECT COUNTS

As it is often impossible to obtain accurate, visual or auditory counts of the animals in a population, wildlife managers use indirect signs of the animals present as *indices* of relative abundance. An index of population indicates relative size of a population and shows population trends (up, down, stable) but does not provide an actual estimate of the number of animals. Examples of indirect counts include counting numbers of muskrat houses, counting scats (fecal pellets) of deer and rabbits, and counting

numbers of nests or den sites in a given area. Sometimes counting the number of birds heard singing is considered an incomplete count and sometimes it is considered an indirect count. Which makes more sense?

One can count fecal pellets of deer or rabbits along transects or in delineated study plots. In either case, the first thing to do after establishing the transects or plots is to remove all old pellets. Then, at a predetermined interval, count all new piles of fecal pellets. This is an index of the number of deer or rabbits in the area: the more animals, the more pellets produced. What assumptions does this index make?

In those areas where muskrats build houses of vegetation in marshes, the number of active, maintained houses in a marsh year to year is an index of the number of muskrats: more muskrats make more houses. If, for a given area, one knows the average number of muskrats living in each house, then the number of houses can be used to estimate the population size. It should be remembered, however, that indirect counts are only indices of population sizes unless other information is known, such as the average number of muskrats living in each house.

MARK-RECAPTURE METHODS

These methods are used extensively to estimate populations of fish, game animals, and many non-game animals. The approach was first used by Petersen (1896) to study European plaice in the Baltic Sea and later proposed by Lincoln (1930) to estimate numbers of ducks. Petersen's and Lincoln's method is often referred to as the Lincoln-Petersen Index, even though it is not an index but a method to estimate actual population sizes. (Should it not be the Petersen-Lincoln Estimate?) Their method involves capturing a number of animals, marking them, releasing them back into the population, and then determining the ratio of marked to unmarked animals in the population. The population (*P*) is estimated by the formula:

$$P = \frac{MC}{R}$$

where *M* is the number of animals marked in the first trapping session, *C* is the number of animals captured in a second trapping session, and *R* is the number of marked animals recaptured in the second trapping session. This is derived from the equation:

$$\frac{P}{M} = \frac{C}{R}$$

which states that the proportion of marked animals captured in the second trapping session is the same as the proportion of total marked animals in the total population. Some of the assumptions behind this method are: 1) mortality is the same for marked and unmarked animals; 2) marked individuals do not lose their marks; 3) marked individuals are caught at the same rate as unmarked individuals (no trap-happy or trap-shy animals); 4) the population has no significant recruitment, or ingress (births or immigration); 5) the population has no significant egress (deaths or emigration); 6) marked animals mixed randomly with unmarked animals; and 7) each trapping session captures a representative sample of various age and sex categories from within the population. Think about these assumptions with respect to wildlife. Assumptions 4) and 5) taken together mean that a population is closed.

The Wildlife Society publication, *Wildlife Management Techniques*, provides methods of estimating 95% confidence limits for Lincoln-Petersen population estimates. Remember, the Lincoln-Petersen method provides and *estimate* of the true population size; it does not state the actual, or true, population size. By calculating the 95% confidence interval, a wildlife manage can learn how confident he or she should be of the accuracy of the population estimate. 95% of the time, the true population size will be within the 95% confidence interval.

Example of the Lincoln-Petersen Index Imagine that you set out live traps in a muskrat marsh. On the first day of trapping you capture 10 muskrats and put eartags in all of them; thus $M = 10$. On the second day of trapping you capture 8 muskrats ($C = 8$), 4 of which are eartagged ($R = 4$). So . . .

$$P = \frac{CM}{R} = \frac{8 \times 10}{4} = 20$$

To express your confidence in this estimate, you calculate the 95% confidence limits for your estimate. The upper and lower 95% confidence limits are

upper: 59
lower: 5.5

This means that if you trap muskrats in this way many, many times, 95% of the time that you obtained an estimate of 20 muskrats, the true population size would be somewhere between 6 and 59 animals. Since you actually captured 14 muskrats, you know that the population size is at least 14.

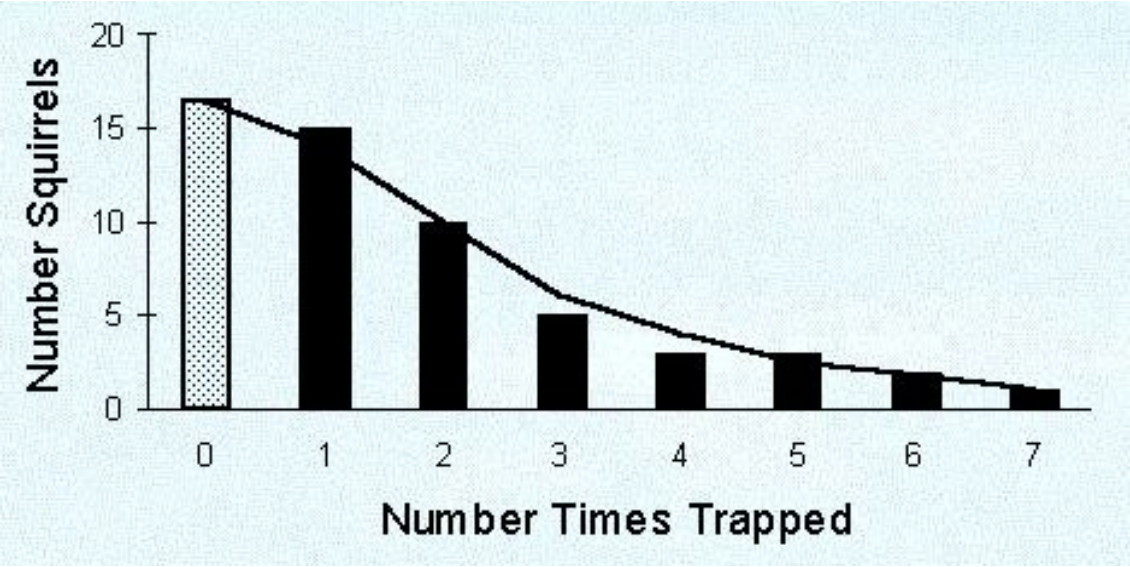
Otis et al. (1978) developed sophisticated modifications of the L-P Estimator that attempt to insure that data are consistent with the assumptions. Several modifications construct stratified indices whereby data are collected separately for specific sub-groups of the population, such as age and sex categories or trap-happy and trap-shy animals. Thus, researchers must uniquely mark each individual captured and record information about that individual, such as sex and age. These modifications also insure an order of magnitude increase in the complexity of the mathematics and are available in computer software, such as *Capture*.

When wildlife managers or researchers establish long-term population studies with frequent samplings, they can estimate not just the population size but the numbers of animals entering and leaving the population (Jolly 1963, 1965; Seber 1973). The Jolly-Seber Method relaxes the assumption that a population is closed. That is, the population can be open and have ingress (births and immigration) and egress (deaths and emigration). By keeping track of capture histories for individual over many capture sessions, ingress and egress can be estimated. Jolly-Seber Estimates can be calculated by hand but the exercise is complicated. Several software packages provide Jolly-Sever Estimates. The *Wildlife Management Techniques* manual shows how to make Jolly-Seber Estimates.

Pollock and his colleagues (Kendall & Pollock 1992, Nichols, et al. 1984, Pollock1991, Pollock & Otto. 1983) developed the Robust Design for estimating animal populations, which incorporates capture-recapture methods for both closed and open populations. In its simplest form, the Robust Design uses an L-P Estimate for total population size during each of several, regularly scheduled trapping sessions and uses of the Jolly-Seber approach to estimate ingress and egress between trapping sessions.

Krebs (1966) formally introduced the Minimum Number Alive (MNA) method, though it had been used by many researchers for years. The MNA method avoids the use of estimators, using instead the minimum number of animals known to be alive during a sampling period as a biased estimator of the population size. Hilborn et al. (1976) tested the sensitivity of this method to five important population parameters in mouse populations. They used simulation models and actual data to estimate the expected error on the MNA in actual studies. Their results showed that the MNA method, though clearly a biased estimator of population size, is an unbiased estimator of critically important population characteristics such as age distribution, pregnancy rate and lactation rate. In addition, in most cases an MNA population estimate is as good as or better than a Jolly-Seber Estimate.

The Frequency of Capture Method (Eberhardt 1969) can be used when capture data are available over several trapping days. Plot the number of times that individuals are captured against the numbers of animals captured each number of times. For example, imagine that you live trap gray squirrels on campus over the course of 2 weeks and trap 15 squirrels only once, trap 10 twice, 5 3-times, 3 4-times, 3 5-times, 2 6-times, and 1 7-times. You plot these captures like so:



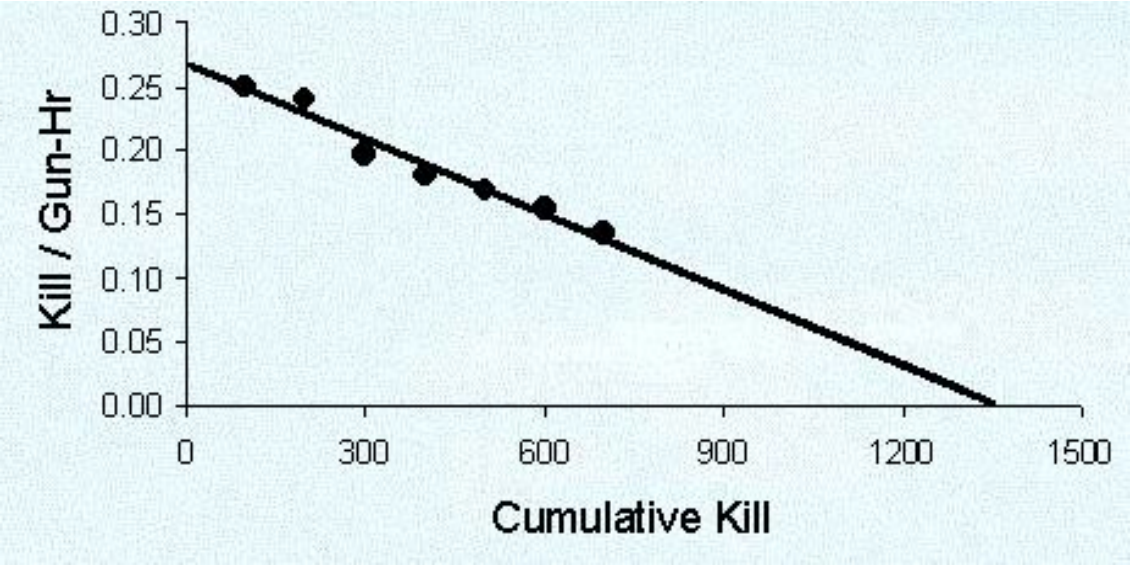
These data are then fit to a statistical distribution to determine how many squirrels were never trapped at all even though they were present. In this case, if we assume that squirrels were captured at random, the estimate of the number never trapped is between 16 and 17. The number never trapped at all but present is added to the MNA to give the Frequency of Capture estimate. In this example, the population size estimate is 55.

Lastly the DeLury Method, first worked out for fish populations, uses kill data to estimate game populations. The critical assumption is that the number of animals killed per unit of hunting time is proportional to the population density; if this assumption is true, then each unit of hunting effort takes a constant proportion of the population. By plotting the kill rate (number of animals killed per unit hunting effort) against the total kill, it is possible to estimate the total population by extending the line to the *X*-axis. The value at the point of intersection is the estimate of the original population, P_o. The validity of this method rests heavily on the assumption of each unit of hunting effort taking a constant proportion of the population. This DeLury Method also assumes that: 1) the population is closed; 2) animal vulnerability remains constant; 3) variable hunter skills average out; and 4) hunting is done individually. Of these assumptions, the one most likely to be violated is constant vulnerability. This can be affected by factors both intrinsic and extrinsic to the hunted population.

Example of the DeLury Method: Imagine that you are a wildlife biologists monitoring the game populations on a designated Wildlife Management Area. Assume further that your Area allows deer hunting for 7 successive days each year and that hunters must apply for a permit to hunt on the Area. Hunters must check in before and after hunting and must report their kill. On each of the 7 successive days, hunters hunt for a total of about 400 hours each day. You record the hours hunted each day, record the number of deer killed each day, and calculate the cumulative kill, producing a table like the following.

Day	Animals Killed	Hours Hunted	Kill/Gun-Hr	Cumulative Kill
1	100	400	.250	100
2	90	375	.240	190
3	81	410	.200	271
4	73	405	.180	344
5	66	390	.170	410
6	59	385	.153	469
7	53	395	.134	522

You then graph kill/gun-hr against cumulative kill to estimate of the initial population size before hunting began. This it the graph you get.



You draw a line through your data points and extend the line to the *X*-axis. Your estimate of P_o , the estimated population size before the hunting season started, is about 1350 on the graph. You also calculate a linear regression through the data points and calculate the *X*-intercept. Here you find that your best estimate of P_o is actually 1335. Because 522 deer were killed, the population after the hunting season is estimated to be 813.

COMPARISONS

Many researchers have used more than method of estimating populations on the same population at the same time. Let us look at 3 of these comparisons.

Morgan & Bourn (1981) compared an Incomplete Count and an L-P Index of the giant tortoise population on Aldabra atoll in the Indian Ocean. To make the incomplete count, the atoll was divided into quadrats 100 m square. All tortoises were counted and marked in 5% of the quadrats and the total number counted was multiplied by 20. The L-P Index was made by counting marked tortoises on transect lines. Morgan & Bourn believed that almost all assumptions for each technique were satisfied, yet the estimates of the population size differed significantly: 87,300 for the incomplete count and 68,100 for the L-P Index. Evidently the assumptions for one or both methods were not met as well as believed. Morgan & Bourn had more confidence in their incomplete count estimate than in their L-P Estimate and cautioned readers about using elaborations on the L-P Index unless *all* assumptions are *completely* met.

Mares et al. (1981) compared the L-P Estimate, the Schnabel estimate (a variation on the L-P Estimate that tends to underestimate population sizes slightly), and a removal estimate on a population of known size of eastern chipmunks in Pennsylvania. The chipmunks, they found, fell into 2 categories: those that readily entered traps and those that were hesitant to enter traps. Thus, all methods tended to estimate the population as being composed mostly of the former group and, thus, all methods tended to underestimate the total population size. The 95% confidence limits for the L-P Estimates on successive days always included the known population size, whereas this was not the case with the Schnabel method. They concluded that, for populations with unequal catchability, the L-P Estimate was the best.

Boufard & Hein (1978) used 7 different methods concurrently for 6 months during 1976 to estimate the size of a gray squirrel population in Pennsylvania. Four of their methods have been discussed (at least briefly) in this handout: Schnabel, Frequency of Capture, Jolly, and MNA. Their results are as follows:

Month	Schnabel Estimate	Frequency of Capture Estimate	Jolly-Seber Estimate	MNA Estimate
June	115 ± 200	392	54	27
July	76 ± 19	96	38	52
August	85 ± 24	82	51	48
September	118 ± 84		35	31
October	159 ± 97	115	111	32
November	179 ± 59	195	20	34

The Schnabel estimates were the most consistent, despite their variability, and the Frequency of Capture estimates were similar to the Schnabel. Twice (July and November), the Jolly-Seber Estimates were less than the MNA.

It is obvious from these comparisons that estimating populations is an exercise fraught with inperfections. The best we can do is to choose the method or methods whose assumptions are best met by the population we wish to study. When possible, one should always collect data in such a way that more than one population estimate can be made. Often, the estimate made using the method whose assumptions are best met turns out not to work as well as anticipated. Having other estimates to augment that "best" one can save the day.

Also note from these comparisons that Lincoln-Petersen Estimates are often reasonably accurate despite violation of their assumptions. I have noted this pattern and, therefore, tend to use L-P Estimates (or variants available through *Capture* software) over other methods when no other method is an obvious choice (for example, using the DeLury Method to estimate the size of a harvested population). Pollock's Robust Design is consistent with my informal observation.

POPULATION DENSITY

Up to now, this handout may appear very straightforward. There is a problem, however. When you determine a wildlife population size, you automatically determine density. What I mean is, you determine the population size in a particular area - and that means you have determined

population

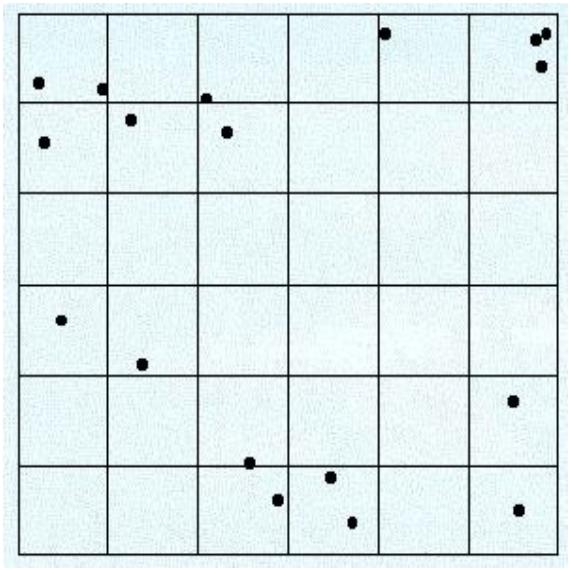
which is defined as density. Lloyd (1967) noted that the number of animals per unit area is a poor measure of density. Read that last sentence again. On first reading that sounds either downright stupid or at least confusing. But what makes the difference is from whose point of view the population is observed: yours or that of the wildlife. If you want to know how many deer are in such-and-such county so that the number harvested can be compared to the number in the population before harvest, then number of animals per unit area is what you need to know. But if you are interested in what the deer herd will do if you institute a management practice designed to increase the population, you need to know more than number of deer per unit area. Deer do not arrange themselves at random across the countryside. And what you measure as animals per unit area may not be what the deer perceive as the population density. Wolff (1980) showed that habitat patchiness and patchy distributions of snowshoe hares have a significant effect on hare population biology. The same is true of other wildlife. For most wildlife, high "densities" lead to reduced birth rates but the densities that are important are the densities that the wildlife experience, not necessarily what we measure. If deer perceive their population density to be higher than we estimate it to be, they will decrease birth rates more than we anticipate. Similarly, if they perceive population density to be lower than we estimate

it to be, they will increase birth rates more than we anticipate. Let's go through an example.

Let's take 18 points placed at random in 36 quadrats. These are random points, so we can take them from a random number generator which ranges from 0 to 99. Let the points be:

X	*Y*
48	10
58	14
93	8
8	43
35	84
5	76
43	17
97	90
16	86
96	95
23	35
68	96
92	28
4	87
39	78
21	80
62	6
98	96

If we plot them they look like this:

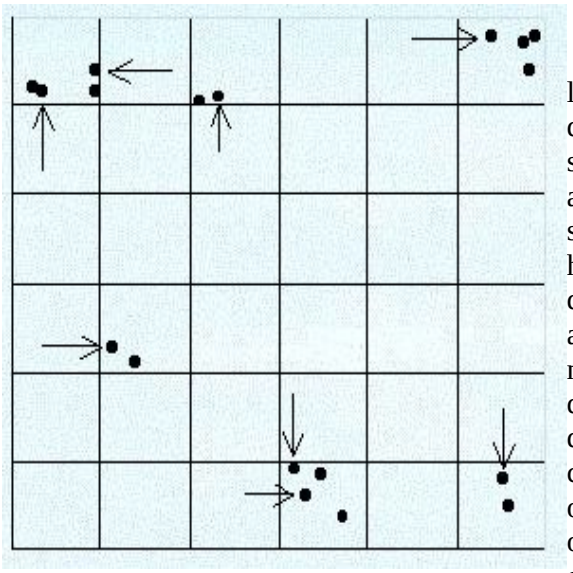


The points are located completely at random. For example, the point in the upper right corner was determined by the last pair of numbers.

Notice how, in this example, the points seem to avoid the center of the graph. Something strange like this nearly always happens in a random distribution. The 'luck of the draw' usually leaves some regions relatively unpopulated. The region is (usually) different each time. *A priori*, each quadrat had an exactly

equal chance of receiving each successive point.

Now, we know that the mean "density", or number of points per unit area of our big square, is 18 points per 36 quadrats or ½ point per quadrat. We can get this in two ways. We can take the total number of points (18) and total number of quadrats (36), divide and get 0.5. Or we can take each quadrat, note its number of points, and then take the average of these numbers. It will come out the same: 0.5



Suppose that these points represent the locations of animals and suppose that 8 or them decide to move closer to a neighbor (movement shown by arrows). We can show that the animals are no long arranged on the graph in a statistically random manner. The distribution has become *patchy*. Obviously, what a patchy distribution means from the point of view of the animals is that, on the average, an animal has more neighbors nearby than is would if the distribution were random. Lloyd (1967) developed a simple measure called "mean crowding", which is the average number of other individuals in the same quadrat averaged over all individuals. This is a measure of what each animal *perceives* as the density of animals around it. Open space with no other animals does little good to the seeker of open space if that open space is far away and hard to find and if all the space close by is filled.

The animals in the random distribution had the following pattern: 1 quadrat had 3 animals, 2 quadrats each had 2 animals, 11 quadrats had isolated individuals, and 2 quadrats were empty. Thus, 3 animals each had 2 others with them in their quadrat; 4 animals each had 1 other with them; and 11 had no others in their quadrat. Mean crowding, $\overline{m}$, is therefore:

Number of animals in quadrat	Number of quadrats
0	22
1	11
2	2
3	1

$$\overline{m} = \frac{(2+2+2+1+1+1+1+1+0+0+0+0+0+0+0+0+0+0+0+0)}{18} = \frac{10}{18} = 0.55$$

Thus mean crowding for this random distribution of animals is 0.55, which is pretty close to 0.5, which is the mean density. In a random distribution, mean crowding is almost always very close to mean density.

In the patchy distribution, however, the 18 individuals were grouped differently: 12 animals each had 3 others in its quadrat and 6 animals each had 1 other. For this example, then, mean crowding is:

Number of animals in quadrat	Number of quadrats
0	30
1	0
2	3
3	0
4	3

$$\overset{*}{m} = \frac{(3+3+3+3+3+3+3+3+3+3+3+3+1+1+1+1+1+1)}{18} = \frac{42}{18} = 2.33$$

which is considerably greater than mean density, which is still 0.5. In a much larger population distributed at random with a mean density of 2.33, the average animal would be no more crowded by others than is an animal in out patchy population whose mean density is only 0.5.

The algebraic expression for mean crowding is

$$\overset{*}{m} = \frac{\Sigma x_i(x_i - 1)}{\Sigma x_i}$$

where x is the number of individuals in quadrat i and the summation is over all quadrats.

So, you can see that for species that react to crowding, measuring mean density (the number of animals per unit area) can give you a very different measure of crowding than the animals actually perceive.

Now there is a major problem with mean crowding. Since Lloyd published his paper on mean crowding years ago, few wildlife biologists have realized its implications. Therefore, little work has been done to develop methods of measuring mean crowding in wildlife populations. Here are some major questions that, at present, have not been answered satisfactorily.

How can wildlife populations be sampled to measure mean crowding?

What is that proper quadrat size?

How can we determine whether a wildlife species reacts to crowding?

NECROPSY – FOCUS ON MAMMALS

Introduction and General Information

- *Post mortem* examination (i.e. necropsy) is an extremely valuable tool in disease investigation and management. It is important to approach each carcass with an open mind, not assuming that the cause of death is known, even if there are obvious external lesions or a known on-going disease problem.

- Before starting a necropsy, consider whether the skin or skeleton is important for museum-based studies. If it is, a cosmetic post mortem is required.

- **Reasons for performing a *post mortem* examination** include "*finding the cause of death, confirming a diagnosis, investigating unsuccessful therapy, increasing knowledge*" and for the detection of sub-clinical disease.
    - *Post mortem* examination is particularly important for animals which die in quarantine in preparation for introduction to a collection, translocation or reintroduction program.
- *Post mortem* examinations may be performed in the field or laboratory; by the case clinician or by a specialist pathologist, dependent on circumstances.
    - Where the gross *post mortem* is performed by an individual other than the pathologist who will perform the further examinations on samples provided, communication between the two parties is essential to ensure that optimal samples are taken (tissue type, volume/ weight, storage, transport, temperature etc.).
    - Inadequate or incorrect sample taking may reduce the likelihood of reaching an accurate diagnosis.
    - Forensic *post mortems* for legal investigation should be performed by an experienced wildlife pathologist, since the credentials of the pathologist will be assessed as part of the case.
- Similar protocols should be used for the *post-mortem* examination of domestic, free-ranging or captive wild mammals.
- Post-mortem examination should be conducted in good daylight whenever possible.
- If possible, findings should be dictated during the examination, or, as an alternative, noted down at the time of the examination.
- **When performing a necropsy or *post mortem* examination**, **it is important to:**

    - first consider the history of the animal (where available). Note the reported clinical signs, treatment, diagnostic tests, possible differential diagnoses, number of animals involved, etc. Communication between the pathologist and the case clinician, where available, is recommended.
    - consider recent and historical disease problems in the collection (captive animal), region (free-ranging), in-contact domestic animal and human populations.
    - examine the site where the carcass was found if possible (e.g. evidence of agonal movements, convulsions disturbing the local area; piles of faeces and urine around the hindquarters suggestive of prolonged.
    - have a systematic approach, whether head-to-tail, system by system (digestive system, respiratory system etc.), or any other.
    - recognize the normal anatomy, normal appearance of organs/tissues and anatomical variation between species.
    - have knowledge of seasonal differences in the body condition and reproductive system which are normal for the species under examination.
    - have knowledge of the expected variations between individuals of the same species dependent on whether they are captive or free-ranging. (e.g. obese body condition may be seen in captive animals but is unlikely in free-ranging individuals; ectoparasite and endoparasite burdens may be expected to be

greater in free-ranging wild animals than those under captive management.)
    - have knowledge of the method, route and time of euthanasia if performed.
    - have knowledge of potential artefactual findings e.g. hypostatic congestion (pooling of blood in organs under the effects of gravity which can be mistaken for pathological congestion), barbiturate crystals from euthanasia solution which can be mistaken for gout (See: **Gout in Waterfowl**), pseudo-prolapse of the anus or vagina as a result of increased pressure within the abdomen caused by gas production after death.)
    - accurately describe lesions/abnormalities.
    - record both positive and negative findings.
    - keep accurate records, including a unique identifying number for each carcass and for samples from that carcass.
    - keep detailed notes on all findings and procedures for forensic post mortems, written in non-technical language wherever possible, for use in court.
    - avoid the use of non-standard abbreviations in permanent records.
    - take photographs (include case identification details) for animal identification and illustration of gross pathology, particularly if the case may be involved in a prosecution enquiry.
    - preserve samples (tissues, parasites etc.) for further testing and future reference/research.
        - A full spectrum of samples should be taken, where possible, at the initial examination if possible and stored appropriately.
        - Further investigations, at first, may be directed at the samples thought most likely to be important in revealing the cause of death. However, if further samples are needed subsequently, the full spectrum are available in store.
        - Where time or financial constraints limit sample taking, a short list of standard tissues should be sampled, in addition to those with apparent gross pathology.
        - In some circumstances it may be advisable to keep the entire carcass for a period following the post-mortem examination, refrigerated in the short term and frozen in the long term, to provide samples in the future if required.
    - consult the appropriate regional authority if a notifiable disease (e.g. **Foot-and-Mouth Disease**) is suspected *before* progressing with the *post mortem* examination,
        - Consult the List A and List B of notifiable diseases made available by the Office International des Epizooties - World Organisation for Animal Health

- Carcass location and body size may dictate whether transport to the laboratory facility for examination is possible, or whether the *post mortem* must be performed in the field.
- Where field *post mortem* examination is unavoidable, attention should be paid to the risk of spread of infection to wild or domestic animals through opening of the carcass and available methods for carcass disposal (e.g. pit, cremation).
- Autolysis of the organs occurs with variable speed; the adrenal medullae, gastro-intestinal mucosa, pancreas, liver, kidney and central nervous system develop autolytic changes particularly quickly.
- ***Post mortem* examination should be performed as quickly as possible after death has occurred** and has been confirmed. However this may not always be possible, and

carcass cooling to slow the rate of autolysis should be practised.
    - Some authors suggest soaking of the fur in cold water with a small amount of detergent to aid in wetting of the skin.
    - The carcass should be placed within a sealed plastic bag, clearly labelled, with excess air removed, and be refrigerated if its body size allows.
    - Carcasses preferably should be refrigerated while awaiting examination.
        - Where sufficiently large refrigeration facilities are unavailable, the carcass should be moved to as cool an area as is available.
    - With very large mammals, cooling of central organs will not occur sufficiently quickly to prevent autolysis; priority should be given to performing the *post mortem* as soon as possible; opening the abdomen may help lower the core temperature as quickly as possible.
    - Where *post mortem* examination must be delayed until 72-96 hours after death, the carcass should be refrigerated only. However if the examination must be delayed over 96 hours *post mortem,* it is recommended to freeze the carcass immediately.
- When transporting a carcass or pathological sample to a laboratory for analysis, attention should be paid to temperature control in transit. Insulated containers should be chosen, ice packing of frozen samples may be used and times when postal delays may be expected should be avoided (e.g. weekend, public holidays, strikes).
    - Local regulations governing the postage of pathological samples should be consulted (labelling, courier, container type etc.)
- In the event of a die-off (mass mortality event) it is important to examine fresh carcasses of a number of individuals, representative of the range of species affected and the ages of individuals affected, and to remember that more than one disease process may be acting at any one time and that the major cause(s) of death may change during a prolonged die-off.
- The results of the *post mortem* examination should be used in conjunction with the history of the mammal or mammals and assessment of the environment to help determine their significance and recommended future action.
- In areas where rabies infection (See: **Rabies)** is enzootic, all mammals found dead, and particularly those with a clinical history of abnormal behaviour or neurological signs, should be carefully examined and considered as potentially infected until proven otherwise.
- **Suspect cases of sudden death should have peripheral blood smears taken to exclude anthrax infection as a differential before the carcass is opened.** Bloody discharges should direct the examiners' attention to the need to exclude anthrax infection before continuing with the examination. Dependent on region, specialist veterinary staff may be legally required to carry out the anthrax testing process.
    - Samples should be taken by nicking the dependent ear or from the coronary band.
    - In wild equids (**Equidae - Horses (Family)** - horses and zebras), wild pigs (**Suidae - Pigs (Family)**) and carnivores (**Carnivora - Carnivores (Order)**), anthrax bacilli may not be present within the blood, therefore examination of a smear made from the cut surface of a lymph node (usually submandibular) is recommended in addition.
    - Tissue and blood smears should first be air dried and then be fixed in methanol.
    - Staining should be performed for two minutes with polychrome methylene blue, or Giemsa stain.
    - Samples should be examined under oil immersion microscopy for evidence of

anthrax bacilli.

- **If anthrax infection is confirmed, careful attention must be paid to quick and effective carcass disposal. Regional authorities responsible for disease control should be notified and action taken as appropriate.**
- If anthrax infection is excluded, the *post mortem* examination should proceed.

- Estimation of time of death is not as widely a developed skill in wildlife as with human pathology; forensic entomology has not been used extensively in wildlife cases to date.
- Detailed knowledge of ballistics (shot gun, air gun, arrow) and the typical wounds that they cause is useful, particularly for forensic *post mortem* examination.
- Knowledge of the species of common predators for the animal under *post mortem* examination in that region is useful. An understanding of the distribution of the wounds that they typically inflict can be a useful aid for identification of cause of death or scavenging.
- **Note:** If poisoning (e.g. plant poisoning) is suspected, the pathologist should be informed of this suspicion **before** the necropsy is carried out.
- **For information on carrying out a Cosmetic Post Mortem, to enable the skin and skeleton to be used for museum-based studies**

WILDLIFE TELEMETRY

Accuracy of Locations

The accuracy of a radio-location varies with habitat type and may result in biased estimates of observed habitat use. A common source of error is signal bounce. Signal bounce occurs most frequently in mountainous terrain where a signal is deflected by a mountain, resulting in potential errors of many kilometres. The most effective way to overcome signal bounce during ground tracking is to take many bearings from several different places. When all signals appear to be coming from the same point then there is a good chance that the animal has been located correctly. However, if the signals are coming from a number of different points then signal bounce is likely still occurring (White and Garrott 1990).

Visual observations of radio-located animals provide the best confirmation of the accuracy of the relocation data. For large animals, a reasonable proportion of locations should be confirmed by direct visual observations (some biologists use >30% as a general rule; however, this may not be practical in all cases). In new study areas or with species which cannot be observed on a regular basis, it is strongly recommended that triangulation be used with an assessment of aerial fixes made using collars placed in known locations. Such trials can test the consistency and accuracy of triangulation using various personnel and methods under various environmental conditions. Results of the trials can be used to identify problems (e.g., signal bounce) and ensure that methods are adjusted to reliably obtain accurate radio locations.

When relocating wildlife in the field, most users judge the angle over which the signal sounds loudest, determine a bearing by mentally bisecting that angle, and follow the bearing to move closer to the signal. The process is repeated until the animal can be seen or its location can be inferred. The latter may be accomplished by circling the signal to determine a

bounded area in which the focal animal must occur, tracking the animal to an obvious habitat or landscape feature, or by sandwiching the animal between the receiver and an apparent obstacle.

Alternatively, if the researcher wishes to avoid disturbing the animal, or if locations must be determined at night, the process of triangulation may be used. This requires finding the intersection of two or more bearings to determine one location. An error polygon can be calculated around the point estimate, resulting in a measure of precision equivalent to the area of the polygon. The size and shape of the error polygon is determined by:

1. the accuracy of the directional antennae;
2. the distance between the two receiving points;
3. the distance of the transmitter from the receiving points;
and
4. the angle of the transmitter from the receiving points.

The most accurate estimate of an animal's location is obtained by receiving fixes that are closest to the animal and at 90o from each other. To reduce the size of the error polygon, three bearings can be taken and the animal's location estimated from the centre of the intersections. The error polygon formed by three radio bearing lines should be small enough to accurately place the animal in a single habitat polygon. If the location is near an edge, additional bearings should be obtained to accurately locate the animal on the map. Where possible, standard telemetry base points should be established, marked and numbered by personnel experienced in use of radio-telemetry equipment. New observers should be familiarized with the base points and standard triangulation procedures by an experienced person. Triangulation of animals which are moving will produce large polygons (less accurate locations). For this reason, it is difficult to accurately determine locations of fast-moving nocturnal wildlife such as owls. If triangulation is used to determine wildlife positions, error measures should be calculated and reported along with the study results (Springer 1979; Saltz and Alkon 1985; Schmutz and White 1990; Saltz 1994). White and Garrott (1990) provide a useful compilation of error calculations fortelemetry.

Protocols

General Guidelines

The following guidelines should be adhered to when relocating animals (adapted from Page 1982):

1. Ensure that you use an antenna which is matched to the frequency transmitted.
2. As a general rule, the antenna elements should be oriented in the samedirection as the transmitter antenna (i.e., when relocating a caribou wearing a radio-collar with a vertically-oriented whip antenna, the receiving elements should also be held vertically).
3. Hold antennas as high as possible or mount them on poles. Keep antennas at least 2 m away from all other objects, especially those which are large and metal objects, as these will cause detuning of the antenna.
4. Make use of null signals as well as peak signals to determine the direction to a transmitter. Using a 2-element antenna, the signal should be weakest when the tips of the elements point directly at the transmitter.
5. Make use of hills and other places of high elevation from which to receive signals.
6. Know your study area. Whenever possible take bearings through the flattest terrain with the least vegetation.
7. Take repeat bearings over a short time period, especially if the animal is active.
8. Get as close to the animal as possible. Attempt to confirm locations with direct observations.

9. Avoid sources of interference.
10. Take as many bearings as practical.

Aerial Surveys

The following guidelines for aerial surveys are adapted from Gilmer *et al.* 1981.

Equipment

 Safety equipment (First aid kit, survival kit, etc.)
 List of animals and frequencies to be located during flight (include notes on frequency drift)
 Clipboard, pencils, pens
 Maps and RIC data forms (or other with similar fields)
 Camera and film
 Communication radio (air to ground)
 Test signal transmitter
 Timepiece
 Receiver
 Scanner
 Auxiliary power supply
 Headphones
 Switchboxes
 Extra coaxial cable (proper length with connectors)
 Antennas and mounts
 Tool kit for mounting antennas (wrenches, screwdriver, pliers)
 Duct tape, electrical tape
 Extra bolts, washers

Procedures - Pre-flight

1. Obtain a good set of maps and air photos of the project area. It can be useful to have both large and small scales.
2. Define the area which is to be searched for animals before the flight. If the Project Area is large, it may be useful to break it down into smaller Study Areas which can be effectively searched within an allotted time.
3. Primary power sources for receivers should be fresh and fully charged at the start of the survey.
4. An up-to-date list of transmitter frequencies should be carried, including the location of each animal from the previous search (as this may be a useful starting point).
5. Set up receiving equipment (this should be done with the pilot who has the ultimate responsibility for its safety):

 Attach mount with antennas to aircraft.
 Run coaxial cables into cabin.
 Hook up switch boxes and receiver.
 Use duct/electrical tape to secure connections and cables where appropriate.
 Test system, make sure switch box functions correctly.
 Check programmed frequencies and dwell time.

Procedures - In Flight

1. Begin the search with the switch box set to “both” allowing the crew to listen for animals on either side of the aircraft.
2. At the outset of the flight, it may be beneficial to test equipment by makinguse of a test transmitter which is left at a known location on the ground.
3. Depending on the nature of the focal species and the objectives of the study, it may be useful to begin searching at the last recorded location for each animal. If this is unsuccessful, a more systematic, transect-based search design should be utilized (see item 8).
4. When a signal is detected, the control switch should be moved to “left” and then to “right” to determine from which side of the aircraft the signal is coming.
5. Once the direction has been determined, the pilot should turn the aircraft in the direction of the transmission. This will result in a temporary “null” signal until the aircraft flies close enough to the transmitter that the signal becomes audible again.
6. At this point, it should be possible to “home-in” on the transmitter position. Again, the operator changes the switch box from “left” to “right” to determine which side of the aircraft the transmitter is on. The operator will then identify an area on the appropriate side over which the pilot shouldbegin a wide circle.
7. By moving the switch “right” and “left”, the operator should be able to determine if the transmitter is within the area being circled. The circle maythen be tightened, and focused based on the strength of the signal and theknowledge of the species habitat preferences.
8. Whenever possible, flight crews should attempt to verify an animals presence through direct observation.
9. For searches of a large number of highly mobile animals over a large area, it may be more appropriate to use a systematic method, using a scanner and parallel transects. For such searches, biologists should be aware of the limitations of receiving equipment to effectively scan for animals in a fastmoving aircraft. To this end, the formula below will calculate the maximum number of animals that may be effectively scanned for on a survey flight (for more information, see Gilmer *et al.* 1981).

$$NC = \frac{MD \times GS \times}{SR} = 3600$$

where: NC=maximum number of animals which can be searched for
MD=minimum detection distance parallel to the aircraft’s direction of movement
SR= receiver scan rate
GS= maximum ground speed of aircraft

STUDY DESIGN

General Considerations

Despite the wide range of possible research questions which a radio-telemetry study can address, the provincial wildlife inventory program requires that all telemetry work be focused on issues of species abundance and distribution.Within the context of the wildlife inventory program, radio-telemetry should be used as part of an inventory project to address at least one of the followingobjectives:

1. Provide information about the use and/or selection of landscape/habitat by a species (section 6.3).
2. Provide locations of key habitat elements (e.g., hibernacula, nests) which are required to facilitate conservation of a species (section 6.3).

3. Provide descriptions of home ranges, including their size, position, density, and/or composition in terms of habitat (section 6.4)
4. Provide an assessment of population size, such as through the use of biascorrecting indices for other RIC-approved surveys, or population dynamics, such as studies of mortality and survival (section 6.5). Assuming research objectives, including hypotheses, have been declared and deemed compatible with the objectives above, a researcher should also consider a number of additional questions before embarking on a study involving radiotelemetry.
 Is radio-telemetry the best method to address the project objective or hypothesis? Are less expensive alternatives available? If so, do the differences in the type and quality of data collected using radio-telemetrywarrant the associated expense?
 How many animals will need to be radio-tagged to provide a meaningful conclusion to the project hypothesis? Can the study species be captured in sufficient numbers to provide an adequate sample size? In the event of low capture success, will the decline in statistical power render the resultsmeaningless?
 Can a radio tag provide a useful measure of the study variable(s) with levels of accuracy which are adequate for the project? Will the resulting data be in format conducive to analysis?
 Can the study species carry a radio tag without undue detrimental effects? Can the study species carry a tag of sufficient size to possess adequate rangeand tag life?
 Is the project's budget sufficient to cover tags and monitoring equipment? Will staffing levels be adequate to ensure proper monitoring of study animals on an adequate monitoring schedule? If the answer is negative or unknown for any of the above, the use of radiotelemetry techniques should be reconsidered. Despite the attraction of radioWildlife Radio-telemetry 32 August 6, 1998 telemetry as an inventory technique, it is very expensive, labour intensive, and potentially stressful to animals. Its application should be limited to those situations where it is most warranted. If the use of telemetry techniques seems valid, the researcher should undertake a literature review of the subject area. S/he should be looking for previous examples of tag types and successful attachment techniques, for the taxonomic group concerned. The investigator should consult with several suppliers of telemetry equipment and researchers experienced in the area in order to determine tag type and attachment method. Much research in telemetry is experimental and many people have not published the results, thus contacting experienced researchers can be very productive.

Sampling Considerations

Radio-telemetry studies must take into account two measures of sample size 1) the number of individuals followed, and 2) the number and timing of relocations of each individual. Data points gathered for individuals are used in home range / habitat use analysis whereas the number of tagged individuals is usually used with more typical statistical testing. This distinction is very important: one cannot expect to answer many questions by obtaining large samples of relocation data for one animal. The number of animals tagged must be adequate for statistical tests since it is the animal and not the number of locations that is the true sample size. There is a compromise to be reached between numbers of animals tagged and numbers of relocations per animal. Biases to one or the other are usually not desired. However, Alldredge and Ratti (1992) stated that 'it is doubtful that random sampling can be achieved in practice in most studies with radio-tagged individuals'.

The number of animals of each age and sex which are sampled is determined by the objective or hypothesis being tested. For example, if only data on mature animals are desired, then the researcher does not have to sample younger animals. Studies of populations which are not easily divided into meaningful sex or age classes are more reliant on assumptions of homogeneous catchability. To illustrate, some wildlife species may be very difficult to sex and/or age underfield conditions. Under these circumstances, the researcher may be forced to

assume that the sample of tagged individuals adequately represents the studypopulation. All such assumptions should be explicitly stated when the study results are reported.

Seasonal considerations are also a factor in many wildlife studies. If the study objectives are to examine winter range size of white-tailed deer, the researcher must monitor study animals throughout the winter. If the objectives are to document total home range size, study animals must be monitored throughout the year. Hibernating species will not require monitoring throughout most of the winter, unless study objectives include the collection of physiological data through biotelemetry. Other species may exhibit circadian patterns in behaviour and/or habitat use. Radio-locations must be a random sample of the animal's behaviour. This can be accomplished by sampling at random times or by sampling at regular intervals.

Researchers must ensure that they collect enough data to address project objectives, and do not rely on general inferences to interpolate between relocation data points when it is inappropriate (e.g., determining home range of a species when data were only collected for a portion of the year). Preliminary sampling (or pilot study) is an excellent way to determine the suitable sample size (relocation points) for a particular project. For example, in a home range study, a researcher can create asymptotic curves (home range size vs. number of data points) to evaluate whether additional relocations of an animal improve the description of its home range. Obviously, the number of relocations which are possible in a project will be dictated by more than good science; logistical considerations such as accessibility, size of study area, staff levels, behaviour of animals (e.g., migratory nature), and other factors will all play a role. Similarly, the number of animals which are tagged (i.e., the sample size needed for statistical testing) will influence the quality of conclusions drawn from a project. The number of individuals required to test a hypothesis should be determined *a priori* using power tests. The number of animals which can actually be tagged will be the product of factors such as the project budget, the number of separate transmitter frequencies possible in the project's assigned frequency range, and the natural history of the organism. As an example of the latter, if the objective is to determine the home range of an animal which lives in social groups such as herds or packs, it may be only necessary to tag one or two individuals in order to document the movements of an entire group. When contingent with objectives, researchers should ttempt to design telemetry projects in a manner that is logistically efficient. Thoughtful planning can help to minimize travel time and maximize relocations collected per trip while taking advantage of existing access within a study area (particularly when locations must be obtained at night). In some cases, this may be accomplished by tagging animals in the same general vicinity so that several radio locations can be obtained in a single outing. Obviously, the relocation efficiency must be properly balanced with study design and objectives. For example, the objective of quantifying turtle nest sites within British Columbia can not be realized by tagging only in the Peace River area. However, it is not always clear how best to distribute radio tags between multiple study areas. Although having numerous study areas will provide more complete coverage of a landscape, in certain situations, having many animals tagged within each study area can allow more information to be obtained for the same time and travel costs.

Data Forms and Data Collection

Provincially-standard protocols for collecting and recording information should always be used. Certain detailed information recorded in the field may depend on the nature of the project, but, at a minimum, should include information specified in the accompanying data forms. This includes detailed physiological information, particularly for large animals which are captured. In addition to information on data forms, the position of each animal should generally beplotted on an air photo or topographic/habitat map. For habitat-use studies,

specific information on the habitats used may also be collected (generally using an Ecosystem Field Form) and, at a minimum, observers should attempt toestimate the Broad Ecosystem Unit in which an animal occurs.

At the end of each field trip, the researcher should review all radio locations to ensure all data are adequately recorded. This may involve coding data into a computer (or the Species Inventory data system) for later analysis. For detailed descriptions of minimum data requirements, see the attached data forms.

In general, data can be subdivided into four groups.

 Physiological data Includes vital signs and morphometric information collected from captured animals.

 Visit Characteristics Includes date and environmental characteristics of relocation trips, such as temperature, cloud cover, wind speed, andprecipitation.

 Observation characteristics Includes information about animals which are relocated. At a minimum, this should include time, animal's UTM grid location, sex/age classification, observation type (air, ground, remote), observation accuracy (sighted, accurate fix, weak signal, vague etc.), and possibly activity, group size, or other characteristics which are specific to anindividual animal (snow depth, snow sinking depth, site position, distance from cover, etc.);

 Habitat information Includes as a minimum, Broad Ecosystem Unit, but may also include biogeoclimatic zone, biophysical habitat class, seral stage, dominant vegetation cover (crops, tree species), and/or reference to a standard ecosystem description form (e.g., Ecosystem Field Form or Ground Inspection Form).

All data should be entered into computer data files on an ongoing basis to determine trends, erroneous data points, number of fixes needed, appropriate time intervals, etc. Periodically (at the end of each seasonal time period, if applicable) data collected should be tabulated and inspected using frequency distribution functions to identify incorrect codes and correct errors. Scatter plots of radio location points should also be examined to identify and verify outliers.

Original notes of personnel collecting the data should be available to assist in correcting ambiguous data. If applicable, radio location data should be compared for males and females, between age groups or between study areas and combined if no significant differences are found. Data should only be pooled where appropriate, which will depend on the specific objectives of the study. Possible pseudoreplication errors may result from:

 inadequate representation of individuals, sexes or ages;
 inadequate representations of different seasons;
 inadequate representation of circadian patterns;
 inadequate representation of different habitats;
 pooling individuals that differ in space use; and
 using autocorrelated data.

Habitat Utilization Studies

Radio-telemetry can provide detailed information about an animal's use of habitat. Expected proportions of use (radio locations) in each habitat are calculated based on the relative availability of each habitat in the study area.

Investigating habitat preference or critical habitat features are common themes among many studies. To make conclusions about how a population uses habitat, a researcher must carefully consider the objectives of the study and ensure adequate representation of all classes of individuals within the study population.

In many studies both sexes must be represented among the tagged animals, as habitat use of males and females may be quite different. Researchers must also ensure that the location of capture is not biasing the selection of individuals forsampling. As an example, if a researcher wished to investigate the choice of roost sites by Barn Owls, a capture program based on mist-netting owls inside buildings would bias the sampling to birds which choose human-made over natural roosts. A better capture method might involve capturing foraging owls at night while they were away from their roost site. Traps set in a particular habitat or at a particular time might be more successful at capturing one sex or age over another. The researcher should carefully classify each animal captured before deciding whether to attach a radio tag. A common practice among many researchers is to randomly sample populations whenever possible.

Some species may exhibit circadian patterns of habitat use, occupying nocturnal or crepuscular habitats which differ substantially from daytime ones. To obtain an accurate picture of habitat use, radio locations may need to be split between day and night within in each season (Beyer and Haufler 1994). Sampling should also be done under all weather conditions and in all seasons as habitat use may vary. Habitat use may also vary between years (Schooley 1994). Ideally, a fixed schedule of sampling should be devised and adhered to. This schedule can still be random. For example, the researcher could randomly select dates within a given period of time to track animals, randomly select areas for capture, etc. The most important thing is that organisms are not being monitored simultaneously with a circadian rhythm of some description. For example, if moose always water at dawn then one would not always collect data at dawn. This would bias the results.

A fundamental feature of many of the parametric analysis programs is independence of radio locations. Several methods or definitions of this concept have been put forth. In one study it is assumed that radio location points are independent if sufficient time has elapsed to allow the animals to redistribute themselves (McNay *et al*. 1994). While another study uses the minimum time it takes for an animal to cross its home range as the basis for a test for the minimum interval between relocations which gives spatial independence (Swihart and Slade 1985). White and Garrott (1990) state that sufficient time must pass between relocations for an animal to move from one end of its home range to the other. More frequent locations or continuous tracking may be required to document intensity of use, dispersal, daily movement patterns, social interactions, weather effects, and for detailed studies on habitat selection where habitat patches are small. For example, more frequent locations may be required to document daily movements from cover to foraging areas and back in early morning.

Habitat classification associated with relocation data points can be measured in several ways. It can be recorded in the field (when the animal is relocated or at a later date when the observer travels to the study area) or one can physically plot the location on a habitat map and transcribe the habitat type from the map. Observers should be trained to identify habitat type in the field. If, for some reason, the this is not possible, then a protocol should be in place to either mark the location physically or have the observer plot the position accurately on a detailed map. This becomes an issue especially during night work. Generally, habitat types should be defined using Broad Ecosystem Units while for more detailed work the Ecosystem Field Form should be used. For more information on standards for habitat description, consult *Species Inventory Fundamentals,*
No. 1.

Although all radio-tracking studies must contend with uncertainty in animal relocations, those which evaluate habitat use/selection must also recognize the potential for error in habitat discrimination and delineation. As the number of habitats increases, multiple comparison error rates also increase so the number of habitats considered should be limited in the study design (Bibby *et al.* 1992).

Data Analysis

Occurrence of animals by habitat type should be summed for each seasonal time period. Expected use should be based on an accurate tabulation of habitat quantities in each study area. Standard use/ availability analyses (Johnson 1980; Marcum-Loftsgaarden 1980; Neu *et al.* 1974) are often used to determine if observed use is significantly different from expected. It is important to be familiar with the assumptions implicit within these types of analysis, particularly as calculating the strength of habitat selection may be a product of the researcher's estimate of how much habitat is actually available. Although statistical techniques required to measure habitat use/availability remain controversial, compositional analysis (Aebischer *et al.* 1993) or standardized selection ratios (Manly *et al.* 1993) are recommended as analytical techniques because they address some of the more serious pitfalls associated with traditional use/availability analysis. For a complete discussion on the advantages and disadvantages of different statistical methods, see Alldredge andRatti (1986, 1992).

Proportions of habitat use are not independent (e.g., if an animal spends more time in habitat A then it must spend less time in habitat B). Therefore, tests that assume independence of habitat cannot be used (Freidmann 1937; Aebischer *etal.* 1993).
Observations gathered during data collection can be most useful to determine the habitat attributes most important to animals. It is easier to understand what animals are doing at the time of field observations than from inspecting a tableof numbers.

Locating Specific Habitat Features

Radio transmitters may be utilized to locate specific habitat features such as nests, dens or roosts for other types of studies. For example, applying temporary tags to birds during the nesting season allows researchers to locate nests easily. The nest can be located in a day or two, and when the tag drops off it can be placed on another bird. This technique may be used to identify similar habitat features for other species, such as bat roosts, snake hibernation dens, or amphibian breeding sites. The use of radio-telemetry to find habitat features may become especially important in British Columbia with the implementation of the Forest Practices Code. Included in the Code are provisions for the protection of specific habitat features which are critical to the viability of certain species and subspecies through the designation of Wildlife Habitat Areas. Under the Code, conservation of these taxa, which include numerous species at risk, is closely tied to habitat, and is contingent on the ability of provincial biologists to locate critical habitat features, such as roosts, hibernacula, or breeding sites. Radiotelemetry may be the most efficient way (and in some cases, the only way) to locate these features. Biologists using telemetry to locate specific habitat features will need to take extra care when attaching transmitters to breeding or reproductive organisms (where this is deemed feasible). Such an exercise should not be taken lightly, as organisms of interest for such study are frequently red- or blue-listed, and poorly planned capture or handling can easily be disruptive to survival and successful reproduction. Additionally, researchers following tagged animals should exercise similar care when making observations in and around critical habitat features. Use of these features frequently occurs at those points in a species life cycle when it is particularly vulnerable to disturbance. It is of little use to locate the new nest of a red-listed bird only to have it abandoned shortly thereafter.

Home Range Determination

Studies of home range size usually seek to obtain a mathematical determination of home range size for representative animals in a population. Sample sizes are dependent on the study objectives, analysis methods and several biological parameters such as social structure. As previously mentioned, which particular subset of the population is sampled is dependent on the research objectives(e.g., whether or not to include different age/sex classes.)

Sample size as it pertains to number of relocations can be determined through pilot studies and asymptotic plots. Locations of each animal are used to calculate a home range size (see Data Analysis) which is recalculated each time the animal is relocated. Graphs for different ages/sexes/individuals may be compared to see how much variation exists between different groups, and what number of relocations is necessary to differentiate between groups. If there is little variation between animals of different sexes or ages, it may not be necessary to allocate sampling effort between different classes (i.e., increase number of tagged individuals). However, if, for instance, males are found to have a significantly larger home range size than females, a study concerned with determining average home range size for the species should allocate sampling efforts between the sexes according to the naturally-occurring sex ratio in the population (this would occur if individuals are randomly sampled). A safeguard against sex/age class differences is to randomly sample the population (this only works if the objective is a general home range over all sex/age classes combined). However, if the objective is to document differences between age/sex classes then equal representation should be met in order to meet requirements of the necessary statistical tests. Preliminary sampling at random or systematic points in time also aids in determining circadian patterns of the study species. Preliminary sampling should be based on the natural history of the organism (i.e., try to sample throughout the time when the animal is active). For example, it may be important to identify the roosts of certain birds; however, prolonged radio tracking of sedentary, roosting birds throughout the night may yield little usefulinformation.

Possible differences due to habitat quality should also be identified during preliminary sampling. A Barn Owl in lush old-field habitat may require a significantly smaller home range than an owl in poorer-quality habitat. Bytesting for differences between characteristics of individuals and characteristics of the habitat, the researcher can often control for many variables other than theone under study.

Some authorities have concluded that approximately 30 relocations per individual will provide an adequate sample size for home range determination for many applications (Kenward 1987). This number is highly variable however so each researcher should test this during their pilot study (the asymptotic plotmethod provides a straightforward approach).

Data Independence

One problem with multiple relocations of the same individual is that the relocations may not be statistically independent, which may lead to underestimations of home range size (McNay *et al.* 1994). Lack of independence may be due to migratory movements or to infrequent movements due to unique places in the home range (e.g., periodic visits to a salt lick), or to sampling protocol (see above). Knowledge of the behaviour of the study species is necessary in order to interpret unusual movements and decide whether or not to include outlying fixes in home range calculations.

Data Analysis

An animal's home range size, shape, and position is often represented by joining the outermost fixes for that animal to form a minimum convex polygon (Mohr 1947). Outlying fixes (representing rare excursions) may unduly influence thepolygon's shape and size to produce a misrepresentation of the space actually used by the animal (McNay *et al.* 1994). Analysis models which allow for data clumping (Don and Reynolls 1983), harmonic mean methods (Neft 1966; Dixonand Chapman 1980; Kenward 1987), ellipses (Jennrich and Turner 1969), cluster analysis, core convex polygons (Kenward 1987) or kernel estimation methods (Naef-daenzer 1993) may provide better representation of the data. The test of any method of depicting home range is the significance of its results in terms of the animal's use of space.

On a related topic, distances between consecutive radio locations of an individual are often used as an index of the total daily movement for that individual (Laundré *et al.* 1987). Rates of movement are often compared between demographic groups or time periods. However, perceived movement distances determined by daily locations may not necessarily be correlated with actual distances moved (Laundré *et al.* 1987). It is recommended that researchers planning to use telemetry data in this way, do preliminary sampling to compare data from once/daily locations with that obtained from round-the clock hourly monitoring. If there is little correlation, the results obtained from daily locations will not be valid.

Demographic Studies

Radio-telemetry may be used to improve the quality of other wildlife surveys which attempt to estimate population size and composition. This is because during a survey, observers will be able to assess the number of unseen radiotagged animals which were known to be in the study area. This knowledge can be used to correct survey results for visibility bias (the failure to observe all animals during an aerial survey). The degree of visibility bias depends on a variety of factors including, the amount of vegetative cover, animal behaviour, animal size and coloration, observers, weather, and equipment. Radio equipped animals allow estimation of this bias since instrumented animals known to be in an area can be recorded as seen or not seen.

Radio-telemetry is often used to improve accuracy of classification counts of species which may be classified by means of survey flights (most big game species). Radio tags are placed on individuals of known sex and age. Classification counts done from aircraft can then be combined with relocation of tagged individuals. Whether or not the tagged animals are visible from the air provides a means of calculating sightability indices to be used as correction factors for the classification counts (Simpson *et al.* 1993). The use of radio collars enhances survey accuracy because:

- radio collared animals can be monitored after the survey to determine whether any left or entered different survey areas;
- the movements and behaviour of collared animals can be monitored to assess their detectability relative to unmarked animals;
- the loss of marked animals can be detected; and
- relocating marked animals immediately after a survey area is completed allows determination of the reasons that animals were missed.

More sophisticated sightability correction models have been developed for surveying large mammals using radio collared animals to correlate sightability with other parameters recorded during surveys such as group size, activity, snow cover and vegetation cover for animals observed (Unsworth and Garton 1991).

Mortality & Survival

In theory, radio-telemetry techniques should enable the importance of causespecific mortality factors to be determined because tagged animals can be located soon after death and the agent of mortality ascertained (Heisey and Fuller 1985). In practice, it is often difficult determine the cause of mortality due to difficulties accessing the carcass soon enough after death and to distinguish mortalities from other tag losses such as tag failure and animal dispersal. It is extremely important that the capture and attachment of a radio tag to the study animal does not affect its probability of death. Researchers engaging in this type of study should employ proper capture methodology and ensure tag attachment does not influence survivability by either evaluating the effects of tag attachment on mortality or using only tag types and attachment methods which have been previously proven to be unbiased. It is also

important for researchers to re-evaluate their methods whenever an iatrogenic or researcherinfluenced mortality occurs.

Defining adequate sample sizes for mortality studies is done by preliminary sampling to determine the variance in survivorship. Survival rates are estimated from the number of transmitter-days, the number of mortalities due to particular causes, and the number of days in the chosen interval of time over which daily mortality rates are assumed to be constant (Heisey and Fuller 1985). A study design described by Pollock *et al.* (1989) allows for new animals to be added to the tagged population after the study has begun.

The fate of lost tags and causes of mortality should be ascertained as closely as possible for results to be credible. Estimates of radio failure rate may be made by keeping accurate records of pulse rates of each transmitter over time, although some transmitters may not change overtly prior to failure, and so this is not always reliable. Kenward (1987) chose to classify as mortalities any tags which were lost a) well before the end of their expected cell life b) with no slowing or irregularity in their signals, and c) without subsequent recapture or resighting. Depending on the situation and the study species, this may not always be appropriate as transmitters will fail without warning and study animals will disperse beyond the limits of the study area. It is important that researchers divulge whatever assumptions they choose to make with regard to mortality, and, in some cases, they will have to accept that the fate of some individuals will remain unknown.