GUIDELINES FOR USE OF LIVE AMPHIBIANS AND REPTILES IN FIELD AND LABORATORY RESEARCH

Second Edition, Revised by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, 2004. (Committee Chair: Steven J. Beaupre, Members: Elliott R. Jacobson, Harvey B. Lillywhite, and Kelly Zamudio).

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justify research with amphibians and reptiles, the use of these animals in scientific research can produce effects that cannot always be predicted. Many investigations may involve simple observations of animals, while others require some form of manipulation, either in the field or in captivity. Such studies can disrupt normal activities, induce stress, or otherwise lead to abnormal behaviors that possibly place individuals at greater risk due to increased susceptibility to predation, accidents, or disease. Thus, just as with other vertebrate groups, the use of amphibians and reptiles in research and teaching raises ethical questions that must be carefully considered prior to the initiation of a project.

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a. Procedures should avoid or minimize distress to the animals, consistent with a conceptually sound research design.

b. Procedures do not constitute unnecessary duplications of previous work.

c. Procedures that may cause more than momentary or slight distress to the animals should be performed with appropriate sedation, analgesia, or anesthesia, except when justified for scientific reasons by the investigator.

d. Animals that would otherwise experience severe or chronic stress or pain that cannot be relieved should be euthanized at the end of the procedure or, if appropriate, during the procedure.

e. Methods of euthanasia will be consistent with recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (Smith et al., 1986), unless deviation is justified for scientific reasons by the investigator. However, the AVMA recommendations cannot be taken rigidly for ectothermic vertebrates; the methods suggested for endothermic birds or mammals are often not applicable to ectotherms, which have significant anaerobic capacities. Additional information on euthanasia of reptiles and amphibians can be found elsewhere (see Cooper et al., 1989; AVMA, 1993; McDiarmid, 1994; Chen and Combs, 1999).

f. The living conditions of animals held in captivity either in the laboratory, at holding facilities, or at field sites should be appropriate for that species and contribute to their health and well being. The housing, feeding, and non-medical care of the animals will be directed by a scientist (generally the investigator) who is trained or experienced in the proper care, handling, and use of the species being maintained or studied. While recognizing that living requirements of amphibians and reptiles may differ dramatically from those conventionally assumed for laboratory mammals, the investigator should ensure that all animals are maintained in a state of cleanliness that promotes good health and a safe and stress-free environment. Feeding intervals, requirements for water, temperature, and humidity levels will vary greatly, and the departure of these parameters from mammalian norms should be carefully explained to IACUC members, attending veterinarians, or other personnel who might not be knowledgeable about the biology of amphibians and reptiles. Some experiments (e.g., competition studies) will require the housing of mixed species, often in the same enclosure. Mixed housing is also appropriate for holding or displaying certain species. Whereas considerable information is available for reptiles/amphibians in captivity in the laboratory, private, and zoological collections, little information is available for housing in the field. It is expected that the investigator working with a species will

have the expertise to construct enclosures suitable for the focal taxon. Enclosing areas where the species occurs naturally is one way to provide a semi-natural environment. Animals held or enclosed in the field should be monitored carefully for natural behaviors and that sufficient food resources are available, either naturally or through supplementation.

Additional general considerations that should be incorporated into any research project using wild amphibians or reptiles include the following:

g. The investigator must have knowledge of all regulations pertaining to the animals under study, and must obtain all necessary federal, state, and local permits for the proposed studies. (See the following for applicable regulations: Estes and Sessions, 1984a; Estes and Sessions, 1984b; King and Schrock, 1985; Levell, 1997; Malaro, 1998; Tompkins, 1998; Simmons 2002; and web resources listed in Appendix A). Researchers working outside the United States should ensure they comply with all wildlife regulations of the country in which the research is being performed. Work with many species is regulated by the provisions of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES; see "CITES" references in Estes and Sessions, 1984a; Estes and Sessions, 1984b; Malaro, 1998; Tompkins 1998; and the CITES home page lister in Appendix A). Regulations affecting a single species may vary with country and with districts or regions.

h. Individuals of endangered or threatened taxa should not be removed from the wild nor imported or exported, except in cases involving conservation efforts that are in full compliance with applicable regulations.

i. Before initiating field research, investigators must be familiar with the target species and its response to disturbance, sensitivity to capture and restraint, and, if necessary, requirements for captive maintenance to the extent that these factors are known and applicable to a particular investigation. Special concern should be shown for species known to remain with nests or young during certain seasons. Removal from the wild of individuals of species known to tend nests should, as a general principle, be avoided during the nesting season, unless such removal is justified for scientific reasons.

j. Every effort should be made prior to removal of animals (if any) to understand the population status (abundant, threatened, rare, etc.) of the taxa to be studied, and the numbers of animals removed from the wild must be kept to the minimum the investigator determines is necessary to accomplish the goals of the study. This statement should not be interpreted as proscribing study and/or collection of

uncommon species. Indeed, collection for scientific study can be crucial to understanding why a species is uncommonly observed.

k. The numbers of specimens required for an investigation will vary greatly, depending on the nature of the questions explored. Certain investigations will require collection of relatively large numbers of specimens, though the actual percent of any population taken will generally be very small. Studies should use the fewest animals necessary to answer reliably the questions being posed. Use of adequate numbers to assure reliability and statistical power is essential, as inadequate studies will ultimately require repetition and can result in wasted animal use. When appropriate, numbers of animals should be justified by specific statistical design requirements, and formal power analysis.

Numerous publications exist that will assist investigators and animal care committees in implementing these general guidelines; a number of useful publications are listed in the literature cited section and in Appendix A.

III. Role of IACUC

Recognizing that the function of the IACUC is to ensure humane use of reptiles and amphibians in laboratory and field research, rather than to curtail it, the IACUC should make every effort to work with Investigators such that their research missions are supported. The role of the IACUC in approving and monitoring laboratory use of reptiles and amphibians includes responsibilities for ensuring that laboratory facilities and housing support the health and well-being of study animals. Furthermore, periodic inspection of such facilities should be the norm, as it is for other traditional research models. Field resources for the care and use of wild vertebrates are very different from laboratory resources, and the role of the IACUC necessarily is limited to considerations that are practical for implementation at locations where field research is to be conducted. Prevailing conditions may prevent investigators from following these general guidelines to the letter at all times. However, the IACUC should expect that investigators will make every effort to follow the spirit of these guidelines. The omission from these guidelines of a specific research or husbandry technique should not be interpreted as proscription of the technique.

The IACUC must be aware that whereas vertebrates typically used in laboratory research represent a small number of species with well understood husbandry requirements, the classes Amphibia and Reptilia contain at least 12,280 distinct species with very diverse and often poorly known behavioral, physiological and ecological characteristics. This diversity, coupled with the diversity of laboratory and field research situations, requires that each project be judged on its own merits. Techniques that are useful and fitting for one taxon, experiment, or field situation may, in another context be counter-productive. Therefore, in most cases, it is impossible to generate specific guidelines for groups larger than a few closely related species. Indeed, the premature stipulation of specific guidelines would “severely inhibit humane care as well as

research" (Guidelines for Care and Use of Lower Vertebrates, 1986). The IACUC must note the frequent use of the word "should" throughout these guidelines, and be aware that this is in deliberate recognition of the diversity of animals and situations covered by the guidelines. Investigators, on the other hand, must be aware that the use of the word "should" denotes the ethical obligation to follow these guidelines whenever realistically possible.

Laboratory studies are generally conducted under relatively controlled circumstances with the purpose of testing specific hypotheses within the framework of a broader scientific investigation. Under such circumstances, the IACUC should reasonably assume that investigators can provide defensible estimates of the numbers of animals required for a study, and outline in detail the conditions under which animals will be housed and manipulated. As practicing herpetologists, we recognize the importance of reptiles and amphibians as model systems for teaching basic biological principles in the classroom laboratory. We also note the social and conservation benefits of public outreach using captive amphibians and reptiles. Therefore, the IACUC should be prepared to approve the humane use of reptiles and amphibians for educational purposes, including both routine use in University teaching laboratories, and maintenance of captive animals for the purposes of general public education. As in laboratory research, the use of reptiles and amphibians in teaching laboratories should be described in sufficient detail to justify numbers and procedures. Of particular importance is the Instructor's assurance that procedures using animals in teaching laboratories yield real pedagogical benefits that cannot be obtained by alternative means (e.g., computer simulations). Collections of animals maintained for educational purposes will vary, but they will be usually field-collected and will likely vary in species composition. The IACUC should be willing and prepared to accept "blanket" protocol applications that involve the short- to long-term captivity of several taxonomically diverse species for the purposes of public outreach as well as exploratory research.

Field investigations very commonly involve studies of interactions among many related or sympatric species, of which a large proportion may be poorly known. There is sound scientific merit in exploratory work, and ample reason for investigators to propose studies of a rather general nature, where opportunity and the flexibility to pursue unanticipated observations may become crucial to the success of the undertaking. New species continue to be discovered in this fashion, and the discovery of novel attributes of known species is to be expected as a consequence of the investigation. The IACUC should recognize that the acquisition of such new knowledge constitutes a major justification for any investigation, and that a corollary of this approach is that protocols may list a large number of individual species, or may refer to taxa above the species level.

When laboratory or field studies on wild vertebrates are to be reviewed, the IACUC must include personnel who can provide an understanding of the nature and impact of the proposed investigation, the housing of the species to be studied, and knowledge concerning the risks associated with maintaining certain species of wild vertebrates in captivity. Each IACUC should therefore include at least one institution-appointed member who is experienced in zoological field investigations. Such personnel may be appointed to the committee on an ad hoc basis to provide

necessary expertise. When sufficient personnel with the necessary expertise in this area are not available within an institution, this ad hoc representative may be a qualified member from another institution. Alternatively, when the IACUC lacks the expertise to evaluate and approve specific procedures there are several potential remedies including, but not limited to, educational demonstrations by the principle investigator and external review by expert. We note however, that external review can be time consuming and should not cost legitimate field researchers opportunities to conduct seasonally-sensitive research. The IACUC should be sensitive to the importance of timing in field research with amphibians and reptiles and make every effort to approve legitimate protocol applications.

Field research on native amphibians and reptiles requires permits from state and/or federal wildlife agencies. These agencies review applications for their scientific merit and their potential

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b. Live capture, (trapping and other methods)

Live Capture. - Investigators should be familiar with herpetological capture techniques (Dunham et al., 1988; Heyer et al., 1994; Brown, 1997; Simmons, 2002) and should choose a method suited to both the species and the study. Live-capture techniques should prevent or minimize damage to the animal. In addition, live-capture techniques for venomous or otherwise hazardous species should be carefully chosen so as to minimize risk to animals and researchers.

Trapping. - Traps of various kinds are often necessary to obtain unbiased samples of secretive, nocturnal or infrequently active species (Corn, 1994). The interval between visits to traps should be as short as possible, although it may vary with species, weather, objectives of the study, and the type of trap. Traps should be checked daily when weather conditions threaten survival of trapped animals. Investigators must make every effort to prevent trap deaths from exposure, drowning, cardiogenic shock, or capture myopathy (Young, 1975). Traps should be sheltered from environmental extremes and care should be taken to reduce predation in pitfall traps (Gibbons and Semlitsch, 1981). Pitfall traps set during extremely dry or wet periods should be equipped so as to prevent desiccation and/or drowning of captured reptiles and amphibians (Corn, 1994). Traps should be tightly covered between sampling periods and removed at conclusion of a study.

Under some circumstances, study animals may be acquired through commercial suppliers for laboratory studies and teaching applications. Generally speaking, licensed amphibian and reptile dealers acquire their specimens through field capture, trade, or captive breeding. Upon receipt of commercial specimens, and prior to introduction to any existing laboratory colonies, commercial specimens should be subjected to careful inspection for potential health problems or known pathogens. If feasible, a quarantine period may be advisable.

2. Restraint, Handling, and Anesthesia

a. General principles: manual versus chemical

conditions. Investigators must understand the specific action of restraint chemicals on the taxa studied. The investigator also should be prepared, if necessary, to hold the animal overnight until recovery is complete. A partially recovered animal may be at risk for injury, overheating, freezing, or predation.

Chemical Restraint. - Many chemicals used for restraint or immobilization of amphibians or reptiles are controlled by the Federal Bureau of Narcotics and Drugs. Permits are generally

is to minimize disturbance for observing natural behavior, the use of anesthetics may be undesired in order to minimize handling time. The IACUC should be receptive to reasonable justification of such procedures (e.g., toe-clipping, venipuncture).

The potent drugs available for wildlife immobilization when properly used are relatively safe (with the exception of succinylcholine) for target animals, but can be extremely dangerous if accidentally administered to humans. Succinylcholine has been used for immobilization of crocodilians and large chelonians. While capable of immobilizing an animal through its depolarizing effects at neuromuscular junctions, this chemical belongs to a class of drugs that have no analgesic properties at all. They should never be used as a means for collecting biopsies or performing any painful procedures. More effective chemicals are available for immobilizing most amphibians (Fellers et al., 1994; Wright, 2001a) and reptiles (Heard, 2001). The degree of danger varies according to the drug, and users must be aware of the appropriate action to take in the event of accident (Parker and Adams, 1978). Several common local anesthetics (e.g., Tetracaine, Lidocaine, Piperocaine, etc.) can be used for collecting biopsies. Lidocaine has been used most commonly and is generally infiltrated around the biopsy site. In small species these drugs may have systemic effects and animals treated with these drugs should be observed before release to the wild to be certain that behavior approximates normal. Investigators should choose the chemical for immobilization with consideration of the effects of that chemical on the target organism and in consultation with researchers that have relevant experience.

b. Hazardous Species

Venomous snakes and lizards, certain large non-venomous lizards and snakes, some colubrid snakes (McKinstry, 1983), highly poisonous frogs, crocodilians, and some large turtles are potentially dangerous, and require special methods of restraint and handling as a compromise between potential injury to handlers and injurious restraint of the animal. The particular method chosen will vary with species and the purpose of the project. There are three elements to successful and safe handling of hazardous species; attention, equipment, and distance. Investigators should never rely on any one of these three elements alone, safety can only be achieved by the simultaneous application of all three.

(1) Attention: Hazardous wild animals are unpredictable. Investigators should always maintain concentration, and their attention on the animal while handling. Never work with hazardous species under distracting circumstances.

(2) Equipment: Using equipment such as tongs, tubes and squeeze boxes (Quinn and Jones, 1974) places a barrier between the investigator and the animal. A barrier is critical to keeping the investigator safe, but should never be trusted completely. For example, the use of heavy leather welding gloves to handle small venomous snakes is a technique that has resulted in some cases of accidental envenomation when fangs penetrate the glove. Gloves give researchers a false sense of

security (which causes lapse of attention), and if they fail, the investigator is not protected by attention or distance.

(3) Distance: Attention and equipment cannot prevent accidents if they lapse; however, distance is sure to prevent injury. Investigators should always keep hazardous species at a safe distance from body and extremities, even when they are controlled by equipment.

Adherence to the following general guidelines is recommended when housing and working with hazardous species (Gans and Taub, 1964):

a. Procedures chosen should minimize the amount of handling time required, and reduce or eliminate contact between handler and animal. For example, bare-handing venomous snakes is a practice that is entrenched in some areas of herpetological research and husbandry despite the fact that injury to snakes and their handlers are common. The availability of tongs, tubes, and other handling devices renders direct contact between investigators and the head or neck of a venomous snake unnecessary. There are few, if any, legitimate circumstances where venomous snakes must be handled with the investigator's bare hand at the head or neck.

b. Those handling venomous snakes or lizards should be knowledgeable concerning the proper methods and tools for handling these animals. A training plan should be in place that emphasizes safe procedures and responsibility.

c. Animal technicians should be aware of emergency procedures to be instituted in case of accidental envenomation. Location of a nearby hospital with a supply of antivenin and of a physician with knowledge of envenomation treatment should be ascertained in advance. At a minimum, emergency procedures should include first aid measures, an evacuation plan (for field and laboratory), the logging of relevant data (species, time of envenomation, circumstances), and contact numbers for relevant medical professionals (personal physicians, nearest Poison Control Center). We also recommend the use of cell phones for both field and laboratory activities.

d. One should avoid working alone. A second person, knowledgeable of capture/handling techniques and emergency measures, should be present whenever possible.

e. Prior consultation with workers experienced with hazardous species, and review of the relevant literature, is of particular importance because much of the information on handling dangerous species is not published, but is passed simply from one investigator to another. Laboratories that work with hazardous species often have handling protocols written in formal manuals that can be obtained by request (e.g., Beaupre, 1999). Some institutions may require written handling protocols for hazardous species.

f. Housing of hazardous species requires special care to avoid escape. Hazardous species should be kept in locking cages (i.e., cages with locking mechanisms that do not rely on

Chelonians (turtles and tortoises)

Several sites can be used in obtaining blood from chelonians, each having advantages and disadvantages. Sites include the heart, jugular vein, brachial vein, ventral coccygeal vein, orbital sinus, and trimmed toenails (Gandal, 1958; Dessauer, 1970; McDonald, 1976; Maxwell, 1979; Taylor and Jacobson, 1981; Rosskopf, 1982; Stephens and Creekmore, 1983; Avery and Vitt, 1984; Nagy and Medica, 1986; Jacobson, 1987).

Cardiac sampling, although not recommended, has been utilized. In young chelonians, before the shell has calcified, a needle can be passed through the plastron into the heart. Older tortoises with calcified shells requires either drilling a hole through the plastron over the heart, or using a spinal needle for percutaneous sampling through soft tissue in the axillary region at the base of the forelimbs. In all situations, a sterile technique is necessary since contamination of the pericardial sac with bacteria and other potential pathogens can lead to pericarditis and death of the turtle. A sterile drill bit should be used to create a hole, and the hole should be sealed with an appropriate sealant such as bone wax (Johnson and Johnson Co., Somerville, N.J., USA) and a methacrylate resin (Cyanoveneer, Ellman International Mfg., Inc., Hewlett, N.Y. USA).

In turtles and tortoises orbital sinus sampling can be used for collecting small volumes of blood in capillary tubes (Nagy and Medica, 1986). However, in order to prevent damage to periocular tissues and possible trauma to the cornea a moderate amount of care must be taken when using this technique. The end of the capillary tube is placed into the lateral canthus of the orbit and utilizing a gentle twisting motion blood can be collected. A further problem with this technique is that dilution of the blood sample with extravascular fluids and secretions may alter composition of plasma and effect volume percentages of cellular components. Blood samples are also commonly obtained from the scapular vein, brachial vein and brachial artery of chelonians (Rosskopf, 1982; Avery and Vitt 1984). However, vessels associated with limbs can rarely be visualized through the skin, and sampling is usually blind. In addition, since lymphatics are well developed in chelonian forelimbs (Ottaviani and Tazzi, 1977), obtaining blood samples from these vessels may result in hemodilution with lymph. At times pure lymph may be obtained.

One of the authors (E. Jacobson) has found that the only peripheral blood vessels which can be consistently visualized in many small and moderate sized tortoises is the jugular vein and carotid artery (Jacobson et al., 1992). The major problem encountered when sampling from these vessels is that manual extension and restraint of the head of the tortoise beyond the margins of the plastron is required, which at times may be difficult or impossible. One method is to push in or lightly touch the rear limbs, which usually causes the tortoise to extend its head from the shell, and allows the sampler to restrain the tortoise's head. Once grasped, the head is pulled out with one hand, and while sitting, the sampler positions the tortoise between the knees, with the tortoise's head pointing toward the sampler's body. The jugular vein and carotid artery are well developed on both right and left sides of the neck. Once the head is extended, the jugular can often be seen as a bulge through the cervical skin, coursing caudal from the level of the tympanic membrane to the base of the neck. The carotid artery is deeper and more difficult to visualize and is located ventral and parallel to the jugular vein. Once either vessel is identified, the skin over the puncture site should be cleaned with 70% ethanol and a 23 or 25 gauge butterfly catheter can be used for obtaining the sample. With the cap removed from the end of the tube, blood will flow down the tube once the needle is inserted into the vessel. The technique described above can be

used in Mediterranean tortoises (*Testudo* spp.), but is not always successful (E. Jacobson, pers. obs).

Crocodilians (crocodiles, alligators, gharial)

Blood samples can be obtained from the supravertebral vessel located caudal to the occiput and immediately dorsal to the spinal cord (Olson et al., 1975). The skin behind the occiput is cleansed with an organic iodine solution and 70% ethanol. A 3.75-cm, 22- or 23-gauge needle is inserted through the skin in the midline directly behind the occiput and is slowly advanced in a perpendicular direction. As the needle is advanced, gentle pressure is placed on the plunger. If the needle is passed too deep, the spinal cord will be pithed. Other sites of blood collection that are commonly used include the heart (via cardiocentesis) and ventral coccygeal vein (Jacobson, 1984). The heart is located in the ventral midline, approximately 11 scale rows behind the forelimbs. In collecting blood from the coccygeal vein, the crocodilian is placed in dorsal recumbency and the needle is inserted through the skin toward the caudal vertebrae.

Lizards

Blood samples can be obtained from several sites. In large lizards, blood is easily obtained from the ventral tail vein (Esra et al., 1975). Toenails can be clipped, and blood can be obtained in a microcapillary tube (Samour et al., 1984). Microcapillary tubes also can be used to obtain blood samples from the orbital sinus (LaPointe and Jacobson, 1974), in a similar fashion for collecting blood from mice.

Snakes

Blood samples can be obtained from a variety of sites, including the palatine veins, ventral tail vein, and via cardiocentesis (Olson et al., 1975; Samour et al., 1984). Some prefer heart puncture to other methods, and as long as the heart is not excessively traumatized with multiple attempts at sampling, the procedure is safe and effective. This method should be limited to those snakes over 300 grams (Jackson, 1981). Essentially, the heart is located either directly by seeing it beating through ventral scales or by palpation. The heart is relatively moveable within the

can be collected, these are more often collected for disease studies rather than biologic studies. The focus here will be skin biopsies.

Amphibians

Skin biopsies are easy to obtain in most species. Lidocaine can be used as a ring block around the biopsy site. The portion of skin is elevated with a forceps and a fine surgical scissor should be used for cutting the tissue. Wound glue or an appropriate suture material (see below) should be used to close the incision.

Chelonians

Of all the reptiles, chelonians present the greatest challenge for biopsy, especially when lesions involve the shell. The reptile shell is a very hard biological structure that makes biopsy somewhat difficult. While under anesthesia, a rotary power saw (Dremel Mototool, Dremel Mfg. Co., Racine, Wisconsin, USA) or bone trephine can be used to cut a wedge out of the shell. Ideally, the biopsy should include normal tissue along with the diseased component. A piece should be fixed in neutral buffered 10% formalin for histopathological evaluation and a piece (with the most superficial contaminated portion removed) submitted for microbial culture. For initial attempts at isolation, the author often uses a broth such as tryptic soy broth. The defect created in the shell should be filled with calcium hydroxide dental paste (Root-Cal, Ellman International Mfg., Inc., Hewlett, New York, USA) and covered over with a methacrylate resin (Cyanoveneer, Ellman International Mfg., Inc., Hewlett, New York, USA). This technique is routinely used in repair of the chelonian shell.

For biopsy of soft tissue, a 2% xylocaine block is satisfactory and can be infiltrated around the biopsy site and the skin cleaned with 70% ethanol and allowed to dry. If the sample is to be cultured, sterile saline is used instead of ethanol. If there is epidermal involvement, a biopsy punch can be used for collecting the sample. Following punch biopsy, the skin may require a single suture for closure. Monofilament nylon is routinely used. If a subcutaneous mass is present, fine-needle aspiration can be performed. This is a rapid method, resulting in minimal trauma to the patient. A 22-gauge needle is inserted into the mass and using a 6 to 12 ml syringe, full negative pressure is developed by quickly pulling back on the plunger. While maintaining negative pressure on the syringe, the needle is moved throughout the mass in multiple planes. After several passes through the mass, the plunger is released and the needle removed from the mass. Negative pressure should not be applied to the plunger while removing the needle from the mass since this will cause the sample to be aspirated into the syringe barrel. The specimen may then be used in culture, cytological preparations, or histopathology (Jacobson, 1992).

Crocodilians and Lizards

A full-thickness biopsy may be difficult in those areas of the crocodilian integument having osteoderms. Small crocodilians and most lizards can be manually restrained, whereas large crocodilians and large monitors must be chemically immobilized. The area around the biopsy site should be infiltrated with 2% xylocaine and a full-thickness skin incision taken with a biopsy punch. As with chelonians, a minimum of two biopsies should be taken, one for

histopathology and one for microbiology. For microbial culture, the lesions can be ground in a sterile tissue grinder and samples applied to appropriate media. This appears to be particularly important for isolation of fungi from reptile skin lesions. The author has had more success in isolating fungi when the skin is ground prior to attempts at isolation (E. Jacobson, pers. obs.).

Snakes

Snakes are ideally suited for skin biopsy. Harmless species can be manually restrained, and venomous species can be guided into a plexiglass tube for restraint or anesthesia. Affected scales can be removed with a scalpel blade, or a sterilized one-hole paper punch can be utilized for biopsies of individual scales. In such cases, the area around the lesion should be infiltrated with 2 per cent xylocaine hydrochloride. In certain skin diseases, such as vesiculating skin lesions, larger samples may be needed. Similarly, for sampling subcutaneous masses, 2 per cent xylocaine can be infiltrated subcutaneously around the mass. Once removed, the mass should be split into several portions for various diagnostic evaluations.

5. Surgical Procedures

a. General principles. With any invasive procedure, standard aseptic technique (Powers, 1985) is essential. Amphibians, because of the structure of the skin, may need special considerations. For instance, prior to preparation of the surgical site, a commercially available artificial slime can be used to coat the skin (Wright, 2001c). While most liquids used in preparation of the surgical site are not absorbed by reptile skin, amphibian skin is permeable, and will be affected by most topical applications. Surgical scrubs and organic iodines, both solutions and soaps, are routinely used in reptiles. But in amphibians they are toxic and therefore must be avoided. In amphibians the two most commonly used disinfectants are chlorhexidine and benzalkonium chloride. While reptile skin is easily draped using either cloth drapes or plastic drapes, amphibian skin needs special attention. First, since amphibians are often anesthetized in a solution of MS222, this chemical needs to be applied to the skin of amphibians throughout the procedure. Aquatic amphibians having gills can be placed on a Styrofoam board with a section cut out for the head, and with the board floating in a solution of MS222, the head is placed in the solution. Sterile cloth soaked in an anesthetic solution of MS222 can be used to cover the animal's body (except for the surgical site). A plastic drape then can be used to completely cover the amphibian.

1. Equipment. The surgical equipment used will depend on the size of the animal. Standard surgical equipment can be used for mid-sized to large sized reptiles. Microsurgical equipment, while expensive, is preferred for use in small reptiles and amphibians (Bennett,

2. Suture material. Suture material used in mammals and birds are used for similar procedures in amphibians and reptiles. The size of the material used will depend upon the size of the patient. For most small amphibians and reptiles, the size will range from 4-0 to 8-0. Larger sized material is available for large animals. Absorbable material such as polyglycolic acid and polydioxanone, are absorbed at a slower rate compared to birds and mammals. If used for closing skin, it may have to be removed following healing of the incision site. Nylon is most commonly used for closing skin. Gut suture material, especially chromic gut is to be avoided since it induces a major inflammatory response in amphibians and reptiles (Jacobson et al., 1985; Bennett, 2000a).

b. Procedures. A wide variety of surgical procedures have been described for amphibians (Wright, 2001c, Wright, 2000) and reptiles (Bennett, 2000a,b; Lock, 2000). In amphibians the most common minor surgical procedures are toe clipping or placement of subcutaneous or intracoelomic passive integrated transponder (PIT) tags (discussed below). Major surgical procedures in amphibians include placement of intravascular catheters for chronic blood sampling, laparoscopy, celiotomy, ovariectomy, and organ biopsy. In reptiles, toe clipping also has been used for identification, particularly in lizards, and the same principles discussed below similarly apply. PIT tags are also commonly used for identification. Radio transmitters (discussed below) have been surgically implanted in snakes, lizards, and crocodilians for tracking studies. These are generally implanted into the coelomic cavity following surgical procedures used for celiotomies in general (Bennett, 2000b). Other surgical procedures include implantation of intravascular catheters for chronic blood sampling and blood pressure recording studies, laparoscopy, endoscopy, ovariectomy, ovariosalpingohysterectomy (removal of the ovary, ovidict, shell gland, uterus), orchidectomy, and organ biopsy (Lock, 2000).

6. Animal Marking and Telemetry

Marking animals for laboratory or field recognition is an essential technique in biological research. Important considerations in choosing a marking technique concern effects on behavior, physiology, and survival of the animal. The utility of any technique varies with the species under study; tissue-removal techniques may pose less long-term survival threat to some species than certain tagging methods. Marking techniques for amphibians and reptiles have been reviewed extensively (Ferner, 1979; Dunham et al., 1988; Donnelly et al., 1994). Although field observation indicates that individual wild animals can survive extensive tissue damage from natural causes (Brunson, 1986), the effect of most tissue-removal marking techniques on survival and fitness is not adequately known and is a topic worth investigating.

When choosing an acceptable marking technique, investigators must consider the nature and duration of restraint, the amount of tissue affected, whether pain is momentary or prolonged, whether the animal will be at greater than normal predation risk, whether the animal's ability to mate is reduced, and whether the risk of infection is minimal. Careful testing of unproven marking techniques on captive animals before use on free-ranging animals may reveal potential problems and is recommended. It may be desirable to use redundant techniques to assure accuracy during a study.

a. Passive Integrated Transponders. -
Passive integrated transponders (or PIT tags) represent a recent advance in animal marking techniques (Camper and Dixon, 1988). The tag itself is a small cylinder that can be injected into the animal either subcutaneously or intraperitoneally. The tags are read by a scanning device that provides electromagnetic energy to the tag, which then reflects a unique series of numbers and/or letters. The injection of the tag is a relatively simple procedure, however, aseptic procedures are needed. Surgical glue can be used to cover the site where the trochar (used for insertion of the PIT tag) is inserted through the skin. Studies that assess the impact of PIT tags on behavior, growth and survival are rare, however, available data suggests no strong evidence for lasting detrimental effects in frogs (Brown, 1997), salamanders (Ott and Scott, 1999) or snakes (Keck, 1994; Jemison et al. 1995). In some cases, PIT tags are not retained as reliably as other marking techniques (Germano and Williams, 1993; Ott and Scott, 1999), and the high cost of individual tags (approximately $5.00 each) and tag readers may render this technique uneconomical for some research programs. In addition, although tags are small, they are clearly inappropriate for small species.

b. Toe Clipping. - Toe clipping, a ubiquitous technique (Dunham et al., 1988), may be used for general marking of free-ranging animals when toe removal is not judgleaividualih -0.iSsl,pvahe

risky. Conversely, the less permeable skin of reptiles may reduce the effectiveness of topical products.

d. Tattoos and Dye Markers. - Tattooing has been used with success on both amphibians and reptiles. Two potential problems should be resolved prior to tattooing: 1) selection of a dye which will contrast with the normal skin pigmentation; and 2) loss of legibility due to diffusion or ultraviolet degradation of the dye. Paint should not be used to mark the moist and permeable skin of amphibians. Reptile skin permeability is quite variable, and paint or paint solvents may be absorbed and cause death of the animal. Paints with non-toxic pigments, bases, and solvents must be used. When toxicity is unknown, laboratory trials, even if limited, should be done before field use. Very tenacious paints may, if applied across shell sutures, severely distort ' the normal shell growth of turtles, especially sub-adults. Paint should not be applied to sutures of turtle shells. Two procedures for tagging amphibians for individual identification have recently been evaluated, or applied to large-scale field studies. Both procedures involve marking different regions of the body of amphibians with colored dyes; the combination of location and color provides a large number of potential unique identifiers. Of these, the most promising seems to be visible implant fluorescent elastomers (VIE) that are injected sub-cutaneously and either visualized with the naked eye (in lighter skinned animals) or with a black light that causes the dyes to fluoresce (Anholt and Negovetic, 1998; Jung et al., 2000; Nauwelaerts et al., 2000). A second method uses pressurized application of inert fluorescent powder (Nishikawa and Service, 1988; Schlaepfer, 1998). Both methods have been used successfully to mark caudates and anurans.

e. Banding and Tagging. - The size, shape and placement of tags should be appropriate to permit normal behavior of the animal marked. Bands and tags projecting from the body may produce physical impairment or enhance the risk of entanglement in undergrowth or aquatic cover.

into natural systems and for disposal of waste material. The pros and cons of using strong emitters must be assessed in terms of possible deleterious effects on the animal, to predators that might ingest isotope-labeled animals, and potential hazard to the public.

g. Radiotelemetry

circumstances require that specimens (very small or larval animals, for example) be formalin-fixed without prior anesthetic killing, prior light anesthetization with an anesthetic such as MS-222 is recommended (Fowler, 1986).

8. Museum Specimens

The collection of samples for museum preparation from natural populations is critical to: 1) understanding the biology of animals throughout their ranges and over time; 2) recording the biotic diversity, over time and/or in different habitats; and 3) establishing and maintaining taxonomic reference material essential to understanding the evolution and phylogenetic relationships of amphibians and reptiles. The number of specimens collected should be kept to the minimum the investigator determines necessary to accomplish the goal of a study. Some studies (e.g., diversity over geographic range or delineation of variation of new species) require relatively large samples (Reynolds et al., 1994).

Museum Specimens and Other Killed Specimens. - The collection of live animals and their preparation as museum specimens is necessary for research and teaching activities in Systematic Zoology. Such collections should further our understanding of these animals in their natural state and do not serve merely as tools for teaching specimen preparation techniques. Herpetological collecting techniques and representative practices of collection management have been compiled (Simmons, 2002), as have references to field techniques (Thomas, 1977). Whenever amphibians or reptiles are collected for museum deposition, specimens should be fixed and preserved according to accepted methods (McDiarmid, 1994; Jacobs and Heyer, 1994; Simmons, 2002) to assure the maximum utility of each animal and to minimize the need for duplicate collecting. In principle, each animal collected should serve as a source of information on many levels of withouc (a* -0.033 In)Trved

dangerous species. We recommend locking containers that do not rely on weighted lids or other hastily constructed alternatives. Cages should be constructed of materials that do not absorb water so that they can be easily cleaned, disinfected and dried when appropriate. Likewise, caging materials should not present hazards such as rough edges or surfaces that can damage animals as they search for escape routes. Cages for dangerous species should be transparent so that the position of the animal can be visually assessed.

Schedules of cage cleaning should represent a tradeoff between cleanliness and disturbance. In some cases, small amounts of fecal material and pheromones deposited in the cage may be beneficial to behavior and stress levels (reviewed in Pough 1992; and taxon-specific chapters in Schaeffer et al., 1992, Greene, 1996).

b. Thermal requirements

Because of their ectothermic nature, thermal considerations are paramount to the health and well-being of amphibians and reptiles (Frye, 1991; Pough, 1992). Taxon-specific ranges of preferred temperature can be obtained from primary literature (reviewed in Frye, 1991; Pough, 1992). Frye (1991) provides general guidelines for estimating preferred temperature ranges based on characteristics of natural habitat. Every effort should be made to ensure that caging environment provides thermal conditions that enhance behavioral and physiological function. Pough (1992) recommends cage designs that provide thermal gradients and ample opportunity for animals to behaviorally thermoregulate by choosing from diverse microenvironments. Such caging arrangements may require heat lamps or tapes, and a diversity of perch and retreat sites. For some larger or more eurythermic species, providing such a diversity of thermal microhabitats may be both unnecessary and/or impractical. In such circumstances, adequate control over room temperature can be substituted. Most sources recommend that captive reptiles and amphibians be subjected to thermal cycles around the “preferred” temperature. Where possible, such cycles should be based on natural thermal variation during the normal active season of the organism (provided that natural variation does not exceed the critical thermal limits of the animal).

c. Lighting

Photoperiod is another important factor that must be considered for captive reptiles and amphibians. Many reptiles obtain physiological cues from light:dark cycles. In addition, many species (especially lizards, and some amphibians), but not all (e.g., most snakes) require an ultraviolet light source for normal calcium metabolism and Vitamin D synthesis (reviewed in Pough, 1992). Principal investigators should research their organisms to determine if UV or full-spectrum lighting is required (reviewed by Pough, 1992). Constant light (or dark) environments should be avoided because they may induce stress (Frye, 1991).

d. Air changes and humidity

As previously indicated, and reviewed in detail by Pough (1992), ectotherms are generally small, have low metabolic rates, and therefore, low rates of gas exchange and waste production. Thus,

mammalian or avian standards for room air changes are generally excessive for reptiles and amphibians. In addition, high humidity is necessary for some species; a condition that is more practical and economical with lower air turnover rates (Pough, 1992), and which may require virtually sealed containers for some amphibians (Jaeger, 1992). Humidity requirements should be considered on a case-by-case basis. It is reasonable for the IACUC to request references that recommend specific humidity guidelines for particular taxonomic groups.

e. Food and water

General nutritional requirements (e.g., herbivory, omnivory, insectivory, carnivory) are well-known for most amphibians and reptiles (Pough, 1992; Schaeffer et al., 1992). For particular species, there is often information available in the primary literature regarding natural diets. Captive diets should mimic natural diets to the closest extent possible, but this is often difficult and substitute foods must be used. Amphibians and reptiles should be fed appropriate foods on schedules that maintain normal growth and/or maintenance depending on the needs of specific studies. Because of their low energy requirements, ectotherms do not usually need frequent feedings, at least in comparison to mammals and birds. The key criteria for feeding schedules should be maintenance of weight and general health. Some reptiles and amphibians may require vitamin supplements (reviewed in Pough, 1992).

Water requirements are also variable and species-specific. Water should be provided with knowledge of a species natural history as a guide (Frye, 1991; Pough, 1992; Greene, 1996). For

outside and move internally in a methodical manner. The exterior of the animal should be

A standard operating procedure for the disposal of animal carcasses from medical or agricultural studies is incineration. In many cases carcasses of amphibians and reptiles used in research retain scientific or educational value, and incineration may be inappropriate. The decision to utilize incineration as a means of disposal should be considered on a case-by-case basis.

b. Donation to teaching or museum collections

When possible, specimens that retain scientific or educational value should be properly preserved (Simmons, 2002) and donated to teaching or museum collections.

2. Living animals

In cases where animals are not sacrificed as a study endpoint, and they are pathogen-free and in good health, there are several options to consider for disposition. Consistent with the concept of minimizing ecological impact and obtaining maximum use out of living organisms, and especially those that were captured from field populations, transfer to other studies, adoption by zoos, museums, or individuals, and/or repatriation into the wild should be considered.

a. Transfer to other studies

In many cases, at the completion of studies, animals retain value for continued research. The IACUC should be receptive to the transfer of healthy valuable organisms both within and between institutions for the purposes of continued study. This is especially important from the standpoint of reducing the need to collect animals from the wild. Such transfers should be accompanied by full documentation and should adhere to applicable local, State and National laws governing possession and transfer of reptiles and amphibians. Appropriate quarantines should be applied (Jacobson, 1993; Woodford, 2001).

b. Adoption

In many cases, healthy animals retain significant educational value and can be constructively donated for adoption by zoos, museums, and even private individuals that support educational or captive breeding programs.

c. Repatriation into the wild

Repatriation of research animals into the wild is a controversial issue. Pough (1992) argues that release of reptiles and amphibians held in captivity "...should be prohibited in almost all cases", due to risk of pathogen introduction, and potential effects on natural gene pools. However, under some circumstances, especially with respect to ecological studies that involve integrated laboratory and field components, repatriation of captive animals may be a necessary element to a successful research program. Release of research animals needs to be considered and

incorporated into the design of the study from its inception. Releases should be limited to cases of short-term captivity where healthy animals are released at their capture location. Furthermore, published protocols for planned releases should be followed (Jacobson, 1993; Woodford, 2001). As a general rule, field-trapped animals should be released only:

(i) If release is not specifically prohibited by national, state, or local law.

(ii) If they are currently healthy and have been held in isolation from exotic species and other research collections. Animals returned to the wild should never be in contact with other species, especially exotics. Two major pathogens in amphibians, chytrid fungi and ranavirus may have been introduced into wild populations by humans (Daszak et al., 1999). Relatively few infectious diseases have been studied in wild amphibians and reptiles and the exact origin of these pathogens is unknown. Captive amphibians and reptiles can harbor pathogens that were acquired in captivity and may serve as a vector for infecting wild populations.

(iii) At the original site of capture. Preservation of the integrity of natural gene pools should be paramount. Conservation efforts or safety considerations may dictate that animals be translocated. For these exceptional circumstances, prior approval of relocation should be obtained from appropriate state and/or federal agencies, and approved relocations should be noted in subsequent publication of research results.

(iv) If their ability to survive in nature has not been irreversibly impaired.

(v) Where there is reasonable expectation that the released animal will re- establish its former social status.

(vi) When local and seasonal conditions are conducive to survival.

VIII. Preparation and Revisions of These Guidelines

The initial draft of these guidelines was prepared by George R. Pisani (SSAR), Stephen D. Busack (HL) and Herbert C. Dessauer (ASIH). Victor H. Hutchison prepared the formal copy and Gary D. Schnell the camera-ready copy. The guidelines were revised and expanded to include laboratory as well as field studies in 2004 by Steven J. Beaupre (ASIH), Elliott Jacobson, Harvey Lillywhite (ASIH) and Kelly Zamudio (ASIH). The current product represents the collective efforts of over 75 persons and the societies extend sincere thanks to all participants.

Periodic revision of these guidelines is expected. Investigators are encouraged to send constructive criticisms or applicable new information to officers of the societies.

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