GUIDELINES FOR THE CAPTURE, HANDLING, AND CARE OF MAMMALS

AS APPROVED BY

THE AMERICAN SOCIETY OF MAMMALOGISTS

Prepared by the

Animal Care and Use Committee

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INTRODUCTION

Background and History

The American Society of Mammalogists (ASM) is the oldest scientific organization devoted to the study of mammals. Founded in 1919, the American Society of Mammalogists currently has 3,700 members residing in ca. 70 countries. Historically, the ASM has encouraged its members to maintain high professional standards and has attempted to aid them in doing so by preparing guidelines related to various professional activities. Previous ASM guidelines have dealt with curation of systematic collections (Advisory Committee for Systematic Resources in Mammalogy, 1974; Committee on Systematic Collections, 1975, 1978), scientific permits (unpublished report of Committee on Legislation and Regulations, 1977), the use of mammals in research (<u>ad hoc</u> Committee for Animal Care Guidelines, 1985), and acceptable field methods in mammalogy (<u>ad hoc</u> Committee on Acceptable Field Methods in Mammalogy, 1987).

The objective of the Society's 1987 guidelines for acceptable field methods in mammalogy was to identify field methods in mammalogy that would meet standards of the American Society of Mammalogists. The guidelines were formulated with ASM ACUC Guidelines

consideration for both the welfare of subject animals and the research needs of field investigators for whom guidelines for laboratory animal care generally do not apply. These published guidelines have served ASM members and non-members well during the past decade; however, the passage of time has seen advances in technology (e.g., passive integrated transponders - PIT tags- for marking animals) that need to be addressed. In addition, the past decade has produced increased recognition of the potential risks to field investigators from handling live and dead mammals, as well as heightened concern within and outside the scientific community regarding the humane treatment of mammals collected and used in scientific research.

As a consequence, the ASM Committee on Animal Care and Use was assigned the responsibility of updating and expanding the preliminary guidelines published in 1987. This committee is a descendant of the <u>ad hoc</u> Committee on Acceptable Field Methods in Mammalogy and was established as a standing committee in 1992. Its current members include G. L. Kirkland, Jr. (chair and editor), G. W. Barrett, M. A. Bogan, J. E. Childs, G. Glass, A. Krevitz, L. R. Heaney, T. H. Horton, T. H. Kunz, T. J. McIntyre, G. R. Michener, J. N. Mills, R. R. Parmenter, A. J. Pinter, J. H. Shaw, S. D. Thompson, B. A. Wunder, and T. L. Yates.

Similar guidelines pertaining to animals, including mammals, have been established by other organizations (for example, the

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Association for the Study of Animal Behaviour, 1996) and should be consulted as needed. It is not our intention to provide a detailed "how-to" publication for field researchers in mammalogy. Such a publication is beyond the scope of the committee's mandate. Furthermore, several excellent references of this type have been published and should be consulted for hands-on details. Two noteworthy and recommended volumes are Kunz (1988) for coverage of techniques for the study of bats, and Wilson et al. (1996) for techniques appropriate for the study of a broad spectrum of mammals.

We emphasize that the guidelines of the American Society of Mammalogists are not intended to obstruct ingenuity in research design or to stifle the quest for newer, better research methods or materials. We believe that the ultimate responsibility for design of research and selection of techniques and study organisms must rest with the investigator. Although the ASM's guidelines have been prepared by mammalogists primarily for use by mammalogists (broadly defined as anyone studying any aspect of mammalian biology), they also may be of interest to governmental agencies that establish, administer, or enforce policies and regulations relating to mammals, to agencies responsible for funding scientific research on mammals, and to institutional committees that oversee the use of animals in education and research.

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 of systematic and evolutionary relationships among species, various genetic phenomena, population dynamics, community structure and 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, ASM ACUC Guidelines

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; ASM ACUC Guidelines

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. <u>The Federal Register</u> 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 diseasecarrying 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 ASM ACUC Guidelines

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 (see HEALTH PRECAUTIONS).

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 (see METHODS OF EUTHANASIA). 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 ASM ACUC Guidelines

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) quidelines 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"

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(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" (<u>ad hoc</u> Committee for Animal Care Guidelines, 1985).

Specific safety guidelines have been published for handling and sampling small mammals that are known reservoirs for agents that can cause severe human diseases such as hemorrhagic fever or hantavirus pulmonary syndrome (Mills et al., 1995<u>a</u>, 1995<u>b</u>). 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).

RESPONSIBILITY FOR DEPENDENT OFFSPRING

Field study of mammals is most frequently carried out during warmer months of the year, which correspond to the reproductive season of many species. As a consequence, there is a high probability that captured females may be lactating and, therefore, have dependent young, which are at risk. Investigators should recognize the dependent relationship between suckling infants and their mothers, and whenever possible should design sampling procedures to minimize the possibility of removing or killing lactating females. When this cannot be avoided or when orphaned young are found, the researcher should assume responsibility for such young, most commonly by killing them quickly and humanely. Live-trapping should be designed to minimize the time lactating females are removed from their dependent offspring.

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 plaque 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 be confirmed.

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 <u>Peromyscus leucopus</u> this results in higher infestations of ticks (<u>Ixodes scapularis</u>), 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 (Code of Federal Regulations, Title 10, Part 20).

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 ASM ACUC Guidelines

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 natural conditions 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 <u>Histoplasma</u> <u>capsulatum</u> (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.

PUBLIC RELATIONS IN THE FIELD

Some field methods, although completely legal and humane, may be misunderstood by the public. Therefore, researchers should to be discreet in all activities that may affect the sensibilities of the public. In general, taking time to explain field activities to interested or concerned individuals is a valuable practice.

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