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Guidelines of the American Society of Mammalogists for the use of wild mammals in research

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Guidelines for use of wild mammal species are updated from the American Society of Mammalogists (ASM) 2007 publication. These revised guidelines cover current professional techniques and regulations involving mammals used in research and teaching. They incorporate additional resources, summaries of procedures, and reporting requirements not contained in earlier publications. Included are details on marking, housing, trapping, and collecting mammals. It is recommended that institutional animal care and use committees (IACUCs), regulatory agencies, and investigators use these guidelines as a resource for protocols involving wild mammals. These guidelines were prepared and approved by the ASM, working with experienced professional veterinarians and IACUCs, whose collective expertise provides a broad and comprehensive understanding of the biology of nondomesticated mammals in their natural environments. The most current version of these guidelines and any subsequent modifications are available at the ASM Animal Care and Use Committee page of the ASM Web site (http://mammalsociety.org/committees/index.asp).

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

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ORGANIZATION OF GUIDELINES

Introduction

Advances in the study of mammals, 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 various management tactics for park managers to follow, 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 they study.

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. In medical research artificially 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. 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 ASM publication *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 experienced, professional mammalogists 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). It stated that the investigator should exercise good judgment and prudence when using animals in research. More complete guidelines were published by the 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), 1998, and again in 2007. Resources for the various editions of these guidelines included information from the United States, other governments (e.g., Canadian Council on Animal Care—Olfert et al. 1993), other professional societies, such as the Society for the Study of Animal Behaviour (2006), the American Veterinary Medical Association (AVMA 2007) *AVMA Guidelines on Euthanasia*, and various publications on trapping methods. In essence, earlier versions of the ASM guidelines provided highlights of more complete information available from either the *Guide for the Care and Use of Laboratory Animals* (hereinafter *Guide*—National Research Council [NRC] 1996) or the AWA; these were, minimize numbers taken, reduce pain or distress of captive animals, and provide humane euthanasia where death was the endpoint. An overview of the development of the ASM guidelines through their various iterations is provided in the 2007 publication (Gannon et al. 2007) and is not repeated here.

These newly revised guidelines 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 up-to-date general and specific guidance on ethical 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 of a project to justify deviations from federal guidelines during submission of a protocol to an IACUC. Institutions have various requirements for animal use and care, but as scientists we have developed an ethos toward 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 document 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 for investigators, educators, and oversight bodies regarding use of wild mammals in research and teaching, particularly in those instances where difficulties might arise in defining what is appropriate when dealing with nondomesticated mammals and field procedures. We emphasize 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 is arguably 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 address 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 a wild-caught animal into an 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—Office of Laboratory Animal Welfare 2002a). Use of sedatives, analgesics, and anesthetics often is under federal and state control. Investigators must consult with federal and state drug enforcement agencies and obtain appropriate licenses during the design stage of a study. 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 vertebrate animals. Some training is available online or is organized by IACUCs at universities and other institutions. Other training is provided by laboratory-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 document we outline issues associated with research involving mammals and provide a framework for addressing those issues based on animal welfare regulations, scientific studies, and our 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. Wild vertebrates do not inhabit antiseptic, stress-free environments with ad libitum food. With these differences in mind, investigators should serve as resources to their IACUCs and institutional veterinarians.

Compliance with Laws and Regulations

Mammalogists conducting research associated with a college, university, or museum that receives federal grant funding are advised to seek approval from their IACUCs and to obtain proper permits from local and federal agencies before conducting any procedure involving live animals. These permit requirements apply whether the principal investigator is working within the United States or elsewhere. The AWA authorizes the USDA/APHIS to regulate vertebrates 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 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 (Office of Laboratory Animal Welfare 2002a). In this context, ''permit'' is any document designated as a ''permit,'' ''license,'' ''certificate,'' or any other document issued by the United States Fish and Wildlife Service to authorize, limit, or describe an activity and signed by an authorized official of the United States Fish and Wildlife Service. Although the focus of this section is on federal and state regulations in the United States, investigators, regardless of their nationality or location of their research, should understand that 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. 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 sera.

When moving specimens of mammals into or out of the United States, researchers are required to file United States Fish and Wildlife Service Form 3–177—currently the electronic declaration form (e-Dec) available at www.fws.gov is preferred and may be mandatory at the regional office or port of entry—and any necessary permits from the Convention on International Trade in Endangered Species if species are listed in Convention on International Trade in Endangered Species appendices I–III. Investigators working outside the United States should expect similar regulations in other countries and ensure compliance 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, Fish and Wildlife Service, United States Department of the Interior, 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 as well as international regulations. International Union for the Conservation of Nature, United States Fish and Wildlife Service, and Convention on International Trade in Endangered Species status is indicated in Wilson and Reeder (2005), but investigators should check for updates. Lists of all mammals (and other animals and plants) that are regarded as endangered, threatened, or species of special concern, along with other pertinent information, are maintained by the United States Fish and Wildlife Service. Additional information is available on the International Union for the Conservation of Nature *Red List* (http://www.iucnredlist.org/) and from Convention on International Trade in Endangered Species (http://www.cites.org/). States, 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, and state, provincial, and federal governments, have regulations or recommendations concerning handling and sampling rodents or other mammals that might be carriers of zoonotic 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 even a more detailed examination.

Categorization of Animal Use for USDA Compliance

[Note: In 2010 the ASM, in conjunction with the Ornithological Council, reviewed guidance documents available to institutions and developed a joint position regarding categorization of animal use for USDA compliance. This text was 1st disseminated as a position statement and addendum to the 2007 version of these guidelines in 2010. The portions of this joint position relevant to work with mammals are included here.]

Two aspects of animal usage classification can cause confusion where activities involving wild animals are concerned: classification of the capture of free-ranging animals within the USDA reporting categories of pain and distress; and identification of field studies for the purpose of determining when IACUC protocol review and IACUC site inspection are required.

United States Department of Agriculture reports: pain and distress categories.—The AWA (7 USC 2143(b)(3)(A)) and the implementing regulation (9 CFR 2.36) require that research facilities in the United States subject to these laws file an annual report with the USDA Animal Care Regional Office documenting their research and teaching activities that used live animals covered by the AWA and its implementing regulations. A component of this report is classification of animal usage into categories intended to describe the absence, presence, or extent of pain or distress and the use of drugs to alleviate these conditions.

United States Department of Agriculture descriptions for animal reporting categories as defined on the reporting form (APHIS Form 7023) are:

C—Animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.

D—Animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.

E—Animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, or experiments, surgery, or tests. (An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be attached to the report.)

Guidance for classifying painful procedures is provided in Policy 11 of the *Animal Care Resource Guide: Animal Care Policy Manual* published by the Animal Care Program of the USDA, APHIS (1997). However, this minimal guidance and the examples given therein pertain to procedures conducted in a laboratory setting, usually in the context of biomedical research.

Classification becomes especially problematic when institutions are faced with applying regulations intended primarily for laboratory settings to the very different context of free-ranging animals. The 2 critical terms in these descriptions are ''pain'' and ''distress.'' According to the *Animal Care Resource Guide: Animal Care Policy Manual* (Animal Care Program, USDA, APHIS 1997), Policy 11, a painful procedure is defined as one ''that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied, that is, pain in excess of that caused by injections or other minor procedures.'' Distress is not defined in current policy except by example: ''Food or water deprivation beyond that necessary for normal presurgical preparation, noxious electrical shock that is not immediately escapable, paralysis or immobility in a conscious animal.'' The principal investigator and the institution must then contend with the task of determining the appropriate classification of captured mammals.

United States Department of Agriculture classifications as applied to animal capture and noninvasive field procedures.—Mammal capture devices are designed either to hold the animal unharmed (live traps) or to kill the animal outright upon capture. Barring mechanical malfunctions and with appropriate placement and trap checking frequency, animals captured in live traps or nets are simply held without injury until removal. Appropriate training is essential for setting capture devices and for removing animals from those devices. Pain or distress, as described in the *Animal Care Resource Guide: Animal Care Policy Manual* (Animal Care Program, USDA, APHIS 1997), is unlikely to result from the simple capture of free-ranging mammals using most live traps or capture techniques approved by the ASM, so animal usage in these instances is consistent with USDA Category C.

Most tissue sampling and marking techniques in the field also are consistent with USDA pain Category C provided that procedures are not more invasive than peripheral blood sampling. Support for this classification is provided in the *Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain and Distress Categories* (National Institutes of Health, Office of Animal Care and Use 2009). This document is distributed by the National Institutes of Health Office of Animal Care and Use, which is the oversight office for intramural research. This guidance expressly states that Category C includes most blood-collection procedures and tissue-collection procedures that involve no or only momentary or slight pain. Based on these same National Institutes of Health guidelines, USDA Category C is also appropriate in instances where protocols requiring peripheral tissue sampling or tagging and release of free-ranging animals necessitate chemical immobilization to conduct the procedures, provided that immobilization is performed only to facilitate the procedure and protect the animal and the researcher from injury rather than to alleviate pain or distress induced by the procedure.

Free-ranging mammals captured in live traps and subsequently euthanized as part of the research study or that are taken in properly functioning kill traps meet the standards for either USDA Category C or Category D; the distinction between these reporting categories depends upon how the animal is killed. Category C appropriately applies to animals taken in live traps if the animals show no obvious signs of pain or distress. The same category applies to animals trapped and then subsequently euthanized using accepted methods that avoid inducing pain or distress and those taken in properly functioning kill traps. These conclusions are consistent with example 4 in the *Animal Care Resource Guide: Research Facility Inspection Guide* (Animal Care Program, USDA, APHIS 2001), Section 14.1.10, except that death is intentional rather than unexpected. The *Research Facility Inspection Guide* pertains to laboratory animals rather than free-ranging wildlife, but euthanasia following a live capture that does not result in pain or distress is analogous to this example.

The *Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain and Distress Categories* (National Institutes of Health Office of Animal Care and Use 2009) make clear that assignment of animals to a reporting category is retrospective. Even though a trapping method ordinarily might comprise Category C, if a problem occurred in the field that resulted in pain or suffering necessitating pain alleviation, Category D is the appropriate reporting category for that particular animal. If livetrapping brings about pain or suffering that necessitates euthanasia, or if kill-trapping fails to bring about swift death and leaves a conscious animal in pain or distress, Category D is the appropriate reporting category. These situations are analogous to example 3 in *Animal Care Resource Guide: Research Facility Inspection Guide* (Animal Care Program, USDA, APHIS 2001) depending upon trap type, trap specificity, and trapping technique.

Field studies.—Considerable misunderstanding has surrounded the application of the AWA to field studies. Regulations promulgated by the USDA under the AWA exempt field studies from IACUC review (9 CFR 2.31(d)), where field study is defined as ''any study conducted on free-living wild animals in their natural habitat that does not harm or materially alter the behavior of the animal under study'' (9 CFR 1.1). None of these terms is defined in the regulation or in guidance documents issued by the Animal Care Program. The same regulation exempts from the inspection requirement of 9 CFR 2.31 ''animal areas containing free-living wild animals in their natural habitat.''

With regard to IACUC protocol review, the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Office of Laboratory Animal Welfare 2002a) makes no distinction between laboratory and field studies. Guidance from the National Institutes of Health, Office of Laboratory Animal Welfare (http://grants.nih.gov/grants/olaw/faqs.htm#ab) states, ''If the activities are PHS-supported and involve vertebrate animals, then the IACUC is responsible for oversight in accordance with PHS policy. IACUCs must know where field studies will be located, what procedures will be involved, and be sufficiently familiar with the nature of the habitat to assess the potential impact on the animal subjects. Studies with the potential to impact the health or safety of personnel or the animal's environment may need IACUC oversight, even if described as purely observational or behavioral. When capture, handling, confinement, transportation, anesthesia, euthanasia, or invasive procedures are involved, the IACUC must ensure that proposed studies are in accord with the *Guide*.'' Other federal agencies have voluntarily adopted these same rules. For instance, the *National Science Foundation Award and Administration Guide* (http://www.nsf.gov/pubs/policydocs/pappguide/nsf10_1/aagprint.pdf) states, ''Any grantee performing research on vertebrate animals shall comply with the Animal Welfare Act (7 U.S.C. 2131 *et seq.*) and the regulations promulgated thereunder by the Secretary of Agriculture (9 CFR 1.1–4.10) pertaining to the humane care, handling, and treatment of vertebrate animals held or used for research, teaching, or other activities supported by federal awards. The awardee is expected to ensure that the guidelines described in the National Academy of Science publication *Guide for the Care and Use of Laboratory Animals* (NRC 1996) are followed and to comply with the *Public Health Service Policy* and *Government Principles Regarding the Care and Use of Animals* (included as Appendix D to the NAS *Guide*).''

How the definition of field study corresponds to the USDA reporting categories is unclear. In most instances protocols involving only procedures classified as Category C are consistent with the regulatory definition of a field study. However, lack of definition of key terms in the definition of field study—harm, material alteration of behavior, and invasiveness—introduce sufficient ambiguity in application of the definition that further guidance from the Animal Care Program would benefit the research community.

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 relevant 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 animal numbers with procedures or research aims. Frequently, field researchers do not know how many individuals will be needed or sampled; 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 by the IACUC. 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.''

The numbers of animals required in field studies will vary greatly depending on study design, species' life-history characters, and questions posed. Behavioral studies might involve capture of only a few animals where the focus is on a specific behavior, or an entire population to mark all individuals. In the latter 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.’’ Genetic, taxonomic, ecological, and other studies require a minimum sample size for statistical analyses. 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 the results cannot be applied to test a hypothesis. A power analysis might be performed to estimate the 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.

Institutional animal care and use committees also are charged with approving the 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 ‘‘knockouts’’ 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 in which the investigator provides an adequate description of the study methods, experimental design, and expected results and a summary of related, previous studies. The IACUC might query 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 unsatisfactory, response to an IACUC because it indicates that the investigator has not considered methods of treatment or euthanasia in the event of an unexpected injury.

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. IACUCs that deal primarily with biomedical protocols sometimes have difficulty evaluating the merit of protocols of field studies. 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), but many institutions interpret AWA in a broader sense and require IACUC review of all laboratory, classroom, and fieldwork involving vertebrate animals. For those studies that require review and approval by the IACUC, many field procedures for mammals are available (e.g., Kunz and Parsons 2009; Martin et al. 2000); these sources should be consulted by the investigator during protocol preparation and referenced as needed. Further, some institutions may have standard procedures available to all investigators preparing protocols.

Considerations for Capturing Mammals

A variety of methods and devices are available for trapping wild 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 can use devices to obtain acoustic signatures (ultrasonic detectors), visual data (still or video cameras), or sticky devices to remove hair from free-ranging mammals. Common reasons to capture mammals include livetrapping to tag (with radiotransmitters, necklaces, ear tags, or passive integrated transponder tags), mark (number, band, hair color, freeze brand, ear tag, or toe clip), or collect tissue. Regardless of the approach, potential for pain, distress, or suffering must be considered. When livetrapping, adequate insulation and food must be provided, and temperature extremes avoided. Kill-trapping methods must provide an efficient and quick death that minimizes pain. In general, 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 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). Individual IACUCs and institutional policies vary widely regarding exemptions for observational studies, so investigators should 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 foothold traps, snares, corrals, cannon nets, culvert traps, and darting), bats (mist nets, harp traps, and bags), and fossorial mammals (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 foothold traps for capturing animals alive must be approached cautiously because of potential for injury or capture of nontarget species (Kuehn et al. 1986). For some taxa foothold traps, including leg snares, might present the only means of capture available and 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 use steel foothold traps of a sufficient size and strength to hold the animal 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 foothold 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, but their release, as with target species, might require chemical immobilization to prevent injury to the animal or researcher.

The number of traps set at a particular time and location should not exceed the ability of the investigator to monitor them at reasonable intervals. Because prompt and frequent checks of traps is the most effective way to minimize mortality or injury to animals in live traps, the investigator should consider staking or visibly flagging a trapline (or otherwise devising some effective system) to ensure that all traps are recovered 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 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 at dawn or early morning in areas that remain shaded or under trap shelters (Kaufman and Kaufman 1989) and checked every few hours in warm weather. Traps then should be retrieved or closed at dusk to prevent unintended capture of nocturnal taxa.

Thermoregulatory demands, especially for small mammals, can induce stress 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 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 could be useful to contract with a veterinarian to assist with any injured or stressed animals. Depending on the nature of the activity, individuals captured using these techniques might need to be sedated or have their eyes covered until the investigator's work is completed (Braun 2005).

Large mammals also can be captured by delivering a sedative into the hip or shoulder musculature using a dart gun. Chemical immobilization, whether for capture or sedation, requires training by a wildlife veterinarian and thorough knowledge of proper dosage, antidote, and sedative effect. 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 during procedures 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. Baits laced with tranquilizer have been described (Braun 2005), but these should be used with caution to prevent sedating nontarget species.

Bats can be captured effectively and humanely with mist nets, harp traps, bag traps, or by hand (Kunz and Parsons 2009). Mist nets should not be left unattended for >15 min when bats are active. Captured bats should be removed from nets immediately to minimize injury, drowning, strangulation, or stress. Bats must be removed carefully from mist nets 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 (e.g., at cave entrances) because numbers can quickly overwhelm the ability of investigators to remove individuals efficiently and safely. 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 or biting captured bats, predation of 1 bat species on another, rabies transfer, or suffocation due to large numbers of bats caught in a short time (Kunz and Parsons 2009).

To minimize stress on captured bats the number of mist nets operated simultaneously should not exceed the ability of investigators to check and clear nets of bats. Nets should not be operated in high winds because these conditions can place 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 gloved hand. Gloves should 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 Parsons 2009).

Investigators should consider that the time of year that bats are studied can impact their survival. Large or repeated disturbance of maternity colonies can cause mortality of offspring and colony abandonment (O'Shea and Bogan 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 animal's eyes might reduce its 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. The 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 transport.

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 shot (AVMA 2007). 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 2007; 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 foothold 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).

Pitfall kill traps can be the best trapping option for some small mammals because the smaller species of rodents and shrews are much more effectively captured with pitfalls than by other means. These traps are particularly efficient where trapping must be continuous or extensive in a way that cannot be achieved with live traps or snap traps that need continual resetting. Pitfalls used with drowning fluids add a measure of preservation and thus can have added utility for scientific collections and detailed study of organs. Additional instances where pitfalls are optimal are outlined in Beacham and Krebs (1980) and Garsd and Howard (1981). Ethical use of pitfall kill traps should minimize struggling and suffering. The pitfall designed by Howard and Brock (1961) does this by using 70% ethanol (or similar alcohol) as the main ingredient of a drowning fluid. Evaporation of the alcohol is retarded by a thin layer of light mineral oil and hexane (2:1) added to the solution. Small mammals falling into the trap and hence into the drowning fluid lose buoyancy almost immediately due to the surfactant action of hexane and mineral oil and thus their ability to swim effectively so that submergence and death occur rapidly. Alcohol then infuses the body and acts as a preservative. As long as the solution is deeper than the head–body length of the animals, they cannot struggle by standing on the bottom and quickly drown. Using pitfall traps as kill traps by placing formalin or ethylene glycol in the bottom, however, is not approved or acceptable to the ASM. Pitfalls used as kill traps should have covers or other means of excluding nontarget species. If the traps will not be operational for extended periods, they should be constructed such that the kill jar and its fluid can be removed to prevent unwanted captures. As with any procedure or experimental protocol, an IACUC might find submersion trapping systems, including pitfalls with drowning fluids for small mammals, acceptable with justification.

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 an animal is still alive, it should be immediately dispatched according to AVMA guidelines (AVMA 2007). The AVMA offers these recommendations regarding kill traps (AVMA 2007:16): ''Mechanical kill traps are used for the collection and killing of small, free-ranging mammals for commercial purposes (fur, skin, or meat), scientific purposes, to stop property damage, and to protect human safety. Their use remains controversial, and the panel recognized that kill traps do not always render a rapid or stress-free death consistent with criteria for euthanasia found elsewhere in this document. For this reason, use of live traps followed by other methods of euthanasia is preferred. There are a few situations when that is not possible or when it may actually be more stressful to the animals or dangerous to humans to use live traps.''

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 swiftly 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 Web site (http://www.mmc.gov/legislation/). These acts prohibit any form of ''take,'' including terminal capture, live capture, and tagging, 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 Web site (http://www.nmfs.noaa.gov/prot_res/overview/permits.html) and at the United States Fish and Wildlife Service 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 dolphin 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). Additionally, the Society for Marine Mammalogy has developed guidelines for the treatment of marine mammals in field research. The most current version of these guidelines is available at http://marinemammalscience.org/images/stories/file/ethics/ethics%20guidelines.pdf.

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 USDA APHIS. 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).

Tissue Sampling and Identification

Tissue Sampling

The collection of small amounts of tissue from wild mammals is often required for studies involving DNA, various proteins (e.g., hemoglobins, albumins, and enzymes), or physiological assays (e.g., hormonal levels and antibody titers). Tissue samples frequently are obtained in conjunction with some marking procedures (e.g., toe clips or wing or ear punches). Even where these techniques are not required for identification, small external tissue samples are frequently taken from unsedated animals with little difficulty. Where blood is required, many procedures do not require anesthesia of the animal and can be conducted in the field by appropriately trained personnel. After tissue collection and prior to release, individuals should be observed to ensure that no trauma or adverse reaction has occurred as a consequence of capture, handling, or tissue or blood removal.

Multiple factors must be considered when determining the most appropriate method for obtaining blood samples. Various morphological attributes (e.g., size of the orbit, absence of tail, or presence of cheek pouches) characteristic of the species can limit potential sites of blood collection. The size of the animal also might restrict collection sites and limit the quantity of blood ($\leq$1.5% of body mass) that can be removed. The training and experience of the individuals performing the procedure is important, because unskilled personnel can cause significant trauma with some techniques. The procedures for blood collection and the qualifications of study personnel must be reviewed by the principal investigator's IACUC.

Obtaining blood from the facial vein.—This technique, which has been used on laboratory mice for many years, allows collection of 4–10 drops of blood with minimal discomfort to the animal (see USDA news release at www.ars.usda.gov/is/pr/2005/050921.htm). The procedure is described (in text, photos, and video) at www.medipoint.com/html/directions_for_use1.html. [Note: No endorsement of this particular commercial product is intended by the ASM.]

Obtaining blood from the caudal vein.—Extracting blood from the caudal vein is a relatively simple procedure that involves the use of a needle (more difficult in small rodents) or nicking of the caudal vein with a lancet. Alternatively, excising the distal 1–2 mm of the tail can yield a small amount of blood and can be used for DNA extraction.

Obtaining blood from the retro-orbital sinus.—Retro-orbital bleeding should be used when less-invasive blood-collection methods have been considered and are not suitable. To minimize the chances of damage to the eye, this technique should be performed by trained and experienced individuals. The use of very short-acting anesthesia (e.g., isoflurane or sevoflurane) in a plastic bag will immobilize rodents in 15–20 s, thereby making the procedure safer for the rodent and the handler.

External Marks

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. 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), often suffices for permanent identification at a distance.

Where naturally occurring identifying marks are not available, external dyes, freeze brands, 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 can be made with nontoxic hair dyes or paint. Care should be taken to ensure that substances used for external marks are nontoxic and otherwise do not 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.

Metal 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 frequently are 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). Ear tags also might affect the Preyer reflex in free-ranging animals. 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 forelimb, which may necessitate cutting a slit in the wing membrane in some cases. Another external marking option for bats is a carefully sized bead-chain necklace (Barclay and Bell 1988).

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) or terminal phalanges and provides a permanent identifying mark. These marking methods necessarily involve recapture because neither is generally suitable for identification at a distance. Further, 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 both of these methods involve removal of a small amount of tissue, they might be especially appropriate in studies where tissue samples also are required.

Because it is more invasive and addressed specifically in the *Guide* (NRC 1996), toe clipping requires 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. 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. 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*, *Peromyscus*, *Reithrodontomys*, and *Sorex*) and in neonates of larger taxa. Toe clipping and ear punches should not be used for marking bats; bats can be wing punched or freeze branded effectively. Toe clips and ear punches should be performed with sharp, sterilized instruments. Anesthetics and analgesics generally are not recommended because prolonged restraint of small mammals to administer 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 body cavity. External attachment often can be accomplished in the field (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, which should be performed by a veterinarian or individuals who have received specialized training, usually require a suitable recovery period before the animal can be released. Before using radiotransmitters, an investigator should consider the mass 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 mass (Wilson et al. 1996). As an alternative to radiotransmitters, light-emitting diodes, or similar markers might be fastened externally to some species.

Internal Tags

Passive integrated transponder 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 passive integrated transponder tags (about the size of a grain of rice) can be too large for some individuals, so their use in very small individuals should be approached cautiously. Currently available passive integrated transponder 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 ^{32}P) in bait can be used to mark feces for studies of movements of individuals or groups of individuals but is of limited use for uniquely marking a large number of individuals.

Chemical Immobilization for Application of Marks and Tissue Sampling

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 or taking noninvasive tissue samples without immobilization. Whether immobilization is required must be considered on a case-by-case basis. If pain is slight or momentary, anesthesia is not recommended so that the animal can be released immediately. Procedures that can 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). In these instances field-portable anesthetic machines allow use of isoflurane 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. 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). 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 and anesthetics and analgesics used in related species (Kreeger 1996). Physiological measurements required for experimental purposes also can 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 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 present, 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 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, cabbage, 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, provide adequate ventilation, and keep them calm. 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 air flow is sufficient and temperatures are not extreme.

Free-ranging mammals might carry 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 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, the caging and holding environment must be selected carefully to accommodate species-specific requirements and to minimize stress. 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. Because of the great variety of mammalian 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 might not be appropriate for wild-caught individuals and might even constitute inhumane treatment. Because of their capture 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.amsjournals.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 consult 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 policy on use of laboratory animals (Office of Laboratory Animal Welfare 2002a), 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 as closely as possible the natural environment. Appropriate materials might include sand or fine wood chips for desert species, soil and leaf litter 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 concealed when possible because the availability of refuges influences behavior (Rusak and Zucker 1975).

Olfactory cues are a fundamental component of the natural environment of most mammals, and the design of husbandry practices should incorporate the maintenance of familiar scents to maximize animal comfort. Individuals frequently scent mark to establish possession and boundaries of a territory. Regular changing of bedding and washing of the cage and associated equipment eliminates normal scent cues and places captive individuals in a novel and potentially stressful 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 can mix a small amount of the old bedding with fresh bedding. Species adapted to arid conditions (e.g., *Onychomys*) likely will perform best when bedding changes occur every 10–14 days, or even less frequently, 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. Most mammals are best maintained with liquid water provided in various containers or via lickable watering systems. However, 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 small amounts of cabbage, 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. 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 confound some studies.

Because the lack of stimulation in a captive environment can result in development of stereotypic behaviors that confound research interests, environmental enrichment can be a critical component of husbandry for nondomesticated mammals. 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 these 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 (*Tamias*) 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 substrate 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 2002a, 2002b; 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 co-occur in nature and often can be housed in the same room without difficulty.

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 that 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).

Release of Captive Mammals

Release of wild-caught mammals held in captivity might be justified in the case of endangered or threatened species or species of special concern because of population levels or population dynamics, or for individuals held for only short periods. Research designs that require release of captive animals as part of a manipulation must be planned to minimize potential impact on the local population and stress to the released individuals.

Concerns regarding release of individuals held in captivity for more than short periods include the following:

- Introduction of individuals into an area without available dens and resources (especially problematic with highly territorial species)
- Alteration of population genetics
- Introduction of individuals not acclimated to the local environment
- Introduction to wild populations of pathogens acquired in a captive environment
- Stress on local populations and released individuals
- Excessive exposure to predation of released individuals due to inappropriate foraging cycles (entrained by captive light cycles or environments), extensive foraging due to not having caches built up for winter months, or lack of familiarity with local resources
- Disruption of social systems
- Animals losing or not learning foraging skills
- Legality of reintroduction of captive animals (varies with state and country)

Decisions on release and permissible duration of captivity before release are often species-specific and must be made on a case-by-case basis. Holding individuals of a given species for one or a few days to recover from surgical implantation of a transmitter or data logger is usually appropriate. In contrast, release of highly territorial species held for even short periods into the same environment from which they were captured can be problematic because vacant territories can be usurped, and reintroduction of the resident virtually guarantees a conflict that would not have occurred had the resident not been removed. For additional information regarding the potential release of marine mammals, investigators are referred to the best practices for these taxa developed by the National Marine Fisheries Service (http://www.nmfs.noaa.gov/pr/pdfs/health/release_guidelines.pdf). Final disposition of captive individuals is of concern, but the integrity of natural populations must be the highest priority in project design and IACUC deliberations.

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 can be accomplished with a single agent, the primary 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 because of irritation of mucosal linings (Leach et al. 2002). 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 Guidelines on Euthanasia* (AVMA 2007) 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, compatible with study design and size, behavior, and species of animal. When nothing can be done to relieve pain or distress or when recovery is not expected, euthanasia is indicated. Except when specifically excluded by permit or law (e.g., with endangered species), protocols involving fieldwork should explicitly indicate the circumstances for and method of euthanasia for voucher and distressed or injured animals, even when animal mortality is not an anticipated outcome, to accommodate unplanned injuries.

Euthanasia must be conducted 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 2007). 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 2007). 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.

Although euthanasia of small mammals in field settings can be accomplished using any of the techniques approved by the AVMA, field settings pose challenges because use of injectable controlled substances or inhalants can present additional risks to investigators and stress to the animals. Thoracic compression offers an acceptable alternative under these circumstances. Thoracic compression is an approved method of euthanasia for small birds (AVMA 2007) and has been used effectively for decades by practicing mammalogists. The AVMA lists advantages of thoracic compression as speed of euthanasia, apparent painlessness, and maximizing use of the carcass. Cervical dislocation and other mechanical techniques are of limited utility in many of these same instances because of logistical considerations and because they distort important body measurements, destroy needed tissues and skeletal elements, and alter hormonal profiles through contamination by blood. The ASM considers thoracic compression an acceptable form of euthanasia when the investigator is skilled in the procedure and when the individual mammals to be handled are sufficiently small that the thoracic cavity can be collapsed to prevent inspiration.

Acceptable methods of euthanasia—their advantages, disadvantages, and effectiveness—are reviewed in the *AVMA Guidelines on Euthanasia* (AVMA 2007). 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 2007). Recently, the American College of Laboratory Animal Medicine 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 the American College of Laboratory Animal Medicine (www.aclam.org/pdf/newsletter2005-12.pdf) provide appropriate directives on these topics. For collecting methods using kill traps it is important to recall the AVMA position that, although kill traps do not always render a rapid or stress-free death consistent with their criteria for euthanasia, situations exist when use of live traps and subsequent euthanasia are not possible or when it might be more stressful to the animals or dangerous to humans to use live traps as opposed to kill traps (AVMA 2007).

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 2007). Thus, euthanasia should be done outside the perceptive range of other captive individuals.

Vouchering of Specimens and Ancillary Materials

Investigators always must 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, involves 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 the associated hazards (e.g., bites and exposure to body fluids) and requirements of the target species (e.g., bats—Constantine 1988). With appropriate preparation and training, investigators can adequately protect themselves and collaborators while conducting fieldwork with mammals (Kunz et al. 1996).

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 provide strategies for avoiding biological, chemical, and other hazards. Sources such as the Centers for Disease Control and Prevention (1998, 1999; http://www.cdc.gov/) or state health departments can provide current information and precautions for personnel conducting epidemiological studies or working with populations suspected of posing specific health risks. Additionally, the ASM provides updated guidelines relative to hantavirus pulmonary syndrome for mammalogists and wildlife researchers conducting work on rodents that should be broadly applicable for field studies (Kelt et al. 2010). These guidelines also make the important clarification that earlier published guidelines by the Centers for Disease Control and Prevention (1998, 1999) never were intended to apply to field investigators conducting nonviral-based research on rodents. Special precautions (e.g., vaccinations) to ensure human safety might be necessary when transporting individuals known or suspected of carrying potentially lethal pathogens such as hantavirus or the rabies virus. In areas where zoonotics are known to occur bagging traps with a gloved hand and bringing them 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 disease.

Many IACUCs will require the investigator to document their protocols for human health and safety while working with wild-caught mammals. However, investigators and IACUC members should remain cognizant that risks from zoonoses vary depending on mammalian species, local environmental conditions, 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 the scientific research objectives to be completed successfully while complying with animal welfare regulations. A rational, well-justified protocol, written succinctly and completely, will facilitate 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 and work with researchers and educators to develop appropriate protocols. IACUCs must be strong advocates for animal welfare and also animal use in research and education, especially when investigators provide clear justification for animal use and expertise upon which the IACUC can rely. These interactions foster strong, positive, and professional relationships between the IACUC and the investigator.

From initial design to completion of a study, investigators should exercise good judgment and prudence when using animals in research. IACUCs appreciate working with investigators who provide details of their research designs and goals. The ''3 Rs'' of Reducing the number of individuals without compromising statistical validity or biological significance, Replacing ''higher'' animals with ''lower'' ones, and Refinements of techniques and care to minimize 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, a sufficient number of animals (i.e., the number needed to meet research goals) must be clearly requested and justified. ''Replacement'' in mammals might be achieved by using cell lines, voucher materials from previous studies, or computer 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 ''Refinement'' might include using behavioral responses as indicators of social dominance rather than outcomes of physical combat.

Most field investigators already embrace 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 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, are the responsibility of the investigator. Guidelines can provide current information on 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 a partir de la previa versión de la Sociedad Americana de Mastozoología (ASM) (Gannon et al. 2007). Esta versión actualizada las técnicas profesionales mas actuales y reglamentaciones relacionadas al uso de ammiferos en investigación y enseñanza. Se incluyen recursos adicionales, resúmenes de procedimientos y requisitos de informes que no eran parte de versiones previas. Asimismo, incluimos detalles sobre el marcado, alberges, captura y colecta de mamíferos. Se recomienda que todo comité institucional para el cuidado y uso de animales, agencias regulatorias e investigadores usen estas guias al desarrollar protocolos de trabajo con animales salvajes. Estas guias 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. la versión mas recioente de estas pautas y todas las modificaciones subsequentes están disponibles en la pagina de la web del comité para el cuidado y uso de animales - ASM Animal Care and Use Committee page of the ASM website (http://mammalsociety.org/committees/index.asp).

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