



OPEN SPACE RESEARCH AND MONITORING APPLICATION INSTRUCTIONS

Research and Monitoring on the open space is subject to landowner review and approval. The Research and Monitoring Application is a mechanism for ensuring that established Best Practices are committed and adhered to while such activities are being conducted on OC Parks and City of Irvine open space lands. Best Practice policies and procedures are necessary to protect open space resources in the public trust and minimize liability to the resources, the public and the landowners. OC Parks and the City and reserve the right to review and evaluate the value of any project proposed for the open space, and issuance of a permit is at their respective discretion alone.

Best Practices for habitat management are mandated by the Orange County Central Coastal Natural Community Conservation Plan (NCCP) & Habitat Conservation Plan (HCP), (County of Orange, USFWS & CDFG 1996). They also follow the intent of the Habitat Management Program described in Chapter 5 of the NCCP agreement. (County of Orange, USFWS & CDFG 1996, Section 5.5). Open space owners maintain discretion as to which research and monitoring techniques and activities are appropriate on their lands (County of Orange, USFWS & CDFG 1996, Section 5.4.7).

The terms of the Research and Monitoring Application and associated policies have been assembled from those of established research institutions such as the University of California Reserve System, The National Park Service, The National Academy of Sciences, and others. The City and County use the Research and Monitoring Application to verify that proposed projects follow the highest standards of conduct and practice. Accordingly, all projects involving research and monitoring on City and County lands are required to complete an Application. Projects that involve observational monitoring only or surveys that do not include manipulation, collection or handling may be subject to abbreviated review. Projects that combine observational monitoring with manipulation will receive normal review. In addition to the Research and Monitoring Application, project sponsors must also obtain any needed Entry Permits from landowners as well as State and Federal permits if applicable.

The Research and Monitoring Application is intended, among other things, to ensure that:

- 1. Proposed activities and level of use are consistent with land owner, NCCP/HCP, USFWS, and CDFG policies and regulations;
- 2. The activities contribute directly or indirectly to understanding and management of the open space lands;
- 3. The activities are best conducted on the open space lands and cannot be conducted as well or better at another location;
- 4. Activities do not impact the public's passive use of the wildlands or conflict with other ongoing management and research projects;





Guidelines and Supplemental Information for Researchers and Monitors

The following guidelines are neither exhaustive nor prescriptive. They are intended to promote general principles for reducing the impact on wildlife during surveys, to promote Best Practices in management and research, and to raise issues which should be considered when evaluating research and monitoring proposals. This information is derived from peer-reviewed literature, published guidelines from credible scientific bodies, and from animal care statements provided by existing license holders. Researchers who do not comply with agreed upon scientific methods established in their Entry Permit, and the conditions described in the Research and Monitoring Application are subject to loss of access and research privileges.

1. Professional Ethics for Scientists and Managers

All researchers and managers are strongly encouraged to adhere to the following (adapted from the Society for Conservation Biology Code of Ethics 2004):

- a) Actively disseminate information to promote understanding of and appreciation for biodiversity and the science of conservation biology and management.
- b) Advocate the use of reliable information, rigorous scientific methodology, and credible inference in management decisions affecting biodiversity.
- c) Recognize that uncertainty is inherent in managing ecosystems and species and encourage application of the precautionary principle in management and policy decisions affecting biodiversity.
- d) Recognize their responsibility to conservation and scientific honesty, and inform other scientists, the public, and prospective clients or employers of this responsibility.
- e) Avoid actions or omissions that may compromise their responsibility to conservation and science.
- f) Be willing to volunteer their services for the public good at a level appropriate to their financial abilities.
- g) Perform professional services or peer reviews only in their areas of competence, cooperate with other professionals in the best interest of conservation, and refer clients to other professionals with appropriate expertise.
- h) Refuse to allow personal interests, compensation, or personal relationships to interfere with their professional judgment or advice.
- i) Scrupulously avoid plagiarism; acknowledge the limitations of their research design, data, and interpretation of results; disclose conflicts of interest; honestly discuss their findings; and attempt to correct misrepresentation of their research by others.

- j) Claim authorship of a publication or report only when they have contributed substantially to the conception, design, data collection, analysis, or interpretation, or have helped draft or revise the article, and approve of the published version.
- When working professionally, interact and collaborate with counterparts, confer regularly with appropriate officials, share information, involve colleagues and students in professional activities, contribute to local capacity-building, and equitably share the benefits arising from the use of local knowledge, practices, and genetic resources.
- 1) Treat colleagues and professional contacts respectfully and support fair standards of employment and treatment for those engaged in the practice of conservation biology and ecosystem management.
- m) Work to ensure that no colleague is unjustly deprived of his or her job, reputation, ability to publish, or scientific freedom as a result of his or her conservation efforts.
- n) Protect the rights and welfare of human subjects used in research and obtain the informed consent of those subjects.
- o) Adhere to the highest standards for treatment of animals used in research in a way that contributes most positively to sustaining natural populations and ecosystems.

2. Permitting

The following is provided to assist in identifying appropriate permits for research:

- a) If the project involves taking, collecting (including banding and/or color marking), capturing, marking, or salvaging, mammals (except marine mammals), birds and their nests and eggs, reptiles, fishes, invertebrates and marine algae, a California Department of Fish and Game Scientific Collecting Permit (*Form fg1379 Scientific Collecting Laws and Regulations*) must be obtained. Please provide documentation of permit.
- b) If the project involves working with: State Listed Species; State Fully-Protected or Special-Concern species; pelicans, herons, egrets, swans, vultures, raptors, bird nests or eggs, bats, carnivores (including seals or sea lions), or deer, a California Department of Fish and Game (CDFG) Scientific Collecting Permit Attachment (*Form fg1379e*) and Memorandum of Understanding is required. Please append MOU and related permit.
- c) If the project involves collection, possession, transplantation or propagation of rare, threatened or endangered plants or manipulation of their habitat, a CDFG Plant Research Permit is required. If the project involves collecting voucher specimens only, a CDFG Rare, Threatened or Endangered Plant Collecting Permit (*Form fg11731 – Conducting Research on Rare, Threatened, or Endangered Plants*) is required. Please append the relevant permit.
- d) If the project involves working with plants or animals listed as federally threatened or endangered, a United States Fish and Wildlife Service (USFWS) Recovery Permit (*Form 3-200-55, OMB No. 1018-0094*) is required. Please append permit.

- e) If the project involves marine mammals, sea turtles, anadromous fish or any listed threatened or endangered marine species, then a NOAA Fisheries Scientific Research and Enhancement Permit (*Marine Mammals Permits and Authorizations; Permit to Take Marine Mammals for Scientific Research and/or Enhancement [includes Level A Harassment]; OMB No. 0648-0084*) is required. Please append permit.
- f) If the project involves taking, transporting, or possessing migratory birds, their parts, nests, or eggs, a USFWS Migratory Bird Scientific Collecting Permit (*Form 3-200-7, OMB No. 1018-0022; Birds Protected by the Migratory Bird Treaty Act*) is required. Please provide documentation of permit.
- h) If the project involves banding migratory birds, a Bird Marking and Salvage Permit issued by the Bird Banding Laboratory is required. Note: If the banding project involves auxiliary markers, mist nets, rocket nets, chemicals, federal or state listed species, eagles, waterfowl, hummingbirds, or blood and feather sampling, an Additional Authorization from the Bird Banding Laboratory is required.

NOTE: STATE AND FEDERAL PERMITS CAN TAKE UP TO 6 MONTHS TO BE APPROVED.

3. Animal Care

Three fundamental concepts for improving the welfare of animals are recommended for scientific research. These are:

- The Replacement of animals with other methods
- The Reduction of the number of animals used.
- The Refinement of the techniques used to reduce the impact on animals.

The *Three Rs* are equally relevant to wildlife research as to laboratory studies of animals.

Researchers should follow the guidelines for capture, handling, and care of animals as set forth below. Failure to follow with these guidelines may result in suspension or revocation of research and entry permits. Changes to these practices may be proposed at any time based on advances in accepted techniques and methodologies.

1. Mammals & Other Vertebrates

Researchers should follow the guidelines for capture, handling, and care of mammals set forth in *Guidelines for the capture, handling and care of mammals as approved by the American Society* <u>of Mammalogists</u> (1998) at: http://www.mammalsociety.org/committees/commanimalcareuse/98acucguidelines.pdf

The 1998 guidelines update those of the following:

The American Society of Mammalogists. 1987. Acceptable Field Methods of Mammalogy, Preliminary Guidelines prepared by the American Society of Mammalogists. *Journal of Mammalogy* Supp. Vol 68, No. 4. p.13.

Toe-clipping and ear-clipping of small mammals is discouraged and alternative methods of marking individuals should be employed, wherever possible. Permission for employing toe-clipping or ear-clipping markings must be obtained in advance.

2. <u>Birds</u>

Gaunt, AS, Oring, LW. 1997. Guidelines to the Use of Wild Birds in Research. The Ornithological Council, Washington DC.

The guidelines above refine the following:

American Ornithologists Union.1988. Report of committee on use of wild birds in research. *Auk* 105 (1 Supplement) 1A-41A.

3. <u>Reptiles & Amphibians</u>

American Society of Ichthyologists and Herpetologists, the Herpetologists League and the Society for the Study of Amphibians and Reptiles. 2001. <u>Guidelines for the use of live</u> <u>amphibians and reptiles in field research</u>. Available online: http://www.asih.org/files/hacc-final.pdf

4. <u>Fish</u>

DeTolla LJ, Srinivas S, Whitaker BR, Andrews C, Hecker B, Kane AS, Reimschuessel R. 1995. *Guidelines for the Care and Use of Fish in Research*. ILAR Journal 37:159-173.

Fisheries Society of the British Isles. *Fish Welfare Briefing Paper 2*. Available online.

Orlans, FB. 1988. *Field research guidelines*. Impact on animal care and use committees. Scientist's Centre for Animal Welfare, Bethesda, Maryland.

5. <u>Feral Animals</u>

Feral literally means "wild" animal. The term "introduced vertebrate pest species" is more accurate and will be used here, as it differentiates so called feral animals, birds and fish from native fauna.

There is a need for special consideration of the ethics of vertebrate pest research for two reasons. Firstly, feral animals are described and legislated in policy as both "pests" and "noxious animals". Also, in much of the human community, there is a perception that there is a reduced requirement for animal welfare and ethical consideration in dealing with these animals.

Areas of Introduced Vertebrate Research – to help guide appropriate considerations

- Processes for Effective Control
 - Methods of capture (e.g., trapping, netting)
 - Methods of killing
 - physical methods

- chemical methods
- biological methods
- Controlling fertility
 - chemical contraception
 - immunocontraception
- Other methods of control
 - habitat destruction
 - fencing in or out of control areas.
 - electronic deterrents
- Interaction with other species
 - predator-prey studies
 - o competition for resources and/or habitat
- Utilization
 - o harvesting methods of capture, transportation, and slaughter
 - o farming nutritional requirements

Animal Welfare and Ethical Concerns in Feral Animal Research

Methods of Killing

Any study of methods of killing generally requires that death is used as the end point of the experiment. Investigators should avoid using death as an experimental end-point whenever possible. The planned end-point and the reason for its choice should be given and justified. If death as an end-point cannot be avoided, it should be justified. When death is essential as the end-point, the study should be designed to result in the deaths of as few animals as possible. In these circumstances the number of animals involved should also be determined with proper consideration for scientific validity.

As there is a special requirement of justification of any proposed research into methods of killing of feral animals, the justification should be from both a broad perspective, for example the effect of the feral animal species on the environment, as well as the specific requirement to do the research (e.g., development of a new lethal disease in the species). The proposed research should include in the justification a significant animal welfare component (i.e., improvement on current practices, whether the killing is by physical, chemical or biological methods)

Methods of Capture

Proposed research into methods of capturing should include in the justification a significant animal welfare component (i.e., improvement on current practices). Current methods of capture may be used in Capture Studies as a "control" in the evaluation of new techniques.

Interaction Studies

Current control methods may be used to reduce feral animal numbers in interaction studies.

Field Studies

The animal experimentation ethics requirements for the use of feral animals in field studies should be the same as for wildlife.

Use as Laboratory Animals

Feral animals may be used in a manner similar to laboratory animals in some studies, requiring close containment either out-doors on a research site or in a research animal house or a designated containment facility. Animals should be taken from natural habitats only if animals bred in captivity are unsuitable for the specific scientific purpose. Feral animals captured as free-living animals should be considered to be non-domesticated and may require special housing, care and handling.

Euthanasia of Feral Animals

All feral animals used in research either in the laboratory or in the field must be euthanized at the conclusion of the research, in accordance with the principles set out in the *Euthanasia* section below.

6. Emergency Procedures

Applications for research should include a detailed description of emergency procedures. The purpose of these is to ensure that threats to the welfare of animals resulting from emergencies are mitigated. In the context of wildlife surveys, emergencies include events such as injuries to animals, inclement weather, floods, wildfires, and the illness or injury of the surveyor. Issues particularly relevant to wildlife surveys include the following.

- Arrangements should be made to clear and close all traps in the event of inclement weather, floods, and wildfires.
- Arrangements should be made to clear and close traps in the event that illness or injury removes the investigator from the field.
- Investigators should have the appropriate skills and equipment to euthanize seriously injured animals in the field should this be necessary. Euthanasia must be by an approved method (see the *Euthanasia section*).
- Arrangements should be made to appropriately transport seriously injured animals to the nearest veterinarian for treatment, noting that injured animals should be taken to veterinarians initially rather than to wildlife carers.
- Any unexpected problems should be reported to the Irvine Ranch Research Committee as soon as possible, including mortalities and injuries to animals. Future surveys may need to be modified in the light of these problems.

7. <u>Euthanasia</u>

Appropriate guidelines and practices for euthanasia of wildlife outlined in the document below should be followed:

Information Resources for Institutional Animal Care and Use Committees. 1985-1999. Techniques for Euthanizing Wildlife in the Field; available online at: <u>http://www.nal.usda.gov/awic/pubs/IACUC/wild.html</u>

Additional information is available in the following:

American Veterinary Medical Association. 2000. <u>2000 Report of the AVMA Panel on</u> <u>Euthanasia. Journal of the American Veterinary Medical Association 218:669-696.</u>

- Methods should be humane and produce a painless death as rapidly as possible.
- Methods that are acceptable are further described in Reilly (2001), UFAW/WSPA (1989), UFAW (1996 and 1997) and AVMA (2000).
- Methods which are not acceptable include car exhaust fumes, cervical dislocation in animals larger than 150 g, drowning and freezing. Note that cooling reptiles and amphibians to make them easier to handle is acceptable but, even after cooling, freezing is not an acceptable method of euthanasia.
- Surveyors should be trained and competent in the use of the acceptable methods of euthanasia.

8. <u>Survey Planning</u>

The existing knowledge of the fauna in the proposed study area should be used to determine if a new survey is both necessary and justified. The outcome of this background research will reveal the extent of the proposed survey necessary to meet the project objective. For example, there may already be sufficient information about some species, in which case there will be no need to re-survey these, thus *reducing* animal usage.

Reduction in animal usage by the application of existing knowledge depends on the availability and accessibility of that knowledge. For that reason, wildlife surveyors are encouraged to publish survey information whenever possible, and to lodge results where they can be accessed in the future.

9. <u>Survey Design and Methodology</u>

The intent of these guidelines is to describe how the welfare of animals can be increased during surveys by employing appropriate design and methodology. This section is a general discussion of these issues. In later sections more specific suggestions are made.

The following points should be considered when designing a wildlife survey:

- It should be appropriate to the objectives of the project
- It should be based on sound scientific and statistical principles so that the results are valid
- It should minimize the impact on animals
- Sample sizes should be kept to the minimum required and that number justified.
- The following general points should be considered when determining the methods to be used in a wildlife survey.

- Surveyors should have practical training and be experienced and competent in all the techniques they intend to use.
- Whenever possible, methods that do not require animals to be captured should be used (for example, spotlight counts, AnaBat TM detectors, hair tubes and playback calls).
- If animals must be captured, the least stressful methods available should be used. Consider the biology of the animal in relation to the time of year of the survey and the time of day of capture and release of the animals. Avoid periods when there are high environmental stresses. Ensure that animals are captive in traps for the minimum time.
- Animals that have to be handled should be restrained gently and the procedures completed as quickly as possible.
- Animals that have to be temporarily held after capture should be housed in a way appropriate to their biology and as free from environmental stresses as possible.
- If identification is necessary, methods used should be non-invasive and temporary whenever possible, and should not adversely interfere with the normal functioning of the animal.

Careful selection of a survey design and methodologies can greatly improve the welfare of animals during wildlife surveys. As examples, a good experimental design can *reduce* the number of animals necessary to achieve a valid result; the use of indirect survey methods such as spotlight counts, AnaBatTM detectors, hair tubes and playback calls *replace* and *reduce* the number of animals used while the use of the least intrusive methods and short handling times *refines* the use of animals.

4. Research Techniques

Research technique guidelines that are provided below should be followed, unless otherwise approved by the landowner. Failure to follow these guidelines may result in suspension of research and entry permits.

1. Radio Tracking

Radio tracking transmitters should only be used by individuals with extensive expertise and in exceptional circumstances. The relative high cost precludes their use except when other methods are totally unsuitable for rare, endangered or vulnerable species. Full justification and a detailed description of the methods, equipment, monitoring and impact on the animals will be required. Use alternative methods wherever possible.

- The methods used, including weight and attachment should be one that has been previously used on the same or similar species and has been proved to be satisfactory.
- Total package weight (collar, transmitter, battery, aerial and bonding material) should ideally be less than 5% of the animal's bodyweight and no greater than 10%, American Society of Mammalogists recommend less than 10%, for terrestrial mammals and less than 5% for bats weighing less than 70 g.
- Harnesses should only be used where the shape of the animal's head/neck means that a collar can be removed by the animal.

- Surgical grade 'super glue' should be used instead of a collar or harness in smaller species, and in platypus or other aquatic animals to attach the transmitter directly to the animals' fur, scales or feathers and to ensure that attachment is temporary only.
- Whip antennae should be incorporated into the collar wherever possible. Where freely attached, antennae should cause minimum disruption to the movement of the animal and the animal should be closely tracked for the first 24 hours.
- All reasonable attempts should be made to remove any attachments immediately if they are found to be causing distress to the animal, (unless the removal procedure is deemed to cause more stress to the animal).
- Transmitters should be removed from all animals at the end of the survey, unless otherwise approved.
- Collars or harnesses should not be used in species where they would interfere with locomotion (e.g., aquatic, burrowing animals).
- In areas where ticks occur, care should be taken in using collars as they may prevent the animal from grooming normally and removing the tick, alternatives to collars should be used.
- Some recommended sites for attachment include intra-abdominally for frogs, using a micro transmitter, and for bats, mid-dorsally using surgical glue.
- Alternatives to radio transmitters include low level radioactive tags e.g., for burrowing animals (these must be removed), LEDs, beta lights, and chemical light tags

2. <u>Use of Pitfall Traps</u>

Pitfall trapping is a sampling technique which is widely used in studies of seasonal occurrence, to examine spatial distribution patterns, to compare relative abundance in different micro-habitats, to study daily activity rhythms, and in community surveys.

The use of formalin as a killing agent in wet pitfall traps must be approved. Solutions such as formalin are used in wet pitfall traps because they preserve the specimen, not because they are humane. There is no rapid loss of consciousness before drowning and preserving.

<u>Usage</u>

Pitfall traps are used for sampling animal populations by:

- capturing species which are difficult to obtain by other methods;
- estimating relative abundances and species richness or for catching particular types of animals;
- determining movement patterns of individual animals.
- The pitfall trap is a relative method of estimating animal numbers and species, thus it cannot be used to estimate absolute population sizes or overall species richness of an area. It produces an "index" by which several areas can be compared. It is a "passive" form of sampling which relies on the animal rather than the observer making the action that leads to capture and enumeration.

Three Approaches to Pitfall Traps

- 1. For survey work, traps that catch the animal randomly animals foraging on the ground 'accidentally' fall into the trap.
- 2. Traps that are used in conjunction with barriers a 'drift fence' barrier can be used to direct foraging animals towards the trap; traps set up on known runs, to collect specific animals; or adaptations such as a lid or cover that encourages behavioral responses in certain animals to take refuge and therefore fall in.
- 3. Baits used to attract certain species or animal groups.

Dry Pitfall Traps

The pitfall trap is a research adaptation of a common hunting technique: the use of a pit in the ground into which an animal falls and cannot escape. The researcher's pitfall trap consists of a container sunk into the soil so that the mouth is level with the soil surface. Many ground dwelling animals fall into the trap and are unable to escape.

Dry pitfall traps used to collect reptiles or frogs generally consist of jars, tins or drums which are buried in the ground with their lips flush with the ground's surface. The openings are covered by a slightly raised lid or stone, or other object to keep out predators and prevent trapped animals from being overheated (during the day) or drowned (when it rains).

To be effective, they should be placed along known 'runs', where they are most likely to be encountered by the animals to be trapped. In addition to being positioned along known 'runs', traps are often used in conjunction with drift fences for enhanced effectiveness.

In certain isolated locations, dry pitfall traps are the only practical method of catching small, ground dwelling vertebrates and invertebrates. An example is ground dwelling spiders.

The advantages of using these traps include the following:

- they are simple, cheap and cost effective;
- have no moving parts;
- do not kill the animals (except inadvertently);
- collect large numbers of animals;
- are safe for the operator;
- are often the only practical alternative.

The disadvantages include the following, that:

- they require deactivating;
- they are fairly non-selective;
- they do not prevent trapped animals from killing each other;
- catch size is influenced by population sizes, activity levels, weather, size and nature of trap.

Management of Dry Pitfall traps

Dry pitfall traps must be managed to minimize the impact on trapped animals by taking into account issues such as:

- time animals will spend in the trap
- the possibility of trapping animals which may prey upon or parasitize other trapped animals
- environmental effects such as dehydration and hyperthermia in hot weather, hypothermia or drowning
- deprivation of food and water
- deactivation of traps when no longer required
- appropriate size of trap diameter, depth
- construction of trap conformation of the walls, lids, covers or grids
- possible non-target species bearing in mind that small vertebrates may in fact be smaller than large invertebrates
- traps should not be set in areas where there is a possibility of them filling with water such as low lying areas or wetlands

Wet Pitfall Trap

A wet pitfall trap is defined as a dry pitfall trap containing a solution designed to trap, kill and preserve an animal or animals. Aqueous solutions used in these traps include; formalin (10% formaldehyde), alcohol, methylated spirits, trisodium phosphate and picric acid.

Wet pitfall traps are routinely used to trap invertebrates, and are acceptable for this purpose. They are currently unacceptable for vertebrates, however, as the preservative solutions used do not kill humanely. Furthermore, traps used for invertebrates can pose a significant risk to small non-target vertebrates, such as lizards, frogs and even small mammals.

We consider that the designs of wet pitfall traps and the solutions in current use are unacceptable for vertebrates because they cause an inhumane death. When used for the capture of invertebrates these traps should be managed so as to minimize the inadvertent capture of vertebrates.

Modifications to enhance the operation of traps

- pitfall traps may be fitted with rain guards to prevent flooding and polystyrene "floats"
- shade covers reduce midday pit temperatures (but may reduce trap success)
- traps may have "exclusion barriers" such as a selective grid or "roof" to exclude unwanted fauna (predators, non-target species)
- leaf litter added to the trap from the site provides shelter and moisture which prolongs survival of trapped animals. A saturated sponge provides high moisture levels for trapped amphibians
- PVC tubing can be used to provide shelter inside the trap
- insecticides may be used where ants are prevalent and cause a problem by attacking trapped animals, for example, Rid Roll on around the rim of the trap. However, as the effects of insecticides on most reptiles and amphibians are not known, insecticides should be used with caution

General Pitfall Trap References

Southwood, TRE. 1978. Ecological methods. Chapman and Hall. London. 524pp.

Gist, CS, and Crossley, DA. 1973. A method for quantifying pitfall trapping. Environmental Entomology 2:951-2.

Greenslade, P, and Greenslade, PJM. 1971. The use of baits and preservatives in pitfall traps. Journal of the Australian Entomological Society. 10:253-60.

Hobbs, TJ. In prep. Influence of shade covers for pitfall traps on reptile and small mammal capture success in Arid Australia. Wildlife Research.

Jansen, MJW, and Metz, JAJ. 1979. How many victims will the pitfall make? Acta Biotheoretica 28:98-122.

Luff, ML. 1973. Some features influencing the efficiency of pitfall traps. Oecologia 19: 345-57.

Milton, DA. 1980. A comparison of three techniques used in a reptile survey of the Conondale Ranges. Victorian Naturalist. 97:26-31.

Upton, MS.1991. Methods for collecting, preserving, and studying insects and allied forms. The Aust. Entomological Soc. Miscellaneous Publication No. 3, 4th edition.

3. <u>Voucher Specimens</u>

The collection of voucher specimens is a traditional part of scientific research. However, it not always appropriate and may be a practice of concern particularly regarding sensitive species or populations. Researchers intending to collect voucher specimens should consult with the City or County staff or their designee on appropriate procedures. Some important considerations are listed below:

- The collection of voucher specimens should be fully justified, the number of specimens collected kept to a minimum and the collection of animals from more than one site should be justified.
- Voucher specimens should not be routinely collected for species that are readily identifiable in the field. Where only confirmation of the field identification is necessary, this might be possible by other means. Examples include hair samples, photographs and sound recordings.
- The City or County or their designee will consider the potential conservation impact of collection as part of the justification for voucher specimens.
- The animal welfare requirements for the capture of voucher specimens are no different from those for animals that will be released.
- Euthanasia of animals to be used for voucher specimens must be by an approved method (see Euthanasia section).
- Voucher specimens must be fully and correctly documented and lodged with a publicly accessible scientific collection, and the City or County management staff should be informed of their placement.

4. Surveys of Terrestrial and Arboreal Mammals

Catling et al. (1997) provides general information on surveying mammals.

Methods not involving animal capture

a) Animal signs

Some mammal species leave signs (scats and tracks) sufficiently distinctive to provide positive identification. Signs which indicate the presence of species or groups of species should be used in surveys wherever possible.

b) Hair tubes

The use of hair tubes is described by Scotts and Craig (1988), Lindenmayer et al (1999) and Mills et al (2002). Points to consider are as follows:

- Ensure that the floor of the tube is free of adhesive tape to prevent small lizards and frogs becoming stuck.
- If an animal does become stuck to the tape, do not try to pull the tape off, as this may seriously damage the skin. Either carefully trim the tape on the animal to as small a size as possible (the remaining tape will be shed during normal skin replacement) or gently ease vegetable oil under the tape and slide it off.
- Slope hair tubes with the entrance pointing slightly downwards to ensure drainage.

c) Spotlight counts

When spotlighting animals:

- Avoid prolonged exposure to light (i.e., more than 2 minutes).
- Use a light with a narrow beam.
- When practical, use a red filter or, preferably, a dimmer switch to reduce light intensity for prolonged observations once the animal has been spotted.

Methods involving animal capture

d) Trapping-general

In general, the following points apply to the use of traps:

- Use the trapping method with the least impact.
- Whenever possible, avoid trapping at times of the year when animals may be susceptible to greater stress, such as during breeding seasons or droughts. If animals are breeding, minimize their time in traps by checking more frequently and releasing pregnant or lactating females as a matter of priority.
- Select the type of trap which is appropriate to the species being targeted.
- Ensure all traps are in good working order and checked immediately prior to use.

- Limit the number of traps set per field worker to that which can be cleared in two hours.
- At any one site, unless justified otherwise, limit trapping periods to no more than four consecutive nights with a minimum of three nights between trapping periods to avoid continually trapping the same individuals.
- Use a bait appropriate to diet of the target species. The bait should not only lure the animal into the trap, but should also replace the food and moisture it would have consumed had it not been trapped. This is particularly important for small mammals which have high metabolic rates.
- Locate each trap to reduce exposure of trapped animals to the sun, wind, rain, etc. (for example, place traps under shrubs or beside logs).
- Avoid placing traps in areas of high ant activity.
- Do not trap during periods of inclement weather.
- Ensure all traps are located and checked each time a trap line is checked and that all traps are removed from the field or closed at the end of the trapping period. If individual traps are numbered and set in order, it makes it easier to ensure that all traps are checked.
- For nocturnal species, begin clearing traps at first light and where practical leave the traps closed until late afternoon. During periods of extremely cold weather, cease trapping completely or clear and close traps by 0200 hrs each day.
- For diurnal species, have an inspection schedule which minimizes the impact on any trapped animals and locate the traps so as to minimize the possibilities of heat or cold stress.
- Release animals as soon as possible and where they were caught.
- Cease trapping immediately if there has been an unusually high mortality of animals.

e) Box traps (also known as Elliott traps)

In addition to the general points above, the following need to be considered:

- Provide bedding in the traps. Dry leaf litter and Dupont Hollofill TM are suitable materials, although the latter sometimes wraps around the animals' feet. Cotton wool should not be used because it absorbs moisture, increasing the risk of hypothermia.
- In areas with wetter climates, place traps in a plastic bag, taking care to ensure adequate drainage (slope traps at 10° to the horizontal to allow drainage during rain).
- During periods of high temperatures in areas where traps cannot be sheltered from the sun, close traps during the day.
- Traps set in trees should be on the opposite side of the tree to the morning sun.

f) Cage traps

In addition to the general points above, the following need to be considered:

- Set traps in sheltered positions.
- Provide shelter for trapped animals by covering the trap with opaque plastic (cooler areas) or with shade cloth (hotter areas).
- If traps cannot be sheltered from the sun, they should be closed during the day if temperatures are high.

5. <u>Surveys of Bats</u>

A description of bat survey methods can be found in Helman and Churchill (1986). Surveys for bats should be carried out by an experienced bat investigator.

Methods not involving animal capture

Ultrasound detectors (for example, the AnaBat TM) can be used to detect bats without any impact and should be used whenever possible.

Methods involving animal capture

The following general points need to be considered when trapping bats:

- Whenever possible avoid trapping during the breeding season.
- Bats should be released at the point of capture as soon as possible. However, they should not be released in daylight. Those which cannot be released before dawn should be held until the following dusk.
- When necessary, bats should be held separately in suspended cloth bags in a dark, quiet and warm place.
- Bats may go into torpor in the trap or while held in bags and will need to be re-warmed before release.
- Care should be taken when handling bats, due to the zoonotic disease

Harp traps

A description of the use of harp traps can be found in Tidemann and Woodside (1978). Points additional to those in above that need consideration are:

- Set traps in a sheltered spot in potential flyways.
- Clear within two hours of dusk and again after dawn but before the sun begins to warm the hessian.
- Harp traps must not be used where large numbers of bats could be caught (for example at entrances to roost sites) to avoid the overheating of bats in the collection bag.

Mist nets

Because of the high risk of injury and death to bats, mist nets should only be used where other methods have already been rejected as unsuitable.

- Mist nets should only be used by trained and competent personnel.
- Only use mist nets after dark to avoid catching birds.
- The net should be attended at all times and captured bats removed immediately.
- Mist nets should not be used in areas where large numbers of bats could be caught (e.g., at entrances to roost sites).
- Nets should be closed when not attended and during the day.

Trip lines

- Due to the risks of injury to bats, use other methods whenever possible.
- Monitor continually whenever the line is deployed.

- Be prepared to enter the water to rescue bats, if necessary.
- Have at least one low-powered torch to collect bats since they will swim away from bright lights.

6. Surveys of birds

Methods not involving capture

Direct Observation

- Avoid close range inspection during breeding and feeding.
- Carry out searches for nests, mounds, display areas, characteristic scrapes and scratchings, visual and auditory searches such as breeding calls.

Playback calls

- Avoid prolonged exposure by limiting calling sessions to two 15 minute periods per night.
- Use of play back calls during the species' breeding season should be done with care so as not to disrupt the breeding of the resident pair.

Spotlighting owls

Examples of accepted techniques to census owls can be found in Kavanagh and Peake (1993).

Methods involving animal capture

Mist nets

- Because of the high risk of injury and death to birds, mist nets should only be used where other methods have already been rejected as unsuitable.
- Mist nets should be attended at least every 30 minutes and captured birds removed immediately.
- Nets must be closed when not attended.

7. Surveys of Reptiles and Amphibians

A summary of survey methods for reptiles can be found in Blomberg and Shine (1996). Choosing the correct season is critical for effective surveys of amphibians (and to a lesser extent with reptiles). Outside of their active season many frogs aestivate or go into torpor, usually in burrows, hollows in trees, crevices in timber or rocks or under loose soil. When in torpor, they are undetectable. To a lesser extent this may also occur during the active season when weather conditions are unsuitable (e.g., dry).

Methods not involving animal capture

Spotlighting amphibians with or without using playback

- Avoid excessive foot traffic around the water body.
- Keep exposure to a minimum to prevent overheating.
- Use a lower intensity light held at a distance for further observations.

Methods involving animal capture

- Consider that hand searches carried out by experienced personnel under suitable conditions will locate nearly all species of reptiles and amphibians in an area within a short period of time which may mean that fewer traps or no traps at all need be set.
- Frogs should be handled as little as possible because handling removes skin secretions and predisposes the frog to fungal infections (White 1990), while continuous holding in the hand can result in overheating.
- Hygiene precautions should be observed when handling frogs and tadpoles, including the use of gloves.
- Gloved hands should be wetted in the local water or in wet grass/vegetation so that loss of skin secretions is minimized when frogs are first picked up.
- Frogs should be moistened with rainwater or water from the stream being surveyed after holding or can be held separately temporarily (up to 24 hours) in a new moist plastic bag containing some vegetation (although, in the dark, vegetation will absorb oxygen).
- Reptiles should be held separately in appropriately sized secure bags or boxes with some vegetation, or a moist paper towel, as appropriate, in a cool place.
- Tadpoles are often easier to find than adults and provide important information about habitats used and other measures of environmental quality. However, care needs to be taken when handling tadpoles, as handling can result in a high level of injury and death of the tadpoles.

Hand searching for reptiles and amphibians

- Take care to uncover and reposition rocks and logs to prevent animal injuries and to avoid causing habitat disturbance which may affect the subsequent abundance of the species.
- Wash hands without soap (for instance in the water of the water body being surveyed or with rainwater) to reduce contamination from chemicals.
- Noose type devices to catch large reptiles should be used with care and sticks to pin snakes need to be padded to avoid causing damage.
- While all personnel must use gloves to handle frogs, smokers must use gloves when handling any amphibians to prevent absorption of nicotine through the animals' skin.

Methods of Surveying for Turtles

Freshwater turtles

- Set traps with an air space to prevent drowning of turtles or by-catch such as platypus, water rats or water birds. The air space can be maintained by use of a float (e.g. an empty drink container) or by tying the trap to an overhanging tree or log. Opera-house style traps can be tied to a stake on the bank.
- Traps should be checked at least at dawn and dusk. They should be checked more frequently if turtle numbers are high and during summer.
- Transport animals separately to avoid the risk of shell damage and hence infection. Keep cool during transport to avoid heat stress.

Marine turtles

- Marine turtles are very susceptible to heat stress, especially during transport. They can be cooled by the use of wet hessian bags.
- Confining the animals in small spaces increases the risk of abrasions, and hence infections. Marine turtles are best restrained by placing them on their backs in a cool place.
- During transport, insulate from heat and also from vibration. They are best transported within a vehicle rather than in the tray of a utility.

8. <u>Surveys of fish</u>

General information is available in Barker et al (2002) and Merrick 1990.

- Consider that fish are usually in their best condition in spring and early summer and will be able to cope with the shock of capture and recover more quickly than in the winter or in mid-summer after spawning.
- Use nets with soft mesh (for example, cotton or nylon) to reduce damage to the fish.
- Use appropriately sized and weighted traps to reduce the risk of non-target animals being caught.
- Fyke nets should have an air space by being set partially out of the water to prevent drowning of trapped mammals or waterfowl. Otherwise they should have a means of escape.
- If possible, avoid using gill nets because fish caught in these often die (or are so damaged during removal that they are unlikely to survive) and because they have the potential to trap many non target species.
- Check and empty traps regularly.
- Handle the fish as little as possible.
- Minimize the removal of the fish's protective mucous covering and reduce temperature shock by wetting hands first in the water from which the fish was caught.
- If electro-fishing is being used for sampling, operators should have appropriate training and follow guidelines.

5. Other Considerations

1. Herbicides, Pesticides, Insecticides and other Toxins

Researchers must get approval from the IRRC to use herbicides, pesticides, insecticides, or other toxins in their research.

2. Seed Collection

Collecting seeds and/or seedlings is destructive sampling with high potential impact for many plant species. To collect seeds or seedlings, ask for permission in your proposal.

3. Long-Term Plots (i.e., plots maintained for more than months or to be left in absence.) Establishment of long-term plots requires Irvine Ranch Research Committee (IRRC) approval, in most cases. If you anticipate the need for the long-term allocation of any site, contact the IRRC ahead of time to avoid siting conflicts with other projects.

4. Large-Scale and Intensive Habitat Manipulation

Large-scale and intensive habitat manipulation, such as bulldozing, controlled burns, mowing, etc. need to be approved by the IRRC in advance. Any significant infrastructure to be placed in the field needs to be approved by the IRRC in advance.

6. Human Health Considerations for Working with Birds

The following health considerations should be reviewed by all researchers interested in working on birds on the Wildlands. They are adapted from the following:

Ornithological Council. 2003. *West Nile Virus: What ornithologists and bird banders should know*. http://www.nmnh.si.edu/BIRDNET.

a) West Nile Virus: what ornithologists and bird banders should know

Introduction

West Nile Virus (WNV) was first isolated in 1937 in Uganda. There have been outbreaks in Israel (1951-1954), France (1962, 2000), and South Africa (1974). It appeared in Western Europe in the mid-1990s and traveled to the United States in 1999, where researchers – and their universities, government research agencies, and other research organizations – became concerned about the risk to field biologists, students, and others. Perhaps out of an abundance of caution and spurred by constant media attention to WNV, one university cancelled field research and field biology classes that involved bird banding. The Ornithological Council—a consortium of 11 scientific ornithological societies in the Western Hemisphere—consulted with a number of experts to compile this fact sheet about the risks of WNV to ornithologists and bird banders and to provide the most up-to-date public health recommendations for those handling live birds, carcasses, or tissues that are potentially infected with WNV.

Understand the risk

All research involves risk. Know the risks and take reasonable precautions. West Nile Virus should be no more of a deterrent to ornithological research and education than any other risk encountered in scientific research.

According to the Centers for Disease Control (as of 11 April 2003; see <u>http://www.cdc.gov/ncidod/dvbid/westnile/qa/symptoms.htm</u>)

- Most mosquitoes bites will not lead to a WNV infection
- Most people who are infected with WNV do not develop any type of illness
- It is estimated that 20% of the people who become infected will develop West Nile fever: mild symptoms, including fever, headache, and body aches, occasionally with a skin rash on the trunk of the body and swollen lymph glands.
- About 1 of each 150 infected persons becomes seriously ill with central nervous system infection (encephalitis &/or meningitis)

• About 6.6% of the 4,161 cases of the laboratory-positive 2002 WNV cases in US were fatal.

For young/healthy researchers who are not immuno-compromised, West Nile Virus is unlikely to cause much more than a mild illness—typically "flu-like symptoms." A more serious case of West Nile Virus in humans results in fever, disorientation, muscle weakness, neck stiffness, headache, nausea. Persons over 50 years of age are at increased risk of severe disease. An analysis of attack rates per million persons during the 1999 New York City outbreak showed that compared with persons 0 to 19 years of age, the incidence of severe neurologic disease was 10 times higher in persons 50 to 59 years of age and 43 times higher in those at least 80 years of age. However, although older persons are at greater risk for West Nile meningoencephalitis or death, persons of any age might develop severe neurologic disease (Nash et al. 2001). CDC recommends that persons with severe or unusual headaches seek medical attention as soon as possible.

In the lab

As of February 2003, there were only two documented cases of researchers contracting West Nile Virus in the course of conducting research. Both cases involved microbiologists. One was infected from an accidental needle puncture in the finger while working with live virus while the other was infected through an accidental scalpel cut while performing a necropsy on a dead Blue Jay (CDC Morbidity and Mortality Weekly Review, 20 December 2002)

It is best to assume that any specimen could be infectious and to take proper precautions at all times. Specifically:

- Neither refrigeration nor freezing will kill the virus. Ornithologists working with thawed tissue or specimens should assume that this material contains live virus.
- Ornithologists preparing specimens or working with tissue from fresh (never frozen) birds should be aware that the virus will remain viable in dead birds for several days.
- Ornithologists preparing specimens should take care to avoid scalpel cuts and punctures. If they occur, cleanse the area promptly and thoroughly, apply antiseptic and report the incident to a supervisor. If signs of illness occur within two weeks of exposure, prompt medical evaluation and consultation with public health authorities should be sought.

Standard measures to minimize exposure to fluids or tissues during handling of potentially infected tissue comprise standard droplet and contact precautions. These include:

- barrier protections such as gloves, masks, and eyewear
- proper use and disposal of needles, scalpels, and other sharp instruments
- minimizing the generation of aerosols (such as vigorous spraying of water on carcasses or work surfaces).

While wearing gloves, be careful not to handle anything but the materials involved in the procedure. Touching equipment, phones, wastebaskets or other surfaces may cause contamination. Be aware of touching the face, hair, and clothing as well. Researchers who use gloves should learn the proper way to remove and dispose of gloves and should avoid touching unprotected skin with the gloved hand. Consult your safety officer or safety manual. Typical instructions say to remove the first glove by grasping the cuff – being careful to avoid touching the bare skin or the wrist of arm - and peeling

the glove off the hand so that the glove is inside out. Repeat this process with the second hand, touching the inside of the glove cuff, rather than the outside. Wash hands immediately with soap and water.

Although the isolate of WNV is classified as a Biosafety Level 3 agent, it is considered acceptable practice to work with specimens and tissue in a Biosafety Level 2 laboratory conditions. See Biosafety in Microbiological and Biomedical Laboratories 4th ed. [http://bmbl.od.nih.gov] for details.

In the field

Although there are no documented cases of ornithologists or bird banders contracting WNV from handling living or dead birds, there has been no surveillance of ornithologists or bird banders to determine the presence/absence or prevalence of the disease. Even if such surveillance were to be implemented, it would be difficult to know if the disease had been contracted through contact with bird feces or saliva or if it had been contracted from an insect bite – at the research site or elsewhere.

- It has been confirmed that WNV may be shed from the cloacal and oral cavities (Komar et al. 2002). Therefore, contact with droppings, dropping-contaminated feathers, or the cloaca may result in exposure to WNV.
- Be sure to have antiseptic (not antibacterial or antimicrobial) available for hand washing and first aid for cuts or punctures sustained while handling birds.
- Reasonable precautions include the use of antiseptic wipes. This will protect both the researcher and the birds subsequently handled by the researcher.
- Avoid contact with bird feces.
- If bitten by a bird, wash hands (when possible) or use antiseptic (not antibacterial or antimicrobial) wipes or even a small amount of fresh bleach.
- Since ornithologists often use needles to take blood samples, extra care should be taken to avoid needle sticks.

Public health officials consider gloves to be an appropriate precaution but ornithologists rarely wear gloves when handling birds, particularly in the field. If gloves are worn, they should be changed or decontaminated with 70% ethanol or other appropriate substance after handling each bird to avoid transmission from one bird to another. Again, be familiar with proper glove removal and disposal. Other barrier protections such as goggles and masks are standard precautions against inadvertent exposure to droplets and fluids.

Ornithologists and bird banders should take the same reasonable precautions to minimize risks of various diseases posed by mosquito bites. Reasonable measures include protective clothing (long sleeves, long pants, socks), and the use of DEET or other insect repellants, with repeated applications over time. For detailed information about the proper use of DEET and summary of a recent assessment of the efficacy and safety of DEET, see below).

Precautions against transmission to birds and other wildlife

Ornithologists and bird banders should not re-use contaminated bags, boxes or other holding/carrying devices and other devices used to restrain birds during processing. The North American Banding Council manual states, "Launder bird bags frequently, as they should be kept clean," and "If a diseased bird is caught, it is extremely important to put that bag aside until it has been washed and disinfected." However, as it is not possible to determine if a bird is shedding virus, the better practice would be to carry an ample supply of bags or other holding/carrying devices so that no bag or other holding device is used more than once before laundering.

- When preparing specimens in the field, place waste material in a biosafety bag, seal it, and burn it, or carry it out with you.
- Never re-use needles or scalpel blades unless decontaminated with a fresh 10% bleach solution.

References

Centers for Disease Control Morbidity and Mortality Weekly Review, 20 December 2002. [http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5150a2.htm].

Komar, Nicholas, Robert Lanciotti, Richard Bowen, Stanley Langevin, and Michel Bunning. 2002. Detection of West Nile Virus in Oral and Cloacal Swabs Collected from Bird Carcasses. Emerging Infectious Diseases 8: 741-742.

Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. New England Journal of Medicine 344:1807-1814.

b) The Proper Use of DEET and an Assessment of the Risks of the Use of DEET

To determine the relative efficacy of DEET versus other insect repellants, Fraidin et al. tested the relative efficacy of seven botanical insect repellents; four products containing N,N-diethyl-m-toluamide, now called N,N-diethyl-3-methylbenzamide (DEET); a repellent containing IR3535 (ethyl butylacetylaminopropionate); three repellent-impregnated wristbands; and a moisturizer that is commonly claimed to have repellent effects. These products were tested in a controlled laboratory environment in which the species of the mosquitoes, their age, their degree of hunger, the humidity, the temperature, and the light–dark cycle were all kept constant.

They found that DEET-based products provided complete protection for the longest duration. Higher concentrations of DEET provided longer-lasting protection. A formulation containing 23.8 percent DEET had a mean complete-protection time of 301.5 minutes. A soybean-oil-based repellent protected against mosquito bites for an average of 94.6 minutes. The IR3535-based repellent protected for an average of 22.9 minutes. All other botanical repellents they tested provided protection for a mean duration of less than 20 minutes. Repellent-impregnated wristbands offered no protection.

They concluded that currently available non-DEET repellents do not provide protection for durations similar to those of DEET-based repellents and cannot be relied on to provide prolonged protection in

environments where mosquito-borne diseases are a substantial threat.

Depending on the time in the field, the temperature, exposure to water, perspiration, or concentration of DEET in the product, you may need to re-apply. This study found that a product containing 23.8% DEET provided an average of 5 hours of protection against mosquito bites. A product containing 20% DEET provided almost 4 hours of protection, and a product with 6.65% DEET provided almost 2 hours of protection. DEET may be washed off by perspiration or rain, and its efficacy decreases dramatically with rising outdoor temperatures.

Much has been said about the safety of DEET usage. The Fraidin paper addressed this issue:

Despite the substantial attention paid by the lay press every year to the safety of DEET, this repellent has been subjected to more scientific and toxicologic scrutiny than any other repellent substance. The extensive accumulated toxicologic data on DEET have been reviewed elsewhere. DEET has a remarkable safety profile after 40 years of use and nearly 8 billion human applications. Fewer than 50 cases of serious toxic effects have been documented in the medical literature since 1960, and three quarters of them resolved without sequelae. Many of these cases of toxic effects involved long-term, heavy, frequent, or whole-body application of DEET. No correlation has been found between the concentration of DEET used and the risk of toxic effects. As part of the Reregistration Eligibility Decision on DEET, released in 1998, the Environmental Protection Agency reviewed the accumulated data on the toxicity of DEET and concluded that "normal use of DEET does not present a health concern to the general U.S. population." When applied with common sense, DEET-based repellents can be expected to provide a safe as well as a long-lasting repellent effect. Until a better repellent becomes available, DEET-based repellents remain the gold standard of protection under circumstances in which it is crucial to be protected against arthropod bites that might transmit disease.

Fradin, M.D., Mark S. and John F. Day, Ph.D. 2002. Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine 347:13-18; available online at http://content.nejm.org/cgi/content/full/347/1/13>.

c) Avian Influenza

Guidelines regarding reducing risks of exposure to Avian Influenza are provided in the following:

Dierauf, L. 2005. Interim Guidelines for the Protection of Persons Handling Wild Birds With Reference to Highly Pathogenic Avian Influenza H5N1. Wildlife Health Bulletin #05-03. USGS National Wildlife Health Center.

References for Research Techniques

Blomberg S., Shine R. in Sutherland, J (ed.) (1996) *Ecological Census Techniques: a handbook*. Cambridge University Press pp. 218 - 226.

Catling PC., Burt RJ., Kooyman R. (1997) A comparison of techniques used in survey of the ground-dwelling and arboreal mammals in forests in north-eastern New South Wales. *Wildlife Research* 24: 217-432

Helman P., Churchill S. (1986) Bat capture techniques and their use in surveys. Macroderma 2:32-53.

Kavanagh R., Peake P. (1993) Survey procedures for nocturnal forest birds: an evaluation of the variability in census results due to temporal factors, weather and technique in P. Olsen (editor) *Australian Raptor Studies*. Australian Raptor Association, RAOU, Melbourne, pp 86-100.

Lindenmayer DB., Incoll RD., Cunningham RB., Pope ML., Donnelly CF., MacGregor CI., Tribolet C., Triggs BE. (1999) Comparison of hairtube types for the detection of mammals. *Wildlife Research* 26:745-753

Merrick JR. in Hand S. (ed) (1990) *Care and Handling of Australian Native Animals: Emergency Care and Capture Management*. Surrey Beatty and Sons, pp. 7 - 15.

Mills DJ., Harris B., Claridge AW., Barry SC. (2002) Efficacy of hair-sampling techniques for the detection of medium-sized terrestrial mammals. I. A comparison between hair-funnels, hair tubes and indirect signs. *Wildlife Research* 29:379-387

NSW Fisheries (1997) Australian Code of Electrofishing Practice. NSW Fisheries Management Publication No. 1

Reilly J. (ed) (2001) *Euthanasia of animals used for scientific purposes*. ANZCCART, Glen Osmond, South Australia

Scotts DJ, Craig SA. (1988) Improved hair sampling tube for detection of rare mammals. *Australian Wildlife Research* 15: 469-72.

Universities Federation for Animal Welfare (1996) *Recommendations for euthanasia of experimental animals:* Part 1. Laboratory Animals 30:293-316

Universities Federation for Animal Welfare (1997) *Recommendations for euthanasia of experimental animals*: Part 2. Laboratory Animals 31:1-32

Universities Federation for Animal Welfare /World Society for the Protection of Animals (1989) *Euthanasia of Amphibians and Reptiles:* Report of a Joint UFAW/WSPA Working Party. UFAW, South Mimms, Potters Bar, Herts, UK. And WSPA, London, UK.

- 5. Manipulative treatments and study sites receive specific prior review and approval;
- 6. Field markers are approved, sites are restored and markers removed upon completion of the activity;
- 7. Project sponsors provide data and reports, as well as any associated GIS files to the landowners;
- 8. Researchers follow the Best Practice Guidelines and policies stated in the Research and Monitoring Application and associated Entry Permit for Open Space.

Depending on the location and subject of the proposed research activities, additional documentation may be required of researchers and monitors.

OC Parks and the City of Irvine encourage applicants to do the following:

- 1. **Plan Ahead**. Please submit application documents to the permitting staff as early as possible 30 calendar days in advance is requested.
- 2. Thoroughly Complete Application and Describe the Proposed Work. Permits will be processed expeditiously for projects and activities that meet permit conditions. However, it is the applicant's obligation to fully describe the activities, sites, conditions, contractors, and other essential information required in the Application. Incomplete project descriptions and undetermined sites, activities or contractors cannot be issued a permit and will result in delays.
- 3. **Provide Maps of Project Areas and Sites**. A core element of the Research and Monitoring Application is mapping of areas and specific sites of proposed activities. It is the applicant's obligation to provide these along with the proposed project descriptions. If specific sites cannot be identified at the time of submission of the application then the proposed project should include site scoping provisions. When sites are eventually selected the application and associated Entry Permit should be updated to specify and map those sites.

Multiple Project Permits

Permits may be obtained for multiple projects that are each clearly identified and provide required information. It is the objective of the OC Parks and the City of Irvine to create a "Master Permit" approach wherever possible for longer term partners, where liability insurance, standard conditions, and other basic terms can be established and reviewed annually, and then individual projects can be reviewed and authorized with minimal effort. Such a master permit would not be an umbrella under which a multitude of unplanned projects could be authorized up front, but instead a mechanism for establishing the basic terms and standard conditions and provide a programmatic approach to permitting as new projects emerge. Applicants who anticipate proposing multiple projects on County or City open space lands are encouraged to consider this approach.



APPENDIX 1: SUPPORTING INFORMATION

Best Practices Guidelines for Researchers & Monitors

The following guidelines are neither exhaustive nor prescriptive. They are intended to promote general principles for reducing the impact on wildlife during surveys, to promote Best Management Practices, and to raise issues which should be considered when evaluating research and monitoring proposals. This information derived from the peer-reviewed literature, published guidelines from credible scientific bodies, and from animal care statements provided by existing license holders. Researchers who do not comply with agreed upon scientific methods, and the regulations described in the Research and Monitoring Application are subject to loss of access and research privileges.

1. Professional Ethics for Scientists and Managers

All researchers and managers are strongly encouraged to adhere to the following (adapted from the Society for Conservation Biology Code of Ethics 2004):

- a) Actively disseminate information to promote understanding of and appreciation for biodiversity and the science of conservation biology and management.
- b) Advocate the use of reliable information, rigorous scientific methodology, and credible inference in management decisions affecting biodiversity.
- c) Recognize that uncertainty is inherent in managing ecosystems and species and encourage application of the precautionary principle in management and policy decisions affecting biodiversity.
- d) Recognize their responsibility to conservation and scientific honesty, and inform other scientists, the public, and prospective clients or employers of this responsibility.
- e) Avoid actions or omissions that may compromise their responsibility to conservation and science.
- f) Be willing to volunteer their services for the public good at a level appropriate to their financial abilities.
- g) Perform professional services or peer reviews only in their areas of competence, cooperate with other professionals in the best interest of conservation, and refer clients to other professionals with appropriate expertise.
- h) Refuse to allow personal interests, compensation, or personal relationships to interfere with their professional judgment or advice.
- i) Scrupulously avoid plagiarism; acknowledge the limitations of their research design, data, and interpretation of results; disclose conflicts of interest; honestly discuss their findings; and attempt to correct misrepresentation of their research by others.

- j) Claim authorship of a publication or report only when they have contributed substantially to the conception, design, data collection, analysis, or interpretation, or have helped draft or revise the article, and approve of the published version.
- When working professionally, interact and collaborate with counterparts, confer regularly with appropriate officials, share information, involve colleagues and students in professional activities, contribute to local capacity-building, and equitably share the benefits arising from the use of local knowledge, practices, and genetic resources.
- 1) Treat colleagues and professional contacts respectfully and support fair standards of employment and treatment for those engaged in the practice of conservation biology and ecosystem management.
- m) Work to ensure that no colleague is unjustly deprived of his or her job, reputation, ability to publish, or scientific freedom as a result of his or her conservation efforts.
- n) Protect the rights and welfare of human subjects used in research and obtain the informed consent of those subjects.
- o) Adhere to the highest standards for treatment of animals used in research in a way that contributes most positively to sustaining natural populations and ecosystems.

2. Permitting

The following is provided to assist in identifying appropriate permits for research:

- a) If the project involves taking, collecting (including banding and/or color marking), capturing, marking, or salvaging, mammals (except marine mammals), birds and their nests and eggs, reptiles, fishes, invertebrates and marine algae, a California Department of Fish and Game Scientific Collecting Permit (*Form fg1379 Scientific Collecting Laws and Regulations*) must be obtained. Please provide documentation of permit.
- b) If the project involves working with: State Listed Species; State Fully-Protected or Special-Concern species; pelicans, herons, egrets, swans, vultures, raptors, bird nests or eggs, bats, carnivores (including seals or sea lions), or deer, a California Department of Fish and Game (CDFG) Scientific Collecting Permit Attachment (*Form fg1379e*) and Memorandum of Understanding is required. Please append MOU and related permit.
- c) If the project involves collection, possession, transplantation or propagation of rare, threatened or endangered plants or manipulation of their habitat, a CDFG Plant Research Permit is required. If the project involves collecting voucher specimens only, a CDFG Rare, Threatened or Endangered Plant Collecting Permit (*Form fg11731 – Conducting Research on Rare, Threatened, or Endangered Plants*) is required. Please append the relevant permit.
- d) If the project involves working with plants or animals listed as federally threatened or endangered, a United States Fish and Wildlife Service (USFWS) Recovery Permit (*Form 3-200-55, OMB No. 1018-0094*) is required. Please append permit.

- e) If the project involves marine mammals, sea turtles, anadromous fish or any listed threatened or endangered marine species, then a NOAA Fisheries Scientific Research and Enhancement Permit (*Marine Mammals Permits and Authorizations; Permit to Take Marine Mammals for Scientific Research and/or Enhancement [includes Level A Harassment]; OMB No. 0648-0084*) is required. Please append permit.
- f) If the project involves taking, transporting, or possessing migratory birds, their parts, nests, or eggs, a USFWS Migratory Bird Scientific Collecting Permit (*Form 3-200-7, OMB No. 1018-0022; Birds Protected by the Migratory Bird Treaty Act*) is required. Please provide documentation of permit.
- h) If the project involves banding migratory birds, a Bird Marking and Salvage Permit issued by the Bird Banding Laboratory is required. Note: If the banding project involves auxiliary markers, mist nets, rocket nets, chemicals, federal or state listed species, eagles, waterfowl, hummingbirds, or blood and feather sampling, an Additional Authorization from the Bird Banding Laboratory is required.

NOTE: STATE AND FEDERAL PERMITS CAN TAKE UP TO 6 MONTHS TO BE APPROVED.

3. Animal Care

Three fundamental concepts for improving the welfare of animals are recommended for scientific research. These are:

- The Replacement of animals with other methods
- The Reduction of the number of animals used.
- The Refinement of the techniques used to reduce the impact on animals.

The *Three Rs* are equally relevant to wildlife research as to laboratory studies of animals.

a. Capture, Handling, Care of Animals

Researchers are required to follow the guidelines for capture, handling, and care of animals as set forth in the following documents. Failure to comply with these guidelines may result in immediate suspension of research and access/entry permits. Changes to these practices can be proposed to the Irvine Ranch Research Committee for a decision.

Mammals & Other Vertebrates

Researchers should follow the guidelines for capture, handling, and care of mammals set forth in *Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists* (1998) that can be found at:

http://www.mammalsociety.org/committees/commanimalcareuse/98acucguidelines.pdf

The 1998 guidelines update those of the following:

The American Society of Mammalogists. 1987. Acceptable Field Methods of Mammalogy, Preliminary Guidelines prepared by the American Society of Mammalogists. *Journal of Mammalogy* Supp. Vol 68, No. 4. p.13.

Toe-clipping and ear-clipping of small mammals is discouraged and alternative methods of marking individuals should be employed, wherever possible. Permission for employing toe-clipping or ear-clipping markings must be obtained in advance.

Birds

Gaunt, AS, Oring, LW. 1997. Guidelines to the Use of Wild Birds in Research. The Ornithological Council, Washington DC.

The guidelines above refine the following:

American Ornithologists Union.1988. Report of committee on use of wild birds in research. *Auk* 105 (1 Supplement) 1A-41A.

Reptiles & Amphibians

American Society of Ichthyologists and Herpetologists, the Herpetologists League and the Society for the Study of Amphibians and Reptiles. 2001. <u>Guidelines for the use of live</u> <u>amphibians and reptiles in field research</u>. Available online: <u>http://www.asih.org/files/hacc-final.pdf</u>.

Fish

DeTolla LJ, Srinivas S, Whitaker BR, Andrews C, Hecker B, Kane AS, Reimschuessel R. 1995. *Guidelines for the Care and Use of Fish in Research*. ILAR Journal 37:159-173.

Fisheries Society of the British Isles. *Fish Welfare Briefing Paper 2*. Available online.

Orlans, FB. 1988. *Field research guidelines*. Impact on animal care and use committees. Scientist's Centre for Animal Welfare, Bethesda, Maryland.

Feral Animals

Feral animal literally means "wild" animal. The term introduced vertebrate pest species is actually more accurate and will be used here, as it differentiates so called feral animals, birds and fish from native fauna.

There is a need for some special consideration of the ethics of vertebrate pest research for two reasons. Firstly, feral animals are described and legislated for as both "pests" and "noxious animals". Also, in much of the community, there is a perception that there is a reduced requirement for animal welfare and ethical consideration in dealing with these animals.

Areas of Introduced Vertebrate Research Processes for Effective Control Methods of capture (e.g., trapping, netting)

Methods of killing
physical methods
chemical methods
biological methods
Controlling fertility
chemical contraception
immunocontraception
Other methods of control
habitat destruction
fencing in or out of control areas.
electronic deterrents
Interaction with other species
predator-prey studies
competition for resources and/or habitat
Utilization
harvesting methods of capture, transportation, and slaughter
farming nutritional requirements

Some areas of Animal Welfare and Ethical Concerns in Feral Animal Research

Studies of Methods of Killing

Any study of methods of killing generally requires that death is used as the end point of the experiment. Investigators must avoid using death as an experimental end-point whenever possible. The planned end-point and the reason for its choice must be given and justified. If death as an end-point cannot be avoided, it must be justified. When death is essential as the end-point, the study must be designed to result in the deaths of as few animals as possible. In these circumstances the number of animals involved should also be determined with proper consideration for scientific validity.

As there is a special requirement of justification of any proposed research into methods of killing of feral animals, the justification should be from both a broad perspective, for example the effect of the feral animal species on the environment, as well as the specific requirement to do the research (e.g., development of a new lethal disease in the species). The proposed research should include in the justification a significant animal welfare component (i.e., improvement on current practices, whether the killing is by physical, chemical or biological methods)

Studies of Methods of Capture

Proposed research into methods of capturing should include in the justification a significant animal welfare component (i.e., improvement on current practices). Current methods of capture may be used in Capture Studies as a "control" in the evaluation of new techniques.

Interaction Studies

Current control methods may be used to reduce feral animal numbers in interaction studies.

Field Studies

The animal experimentation ethics requirements for the use of feral animals in field studies should be the same as for wildlife.

Use as Laboratory Animals

Feral animals may be used in a manner similar to laboratory animals in some studies, requiring close containment either out-doors on a research site or in a research animal house or a designated containment facility. Animals should be taken from natural habitats only if animals bred in captivity are unsuitable for the specific scientific purpose. Feral animals captured as free-living animals must be considered to be non-domesticated and may require special housing, care and handling.

Euthanasia of Feral Animals

All feral animals used in research either in the laboratory or in the field must be euthanized at the conclusion of the research, in accordance with the principles as set out in the *Euthanasia* section below.

Emergency Procedures

All applications for wildlife research require a detailed description of emergency procedures. The purpose of these is to ensure that threats to the welfare of animals resulting from emergencies are mitigated. In the context of wildlife surveys, emergencies include events such as injuries to animals, inclement weather, floods, wildfires, and the illness or injury of the surveyor. Issues particularly relevant to wildlife surveys include the following.

- Arrangements must be made to clear and close all traps in the event of inclement weather, floods, and wildfires.
- Arrangements must be made to clear and close traps in the event that illness or injury removes the investigator from the field.
- Investigators should have the appropriate skills and equipment to euthanize seriously injured animals in the field should this be necessary. Euthanasia must be by an approved method (see the *Euthanasia section*).
- Arrangements must be made to appropriately transport seriously injured animals to the nearest veterinarian for treatment, noting that injured animals should be taken to veterinarians initially rather than to wildlife carers.
- Any unexpected problems should be reported to the Irvine Ranch Research Committee as soon as possible, including mortalities and injuries to animals. Future surveys may need to be modified in the light of these problems.

Euthanasia

Appropriate guidelines and practices for euthanasia of wildlife outlined in the document below should be followed:

Information Resources for Institutional Animal Care and Use Committees. 1985-1999. Techniques for Euthanizing Wildlife in the Field; available online at: <u>http://www.nal.usda.gov/awic/pubs/IACUC/wild.htm</u>. Additional information is available in the following:

American Veterinary Medical Association. 2000. <u>2000 Report of the AVMA Panel on</u> <u>Euthanasia. Journal of the American Veterinary Medical Association 218:669-696.</u>

- Methods must be humane and produce a painless death as rapidly as possible.
- Methods that are acceptable are further described in Reilly (2001), UFAW/WSPA (1989), UFAW (1996 and 1997) and AVMA (2000).
- Methods which are not acceptable include car exhaust fumes, cervical dislocation in animals larger than 150 g, drowning and freezing. Note that cooling reptiles and amphibians to make them easier to handle is acceptable but, even after cooling, freezing is not an acceptable method of euthanasia.
- Surveyors must be trained and competent in the use of the acceptable methods of euthanasia.

Survey Planning

The existing knowledge of the fauna in the proposed study area should be used to determine if a new survey is both necessary and justified. The outcome of this background research will reveal the extent of the proposed survey necessary to meet the project objective. For example, there may already be sufficient information about some species, in which case there will be no need to re-survey these, thus *reducing* animal usage.

Reduction in animal usage by the application of existing knowledge depends on the availability and accessibility of that knowledge. For that reason, wildlife surveyors are encouraged to publish survey information whenever possible, and to lodge results where they can be accessed in the future.

Survey Design and Methodology

It is not the purpose of these guidelines to provide detailed technical advice about the design and methodology of wildlife surveys. Rather, the intent is to show how the welfare of animals can be increased during surveys by employing appropriate design and methodology. This section is a general discussion of these issues. In later sections more specific suggestions are made.

The following points should be considered when designing a wildlife survey:

- It should be appropriate to the objectives of the project
- It should be based on sound scientific and statistical principles so that the results are valid
- It should minimize the impact on animals
- Sample sizes should be kept to the minimum required and that number justified.
- The following general points should be considered when determining the methods to be used in a wildlife survey.
- Surveyors must have practical training and be experienced and competent in all the techniques they intend to use.
- Whenever possible, methods that do not require animals to be captured should be used (for example, spotlight counts, AnaBat TM detectors, hair tubes and playback calls).
- If animals must be captured, the least stressful methods available should be used. Consider the biology of the animal in relation to the time of year of the survey and the

time of day of capture and release of the animals. Avoid periods when there are high environmental stresses. Ensure that animals are captive in traps for the minimum time.

- Animals that have to be handled should be restrained gently and the procedures completed as quickly as possible.
- Animals that have to be temporarily held after capture should be housed in a way appropriate to their biology and as free from environmental stresses as possible.
- If identification is necessary, methods used should be non-invasive and temporary whenever possible, and must not adversely interfere with the normal functioning of the animal.

Careful selection of a survey design and methodologies can greatly improve the welfare of animals during wildlife surveys. As examples, a good experimental design can *reduce* the number of animals necessary to achieve a valid result; the use of indirect survey methods such as spotlight counts, AnaBatTM detectors, hair tubes and playback calls *replace* and *reduce* the number of animals used while the use of the least intrusive methods and short handling times *refines* the use of animals.

b. Research Techniques

Research technique guidelines that are provided below must be followed, unless otherwise approved by the IRRC. Failure to follow these guidelines may result in immediate suspension of research and entry/access permits.

Radio Tracking

Radio tracking transmitters should only be used by individuals with extensive expertise and in exceptional circumstances. The relative high cost precludes their use except when other methods are totally unsuitable for rare, endangered or vulnerable species. Full justification and a detailed description of the methods, equipment, monitoring and impact on the animals will be required. Use alternative methods wherever possible.

- The methods used, including weight and attachment should be one that has been previously used on the same or similar species and has been proved to be satisfactory.
- Total package weight (collar, transmitter, battery, aerial and bonding material) should ideally be less than 5% of the animal's bodyweight and no greater than 10%, American Society of Mammalogists recommend less than 10%, for terrestrial mammals and less than 5% for bats weighing less than 70 g.
- Harnesses should only be used where the shape of the animal's head/neck means that a collar can be removed by the animal.
- Surgical grade 'super glue' should be used instead of a collar or harness in smaller species, and in platypus or other aquatic animals to attach the transmitter directly to the animals' fur, scales or feathers and to ensure that attachment is temporary only.
- Whip antennae should be incorporated into the collar wherever possible. Where freely attached, antennae should cause minimum disruption to the movement of the animal and the animal should be closely tracked for the first 24 hours.

- All reasonable attempts should be made to remove any attachments immediately if they are found to be causing distress to the animal, (unless the removal procedure is deemed to cause more stress to the animal).
- Transmitters should be removed from all animals at the end of the survey, unless otherwise approved.
- Collars or harnesses should not be used in species where they would interfere with locomotion (e.g., aquatic, burrowing animals).
- In areas where ticks occur, care should be taken in using collars as they may prevent the animal from grooming normally and removing the tick, alternatives to collars should be used. Any problems which are encountered should be reported to the Irvine Ranch Research Committee.
- Some recommended sites for attachment include intra-abdominally for frogs, using a micro transmitter, and for bats, mid-dorsally using surgical glue.
- Alternatives to radio transmitters include low level radioactive tags e.g., for burrowing animals (these must be removed), LEDs, beta lights, and chemical light tags

Use of Pitfall Traps

Pitfall trapping is a sampling technique which is widely used in studies of seasonal occurrence, to examine spatial distribution patterns, to compare relative abundance in different micro-habitats, to study daily activity rhythms, and in community surveys.

The use of formalin as a killing agent in wet pitfall traps must be approved. Solutions such as formalin are used in wet pitfall traps because they preserve the specimen, not because they are humane. There is no rapid loss of consciousness before drowning and preserving.

Usage

Pitfall traps are used for sampling animal populations by:

- capturing species which are difficult to obtain by other methods;
- estimating relative abundances and species richness or for catching particular types of animals;
- determining movement patterns of individual animals.
- The pitfall trap is a relative method of estimating animal numbers and species, thus it cannot be used to estimate absolute population sizes or overall species richness of an area. It produces an "index" by which several areas can be compared. It is a "passive" form of sampling which relies on the animal rather than the observer making the action that leads to capture and enumeration.

There are three basic approaches to using pitfall traps

(i) For survey work, traps that catch the animal randomly - animals foraging on the ground 'accidentally' fall into the trap.

(ii) Traps that are used in conjunction with barriers - a 'drift fence' barrier can be used to direct foraging animals towards the trap; traps set up on known runs, to collect specific animals; or adaptations such as a lid or cover that encourages behavioral responses in certain animals to take refuge and therefore fall in. (iii) Baits used to attract certain species or animal groups.

Dry Pitfall Traps

The pitfall trap is an adaptation by the ecologist of a common hunting technique: the use of a pit in the ground into which an animal falls and cannot escape. The ecologist's pitfall trap consists basically of a glass, plastic or metal container, sunk into the soil so that the mouth is level with the soil surface. Many ground dwelling animals fall into the trap and are unable to escape.

Dry pitfall traps used to collect reptiles or frogs generally consist of jars, tins or drums which are buried in the ground with their lips flush with the ground's surface. The openings are covered by a slightly raised lid or stone, or other object to keep out predators and prevent trapped animals from being overheated (during the day) or drowned (when it rains).

To be effective, they should be placed along known 'runs', where they are most likely to be encountered by the animals to be trapped. In addition to being positioned along known 'runs', traps are often used in conjunction with drift fences for enhanced effectiveness.

In certain isolated locations, dry pitfall traps are the only practical method of catching small, ground dwelling vertebrates and invertebrates. An example of this would be trapping for ground dwelling spiders

The advantages of using these traps include the following:

- they are simple, cheap and cost effective;
- have no moving parts;
- do not kill the animals (except inadvertently);
- collect large numbers of animals;
- are safe for the operator;
- are often the only practical alternative.

The disadvantages include the following, that:

- they require deactivating;
- they are fairly non-selective;
- they do not prevent trapped animals from killing each other;
- catch size is influenced by population sizes, activity levels, weather, size and nature of trap.

Management of Dry Pitfall traps

Dry pitfall traps must be managed to minimize the impact on trapped animals by taking into account issues such as:

- time animals will spend in the trap
- the possibility of trapping animals which may prey upon or parasitize other trapped animals
- environmental effects such as dehydration and hyperthermia in hot weather, hypothermia or drowning
- deprivation of food and water
- deactivation of traps when no longer required
- appropriate size of trap diameter, depth
- construction of trap conformation of the walls, lids, covers or grids

- possible non-target species bearing in mind that small vertebrates may in fact be smaller than large invertebrates
- traps should not be set in areas where there is a possibility of them filling with water such as low lying areas or wetlands

Wet Pitfall Trap

A wet pitfall trap is defined as a dry pitfall trap containing a solution designed to trap, kill and preserve an animal or animals. Aqueous solutions used in these traps include; formalin (10% formaldehyde), alcohol, methylated spirits, trisodium phosphate and picric acid.

Wet pitfall traps are routinely used to trap invertebrates, and are acceptable for this purpose. They are currently unacceptable for vertebrates, however, as the preservative solutions used do not kill humanely. Furthermore, traps used for invertebrates can pose a significant risk to small non-target vertebrates, such as lizards, frogs and even small mammals.

We consider that the designs of wet pitfall traps and the solutions in current use are unacceptable for vertebrates because they cause an inhumane death. When used for the capture of invertebrates these traps must be managed so as to minimize the inadvertent capture of vertebrates.

Modifications to enhance the operation of traps

- pitfall traps may be fitted with rain guards to prevent flooding and polystyrene "floats"
- shade covers reduce midday pit temperatures (but may reduce trap success)
- traps may have "exclusion barriers" such as a selective grid or "roof" to exclude unwanted fauna (predators, non-target species)
- leaf litter added to the trap from the site provides shelter and moisture which prolongs survival of trapped animals. A saturated sponge provides high moisture levels for trapped amphibians
- PVC tubing can be used to provide shelter inside the trap
- insecticides may be used where ants are prevalent and cause a problem by attacking trapped animals, for example, Rid Roll on around the rim of the trap. However, as the effects of insecticides on most reptiles and amphibians are not known, insecticides should be used with caution

General Pitfall References

Southwood, TRE. 1978. Ecological methods. Chapman and Hall. London. 524pp.

Gist, CS, and Crossley, DA. 1973. A method for quantifying pitfall trapping. Environmental Entomology 2:951-2.

Greenslade, P, and Greenslade, PJM. 1971. The use of baits and preservatives in pitfall traps. Journal of the Australian Entomological Society. 10:253-60.

Hobbs, TJ. In prep. Influence of shade covers for pitfall traps on reptile and small mammal capture success in Arid Australia. Wildlife Research.

Jansen, MJW, and Metz, JAJ. 1979. How many victims will the pitfall make? Acta Biotheoretica 28:98-122.

Luff, ML. 1973. Some features influencing the efficiency of pitfall traps. Oecologia 19: 345-57.

Milton, DA. 1980. A comparison of three techniques used in a reptile survey of the Conondale Ranges. Victorian Naturalist. 97:26-31.

Upton, MS.1991. Methods for collecting, preserving, and studying insects and allied forms. The Aust. Entomological Soc. Miscellaneous Publication No. 3, 4th edition.

Voucher Specimens

The collection of voucher specimens is a traditional part of scientific research. However, it is a practice of concern to some sections of the community. Wildlife surveyors intending to collect voucher specimens should consult with the Irvine Ranch Research Committee on what is appropriate. Briefly, some important points are listed below:

- The collection of voucher specimens must be fully justified, the number of specimens collected kept to a minimum and the collection of animals from more than one site must be justified.
- Voucher specimens should not be routinely collected for species that are readily identifiable in the field. Where only confirmation of the field identification is necessary, this might be possible by other means. Examples include hair samples, photographs and sound recordings.
- The Irvine Ranch Research Committee must consider the potential conservation impact as part of the justification for collection of voucher specimens.
- The animal welfare requirements for the capture of voucher specimens are no different from those for animals that will be released.
- Euthanasia of animals to be used for voucher specimens must be by an approved method (see the Euthanasia section).
- Voucher specimens must be fully and correctly documented and lodged with a publicly accessible scientific collection, and the Irvine Ranch Research Committee should be informed of their placement.

Surveys of Terrestrial and Arboreal Mammals

Catling et al. (1997) provides general information on surveying mammals.

Methods not involving animal capture

a) <u>Animal signs</u>

Some mammal species leave signs (scats and tracks) sufficiently distinctive to provide positive identification. Signs which indicate the presence of species or groups of species should be used in surveys wherever possible.

b) <u>*Hair tubes*</u>

The use of hair tubes is described by Scotts and Craig (1988), Lindenmayer et al (1999) and Mills et al (2002). Points to consider are as follows:

- Ensure that the floor of the tube is free of adhesive tape to prevent small lizards and frogs becoming stuck.
- If an animal does become stuck to the tape, do not try to pull the tape off, as this may seriously damage the skin. Either carefully trim the tape on the animal to as small a size as possible (the remaining tape will be shed during normal skin replacement) or gently ease vegetable oil under the tape and slide it off.
- Slope hair tubes with the entrance pointing slightly downwards to ensure drainage.

c) <u>Spotlight counts</u>

When spotlighting animals:

- Avoid prolonged exposure to the light (i.e., more than 2 minutes).
- Use a light with a narrow beam.
- When practical, use a red filter or, preferably, a dimmer switch to reduce light intensity for prolonged observations once the animal has been spotted.

Methods involving animal capture

Care should be taken when handling bats, due to the zoonotic disease

d) <u>Trapping-general</u>

In general, the following points apply to the use of traps:

- Use the trapping method with the least impact.
- Whenever possible, avoid trapping at times of the year when animals may be susceptible to greater stress, such as during breeding seasons or droughts. If animals are breeding, minimize their time in traps by checking more frequently and releasing pregnant or lactating females as a matter of priority.
- Select the type of trap which is appropriate to the species being targeted.
- Ensure all traps are in good working order and checked immediately prior to use.
- Limit the number of traps set per field worker to that which can be cleared in two hours.
- At any one site, unless justified otherwise, limit trapping periods to no more than four consecutive nights with a minimum of three nights between trapping periods to avoid continually trapping the same individuals.
- Use a bait appropriate to diet of the target species. The bait should not only lure the animal into the trap, but should also replace the food and moisture it would have consumed had it not been trapped. This is particularly important for small mammals which have high metabolic rates.
- Locate each trap to reduce exposure of trapped animals to the sun, wind, rain, etc. (for example, place traps under shrubs or beside logs).
- Avoid placing traps in areas of high ant activity.
- Do not trap during periods of inclement weather.
- Ensure all traps are located and checked each time a trap line is checked and that all traps are removed from the field or closed at the end of the trapping period. If individual traps are numbered and set in order, it makes it easier to ensure that all traps are checked.

- For nocturnal species, begin clearing traps at first light and where practical leave the traps closed until late afternoon. During periods of extremely cold weather, cease trapping completely or clear and close traps by 0200 hrs each day.
- For diurnal species, have an inspection schedule which minimizes the impact on any trapped animals and locate the traps so as to minimize the possibilities of heat or cold stress.
- Release animals as soon as possible and where they were caught.
- Cease trapping immediately if there has been an unusually high mortality of animals.

e) <u>Box traps (also known as Elliott traps)</u>

In addition to the general points above, the following need to be considered:

- Provide bedding in the traps. Dry leaf litter and Dupont Hollofill TM are suitable materials, although the latter sometimes wraps around the animals' feet. Cotton wool should not be used because it absorbs moisture, increasing the risk of hypothermia.
- In areas with wetter climates, place traps in a plastic bag, taking care to ensure adequate drainage (slope traps at 10° to the horizontal to allow drainage during rain).
- During periods of high temperatures in areas where traps cannot be sheltered from the sun, close traps during the day.
- Traps set in trees should be on the opposite side of the tree to the morning sun.

f) <u>Cage traps</u>

In addition to the general points above, the following need to be considered:

- Set traps in sheltered positions.
- Provide shelter for trapped animals by covering the trap with opaque plastic (cooler areas) or with shade cloth (hotter areas).
- If traps cannot be sheltered from the sun, they should be closed during the day if temperatures are high.

Surveys of Bats

A description of bat survey methods can be found in Helman and Churchill (1986). Surveys for bats should be carried out by an experienced bat investigator.

Methods not involving animal capture

Ultrasound detectors (for example, the AnaBat TM) can be used to detect bats without any impact and should be used whenever possible.

Methods involving animal capture

<u>General</u>

The following general points need to be considered when trapping bats:

- Whenever possible avoid trapping during the breeding season.
- Bats should be released at the point of capture as soon as possible. However, they should not be released in daylight. Those which cannot be released before dawn should be held until the following dusk.

- When necessary, bats should be held separately in suspended cloth bags in a dark, quiet and warm place.
- Bats may go into torpor in the trap or while held in bags and will need to be re-warmed before release.
- Care should be taken when handling bats, due to the zoonotic disease

<u>Harp traps</u>

A description of the use of harp traps can be found in Tidemann and Woodside (1978). Points additional to those in above that need consideration are:

- Set traps in a sheltered spot in potential flyways.
- Clear within two hours of dusk and again after dawn but before the sun begins to warm the hessian.
- Harp traps must not be used where large numbers of bats could be caught (for example at entrances to roost sites) to avoid the overheating of bats in the collection bag.

<u>Mist nets</u>

Because of the high risk of injury and death to bats, mist nets should only be used where other methods have already been rejected as unsuitable.

- Mist nets must only be used by trained and competent personnel.
- Only use mist nets after dark to avoid catching birds.
- The net must be attended at all times and captured bats removed immediately.
- Mist nets should not be used in areas where large numbers of bats could be caught (e.g., at entrances to roost sites).
- Nets should be closed when not attended and during the day.

Trip lines

- Due to the risks of injury to bats, use other methods whenever possible.
- Monitor continually whenever the line is deployed.
- Be prepared to enter the water to rescue bats, if necessary.
- Have at least one low-powered torch to collect bats since they will swim away from bright lights.

Surveys of birds

Methods not involving animal capture Direct Observation

- Avoid close range inspection during breeding and feeding.
- Carry out searches for nests, mounds, display areas, characteristic scrapes and scratchings, visual and auditory searches such as breeding calls.

<u>Playback calls</u>

• Avoid prolonged exposure by limiting calling sessions to two 15 minute periods per night.

• Use of play back calls during the species' breeding season should be done with care so as not to disrupt the breeding of the resident pair.

Spotlighting owls

Examples of techniques to census owls can be found in Kavanagh and Peake (1993).

Methods involving animal capture Mist nets

- Because of the high risk of injury and death to birds, mist nets should only be used where other methods have already been rejected as unsuitable.
- Mist nets should be attended at least every 30 minutes and captured birds removed immediately.
- Nets must be closed when not attended.

Surveys of reptiles and amphibians

General

A summary of survey methods for reptiles can be found in Blomberg and Shine (1996). Choosing the correct season is critical for effective surveys of amphibians (and to a lesser extent with reptiles). Outside of their active season many frogs aestivate or go into torpor, usually in burrows, hollows in trees, crevices in timber or rocks or under loose soil. When in torpor, they are undetectable. To a lesser extent this may also occur during the active season when weather conditions are unsuitable (e.g., dry).

Surveys not involving animal capture

Spotlighting amphibians with or without using playback

- Avoid excessive foot traffic around the water body.
- Keep exposure to a minimum to prevent overheating.
- Use a lower intensity light held at a distance for further observations.

Surveys involving animal capture

<u>General</u>

- Consider that hand searches carried out by experienced personnel under suitable conditions will locate nearly all species of reptiles and amphibians in an area within a short period of time which may mean that fewer traps or no traps at all need be set.
- Frogs should be handled as little as possible because handling removes skin secretions and predisposes the frog to fungal infections (White 1990), while continuous holding in the hand can result in overheating.
- Hygiene precautions must be observed when handling frogs and tadpoles, including the use of gloves.
- Gloved hands should be wetted in the local water or in wet grass/vegetation so that loss of skin secretions is minimized when frogs are first picked up.
- Frogs should be moistened with rainwater or water from the stream being surveyed after holding or can be held separately temporarily (up to 24 hours) in a new moist plastic bag containing some vegetation (although, in the dark, vegetation will absorb oxygen).

- Reptiles should be held separately in appropriately sized secure bags or boxes with some vegetation, or a moist paper towel, as appropriate, in a cool place.
- Tadpoles are often easier to find than adults and provide important information about habitats used and other measures of environmental quality. However, care needs to be taken when handling tadpoles, as handling can result in a high level of injury and death of the tadpoles.

Hand searching for reptiles and amphibians

- Take care to uncover and reposition rocks and logs to prevent animal injuries and to avoid causing habitat disturbance which may affect the subsequent abundance of the species.
- Wash hands without soap (for instance in the water of the water body being surveyed or with rainwater) to reduce contamination from chemicals.
- Noose type devices to catch large reptiles should be used with care and sticks to pin snakes need to be padded to avoid causing damage.
- While all personnel must use gloves to handle frogs, smokers must use gloves when handling any amphibians to prevent absorption of nicotine through the animals' skin.

Surveys of turtles

Freshwater turtles

- Set traps with an air space to prevent drowning of turtles or by-catch such as platypus, water rats or water birds. The air space can be maintained by use of a float (e.g. an empty drink container) or by tying the trap to an overhanging tree or log. Opera-house style traps can be tied to a stake on the bank.
- Traps should be checked at least at dawn and dusk. They should be checked more frequently if turtle numbers are high and during summer.
- Transport animals separately to avoid the risk of shell damage and hence infection. Keep cool during transport to avoid heat stress.

Marine turtles

- Marine turtles are very susceptible to heat stress, especially during transport. They can be cooled by the use of wet hessian bags.
- Confining the animals in small spaces increases the risk of abrasions, and hence infections. Marine turtles are best restrained by placing them on their backs in a cool place.
- During transport, insulate from heat and also from vibration. They are best transported within a vehicle rather than in the tray of a utility.

Surveys of fish

General information is available in Barker et al (2002) and Merrick 1990.

- Consider that fish are usually in their best condition in spring and early summer and will be able to cope with the shock of capture and recover more quickly than in the winter or in mid-summer after spawning.
- Use nets with soft mesh (for example, cotton or nylon) to reduce damage to the fish.
- Use appropriately sized and weighted traps to reduce the risk of non-target animals being caught.

- Fyke nets should have an air space by being set partially out of the water to prevent drowning of trapped mammals or waterfowl. Otherwise they should have a means of escape.
- If possible, avoid using gill nets because fish caught in these often die (or are so damaged during removal that they are unlikely to survive) and because they have the potential to trap many non target species.
- Check and empty traps regularly.
- Handle the fish as little as possible.
- Minimize the removal of the fish's protective mucous covering and reduce temperature shock by wetting hands first in the water from which the fish was caught.
- If electro-fishing is being used for sampling, operators should have appropriate training and follow guidelines.

Herbicides, Pesticides, Insecticides and other Toxins

Researchers must get approval from the IRRC to use herbicides, pesticides, insecticides, or other toxins in their research.

Seed Collection

Collecting seeds and/or seedlings is destructive sampling with high potential impact for many plant species. To collect seeds or seedlings, ask for permission in your proposal.

Long-Term Plots (i.e., plots maintained for more than months or to be left in your absence.) Establishment of long-term plots requires Irvine Ranch Research Committee (IRRC) approval, in most cases. If you anticipate the need for the long-term allocation of any site, contact the IRRC ahead of time to avoid siting conflicts with other projects.

Large-Scale and Intensive Habitat Manipulation

Large-scale and intensive habitat manipulation, such as bulldozing, controlled burns, mowing, etc. need to be approved by the IRRC in advance. Any significant infrastructure to be placed in the field needs to be approved by the IRRC in advance.

References for Research Techniques

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4. Human Health Considerations for Working with Birds

The following health considerations should be reviewed by all researchers interested in working on birds on the Wildlands. They are adapted from the following:

Ornithological Council. 2003. *West Nile Virus: What ornithologists and bird banders should know*. http://www.nmnh.si.edu/BIRDNET.

a) West Nile Virus: what ornithologists and bird banders should know

Introduction

West Nile Virus (WNV) was first isolated in 1937 in Uganda. There have been outbreaks in Israel (1951-1954), France (1962, 2000), and South Africa (1974). It appeared in Western Europe in the mid-1990s and traveled to the United States in 1999, where researchers – and their universities, government research agencies, and other research organizations – became concerned about the risk to field biologists, students, and others. Perhaps out of an abundance of caution and spurred by constant media attention to WNV, one university cancelled field research and field biology classes that involved bird banding. The Ornithological Council—a consortium of 11 scientific ornithological societies in the Western Hemisphere—consulted with a number of experts to compile this fact sheet about the risks of WNV to ornithologists and bird banders and to provide the most up-to-date public health recommendations for those handling live birds, carcasses, or tissues that are potentially infected with WNV.

Understand the risk

All research involves risk. Know the risks and take reasonable precautions. West Nile Virus should be no more of a deterrent to ornithological research and education than any other risk encountered in scientific research.

According to the Centers for Disease Control (as of 11 April 2003; see <u>http://www.cdc.gov/ncidod/dvbid/westnile/qa/symptoms.htm</u>)

- Most mosquitoes bites will not lead to a WNV infection
- Most people who are infected with WNV do not develop any type of illness
- It is estimated that 20% of the people who become infected will develop West Nile fever: mild symptoms, including fever, headache, and body aches, occasionally with a skin rash on the trunk of the body and swollen lymph glands.
- About 1 of each 150 infected persons becomes seriously ill with central nervous system infection (encephalitis &/or meningitis)
- About 6.6% of the 4,161 cases of the laboratory-positive 2002 WNV cases in the United States were fatal.

For young/healthy researchers who are not immuno-compromised, West Nile Virus is unlikely to cause much more than a mild illness—typically "flu-like symptoms." A more serious case of

West Nile Virus in humans results in fever, disorientation, muscle weakness, neck stiffness, headache, nausea. Persons over 50 years of age are at increased risk of severe disease. An analysis of attack rates per million persons during the 1999 New York City outbreak showed that compared with persons 0 to 19 years of age, the incidence of severe neurologic disease was 10 times higher in persons 50 to 59 years of age and 43 times higher in those at least 80 years of age. However, although older persons are at greater risk for West Nile meningoencephalitis or death, persons of any age might develop severe neurologic disease (Nash et al. 2001). CDC recommends that persons with severe or unusual headaches seek medical attention as soon as possible.

In the lab

As of February 2003, there have been only two documented cases of researchers contracting West Nile Virus in the course of conducting research. Both cases involved microbiologists. One was infected from an accidental needle puncture in the finger while working with live virus while the other was infected through an accidental scalpel cut while performing a necropsy on a dead Blue Jay (CDC Morbidity and Mortality Weekly Review, 20 December 2002)

It is best to assume that any specimen could be infectious and to take proper precautions at all times. Specifically:

- Neither refrigeration nor freezing will kill the virus. Ornithologists working with thawed tissue or specimens should assume that this material contains live virus.
- Ornithologists preparing specimens or working with tissue from fresh (never frozen) birds should be aware that the virus will remain viable in dead birds for several days.
- Ornithologists preparing specimens should take care to avoid scalpel cuts and punctures. If they occur, cleanse the area promptly and thoroughly, apply antiseptic and report the incident to a supervisor. If signs of illness occur within two weeks of exposure, prompt medical evaluation and consultation with public health authorities should be sought.

Standard measures to minimize exposure to fluids or tissues during handling of potentially infected tissue comprise standard droplet and contact precautions. These include:

- barrier protections such as gloves, masks, and eyewear
- proper use and disposal of needles, scalpels, and other sharp instruments
- minimizing the generation of aerosols (such as vigorous spraying of water on carcasses or work surfaces).

While wearing gloves, be careful not to handle anything but the materials involved in the procedure. Touching equipment, phones, wastebaskets or other surfaces may cause contamination. Be aware of touching the face, hair, and clothing as well. Researchers who use gloves must learn the proper way to remove and dispose of gloves and must avoid touching unprotected skin with the gloved hand. Consult your safety officer or safety manual. Typical instructions say to remove the first glove by grasping the cuff – being careful to avoid touching the bare skin or the wrist of arm - and peeling the glove off the hand so that the glove is inside out. Repeat this process with the second hand, touching the inside of the glove cuff, rather than the outside. Wash hands immediately with soap and water.

Although the isolate of WNV is classified as a Biosafety Level 3 agent, it is considered acceptable practice to work with specimens and tissue in a Biosafety Level 2 laboratory conditions. See Biosafety in Microbiological and Biomedical Laboratories 4th ed. [http://bmbl.od.nih.gov] for details.

In the field

Although there are no documented cases of ornithologists or bird banders contracting WNV from handling living or dead birds, there has been no surveillance of ornithologists or bird banders to determine the presence/absence or prevalence of the disease. Even if such surveillance were to be implemented, it would be difficult to know if the disease had been contracted through contact with bird feces or saliva or if it had been contracted from an insect bite – at the research site or elsewhere.

- It has been confirmed that WNV may be shed from the cloacal and oral cavities (Komar et al. 2002). Therefore, contact with droppings, dropping-contaminated feathers, or the cloaca may result in exposure to WNV.
- Be sure to have antiseptic (not antibacterial or antimicrobial) available for hand washing and first aid for cuts or punctures sustained while handling birds.
- Reasonable precautions include the use of antiseptic wipes. This will protect both the researcher and the birds subsequently handled by the researcher.
- Avoid contact with bird feces.
- If bitten by a bird, wash hands (when possible) or use antiseptic (not antibacterial or antimicrobial) wipes or even a small amount of fresh bleach.
- Since ornithologists often use needles to take blood samples, extra care should be taken to avoid needle sticks.

Public health officials consider gloves to be an appropriate precaution but ornithologists rarely wear gloves when handling birds, particularly in the field. If gloves are worn, they should be changed or decontaminated with 70% ethanol or other appropriate substance after handling each bird to avoid transmission from one bird to another. Again, be familiar with proper glove removal and disposal. Other barrier protections such as goggles and masks are standard precautions against inadvertent exposure to droplets and fluids.

Ornithologists and bird banders should take the same reasonable precautions to minimize risks of various diseases posed by mosquito bites. Reasonable measures include protective clothing (long sleeves, long pants, socks), and the use of DEET or other insect repellants, with repeated applications over time. For detailed information about the proper use of DEET and summary of a recent assessment of the efficacy and safety of DEET, see below).

Precautions against transmission to birds and other wildlife

Ornithologists and bird banders should not re-use contaminated bags, boxes or other holding/carrying devices and other devices used to restrain birds during processing. The North American Banding Council manual states, "Launder bird bags frequently, as they must be kept clean," and "If a diseased bird is caught, it is extremely important to put that bag aside until it has been washed and disinfected." However, as it is not possible to determine if a bird is shedding virus, the better practice would be to carry an ample supply of bags or other holding/carrying devices so that no bag or other holding device is used more than once before laundering.

- When preparing specimens in the field, place waste material in a biosafety bag, seal it, and burn it, or carry it out with you.
- Never re-use needles or scalpel blades unless decontaminated with a fresh 10% bleach solution.

References

Centers for Disease Control Morbidity and Mortality Weekly Review, 20 December 2002. [http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5150a2.htm].

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Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. New England Journal of Medicine 344:1807-1814.

b) The Proper Use of DEET and an Assessment of the Risks of the Use of DEET

To determine the relative efficacy of DEET versus other insect repellants, Fraidin et al. tested the relative efficacy of seven botanical insect repellents; four products containing N,N-diethyl-m-toluamide, now called N,N-diethyl-3-methylbenzamide (DEET); a repellent containing IR3535 (ethyl butylacetylaminopropionate); three repellent-impregnated wristbands; and a moisturizer that is commonly claimed to have repellent effects. These products were tested in a controlled laboratory environment in which the species of the mosquitoes, their age, their degree of hunger, the humidity, the temperature, and the light–dark cycle were all kept constant.

They found that DEET-based products provided complete protection for the longest duration. Higher concentrations of DEET provided longer-lasting protection. A formulation containing 23.8 percent DEET had a mean complete-protection time of 301.5 minutes. A soybean-oil-based repellent protected against mosquito bites for an average of 94.6 minutes. The IR3535-based repellent protected for an average of 22.9 minutes. All other botanical repellents they tested provided protection for a mean duration of less than 20 minutes. Repellent-impregnated wristbands offered no protection. They concluded that currently available non-DEET repellents do not provide protection for durations similar to those of DEET-based repellents and cannot be relied on to provide prolonged protection in environments where mosquito-borne diseases are a substantial threat.

Depending on the time in the field, the temperature, exposure to water, perspiration, or concentration of DEET in the product, you may need to re-apply. This study found that a product containing 23.8% DEET provided an average of 5 hours of protection against mosquito bites. A product containing 20% DEET provided almost 4 hours of protection, and a product with 6.65% DEET provided almost 2 hours of protection. DEET may be washed off by perspiration or rain, and its efficacy decreases dramatically with rising outdoor temperatures.

Much has been said about the safety of DEET usage. The Fraidin paper addressed this issue:

Despite the substantial attention paid by the lay press every year to the safety of DEET, this repellent has been subjected to more scientific and toxicologic scrutiny than any other repellent substance. The extensive accumulated toxicologic data on DEET have been reviewed elsewhere. DEET has a remarkable safety profile after 40 years of use and nearly 8 billion human applications. Fewer than 50 cases of serious toxic effects have been documented in the medical literature since 1960, and three quarters of them resolved without sequelae. Many of these cases of toxic effects involved long-term, heavy, frequent, or whole-body application of DEET. No correlation has been found between the concentration of DEET used and the risk of toxic effects. As part of the Reregistration Eligibility Decision on DEET, released in 1998, the Environmental Protection Agency reviewed the accumulated data on the toxicity of DEET and concluded that "normal use of DEET does not present a health concern to the general U.S. population." When applied with common sense, DEET-based repellents can be expected to provide a safe as well as a long-lasting repellent effect. Until a better repellent becomes available, DEET-based repellents remain the gold standard of protection under circumstances in which it is crucial to be protected against arthropod bites that might transmit disease.

Fradin, M.D., Mark S. and John F. Day, Ph.D. 2002. Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine 347:13-18; available online at http://content.nejm.org/cgi/content/full/347/1/13>.

c) Avian Influenza

Guidelines regarding reducing risks of exposure to Avian Influenza are provided in the following:

Dierauf, L. 2005. Interim Guidelines for the Protection of Persons Handling Wild Birds With Reference to Highly Pathogenic Avian Influenza H5N1. Wildlife Health Bulletin #05-03. USGS National Wildlife Health Center.