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GUIDELINES OF THE AMERICAN SOCIETY OF MAMMALOGISTS FOR THE USE OF WILD MAMMALS IN RESEARCH

WILLIAM L. GANNON,* ROBERT S. SIKES, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS

Department of Biology and Office of the Vice President for Research and Economic Development, Research Ethics and Compliance Services, MSC05 3180, University of New Mexico, Albuquerque, NM 87131, USA (WLG)
University of Arkansas at Little Rock, Basic Animal Services Unit and Department of Biology, 2801 South University Avenue, Little Rock, AR 72204, USA (RSS)

General guidelines for use of wild mammal species are updated from the 1998 version approved by the American Society of Mammalogists (ASM) and expanded to include additional resources. Included are details on marking, housing, trapping, and collecting mammals. These guidelines cover current professional techniques and regulations involving mammals used in research. Institutional animal care and use committees, regulatory agencies, and investigators should review and approve procedures concerning use of vertebrates at any particular institution. These guidelines were prepared and approved by the ASM, whose collective expertise provides a broad and comprehensive understanding of the biology of nondomesticated mammals in their natural environments.

Key words: animal capture, animal care, animal housing, animal marking, animal use ethics, federal regulation, Institutional Animal Care and Use Committee, trapping

ORGANIZATION OF GUIDELINES

* *Correspondent: wgannon@unm.edu*

Introduction

Advances in mammalogy, from exploring physiological functions to understanding evolutionary relationships and developing management strategies, are predicated on responsible use of mammals in research. Founded in April 1919, the American Society of Mammalogists (ASM) has long been concerned with the welfare of mammals, and in particular, natural communities. In 1928, one of the founders of the ASM, Joseph Grinnell, instructed administrators of Yosemite National Park to maintain the Park as a natural mammalian community without unnecessary or destructive development. Grinnell (1928:76) described a number of management tactics that should be considered by Park managers, but in particular, he advised that to address an unwanted increase in the bear population Park officials needed to ''devise [some] means whereby troublesome individual bears could be discouraged from raiding food-stores, without doing them serious bodily harm. But I recommend that exceeding care be taken in such procedure, not to rouse, unnecessarily, adverse public opinion, and not to drive away the bears altogether, for they constitute a particularly valuable element in the native animal life of the valley.'' Thus, Grinnell made informed management recommendations and also advocated animal care and use with sensitivity toward public opinion. The same is true today because mammalogists care deeply about the sentient organisms that they study.

In medical research, highly selected, domesticated strains are used to reduce differences among individuals. In this research, the mammalian model (usually *Mus* or *Rattus*) frequently is considered more the vessel, vehicle, or source of tissue for the drug study or neuroscience investigation. In contrast, field researchers usually are interested in the mammals themselves as the focus of study, and variation among individuals and natural behaviors are of fundamental interest and importance. Differences between medical research and basic research on mammals frequently pose problems for field researchers because regulations developed for laboratory environments and domesticated taxa are increasingly and inappropriately extrapolated to the field and to wild taxa even though conditions and context are dissimilar. Guidelines for animal protocols have become more important with increasing use of native animal models in research. The Animal and Plant Health Inspection Service (APHIS) within the United States Department of Agriculture (USDA) unit has amended the Animal Welfare Act (AWA—USDA 2005; http://www.access.gpo.gov/uscode/title7/chapter54.html) to oversee field studies, which are defined as studies conducted on free-living wild animals in their natural habitat.

The *Guidelines for the Use of Animals in Research* (*ad hoc* Committee for Animal Care Guidelines 1985) was the 1st effort to codify the expertise and philosophy of the ASM on use of mammals in research. This single-page statement broadly listed considerations, such as concern for number of animals used, and highlighted laws that regulated use of animals (including Convention on International Trade in Endangered Species [CITES]). It stated that the investigator should use good judgment and be prudent when using animals in research.

A more detailed set of guidelines was published by ASM in 1987 with *Acceptable Field Methods in Mammalogy*: *Preliminary Guidelines Approved by the American Society of Mammalogists* (*ad hoc* Committee on Acceptable Field Methods in Mammalogy 1987). In the same year, the Animal Care and Use Committee was designated a standing committee of the ASM. These guidelines provided an explanation of fieldwork in mammalogy and rationale for collecting specimens. Guidance also was given on adequate sample sizes, and summaries of laws and regulations that pertained to mammals were detailed. The utility of this publication was perhaps best illustrated in the section on methods, where details were provided on use of kill-trapping and shooting, livetrapping, mistnetting (for bats), and methods relatively new to mammalogists, such as harvesting or sampling of tissues. Acceptable methods of marking, tracking, holding, transporting, and releasing mammals were described in the 1987 version. Issues concerning holding of mammals in captivity, administering humane euthanasia, and listing health precautions for researchers also were covered. Resources for these guidelines included information from other professional societies, such as the Society for the Study of Animal Behaviour (updated 2006), the American Veterinary Medical Association (AVMA) Panel on Euthanasia (updated 2001), and several publications on trapping methods. Ten of the 15 citations listed in the 1987 publication were papers either in press or unpublished, or were committee reports. Much of the information provided in the 1987 guidelines was from comments by investigators and researchers who were conducting fieldwork.

The 1987 guidelines were again updated and expanded by the Animal Care and Use Committee in 1998. In captive situations, the ASM suggested (Animal Care and Use Committee 1998) that researchers read and follow guides published by the United States and Canadian governments (Canadian Council on Animal Care 1993). In essence, ASM guidelines provided highlights of more complete information available from either the *Guide for the Care and Use of Laboratory Animals* (National Research Council; hereinafter *Guide*—NRC 1996) or the AWA; that was, minimize numbers taken, reduce pain or distress of captive animals, and provide humane euthanasia for studies where death was the endpoint. Also, there were concerns that people not familiar with research might view use of animals unfavorably. Consequently, researchers were advised to be prepared to justify their project and use of animals to an inquiring public.

The guidelines herein are intended to provide investigators and those charged with evaluating animal use in research (institutional animal care and use committees [IACUCs], reviewers and editors of research manuscripts, management agency personnel, graduate committees, and the public) with general and specific guidance on mammal care and use issues and health, safety, and environmental concerns particular to nondomesticated mammals. We emphasize that these guidelines are not intended to constrain ingenuity in meeting research demands, but rather to bring relevant safety, regulatory, and ethical concerns regarding animal use to the attention of investigators. It is the responsibility of the principal investigator

(PI) of a project to justify deviations from federal guidelines during the submission process of a protocol to an IACUC. Institutions have varying requirements for animal use and care, but as scientists, we have developed an ethos towards animal use. ''Ethics'' typically is defined as a study of moral values, that is, expectations about beliefs and behaviors by which we judge ourselves and others (Macrina 2005). All research procedures commonly used today must be considered and discussed by IACUCs as to whether they cause even momentary pain and distress.

This paper was prepared and approved by the ASM, whose collective expertise provides a broad and comprehensive understanding of the biology of nondomesticated mammals in their natural environments. It is intended to be a resource in those instances where difficulties arise in defining what is appropriate when dealing with nondomesticated mammals. It must be explicitly clear that this document is not intended to be an exhaustive catalog of procedures and that final approval of any protocol rests with the IACUC.

General Guidelines

Fieldwork with Mammals

Fieldwork might be the most difficult issue for IACUCs and others who typically evaluate use of animals in laboratory-based studies. Fieldwork in mammalogy involves designing and conducting research to answer scientific questions by working with mammals in their natural habitats. This process might involve capturing an animal to obtain reproductive and other data and subsequently releasing it to obtain additional information on population dynamics, movements, and habitat relationships. In some cases, the investigator might bring an animal back to the animal resource facility for further study. In the United States, field and laboratory researchers who receive federal support must comply with relevant provisions of the United States Public Health Service policies on humane care and use of laboratory animals (Office of Laboratory Animal Welfare, National Institutes of Health [OLAW/NIH 2002]). Use of sedatives, analgesics, and anesthetics often is under federal and state control. Investigators must check with federal and state drug enforcement agencies and obtain appropriate licenses during a study's design stage. Some drugs (e.g., narcotics) must have strict inventory logs and be stored in doubly locked areas to prevent unauthorized access.

Training—especially in the rapidly changing area of compliance—is extremely important for all individuals handling animals. Some training is available online or is organized by IACUCs at universities and other institutions. Other training is provided by lab-animal veterinarians or technicians experienced in research-oriented procedures. Training provides the investigator with experience in acceptable methods of restraining, marking, monitoring vital signs, administering injections, taking blood samples, and assessing stress or signs of pain or distress. The investigator is responsible for knowing how to perform procedures in the appropriate setting (field, laboratory, etc.) for which their protocol was approved. In this paper, we outline issues associated with research involving mammals and provide a framework for addressing those issues based on animal welfare regulation, scientific studies, and experiences as mammalogists.

The IACUCs are urged to recognize the investigator as a cooperator versed in the biology of the taxa used in their research. Wild vertebrates, particularly mammals, are vastly different in physiology and behavior from their usually highly inbred conspecifics used in biomedical research settings. Wild vertebrates do not inhabit antiseptic, stress-free environments. Investigators should be prepared to serve as resources to their IACUCs and institutional veterinarians.

Compliance with Laws and Regulations

Mammalogists conducting research associated with a college or university that receives federal grant funding are advised to seek approval from their IACUCs and obtain proper permits from local and federal agencies before conducting any procedure involving live animals. This is true whether the PI is working within the United States or elsewhere. The AWA authorizes the USDA/APHIS to regulate animals used (or intended for use) in research, testing, experimentation, or exhibition purposes, or as pets, regardless of whether animals are maintained in a laboratory or farm setting. However, the USDA/APHIS does not regulate animals used for food or fiber (or for improving quality of food or fiber), or for improvement of animal nutrition, breeding, management, or production efficiency.

The United States Fish and Wildlife Service (USFWS) defines a mammal as any member of the class Mammalia, including any part, product, egg or offspring, or the dead body or parts thereof (excluding fossils), whether or not included in a manufactured product or in a processed food product (OLAW/NIH 2002). In this context, ''permit'' is any document designated as a ''permit,'' ''license,'' ''certificate,'' or any other document issued by the USFWS to authorize, limit, or describe an activity and is signed by an authorized official of the USFWS. Although the focus of this section is on federal and state regulations in the United States, investigators, regardless of nationality or location of their research, should be aware that wherever they are working, local, state–provincial, federal–national, or international laws or regulations likely exist that pertain to scientific collecting, transport, possession, sale, purchase, barter, exportation, and importation of specimens or parts thereof, or other activities involving native or nonnative species of mammals. Therefore, each investigator must have knowledge of, and comply with, all relevant laws and regulations pertaining to field collection of mammals. Federal regulations exist in the United States that pertain to collection, import, export, and transport of scientific specimens of mammals, and ignorance of the law or even inadvertent violation of regulations could result in prosecution (Choate and Genoways 1975). Researchers living in or conducting research in the United States must obtain permits issued by federal agencies to import or export specimens of nonendangered species through a nondesignated port of entry; import or export endangered wildlife through any port; import injurious wildlife; import, export, ship interstate, take, or possess endangered species or

parts thereof for research or propagation; take, harass, possess, or transport marine mammals; import or transfer etiological agents or vectors of human disease and living nonhuman primates; collect scientific specimens on national wildlife refuges; import ruminants and swine, including parts, products, and by-products; and import organisms or vectors, tissue cultures, cell lines, blood, and serum.

When moving specimens of mammals into or out of the United States, researchers are required to file USFWS form 3-177 (currently the electronic declaration form available at www.fws.gov is preferred and may be mandatory at the regional office or port of entry) and any necessary permits from CITES if species are listed in CITES appendices. Investigators working outside the United States should expect similar regulations in other countries and ensure that they comply with all applicable regulations dealing with species of special concern. Investigators also must ascertain whether additional permits are needed when they review state–provincial and federal–national laws and regulations that relate to their planned field investigations. Further, investigators must be familiar with current lists of mammalian species deemed threatened or endangered by appropriate state–provincial or federal–national governments and comply with all laws and regulations pertaining to capture of these and other categories of protected mammals. A list of threatened or endangered species and 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, D.C. 20240 (http://www.fws.gov/endangered/wildlife.html). Regulations relevant to these taxa are published in the Code of Federal Regulations, Title 50, Chapter 1; amendments to regulations under Title 50 also are published in the Federal Register (USDA 2005).

Most states and provinces require scientific collecting permits, and investigators must comply with this requirement and other regulations imposed by agencies in the states or provinces in which they conduct fieldwork. World Conservation Union (IUCN), USFWS, and CITES status is indicated in Wilson and Reeder (2005), but investigators should check for updates. Lists of all mammals (as well as other animals and plants) that are regarded as endangered, threatened, or species of special concern, along with other pertinent information, are maintained by the USFWS. Additional information is available on the IUCN *Red List* (http://www.iucnredlist.org/) and from CITES (http://www.cites.org/). Cities, counties, national and state parks, or other organizations might have additional regulations regarding scientific uses of wildlife on lands under their jurisdiction. Compliance with these regulations is essential. Finally, the investigator should obtain permission of the owner, operator, or manager of privately owned land before commencing fieldwork thereon.

Many institutions, as well as state, provincial, and federal governments, have regulations or recommendations concerning handling and sampling rodents or other mammals that might be carriers of human diseases. Investigators must ensure their own safety and that of employees or students by understanding the disease-carrying potential of the mammals they study. Additionally, as part of their charge of reducing institutional liability, most IACUCs have adopted some form of occupational health screening for all persons involved with animal research. Screening might involve completion of a check-off form inquiring about allergies or other health conditions of investigators, students, and employees or a more detailed examination.

Numbers and Species (Including Endangered Taxa)

The *Guide* (NRC 1996) requires that protocols include details concerning the numbers of animals to be used. These details are of considerable importance during IACUC discussions. The 3 Rs outlined in the *Guide* (Reduction, Refinement, and Replacement—NRC 1996) direct IACUC committee members to determine if the smallest number of animals necessary to accomplish research goals is being used. Further, oversight agencies such as the USDA focus on clear association of individual numbers with procedures or research aims. Frequently, field researchers do not know how many individuals will be needed; this is especially true in the case of surveys or other exploratory work common in mammalogy. Statements in protocols such as, ''it is unknown how many animals we will capture'' are generally not well received. For IACUC protocols, the investigator can provide generalized statements such as: ''In this survey we expect to collect different species of *Oryzomys* and will sample an estimated 25 localities. We will not exceed 20 specimens/species of *Oryzomys*/locality. It is anticipated that the total number of specimens collected during this study will not exceed 500 individuals/year.''

Genetic, taxonomic, ecological, and other studies require a minimal sample size for statistical analyses. In contrast, behavioral studies might involve capture of an entire population to mark all individuals. In this case, the investigator can provide a statement that ''all animals in the population will be captured, marked, and released, and it is estimated that this will not exceed 200 individuals/year.'' Members of the IACUC also care if too few animals are being sampled. Too few animals might not allow the investigator to address research questions with sufficient scientific rigor and, subsequently, will result in a waste of animals if results cannot be applied to test a hypothesis. A power analysis might be performed to estimate number of animals required to obtain statistical significance for a given level of variance and a minimum difference between samples. The NRC (2003) provides guidelines for determination of sample size and estimation of animal numbers for laboratory studies. Numbers in field studies will vary greatly but should be considered in the context of study design, species' life-history characters, and questions posed.

The IACUCs also are concerned with particular species of mammals involved in a project. Again, medically oriented protocols commonly use laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*) bred for many generations by animal resource facilities. Recent additions to laboratory mice and rats are these same species bred as ''knock outs'' or transgenics (NRC 2003). Laboratory animals are bred for genetic manipulations that produce disease conditions upon which treatments can be tested. In addition to laboratory mice and rats, more than

5,400 species of mammals occur globally that field investigators might study scientifically (Wilson and Reeder 2005). For such studies, the IACUC will require a protocol for the committee to evaluate in which the investigator provides an adequate description of the study methods, experimental design, and expected results as well as a summary of related, previous studies. The IACUC also might ask investigators about planned methods of euthanasia even if the proposed study involves only observing or catching and releasing animals. ''We are not killing any animals'' is a frequent, but unsuitable, response to an IACUC because it indicates that the investigator has not considered methods of treatment or euthanasia in the event of an accident.

The investigator must provide assurance to the IACUC that permits necessary for use of wild mammals have been issued for the proposed project; copies of permits might be requested by the IACUC. Although most IACUCs usually do not focus on scientific merit, it is required by federal regulations in the United States that the IACUC ask that scientific merit has been assessed. Peer review of scientific proposals, approval of project permits by resource agencies, and support from academic departmental chairs can provide assurance to the IACUC that the project is sound and use of animals justified. Although rare, the IACUC might seek an outside assessment or request evidence of peer review to evaluate scientific merit.

Trapping Techniques

Oversight of Field Studies

Field studies not involving invasive procedures that harm or significantly alter behavior of an animal are exempt from IACUC review (Section 2.31 (d) IACUC review of activities involving animals (1) ''field studies . . . are exempt.''—USDA 2005:21), but many institutions interpret AWA in a broader sense and ask their IACUCs to review all laboratory or fieldwork that involves any kind of animal use conducted by researchers. For those studies that require review and approval by the IACUC, many field procedures for mammals are available (Martin et al. 2000); these sources should be consulted by the investigator during protocol preparation and referenced if needed. Further, some institutions may have standard procedures available to all investigators preparing protocols (e.g., http://hsc.unm.edu/som/research/acc/).

Considerations for Capturing Mammals

Techniques for capture of specific species of mammals are detailed in summary sources (Wilson et al. 1996), internet sites devoted to specific subsets of mammals (http://www.furbearermgmt.org/resources.asp#bmps), and especially in articles from the primary literature. Trapping can include live traps (e.g., Sherman, box, mist nets, snares, Tomahawk, Hav-A-Hart, pitfall, nest box, and artificial burrow), kill traps (Museum Special, rat traps, and pitfalls), and other specialty traps for particular species or purposes. Shooting might be necessary to obtain specimens of some species. Sometimes physical capture of animals is not essential, and investigators may use devices to obtain acoustic signatures (ultrasonic detectors), visual data (still or video cameras), or sticky devices to remove hair from free-ranging animals. Common reasons to capture mammals include livetrapping to tag (with radiotransmitters, necklaces, ear tags, or passive integrated transponder [PIT] tags), mark (number, band, hair color, freeze brand, ear tag, or toe clip), or tissue collection. Regardless of the approach, potential for pain, distress, or suffering must be considered. When livetrapping, adequate insulation, food, and avoidance of temperature extremes must be provided. Kill-trapping methods must provide an efficient and quick death that minimizes pain. For the most part, observational techniques are not of concern to IACUCs unless they involve capture (e.g., capturing bats in mist nets to identify species before animals are released and recorded as they retreat, or use of artificial burrows or nest boxes to facilitate capture), harassment, or visiting nest sites during critical times in a species' life cycle (e.g., bat nursery roost or seal pup nursery). It should be noted that individual IACUCs and institutional policies vary widely regarding exemptions for observational studies, so investigators are urged to become familiar with their institutional policies before beginning any work with mammals.

Live Capture

Investigators conducting research requiring live capture of mammals assume the responsibility for using humane methods that respect target and nontarget species in the habitats involved. Methods for live capture include those designed for small mammals (Sherman, Tomahawk, and Hav-A-Hart traps, pitfalls, artificial burrows, and nest boxes), medium-sized to large mammals (Tomahawk, Hav-A-Hart, and foot-hold traps, snares, corrals, cannon nets, culvert traps, and darting), bats (mist nets, harp traps, and bags), and fossorial mammals (e.g., Baker and Williams 1972; Hart 1973). Methods of live capture should not injure or cause excessive stress to the animal. Adequate measures should be taken to ensure that the animal is protected from predation and temperature extremes and has food and water available, as needed, until it is released. For permanent trapping grids or webs, the investigator might provide shelters over traps to protect captured animals from extreme temperatures and precipitation (Kaufman and Kaufman 1989).

Use of steel foot-hold traps for capturing animals alive must be approached cautiously because of potential for injury and capture of nontarget species (Kuehn et al. 1986). For some taxa, foot-hold traps, including leg snares, might present the only means of capture available and indeed might be most effective (Schmintz 2005; see also http://www.furbearermgmt.org/resources.asp#bmps for specific techniques). When their use is appropriate, investigators have an ethical obligation to employ steel foot-hold traps of a sufficient size and strength that the animal is held firmly. Traps other than snares with rubber padded or offset jaws should be used to minimize potential damage to bone and soft tissue. Snares or spring foot-hold traps must be checked frequently (perhaps twice daily or more often depending upon target species and potential for capture of nontarget species) and captured animals assessed carefully for injury and euthanized when necessary. Nontarget species, if uninjured, should be released immediately.

To facilitate prompt checking, the number of traps set at a particular time and location should not exceed the ability of the investigator to monitor traps at reasonable intervals. Because the most effective way to prevent mortality or injury to animals in live traps is prompt and frequent checks of traps for captured animals, the investigator should consider staking or visibly flagging a trap line (or otherwise devising a system) to ensure that all traps are checked and removed reliably and efficiently. Regular monitoring ensures that target animals remain in good condition while in traps and allows prompt release of nontarget species with no ill effects caused by capture. Examination intervals vary and are dependent on target species, type of trap, weather, season, terrain, and number and experience of investigators. Generally, live traps for nocturnal species are set before dusk and checked at dawn. Traps are then retrieved or closed during the day to prevent capture of diurnal, nontarget taxa. However, live traps for small mammals, particularly shrews, should be checked more frequently (e.g., every 1.5 h—Hawes 1977) to minimize mortality due to the higher metabolism of these animals. Similarly, species of larger size with high metabolic rates (e.g., *Mustela*) also require shorter intervals between checking traps. Live traps for diurnal species should be set in areas shaded at dawn or early morning or under trap shelters (Kaufman and Kaufman 1989) and checked every few hours in warm weather. Traps should then be retrieved or closed at dusk to prevent accidental capture of nocturnal taxa.

Thermoregulatory demands, especially for small mammals, can stress an animal even if duration of captivity is short. Thermoregulatory stress can be minimized by providing an adequate supply of food and nesting material in the live trap. Because most live traps for small mammals are constructed of metal and conduct heat readily, it might be necessary to insulate traps to minimize hypo- and hyperthermia in captive animals. Insulation can be accomplished by using such items as cotton or synthetic fiber batting, leaves, or twigs to provide dead air space between the animal and conducting surface and to provide escape from the temperature extremes. Critical temperature tolerance limits vary with species and environmental conditions. Investigators must be responsive to changing conditions and modify trapping procedures as necessary to minimize thermal stress.

If disturbance (removal of animal or trap damage) of live traps for small mammals by larger species of carnivores, birds, and others is problematic, trap enclosures (Getz and Batzli 1974; Layne 1987) or other methods to secure traps might be required. Pitfall traps can be fitted with raised covers to minimize capture of nontarget species, provide cover from rain and sun, and prevent predation from larger animals. Pitfall traps used for live capture might require small holes in the bottoms to allow drainage in rainy weather, or enhancements such as small sections of polyvinyl chloride (PVC) pipe to provide escape from other captured animals.

Traps used for live capture of larger mammals include box traps, clover traps, and culvert traps. Some large mammals (e.g., ungulates and kangaroos) can be herded along fences into corrals or captured with cannon nets or drop nets projected from helicopters using net guns. These methods require immediate attention to the animals by trained personnel to prevent injury and can cause substantial distress in some species. With a large-scale capture, it may be useful to have a veterinarian on hand to assist with any injured or stressed animal. Depending on the nature of the work, individuals captured using these techniques may need to have their eyes covered or be sedated until the investigator's work is completed (Braun 2005).

Large mammals also can be captured by shooting a sedative into the hip or shoulder musculature using a dart gun. Baits laced with tranquilizer have been described (Braun 2005), but these should be used with caution to prevent sedating nontarget species. Chemical immobilization, whether for capture or sedation, requires training by a wildlife veterinarian and thorough knowledge of proper dosage, antidote, and sedative effect before use. An excellent reference for chemical immobilization of mammals is Kreeger (1996). Local and national regulations may restrict use of certain drugs (e.g., narcotics). Location of the animal within the habitat should be considered in light of time necessary for sedation and recovery to avoid injury or drowning of the sedated mammal. Further, sedated mammals must be monitored closely and observed after release until they regain normal locomotion. In no instance should sedated animals be left in proximity to water or exposed to potential predators while under the influence of immobilizing drugs.

Bats can be captured effectively and humanely with mist nets, harp traps, bag traps, or by hand (Kunz and Kurta 1988). Mist nets should not be left unattended for >15 min. Captured bats should be removed from nets immediately to minimize injury, drowning, strangulation, or stress. Removal of bats from mist nets must be done carefully to minimize stress and avoid injury to delicate wing bones and patagia. If a bat is badly tangled, it can be removed by cutting strands of the net. Mist nets should not be used where large numbers of bats might be captured at once, such as at cave entrances, because numbers can quickly overwhelm the ability of investigators to remove individuals efficiently. In these situations, harp traps or sweep nets are preferred (Wilson et al. 1996). Although harp traps do not require constant attention, they should be checked regularly, especially when a large number of captures is expected in a short period of time. Investigators using harp traps should guard against predators entering the trap bag, biting, predation of 1 bat species on another, rabies transfer, or suffocation due to large numbers of bats caught in a short time (Kunz and Kurta 1988).

As with traps for terrestrial mammals, to minimize stress on captured bats, the number of mist nets operated at one time should not exceed the ability of the investigators to check and clear nets of bats. Nets should not be operated in high winds because these conditions can put undue stress on bats entangled in nets. Mist nets should be operated only at night or during crepuscular periods and closed during the daytime to prevent capture of nontarget taxa (e.g., birds).

Roosting bats sometimes can be removed by hand. Gloves should be used that offer protection from bites but still allow

the investigator to feel the body and movements of the bat to prevent injury to the animal. Long, padded tissue forceps might be used to extract bats from crevices, but extreme care should be taken to avoid injury to delicate wing bones and membranes (Kunz and Kurta 1988).

The time of year that bats are studied must be considered and may be crucial to their survival. Large or repeated disturbance of maternity colonies might cause mortality of offspring and colony abandonment (Kunz 2003). Also, repeated arousal of hibernating bats can lead to mortality because of depletion of critical fat stores (Thomas 1995).

Captured small and medium-sized mammals should be handled by methods that control body movements without restricting breathing. Covering an individual's eyes might reduce the animal's struggle to escape. Restraint by a mesh or cloth bag allows the investigator to mark, measure, or otherwise sample an individual through mesh or the partially opened end of the bag (e.g., *Cynomys gunnisoni*—Davidson et al. 1999). Some small mammals also can be transferred directly from a trap to a heavy-duty plastic or cloth bag for transport. Design of some traps (e.g., box-type traps such as Sherman or Tomahawk live traps) also allows them to be used as a temporary cage for easy and safe transportation.

Kill-Trapping and Shooting

When study design requires that free-ranging mammals be euthanized to collect specific types of data or samples, individuals may be live trapped and then euthanized humanely, trap-killed, or killed by shooting (AVMA 2001). When this type of sampling is required, the investigator must 1st consider the goals of the study and the impacts that removing a number of animals will have on the natural population. Animals should be euthanized as quickly and as painlessly as possible (see methods below) without damaging materials needed for research.

Traps suitable for kill-trapping include snap traps (e.g., Victor and Museum Special) for rat- and mouse-sized mammals, kill traps (e.g., Macabee) designed for subterranean species, harpoon traps for moles, snares for carnivores and furbearers, and Conibear or similar body-grip traps for medium-sized mammals. Some trapping techniques that use drowning as a means of euthanasia have been described as inhumane or unethical because time to unconsciousness exceeds 3 min (AVMA 2001; Powell and Proulx 2003). However, submersion trapping systems might be quite effective for furbearers found in or near waterways. Such systems rely on equipment (e.g., steel foot-hold traps with 1-way cable slides and locks) or techniques that cause the furbearer, upon capture, to quickly and irreversibly submerge until death (http://www.furbearermgmt.org/resources.asp#bmps). As with any procedure or experimental protocol, an IACUC might find submersion trapping systems acceptable with justification, depending upon circumstances.

Investigators should strive to use the trap that will inflict the least trauma and result in a clean, effective kill. Most traps should be checked at least once a day and, in the event that an animal is still alive, it should be immediately dispatched according to AVMA guidelines (AVMA 2001). Using pitfall traps as kill traps by placing formalin or ethylene glycol in the bottom is not approved or acceptable to the ASM. Further, the AVMA offers these recommendations (AVMA 2001:684): ''Kill traps do not always meet the panel's criteria for euthanasia. At the same time, it is recognized that they can be practical and effective for scientific animal collection when used in a manner that ensures selectivity, a swift kill, no damage to body parts needed for field research, and minimal potential for injury of non-target species.''

An effective way (sometimes the only way) to collect certain species of mammals is by use of a firearm. Investigators using this method must be experienced in safe handling of firearms and adhere strictly to laws and regulations related to their possession and use. The firearm and ammunition should be appropriate for the species of interest so that the animal is killed immediately without excessive damage to the body. A .22-caliber rifle loaded with bullets or shotguns loaded with appropriate shot sizes are suitable for medium-sized mammals. Generally, small mammals (chipmunk size or smaller) can be taken with .22-caliber rifle or pistol loaded with #12 (dust) shot, whereas animals the size of rabbits can be taken with shotguns loaded with #6 shot. Large mammals should be taken with a high-velocity rifle, where legal, or shotguns using appropriate ammunition (e.g., rifled slugs or larger shot). After the animal has been shot, it should be retrieved quickly.

Marine Mammals

All marine mammals in United States territorial waters are protected by the Marine Mammal Protection Act of 1972. Some species also are protected by the Endangered Species Act of 1973. The latest versions of both acts can be found at the United States Marine Mammal Commission's Web site (http://www.mmc.gov/legislation/). These acts prohibit any form of ''take,'' including terminal capture, live capture, tagging, and so on, of marine mammals without appropriate federal permits. Exceptions are made for certain aboriginal or traditional harvests of marine mammals and for commercial fisheries that might take marine mammals incidental to normal fishing operations. Permit application forms and instructions can be found on the National Marine Fisheries Service's Web site (http://www.nmfs.noaa.gov/prot_res/overview/permits.html) and at the USFWS's Web site (http://permits.fws.gov/).

Methods of live capture for marine mammals include nets (ranging from purse seines to small, handheld hoop nets) and mechanical clamps with lines that are placed over an animal's peduncle while it rides the bow pressure wave of a vessel. Many live capture techniques for smaller cetaceans are reviewed by Asper (1975). Some dolphins or small whales (e.g., *Phocoena* and *Delphinapterus*) can be captured by hand in shallow water (Walker 1975). Although polar bears (*Ursus maritimus*) and some species of pinnipeds (e.g., northern elephant seal [*Mirounga angustirostris*]) might be captured using remotely injected chemicals, chemical immobilization of marine mammals for capture risks losing animals by drowning or overdose (Dierauf and Gulland 2001). Euthanasia for marine mammals was reviewed by Greer et al. (2001).

Holding of marine mammals in captivity is regulated by the Marine Mammal Protection Act, the Endangered Species Act, and the AWA. The latter is administered by APHIS of the USDA. The AWA regulations include species-specific criteria for pool and pen sizes and construction, water quality, food storage and handling, and routine health care. The most current AWA regulations can be found on the APHIS Web site (http://www.aphis.usda.gov/ac/cfr/9cfr3.html#3.100).

Marking for Identification

Individual identification of mammals is necessary for many types of studies, both in the laboratory and field. Identification marks can be natural (stripe pattern, color, or mane patterns) or those applied by the investigator. Further, marks may be temporary or permanent, and external or internal. Of primary concern is the distance from which the animal must be identified. On large species, cataloging natural variations in fur or whisker patterns (West and Packer 2002), or previously sustained injuries on body parts (such as to wing, ears, or flukes) might be sufficient for permanent identification at a distance.

External Marks and Tags

Where naturally occurring identifying marks are not available, external dye, freeze-branding, or paint marks might provide the degree of longevity required. Dye marks on juveniles or subadults are of more limited duration because of rapid molting. Identification marks might be made with nontoxic hair dyes or paint. Care should be taken to ensure that substances used for external marks are nontoxic and do not otherwise alter the behavior of animals or subject them to increased predation. Freeze branding is an effective means of marking bats and other species and marks might last several years (Sherwin et al. 2002). Tattooing and ear punches provide a permanent means of identification but require handling of individuals for individual recognition.

Metallic or plastic tags and bands or collars are cost-effective and might be suitable for identification at appreciable distance on large terrestrial species. Tags typically are applied to the ears of terrestrial mammals and to flippers of seals and sea lions. Use of individually numbered tags on small mammals necessitates handling the animal each time an individual is to be identified. Although they are frequently used with a high degree of success, ear tags might inhibit grooming of ears and promote infection by parasites in some rodents (Ostfeld et al. 1996), although potential for infection likely varies with species and environment. Further, unless carefully sized, tags might snag, either during grooming or by vegetation in free-ranging animals, and can be lost (Wood and Slade 1990). Many of the problems associated with ear tags are reduced in laboratory settings, where ear tags might be especially useful for long-term identification. Ear tags are not an option for species with greatly reduced pinnae (e.g., shrews). Wing bands for bats should be applied so that they slide freely along the forearm, which may necessitate cutting a slit in the wing membrane in some cases. Another external marking option for bats is a bead-chain necklace (Barclay and Bell 1988), although these necklaces must be sized carefully.

Individuals of some taxa might be identified by unique patterns of ear punches (where a small amount of tissue is removed from external pinnae using some type of hole punch) or toe clips. Toe clipping involves removal of 1 or more digits (generally only 1 per foot) and provides a permanent identifying mark. Because both of these methods involve removal of a small amount of tissue, they might be especially appropriate in studies where tissues samples also are required. Neither of these methods is generally suitable for identification at a distance, and ear punches might become unidentifiable through time in free-ranging individuals because of healing, subsequent injuries sustained in the field, or being obscured by hair.

Because it is more invasive and addressed specifically in the *Guide* (NRC 1996), toe clipping requires considerable justification to the IACUC. Justification for toe clipping as a means of identification should include consideration of the natural history of the species, how the feet are used in the animal's environment, and the size of the toe of the organism. Digits generally should not be removed from the forefeet of subterranean or fossorial taxa where they are used for digging. Nor should primary digits be removed from arboreal or scansorial taxa where they are used for climbing. Further, the size of the toe is related to body size of the animal; toe clipping in species with fleshy digits should be avoided. Toe clipping might be especially suitable for permanent identification in small species (e.g., *Chaetodipus*, *Perognathus*, *Reithrodontomys*, and *Sorex*) and in neonates of larger taxa. Toe-clipping and ear punches should not be used for marking bats; bats can be effectively wing punched or freeze branded. Toe clips and ear punches should be performed with sharp, sterilized instruments. Anesthetics and analgesics generally are not recommended; prolonged restraint of small mammals to apply these substances and consumption of the analgesic substances (e.g., creams) via licking likely cause more stress and harm than conducting the procedure without their use.

Radiotransmitters provide a mechanism to monitor movements and survival of individuals and, therefore, also serve to identify an individual. Transmitters can be attached externally with surgical or skin glue or a collar, or implanted into the animal's body cavity. External attachment often can be accomplished in the field (e.g., Munro et al. 2006; Rothmeyer et al. 2002), whereas more invasive implantation might require transport to a laboratory where sterile conditions can be arranged. Investigators using collars should take into account potential for growth of an animal or seasonal changes in neck circumference (e.g., male cervids) and use devices designed to accommodate such changes (Strathearn et al. 1984). If external transmitters are attached using glue, individuals of some species will groom each other excessively to remove adhesive from their fur (Wilkinson and Bradbury 1988). Surgical implantation and more invasive procedures should be performed by a veterinarian or individuals who have received specialized training and usually require a suitable recovery period before the animal may be released. Before using radiotransmitters, an investigator should consider the weight of the

transmitter relative to the body mass of the target species or individual. Generally, the transmitter should represent <5–10% of the individual's body weight (Wilson et al. 1996). As an alternative to radiotransmitters, light-emitting diodes (LED) or similar markers might be fastened externally to some species using similar considerations.

Judgment about 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 and distress to the animal. If pain is slight or momentary, anesthesia is not recommended so that the animal can be released immediately. If pain is more than slight or momentary, field-portable anesthetic machines allow use of isoflorane and similar inhalants to provide a reliable anesthetic and rapid recovery after the animal is no longer exposed to the gas. Use of anesthesia for blood sampling will depend on data needed and species requirements. For example, some anesthetics (e.g., ketamine) depress blood pressure and make blood collection lengthier and potentially dangerous. Anesthesia also might alter the blood component (e.g., cortisols) under investigation. Use of anesthesia should be weighed against risk of mortality because some species are very sensitive to anesthesia (e.g., felids—Bush 1995; Kreeger 1996).

Internal Tags

Passive integrated transponder (PIT) tags are electronic devices encased in glass or resin capsules. They do not emit constant signals but can be interpreted with a remote reader in much the same way that bar codes are scanned. Tags are injected subcutaneously by using a modified large-bore hypodermic syringe and are suitable for many field and laboratory identification needs. Tags should be massaged away from the point of insertion subdermally to prevent loss. Even the smallest PIT tags (about the size of a grain of rice) may be too large for some individuals, so their use in very small individuals should be approached cautiously. Currently available PIT tag readers must be in reasonably close proximity to the tag (~ 10 cm) for reading, so their use with large aggressive taxa (e.g., *Procyon* and *Lynx*) might require anesthesia both for application of the tag and for subsequent reading to prevent injury to the animal and investigators. Because of stress for both subject and investigator, other methods of tagging large mammals, such as using radiotransmitters or naturally occurring markers, might be preferable. Ingestion of colored plastic particles or radioactive isotopes (such as P^{32}) in bait can be used to mark feces for studies of movements of individuals or groups of individuals, but is of limited utility to mark uniquely a large number of individuals.

Immobilization for Application of Marks

Depending on the biology of the target species, its size, and goals of the study, captured animals might require chemical immobilization for handling. Investigators should bear in mind that stress and restraint associated with immobilization might be greater than applying or reading a particular mark without immobilization. Whether or not immobilization is required must be considered on a case-by-case basis. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia (article V, United States government principles for the utilization and care of vertebrate animals used in testing, research, and training; http://grants.nih.gov/grants/olaw/references/phspol.htm). Selection of anesthetics and analgesics for specific mammals should be based on evaluation by a specialist, such as a wildlife veterinarian, knowledgeable about the use of anesthesia in species of mammals other than standard laboratory or pet taxa. The investigator should conduct a literature review for alternatives as well as anesthetics and analgesics used in related species (Kreeger 1996). Physiological measurements required for experimental purposes also may affect the choice of anesthesia. Sedatives, anxiolytics, and neuromuscular blocking agents are not analgesic or anesthetic and hence do not relieve pain; these substances must be used in combination with a suitable anesthetic or analgesic (NRC 1996).

Maintenance of Wild-Caught Mammals in Captivity

Procurement and Holding Conditions

Any time that wild-caught individuals are to be held or transported, the investigator must consider the transport or holding cage, appropriate and sufficient food and moisture for the captured animal, ambient environment, ecto- or endoparasites potentially associated with the specimen, and safety of the investigators (see section on human safety). Cages must be constructed to minimize possibility of injury, provide adequate ventilation, allow for protection from wastes, and generally should be of sufficient size to permit the captive individual to make appropriate postural adjustments (NRC 1996). Some types of live traps (e.g., Sherman live traps and Tomahawk traps) can be used as holding or transport cages for short periods of time for appropriate species.

Captive mammals held for any length of time (>12 h for USDA regulated species and >24 h for all others) must be provided with suitable sources of food and moisture. Food can be provided at the time of capture. For many small mammals, especially rodents, fruits or vegetables (e.g., grapes, celery, lettuce, or slices of apple or potato) with high moisture content will suffice during transport or short periods of captivity until more permanent housing, food, and water provisions can be provided. Water bottles generally should be avoided during transport because they will leak and dampen bedding.

Care must be taken in transporting captive animals to prevent their exposure to temperature extremes or precipitation and to provide adequate ventilation. Regardless of cage construction, the more quietly the animal can be maintained in appropriate caging, the better. Minimizing disturbance and placing transport cages in cool, darkened settings is best. In some instances, these conditions can be achieved simply by placing a drape over the cage, provided there is sufficient air flow and temperatures are not extreme.

Free-ranging mammals might carry a number of diseases and almost certainly harbor ecto- and endoparasites. Some facilities require treatment for ectoparasites before transport, and most will require quarantine of newly captured individuals before entering an animal resource facility. Even if these are not required, the investigator should take appropriate steps to minimize potential impacts to other captive species and to humans. Most ectoparasites can be controlled by dusting with commercial flea and tick powder. Treatments for endoparasites are more involved and generally should be pursued after consultation with a veterinarian. Investigators should contact the local institutional occupational health office for information on risks to humans from species of mammals under consideration before transport.

Maintenance Environments

When individuals of wild species are to be maintained in captivity for >12 h, particular care must be taken to minimize distress. Cages or pens of an appropriate size and construction must adequately contain animals for their health and safety and that of investigators and animal care personnel. Given the variety of species that might be maintained, no specific guidelines for cage materials or size are possible, but considerations should be given to all aspects of ecology, physiology, and behavior of target species. Guidelines developed for husbandry of domesticated species usually are not appropriate for wild-caught individuals and might even constitute inhumane treatment. Because of their being captured as free-ranging individuals, nondomesticated species might perform better in larger cages or pens than those used for similar-sized domesticated species (Fowler 1995). Temperature, humidity, lighting, and noise levels also must be within appropriate limits. An excellent source of information on the specific needs of wild-caught species is the ASM's *Mammalian Species* series (http://www.asmjournals.org). Additional valuable information usually can be obtained directly from investigators or animal-care staff familiar with particular species. Investigators proposing to maintain wild-caught mammals in captivity are encouraged to contact other researchers or institutions experienced with the taxa in question and to work with the IACUC's attending veterinarian before submitting a protocol. However, investigators should realize that departures from the *Guide* (NRC 1996) or the Public Health Service policies on animal use (OLAW/NIH 2002), even if optimum for the proper maintenance of nondomesticated taxa, will require justification to the IACUC.

Careful selection of bedding materials and substrate is critical to meet the needs of the target species; materials used should simulate their natural environment. Appropriate materials might include sand or fine woodchips for desert species, soil for shrews and fossorial forms, and hay or straw for other species of rodents. The quantity of bedding also might be important if a dense covering (e.g., straw) allows establishment of runways that are components of the natural environment of the target species. Refuges should be provided where captive individuals can remain out of sight when possible because their availability influences behavior (Rusak and Zucker 1975).

Olfactory cues are a fundamental component of the natural environment of most mammals. Individuals frequently scent mark to establish possession and boundaries of a territory. Regular changing of bedding and washing of the cage and its equipment eliminates normal scent cues and places captive individuals in a novel environment. Investigators can reduce stress that accompanies cleaning by changing bedding and cage equipment on a less frequent cycle than typically used for domesticated species (often 1 or 2 times weekly). Investigators also might carry over some of the old bedding and place it with the fresh bedding. Species adapted to arid conditions (e.g., *Onychomys*) likely will perform best when bedding changes occur every 10–14 days, or even longer, whereas others (e.g., *Sigmodon*) might require weekly changes. Because scent marks often are deposited on watering devices or cage lids, disturbance associated with being placed into a novel environment can be reduced by changing these devices on a schedule different from that of the cage and bedding so that captive individuals are not regularly placed in an environment completely devoid of familiar scents. The importance of establishing and maintaining familiar surroundings, especially as identified by olfactory cues, cannot be overemphasized.

All species of mammals require some source of water in captivity, although water sources and requirements vary widely among species. For example, kangaroo rats (*Dipodomys*) and pocket gophers of various genera live without free water in the wild because they get water directly from their food and retain metabolic water (Boice 1972). These taxa can be maintained in captivity by periodically feeding of small amounts of lettuce, celery, or apple. The frequency of these supplemental feedings is dependent upon the ambient humidity in their environment. Adult heteromyids (e.g., *Dipodomys*) seldom even require these. In fact, if provided with ad libitum access to free water, xeric-adapted species can become dependent upon these sources (Boice 1972), which can result in changes in physiological functions that might in turn pose problems for some studies.

Environmental enrichment for nondomesticated mammals should be considered; lack of stimulation in a captive environment can result in development of stereotypic behaviors that confound research interests. Enrichment might be as simple as increasing structural complexity in the cage or providing additional materials for manipulation. For example, the captive environment of woodrats (*Neotoma*) kept in false-bottom cages can be improved by providing rodent chow directly in the cage rather than in a feeder attached to the cage front. This allows the natural hoarders to regularly rearrange food within their cage. Their environment can be improved even more by providing strips of cardboard that will simulate the woody debris they use to construct nests in the wild. Other species of rodents also can benefit from inclusion of fibrous materials from which to construct nests. Chipmunks (*Neotamias*) and red squirrels (*Tamiasciurus*) are very active and can be difficult to maintain in captivity, but they can be housed by using cages that incorporate 3-dimensional structures (e.g., hanging branches and perches) along with a bottom sufficient for digging and caching food. For some species, hiding food in cardboard boxes allows the animal to ''forage.''

Social structure of the target species also must be taken into account when housing captive mammals. Captive situations that permit an approximation of the natural social structure of the target species are likely to be most successful and minimize distress. Individuals of species that are social or gregarious should be housed with other individuals. Of course, investigators must be aware of seasonal changes in social structure and modify housing environments accordingly.

Separation of Taxa and Minimizing Stress

The AWA and Animal Regulations (Office of Laboratory Animal Welfare 2002; USDA 2005) state that animals housed in the same primary enclosure must be compatible. That is, prey species should not be maintained near carnivores in the same animal room, and diverse taxa of carnivores generally should not be housed together. Closely related species of some rodents frequently occur together in nature and often can be housed without difficulty in the same room.

The general principles for identifying captive mammals in pain or distress are abnormal appearance or behavior. Normal appearances and behavior are determined by species-specific characteristics and personal experience of the handlers. Because behavioral changes are the means to identify pain or distress, all personnel involved with animals should understand the normal behavioral patterns of the species they are housing. Thus, all animals should be monitored regularly by trained staff.

A source of pain generally is easy to identify if it is a physical abnormality, but stress or distress might not be due to pain and is not immediately recognizable. IACUCs generally consider procedures that cause pain or distress in humans likely also will cause pain or distress in other animals. Characteristics of an animal in pain include, but are not limited to, diarrhea or vomiting; poor coat; inflammation or bleeding; hair loss; abnormal posture; incessant scratching; self-aggression; lameness; whining; weight loss (20–25% of baseline); decreased food or water consumption (dehydration); decreased activity; or changes in body temperature, pulse, or respiratory rate (NRC 1992). Behaviors that might signal pain or distress include listlessness or lethargy, lying on the side for extended periods, inability to reach food or water, or unusual or prolonged vocalizations (NRC 1992).

EUTHANASIA

The *Guide* defines euthanasia as ''the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress'' (NRC 1996:65). Euthanasia is a 2-step process that involves use of an agent to depress or eliminate the function of the central nervous system and a 2nd step to stop the heart. The 1st action causes the animal to become unconscious and insensitive to pain. Although both of these goals may be accomplished with a single agent, the main concern is alleviating pain immediately.

Inhalation of carbon dioxide (hypoxia) commonly is used as a method of euthanasia in the United States. Although euthanasia by carbon dioxide has been the accepted method of choice in laboratory settings for the past 2 decades, it recently has been shown that some species display a high degree of avoidance of concentrations of carbon dioxide (Leach et al. 2002) because of irritation of mucosal linings. Alternatively, argon gas has been used in the European Union for laboratory mice (*M. musculus*). Euthanasia techniques are reviewed and approved by the IACUC during review of the animal care and use protocol. Investigators should be aware that animal welfare regulations urge following the most current AVMA Panel of Euthanasia report (AVMA 2001) and that deviations from these guidelines must be justified. Justification for deviations can include citation of published literature or results from pilot studies.

Mammals must be euthanized humanely when live-caught individuals are retained as voucher specimens or when individuals are injured or distressed and cannot be released. Field methods for euthanasia should be quick and as painless as possible, and compatible with study design and size, behavior, and species of animal. When nothing can be done to relieve pain or distress or recovery is not expected, euthanasia is indicated.

Euthanasia must be carried out by personnel properly trained in the procedure being used. Proper euthanasia technique includes a follow-up examination to confirm the absence of a heartbeat. Standard tests for successful euthanasia include a toe pinch, dilated pupil (lack of response to touch on eye), and absence of heartbeat; cessation of breathing is not a sufficient criterion. Decapitation, cervical dislocation, or thoracotomy (open biopsy of lung, pleura, hilum, and mediastinum) should be administered after euthanizing drugs to insure that animals do not revive (AVMA 2001). Although decapitation and cervical dislocation might be humane when administered by properly trained personnel, protocols proposing these techniques in the laboratory must justify these methods if sedation or anesthesia are not administered (AVMA 2001). Investigators also should be aware that adding steps of sedation and anesthesia before euthanasia might add distress and even impose additional pain to the animal. For many species of small body size, euthanasia (e.g., cervical dislocation) can be done efficiently in the field without sedation by experienced personnel.

Acceptable methods of euthanasia—their advantages, disadvantages, and effectiveness—are reviewed in the 2000 report of the AVMA Panel on Euthanasia (AVMA 2001). The report also provides information on inhalant agents, noninhalant pharmaceutical agents, and physical methods used in euthanasia. Unacceptable methods generally include air embolism, blow to the head, burning, chloral hydrate, cyanide, decompression, drowning, exsanguination (unless blood is collected from the unconscious animal as part of the approved protocol), formalin, various household products, hypothermia, neuromuscular blocking agents, rapid freezing, strychnine, and stunning (Appendix 4—AVMA 2001). Recently, the American College of Lab Animal Medicine (ACLAM) evaluated rodent euthanasia. They had 3 issues of concern: euthanasia of fetal and neonatal rodents, use of carbon dioxide for euthanasia, and impact of euthanasia techniques on data collection. Publications by ACLAM (www.aclam.org/PDF/newsletter2005-12.pdf) provide good directives on these topics.

Finally, euthanasia must be performed with a conscious respect for its effect on other animals (including human observers). Fear in other animals can be triggered by distress vocalizations, fearful behavior, and release of odors and pheromones by a frightened animal (AVMA 2001). Thus, euthanasia should be done outside the perceptive range of other captive individuals.

Vouchering of Specimens and Ancillary Materials

It is especially important that investigators plan what to do with animals from wild populations when their study is completed or when animals are procured unexpectedly during the study. The latter might result from incidental deaths when animals are found dead in traps or on roadways. All specimens and ancillary material generated from field studies should be deposited with relevant data into an accredited research collection. The ASM Systematic Collection Committee has compiled a list of accredited collections in the Western Hemisphere (Hafner et al. 1997). The information is available online at http://www.mammalsociety.org/committees/index.asp. Deposition of specimens and ancillary materials in permanent collections maximizes benefits from each specimen collected, ensures access to valuable data by future investigators, and serves as a voucher for individuals or species used in published research. Further, in some instances archived specimens might be used in lieu of sacrificing individuals in future studies.

Human Safety

Working with wild mammals, particularly in field situations, comes with inherent risks, both biotic (e.g., bites, pathogens, parasites, and venomous plants and animals) and abiotic (e.g., lightning and exposure). Fortunately, most of these risks can be minimized with basic training, planning, mentoring, and experience. Investigators have the responsibility to ensure that personnel handling, transporting, or maintaining wild-caught mammals are qualified and familiar with requirements of the target species (e.g., bats—Constantine 1988) and hazards (e.g., bites and exposure to body fluids) associated with them. With appropriate preparation and training, investigators can adequately protect themselves and collaborators while conducting fieldwork with mammals (Kunz et al. 1997).

Many universities and other institutions offer field courses, workshops, and online programs for investigators and students to achieve the proper training in fieldwork and in working with wild-caught mammals. Occupational health medical staffs also are available and aware of ways to avoid biological, chemical, and other hazards. Sources such as the Centers for Disease Control and Prevention (CDC 1998, 1999; http://www.cdc.gov/) or state health departments can provide current information for precautions against specific risks. Special precautions to ensure human safety might be necessary when transporting taxa known or suspected of carrying potentially lethal pathogens such as *Hantavirus* or rabies. In areas where zoonotics are known to occur, bagging traps with a gloved hand and bringing them back to a central processing area that follows institutional biosafety recommendations might be sufficient. Additional precautions might be required at the time of final processing of the captured animal depending on data required. Although chloroform is considered highly hazardous to personnel with attendant health risks of cancer and liver toxicity (http://www.osha.gov/SLTC/healthguidelines/chloroform/recognition.html), under open-air field conditions, its use might be appropriate because it kills ectoparasites that might pose greater risks to the researcher through transmission of diseases such as plague, typhus, or agents such as *Hantavirus*.

Many IACUCs will require the investigator to document their protocols for human health and safety while working with mammals. However, investigators and IACUC members should remain cognizant of the fact that risks from zoonoses vary depending on local environmental conditions, species of mammals handled, and the potential pathogens. Safety precautions should match perceived risks.

Summary

These updated guidelines on the use of mammals, including wild species, emphasize that investigators are responsible for compliance with federal and state guidelines regulating care and use of animals in research, display, and instruction. Investigators should work with IACUCs to develop research protocols that allow investigators to carry out scientific research objectives while complying with animal welfare regulations. A rational, well-justified protocol, written succinctly and completely, will open a positive and productive dialog with the IACUC. The task of the IACUC is to provide assurance to federal regulatory agencies and the public that animal research at an institution is being accomplished in accordance with the regulations and intent of the AWA. Therefore, the IACUC is a strong advocate for animal welfare and also animal use and research, especially when investigators provide clear justification for animal use and expertise upon which the IACUC can depend. These interactions produce strong, positive, and professional relationships.

From the initial design of a study to its completion, investigators should exercise good judgment and be prudent when using animals in research. IACUCs appreciate working with investigators who try to provide details of their research designs and goals. The 3 Rs of Reducing the numbers of animals without compromising statistical validity, Replacing ''higher'' animals with ''lower'' ones, and Refinements of techniques and care to lessen pain or distress to animals (NRC 1996) are important goals for field mammalogy. Even in faunal surveys, a cap on the number of animals collected usually is imposed by the permitting agency and likewise is expected by the IACUC. Underestimates of the number of animals needed for a study might invalidate results. Therefore, sufficient numbers of animals, the number needed to meet research goals, must be clearly requested and justified. ''Replacement'' in mammals might be achieved by using cell lines or simulations where possible. Further, larger mammals usually are not

collected in surveys or for genetic work. Rather, they can be subsampled by ear punch or hair combs, or tissues might be requested from mammalian research collections where much of this material might already be archived as specimens. Other alternatives include using carcasses of species of interest (especially larger carnivores or ungulates) that have been trapped or hunted for other purposes. However, investigators are reminded that such sources may introduce undesirable biases associated with age, sex, or size. Finally, an example of a refinement might include using behavioral responses as indicators of social dominance rather than outcomes of physical combat.

Most field investigators already consider the ethical treatment of animals because of their respect for nature and their dedication to study wild species. These guidelines were developed to assist investigators in maintaining compliance and understanding the evolving suite of regulations. How we view use of mammals in research does not differ much from that of Joseph Grinnell when he walked Yosemite Valley nearly 100 years ago. Knowledge of most aspects of mammalian biology has advanced, but we still struggle with a basic understanding of our place in nature. Mammalogists continue to explore the farthest reaches of the Earth. In contrast, the public and even some scientists in other fields have become removed sufficiently from what is wild that we still must be prepared to answer the question ''what good is it?'' That is, we must be able to communicate to a broad audience the applied and theoretical values of research on wild mammals. Proactive consideration of humane treatment of our study animals will help to prevent retroactive criticism of our ethics and the research itself. With this in mind, the ultimate design of research objectives, and the methods and techniques to address those objectives, is the responsibility of the investigator. Guidelines can provide current information of ethical and regulatory standards, but they cannot replace individual judgment. Moreover, it is the investigator who has the drive, ingenuity, and freedom to seek novel and insightful advances in science.

Resumen

Las pautas generales para el uso de especies de mamíferos silvestres son actualizadas de la versión de 1998 por la Sociedad Americana de Mastozoología (ASM) y han sido expandidas para que incluyan recursos adicionales. Se incluyen detalles sobre el marcaje, los refugios, la captura y la colecta de mamíferos. Estas pautas cubren técnicas profesionales actuales y regulaciones que implican a los mamíferos utilizados en la investigación. Los comités institucionales de uso y cuidado animal, las agencias reguladoras y los investigadores, deben revisar y aprobar los procedimientos concernientes al uso de vertebrados en cualquier institución particular. Estas pautas fueron preparadas y aprobadas por la ASM, cuya experiencia colectiva provee un entendimiento amplio y comprensivo de la biología de los mamíferos no domesticados en su ambiente natural.

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