



THE ORNITHOLOGICAL COUNCIL

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GUIDELINES TO THE USE OF WILD BIRDS IN RESEARCH

Edited by
Abbot S. Gaunt & Lewis W. Oring

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THE ORNITHOLOGICAL COUNCIL

The founding premise of the Ornithological Council is that the ability to make sound policy and management decisions regarding birds and their habitat is dependent on the application of impartial scientific data and the continued collection of such data. The Council works to support this important mission. It serves as a conduit between ornithological science and legislators, land managers, conservation organizations and private industry. It provides scientific facts and expert analyses on birds to those in need of the information.

The Council was founded by seven ornithological societies in North America: American Ornithologists' Union (AOU), Association for Field Ornithology (AFO), Colonial Waterbird Society (CWS), Cooper Ornithological Society (COS), Pacific Seabird Group (PSG), Raptor Research Foundation (RRF) and Wilson Ornithological Society (WOS). Each society has two representatives on the Board of Directors. In 1998, the Society for Caribbean Ornithology (SCO) and Sección Mexicana del Consejo Internacional para la Preservación de las Aves (CIPAMEX) joined the Ornithological Council. In 1999, the Colonial Waterbird Society became known as the Waterbird Society and the Society of Canadian Ornithologists/La Société des Ornithologistes du Canada became the tenth member of the Ornithological Council.

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Table I

Abbreviations Used in Text

AAALAC	American Association for Accreditation of Laboratory Animal Care
ABA	American Birding Association
ABS	Animal Behavior Society
ASAB	Association for the Study of Animal Behaviour
AOU	American Ornithologists' Union
APHIS	Animal and Plant Health Inspection Service
AVMA	American Veterinary Medical Association
AWA	Animal Welfare Act
BBL	Bird-Banding Laboratory
BRD	Biological Resources Division (of USGS: was NBS)
CCAC	Canadian Council on Animal Care
CFR	Code of Federal Regulations
CITES	Convention on International Trade in Endangered Species of Wild Flora and Fauna
CWS	Canadian Wildlife Service
DVM	Doctor of Veterinary Medicine
ESA	Endangered Species Act
<i>Guide</i>	<i>Guidelines for the Care and Use of Laboratory Animals</i>
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
MTAB	Memorandum to Banders
MTBA	Migratory Bird Treaty Act
NBS	National Biological Survey (now BRD)
NIH	National Institutes of Health
NSF	National Science Foundation
NWHC	National Wildlife Health Center
OMA	Office of Management Authority (of USFWS)
OSNA	Ornithological Societies of North America
USDA	United States Department of Agriculture
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey

PREFACE

In 1988, the American Ornithologists' Union, Cooper Ornithological Society, and Wilson Ornithological Society, with encouragement and financing from the National Science Foundation, published the first edition of *Guidelines for the Use of Wild Birds in Research*. That publication proved extremely useful to many ornithologists and to the regulatory community (e.g., American Association of Accreditation of Laboratory Animal care and Institutional Animal Care and Use Committees). Advances in techniques over the past decade have made it appropriate upgrade this valuable reference. The current edition, published by the Ornithological Council (a consortium of the original publishers plus several other professional ornithological societies), greatly expands the previous edition, updates references, and adds several new sections. Among the major additions are a discussion of the role of AAALAC and IACUCs, a description of the responsibilities of both regulators and scientists, information on research permits, and a section on the care and housing of aquatic species. Sections are written to be complete in themselves so that the entire document need not be read to understand any one section. We have also included many cross-references, *[in italicized brackets]*

Most of the authors of the first edition helped with the revision. In addition, each section has been reviewed by a wide range of colleagues in both the ornithological and veterinary communities. This information is intended to indicate to IACUCs or other regulating groups the general procedures that are appropriate for birds. These procedures may be quite different from those appropriate for mammals. It is not intended to be a complete reference on techniques and procedures, nor, except where specified, to set limits on which procedures are allowed: these are guidelines, not laws. The procedures discussed herein are by no means intended to be exhaustive. Variations from procedures described herein may well be acceptable, *provided that the investigator presents an adequate justification of the scientific value of the requested variant*. It is the professional responsibility of all researchers and regulators to stay informed of new developments in their field of research. The Ornithological Council solicits comments and recommendations on all aspects of this publication.

This publication has been reviewed and endorsed by the following ornithological societies:

American Ornithologists' Union

Association of Field Ornithologists

Colonial Waterbird Society

Cooper Ornithological Society

Pacific Seabird Group

Raptor Research Foundation

Wilson Ornithological Society

1. INTRODUCTION

A. Overview

Consistent with long standing interests in conservation, education, research, and the well-being of birds, the Ornithological Council endorses the following guidelines and principles for scientists conducting research on wild birds. These Guidelines are formulated with consideration of animal welfare and research needs. Guidelines for the care of laboratory mammals [see I,B] often are not appropriate to wild vertebrates, even those held in captivity. On the other hand, the uses of wild animals have aspects that are not encountered in laboratory situations. The first of these is investigations that may affect populations. Investigations often involve, or necessitate, risk of injury or death of the experimental subject. Risks to individual animals should be minimized, but cannot be entirely eliminated. Risks that threaten the health or existence of populations are far more serious. It is these with which the ornithological and conservation community must be most concerned. Except under extraordinary circumstances, **experiments that threaten the stability or existence of populations are proscribed.**

A second difference between ornithological practice and biomedical research is that the latter rarely involves amateurs and has no recreational component. In contrast, amateur ornithologists often engage in research, and recreational uses of birds (including aviculture, bird watching, falconry, and hunting) are enjoyed by millions of persons. Such activities are beyond the scope of these Guidelines, but we do include the ABA's Code of Ethics (Appendix B).

Humane treatment of wild vertebrates in field research is essential for ethical, scientific, and legal reasons (Young 1975; ASAB and ABS 1993; Peck and Simmonds 1995). Traumatized animals do not behave normally and are more susceptible to predation or accidental injury than untraumatized conspecifics. Disturbance of animals or microhabitat may compromise observations and survivorship calculations.

Acquisition of new knowledge and understanding constitutes a major justification for any investigation. All effects of possibly valuable new research procedures (or new applications of established procedures) cannot be anticipated. The description and geographic distribution of newly discovered species often justifies studies of organisms that are poorly known. Many better known species of birds are used widely for a variety of studies in basic and applied biology. It is impossible to predict all potential observation or collection opportunities at the initiation of most field work, yet the observation or acquisition of unexpected taxa may be of considerable scientific value. Field studies of birds often involve many species, some of which may be unknown to science before onset of a study. A consequence of these points is that investigators frequently must refer to taxa above the species level, as well as to individual species, in their research protocols.

Researchers studying wild vertebrates generally recognize the necessity for collaboration among biologists, conservationists, veterinarians, and others concerned about the survival and well-being of wildlife. The following guidelines parallel those prepared by the Canadian Council on Animal Care for wild vertebrates (last revised 1991). To those who adhere to the precepts of careful field research, these Guidelines may seem to be simply a formal statement of the obvious.

These Guidelines have been prepared to include current information about techniques relevant to birds; advances in methods will require future amendments. Due to the considerable anatomical, behavioral, and physiological diversity of the many species covered by these Guidelines, and to the fact that usually the investigator will be an authority on the requirements and tolerances of the species under study, ultimate responsibility for certain techniques or procedures may best be left to the investigator.

B. Relationships Among Concerned Organizations

Beyond ethical considerations, the proper care and use of animals in research has a more formal framework. Most research on wild birds falls under the aegis of the Migratory Bird Treaty Act, which is enforced by USFWS, CWS, and state and provincial wildlife agencies. [see II.A]. Much of the legal background for current practices involving laboratory animals in the United States stems from the Animal

Welfare Act of 1970 (P.L. 91-579) and 1976 Amendments to the Animal Welfare Act (P.L. 94-279). Oddly, AWA does not consider rats, mice, or birds to be "animals," and it excludes field studies that "do not materially alter the behavior of the animals under study." However, both U.S. Departments of Agriculture and of the Interior (hence, USFWS and BRD), together with NIH and NSF, are signatories of the Interagency Research Animal Committee's Principles for the Utilization of Vertebrate Animals Used in Testing, Research and Training. Hence, all Federal personnel as well as recipients of grant monies from these or other government agencies must conform to established criteria for laboratory animals. The criteria are presented in the *Guide for the Care and Use of Laboratory Animals (=Guide)*, of the National Research Council, first published by NIH in 1962 and revised periodically; the latest revision is 1996. This publication is an essential reference for all researchers dealing with live animals.

Several organizations play roles in the implementation of the *Guide*, but the principal one is the American Association for Accreditation of Laboratory Animal Care (AAALAC), which deals with the use of *all* vertebrates (except humans) used in research. AAALAC is an oversight group to which virtually all U. S. academic and research institutions voluntarily subscribe. It is responsible for the accreditation of animal research facilities. AWA mandates every institution that performs research with *live* animals must establish an Institutional Animal Care and Use Committee (IACUC) that is responsible for providing institutional oversight and assuring compliance with AWA and any other regulations that may apply.

The *Guide* is concerned primarily with laboratory animals and does not specifically address the husbandry and care of wild birds. Areas of the *Guide* that deal with program and facility-wide issues are intended to be applied with professional judgment, exercised via the IACUC. [see D] The new *Guide* stresses performance-based standards for all species, i.e., based on professional judgment. In the case of wild birds, such judgment requires familiarity with the needs of the species in question. Deviations from standard procedures may be acceptable *if the scientific value of the variant is properly justified*. It is the responsibility of the investigator to provide such justification, together with documentation and data as may be applicable. Many sources of information concerning wildlife (e.g., Friend et al. 1994; Orleans et al. 1987; Giron Pendleton et al. 1987; or the detailed and still useful *Laboratory Animal Management: Wild Birds*, King et al. 1977), are either very general or oriented toward mammals or particular species of birds, or are dated. Hence, these *Guidelines for Use of Wild Birds in Research* are intended to provide avian-specific, current information to both the IACUC and investigators in order to facilitate their interaction.

C. General Considerations

Many applications and proposals for research grants now require that each investigator provide written assurance that field research with birds will meet the following requirements:

- a. Procedures with animals must avoid or minimize distress and pain to the animals, consistent with sound research design, [see IX]
- b. Procedures that may cause more than momentary or slight pain or distress to the animals should be performed with appropriate sedation or analgesia, except when justified for scientific reasons in writing by the investigator in advance, [see IX.C]
- c. It is unethical to allow an animal to suffer severe or chronic pain that cannot be relieved. If a procedure is likely to induce such a condition the animal must be euthanized at the end of the procedure, [see IX.E]
- d. Methods of euthanasia will be consistent with recommendations of the AVMA Panel on Euthanasia (Andrews et al. 1993) unless deviation is justified for scientific reasons in writing by the investigator, [see IX.E]
- e. The living conditions of animals held in captivity at field sites should be appropriate to satisfy the standards of hygiene, nutrition, group composition and numbers, refuge-provision, and protection from environmental stress necessary to maintain that species in a state of health and well-being. The housing, feeding, and non-veterinary care of the animals will be directed by a person (generally the investigator) trained and experienced in the proper care, handling, and use

of the species being maintained or studied. Some experiments (e.g., competition studies) will require the housing of multiple species, possibly in the same enclosure. Mixed housing is also appropriate for holding or displaying certain species, [see VII]

Additional general considerations that should be incorporated into any research design using wild birds include the following:

f. Taxa chosen should be well-suited to answer the question(s) posed.

g. The investigator must have knowledge of all regulations pertaining to the animals under study, and must obtain all permits necessary for carrying out proposed studies. Researchers working outside the U.S. should ensure that they comply with all wildlife regulations of the country in which the research is being performed. Transportation of many species is regulated by the provisions of CITES. Regulations affecting a single species may vary with country. Local regulations at state, county, or city levels may also apply, [see II] Authors should present evidence that research reported in submitted manuscripts or for presentation at meetings was conducted under the auspices of all proper permits, [see II.A]

h. individuals of endangered or threatened taxa should neither be removed from the wild (even in collaboration with conservation efforts), nor imported or exported, except in compliance with applicable regulations, [see II]

i. Before initiating field research, investigators must be familiar with the study species and its response to disturbance, sensitivity to capture and restraint, and, if necessary, requirements for captive maintenance to the extent that these factors are known and applicable to a particular study. Removal from the wild of possibly nest- or young-tending individuals should, as a general principle, be avoided unless justified for scientific reasons, [see III].

j. Every effort should be made prior to any removal of animals to understand the population status (abundant, threatened, rare, etc.) of the taxa to be studied, and the numbers of animals removed from the wild must be kept to the minimum the investigator determines is necessary to accomplish the goals of the study. This statement should not be interpreted as discouraging study or collection of uncommon species. Collection for scientific study can be crucial to understanding why a species is not abundant.

k. Procedures that are likely to have lasting effects on populations should be undertaken with the utmost caution. **Except in the most extraordinary circumstances, procedures likely to affect the stability or existence of a population are proscribed.** In such instances, the investigator must demonstrate the concurrence of recognized experts that the procedure is necessary.

l. The number of specimens required for an investigation will vary greatly, depending upon the questions being explored. As discussed later [see IV.B], certain kinds of investigations require collection of relatively large numbers of specimens, although the actual percentage of any population taken will generally be very small. In the case of accidental mortality, it is desirable to save specimens for deposit in museums or teaching collections. Studies should use the fewest animals necessary to answer reliably the questions posed. Use of adequate samples at the outset will prevent unnecessary repetition of the study, resulting in waste or increased distress to the birds.

m. The usefulness of specimens should be maximized by preserving not only skins but also carcasses, skeletons, DNA samples, and specific tissues.

n. The principal investigator must ensure that all personnel associated with the project have been properly trained. Students and technicians are obligated to seek advice when in doubt. Anyone wishing to use an unfamiliar technique is obligated to seek advice from an expert, and, if possible, to visit and practice with that expert. Appropriate expertise may exist outside the academic or wildlife communities, e.g., both private (hobby) and professional (zoo) aviculturists possess useful skills and information.

D. Institutional Animal Care and Use Committee

Every educational and research facility in the U.S. that has research programs involving animals must have an Institutional (Laboratory) Animal Care and Use Committee. For an extensive discussion of the duties of the IACUCs, see Orlans et al. (1987).

The *legal* role of IACUCs with regard to field studies is, at the time of this writing, slightly ambiguous, in part because, in some legal contexts, birds are not considered "animals,"¹ and because I(Laboratory)ACUC jurisdiction is supposedly directed toward laboratory animals. Further, "field studies," defined as in the Animal Welfare Act as "any study conducted on free-living wild animals in their natural habitat, which does not involve invasive procedure, and which does not harm or materially alter the behavior of the animals under study," would seem to be excluded from IACUC jurisdiction.² However, discussions of the legality the definition of "animal" or of IACUC authority over field studies are largely irrelevant. First, the respectful and ethical treatment of animals is not dependent on legality. Second, almost all granting agencies require an IACUC approved protocol as part of the application. We recommend that an IACUC protocol be sought for any experiments in which birds are handled or otherwise manipulated, certainly if invasive procedures are involved.

Field work, which by its very nature deals with largely uncontrolled environments, is fundamentally different from laboratory work in many respects. Hence, the IACUC must necessarily consider procedures and techniques that are practical for implementation at the site of the research. However, that there is consensus that IACUC approval of invasive procedures in the field does **not** require inspection of the "surgical" site! Prevailing conditions may prevent investigators from following even these Guidelines to the letter at all times. Investigators must, however, make a good faith effort to follow the spirit of these Guidelines and to justify deviations when they can be foreseen. The omission from these Guidelines of a specific research or husbandry technique (or their application to particular species) must not be interpreted as proscription of the technique. IACUCs must be aware that, although vertebrates typically used in laboratory research represent a small number of species with well understood husbandry requirements, the class Aves contains at least 9,000 species with very diverse and often poorly known behavioral, physiological, and ecological characteristics. This diversity, coupled with the diversity of field research situations, requires that each project be judged on its own merits. Techniques that are useful and fitting for one taxon, experiment, or field situation may be less useful in another time, place, or

¹ In a Federal Register notice dated 28 January 1998 (64 FR 4356), APHIS announced that a petition had been filed by several private organizations and individuals, seeking to compel APHIS to amend the definition of "animal" in the AWA to eliminate the express exclusion of birds, rats, and mice. As of 1 March 1999, no final decision had been announced by APHIS. The Ornithological Council is following this matter and will inform ornithologists of the decision, when made by APHIS, on the BIRDNET website (www.nmnh.si.edu/BIRDNET- refer to the "Ornithology and Society page or the "All about Permits" page). Ornithologists are also urged to consult APHIS.

²On 31 July 1998 (63 FR 40844) the Animal and Plant Health Inspection Service (APHIS) proposed to amend the Animal Welfare Act regulations by "clarifying the definition of the term field study." The stated purpose of the definitional change is to make clear that if a study includes *any one* of the three conditions - harm, invasive procedures, or material alteration of behavior - the study is not considered a "field study" and is therefore subject to IACUC review. It is not necessary that a study include all three conditions in order to be subject to IACUC review. The definition would also be amended to add the words "potential to" harm or materially alter the behavior of an animal under study. As of 1 March 1999, no final decision had been announced by APHIS. The Ornithological Council is following this matter and will inform ornithologists of the decision, when made by APHIS, on the BIRDNET website (www.nmnh.si.edu/BIRDNET - refer to the "Ornithology and Society" page or the "All About Permits" page.). Ornithologists are also urged to consult APHIS.

design. Therefore, in most cases it is impossible to generate specific guidelines for groups larger than a few closely related species. The premature stipulation of specific guidelines could severely inhibit humane care, as well as research. Further, the assessment of stress in field situations is a complex issue. Animals behave in ways that promote their own survival or the survival of their own genes, often in ways that appear "cruel." Furthermore, people of good will may evaluate a situation quite differently (compare Bekoff 1993 with Emlen 1993). IACUCs must note the frequent use of the word "should," throughout these Guidelines, and be aware that this is in deliberate recognition of the diversity of animals and situations covered by the Guidelines. Investigators, must be aware that use of the word "should" denotes the ethical obligation to follow these Guidelines when realistically possible. Before approving applications and proposals or proposed significant changes in ongoing activities, the IACUC shall conduct a review of those sections related to the care and use of animals and determine that the proposed activities are in accord with these Guidelines, or that justification for a departure from these Guidelines for scientific reasons is required.

When studies on wild vertebrates are to be reviewed, the IACUC must include personnel who can provide an understanding of the nature and impact of the proposed field investigation, the housing of the species to be studied, and knowledge concerning the risks associated with maintaining certain species of wild birds in captivity. Each IACUC should, therefore, include at least one institution-appointed member who is experienced in zoological field investigations. Such personnel may be appointed to the committee on an *ad hoc* basis to provide necessary expertise. When sufficient personnel with the necessary expertise in this area are not available within an institution, a consultant qualified to address these issues should be requested by the IACUC. The AWA states that such a consultant may be asked for information, but may not vote. If manipulation of parameters of the natural environment (e.g., day length) is not part of the research protocol, field housing for wild birds held for an extended period of time should approximate natural conditions as closely as possible while adhering to appropriate standards of care (e.g., Nace 1974). Caging and maintenance should provide for the safety, health, and well-being of the animal, while adequately allowing for the objectives of the study, [see VII]

The role of IACUCs in overseeing classroom uses of animals varies somewhat among institutions (Elliott 1995). Field exercises in which animals are observed but not manipulated should not require an IACUC approved protocol. Ethical behavior for such exercises has been developed by ABA (Appendix B, especially section 4). Most institutions require that any exercise involving manipulation of living vertebrates be cleared with IACUC, and if the manipulation is extensive, an approved protocol may be requested. As the rules or guidelines concerning animal use are under constant refinement, it is *the instructor's* responsibility to keep abreast. Keeping current may be facilitated by consulting e-mail and web-site sources.

II. PERMITS

A. Overview

Through numerous and complex laws, regulations, and policies (for convenience, here called "rules") among administrative units at various levels (national, state, county, and even campus), wild birds are among the most rigidly protected taxa. Only threatened or endangered species are afforded greater protection. Although the principal statutory authority at the national level is the Migratory Bird Treaty Act (MBTA), the term "migratory" should not be taken literally. In legal terms, "migratory" birds are any species named in the regulations (50 CFR 10). For practical purposes, the MBTA list includes all native species except galliform birds, [see below]. Statutes such as the MBTA give agencies the authority to make the regulations that prescribe the specific requirements for obtaining permits and limitations for working with wild birds. Regulations at the federal and state levels are occasionally reviewed and revised for greater clarity and simplicity to applicants. USFWS and some state agencies are working with the Ornithological Council to assure that changes in regulations or procedures are rapidly disseminated to wild-bird researchers through the *Ornithological Newsletter* as well as the Federal Register (FR). The FR, which is published every weekday, is the official listing of all proposed and adopted changes to the Code of Federal

Regulations, which is published annually. When those proposed changes are made final, another notice, giving the effective date, which is usually 30 days after the rule is made final, appears in the FR. It is therefore important that you determine if any changes have been made to the rule. As searching the FR can be time-consuming and it is possible that you may still miss pertinent notices, it may be best to ask the permitting official for a copy of the most recent version of the regulations that apply to the permits you are requesting.

Wildlife managers and conservationists take the task of natural resource preservation seriously. Therefore, many rules designed to protect wild bird populations have been promulgated. All researchers must be aware of the regulations that protect wild birds and obtain the necessary permits for their work. To do so requires a knowledge of the rules. As the results of research may have a direct bearing on conservation efforts, and thereby on the rules, researchers and law enforcement officials should consider themselves as a team. Regulatory agencies should ensure that information concerning permit requirements is readily available and easily interpretable by those not accustomed to regulatory language. Requested assistance to researchers should be provided in a friendly and prompt manner by the enforcing agencies.

As not every research project will have direct application to conservation and management, enforcement agencies must recognize the value and need for both basic and applied research. Agencies should seek the advice of recognized field researchers and their professional experiences in making decisions regarding the granting of unusual requests. Agency personnel should help to expedite scientific research on wild birds, although the *conservation* of biological resources must always be given first priority in the issuance of permits and in the conduct of scientific field and laboratory research on wild birds.

Any possession, capture, handling, collecting (totally or of parts), marking, or disturbing of native wild birds, their nests, or their eggs requires some kind of special licenses or permits. Nearly all bird species in North America are protected by a large variety of laws (see Lund 1980 for a review of the philosophy of this legal protection system). Working on public lands or lands managed by private organizations also may require permits. Some examples of agencies and organizations that need to be consulted include: U.S. National Park Service (National Parks and Monuments), USFWS (National Wildlife Refuges), U.S. Department of Defense (military bases and lands), U.S. Forest Service (National Forests), U.S. Bureau of Land Management (public lands), state agencies and organizations, and private land managers (The Nature Conservancy and National Audubon Society (nature reserves), all the way to the individual landowner.

Work on public lands may require permission of several administrative levels (such as regional and local offices). Often the best policy is to start "low" in the bureaucracy and work up. Assuming that the researcher has all necessary permits, the local manager or landowner may be the only person whom a researcher needs to contact to acquire approval for using various lands for a study area. Researchers should keep in close contact with the local officials and keep them informed of the progress of the research on a regular basis.

Research that requires collecting, handling, disturbing, holding captive or in any way manipulating wild birds generally requires written approval from the organizations listed in Table 11-1. Many more details concerning wildlife protection and permit applications are given by Little (1993), which updates Estes and Sessions (1983, 1984) and King and Schrock (1985). The field-researcher should consult those references for additional details.

Current guidelines, as well as advice, concerning permits are available in Little (1993) and directly through various state offices and USFWS regional offices, or their equivalents in other countries. Researchers who intend to handle or collect wild birds or parts thereof, eggs, or nests should contact appropriate agencies (Table 11-1) at least six months prior to proposed field activities. For research within the U.S., researchers should contact the appropriate state agency, appropriate USFWS Regional Office, the specific managing agency if the research is to be conducted on federal or state lands, and the local landowner if the research is proposed for private lands.

For foreign research permits, we suggest that North American researchers contact the USFWS, CWS, or Instituto Nacional de Ecologia, MEXICO for guidance on permit applications in those countries or countries outside their own (see Appendix A for addresses). The embassies of any country where permits for research activities are desired often can provide additional contact sources and give advice on rules and permit application procedures in their countries. The "home" embassy of the researcher should also be contacted. For example, the INE in Mexico requires that U.S. researchers go through the Scientific Affairs Office in the U.S. embassy in Mexico City for permit applications. This office can facilitate the proper and expedient acquisition of permits (see Appendix A for addresses).

Table II-1. Various Types of Permits and Their Issuing Agencies

<u>Type of Permit</u>	<u>Consulting Agency</u>
Local Animal Use and Care Permit	IACUC, USDA
Restricted Area Permit (private and public lands)	Agency or person in charge
State/Provincial Collecting or Banding Permit	State/Provincial Agency
Federal Collecting Permit	Migratory Birds Section of USFWS or CWS office
Federal Banding Permit	BBL (USGS); CWS
Federal Endangered Species Permit	USFWS regional office
Foreign Collecting/Research Permit	The country via U.S. or Canadian Embassy in that country
Foreign Immigration Permit	As above; passport, visa
Foreign Equipment Use Permits	As above; appropriate agency in country
Foreign Export Permit (for specimens)	The country
U.S. Import Permit (for specimens)	USDA; USFWS (OMA)
U.S. Endangered Species Import/Export Permit (CITES)	USFWS (OMA)
U.S. Facilities Permit, restricted materials	USDA
U.S. Facilities Permit, museum as repository	USFWS

State and province permit rules vary extensively, and researchers must be aware of these differences. Collecting of some bird species (usually those considered as "pests") is exempt from rules in some states, but may not be in others. All species included in the Migratory Bird Treaty Act of 1918, as amended through 1972 (16 US Code 703-711; see Code of Federal Regulations [50 CFR 10] for up-to-date lists of migratory birds) require both federal and state/provincial permits for collection and handling. All species included in the Endangered Species Act (Endangered and Threatened Wildlife and Plants, 50 CFR 17.11 and 17.12) require additional permits or cooperative agreement (a form of contract or work agreement between the researcher and USFWS) that outline the activities to be conducted. Some states, however, also require special permits for banding or other forms of potential disturbance. The possession of whole specimens and "parts thereof" is interpreted differently in different states and provinces. Hence, it is critical that the personnel of state and province wildlife management agencies, managers of local management units, and even IACUCs on campuses and other small administrative units be consulted prior to beginning a project.

Publication: Many biomedical publications require that IACUC protocol numbers be included in the Acknowledgments section of any paper reporting research on laboratory animals. To date, no such regulations apply to research on wild animals, nor is acknowledgment of permits required by agencies granting those permits. Yet, such acknowledgments help assure the public at large that the research was performed legally and that the investigators maintain and support high ethical standards in the use of natural resources and care of animals. Further, many agencies require reprints of any publications of

research performed under the auspices of their permit. As with agencies offering financial support, acknowledgment of the cooperation of permit granting agencies is often greatly appreciated. Therefore, we encourage authors of scientific publications on wild birds to acknowledge an IACUC-accepted protocol and any applicable permits in the acknowledgment sections of their manuscripts, and we encourage reviewers of submitted papers to look for such acknowledgments and comment critically when they are absent. Editors *may* then, at their discretion, hold the failure of an investigator to produce evidence that the research in question was legally performed to be a factor in the decision whether or not to publish. Likewise, program officers for ornithological societies *may* justifiably request that similar evidence accompany requests for a place on the program. *However, ultimate responsibility remains with each investigator.*

B. Collecting Permits

A Scientific Collecting Permit allows the permittee to collect (kill), salvage (possess specimens that died either from causes other than the permittee's action, e.g., road, tower and window kills, or accidentally from the permittee's own actions), and to possess specimens of wild birds. A collecting permit is required if any wild bird specimens (or parts of specimens such as blood samples and other fluids, biopsies, feathers and other hard parts, swabs, etc.) are to be collected, trapped, transported, or otherwise manipulated (some tissues may be collected under the auspices of a Banding and Salvage Permit [see *below*]). The permit usually defines the numbers of specimens that may be collected, the geographic area covered, the means that may be used to obtain the specimens, and the individual (s) who is (are) authorized to collect.

A critical issue is that of "possession", a term that is best interpreted broadly. An investigator may possess specimens for research or educational purposes, but may not establish a private collection. All specimens not destroyed in the process of the research must eventually be donated to a designated depository. Possession may also determine the legality of the activity. For instance, the state of Ohio permits anyone, with or without a permit, to kill any number of some species, e.g., *Passer domesticus*, but possession of a specimen of such a species requires a permit.

An exception to the "possession requires a permit" rule is the case of loaned specimens. Many museums and collections make sets of specimens available for loan to those engaged in educational activities. A teacher, park ranger, or other individual wishing to use such a demonstration set need not have either a collection or salvage permit. Rather, the loaning institution should provide a document (usually a form letter) to accompany the loan indicating the details of the loan. Similarly, individuals seeking a loan of research specimens from a recognized collection need not themselves possess a permit. Research loans are always accompanied by abundant documentation, which should be kept readily available if questions arise.

Federal permit: U.S. federal collecting permits (Migratory Bird Permit) are issued to individuals (including individuals representing organizations) by the Regional Offices of the USFWS: Canadian permits by Regional Offices of the CWS (Appendix A). These permits allow the capture, handling, collection and possession of migratory birds or parts thereof, nests and eggs. Some non-migratory birds, e.g., many gamebirds, require only a state permit for collection and study, unless the study is to be done on federally administered lands. In a few states, both the federal and state collecting permits are combined into one, but the combined permit still requires the approval of both agencies.

State or province permits: Mexico does not currently require state permits, but the U.S. and Canada do. Individual researchers or units should apply to the appropriate division within the state or province departments of Fish and Game or their equivalents. Usually the law enforcement division or the equivalent provides forms and instructions. The local law enforcement official (warden, etc.) from the area where research is proposed will often assist. In all states and provinces, a regular hunting license with appropriate stamps is required if the researcher collects any game species during a regular hunting season.

Foreign permits: Most foreign countries require special permits to conduct research and collect specimens or parts of specimens, and to export them. In addition, many countries require special permits for equipment (airplanes, autos, firearms, etc.) from appropriate offices. If the researcher does not have any contacts in the country of proposed research, then the best place to start is that country's foreign office or embassy. Those who work on birds in Latin American countries should consult Rosenberg and Wiedenfeld (1993). Many countries *require* a foreign counterpart for research in their countries. In some cases, the researcher can collect and do research under their counterpart's permit, provided the name and proposed activities are listed in that permit. The research counterpart can often significantly assist in obtaining any other permits that may be required in her/his country. Foreign research permits often have associated fees. It is a courtesy (and often a requirement) that the authorizing agencies be kept well informed with reports and reprints.

If the researcher intends to collect specimens, firearm and trap permits are also often required. A firearm permit is usually issued by the military and requires special application as well as often a fee. Usually the best starting place is that country's embassy in the United States or Canada. In addition, just being in many foreign countries requires a passport and perhaps a visa or tourist card, and vehicle and boat permits. Proof of citizenship is required to return to the United States, even from Canada or Mexico.

The process of obtaining proper documentation is always time-consuming and sometimes frustrating. However, researchers who remember that they are guests when in a foreign country and who govern their behavior accordingly usually find the system less daunting and the agencies and personnel involved more accommodating.

C. Banding and Salvage Permits

A banding and salvage permit (often called simply Banding Permit) allows the permittee to band birds and salvage, but not collect (kill), specimens. The permit also allows for short-term possession of birds. For the U.S. federal permit, the terms are 24 hours for live birds and six months for salvaged specimens, but it may be considerably shorter for a state permit. These permits are also geographically restricted.

Federal permit: There are three kinds of banding permits, master (personal in Canada), station, and subpermittee. Many U.S. students and most banders in Canada will be subpermittees, working under the direction of the Master Permittee (research director or, often, graduate adviser) or a station permit. Canada provides two kinds of individual permits. One authorizes the use of common cage traps and banding of flightless young; the other authorizes the use of mist nets, auxiliary markers, etc. Such conditions of use are included directly on U.S. permits.

U.S. permits are issued by the BBL, Canadian permits by CWS (Table 11-1 ; Appendix A), A bird-bander must be at least 18 years of age, qualify as an established biologist, and have a good reason to band birds. The purpose of the banding program is to provide various kinds of scientific data on birds. Data on all birds banded in the U.S. and Canada and their recovery information are processed by the combined system of the USGS (BRD) and the CWS. If birds are to be color-marked, radio-tagged, or otherwise manipulated in addition to banding, additional authorization is required. Some states also coordinate radio-telemetry frequencies used on wild birds, so appropriate officials must be notified of activities and frequencies. Some states require additional permission to clear radio-frequencies.

Many researchers wish to obtain blood or feather samples while banding. Such samples can be collected with a modified federal bird-banding and salvage permit, as BBL now has an internal agreement with USFWS Law Enforcement Division to allow authorization of such activities under specific guidelines. The permission requires a special rider attached to the banding permit. Requests for such riders should be directed to BBL.

Field researchers who do not need to collect may wish to use the salvage portion of their permit. Salvaged specimens can provide valuable scientific information and can reduce the need for collecting. Any researcher encountering a source of potentially valuable specimens, but who does not have a collecting or salvage permit authorizing acquisition of such specimens, should attempt to ensure that the

specimens are not lost to science by requesting assistance from state and federal enforcement agencies. Such actions may be especially important when endangered species or mass mortalities (as from weather, disease, or tower-kills) are involved. Ornithology departments in many public museums are legitimate depositories for specimens and may also be able to provide assistance.

State or provincial permits: State and provincial requirements for banding and salvage permission vary. A state may require a banding permit, or require one only if the investigator does not possess a state collecting permit, and may or may not separate permission to salvage into a separate permit.

D. Endangered Species Permits

Any activity with an federally-listed endangered species requires a permit issued by the USFWS regional office (which holds a master Endangered Species Permit itself), [see F] Many states and provinces have their own endangered species lists which may include species not listed on the federal list. Researchers should determine the status of the species at the federal and state or provincial level to determine which permits are needed.

E. Import/export permits

Moving biological specimens, especially living specimens, across an international border is a complex procedure, in part because the laws of more than one nation will be involved, and in part because some pertinent laws may have little to do with research or conservation. Other considerations include whether or not the specimen was originally wild or was captive bred, whether the species is covered by the MBTA, whether the species is endangered, threatened, and/or CITES listed, or whether the species is subject to special health or agricultural concerns. Because most museums and zoos routinely move specimens across borders, they are usually familiar with the procedures. Individual researchers are encouraged to work through such institutions whenever possible. Even so, *it is best to begin by consulting with USFWS or CWS at their national headquarters* (Appendix A).

We assume that most individual researchers collecting specimens in foreign countries will be doing so in connection with some established, institutional collection. Many institutions hold APHIS permits and Certificates of Scientific Exchange under CITES, and/or multiple endangered/threatened species permits that can cover the activities of individual researchers. Although an institutional connection will probably ease the acquisition of appropriate documentation, it nevertheless remains the individual researcher's responsibility to be sure he/she is familiar with the requirements and has all the appropriate permits. Much of what follows is directed toward the individual researcher who may wish to import either individual specimens or parts thereof (including tissue samples), and whose activities are not covered by institutional permits.

Any imports of birds, their parts (including blood and tissue samples), eggs, or nests require specific statements to the effect that the specimens were collected under valid permits in the place from which they are being imported. If the specimen is an endangered species, it will likely require a CITES permit. In addition, in the U.S. an APHIS import permit is required (Appendix A). If the species imported is listed in the Migratory Bird Treaty Act, a collection permit to cover importation and possession will be required. CITES permits are required for the export or import of specimens, tissues, or products made by species listed in the Convention, which are not necessarily the same as those listed in the SEA. If one of the countries is not signatory to the Convention, an "in lieu of" document, which contains the same information as a CITES permit, must be attached to the shipment. As the U.S., Canada, and Mexico are all signatories, permits would be required by any researcher in those countries, regardless of whether the other country is signatory. It should be noted that the Convention contains certain exemptions for educational and scientific institutions that routinely engage in the exchange of specimens across borders.

F. Refuge Permits

Any research involving wild birds on a National Wildlife Refuge or other public lands requires a permit that outlines the proposed activities and the areas where the research is to be conducted. These permits are issued by the manager of the particular refuge.

G. Miscellaneous permits and agreements

Some states require an additional permit issued to an individual or unit for other activities such as trapping, handling, sampling, marking, dying, injecting radioactive tracers, etc. The use of radioactive tracers also requires special permits. Some states combine all these permits (including collecting) into one permit (a practice we endorse).

Written permission is usually required to conduct special activities such as those listed above, as is research on Endangered Species or wild birds otherwise protected in some other way (such as eagles protected under the Bald Eagle Protection Act, 16 U.S.C. 668). Most states have their own lists of restricted species. The federal endangered species list (Endangered and Threatened Wildlife and Plants, 50 CFR 17.11 and 17.12) is usually used as a basic guideline. Local regulations protecting migratory or endangered bird may be more, but not less, restrictive. In many states, work on endangered or specially-protected species is usually additionally permitted by a "memorandum of understanding" between the state agency and the individual or research unit.

H. IACUC Protocols

Research involving birds may require institutional approval in the form of an IACUC protocol. Exceptions to this rule include some research on poultry and such nonmanipulative activities as simple observation, [see *I.D.*] We recommend that such approval be sought for any experiment that requires manipulation of individuals or populations, even if the investigator's institution does not formally require such. Most granting agencies require an IACUC approved protocol as part of the application for funds, and the practice speaks for the investigator's concern for ethical behavior.

I. Records

One of the earliest lessons learned, often the hard way, by every investigator is that you cannot take too many notes or make too detailed records. This maxim is especially true for research requiring permits. Permitted field research always requires regular reports of activities. These reports often require special forms or formats. Further, records involving the permitted activity must be available for inspection at any time. Hence, it is well worth the effort to maintain absolutely current records in the appropriate format.

III. INVESTIGATOR IMPACT

A. Overview

Ornithologists have an obligation to assess their research for potential negative effects on their study populations as well as on the environment in general, and to minimize such effects. Although research may further scientific knowledge, investigators should weigh any potential gain in knowledge against the consequences of disruption (ABS 1986). In assessing the consequences of disruption, however, it should be borne in mind that, although short-term adverse effects may result from research activities, populations usually recover rapidly, and research often yields long-term positive effects for the affected populations.

B. Considerations

Two important aspects of observer-caused disturbance can be recognized. First, disturbances may create biases that affect both the gathering and analysis of data. Second, research activities might have effects on the status and well-being of the study subjects themselves. Both effects vary along a continuum

from obvious to subtle (e.g., MacArthur et al. 1982; Jordan and Burghardt 1986). Although slightly dated and dealing primarily with raptors, Fyfe and Olendorff's (1976) review of this entire topic is well worth the effort to find and read carefully.

C. Investigator Disturbances

Many effects of field investigations are similar to the more overt effects caused by nonscientific human activities such as tourism, general recreational activity, etc., but they almost always occur to a much lesser degree and are usually very local. Although scientific activities may occasionally have severe effects (see reviews by Duffy 1979; Anderson and Keith 1980; and Fetterolf 1983), in other instances, detrimental effects are negligible (Willis 1973). The variation may depend on local conditions, including structure of the habitat (Brown and Morris 1995), or precise point in the breeding cycle (Fyfe and Olendorff 1976; Griere and Fyfe 1987). In field ornithology, adverse effects are most commonly associated with nest visits, aircraft surveillance, working in or passing through sensitive areas, approaching birds closely, and manipulating them. [see IV, V] Once again, responses to any activity vary from species to species, and what may be anathema for one is inconsequential for another. Therefore, neither blanket rules on the part of regulators nor universal research protocols on the part of investigators are appropriate.

Nest Visits: The potential detrimental effects of visits to nests long have been known (e.g., Evans and Wolfe 1967). Problems from nest visitation have resulted in potentially biased data and decreased reproductive success in both terrestrial birds (e.g., Willis 1973; Mayfield 1975; Howe 1979; Lenington 1979; Westmoreland and Best 1985) and aquatic birds (e.g., Hunt 1972; Gillett et al. 1975; Robert and Ralph 1975; Kury and Gochfeld 1975; Fetterolf and Blokpoel 1983; Rodway et al. 1996; see also reviews by Manuwal 1978; Anderson and Keith 1980; Burger 1981a,b; Hockey and Hallinan 1981). Again, however, there are cases in which nest visitation produced no evident adverse effects (Schreiber 1994, 1996, Skagen et al., 1999). Low or ground-level nests should be approached tangentially, with a 3 - 4 meter detour to the nest. The investigator should return along the detour to the tangential path and continue in the same direction. Ground predators can be discouraged by spreading naphthalene crystals along the detour segment. If flagging is used to mark nest sites, care should be taken that the flags do not impede the owner's access to the nest nor draw the attention of predators.

Aircraft Overflights: Low-flying aircraft may be used in censusing birds. Although such flights have the potential for disruption of bird activities, especially in colonial and open-nest species, Dunnet (1977) showed that regular movements of fixed- and rotary-wing aircraft in non-research activities had no observable effect on cliff-nesting seabirds, and Kushlan (1979) observed only minimal effects from carefully conducted helicopter censusing of wading bird colonies. Burger (1981 a) showed that *Larus argentatus* respond differently to various aircraft-related stimuli and that they seem to be more sensitive away from breeding colonies than at the colonies themselves. On the other end of the disturbance continuum, *Pelecanus erythrorhynchos* were seriously affected by low-flying aircraft, indicating that their population status could be affected by chronic disturbance (Bunnell et al. 1981).

Kushlan (1979) recommended the following procedures for aircraft overflights: gradual approach by first circling the study subjects at a distance, flying around the periphery of the sensitive area rather than directly over it, slow and quiet flight, and continual attention for signs of disturbance.

Approach and nearness to sensitive areas: Damage may also occur to species not under study. Hockey and Hallinan (1981) found that both near-approach and passage by people had detrimental effects in penguin colonies. Burger and Gochfeld (1981) demonstrated that *Larus argentatus* and *L. marinus* can discriminate between direct and tangential approaches by investigators, and that these birds more readily abandon nests when investigators looked directly at them. This suggests that investigator attitude and presentation may have an effect in creating or minimizing disturbances.

Researchers should be aware that their activities, if observed, may draw the attention of curious persons. Unfortunately, considerable disturbance may result from altogether innocent attempts of the general public to determine what a researcher is doing. Tourists and photographers may present special

problems. When observation by the public is likely, researchers should consider diplomatic means to discourage invasion of the research area.

D. Suggestions for Field Researchers

Investigators should monitor their studies for adverse effects of disturbance. Wherever possible, action should be taken to alleviate or minimize detrimental activities. Research activities should be consistent with the gathering of adequate samples for valid research results, yet be balanced to minimize adverse effects.

Mineau and Weseloh (1981) outlined a general system of nest-checking for colonial birds that minimizes investigator disturbance while maximizing data yield. Safina and Burger (1983) recommended minimizing visits by use of telescopic observation to look into a colony or sensitive area rather than entering it. Such methods may include the use of powerful lenses, other remote-sensing devices, and, if necessary, blinds that provide a nondisturbance entrance (see Shugart et al. 1981). Other researchers suggest visits timed (within and between days), for example, to minimize loss of regurgitated food by young birds, to avoid disturbance of nests during their most sensitive phenological stages (such as egg laying), and to avoid actions that might cause a chick to become separated from its parents (Parsons and Burger 1982).

Interspecific differences in response to disturbance require that field investigators be familiar with their study species (although personal experience is desirable, familiarity with the literature and consultation with others may suffice in the case of new investigators) such that they can reasonably predict reactions to certain field activities. Because some habituation to investigator disturbance is possible (see Parsons and Burger 1982), consistency in timing and intensity of visits may alleviate some problems. Selection of a study population already habituated to human activity sometimes may eliminate unwanted side effects of scientific research (Burger and Gochfeld 1981).

Finally, investigators should monitor the effects of their activities on a continual basis. Those effects may change with time, perhaps in response to conditions other than the investigator's activity *per se*.

E. Publication

Investigators may be hesitant to publish evidence of investigator induced, negative effects in their own studies. Yet it is essential, both practically and ethically, that the failure of a procedure be made known. Otherwise remedial action becomes more difficult, and other investigators may encounter the same problem. Equally, it is essential that editors be willing to publish such negative results.

F. Conclusion

Elimination of long-term damage to populations or the environment requires a reliance on the ethics of the investigator. Thus, all ornithologists are expected to observe a conservation ethic and to pass on such an ethic to those with whom they work (especially students). Since the ultimate goal of all research is to understand better the natural world, there can be no justification for taking part in long-term destruction of that which we seek to understand. The study of investigator impact on wild birds is, itself, a field that well deserves additional research attention.

IV. COLLECTING AND TRAPPING

A. Why Ornithologists Collect Specimens

Ornithological research often involves the judicious collecting of birds in the field. Information obtained from these specimens enables accurate identification of species and understanding of evolutionary

relationships, genetics of wild populations, population structure and dynamics, comparative anatomy and physiology, adaptation, behavior, parasites and diseases, economic importance, geographic and microhabitat distributions, and ecology of birds in natural or disturbed habitats (AOU 1975). Knowledge from ornithology promotes knowledge in other biological sciences and affects policies concerning game and non-game species, endangered species, economically important species, habitat conservation, ecosystem analysis, pest and disease control, predator control, and domestication.

Most bird specimens removed from the field are deposited in the collections of natural history museums or biological data banks for future studies. Museum collections are managed repositories for whole specimens and their parts, whereas biological banks are collections of histologically or cryobionically preserved organs, tissues (including live cultures), cells (including sperm and ova), or embryos. Both kinds of repositories permit qualified researchers to study their collections. The great value of these collections is demonstrated by the substantial funding that has been provided by federal, state, and private agencies for their care, as well as by the large scientific literature based on collected specimens. Voucher specimens should be retained at the conclusion of field investigations and deposited in collections that meet the minimal standards of maintenance established by the AOU and that have active loan programs, so that the specimens will be available for use by future investigators (Remsen 1991, 1995; Winker et al. 1996).

B. What is an Adequate Sample?

"Regulating agencies are often overly enthusiastic in restricting scientific collecting, which is the only kind of mortality that is so highly controlled and yet from which bird species might derive benefits, whereas the same or sister agencies often permit or even encourage activities that are responsible for massive mortality in bird populations. Given that (1) the goal of scientists, conservation agencies, and governments is protection of populations, not individual birds; (2) scientific collecting has no measurable impact on the vast majority of bird populations; (3) scientific specimens represent an important source of information on bird biology and conservation; and (4) existing scientific collections are largely inadequate for answering many questions that could be answered by greater numerical, seasonal, and geographic representation, then it follows that continued scientific collecting will benefit ornithology and conservation and should, therefore, be encouraged by conservation and government agencies." J. V. Remsen, Jr. (1995).

An adequate sample is the minimal number of specimens or other data necessary to ensure investigative and statistical validity. The sample size required for a study depends on the nature of the investigation and the extent of variation in the parameters being studied. Field studies require larger samples than do laboratory studies, because field investigators have less control over the conditions that produce variation, and many studies requiring specimens are studies of variation *per se*, and thus require large sample sizes. Computer modeling can help define and sometimes reduce sample size requirements.

The collection of scientific specimens typically has no lasting effect on avian populations. Few ornithologists collect more than 100 individuals in a given year, and the total number of specimens collected annually for research purposes in North America is fewer than 10,000. In contrast, an estimated 140 million birds are killed by collisions with vehicles and picture windows, and over 100 million are harvested by hunters (Banks 1979). Domestic cats kill many millions of birds per year (George, 1974; Mitchell, 1992; Jurek, 1994).

C. Methods for Collecting Specimens

Humane scientific methods of trapping and shooting are those that kill the bird instantly but avoid injury to the body parts required for the investigation. Shooting is the most effective way to collect many species. Ornithologists who collect birds with a shotgun should be experienced in the proper and safe use of

firearms and must comply with laws and regulations governing their use. Humane use of firearms necessitates that birds be killed outright, so that the firearm and ammunition load should be appropriate for the species to be collected. Every effort should be made to avoid wounding birds, not only to minimize suffering, but also to maximize the probability of retrieving rather than losing the specimen. Wounded birds should be killed promptly, [see IX.G] The frequently used thoracic (cardiopulmonary) compression is not among the techniques recommended by the AMVA, but may be used when other techniques might damage portions of the carcass critical to the study. This technique is not appropriate for use in the laboratory, for large birds, or for diving birds. If, however, the bird has been rendered unconscious by administration of an anesthetic [see IX.D], then the mechanics by which the euthanasia is accomplished are not important. Prior administration of an anesthetic may be useful when euthanizing large birds under any conditions. Ideally, the collector would carry a supply of a euthanasia compound, but that is not practical (and may be illegal) in many cases. Methods of euthanasia are reviewed by the AVMA Panel on Euthanasia (Andrews et al. 1993), but may not be practical under most field conditions, [see IX.G]

Mist nets are used increasingly to collect specimens in dense vegetation where shooting is less effective, when shot holes will damage the specimen for study, and where use of firearms is prohibited. Mist nets must be checked frequently; therefore, the number of nets set up should reflect the manpower available to check them. Nets set for diurnal species should be closed at dusk to avoid accidental capture of nocturnal species and vice versa. Birds are sensitive to exposure to heat, cold, thirst, or hunger, and consequently should not be left in nets longer than necessary. If the substrate below a net becomes heated by insolation, temperatures lethal to small birds within very short time spans may be achieved. Similarly, extreme cold poses special problems, especially for small species. Nets should be shaded or positioned to avoid full exposure to the sun, and, where possible, trapping or netting should be avoided if the ambient temperature is below 0 EC or above 35 EC, or in windy, or rainy weather. Nets and traps should be watched or checked at least every 20 minutes during the nesting season or when the weather is unfavorable (intense sun or inclement weather), and about every 30 minutes (at least once per hour) during the rest of the year. Captured birds can injure themselves trying to escape, even from metal walk-in traps; these injuries reduce the specimen's scientific value and cause needless trauma. These same humane methods apply even more strongly to the trapping and netting of birds for purposes of marking and release.

Living animals are sometimes used as decoys to attract birds to traps or nets. Pigeons, starlings, or other non-protected species may be used to lure raptors. Conversely, raptors may be used to lure mobbing species. Such practices are invaluable for attracting difficult to capture birds (Bloom 1986). However, those using such procedures are ethically and legally responsible for the well-being of all the animals involved, not just the target species. Although decoys in such situations are clearly subject to stress or even death, everything possible should be done to reduce the stress of the lure species to the minimal level intrinsic to the technique. For example, food and water should be provided to lures in a dho-gaza trap, but cannot be provided to those in a road-drop bal-chatri. [see VIII.H]

D. Habitat and Population Considerations

Each investigator should observe and pass on to students and coworkers a strict ethic of habitat conservation. Because many essential details of life history may remain unknown until a study is well along, collecting should always be conducted so as to leave habitat as undisturbed as possible. Permanent removal of large numbers of animals from any breeding or roosting aggregation should be avoided unless justified in writing for scientific reasons by the investigator. Similarly, relatively large collections of gravid nesting females from any population for destructive sampling should be avoided unless justified for scientific reasons. Systematists should search for suitable specimens in extant collections before conducting field work. If the purpose of an experiment is to alter behavior, reproductive potential, or survivability, the interference should be no more than that necessary to test hypotheses

accurately. Investigators working with populations that are experiencing a short-term die-off or long-term decline must be especially sensitive to the effects of collecting.

V. MARKING PROCEDURES

A. Overview

All laboratory and much field research involving wild birds requires that individuals be marked in some way for future identification. It is essential not only to the welfare of the birds but also to the integrity of the research results that the marking procedure not adversely affect the behavior, physiology, or survival of individuals. Because of the difficulty in providing appropriate controls for the marking method, systematic studies of possible adverse effects of marking procedures are still few, and many of the cautions mentioned here are based on anecdotal observations. In general, investigators should not assume that marking procedures will have no adverse effects on their subjects and should make efforts to evaluate any such influences. Rather, it should be assumed that, if banding is done carefully with appropriately sized bands, harm to target birds will be minimized. Investigators should not allow unsupervised, inexperienced persons to handle birds until the persons have been properly trained to retrieve birds from nets or traps and to hold, handle, and release birds properly. Ultimately, this approach will yield benefits not only to the animal subjects but also to research quality. General references include (Marion and Shamis 1977; Stonehouse 1978; Schemnitz 1980, which includes a useful bibliography of bird-marking techniques; Calvo and Furness 1992). Techniques commonly used for raptors, but also useful for other species, are reviewed by Young and Kochert (1987).

For a marking procedure to be effective, it should meet as many of the following criteria as possible (Marion and Shamis 1977).

- a. The bird should experience no immediate or long-term hindrance or irritation.
- b. The marking should be quick and easy to apply.
- c. The marking code (digits or colors) should be readily visible and distinguishable.
- d. The markings should persist on the bird until research objectives have been fulfilled.
- e. The bird should suffer no adverse effects on its behavior, longevity, or social life
- f. Careful records should be made of all aspects of the marking procedure. These should be maintained in an accessible and safe place.

In special cases it may be possible to identify individuals on the basis of unique markings or vocalizations (see Pennycuick, 1978; Gilbert, et al. 1994) without the necessity of handling or attaching markers to them.

B. Metal Bands

Banding has been called the greatest advance in the study of birds in this century (Coulson 1993). Numbered metal (usually aluminum, but various alloys for special purposes) bands issued by the Bird Banding Laboratory (BBL) of the USGS or by CWS provide the most widely used method of individually marking birds. Animals must usually be recaptured in order for the band numbers to be read. It is imperative that bands of the correct size be used; bands too small for the species in question may cause serious injury to or even loss of the banded leg. Recommended band sizes for all species of North American birds can be found in the Bird Banding Manual (USFWS 1991) and in periodic memoranda to banders (MTAB) issued by BBL. Where appropriate band sizes are used, there are few indications that the application of metal bands produces adverse effects on the subjects (references cited in Marion and Shamis 1977). Species-specific problems are discussed in Marion and Shamis (1977), Henckel (1976), Salzert and Schelshorn (1979), Reed and Oring (1993), Gratto-Trevor (1994), and MTAB from BBL. Banders must know their subject well in order to minimize the likelihood of injury. Injuries may result from a bander's failure to anticipate future growth of young birds, to adequately consider size dimorphism when

choosing band size or the risk of a large band slipping over the foot, or to recognize that determination of how many bands can be safely fitted on one leg is a species-specific issue. Two or more aluminum bands should not be applied to the same leg as they may flange over and injure the leg.

C. Colored Plastic and Celluloid Leg Bands

One or more colored leg bands are often applied to one or both legs of a bird. They provide a means of individually recognizing birds in the field without recapturing them. They are being used increasingly in studies of behavior and ecology, often involving large numbers of individuals. The use of color bands requires special permission from BBL. When used in combination with aluminum bands, plastic bands must be of the same size. When bands of the proper size are used, few adverse effects of color bands have been reported (cf. Nisbet 1991). However, some studies have shown that certain band colors, especially those that are similar to plumage or soft part colors involved in social signals, may affect mating attractiveness, dominance status, or aggression (e.g., Burley 1981, 1985, 1986a,b; Burley, et al. 1982; Johnson et al. 1993; Holder and Montgomerie 1993) in some species. Depending upon the duration of the study, it may be important to consider that some colors of commercially available celluloid bands fade. After two years or so they may be unrecognizable (Anderson 1980; Hill 1992; Lindsey et al. 1995). UV-stable bands are available from several suppliers. Most colors of UV-stable plastic remain bright for several years unless covered with an obscuring substance such as dirt or algae. Blue bands fade relatively quickly. In recent years, students of long distance migrants, especially shorebirds, have employed plastic flags with unique colors representing different countries, e.g., green for U.S.A., and different positions being used to represent points of origin. The flags are larger and more conspicuous than bands, thus allowing sightings over longer distances. Given a philosophy that the manipulation of wild animals should be the minimum necessary for the study, flags should be used only when they provide a substantial advantage over normal color bands. The use of flags on newly hatched chicks is discouraged.

An inexpensive alternative to commercial color bands is described by Hill (1992). A technique for color banding nestling passerines is given by Harper and Neill (1990).

The use of constant numbers and relative positions of bands can help verify the correctness of reports of marked birds.

D. Dyes and Ultraviolet Markers

Dyes applied to the plumage are used extensively on birds, especially colonial water-birds and waders. Water-proof, felt-tip markers are useful for short-term markers, as are tattoo inks, wax cattle-marking sticks, and non-lead paint. Picric Acid, Rhodamine B and Malachite Green are among many frequently used dyes. Picric acid (picronic acid; trinitrophenol; nitroxanthic acid; carbazotic acid; phenoltrinitrate) can be a significant explosion hazard. During extended storage, it may lose water and become unstable. Never open nor touch a bottle of dry or contaminated picric acid; an explosion could result from the friction produced. Crystallized picric acid is a severe explosion risk, is especially reactive with metals or metallic salts, and is also toxic by skin absorption and inhalation. For all these reasons, the use of picric acid is **strongly discouraged**. Methods of dye use are discussed in Kennard (1961), Taber and Cowan (1969) and Day et al. (1980). Recommendations for fixatives to improve retention of the dyes on feathers can be found in Belant and Seamans (1993). Caution must be exercised in applying the dye, especially when contour feathers are extensively colored. The alcohol or detergent base may remove oil from the bird's feathers, and wetting can lead to heat loss. Care should be taken to ensure that dyed birds are thoroughly dry prior to release. A method for color-marking incubating birds by applying dye to their eggs (Paton and Pank 1986; Cavanagh et al. 1992) can result in high rates of egg mortality and should be used only with appropriate cautions (Belant and Seamans 1993). Dyed birds are sometimes treated differently by conspecifics, and may be subject to greater risk from predators (Frankel and Baskett 1963). Investigators should make systematic attempts to evaluate such possible effects because they may influence not only

the welfare of the subjects but the research results as well. Paint of any kind should be used only sparingly on feathers because of its impact on feather structure and function.

Aerial and ground spraying techniques, developed to mass-mark birds in roosting or nesting colonies, employ various colors of fluorescent particles (suspended in a liquid adhesive) that are sprayed from agricultural spray systems (Jaeger et al. 1986; Otis et al. 1986). The marker is visible under long-wave UV light when a bird is examined in hand and is retained for several months or until molt. No adverse effects have been noted, and behavioral changes are not likely because the marker is not visible in daylight. As with any spray application, the nature of the habitat and the composition of the spray formulation should be examined for potential environmental concerns. Fluorescent dyes are also useful for locating and tracking cryptically colored birds (Steketee and Robinson 1996). Applications of mass-marking agents should be coordinated with BBL.

E. Neck Collars

Plastic neck bands or collars have been used extensively for marking waterfowl. Aldrich and Steenis (1955) concluded that properly applied neck bands are effective markers with few adverse effects on geese. In general, neck collars seem to be superior to nasal discs for tagging waterfowl (Sherwood 1966; Raveling 1976: but cf. Helm 1955; Lensink 1968; Ankney 1975; Hawkins and Simpson 1985; Abraham et al. 1983; Macinnes and Dunn 1988; Ely 1990). As with all marking techniques, responses differ among species, and investigators should systematically evaluate any possible influences of the marker.

F. Nasal Discs and Saddles

These are numbered and/or colored plastic discs or plates applied to each side of the bird's bill, fastened together through the nasal opening by various methods (Bartonek and Dane 1964; Sugden and Poston 1968; Doty and Greenwood 1974; Alison 1975). They have been applied primarily to waterfowl. Various undesirable results have been reported, including high rates of marker loss, often with injury to the nares (Sherwood 1966), higher mortality rates attributed to entanglement with submerged vegetation (Sugden and Poston 1968), mortality due to ice accumulation (Byers 1987), and reduced success in obtaining mates (Koob 1981). The data suggest that nasal discs are better suited for larger species of birds that do not dive. Caution is advised in their use, and where practical other marking methods should be substituted.

G. Patagial (Wing) Markers and Leg Tags

Wing tags have been applied to many species of birds. The main advantages are that the tags are highly visible, may be coded for individual recognition, and are retained by birds for relatively long periods of time. They are often useful to the success of studies of social behavior, migration, and natal and winter site fidelity. Descriptions of tag types and evaluations of their effectiveness may be found in Hester (1963), Anderson (1968), Hewitt and Austin-Smith (1966), Southern (1971), Stiehl (1983), Curtis et al. (1983), Sweeney et al. (1985), and Cummings (1987). Some reports indicate that most birds accept patagial tags readily, and adverse effects seem to be minimal (e.g., Maddock and Geering 1994). On the other hand, Kinkel (1989) reported that the survivorship and reproductive behavior and abilities of ring-billed gulls were adversely affected for up to four years after tagging. The effects disappeared when the tags were replaced with colored bands. Tags sometimes result in some callousing of the wing, and feathers in the area of the tag may not be replaced at the time of molt. Investigators should be alert to the possibility of negative behavioral effects (H. Blokpoel, *pers. comm.*, W.H. Drury, *pers. comm.*) and increased predation (D. Lank, *pers. comm.*).

A Velcro™ leg tag developed for marking gull chicks (Wiltsteed and Fetterolf 1986) may not be suitable for all species because of differences in growth rates that require frequent adjustment of the tag (Cavanagh and Griffin 1993).

H. Radio Transmitters

During the past three decades, the attachment of small radio transmitters to free-living birds has become a routine means of enabling investigators to monitor the location and movements of tagged individuals. Transmitters are applied most frequently to large (> 100 g) species, but the development of units weighing 2 g or less has made their application feasible even for small (< 50 g) birds. It is axiomatic that the application of an additional mass to a bird's body will have some effect on its energetics and aerodynamics. That effect presumably increases in proportion to the percent body mass of the transmitter and will be influenced by its placement on the bird's body. The effects are evident even in such medium-sized, strong flyers as homing pigeons (Gessaman and Nagy 1988). For large birds, for which transmitters often amount to less than 1% of body mass, these effects are negligible. With small birds, transmitters are often 5-10% of lean body mass. Transmitters weighing more than 10% of body mass should not be applied to birds released into the field, and where possible transmitters should not exceed 5% (Caccamise and Hedin 1985). As many birds, including some small passerines, accumulate migratory fat deposits that may equal up to 50% of body mass, for specific, short-term (i.e., the bird will be recovered within a short time and the excess weight removed) purposes this weight guideline could be relaxed. The use of such excess weight requires specific justification. Application of radio transmitters can have significant adverse effects on survival, reproductive success, energetics and behavior (Boag 1972; Ramakka 1972; Boag et al. 1973; Greenwood and Sargeant 1973; Gilmer et al. 1974; McCrary 1981; Nesbitt et al. 1982; Morris et al. 1981;

Hooge 1991 and many references cited therein; Foster et al. 1992; Gammonley and Kelley 1994; Ward & Flint 1995; Robb, 1997). Therefore, the use of transmitters should be undertaken with caution and is most appropriate for studies in which the data can be obtained in no other manner or in which data acquisition will be significantly improved. Excellent discussions of methods used in radio tracking studies can be found in Amianer and Macdonald (1980), Cochran (1980), and Kenward (1987).

A wide variety of attachment methods is currently in use, including body harnesses, attachment to the skin of the back with adhesives and/or sutures, neck collars, attachment to rectrices, attachment to the leg, and abdominal or subcutaneous implants. Assuming that transmitters of appropriate size are used, most negative effects of radio transmitters on birds result from the attachment method. Robb (1997) found that mallards fitted with transmitters on Dwyer harnesses survived longer, but behaved differently from other members of the population, including those with collar-mounted transmitters. Investigators desiring to employ telemetry should consult the sources relevant to their species for methods of affixing transmitters. In general, observation of individuals in captivity should be employed in evaluating the efficacy of attachment methods. Many species react negatively to harnesses that in some way go around the wings. Wherever possible, alternative methods of attachment should be employed (e.g., tail-mounting on larger species, suturing and subcutaneous anchors for large species, gluing and alternative harness designs for smaller shorebirds and passerines). Mounting radio transmitters on neck collars has been found to be inappropriate for at least one waterfowl species (Sorenson 1989). Special attention must be given to attachment in species that live in dense vegetation and those that roost or nest in cavities. Consideration should be given to the risk of birds being injured if transmitters become entangled in vegetation (Karl and Clout 1987). The removal of transmitters at the end of the experiment is not mandated, and indeed may not be possible, but investigators should consider whether an attachment that will "self-remove" at the end of the useful life of the transmitter will fit the experimental design. "Self-removal" should be rapid, as a loosely attached, flapping transmitter can be both an impediment to locomotion and an attractant for predators.

I. Electronic Tags

Small chips, each containing an individual digital code, have been developed for injection under the skin (Bio Medic Data Systems, Maywood, NJ). The code can be detected by a scanner that is passed over the animal's body. Although developed for laboratory animals, these chips have been used on wild storks

(Michard et al. 1995) and on burrowing petrels (Mauck et al. 1995). The chips and scanners are expensive, but they offer the enormous advantage that, if the tagged animal can be induced to enter the field of a scanner (e.g., at feeding or nesting sites), various data can be automatically recorded and assigned to specific individuals, thus completely eliminating the need for additional handling. Gluing the chips to feathers permits their recovery at the completion of the experiment, thereby decreasing cost (Mauck et al. 1995).

J. Responsibility of Bander

The primary responsibility for ethical marking lies with the master permit holder under whose auspices the marking takes place. Similarly, the primary ownership of resighting data lies with the master permit holder. At the same time, the master permit holder has a responsibility to inform persons reporting resightings of the nature of the research involved and the status of the birds whose location was reported, should such information be requested.

VI. TRANSPORT OF WILD BIRDS

A. Overview

It is frequently necessary to transport birds, whether as part of an experimental protocol (e.g., in a study of homing behavior) or to move research birds from capture sites to the laboratory or other holding facilities. Transport of warm-blooded animals is covered by provisions of AWA [see *I.B.*]. Regulatory authority under AWA is vested in US DA and implemented by APHIS. Rules pertaining to the transport of birds can be found in the Code of Federal Regulations Title 9, Subchapter A, Part 3, Subpart F, and in 50 CFR Ch. 1, Part 14. These regulations contain very specific requirements (e.g., the total surface area of ventilation openings in transport containers). Anyone transporting wild birds should be thoroughly familiar with these regulations, [see *II. C*] USDA has an Internet site containing current information, and a Voice Response Service (1-800-345-USDA) concerning regulations by state. Information is also available on disc from the Animal Welfare Information Center (301-504-6212; National Agricultural Library, 10301 Baltimore Bldg., Beltsville, MD 20705) and on the Washington University, St. Louis Web site (Appendix A).

The shipper is responsible for providing an appropriate shipping container and a health certificate signed by a USDA veterinarian. Shipping containers and packing of birds varies widely from species to species. The International Air Transport Association publishes species-specific regulations (Live Animals Regulations, 22nd Edition, effective 1 October 1995: may be obtained from IATA, 2000 Peel St., Montreal, Quebec H3A 2R4; 1-800-716-6326) for containers used in air shipment of birds. Similar containers would be appropriate for ground transport as well. Structural strength of the container must be sufficient to contain the birds and withstand the normal rigors of transport. The interior must be free of any protrusions that could cause injury. Openings should be easily accessible at all times for emergency removal of animals.

Ideally, birds should be individually isolated in separate cells within the shipping container. Individuals of the same species may be transported in the same primary enclosure, but it must be determined that frequent fighting will not occur. Each individual should have sufficient space to assume normal postures and engage in comfort and maintenance activities unimpeded by other birds. Tops of containers should be padded when excitable birds or species with delicate bone structures are to be shipped, and padding underfoot is important for species with soft feet. Space sufficient to permit flight usually is not advisable because chances of injury are increased. In addition, it may be necessary to restrain the wings of larger species, but this must be done in a manner to avoid overheating. Adequate ventilation must be provided; the inside of containers should be as dark as possible but still allow birds to find water or food and to move about. For longer journeys, water should be provided in the form of moist sponges, or apple, orange, or cucumber slices. Depending upon the species involved and the duration of travel, food may be scattered within the container. This practice should be avoided if it is likely to foul plumage or permit the growth of

fungi, [see VII.F,G] When transporting chicks, a protected heat source (i.e., one that can't burn the chicks) and room to move away from it should be provided.

Raptors should be shipped in closed, darkened boxes (not commercially available large pet carriers) with plenty of ventilation. The bottom and, if possible, other inner surfaces should be lined with carpet or other padding. A perch is not necessary. If the bird is provided food and water just before departure and immediately on arrival, journeys of 36 hours can be made without provisioning.

In general, animals should be shipped as soon as *possible* after capture. Proper arrangements should be made to ensure that birds arrive at destinations during normal working hours rather than on weekends or holidays. Coordinating date and time of capture to facilitate the timing of both shipping and arrival may be necessary.

B. Air Transport

Wild birds may be shipped by commercial airlines. Regulations are complex and vary from carrier to carrier. Some airlines will allow birds as carry-on baggage, whereas other airlines will not ship wild birds at all. Local agents are often unfamiliar with the necessary procedures. Thus, air shipment by common carrier requires careful advance arrangements. IATA Live Animals Regulations contain all the necessary information for both domestic and international air shipment and should be consulted by anyone contemplating shipping birds by air. IATA regulations have been formally adopted by the U.S. and Canada (along with many other countries) and by CITES and the Office International des Epizooties. The manual contains chapters outlining specific governmental regulations by country, requirements of specific airlines, necessary documents and forms, lists of species protected under CITES, container requirements for all types of birds (adopted by USFWS), and detailed, illustrated descriptions of the containers and their construction, and much more. State permits are required to possess birds both in the state in which birds originate and the state to which they are to be transported, [see II.B]

C. Ground Transport

In motor vehicles, containers of birds should be placed in a well-ventilated area, protected from direct sunlight, and visually isolated from passengers and views through windows. Long trips should be broken up by rest periods during which the birds may feed and drink uninterrupted. If possible, transport diurnally active species at night when they can be kept in darkness, will be inactive, and when ambient temperatures may be more favorable. Bocetti (1994) describes techniques for confining and transporting small insectivorous passerines and for evaluating their condition by periodic examination of feces.

Interstate ground transport requires federal and state authorization for the state of origin, destination, and states traversed in passage as well as an animal health certificate. Information concerning regulations is available by telephone or World Wide Web. [see A]

D. Short-term Captivity

Guidelines for holding wild birds for short periods of time (less than 30 days) will be similar to those outlined under Housing and Captive Breeding [see VII]. Inspections of the shipment should be made upon arrival. Injured or visibly sick birds should be isolated and treated immediately, and dead birds should be necropsied and a veterinarian consulted. Birds should be marked for individual recognition and examined carefully for external parasites or signs of illness. Procedures for initial examination are outlined in Laboratory Animal Management: Wild Birds (National Research Council 1977) and texts on avian medicine. [see IX, A]

Recently captured birds may experience difficulty in adjusting to conditions of captivity. The potential problems will be highly species-specific, and investigators will have to rely on good judgment and the experience of those who have handled the taxa in question. Frequent and careful observation of birds during the adjustment period is necessary to ensure acclimation. When birds are individually housed in small box cages, covering the cage with cloth or newspaper often reduces thrashing and escape attempts that can result in injury. Because hard-sided cages increase the risk of injury from wall-strikes, consideration should be given to the use of soft-sided (net) cages where these are practical. However, net cages may pose cleaning problems. Consideration should be given to the mesh size or distance between bars of small cages. Many species of small birds injure themselves by repeatedly poking their beaks and

heads through cage mesh in attempts to escape. Food and water should be conspicuous and widely scattered to facilitate their discovery by the birds. Birds introduced into social situations should be watched carefully for adverse effects of aggression. Great intraspecific variability is to be expected. Aggression can often be minimized by providing food, water, and hiding places at several locations in the aviary.

VII. HOUSING AND CAPTIVE BREEDING

A. Overview

Maintaining live birds in captivity is expensive, time consuming, and requires special expertise. That expertise can often be found outside the community of academic or governmental ornithologists. Zoos are an obvious source of help and information. Private aviculturists are often eager to share their expertise. Investigators wishing to establish long-term colonies of captive birds are urged to contact their local avicultural societies. The American Federation of Aviculture's monthly magazine, *The Watchbird*, contains articles concerning the husbandry of a wide variety of birds, discussions of health problems and their treatments, and advertisements for many products and services.

Choosing appropriate veterinary assistance is critical. Clearly, a veterinarian with experience in avian medicine is preferred. Sources of information concerning wildlife veterinarians include your local zoo, the American Association of Wildlife Veterinarians, and the American Association of Zoo Veterinarians, and the Wildlife Health Information Palmer-ship. Members of the American Association of Avian Veterinarians may also help, but are more concerned with pet birds. [see Appendix A for Internet sites] Veterinarians who routinely work with wild birds may need a Rehabilitation permit from the USFWS and equivalent state permits, but no but no special permit is required. depending on the exact nature of their work. For routine care and maintenance, there is a distinct advantage in having available the services of an on-staff Registered Animal Health Technician or equivalent (the title varies among states).

The living conditions of birds should be appropriate for each species and contribute to their sound health and comfort. Housing, feeding, and nonveterinary care should be directed by a person (generally the investigator) trained and experienced in the proper care, handling, and use of species being maintained. Investigators maintaining captive flocks of wild birds should refer to the King et al. (1977) or Ritchie et al. (1994) for a thorough listing of husbandry requirements of particular species. The following summary applies to birds being held for 30 days or longer in captivity.

B. Quarantine and Isolation of Animals

Generally, all newly acquired birds shall be kept in strict isolation from other captive populations for a minimum of 30 days. Caretakers should deal with these birds last and not return to other housing areas. The quarantined birds should be observed for symptoms of disease. Fecal examinations for intestinal parasites and visual examination for external parasites should always be performed. Diagnostic procedures for *Salmonella*, *Chlamidia*, tuberculosis, and other significant diseases of concern should be considered. A wildlife health professional should be consulted for assessment and testing. Quarantine regulations for imported birds (except migratory birds between U.S. and Canada) are defined by AWA. Special regulations apply to psittacines and ratites.

Specific protocols may require some modifications of strict quarantine. For example, in song learning experiments, nestlings or fledglings of known age taken in the field may have to be transferred directly into a laboratory experiment such as an anechoic chamber, which should serve as a quarantine space if possible.

C. Prevention, Diagnosis, Treatment, and Control of Animal Diseases

The investigator or other qualified person should observe all laboratory birds daily for clinical signs of illness, injury, or abnormal behavior. All deviations from normal and deaths from unknown causes should be reported at once to the investigator and person responsible for veterinary care. Investigators should be aware that, by the time a birds looks ill, the illness is usually well advanced. Therefore, immediate response to apparent illness is required. The potentially ill bird should be separated (isolated) from healthy birds immediately. Common signs of illness include:

- a. an unwillingness to move; listlessness;
- b. "fluffed" feathers - a bird looking cold when others are fine;
- c. closed or half-closed eyes; an unusually sleepy bird;
- d. drooping wings;
- e. limping or unwillingness to put weight on a foot;
- f. any change in stool consistency;
- g. feces adhering to feathers around vent [*see F: Sick or ailing birds*].

If the illness is contagious, by the time it is detected other birds will likely have been exposed, and additional treatment may be necessary. *Consult a veterinarian immediately.*

All laboratory or aviary birds that die from reasons other than a planned portion of the experimental design should be submitted for to a veterinarian for necropsy.

D. Separation by Species and Source

Several species may be routinely held in a single facility, provided the requirements or habits of the species are not in conflict; e.g., nervous and raucous species should be separated. Although some experiments may necessitate physical separation of species, others may require mixed-species housing (e.g., a study of brood parasitism by viduine finches on estrildids, or a study of interspecific song acquisition).

Studies of social behavior of group-living species may require housing birds in groups in the same enclosure. Because of the diversity of housing needs, the method of housing must rely upon the expertise of the investigator. Care should be taken not to mix species if one may carry a disease that is fatal in the other, e.g., conures and macaws.

E. Daily Care

Staple food: Animals should be fed palatable, uncontaminated, and nutritionally adequate food daily or according to their particular requirements, unless the experimental protocol requires otherwise (*Guide*). Because diets are highly specialized, they must be tailored to the species in question. A zoo nutritionist or veterinarian should be consulted before formulating a diet or adding grit, vitamins, or other supplements to an existing diet.

The form and presentation of the food is important to many species. Some species may become "addicted" to certain foods, e.g., sunflower seeds, and will refuse anything else, even to the point of severe malnutrition. Hence, it is important to establish a healthy, varied diet early in the life of hand-raised birds.

Grit: Many birds may require grit in their gizzards to process their food or as a source of minerals. Commercial sterilized bird grit is available from feed stores or pet stores in bulk. Crushed oyster shell or sterilized crushed hen's egg shells may be mixed in the grit as a source of calcium and other minerals. Grit may help to prevent "egg-binding" during the breeding season. Some investigators may prefer incorporating calcium and minerals directly in the staple diet. Egg shell is sterilized in an oven at 175 EC for 40 minutes before being crushed and provided to the birds.

Vitamins: These should be included depending on the quality of the bird's rations; avoid vitamin toxicities. The pelleted diets available for psittacines contain vitamin and mineral supplements, so additional sources of vitamins and minerals should be provided only after veterinary consultation. Vitamins are available as water-soluble (e.g., Avitron® or Vitapol®) powder from pet stores or feed stores.

Multivitamin powder (especially Avia®) is used by some investigators. Some supplements are meant to be placed in bathing water and ingested during preening. This is a handy technique for finicky eaters.

Water: Fresh water should be given daily for species that require water. [cf. J] For species normally taking water baths, water should be provided in open containers to allow bathing. Some birds may be misted for feather maintenance. Water for drinking may also be provided in commercial bird-drinking tubes (e.g., Edstrom Industries, Inc.). Drinking tubes for small mammals (nipple waterers) may be used if birds will adapt to use them - some birds will refuse to drink from these. Automatic tube watering systems reduce leakage onto cage liner material, thus reducing the growth of fungus, and the main water source can be cleaned without opening the cage. Water containers should be washed daily [cf. J] with soap and water and at least twice weekly with diluted household bleach. Containers should be of non-porous materials, e.g., glass, glazed porcelain, plastic, or stainless steel. Perches should not be placed directly over water receptacles.

Cleaning: Cage liners should be changed often enough to maintain good hygiene. Seed-eaters usually have relatively dry feces, and their cage bottoms may be lined with newspaper and changed twice weekly. Insect and fruit eaters tend to have messier (and smellier) droppings and should have the cage trays (bottoms) cleaned at least every other day depending on how messy the particular species is. [cf. J]

F. Caging, Housing, and General Maintenance

Cages: Stainless steel, galvanized steel, fiber-glass, or plastic cages permit easy cleaning as they may be put in a steam-cleaning machine when necessary. New cages containing galvanized steel or galvanized mesh should be brushed with a wire brush and vinegar solution before they are first used to reduce the possibility of zinc poisoning (Howard 1992). Similarly, any soldered joints should have a protective coating to prevent lead poisoning.

If the birds are easily transferable between cages, then cages should be thoroughly cleaned every three months (once per quarter). Cages should always be thoroughly cleaned with a disinfectant after use by one bird is completed and before another is introduced. Steel (galvanized or plain) showing surface rust should be thoroughly cleaned, buffed, primed, and painted with epoxy paint to prevent rust. If experimental designs require the use of wooden-and-wire cages, then these should be checked for mites. Pyrethrin sprayed into cracks and corners will kill these pests. Cages that have been infested with mites can be treated with boiling water. Cages, runs, and pens should be in good repair and devoid of sharp protrusions that might injure the birds.

Minimum Cage Size: Cages should provide sufficient room for normal maintenance behavior and wing-flapping. Minimum size depends on whether birds are just being maintained in the laboratory or whether breeding is desired. Because of the diversity of avian species, investigators must assume responsibility in determining adequate cage size.

Cage Bottom: Paper, fine sand, wood-shavings, or (ideally) newspaper may be used on cage bottoms. Avoid ground, dried corncobs (Sanicel®), walnut shells, or any other substrate that may promote the growth of fungi, especially *Rhizopus* and *Isospora*. The probability of fungal infections accrues over time, so that even large flight cages or aviaries need to be disinfected at regular intervals. Bocetti and Swayne (1995) recommend disinfecting aviaries annually with a combination of A-33® (Ecolab, Inc), 5% sodium hypochlorite, and a methyl bromide fumigant. Cages with wire bottoms and traps may be used for some species (e.g., some galliforms). They should be avoided for seed-eating song birds as some individuals may knock their entire seed allotment through the wire.

Perches: Perch type should be appropriate to the species. Perches should provide good footing with a minimum of trauma. They should be made of durable and sanitizable materials such as metal, plastic, or PVC, or of economically replaceable material such as wood. Wooden perches are preferred for small birds; ideally, natural branches of different sizes should be used. As long-term use of metal or plastic perches increases the incidence of bumble-foot due to slippage, it may be necessary to wrap the perch with a non-abrasive, non-slip surface, e.g., raptor keepers often wrap rope around a core. Perches should

not be covered with sandpaper. Toenails and beaks should be pared routinely to avoid overgrowth. Investigators should be aware that inappropriately sized perches will lead to leg swelling. A variety of perch sizes is advised.

Cleaning: Water dishes should be washed daily and seed dishes should be washed twice weekly using a safe and effective disinfectant. Such as sodium hypochlorite (household bleach diluted 1/10 (Smith 1990). [cf. K] Quaternary ammonia compounds may be used to disinfect cages, laboratory counters, and floors. Industrial wet/dry vacuum cleaners are useful aids in floor maintenance. Small, hand vacuum cleaners are useful for spot cleaning. Investigators should not use these when birds are breeding, as undue disturbance may cause nest disruption [see 111. C]. Water bottles should be washed twice weekly.

Nest Boxes and Nesting: Although metal boxes can be used for some species (e.g., large psittacines), many species prefer (or require) wicker or wooden nest boxes into which they can carry grass, coconut fibers, excelsior, or feathers. Parrots and parakeets also breed in wooden boxes into which a layer of wood shavings may be introduced. Emberizid finches may build in bushy boughs tied together in a bunch to simulate a bush, or in a potted Boston fern or ornamental bunch grass.

Outdoor and Indoor Aviaries: Climate and facilities permitting, birds may be housed in outdoor aviaries. At least one side of the aviary and part of the roof should be covered to protect birds from wind and rain. Larger outdoor aviaries may contain a permanent covered enclosure to serve this purpose. Perches of different sizes should be provided. Shrubs and trees in pots or planter boxes, or planted on the ground in the aviary, will enable birds to hide when potential predators (cats, raptors, strange humans) are sighted. This gives the birds a sense of security and promotes well-being. Bunches of leafy boughs lashed together with rope or wire and hung on the aviary sides or shelter walls can provide the same effect. Grass may be planted on the aviary bottom if desired. Plantings may attract insects relished by many birds. Half-ripe grass seeds are also a favorite food item. A black-light trap may also be installed to attract live insect food.

Extreme care must be taken with outside cages to prevent access by predators. Climbing predators are especially dangerous. Single raccoons are known to kill confined birds as large as cranes. Further, raccoon droppings may carry parasites (*Baylissascaris procyonotis*) capable of attacking the avian central nervous system (Ritchie et al. 1994).

The floor of indoor aviaries may be covered with newspaper, sand (commercially available), or wood shavings. Sand and wood shavings should be replaced at regular intervals to reduce the build-up of enteric bacteria and fungus. Wood shavings may require prefilters to prevent clogging of air filtration systems. Such systems may rapidly accumulate, and become a source of, fungal spores. They should be changed monthly (Bocetti and Swayne 1995). Environmental enrichment with branches and/or vegetation is desirable. For some species wire floors may be used. Walls of large cages, racks and tables or other furnishings constructed from porous materials should be coated with a durable moisture-proof, seamless substance (e.g., epoxy paint, spar-varnish, etc.). These paints and glazes should resist cleaning agents, disinfectants, and scrubbing.

Lighting: Many aviculturists believe that it is advantageous to use full spectrum (UV) light sources in indoor facilities. These bulbs should be replaced every six months. The practice, however, is controversial, indicating another instance in which consultation with a veterinarian would be useful. A small night light placed near the food source is desirable in cold weather use of outdoor aviaries. A night light is also useful to alleviate stress in recently captured birds and in certain experimental protocols.

Unless experimental protocols dictate otherwise, birds normally should be maintained on photoperiods natural to the species. These may vary with the species, and the schedules of long and short photoperiods must be left to the discretion of the investigator, as these schedules are often tied to an experimental time table and may differ according to species.

Temperature: A temperature range appropriate to the species should be maintained with a thermostat-controlled heating source. Many birds readily acclimate to a wide range of temperatures. However, extreme temperature changes may be lethal at worst and stressful to the immune system at least, and birds should be kept away from areas with appreciable fluctuations in temperature. Normally, room temperatures should be checked daily. In outdoor aviaries, a heat source may be necessary. Infrared bulbs, which will not interfere with light/dark cycles, or non-light radiant heaters are commercially available in pet stores.

Humidity: Humidity should be kept within the range normal to the natural environment of the species if normal behavior and reproductive success are expected. Hatching success of eggs of some species is sensitive to humidity.

Storage of feed and supplies: Supplies and equipment should be stored in cabinets or rooms that can be fumigated and are not used to house animals. These cabinets or rooms may be in or adjacent to aviaries. All feed should be stored in rodent-free, covered containers. Animal keepers may find it convenient to keep some feed in the laboratory. Containers should be plastic or metal but not glass. They may be housed in close proximity to the bird colonies or aviaries. Ideally, food should be kept at temperatures <22 EC or refrigerated at <4 EC. The shelf life recommended by the manufacturer should be noted and containers marked with expiration (discard) dates.

Disposal of waste material: All garbage cans holding waste material (e.g., from cleaning cages) should be kept outside the immediate area of the laboratory. Use of garbage liner bags and daily removal of garbage is encouraged.

Dead animals: Dead birds should be labeled as salvage specimens, placed in plastic bags and refrigerated (frozen if storage is to exceed a 24 hours) away from the holding facilities. The cage should be washed with water and disinfected after the carcass has been removed. We recommend that all dead animals be scheduled for necropsy. "Fresh" necropsies are preferable, but if it is not possible, the animal should be sprinkled with water containing detergent, refrigerated in a sealed plastic bag (after being cooled), and taken to the veterinarian as soon as possible. If a delay of longer than 24 hours is likely, the cadaver may be frozen.

Mopping of floors: Laboratory floors should be swept regularly and should be maintained in a clean condition. [*cf. J*] Sodium hypochlorite (household bleach) diluted 1/10 or other appropriate disinfectant may be used to sanitize floors.

Provisions for emergency care: Names, addresses, and phone numbers of consulting veterinarians, and individuals responsible for the animals should be prominently posted. Provisions should be made for observations and care of animals every day, including weekends and holidays, to safeguard their well-being and to satisfy research requirements. Emergency numbers of both caretakers and physical facilities should be posted in a prominent place.

Sick or ailing birds: An unhealthy bird will usually sit with fluffed feathers, and eyes closed or half-closed. Droppings may look watery, and feathers around the cloaca may be smeared with feces. A sick bird should be moved to a room designated temporarily as a treatment room. A heat-lamp should be provided, but only one corner of the cage should be heated, and the lamp should not be closer than is comfortable for your own hand. The bird must have the opportunity to move away if it gets too hot or it may die of heat-shock. *Seek a veterinarian's help.* Small incubators or commercial brooder units are ideal to hold ailing birds, but be sure that the unit can be disinfected later. Weight gain or loss is a good indicator of general health. A weight loss of more than 10% should be evaluated by a veterinarian.

G. Special Considerations for Aquatic Birds

Aquatic species have needs that are distinct from those of other birds; these mainly have to do with the anatomy of their feet and the importance of waterproofing in their plumage. Species differ widely, so no single prescription will apply to all aquatic birds. Nonetheless, some general rules apply.

Waterproofing of plumage: Maintenance of waterproof plumage is fundamental to the comfort and health of all aquatic birds and requires access to absolutely clean water. Aquatic birds must be allowed to bathe at least once a day. Diving or pelagic birds require cages or enclosures that allow swimming as well as exit from the water.

Access to water: In general, merely providing pans of water in the cage is not acceptable unless the pan is large enough to allow bathing *and* water is changed frequently. How frequently will depend on how rapidly a surface film of dirt, feces or dropped/dunked food forms. Even very light films will interfere with waterproofing. In most cases, pans of water should be changed at least twice daily. If it is possible to provide it, a flow-through system for water is less labor-intensive, more effective, and disturbs the birds less. Such systems should have a constant input of clean water and drain constantly *from the surface*.

Drainage from the surface can be accomplished either by use of a standpipe in the drain, or by overflow over the top edge of the pool/pond/container. Very simple systems can be created by putting a running hose in a commercially available, plastic, child's swimming pool, and letting the water overflow the top. Where standpipes are used, the top of the pipe *must* be covered with screen or netting of small enough mesh to exclude the birds' legs and toes. If drain water is filtered instead of thrown away, filtration must remove bacterial and viral pathogens as well as particles that cause surface films. Rubega and Oring (*pers. obs.*) report excellent results keeping shorebirds in a filtered system that employs activated charcoal and a UV sterilizer, and filters down to 2F. In any flow-through system, feces and food will tend to accumulate at the bottom. These must be removed by siphoning or wet-vacuuming at least twice weekly, but as frequently as is required to prevent decomposition and/or stirring up into the surface layer.

Flooring and foot problems: Aquatic birds are highly susceptible to wounds and infections of the feet and legs. These result primarily from pressure sores developed when the bird is forced to stand for long periods on hard flooring. These sores subsequently become infected when birds walk in feces or dropped food. Infections of this kind are painful and debilitating, and can cause the loss of digits or limbs. Untreated infections occasionally lead to slow and painful death and always lead to some loss of function.

For birds that will be held for more than 2 to 3 days, cage or aviary bottoms must be lined with some resilient material. Sand is commonly used, but must be raked free of feces frequently. Sand must also be completely replaced frequently to avoid creating a bacterial reservoir [*see F: Cage Bottom*]. Rubega (*pers. obs.*) reports good results with shorebirds and gulls by laying down mats of commercially-available rubber or plastic carpet padding, replaced and washed daily. Better results are attained if mats are flushed with constantly running water in the cage. Rubega and Oring (*pers. obs.*) have recently had excellent results with shorebirds by flooring with a commercially-available, slip-proof, rubberized waterproofing system called Tufflex®, also flushed constantly.

Regardless of the flooring used, investigators should be aware of the potential for infections. Any bird that shows signs of limping, reluctance to put weight on a foot or leg, redness, or swelling in the feet or legs should be closely examined immediately. The presence of foot sores requires immediate (and repeated) treatment with a topical disinfectant, isolation from other birds, and modification of cage flooring. In general, unless the investigator is very experienced, advanced cases should be referred to a veterinarian for treatment.

H. Raptors

Raptors have long been maintained in captivity in connection with the sport of falconry. Discussion of techniques associated with that activity is not appropriate here. However, in recent years breeding programs for several species of raptors have been established for management purposes, and research with raptors may involve the use of captive birds. Most procedures outlined in this document apply to these programs. Procedures applicable specifically to raptors are discussed by Carpenter et al. (1987) and Redig et al. (1993) *see also VI.A*].

I. Identification and Records

A waterproof label should be attached to each experimental cage containing the following information:

- a. identification and number of animals;
- b. date experiment started, and projected end (approximate);
- c. any special instructions on feeding (may be in code);
- d. name of responsible investigator.

Records should include source and eventual disposition of each animal. It is recommended that birds be leg-banded with plastic or metal bands to facilitate identification of individuals. Permit and protocol numbers should be prominently displayed in the animal holding room. The investigator is responsible for maintaining records concerning the histories and dispositions of all individual birds as required by local, state, and federal law.

J. Disposition of Birds after Experiments

Upon completion of studies, researchers should release field-trapped specimens whenever this is practical and ecologically appropriate. Exceptions are if national, state, or local laws prohibit release, if release might be detrimental to the existing gene pools in a specific geographic area, or if the specimen has been exposed to potential pathogens that could be released into wild populations.

As a general rule, field-trapped animals should be released only:

- a. at the site of the original capture, unless conservation efforts or safety considerations dictate otherwise. For these latter exceptional circumstances, prior approval of relocation should be obtained from appropriate state and/or federal agencies, and approved relocations should be noted in subsequent publication of research results.
- b. if their ability to survive in nature has not been irreversibly impaired by major structural or physiologic damage, e.g., surgical deafening. Birds that have been so impaired but are otherwise healthy may be donated to zoos or other appropriate organizations.

[see IX. G]

- c. when local and seasonal conditions are conducive to survival.
- d. if there is no chance that they have been exposed to a transmittable disease.

Prior to release, each bird should be examined for signs of injury or disease; birds unlikely to survive should not be released. Birds should be released early in the day and during favorable weather so that they will be able to feed and locate suitable roosting sites before dark. Released birds should not bear the color bands that fit the color sequences allotted to a licensed bird bander. Captive animals that cannot be released should be properly disposed of, either by distribution to colleagues for further study, by donation to a zoo or aviary (it is illegal to distribute migratory birds as pets, and generally inadvisable to distribute even those species common in the pet trade to other than serious aviculturists), or by preservation and deposition as teaching or voucher specimens in research collections.

In both the field and laboratory, the investigator must be careful to ensure that euthanized animals really are dead before disposal. In those rare instances when specimens are unacceptable for deposition as vouchers or teaching purposes, disposal of carcasses must be in accordance with acceptable practices as required by applicable regulations. Animals containing toxic substances or drugs (including euthanasia agents such as barbiturates) must not be disposed of in areas where they may become part of the natural food web.

K. Variations on Standard Procedure

In most experimental protocols it is desirable to keep disturbance due to routine inspection, maintenance, and feeding activities to a minimum. Captive-breeding birds may desert nests if disturbed frequently, and behavioral patterns may be disrupted for several hours (or even permanently) if subjects can detect intrusion or potential intrusion (noise/sight of investigator or animal keeper). In these cases,

routine daily inspections should be suspended, and a schedule should be established for feeding, watering, and cleaning that minimizes interference with data collection but simultaneously ensures health and well-being of the experimental subjects. For example, cages can be cleaned twice weekly instead of daily, conditions can be checked by observation with video monitors or through one-way glass screens. Fresh water and food can be provided to last for several days. Containers can then be removed, washed, and sterilized twice weekly. In some circumstances, an investigator may be able to reduce intrusion even further by employing mechanical means of providing food and water, e.g., automatically filling or rotating food hoppers, drip tubes, etc. Frequency of disturbance can be left to the discretion of the investigator provided that the well-being of the subjects is not compromised and that the procedure has been included in an approved experimental protocol.

L. Zoonoses

Beyond the ethical considerations of handling birds, investigators must be aware that the routine handling of animals incurs certain personal risks. Clearly steps must be taken to protect the handler from dangerous species, or even aggressive species that may attack with painful if not serious results. [see IX.C] Further, a variety of diseases are transmittable from birds to humans (Evans and Carey 1986). Common among these are campylobacteriosis, histoplasmosis, ornithosis, tuberculosis, salmonellosis, and *Yersinia spp.* (enterocolitis and pseudotuberculosis).

The most well known of the above diseases is a form of chlamydiosis known as ornithosis, often, but inaccurately, termed psitticosis or parrot fever. In fact, this highly contagious agent (*Chlamydia psittac*) is known from more than 120 nonpsittacines and several domesticated mammals (Gerlach 1986). Its symptoms are flu-like, and, because it is not a common disease, it is often misdiagnosed. Bird handlers suffering from atypical pneumonia, recurring fever, or from otherwise unaccounted-for chest pain, anorexia, dyspnea, or profuse sweating should inform their physician of the possibility of ornithosis. Note that the standard antibody test is subject to a cross reaction with *Chlamydia trachomatis*, a human venereal disease. See the AVMA publication *Zoonoses Updates* for more recent information.

VIII. Minor Manipulative Procedures

A. Overview

The collection of tissue samples, experimental manipulations using injections and implants of hormones/drugs, playbacks of tape-recorded vocalizations, and presentation of decoys, are fundamental tools for ornithologists. Most if not all of these activities require permits from federal and/or state agencies. [see II]

Clearly, wild birds used in captive studies should be as healthy and free of trauma as possible. Some exceptions to this rule include investigations of the effects of environmental stress. It has been shown that passeriforms require 3-4 weeks to acclimate to captivity before experiments begin. Usually body mass declines after capture, and plasma levels of metabolic and reproductive hormones are often abnormal. After 3-4 weeks body mass returns to that of capture, and hormone levels stabilize (Wingfield et al. 1982). Obviously, housing conditions in captivity are important and depend upon the species to be investigated. Even slight over-crowding can delay acclimation to captivity by several weeks (Wingfield et al. 1982).

B. Collection of Blood Samples

Methods for collecting blood samples from birds have been reviewed by Morton et al. (1993) and Campbell (1994). A video demonstrating blood collection techniques is available from NWHC. [see Appendix A] Common techniques include the use of a syringe for obtaining blood from the jugular vein, occipital venous sinus, or heart puncture (see also Dorrestein et al. 1978; Vuillaume 1983). However, many investigators prefer to obtain small amounts blood from the ulnar (wing) vein or from vessels in the tibio-tarsi. Heart puncture by the furcular route may result in severe debilitation or death, especially among

smaller species. Utter et al. (1971) found that heart puncture via a sternal route was much less severe, and even free-living birds survived well. However, the potential for debilitation is still marked. Toe-clipping is acceptable in the field for very small birds such as hummingbirds. It is generally necessary to clip only the toenail (Leonard, 1969). (N.B. Although toe-clipping may have the helpful side effect of identifying previously sampled birds, it is not an approved procedure for marking birds.)

In most cases a suitable blood sample can be collected from the ulnar vein or tibiotarsal blood vessels. In larger species a syringe and needle is appropriate. For smaller species (e.g., less than 100 g) it is recommended that the vein be punctured with a 26 gauge or smaller needle and blood collected directly into micro-hematocrit capillary tubes. If the animal is not to be killed or incapacitated as part of the experiment, then the volume of blood to be withdrawn is an important issue (McGuill and Rowan 1989). A general rule of thumb is that no more than 2% of the body weight of the animal be collected in any 14 day period, or no more than 1% at any one time (McGuill and Rowan 1989). For a 10 g bird the maximum would be approximately 2-3 capillary tubes (100F of whole blood) and about 10 capillary tubes (500F) from a 50 g bird. These limits apply whether blood is collected for DNA analysis, or whether plasma is harvested for hormone, metabolite studies etc. However, because avian erythrocytes are nucleated, not much blood is needed for most DNA studies. Hence, we recommend quantities of 1/3 to 1/2 capillary tube for birds <7 g, 1 tube for birds 7-15 g, and 2 tubes for larger birds. Once taken, the blood (and other tissue) samples should be properly preserved for survival under field conditions (Seutin et al. 1991).

In recent years, investigations on the response of adrenocortical hormones to a standardized stressor have been used to study adaptation to environment and to monitor species in potentially disturbed habitats (Wingfield 1994). The procedure may have additional useful application in conservation biology by comparing captive breeding individuals with their free-living counterparts. To do this requires holding the individual for a period of 30-60 min and collecting a small blood sample at intervals for measurement of hormones. The standard stress is simply capture, handling and restraint - it is assumed that all individuals of all species will regard capture and handling as stressful (Wingfield 1994). Between samples, many birds can be held in cloth bags, which allow adequate ventilation but prevent injury if the bird struggles. These bags should be placed in a secure place in the shade and sheltered from direct effects of weather. Bags are not an appropriate form of confinement or restraint for species with long necks or long bills. [see IX.C] The combined volume of blood taken during a stress series must not exceed the equivalent of 1% of body mass. With care, sequential blood samples may be taken from the same site such as the ulnar vein without creating multiple puncture wounds. Serial collection of blood samples by heart puncture should not be attempted. This stress series protocol provides highly useful information on hormone changes in response to stress and birds are released unharmed. Care should also be taken to ensure that breeding birds are not withheld from their nests for too long. At other times the 30-60 min holding period is not a problem, unless the individual becomes separated from a flock, or could potentially lose a territory. Investigator discretion is required.

In semi-captive or free-living species, collection of blood does not affect survival (Raveling 1970; Bigler et al. 1977; Wingfield and Farner 1976; Gowaty and Karlin 1984; Frederick 1986). Moreover, normal feeding and brooding activities, molt, and ability to migrate also are not affected (Wingfield and Farner 1976; Frederick 1986). Brown (1995) found that collection of blood samples from the jugular vein of 9-day old *Larus delawarensis* chicks had no effect on survival rates to 21 days of age. The rate of nest desertion by adults was also unaffected. In captivity, wild birds survive well after repeated blood sampling (even at 3-7 day intervals), and body mass and hematocrits remain normal (Wingfield et al. 1982; Stangel 1986). Lanctot (1994) determined that withdrawal of blood from the jugular vein of *Tryngites subruficollis* chicks within 24 hr of hatching had no effect on growth or survival to fledging. Up to 0.05 ml of whole blood was collected. Further, the occurrence of hematomas on the jugular in some chicks did not impair survival. Oring (*pers. obs.*) found little trouble in taking blood from *Charadrius vociferus* chicks in amounts of 100F/10 g. With chicks, the risk of dehydration exceeds that of blood loss, so investigators should take

precautions to provide such birds with fluids. In summary, collection of blood samples from wing and leg veins does not impair behavioral patterns, reproduction and survival of wild birds.

C. Collection of Other Tissues

Techniques in modern physiology and genetics often require biopsy of any of several tissues. Those most commonly sampled (in addition to blood) are adipose tissue, muscle, liver, and gonad. Handling time must be minimized, especially with breeding birds. If periods longer than a few minutes are routinely necessary, as they may be if the sampling procedure is complex, then a justification should be included in the IACUC protocol.

Various studies (e.g., Baker 1981; Westneat 1986; Westneat et al. 1986, and Frederick 1986) show that biopsy has little effect on body condition or survival in either wintering or breeding birds. After prompt handling and release, the bird often returns to normal foraging and breeding activity. Males often sing within minutes of release, and even nestlings that were biopsied showed no debilitation, begged for food, and were fed normally. It should also be noted that biopsy of the pectoralis major muscle does not hinder flight. Samples taken should involve the minimal amount of tissue necessary for scientific validity. IACUC approval is strongly recommended for tissue sampling if anesthesia is not used. [see IX.D]

Feather pulp is also collected for genetic investigations. Plucking a few feathers is usually a relatively innocuous procedure, but care should be taken not to remove so many feathers as to impair flight or other essential functions (this is less of a problem in a captive subject). Note also that removal of growing feathers can result in bleeding, and release should be delayed until this has stopped.

Depending on the nature of the data sought, it may be possible to make certain tissue collections without touching a live animal. For example, DNA can be extracted from feathers or egg shell membranes found in nests, as well as from museum specimens (Ellegren 1991; Morin et al. 1994; Pierce et al. 1997).

D. Collection of Food Samples and Forced Feeding

Obtaining information on a species' diet in the field is often an important component of ecological and nutritional studies. Use of neck ligatures to obtain food samples from nestlings may occasionally be justified. In such cases, the investigator should be careful to ensure normal blood circulation and tracheal function. Further consideration should be given as to whether the procedure will result in unwarranted food deprivation.

In some instances birds are sacrificed for direct observation of stomach contents. In many cases, however, this is not necessary, and collection of fecal samples and regurgitated pellets can provide most, if not all, of the information needed. However, in some species fecal material is not useful (e.g., frugivores). In other cases, such as marine birds at sea, it is not possible to collect fecal samples, although many will regurgitate stomach contents soon after capture. However, others may not, and the use of palpitation techniques and emetics may become necessary. It should be noted that emetics are potent in their action and some mortality results from choking, severe trauma, and shock (Prys-Jones et al. 1974). Wilson (1984) and Ryan and Jackson (1986) have developed a stomach pump that can be used as an alternative to emetics and sacrifice. They showed that the pump gave qualitative and quantitative results (at least in larger birds) comparable to those obtained from sacrificed birds. Pumping also had no apparent ill effects. However, the potential mortality from use of emetics has been greatly reduced by administering small quantities of the emetic through a small tube inserted down the esophagus into the proventriculus (Gionfriddo et al. 1995).

Once again, knowledge of the species to be studied is important in assessing whether it may respond adversely. Grebes, for instance, should not be subjected to any regurgitative techniques because of the feathers in their crops (Jehl, *pers. comm.*)

Nutritional investigations may require force feeding of experimental subjects (usually in captivity). Tube feeding using a soft rubber or atraumatic metal feeding tube of proper size and volumes of food that are

appropriate for the size of the bird is safe and effective. Murphy and King (1986) found that force feeding by inserting a tube down the esophagus was injurious in some cases. Food has to be fed as a slurry, and regurgitation can result in choking (especially in small species). Intubation may also injure the esophageal wall. As an alternative, Murphy and King (1986) suggest feeding pelleted food by placing pellets directly into the pharynx with forceps, thereby inducing reflexive swallowing. Mortality is reduced to near zero, and regurgitated pellets do not result in choking, but use of pellets takes much longer than tubal feeding.

E. Cloacal Lavage

Studies of the mode and timing of insemination are important for analysis of population trends, transfer of genetic information, and mating systems. Cloacal lavage, of both males and females, is a technique to acquire information concerning sperm production and transfer (Quay 1984, 1986a,b,1989). The technique is sometimes extended by the implantation of cloacal microspheres (Quay 1988). Like stomach pumping, lavage is not invasive in the sense that it does not require penetration of an epidermal barrier. As with stomach pumping, it should produce only a slight and temporary discomfort when performed by a properly practiced person.

F. Injections and Insertion of Implants

In the United States, all drugs/medications administered to birds must be used according to the specifications of the Animal Medical Drug Use Clarification Act, which requires that extra-label use must take place under the supervision of a veterinarian.

Injection of experimental substances is widespread in research on birds. Subcutaneous and intramuscular injections are simple in the laboratory and cause little trauma. Intravenous injections require some acquired skill. Intraperitoneal injections require justification because some drugs may irritate the viscera and because of possible mechanical or chemical damage of the viscera.

Under field conditions, most injections (especially subcutaneous injections) appear to have no effect (independent of the substance injected) on survival or normal activities (e.g., Reyer 1984). For longer term studies, however, repeated injections are often necessary, requiring multiple capture at frequent intervals. This in itself may cause serious disruption of normal activities. For these reasons, implants in silicone rubber tubes, pellets, or miniosmotic pumps should be used to provide long term administration of the experimental substance (up to several weeks). Whenever possible, such implants should be made subcutaneously because intraperitoneal implants are often encapsulated by connective tissue. Implants inserted under the skin of the flank or side of the thorax are most effective and are easy to remove after the experiment is terminated. Implants **should not** be placed on the back because they frequently rupture the skin, allowing infection. Implants under the skin of the neck are also not advised: they can penetrate the thoracic cavity, resulting in severe respiratory distress. Custom-made, mini-osmotic pumps are available for odd-sized animals or for administering substances for prolonged periods. (Alza Corporation, Palo Alto, CA. The company provides a free training video for the use of these pumps in rodents; it should be applicable to birds.) As with other invasive procedures, the area of operations should be as sterile as possible.

Timing of implants is also important in some cases. Treatment of free-living birds with hormones usually has no debilitating effect, but some treatments, such as the sex steroids, can disrupt the normal temporal progression of reproductive and associated events. Molt and migration can be abolished by implants of sex steroids. Hence, every effort should be made to remove the implant after the experiment. In those species that breed at high latitude or altitude, the short breeding season allows only a short time for molt. If these functions are disrupted by implants, death may result due to poor plumage and delayed migration. It is recommended that, when possible, all implants be removed from controls and experimentals. However, experimental subjects with control implants, or implants from which all hormone has diffused, do survive over winter at the same rate as unimplanted individuals (Wingfield 1984). Further, the stress of recapture may cause more problems than it solves. A crucial element in assessing

appropriate actions in all of the above is whether the risk induced by the experiment applies primarily to individuals or to the population. [see I.A]

G. Determination of Egg Viability

Certain experimental procedures require an estimation of the number of eggs within a clutch that have viable embryos and the age of embryos. A common technique uses transillumination (candling) to detect the presence of an embryo, but many species have eggs with shells too thick or too heavily pigmented for candling to be useful. Breaking open eggs, with obvious deleterious effects on reproductive success, is justified in some cases, but ultrasonography is the technology of choice. Electronic devices such as doppler stethoscopes that can detect the embryonic heart beat or movements of the embryo within the shell may also be useful. Some of these techniques are being adapted for use in the field (e.g., Mineau and Pedrosa 1986). Floating the egg is useful for some species. If the egg is more than ten days old and does not float, there is no viable embryo.

H. Playback of Tape-Recorded Vocalizations and the Use of Decoys

Playback of tape-recorded vocalizations to free-living birds causes little disturbance or trauma if the duration of the playback is kept within reasonable bounds (normally less than 30 minutes). More prolonged playback may distract subjects from activities that are essential to reproductive success. Unless required for the experiment, speakers **should not** be placed close to the nest, etc. Activity while on the territory should also be minimal to avoid destruction of the local habitat (Johnson et al. 1981; Marion et al., 1981; Baptista and Gaunt, 1997).

Live decoys are frequently used in conjunction with playbacks. The same guidelines hold: minimize investigator activity and avoid placing the decoy close to the nest. Live decoys require particular attention in the field. Birds used in this way should be trained for a day or so prior to onset of the experiment. An untrained bird tends to flail around the cage when placed on another's territory. A decoy habituated to housing in a cage under field conditions provides a more appropriate experimental stimulus and is also subjected to less stress. Note also that the decoy must be provided with food and water at all times. Avoid placing the decoy in exposed situations, especially on hot days. Never expose a decoy to full sunlight without some form of shelter. [see IV.C]

Recorded playbacks are often employed in recreational uses of birds. ABA recognizes that such usage, as well as several other practices, e.g., photography, may affect birds adversely, and have addressed that issue. [Appendix B]

I. Artificial Eggs

The use of artificial eggs is invaluable to many ornithological studies, allowing reduced risk during trapping and providing for the development of eggs of special value (e.g., in the maintenance of threatened populations). Artificial eggs composed of a variety of materials, including wood, paper mache, plastic, and clay, have elicited normal nesting responses. However, egg recognition varies widely among species. In some species, individuals recognize the unique patterns of their own eggs. For others, egg recognition mechanisms may be very general. When eggs are used briefly, e.g., during trapping, a general approximation of real eggs will suffice. However, when it is intended that artificial eggs be incubated for days or weeks, extreme care should be given to the mimicry of the original egg shape, size, pattern and weight. Birds uncomfortable sitting on surrogate eggs may desert.

J. Experimental Manipulation of Plumage

Altering the external appearance of a bird by manipulating the size and color of plumes, waffles, etc. has proved to be a powerful experimental tool in behavioral ecology. Imping, a technique for identifying individuals by "transplanting" one or more feathers from a bird of a different color, extends back into antiquity. Under captive conditions such manipulations are not traumatic unless, as a result, the

experimental subject has difficulty feeding and drinking. Under natural conditions, however, it is important to ensure that such manipulations do not impair flight or other types of locomotion.

IX. MAJOR MANIPULATIONS

A. Overview

Aviculture is one of the largest hobbies in North America, and the veterinary practice devoted to birds is now extensive (Johnston 1982). As a result, the techniques of avian anesthesia and surgery are developing rapidly. Modern techniques are well presented in several recent texts, e.g., Harrison & Harrison 1986; Richie et al. 1994 (encyclopedic); Altman et al. 1997 (organized like a symposium, with many general topics covered in terms of specific studies). New texts appear almost annually. No laboratory involved with invasive studies of birds should be without one of these. However, neither these texts nor anything that follows in these guidelines is meant to serve as a self-training manual. As is the case for all complex procedures, surgery should not be undertaken by novices. It is the investigator's responsibility to obtain training from an expert, either a senior investigator with long practice and experience, or a veterinarian. We here present detailed material concerning commonly used procedures and commonly encountered problems in order to facilitate communication between investigators and their IACUCs, many of which are more familiar with mammals than birds and laboratory conditions than field conditions.

Because the field is not static, we will not attempt to present a catalogue of acceptable techniques. Rather, we shall attempt to establish a philosophy that will help all involved to determine whether a given approach is appropriate. The techniques discussed should be considered as examples. We shall also indicate limitations and unacceptable procedures.

B. Some Primary Considerations

- a. A distressed animal provides poor data.
- b. In cases of doubt, or in the absence of specific justification for relaxed standards, the stricter regime of analgesia and antisepsis should be adopted.
- c. Techniques appropriate for one experiment or at one time may be counterproductive in another.

Any invasive technique is potentially distressful and even dangerous. The subtleties (e.g., angle of introduction of a hypodermic needle, positioning of the subject, position of the investigator's hands) that allow experts to perform these procedures smoothly, rapidly, and with minimum distress to the subject are developed from long practice, in many cases are almost unconscious, and may not be well communicated in text books or instruction manuals. Hence, an investigator wishing to adopt a new technique should seek direct instruction from an expert, and should practice on appropriate models until skilled.

C. Restraint

Invasive procedures clearly require restraint and sometimes immobilization. Restraint may be needed during pre-procedural examination and preparation and during recovery from anesthesia. Each restraint event should be preceded by a determination of necessity. All equipment and supplies for the procedure, data forms or notebooks, lighting, or anything else that may be needed should be positioned to be immediately accessible before the restraint is begun. Birds with long legs and necks must be given special restraint, especially during recovery from anesthesia or at other times when coordination may be disturbed. Some species may be dangerous to the handler. Proper restraint includes protection for the handler as well as the bird, else the bird may be injured during defensive maneuvers. Heavy gloves are appropriate for handling raptors, but these must be cleaned and disinfected lest they promote the spread of disease. Clean towels, ranging in thickness from disposable paper to heavy cloth, may be employed to provide a first barrier and then as a medium of restraint. The same cloth may then be used to give raptors something to grip. Safety goggles should be worn when handling birds with long beaks; ear protectors or

plugs when working near species capable of loud calls. Minimizing external stimuli such as vocalization, rapid changes of light or temperature, touch, etc. helps ensure successful handling. Special training is required for those wishing to handle certain large birds such as raptors, cranes, flamingoes, ratites, large anatids and galliforms, etc.

The nature of the restraint depends on the procedure and the species (Fowler 1978, 1995). For some procedures, especially those using little or no anesthesia, variations of handling techniques used in banding are adequate (Donovan 1958). For such relatively innocuous procedures, large species can often be calmed by enclosing the head in a opaque hood. Hoods are also useful for reducing struggling during pre-surgical evaluation and post-operative recovery. Small to medium sized birds can be enclosed in cardboard or fabric tubes or comparable devices. Care should be taken that the restraint does not: 1) interfere with ventilatory movements of the abdomen and thorax or impede respiratory air flow; 2) enclose the bird so as to induce hyperthermia; 3) expose the bird so as to induce hypothermia, or 4) unduly damage flight feathers. Temperature controlling equipment such as ice, fans, or warming pads (hot-water chemical flask warmer) may be needed.

D. Anesthesia

An anesthetic is an agent that produces 1) analgesia (reduction of pain), 2) immobilization, and 3) loss of consciousness, so that the individual is unresponsive to stimulation. Anesthesia ideally minimizes stress in administration of the drug and eliminates pain during the research procedure. It also ensures the safety of the bird and provides adequate restraint during the procedure. General anesthetics perform all of these functions, but not necessarily equally well, and the effects of many are dose-dependent. Hence, it is important to recognize the level of the effect. Local anesthetics induce analgesia of a specific body area or region, but do not produce unconsciousness or tranquilization.

The choice of agent is based on the animal's general body condition and on working conditions in the laboratory or field. The drug of choice for one species may be ineffective for another, sometimes closely related, species. The dose for a given drug may vary among taxa. Within species, effects may vary with age, sex, season, or fat content of the bird. Prolonged recovery time or the need for special equipment may render an anesthetic of choice in the laboratory totally inappropriate for field use. Every anesthetic agent has specific advantages and disadvantages. The investigator must be fully knowledgeable about the physiologic and pharmacologic characteristics of the avian species to be used as well as the pharmacologic characteristics of the drug or drugs to be used in the research project. AWA mandates consultation with a veterinarian for laboratory animals, and AAALAC supports this practice for all vertebrates. *The most important message on this subject is that there are no easy answers and no single agent that is ideal for all situations. The investigator, in consultation with a practicing avian veterinarian or veterinary anesthesiologist, must take the time to determine which agent or combination of agents is appropriate to the study and to justify that decision.*

The effects of specific drugs have been determined for many, but not all, species; response can be variable among species. The investigator must know the anatomic, physiologic, and pharmacologic characteristics of the species with which they work as well as the pharmacologic profile of the drug or drugs they intend to use in research. For instance, the response of birds differs from that of mammals, especially with regard to opioids such as morphine. (For this reason, when using an opioid, the investigator should use a kappa agonist such as butorphanol, rather than morphine). When information concerning the effect of a drug on the species under consideration is unavailable, pre-experimental testing with low dosages is advised. A good starting point for those working with exotic species is Samour, et al 1984, which surveys the effects of CT-1341, ketamine and ketamine plus xylazine on 154 species in 15 orders. Genevois et al. (1983) also considers several agents and 35 species. Various techniques are summarized in Fedde (1978), Sinn (1994), Chapter 23 of Muir et al. (1995), Heard (1997) and several chapters in Redig et al. (1993). The state of knowledge about avian medicine and anesthesiology is developing rapidly, so even the experienced investigator should take the time to review recent literature

before selecting a specific drug. Among the several recent texts on avian medicine are Ritchie et al. (1994) and Samour (1999).

General anesthetics are administered either as a gas or as an injection. Inhalatory anesthetics have the large advantage that dosage is easily changed during the procedure, and, because of the clearing properties peculiar to the avian respiratory system, recovery can be extremely rapid. Most inhalatory anesthetics must be administered with special equipment for precision delivery. Isoflurane is considered the inhalant of choice in birds. Note that it must be administered with an appropriate vaporizer for safe delivery of anesthetic gas to adequately deliver safe and effective levels of anesthesia. Light-weight, portable systems are available for field use (Exotic Animal Medical Products; Seven Seven Anesthesia). For a practical reference to field use of gaseous anesthetics, see Olsen et al. 1992. Injectable anesthetics may be placed in a muscle mass (I.M.), in a vein (I.V.), or intra-osseous, with proper training in the technique (Heard 1999). Intravenous administration provides more predictable reactions, faster induction and usually faster recovery, and should be used when possible, but it requires some skill even with large species and is inappropriate for small species. The dosage for most injected anesthetics varies inversely with weight (Boever and Wright 1975), i.e., small birds require relatively more. Hence, the weight of the subject must be accurately measured prior to administration of the drug, and the drug may need dilution.

Anesthetics may be combined with each other or other drugs for synergistic or antagonistic effects. Muscle relaxants such as diazepam or midazolam may be used in birds, but only in conjunction with an analgesic agent. Ketamine, which is the injectable anesthetic of choice for many birds, is often used as a sedative to be followed by a gaseous anesthetic using endotracheal intubation. Recovery from ketamine is often violent. Muscle relaxation is poor and analgesia may be inadequate for the drug to be used as a sole agent for painful procedures. A common practice is to mix of ketamine and other products (Heard 1999, Rupiper et al. 1999, Muir and Hubbell 1995). Such complex procedures require special skills. Investigators wishing to use them should consult, or practice with, a veterinarian experienced in working with avian species.¹

Given the difficulties of administering some of the common general anesthetics, the use of local anesthetics is attractive, especially if the procedure is simple and the bird is to be released quickly. However, the advantages may be more apparent than real. Dosages are uncertain, and the effects may be general and prolonged (Graham-Jones 1965). To some extent, the problem is one of size (Gandal 1969; Klide 1973), with small species susceptible to overdose. Extreme care must be taken in calculating dosages. A dose as small as 0.1 ml of 2% lidocaine is a gross (and lethal) overdose for a 30- gram bird. Studies on mammals indicate that several common local anesthetics, including 1% procaine, 0.2% tetracaine, 0.5% lidocaine (with and without epinephrine), 2% chlorprocaine, 0.25% dibucaine, 2% mepivacaine, and 2% pirocaine, have temporary but severe myotoxic effects (Basson and Carlson 1980; Foster and Carlson 1980; Carlson and Rainin 1985). Diluting local anesthetics (with sterile, preservative-free normal saline solution) would increase their margin of safety. The intramuscular use of local

¹ In 1999, Ketamine was re-classified as a Class III controlled substance. Non-veterinarians who register with the Drug Enforcement Administration (DEA) can legally obtain Schedules (Class) II-V controlled substances. Individual researchers and institutional departments can register with the DEA.. "Mid-level practitioners," defined as "other than a physician, dentist, veterinarian, or podiatrist" are permitted to conduct research using Controlled Substances only if expressly permitted by state law. DEA Forms 224 may be obtained at any area office of the Administration or by writing to the Registration Unit, Drug Enforcement Administration, Department of Justice, Post Office Box 28083, Central Station, Washington, DC 20005. DEA Forms 224a, 225a, and 363a will be mailed, as applicable, to each registered person approximately 60 days before the expiration date of his/her registration. Certain record-keeping and inventory requirements must be met. Ornithologists outside the United States should consult with their own law enforcement agencies about access to these substances.

anesthetics should be undertaken with caution. Generally, the preferred short-acting drug is lidocaine and the preferred long-acting drug is bupivacaine.

Chilling has sometimes been used for topical analgesia. Ethyl chloride can temporarily numb a small area for quick incision, such as in laparotomy (Risser 1971). Refrigerants such as dichlorodifluoromethane may also be used for cryosurgery. However, because it is difficult to control the degree of local chilling, and frozen tissue may be permanently damaged or rendered inoperable, and because a relationship between hypothermically induced immobility and analgesia has not been clearly established, the use of chilling as a general anesthetic is strongly discouraged.

For discussions of the complex topic of pain in animals, see Bateson 1991; Elzanowski and Abs 1991; Gentle, 1992; Andrews et al. 1993. The evident psychological component is usually aggravated by fear. Similarly, various species respond to traumatic experiences differently, and either restraint or disorientation may elicit more evident distress than such physical injuries as punctures or small incisions. Unfortunately, an animal's fear of the unknown cannot be lessened by assurances. Hence, anesthetics may be used not only to ameliorate pain but to reduce the total stress of a procedure. It was once thought that in some cases, the stress of a procedure would be increased and/or the bird's chances of survival would decrease as the result of the administration of anesthetic. Current knowledge is to the contrary. Numerous studies show that surgery is far more stressful than anesthesia, at least in the laboratory setting. Birds that have had laparoscopies under anesthesia return to normal behavior sooner and resume eating sooner than do birds that have not been anesthetized. However, in the wild, when birds must be able to avoid predators, find shelter for the night, and survive inclement weather, the lingering effects of anesthesia may be detrimental to the survival of the bird. If anesthesia is used, the bird should not be released until the effects of anesthesia have completely disappeared.

E. Surgery

Avian surgery is considerably different from mammalian surgery (Ritchie et al. 1994; Altman et al. 1997). In part, the differences are due to avian structure, especially the airsacs and flow-through respiratory system, and/or physiology, e.g., blood-pH and proclivity to fall into hypothermia. These can generally be accommodated by altered techniques. However, two differences require further comment. First, birds may not show the same inflammatory response to infection associated with cuts or punctures as is seen in mammals. This does not mean that birds are not subject to infection. The physiological response is simply different from that of mammals, so standards of antisepsis cannot be relaxed. Second, many birds show little overt behavioral evidence of pain or discomfort from punctures or incisions over much of the body, especially in the apteria (Green 1979; Steiner & Davis 1981). The head and bill, scaled portions of the legs, and vent area are exceptions. However, most birds show strong evidence of discomfort from pinching or pulling of the skin or plucking feathers. Because of this lack of response, because anesthesia can be stressful to a bird, and because birds can be severely stressed by prolonged handling (Gandal 1969), many aviculturists and investigators perform some surgical procedures, including laparotomy and muscle biopsy, with little or no anesthesia and close incisions without sutures (Risser 1971; Wingfield & Farner 1976; Baker 1981). Such procedures need not affect the survival or reproductive potential of the subject (Ketterson and Nolan 1986; Westneat 1986; Westneat et al. 1986). Given the availability of local analgesics and the rapidity with which birds can recover from such gaseous general anesthetics as isoflurane, the practice of invasive techniques without the use of anesthesia requires special justification, e.g., the bird is to be released into the wild immediately, [see D]. In no case should such procedures be performed by those who have not developed the necessary skills.

The acceptability of a procedure varies with the experience and skill of the investigator. Any invasive procedure more complicated than a simple injection should be rehearsed with an appropriate model (mock-up, cadaver, generally anesthetized subject), and the most conservative limitations on techniques should be maintained until they can be performed quickly and smoothly. As a major portion of surgical trauma for many birds is the necessary restraint, rapid, but not hasty performance can markedly reduce

distress. Again, individuals who are not familiar with a technique should learn it directly from the tutelage of an expert.

The conditions governing the adoption of procedures may depend on the intended fate of the bird. We can distinguish four categories of subjects:

- a. wild birds in the field that are to be released immediately upon recovery;
- b. wild birds that have been brought into a laboratory and will be released after recovery in a holding facility;
- c. wild or captive bred birds that are to remain captive permanently or for an indefinitely long period after the procedure;
- d. birds that will be euthanized without recovery.

For any animal that is to be released to the wild, the prime consideration shall be that the procedure will have a minimal effect on the subsequent survival and reproductive potential of the subject. If the purpose of the experiment is to alter survivability or reproductive potential, then the interference should be no more than necessary, as judged and justified by the investigator, to test the issue in question.

High standards of antisepsis should be practiced routinely during invasive procedures. Some procedures may need only disinfection of instruments. No single procedure for sterilization is appropriate to all materials and all situations. Procedures for chemical disinfection and sterilization have been reviewed by the American Dental Association (Council on Dental Therapeutics et al. 1985). Precautions should be taken to reduce the possibility of disease transmission. Disposable blades and needles should be used, and instruments should be immersed in a strong disinfectant between subjects (household bleach or 90% ethanol plus flaming).

Instruments should be rinsed in sterile, distilled water after immersion in either bleach or ethanol (unless flamed) or other disinfectants before use. Different disinfectants require different soak times to be effective. There are also a variety of commercially available, cold disinfectants, often conveniently packaged in small quantities. Many of these are less likely to damage tools than bleaching or flaming. A plastic board can serve as a temporary, portable surgical area. This, too, should be sterilized with bleach or ethanol between uses.

Aseptic conditions are not required in the laboratory, but the surgical area, which should be specifically designated and set aside for that use only, should be scrubbed with a strong disinfectant, e.g., dilute sodium hypochlorite (household bleach, dilute 1/10), quaternary ammonium compound, or an iodoform compound (followed by alcohol to remove the residue), before and after procedures. All organic debris from previous procedures must be removed or sterilized. Special precautions, such as color coding and separate storage areas, must be taken to insure that surgical instruments are used for that purpose only. They must not be mixed with autopsy, dissection, or skinning instruments. The investigator should wear *sterile* disposable surgical gloves during the procedure.

AAALAC regulations require that a sterile field be maintained as effectively as possible -even in the field. One can use plastic boards for surgical procedures. These can be disinfected with 1/10 (or even 1/32) dilution of household bleach solution, quaternary ammonium compound, chlorine dioxide based sterilant (Clidox®), or chlorhexidine (Nolvasan®). Alternatively, the board can be wrapped with pre-sterilized cloths or disposable paper covers. Sterilizing instruments under field conditions is a vexing problem. A variety of chemical sterilizing solutions exist, but these may require that the instruments be rinsed in sterile water before use. Techniques that may be appropriate to specific situations can be garnered from any of the publications mentioned above and from studies on rodents (Cunliffe-Beamer 1993; Callahan et al. 1995). The best general solution is to use disposable instruments and blades. As long as they are not contaminated with blood or other tissues, non-disposable instruments can be soaked in 70% ethanol between uses. The investigator should wear sterile surgical gloves, which can be obtained from medical supply stores; they are light and compress into a small storage space for field use. Whenever possible, the procedures should be carried out in some sort of shelter that reduces airflow and the possibility of wind-borne contaminants.

Repeated surgeries on a single subject are discouraged unless they are part of a single experiment and have scientific justification. Although repeated surgeries may be desirable to reduce the number of birds to be removed from the wild, especially if the investigation involves imported species, that argument is not recognized for primates, and approval must be sought from USDA.

Wound closure may present difficulties requiring skill and the proper suture materials. Cyanoacrylic tissue glues (e.g., Tissu-Glu®, Ellman International, or Vetbond®, 3M Corp.; N.B. household super glues are toxic to tissues) may be used. A disadvantage is that the necessary drying time markedly increases handling time. However, such procedures may be useful to protect the cleanliness of the wound if the bird is to be returned to a relatively dirty environment such as a nest or open water. Surgical staples are an effective and rapid means of closing large incisions in medium-sized and large birds. However, as they must be removed mechanically, they are therefore not advised for field use.

Other issues specific to birds:

- a. Because of the high metabolic rates characteristic of birds, pre-surgical fasting is not advised for small birds and should be of a duration sufficient only to empty the crop in large birds (overnight for large birds, 4-6 hrs at most for small birds).
- b. Hypothermia is a common avian response to general anesthesia. Therefore, the surgical recovery areas should be warm, and special heating arrangements may be necessary for prolonged recoveries.
- c. If recovery is prolonged, the bird should be rotated to lie on alternate sides every few minutes. An anesthetized bird should not be allowed to lie on its back except as necessary for the surgery. The cage should be covered to reduce stress.
- d. Some anesthetics, especially ketamine-plus-xylazine, do not induce eye closure. In such cases, the opened eyes should be bathed with an optical wetting agent every few minutes or should be protected with an ophthalmic ointment.

F. Laparotomy and Other Techniques for Sexing

Laparotomy penetrates a body cavity and, thus, is considered a major surgical procedure. Exploratory laparotomy has several uses. It can provide information on sex in monomorphic species and stage of gonadal development, as well as indicate presence of parasites, gross condition, and activity of other organs. Topical application of xylocaine cream may reduce discomfort of laparotomized birds (Ritchie et al. 1994). Many experts perform this procedure with only a topical anesthetic or no anesthetic at all, especially in the field, where speed of operation is important so that the bird can be released quickly and in a condition to avoid predators. [see E] Such usage is not recommended for anyone lacking adequate instruction and abundant practice on anesthetized or recently deceased birds. Even skilled practitioners should practice following any significant hiatus in performance. Several reports have shown that laparotomy has no effect on survival and does not disrupt breeding activity or winter foraging (Bailey 1953; Miller and Miller 1968; Wingfield and Farner 1976; Ketterson and Nolan 1986). In the laboratory, and in the field where practicable, isoflurane is ideal anesthetic for this procedure [see D].

Any unsealed wound can be a route for infection and for herniation of abdominal tissues and organs. Except for a small (2-4 mm) puncture for a laproscope, all laparotomy wounds should be sealed. Surgical glues serve this purpose well. Wounds in waterbirds should be sutured to reduce infection. In those species that dive, the wound must be sealed to avoid penetration of water into the body cavity as pressure increases with depth. [see E] An unpublished study reveals that, after laparotomy of diving alcids and closure of the wound with non-toxic glue, adults returned to the breeding colony and nested normally. Body mass was not reduced, suggesting that the birds were able to dive and feed normally.

Recently, several less invasive techniques have become available for sexing birds. These are summarized in Halverson (1997). Especially promising are techniques using DNA to assess the presence of the W-chromosome, e.g., Quinn et al 1990, as these could be used to sex nestlings and even eggs

(Langenberg et al. 1997; Nuechterlein and Buitron 1997). These appear to be highly effective and accurate. However, the easier and cheaper polymerase chain reaction techniques are species specific (Griffiths and Tiwari 1993). Preparation and testing of a probe may take several trials, even for an expert (P. Parker, *pers. comm.*). Hence, one should be sure that the proper probe is available *before* using the technique on critical specimens. Similarly, flow cytometry is relatively easy, but may not provide totally unambiguous data (Tiersch et al. 1991; McLain and Roth 1997). Fecal sex steroids also provide an alternative to surgical sexing, where it can be certain that the specimen represents an individual bird. All of these techniques require the return of tissue samples to the laboratory and some delay during processing. Nevertheless, investigators are encouraged to explore the appropriateness of these new techniques to their studies.

G. Euthanasia

The technique for euthanasia should not interfere with post-mortem analysis and should be as swift and as painless as possible. The technique adopted will be considerably influenced by what one wishes to do with the cadaver (i.e., use it for a museum specimen or for tissue chemistry or just dispose of it). Many techniques for euthanasia have been reviewed by the American Veterinary Medical Association (Andrews et al. 1993). Relatively few are appropriate for birds, and none consider the needs of field studies. Hence, we make some general comments on the nature of what might be done and list only those procedures that are not acceptable. This is another area in which a close working relationship with an avian veterinarian will be useful.

Generally acceptable techniques involve overdose with an anesthetic, either injected or gaseous (including carbon dioxide) or administration of a specific euthanasia compound (usually based on barbituates). Such procedures pose little problem for laboratory studies, but may be impractical in the field. Field investigators who normally include hypodermic syringes as part of their equipment, e.g., for tissue sampling, will probably find that a small bottle of anesthetic or euthanasia compound adds little burden.

It is necessary to determine the correct dosage. Others may find the advantages of a technique that provides a specimen with minimal damage and can be easily adjusted to any size specimen are attractive. There will remain, however, field conditions in which carrying the equipment for administering a drug is impractical, or perhaps even illegal. Such situations require mechanical means for dispatch. The traditional technique of cardiac (thoracic) compression approaches the limits of present standards of speed and minimal stress and *may* not be accepted by an investigator's IACUC. Thus, although the technique is permissible for field use, we recommend use of an alternative whenever practical. A mechanical alternative is cervical dislocation, in which the neck is quickly stretched (not twisted) until the spinal cord snaps. This technique is easily learned and can be used on birds as large as pheasants or small geese.

Unacceptable methods include curare, decamethonium, gallamine, magnesium or potassium salts, nicotine, pancuronium, strychnine, and succinylcholine. None of these chemicals cause loss of consciousness in a humane manner. Curare, decamethonium, gallamine, pancuronium, succinylcholine, and magnesium produce muscle paralysis in conscious animals, causing death through asphyxiation. Nicotine and strychnine cause painful and protracted convulsions, leading to death by asphyxiation. Potassium salts stop the heart from contracting in conscious animals, causing distress until unconsciousness occurs. Potassium salts are acceptable for euthanasia only when administered to a deeply anesthetized animals. Carbon monoxide and ether are undesirable because of danger to personnel. On the other hand, if an animal has been anesthetized, or is unconscious from trauma, the mechanical means of euthanasia are of less consideration. It is important to remember that the primary purpose of euthanasia is to terminate suffering. Hence, speed is important. An animal that is already dying from severe trauma, e.g., from gunshot, should be terminated by the fastest available method compatible with preserving the desired portions of the specimen.

Finally, euthanasia is not a technique for the disposal of animals at the end of an experiment but a procedure to end chronic distress or pain. Investigators should seek ways to provide healthy experimental subjects with an opportunity for a continued, comfortable existence.

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Appendix A Addresses

Note: Government offices change addresses and phone numbers with amazing frequency. It is, perhaps, best to start by telephoning the office in question to be sure that your request for an application is sent to the correct address.

CANADA

Canadian Bird Banding Office
Canadian Wildlife Service
Environment Canada
Ottawa, Ontario
K1A 0H3

Regional Offices

Atlantic Region

Regional Director: Dr. George Finney
Environmental Conservation Service
Environment Canada
P.O. Box 1590 - 63 East Main St.
SACKVILLE, New Brunswick
E0A 3C0
(506)-364-5011; fx: 364-5062
Exec. Assistant: Lorraine Wheaton
e-mail: WHEATONL@CPDAR.AM.DOE.CA

Ontario Region:

Regional Director: Mr. Simon Llewellyn
Environmental Conservation Service
Environment Canada
4905 Dufferin Street
DOWNSVIEW, Ontario
M3H 5T4
(416)-739-5839/5840; fx: 739-4408
e-mail: LLEWELLYNS@AESTOR.AM.DOE.CA
Exec. Assistant: Lana Birmann

Pacific & Yukon Region

Regional Director: Mr. Brian Wilson
Environmental Conservation Service
Environment Canada
1200 West 73rd Avenue
VANCOUVER, British Columbia
Z6P 6H9
(604) 664-4065067; fx: 664-4068
e-mail: WILSONB@AESVAN.AM.DOE.CA
Exec. Assistant: Kim Colavecchia

Region du Quebec:

Directeur régional: Monsieur Michel Lamontagne
Conservation de l'environnement
Environnement Canada
1141 route de l'Église, 9e étage
Case postale 10 100 Sainte-Foy (Quebec)
G1V 4H5
(514) 283-5869; fx: 283-1719
e-mail: LAMONTAGNM@CPQUE.AM.DOE.CA
Exec. Assistant: Jocelyne Séguin
(418) 648-7808; fx: 649-6591
e-mail: SEGUINJ@CPCSL.AM.DOE.CA

Prairie & Northern Region

Regional Director: Mr. Gerald McKeating
Environmental Conservation Service
Environment Canada
Twin Atria Building, 2nd Floor, 4999 - 98 Avenue
EDMONTON, Alberta
T6B 2X3
(403)-951-8853; fx: 495-2615
e mail: MCKEATING@EDM.AB.DOE.CA
Exec. Assistant: Vi Jespersen

MEXICO

Instituto Nacional de Ecologia
Dirección General de Aprovechamiento Ecológico de los Recursos Naturales
Rio Elba No. 20, 10E Piso
Col. Cuauhtemoc, 06500 Mexico D. F.
MEXICO

Scientific Affairs Office
American Embassy
Reforma 305 06500
Mexico, D. F.
MEXICO

United States

USDA, APHIS-VS
4700 River Road, Unit 38
Riverdale, MD 20737-1231

U.S. Geologic Survey
Bird Banding Office
12100 Beech Forest Rd-4037
Laurel, MD 20708-4037
(301) 497-5790; e-mail BBL@nbs.gov; fax: 497-5717

U.S. Geologic Survey
National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711-6223
(608) 264-5411; e-mail: NWHC@nbs.gov; fax: 264-5431

U.S. Fish and Wildlife Service
Office of Management Authority
4401 N. Fairfax Drive, Room 430
Arlington, VA 22203
800-358-2104 (in U.S.); (703) 358-3210 (outside U.S.); fax: 358-2281

<u>Region</u>	<u>U.S. Fish and Wildlife Service, Migratory Birds Permits Office</u>	<u>U.S. Fish and Wildlife Service Endangered Species Office</u>
1: California, Hawaii, Idaho, Nevada, Oregon, Washington, Guam, American Samoa	Eastside Federal Complex 911 N.E. 11th Avenue Portland, OR 97232-4181 (503) 872-2715 fax: (503) 231-2364	(503) 231-2063 fax: (503) 872-2716
2: Arizona, New Mexico, Oklahoma, Texas	P.O. Box 709 Albuquerque, NM 87103-709 (505) 248-7882 fax: (505) 248-7885	P.O. Box 1306 Albuquerque, NM 87103-1306 (505) 248-6649 fax: (505) 248-6922
3: Illinois, Indiana, Iowa, Michigan, Ohio, Wisconsin	1 Federal Drive BHW Federal Building Fort Snelling, MN 55111 (612) 725-3775/3776 fax: (612) 725-3013	(612) 725-3276/3250 (612) 725-3501

4: Alabama, Arkansas, Florida,
Georgia, Kentucky, Louisiana,
Mississippi, North Carolina,
South Carolina, Tennessee

P.O. Box 49209
Atlanta, GA 30359
(404) 679-7070
fax: (404) 679-7285

1875 Century Blvd.
Atlanta, GA 30345
(404) 679-7110
fax: (404) 679-4006

5: Connecticut, Delaware,
Maine, Massachusetts, New
Hampshire, New Jersey, New
York, Pennsylvania, Rhode
Island, Vermont, Virginia, West
Virginia

P.O. Box 779
Hadley, MA 01035-0079
(413) 253-8698
fax: (413)253-8482

300 Westgate Center Drive
Hadley, MA 01035-9589
(413) 253-8628
fax: (413)253-8482

6: Colorado, Kansas, Montana,
Nebraska, North Dakota, South
Dakota, Utah, Wyoming

6
Box25486
Denver Federal Center
Denver, CO 80225
(303) 236-7890
fax: (303) 236-7901

(303) 236-7400
fax: (303) 236-0027

7: Alaska

1011 East Tudor Road
Anchorage, AK 99503
(907) 786-3300
fax: (907) 786-3313

INTERNET RESOURCES

American Association of Wildlife Veterinarians:
www.emtc.nbs.gov/http_data/whip/aawvnet.html

American Association of Zoo Veterinarians: www.worldzoo.org/aazv/aazv.htm

American Zoo and Aquarium Association, Nutritional advisory group:
www.aza.org/aza/advisory/NUTRI96.htm; a list of other advisory groups can be obtained by deleting NUTRI96.htm99

Association of Avian Veterinarians: www.aav.org

Association for Assessment and Accreditation of Laboratory Animal Care International: www.aaalac.org

National Academy of Sciences, Institute of Laboratory Animal Research, Guide for the Care and Use of Laboratory Animals: <http://www.nap.edu/readingroom/books/labrats/>

National Institutes of Health Office for Protection from Research Risk:
http://grants.nih.gov/grants/oprr/library_animal.htm (includes tutorials for Institutional Animal Care and Use Committees, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, March, 1996, and the 1996 (ILAR) Guide for the Care and Use of Laboratory Animals, National Academy of Sciences.

Ornithological Council: www.nmnh.si.edu/BIRDNET (for updates on regulations and policies affecting ornithological research, please visit the "Ornithology and Society" page or the "All about Permits" page.

Scientists Center for Animal Welfare: <http://www.scaw.com>

U.S. Fish and Wildlife Service: www.fws.gov (includes Office of Migratory Bird Management, Division of Endangered Species, Division of Refuges, and CITES Office of Management Authority)

U.S. Department of Agriculture, Animal and Plant Health Inspection, Animal Care Division
www.aphis.usda.gov (for information regarding Animal Welfare Act regulations and policies)

Wildlife Health Information Partnership: www.emtc.nbs.gov/http_data/whip/whiphmpg.html

Appendix B
American Birding Association
Code of Birding Ethics

1. Promote the welfare of birds and their environment.

1 (a) Support the protection of important bird habitat.

1 (b) To avoid stressing birds or exposing them to danger, exercise restraint and caution during observation, photography, sound recording, or filming.

Limit the use of recordings and other methods of attracting birds, and never use such methods in heavily birded areas. or for attracting any species that is Threatened, Endangered, or of Special Concern, or is rare in your local area.

Keep well back from nests and nesting colonies, roosts, display areas, and important feeding sites. In such sensitive areas, if there is need for extended observation, photography, filming, or recording, try to use a blind or hide, and take advantage of natural cover.

Use artificial light sparingly for filming or photography, especially for close-ups.

1 (c) Before advertising the presence of a rare bird, evaluate the potential for disturbance of the bird, its surroundings, and other people in the area, and proceed only if access can be controlled, disturbance minimized, and permission has been obtained from private land-owners. The sites of rare nesting birds should be divulged only to the proper conservation authorities.

1(d) Stay on roads, trails, paths where they exist; otherwise keep the habitat disturbance to a minimum.

2. Respect the law and the rights of others.

2(a) Do not enter private property without the owner's explicit permission.

2(b) Follow all laws, rules, and regulations governing use of roads an public areas, both at home and abroad.

2(c) Practice common courtesy in contacts with other people. Your exemplary behavior will generate goodwill with birders and non-birders alike.

3. Ensure that feeders, nest structures, and other artificial bird environments are safe.

3(a) Keep dispensers, water, and food clean and free of decay or disease. It is important to feed birds continually during harsh weather.

3(b) Maintain and clean nest structures regularly.

3(c) If you rare attracting birds to an area, ensure that birds are not exposed to predation from cats and other domestic animals, or dangers posed by artificial hazards.

4. Group birding, whether organized or impromptu, requires special care.

Each individual in the group, in addition to the obligations spelled out in Items #1 and #2, has responsibilities as a Group Member.

4(a) Respect the interests, rights, and skills of fellow birders, as well as people participating in other legitimate outdoor activities. Freely share your knowledge and experience, except where code 1 (c) applies.

4(b) If you witness unethical birding behavior, assess the situation, and intervene if you think it is prudent. When interceding, inform the person(s) of the inappropriate action, and attempt, within reason, to have it stopped. If the behavior continues, document it, and notify appropriate individuals or organizations.

Group Leader Responsibilities [amateur and professional trips and tours].

4(c) Be an exemplary ethical role model for the group. Teach through word and example.

4(d) Keep groups to a size that limits impact on the environment and does not interfere with others using the same area.

4(e) Ensure everyone in the group knows of and practices this code.

4(f) Learn and inform the group of any special circumstances applicable to the areas being visited (e.g., no tape recorders allowed).

4(g) Acknowledge that professional tour companies bear a special responsibility to place the welfare of birds and the benefits of public knowledge ahead of the company's commercial interests. Ideally, leaders should keep track of tour sightings, document unusual occurrences, and submit records to appropriate organization.

ABA: (800) 850-2473; fax: (800) 247-3329; e-mail member@aba.org

