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

ROBERT S. SIKES,* WILLIAM L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE[1] OF THE AMERICAN SOCIETY OF MAMMALOGISTS

University of Arkansas at Little Rock, Basic Animal Services Unit and Department of Biology, 2801 South University Avenue, Little Rock, AR 72204, USA (RSS)
Department of Biology and Office of Animal Care, Research Ethics and Compliance Services, University of New Mexico, Albuquerque, NM 87131, USA (WLG)
[1] This committee included Darrin S. Carroll, Centers for Disease Control; Brent J. Danielson, Iowa State University; Jerry W. Dragoo, University of New Mexico; Michael R. Gannon, Pennsylvania State University, Altoona College; William L. Gannon, University of New Mexico; David W. Hale, United States Air Force Academy; Christy McCain, University of Colorado; Daniel K. Odell, Hubbs–SeaWorld Research Institute; Link E. Olson, University of Alaska; Sarah Ressing, Southern Illinois University, Carbondale; Robert S. Sikes (Chair), University of Arkansas at Little Rock; Robert M. Timm, University of Kansas; Stephanie A. Trewhitt, San Jose State University; and Janet E. Whaley, National Oceanic and Atmospheric Administration.

* Correspondent: rssikes@ualr.edu

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

ation (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 w342.

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

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.~~T~~he AWA (7 USC 2143(b)(3)(A)) and the implementing regulation (9 CFR 2.36) require that

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,

pitfalls, artificial burrows, and nest boxes), medium-sized to

immobilizing drugs. Baits laced with tranquilizer have been

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

limit potential sites of blood collection. The size of the animal also might restrict collection sites and limit the quantity of blood (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

these substances and consumption of the analgesic substances

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

with liquid water provided in various containers or via lickable watering systems. However, kangaroo rats (*Dipodomys*) and

a case-by-case basis. Holding individuals of a given species

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.

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 déferas 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 está disponibles en la pagina de la web del comite

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