Guidelines to the Use of Wild Birds in Research

Notes regarding this Reference Resource:

*This reference was adopted by the Council on Accreditation with the following clarification and exceptions:

Clarification: AAALAC International underscores the need for scientific justification and IACUC approval for blood collection by intracardiac route as a survival procedure under general anesthesia (pg 136).

Exception: AAALAC International does not endorse digit amputation as a route for blood collection but endorses nail clipping for blood collection with scientific justification and IACUC approval (pg.138).

Exception: AAALAC International does not endorse chilling of the surgical site as an acceptable analgesic (pg 176).

Exception: AAALAC International does not endorse performing a major invasive procedure (e.g. coeliotomy/laparotomy) without the use of a general anesthetic (pg. 173,174, 183, 186).

(Reference Resource begins next page...)

THE ORNITHOLOGICAL COUNCIL

Providing Scientific Information about Birds

GUIDELINES TO
THE USE OF WILD BIRDS IN RESEARCH

Special Publication 1997

Edited by

Abbot S. Gaunt & Lewis W. Oring

Third Edition 2010

Edited by

Jeanne M. Fair, Editor-in-Chief

Ellen Paul & Jason Jones, Associate Editors

GUIDELINES TO THE USE OF WILD BIRDS IN RESEARCH

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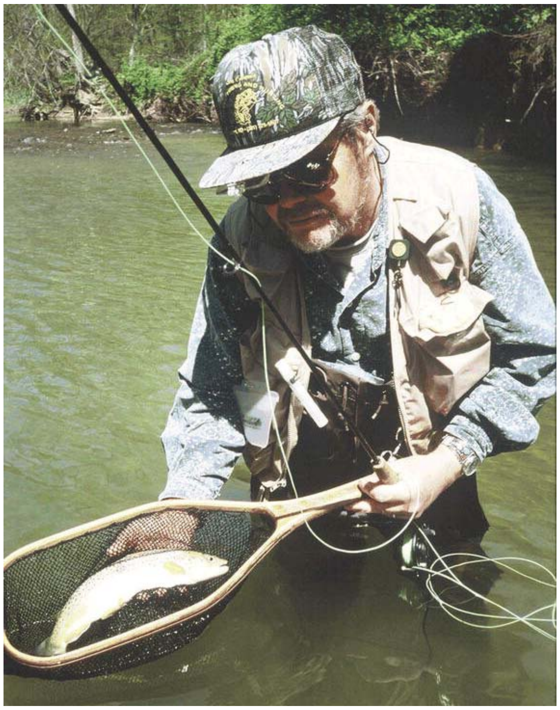
Dedication

The Ornithological Council dedicates this 2010 revision to Lewis W. Oring and the late Abbot (Toby) S. Gaunt, whose commitment to the well-being of the birds for whom ornithologists share a deep and abiding concern has served our profession well for so many years.



Toby Gaunt

Lew Oring

Acknowledgments and disclaimer

Third edition

The Ornithological Council thanks the Office of Laboratory Animal Welfare of the National Institutes of Health for their financial support for the production of this revision. In particular we are grateful to Susan Silk for her patient assistance. We also thank Carol Wigglesworth for helping us to get started. We thank the American Ornithologists' Union, the Cooper Ornithological Society, and the Wilson Ornithological Society for their financial support.

Jeanne M. Fair edited this edition with enormous skill, insight, and style. The expertise and diligent research of our section editors – Anne Barrett Clark, Clara Davie, Jeanne Fair, Jason Jones, and Gary Kaiser – resulted in a thorough and considered treatment of each topic. Additional contributions were made by Adrian del Nevo, Scott Carleton, and Ellen Paul. A number of anonymous reviewers generously devoted the time and expertise to help improve the content and presentation.

We also thank the American Zoo and Aquarium Association for sharing their housing and husbandry manuals with us and the North American Banding Council for allowing us to reprint some of their materials.

Funding for this publication was made possible in part by the Office of Laboratory Animal Welfare, National Institutes of Health, Department of Health and Human Services. Any opinions, findings, and conclusions or recommendations expressed in this publication do not necessarily reflect the views of the DHHS; nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.

First and second editions

We received information and guidance from a wide variety of sources. Among our colleagues, those who provided special help include: Richard Banks, Jim Bednarz, Fred Cooke, Sandra Gaunt, Jerry Jackson, Fred Quimby, J. Van Remsen, Margaret Rubega, and Elizabeth Ann Schreiber. We are especially grateful to specialists Carol Anderson (USFWS), August Battles, D.V.M., Rich Benardski, D.V.M., Christopher Brand, and several anonymous reviewers (NWHC), Donald Burton D.V.M., Mary Gustafson (BBL), Buddy Fazio (USFWS), James M. Harris , D.V.M., Bill Kurrey (USFWS), Sharron Martin, D.V.M., Diana McClure, D.V.M., Dan Petit (then with the USFWS), and Steve Wentz (CWS). The American Birding Association kindly provided us with an updated version of their Code of Birding Ethics even before it had been distributed to their membership. Several veterinarians gave generously of their time and expertise in contributing to the Second Edition: Pauline Wong, D.V.M., John Ludders, D.V.M., Glenn H. Olsen, D.V.M., Ph.D., David Brunson, D.V.M., and F. Joshua Dein, V.M.D.

We are grateful to the Summerlee Foundation of Dallas, Texas for funds to support the production of this document, and to the Biological Resources Division of the U.S. Geologic Survey for funds to support its publication.

When mentioned, brand names are exemplary. Some named products were drawn to our attention by investigators or veterinarians who have individually found them useful, but in no case is an endorsement by the Ornithological Council or any other ornithological society implied.

About 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 requires 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, regulators, land managers, conservation organizations, and private industry to assure tat the scientific information needed for decision making that affects birds is available.

The Council was founded in 1992 by seven ornithological societies in North America: American Ornithologists' Union, Association for Field Ornithology, Cooper Ornithological Society, Pacific Seabird Group, Raptor Research Foundation, Waterbird Society and Wilson Ornithological Society. In recent years, the Society of Canadian Scientists, the Society for the Conservation and Study of Caribbean Birds, the Neotropical Ornithological Society, and CIPAMEX have become members.

Suggested citation

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With the publication of the 2010 revision of Guidelines to the Use of Wild Birds in Research, the print version is discontinued. We encourage you to cite the internet version by including the URL (www.nmnh.si.edu/BIRDNET/guide) and the date accessed, including the given date of any updates.

Questions and comments:

We welcome your comments. Suggestions for substantive changes will be reviewed by a committee and if accepted, will be incorporated into the Guidelines. Questions are also welcome. Send comments to: ellen.paul@verizon.net.

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CHAPTER I. INTRODUCTION

A. Overview

Context: the study of wild birds

Ornithologists study wild birds to fill the need and desire to understand the lives of birds in natural environments. Although some studies of wild birds take place in laboratories and aviaries, a growing number of studies of fundamental scientific issues such as behavioral ecology and ecophysiology are conducted on wild animals under natural conditions, as scientists have come to understand the limitations of laboratory and captive work in those areas. Studies are also undertaken for the express purpose of developing appropriate conservation or management strategies in a world in which most species face challenges resulting from anthropogenic changes to the landscape. In either case, the birds studied benefit from that research, as do other species that share their habitats. Often the individual study animals themselves benefit from the research. Whether the primary motivation of ornithological study is the advancement of scientific knowledge or the acquisition of information used for management purposes, wildlife research yields results that are directly relevant to the welfare and conservation of the species, communities, and ecosystems studied. Indeed, species conservation would not be possible without a solid base of information derived from field studies and it could be argued that conservation decisions and actions made without the benefit of a scientific basis could be ineffective or even harmful.

The deep appreciation of and concern for birds that motivates ornithologists to dedicate their research careers to this underappreciated and underfunded research discipline is also expressed in their concern for the impacts of the research on the birds they study. And from a purely practical standpoint, they also realize that they must minimize the impacts of research methods to ensure that the scientific results are valid. They also realize that their methods will be scrutinized and judged not only by the Institutional Animal Care and Use Committees, but also by journals and by the general public.

These Guidelines are formulated with consideration of animal welfare and research needs in the context of these premises, and in the context of the conditions under which wild birds are studied. Guidelines for the care of animals bred in captivity for use in biomedical research generally are not appropriate to wild vertebrates studied in the field or even in captivity. Studies of wild animals entail conditions that are not encountered in laboratory situations. The ordinary conditions of field work may limit the amount and type of equipment that can be carried or necessitate the use of methods that entail some risk of harm, even when used correctly. Considerations such as the presence of other species and the way the investigation may affect those species, or the way those species may interact with the study species, may arise. Conditions in the field vary so much that it is inappropriate, if not impossible, to identify a best method or unacceptable methods. The guiding principle for these Guidelines and for ornithologists is to always appreciate the potential impacts and select methods that minimize impacts without jeopardizing the ability to collect data needed to answer the research question.

History of Guideline to the Use of Wild Birds in Research

The ornithological profession has long been diligent about assessing the impacts of research methods and has sought to modify methods to reduce impacts or to find alternative methods. Consistent with these interests in the advancement of scientific knowledge, bird conservation, and the well-being of individual birds and bird populations, the American Ornithologists' Union in 1975 first published the *Report of the American Ornithologists' Union ad hoc Committee on the Scientific and Educational Use of Wild Birds*. In 1988, the American Ornithologists' Union, the Cooper Ornithological Society, and the 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*. When the Ornithological Council was formed by these societies, together with the Waterbird Society, the Raptor Research Foundation, and the Association of Field Ornithologists, responsibility for periodic revision of *Guidelines* was assigned to the Ornithological Council. A major revision was published in 1997, followed by a minor revision in 1999. Each iteration has been peer-reviewed, as has this current revision.

In 2007, the Ornithological Council embarked upon this major revision for the following reasons:

1. A clear, strong commitment to humane research methods is necessary to insure the public and policy makers that the profession is adequately self-policing. It is important to address the concerns of oversight organizations and the public fully and fairly. Self-scrutiny and re-assessment are critical in assuring that researchers develop and use the most humane research methods available.

2. The need for science-based standards is increasingly important. Science-based standards will be valuable to federal agencies that regulate wildlife research and to animal care and use committees in determining whether a practice is appropriate.
3. Institutional Animal Care and Use Committees (Institutional Animal Care and Use Committee) are intensifying their scrutiny of research protocols. A published, peer-reviewed source such as *Guidelines* may be their only source of information about research involving wild birds, and especially about ornithological research under field conditions. Thus, it is important that *Guidelines* be as comprehensive and current as possible.
4. New methods and new data on traditional methods for studying wild birds are vital for ornithologists and others who study wild birds. This information will help ornithologists to learn about new or alternative methods that may reduce the impact of the research on wild birds, or to avoid traditional methods that have been determined to have negative impacts that might be avoided. Studies published since the 1999 edition may have assessed impacts that had not been studied previously, or not well-understood.
5. The permitting section, which was incomplete and outdated, is being supplanted by a separate series of publications on permits that provide far more detail than can be accommodated in this document. This series should be complete, or nearly so, by the time this revision has been published. In addition, the Ornithological Council members are from or work in countries throughout the Western Hemisphere. We intend to provide detailed permit information for every country in the Western Hemisphere on BIRDNET, the Ornithological Council Permits webpage.

Outcome-oriented approach

These Guidelines are outcome-oriented. The intent is to examine the kinds of impacts that result from research methods, with the goal of eliminating or minimizing those impacts. Researchers should always strive to use the method that eliminates or reduces impact to the maximum extent, consistent with the purpose of the research. In other words, we advocate the consistent application of the refinement principle: choose methods to lessen or eliminate stress, pain and suffering, and to make the animals more comfortable. This refinement principle is one of the “3 Rs” that became the touchstone of animal research after publication of The Principles of Humane Experimental Technique (Russell and Burch 1959). Based on a scientific study of humane technique pertaining to research involving laboratory animals, the first principle calls for

replacement, which has, in recent years, come to mean replacement with non-animal models such as cells, tissue culture, and computer-based models. This principle has been of some practical application in biomedical research. In wildlife research, of course, animals studied are the objects of the research rather than the subjects. Replacement is rarely an option in wildlife research. Animal ecologists have modeled some aspects of behavioral ecology but at some point, hypotheses developed with models are field-tested to determine the extent to which the model explains behavior of actual animals. When studying endangered species, closely related species are sometimes used. Generally, though, replacement is not an option in wildlife biology. **Reduction** – the second principle – calls for methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures or for obtaining more information from a given number of animals so that fewer animals are needed to complete a given research project. The number of animals used should be the minimum that is consistent with the aims of the experiment. Achieving this end requires careful statistical planning. Those who lack adequate training in biostatistics would need to consult with a biostatistician to determine the appropriate number of animals or samples needed for the study. A study with an inadequate sample size that results in the failure of the study or a study that can't be completed or published has actually increased the number of animals used in research without a gain in knowledge. The third principle – **refinement** –entails the use of methods that alleviate or minimize potential pain and distress and that enhance animal well-being. Refinement should be the guiding paradigm for all wildlife research, which entails choosing the method that will generate the information needed while alleviating or minimizing negative impacts. So, for instance, this might entail a reduction in handling time or the selection of alternate means to obtain material for genetic sampling.

We provide peer-reviewed information about methods to achieve outcomes based on the refinement principle. We do not identify best practices, because research conditions – particularly in field settings - vary greatly, the physiology and behavior of bird species varies greatly, and the purpose of the research may require the use of a particular technique and so no one method or technique is suitable in all circumstances. Where appropriate, we identify practices that require exceptional justification.

Due to the considerable anatomical, behavioral, and physiological diversity of the birds species, 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. This approach is consistent with that taken by the Guide to the Care of Use of Laboratory Animals (ILAR Guide), published by the National Research Council. The ILAR Guide "charges users of research animals with the responsibility of achieving specified outcomes" but leaves it to the researcher to determine how best to achieve that outcome. As the ILAR Guide says, this "performance approach is desirable because many variables (such as the species and previous history of the animals, facilities, expertise of the people, and research goals) often make prescriptive approaches impractical and unwarranted." The performance-based standard necessarily relies upon the professional judgment of the researcher. In these *Guidelines*, then, we report research-based discussion of various methods for the purpose of providing the researcher with the knowledge needed to exercise professional judgment, but the inclusion of a method does not imply that it is appropriate for a given species, set of circumstances, or research questions. Conversely, the omission of discussion of a method does not imply that it is not acceptable. More likely, an omission signifies only the absence of published information. An Institutional Animal Care and Use Committee requires justification of the choice of a particular method, whether it is considered a "standard" method, a variant of a standard method, or a newly developed method.

Most of the scientific papers discussed in the text describe methods, and we provide a resource list that includes many references on field techniques but this Guide is not intended to be a complete reference on techniques and procedures.

Practical limitations and general guidance for application

These Guidelines include current information about techniques relevant to birds and policies relevant to ornithological research. Advances in methods and changes to policy will require future amendments. For that reason, these Guidelines will be supplemented continually through updates (on BIRDNET) as needed. The Ornithological Council will maintain a literature database accessible to individual members of the member societies of the Ornithological Council and to members of Institutional Animal Care and Use Committees and officials of federal and state agencies upon request.

The Ornithological Council will provide the text of Guidelines free of charge and will make a Spanish translation available. We hope to provide other translations if possible.

The Ornithological Council encourages members of Institutional Animal Care and Use Committees to contact the Ornithological Council for information about specific research methods and for referrals to ornithologists with expertise as to specific methods and species.

Discussion of policy and procedure focuses on the United States. Seven of the eleven member societies of the Ornithological Council are based in the United States; though some are international in scope and the members of these scientific societies study birds everywhere in the world. When they conduct research in the United States or receive funding from a federal agency, even if the research takes place outside the United States, they must comply with United States law. The system of statutes, regulations, and procedures in the United States that mandate the scrutiny of research involving animals is perhaps the most elaborate and rigorous in the Western Hemisphere. The basic principles of animal welfare - particularly the reduction, replacement, and refinement principles - are universal, as is the science.

B. Regulatory agencies and other organizations

United States

Government frameworks for the agencies and organizations that regulate or oversee ornithological research vary from one country to another. In the United States, four federal agencies and fifty state agencies oversee research on wild birds. The U.S. Fish and Wildlife Service requires permits pursuant to the mandates of the Migratory Bird Treaty Act or the Endangered Species Act, though the Bird Banding Laboratory of the U.S. Geological Survey issues permits for bird marking. Nearly all of the fifty states require permits for research involving wild birds. The federal and state laws implemented by these agencies are intended to protect bird populations, though the permit regulations in the United States allude very briefly to humane conditions for live wildlife possessed under a permit. Substantial detail about permitting requirements is provided on BIRDNET.

The Animal Welfare Act, as initially enacted by Congress in 1970 (P.L.91-579) and later amended in 1976 (P.L.94-279) and now codified in the U.S. Code at 7 U.S.C. 2131 *et seq.* is intended to “insure that animals intended for use in research facilities or for exhibition purposes or for use as pets are provided humane care and treatment.” The U.S. Department of Agriculture, Animal and Plant Health Inspection Service Animal Care program implements this law by issuing and enforcing regulations (9 C.F.R. 2.1 *et seq.*). As explained more fully below, the regulations do not, at the time of this revision, apply to birds. The Animal Care program is in the process of developing regulations for research pertaining to birds. These regulations likely will be proposed in early 2010 for public comment.

Under the Health Research Extension Act of 1985 (P.L. 99-158, codified at 42 U.S.C.289d), the director of the National Institutes of Health established guidelines for the proper care and use of animals used in biomedical and behavioral research. Though this statute and the guidelines do not apply to most ornithological research, universities adhere to these guidelines and apply them to all research involving live vertebrates because to maintain eligibility to receive grants and contracts from the National Institutes of Health, they must agree to do so. The policy, known as the Public Health Service Policy on Humane Care and Use of Laboratory Animals, is overseen by the Office of Laboratory Animal Welfare of the National Institutes of Health. Other federal funding agencies, such as the National Science Foundation, voluntarily adhere to these standards and compliance is a condition of receiving grants.

The Animal Welfare Act (and its implementing regulations) varies in some respects from the Health Research Extension Act and the Public Health Service Policy, primarily with regard to procedural requirements. To avoid conflict and duplication, the U.S. Department of Agriculture and the National Institutes of Health have, by agreement, assigned oversight of research issues to the National Institutes of Health.

In the United States, federal agencies adhere to an interagency policy known as the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. These principles govern the use of animals in research conducted by federal agencies.

The Institute of Laboratory Animal Research of the National Research Council publishes the *Guide for the Care and Use of Laboratory Animals* (*ILAR Guide*)*,* a leading guidance document that elaborates upon the underlying philosophy and basic principles for appropriate care of research animals. This *Guide* discusses field investigations in a very cursory manner, but is nonetheless used by Institutional Animal Care and Use Committees in assessing research protocols for field studies. At the time of this revision, the *Guide* itself was undergoing revision. The Institute, which also publishes a quarterly journal, has no oversight or regulatory functions

Canada

The Canadian Wildlife Service implements that country's Migratory Birds Convention Act; the Bird Banding Office of the issues permits for marking birds and the provincial and territorial offices issue permits for other research activities.

In Canada, oversight of animal welfare in research falls to the Canadian Council on Animal Care. This non-governmental organization was created when the Medical Research Council of the Canadian Institutes of Health requested that the National Research Council (the Government of Canada's premier organization for research) establish a committee to investigate the care and use of experimental animals in Canada. In 1968, following the Committee's recommendation to create a voluntary control program exercised by scientists in each institution, subject to peer review and committed to implementing the guiding principles of an independent advisory body, the Canadian Council on Animal Care (Canadian Council on Animal Care) was established. The Canadian Council on Animal Care was incorporated as a non-profit, autonomous and independent body in 1982. It receives most of its funding from the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council (NSERC), with additional contributions from federal science-based departments and private institutions.

The Canadian Council on Animal Care mission statement underlines the focus of the organization on the ethical principles of animal-based experimentation:

> The purpose of the Canadian Council on Animal Care is to act in the interests of the people of Canada to ensure through programs of education, assessment and persuasion that the use of animals, where necessary, for research, teaching and testing employs optimal physical and psychological care according to acceptable scientific standards, and to promote an increased level of knowledge, Animal Welfare Act, and sensitivity to relevant ethical principles.

The Canadian Food Inspection Agency enforces the regulations through routine inspections, unannounced site inspections and response to reports of non-compliance. Federal and provincial laws prohibit cruelty to animals; most entail criminal sanctions.

Private organizations

Private organizations also play a role in assuring the welfare of animals studied in scientific research. Principle among them is the Association for the Assessment and Accreditation of Laboratory Animal Care International. Virtually all U. S. academic and research institutions belong to this organization and seek accreditation by meeting its exacting standards. It is the only private accrediting organization recognized by the Public Health Service of the U.S. Department of Health and Human Services. The Scientists Center for Animal Welfare and PRIM&R (Public Responsibility in Medicine and Research; its membership arm known as the Applied Research Ethics National Association is now fully subsumed into PRIM&R) are membership organizations that advance ethical standards in the conduct of research involving live animals through training, workshops, and publications.

International organizations

Efforts to develop international, harmonized standards for the care and treatment of animals used in research are underway. The International Council for Laboratory Animal Science dates to 1955, when the International Union of Biological Sciences appointed an international committee to study the problems that existed within those scientific fields in which live animals were used in experimental procedures. Later that year the United Nations Educational, Scientific and Cultural Organization requested information on the production and use of laboratory animals in various countries. These two initiatives resulted in agreement to establish an independent non-governmental scientific committee with the aim to raise the standards in the use of laboratory animals on a global basis. It was in this way and under the auspices of these two organizations that the International Council for Laboratory Animal Science was established in 1956. The Ornithological Council joined this international body in 2007 to represent scientific ornithology because so many of the members of the societies that comprise the Council conduct their research outside the United States. To date, the International Council for Laboratory Animal Science has focused primarily on biomedical research and on oversight procedures rather than substantive standards, but in anticipation of the eventual inclusion of field biology, the Ornithological Council seeks to become an authoritative source of information for this and other, similar multinational efforts.

C. The oversight of research involving animals: legal basis and implementation

United States

In the United States, protection of animals studied in research is overseen by Institutional Animal Care and Use Committees, who derive their authority from two sources: the Animal Welfare Act (7 U.S.C. 2131 *et seq.*) and the Health Research Extension Act of 1985 (P.L. 99-158) which amended the Public Health Service Act and is now codified at 42 U.S.C. 289(d). When first enacted in 1966, the Animal Welfare Act established a system for inspection of facilities that bred or sold animals for research and of the research labs. Over time, it was amended to include oversight of research methods. The terms of the statute give it very broad and comprehensive application:

"The term ``research facility'' means any school (except an elementary or secondary school), institution, or organization, or person that uses or intends to use live animals in research, tests, or experiments, and that (1) purchases or transports live animals in commerce, or (2) receives funds under a grant, award, loan, or contract from a department, agency, or instrumentality of the United States for the purpose of carrying out research, tests, or experiments…" The Public Health Service Act required the Director of the National Institutes of Health to establish guidelines for the proper care and treatment of animals used in research and also required that every institution receiving funding from the National Institutes of Health to assure that agency that it would comply with those guidelines. In 1986, the National Institutes published those guidelines, known as the Public Health Service Policy on Humane Care and Use of Laboratory Animals. These guidelines, since updated at least twice, require that "In order to approve proposed research projects or proposed significant changes in ongoing research projects, the Institutional Animal Care and Use Committee shall conduct a review of those components related to the care and use of animals and determine that the proposed research projects are in accordance with this Policy. In making this determination, the Institutional Animal Care and Use Committee shall confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the [ILAR] Guide [to the Care and Use of Laboratory Animals] unless acceptable justification for a departure is presented."

Other federal agencies that fund research adopted these rules on a voluntary basis. For instance, the National Science Foundation Award and Administration Guide provides that:

> Any grantee performing research on vertebrate animals shall comply with the Animal Welfare Act [7 U.S.C. 2131 et seq.] and the regulations promulgated thereunder by the Secretary of Agriculture [9 CFR 1.1-4.11] pertaining to the humane care, handling, and treatment of vertebrate animals held or used for research, teaching or other activities supported by Federal Animal Welfare Act. The Animal Welfare Act is expected to ensure that the guidelines described in the National Academy of Science (NAS) [ILAR] Publication, "*Guide for the Care and Use of Laboratory Animals*" (1996) are followed and to comply with the *Public Health Service Policy and Government Principles Regarding the Care and Use of Animals* (included as Appendix D to the NAS Guide).

The National Science Foundation Grant Proposal Guide provides that:

> Any project proposing use of vertebrate animals for research or education shall comply with the Animal Welfare Act [7 U.S.C. 2131 et seq.] and the regulations promulgated thereunder by the Secretary of Agriculture [9 CFR 1.1-4.11] pertaining to the humane care, handling, and treatment of vertebrate animals held or used for research, teaching or other activities supported by Federal awards. In accordance with these requirements, proposed projects involving use of any vertebrate animal for research or education must be approved by the submitting organization's Institutional Animal Care and Use Committee (Institutional Animal Care and Use Committee) before an award can be made. For this approval to be accepted by NSF, the organization must have a current Public Health Service (PHS) Approved Assurance.

The Departments of Defense, the National Aeronautic and Space Administration, the U.S. Department of Agriculture, and other grant-making agencies have similar policies.

Technically, then, if a research project does not involve the transport or purchase of animals across state lines, and if the facility receives no federal funding, then the Animal Welfare Act is not applicable. In that case, while ornithologists or research facilities may not need to follow the procedural mandates of the Animal Welfare Act, they should still adhere to the principles of appropriate care and use. These facilities might also want to consider establishing a review board of the nature of an Institutional Animal Care and Use Committee, with one or more scientists unaffiliated with the facility assessing the research protocols used by the researchers of that facility. The absence of federal requirements should be considered as no more than an absence of paperwork and reporting burdens, but the basic review procedures and substantive standards established by the Animal Welfare Act regulations and by documents such as these *Guidelines* should be considered best practices. A research organization would be well advised to document its self-proscribed procedures and its adherence to those procedures to assure itself and its staff, supporters, and the public that it takes seriously its commitment to the appropriate care and use of the animals studied by its researchers.

Some research facilities that are not legally subject to the requirements of the Animal Welfare Act and the Public Health Service Act have investigated the possibility of asking the Institutional Animal Care and Use Committee of nearby universities or other research organizations to review their research protocols. Most universities are unwilling to do so, in part because their own committees, comprised of volunteers, are already overtaxed. Universities and other research organizations also shy from accepting this responsibility because they are required to provide a formal "assurance" document to the National Institutes of Health (Office of Laboratory Animal Welfare) committing to adhere to and implement numerous laws, regulations, and policies, including review of research protocols, facilities inspections, record-keeping, and reporting requirements. Eligibility for funding from the Public Health Service of the U.S. Department of Health and Human Services is conditioned upon fulfillment of the assurance. A university or research organization, having no authority or oversight over another organization, would not want to risk its eligibility for federal funding by voluntarily accepting any level of responsibility for the activities of that organization.

Are birds covered?

Ornithologists in the United States know that their research has always been regulated, notwithstanding the fact that research involving birds – wild, captive, or bred-in-captivity, is not covered by the Animal Welfare Act or the implementing regulations, though regulations likely will be promulgated by 2009. The regulation of ornithological research in the United States stems from the policies of the Public Health Service, which cover all live vertebrates, and that determine eligibility for federal research funding.

The Animal Welfare Act (Animal Welfare Act) as originally enacted in 1966 (P.L. 89-544) did not include birds. The 1970 amendments (P.L.91-579) defined animals to be covered under the Animal Welfare Act as "any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal, as the Secretary may determine is being used, or is intended for use, for research, testing, experimentation, or exhibition purposes…" Until 1998, the regulations issued by the Secretary of Agriculture excluded rats, mice, and birds from Animal Welfare Act implementation. Litigation filed by an animal welfare organization prompted the Secretary of Agriculture to announce that these taxa would be included and that the Animal and Plant Health Inspection Service would issue implementing regulations. Subsequent legislative directives enacted by Congress halted that process by amending the Animal Welfare Act to made permanent the exclusion of rats, mice, and birds (Pub. L. 107-171, Section 10301), now codified at 42 U.S.C. 2131(g) as follows:

> (g) The term "animal" means any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal, as the Secretary may determine is being used, or is intended for use, for research, testing experimentation, or exhibition purposes, or as a pet; but such term excludes (1) birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research…

This provision was intended to codify the original regulation promulgated by the Department of Agriculture to exclude rats, mice, and birds. Unfortunately, a printer's error, in the form of an insertion of a comma prior to the word "bred" caused the Animal and Plant Health Inspection Service Animal Care staff to interpret the new statutory language to mean that the condition "bred for use in research" applied to birds as well as rats and mice. The USDA then prepared to promulgate regulations accordingly. The Ornithological Council sought to have this error corrected by way of a revision in the 2007 Farm Bill; counsel for the Senate Agriculture Committee agreed that it had been a printer's error and should be corrected. Despite strenuous efforts by the Ornithological Council, the Congress declined to correct this error. The USDA will now proceed to draft new regulations pertaining to research involving wild birds, whether studied in the field or the lab. The proposed regulation should be published for comment in 2010.

The Ornithological Council believes strongly that birds, both wild and captive-bred, should be treated humanely, both in the laboratory and in research conducted in the wild. It is for this reason that we publish this peer-reviewed Guidelines to the Use of Wild Birds in Research. Our objection to the inclusion of birds in the Animal Welfare Act regulations is based solely on the fact that it is likely to impose additional burdens on research without producing an *improvement in the humane treatment of birds, because, as explained below, this research is already regulated under the Health Research Extension Act of 1985, which makes the Animal Welfare Act applicable to all vertebrates. We object only to duplicative and potentially conflicting sets of regulations and burdensome procedural compliance, without contributing to the humane treatment of birds in research.*

Are field studies covered?

The Animal Welfare Act regulations define "field study" as a study conducted on free-living wild animals in their natural habitat. Under the implementing regulations, this definition excludes any study that involves an invasive procedure, harms, or materially alters the behavior of an animal under study" (9 CFR 1.1). The U.S. Department of Agriculture has declined to define the terms "invasive procedure," "harms," and "materially alters the behavior." Read broadly, only purely observational studies would constitute field studies.

Field studies are, under the Animal Welfare Act regulations [9 CFR 2.31(c)(2) and 9 CFR 2.31(d)] exempt from the site inspection and protocol review procedures. However, ornithologists will nearly certainly find that their institutions require review of all studies, even class bird walks. Ornithologists should understand that institutions receiving federal funding are required under the PHS policy to "assure" that *all* of the institution's programs and facilities have been evaluated. To comply with the terms of the "assurance" the institution must require that all protocols be submitted, even if the specific study methods are not further evaluated. Also note that the PHS policy does not exclude field study. According to the Office of Laboratory Animal Welfare:

> If the activities are PHS-supported and involve vertebrate animals then the Institutional Animal Care and Use Committee is responsible for oversight in accord with PHS Policy. Institutional Animal Care and Use Committees must know where field studies will be located, what procedures will be involved, and be sufficiently familiar with the nature of the habitat to assess the potential impact on the animal subjects. Studies with the potential to impact the health or safety of personnel or the animal's environment may need Institutional Animal Care and Use Committee oversight, even if described as purely observational or behavioral. When capture, handling, confinement, transportation, anesthesia,

euthanasia, or invasive procedures are involved, the Institutional Animal Care and Use Committee must ensure that proposed studies are in accord with the Guide for the Care and Use of Laboratory Animals (the "ILAR Guide"). The Institutional Animal Care and Use Committee must also ensure compliance with the requirements of pertinent state, national and international wildlife regulations.

The National Science Foundation Award and Administration Guide expressly includes field study without defining the term: "The grantee is responsible for the humane care and treatment of any vertebrate animal used or intended for use in such activities as field or laboratory research, development, training, experiments, biological testing or for related purposes supported by NSF grants."

Discussions of the legality of authority over field studies are largely irrelevant. The respectful and ethical treatment of animals does not depend on legality. And in practice, research institutions require the submission for review and approval of all research protocols. Ornithologists sometimes chafe about being required to submit protocols for purely observational work, such as point counts and song recording – or even bird walks for students, which involve no research whatsoever. Realize that the institution is taking measures that it perceives to be necessary to comply with the terms of its assurance to the National Institutes of Health, and thus to maintain its eligibility for federal funding. The purpose of requiring review of proposals for purely observational work is to assure that in fact the work is observational in nature and that no further review is needed. Unless the protocol is submitted for review, the research institution cannot know what research is being conducted. However, the purpose of these reviews is to determine that no further review is needed; there is rarely additional scrutiny.

Generally, these agencies and the Institutional Animal Care and Use Committees do not require inspection of field study sites, partly because it would be impractical, if not impossible, to send Institutional Animal Care and Use Committee members to field sites, which may be very distant from the university and that may not be stationary. Furthermore, study site inspection is, under the regulations, limited to "any building room, area, enclosure, or other containment outside of a core facility or centrally designated or managed area in which animals are housed for more than 12 hours." Researchers should know, however, that universities often regard these mandates as minimum standards and not as constraints, and so frequently require more of the researcher than the law suggests. So, for instance, some Institutional Animal Care and Use Committees ask researchers to carry videotape equipment into the field to record one or more actual procedures. This request may be burdensome, in that researchers may not have enough field assistants to carry additional equipment and to videotape procedures. Researchers may want to consider proposing alternative demonstrations, such as a mist-netting and banding demonstration in an area near the campus.

Application of the Animal Welfare Act outside the United States

Researchers receiving funding from an agency of the United States government, or working at institutions that receive federal funding should note that even if research takes place outside the United States, protocol review and approval by the Institutional Animal Care and Use Committee. The Grant Policy of the National Science Foundation expressly provides that, "(iv) awards to U.S. grantees for projects involving the care or use of vertebrate animals at a foreign institution or foreign field site also require approval of research protocols by the U.S. grantee's Institutional Animal Care and Use Committee. If the project is to be funded through an award to a foreign institution or through an individual fellowship award that will support activities at a foreign institution, NSF will require a statement of compliance that the activities will be conducted in accordance with all applicable laws in the foreign country and that the International Guiding Principles for Biomedical Research Involving Animals will be followed." See "Vertebrate Animals" in the National Institutes of Health Animal Award and Administration Guide. The Public Health Service Policy on the Humane Care and Use of Laboratory Animals issued by the National Institutes of Health provides that, "This Policy is applicable to all PHS-conducted or supported activities involving animals, whether the activities are performed at a PHS agency, an awardee institution, or any other institution and conducted in the United States, the Commonwealth of Puerto Rico, or any territory or possession of the United States. Institutions in foreign countries receiving PHS support for activities involving animals shall comply with this Policy, or provide evidence to the PHS that acceptable standards for the humane care and use of the animals in PHS-conducted or supported activities will be met. No PHS support for an activity involving animals will be provided to an individual unless that individual is affiliated with or sponsored by an institution which can and does assume responsibility for compliance with this Policy, unless the individual makes other arrangements with the PHS."

Overview of the Institutional Animal Care and Use Committee system

The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires that all institutions subject to the Policy (those receiving funding from the National Institutes of Health; as noted above, other federal funding agencies have adopted this same policy) establish an Institutional Animal Care and Use Committee consisting of five members including a veterinarian, a scientist experienced with animal research, a nonscientist (such as a lawyer, an ethicist, or a member of the clergy), and an individual who is not affiliated with the institution in any way. The Institutional Animal Care and Use Committee must review all protocols for research supported by agency funding to " confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the Guide unless acceptable justification for a departure is presented. The regulations (9 CFR 2.31) that implement the Animal Welfare Act establish the specific issues to be considered by the Institutional Animal Care and Use Committee in reviewing research protocols. These considerations, which may not be applicable in some field research situations, are as follows:

a. Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.

b. The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources used to determine that alternatives were not available;

c. The principal investigator has provided written assurance that the activities do not unnecessarily duplicate previous experiments;

d. Procedures that may cause more than momentary or slight pain or distress to the animals will:

(1) Be performed with appropriate sedatives, analgesics or anesthetics, unless withholding such agents is justified for scientific reasons, in writing, by the principal investigator and will continue for only the necessary period of time;

(2) Involve, in their planning, consultation with the attending veterinarian or his or her designee;

(3) Not include the use of paralytics without anesthesia;

e. Animals that would otherwise experience severe or chronic pain or distress

that cannot be relieved will be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

f. The living conditions of animals will be appropriate for their species and contribute to their health and comfort. The housing, feeding, and nonmedical care of the animals will be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied.

g. Medical care for animals will be available and provided as necessary by a qualified veterinarian.

h. Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures.

i. Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques. Major operative procedures on non-rodents will be conducted only in facilities intended for that purpose which shall be operated and maintained under aseptic conditions. Non-major operative procedures and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic procedures. Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures.

j. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless:

(1) Justified for scientific reasons by the principal investigator, in writing;

(2) Required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian; or

(3) In other special circumstances as determined by the Administrator on an individual basis. Written requests and supporting data should be sent to the Animal and Plant Health Inspection Service, Animal Care, 4700 River Road, Unit 84, Riverdale, Maryland 20737-1234;

k. The humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death, unless a deviation is justified for scientific reasons, in writing, by the investigator. In practice, methods of euthanasia consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia are considered acceptable.

Significant changes to ongoing research must also be reviewed and approved.

To these considerations, the Ornithological Council suggests that ornithologists also consider these issues when developing their research protocols

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

b. 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 in the country where the research is to be conducted. Authors should include in all published papers, reports, and presentations a statement that the necessary permits were obtained.

c. 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 are applicable to a particular study. Removal from the wild of adults that may be tending nests, chicks, or dependent fledglings should, as a general principle, be avoided unless justified for scientific reasons.

d. Studies should use the fewest animals necessary to reliably answer the questions posed. An adequate sample size will prevent unnecessary repetition of the study, thus avoiding additional impacts on wild birds.

e. Every effort should be made prior to removal of animals to understand the population status of the taxa to be studied. The number of individuals removed from the wild must be kept to the minimum determined by the investigator to be 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. This issue is discussed in more detail in the section on Scientific Collecting.

f. Procedures that are likely to have lasting effects on populations should e undertaken with 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.

g. Researchers should plan to salvage birds where accidental mortality occurs, for deposit as specimens in museums or teaching collections. The usefulness of specimens should be maximized by saving as much material as possible, including skins, carcasses, skeletons, fluids, tissues, and DNA samples. Researchers should learn methods for preserving and labeling specimens and should have the necessary materials and equipment available.

h. The principal investigator must ensure that all personnel associated with the project have been properly trained. Students and technicians must be required to ask questions and seek assistance. Anyone wishing to use an unfamiliar technique must seek advice from an expert and, if possible, to visit that expert and practice the technique under the guidance of the expert. Appropriate expertise may be found in the academic and wildlife management communities, the zoo and aquarium communities, and among aviculturists.

Standards of review for field studies: a note for ornithologists

Most guidance available to Institutional Animal Care and Use Committees pertains primarily to biomedical research or research in the controlled environment of a laboratory. The Institute for Laboratory Animal Research of the National Research Council of the National Academies of Science publishes the Guide for the Care and Use of Laboratory Animals. As the title suggests, the discussion pertaining to wild animals studied in the field or in the laboratory is minimal. Nonetheless, this resource, together with the Institutional Animal Care and Use Committee

Guidebook published jointly by the Applied Research Ethics National Association and the Office of Laboratory Animal Welfare of the National Institutes of Health is the primary source of standards and guidance for Institutional Animal Care and Use Committees. This Guidebook, which suggests that Institutional Animal Care and Use Committees consult relevant experts, alludes to these Guidelines and to the Ornithological Council. Ornithologists are encouraged to ask Institutional Animal Care and Use Committee members to consult with the Ornithological Council should the Institutional Animal Care and Use Committee desire additional information about a proposed research method; we will refer them to ornithologists with relevant expertise and provide literature and other information as may be available.

For various reasons, field biologists rarely serve on Institutional Animal Care and Use Committees. As a result, it is often necessary for the ornithologist to help Institutional Animal Care and Use Committee members to understand the nature of research in field conditions. In addition, the ornithologist should be prepared to provide evidence – from these *Guidelines* and the supporting literature – of the known impacts (or lack of impacts) of field research methods. In other words, the ornithologist should regard the protocol review as an opportunity to impart information and to educate. Approaching the protocol review as an adversarial proceeding serves no purpose, and is generally detrimental.

The Ornithological Council strongly encourages field biologists to serve on these committees, which are intended to allow scientific research to be assessed through a peer review system. If field biologists do not serve, there may be no committee members who have the expertise to serve as true peers.

Standards of review for field studies: a note for Institutional Animal Care and Use Committees

Field biology takes place in uncontrolled and usually uncontrollable environments that differ drastically from laboratory work. The Institutional Animal Care and Use Committee must necessarily consider procedures and techniques that are practical for implementation at the site of the research. 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. Vertebrates typically used in laboratory research represent a small number of species with well understood husbandry requirements. By contrast, the class Aves contains at least 10,000 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 design. Therefore, in most cases it is impossible to generate specific guidelines for groups larger than a few closely related species. The stipulation of specific guidelines could actually inhibit humane care, as well as research, by imposing inappropriate requirements. 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).

When studies on wild birds are to be reviewed, the Institutional Animal Care and Use Committee should attempt to include personnel who understand 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 Institutional Animal Care and Use Committee should, therefore, attempt to include at least one institution-appointed member who is experienced in wildlife biology. 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 Institutional Animal Care and Use Committee, though such consultants are not permitted to vote. The Ornithological Council is willing and able to identify experts for consultation with Institutional Animal Care and Use Committees.

Population-level impacts

A particular subject of concern involves oversight of the impact of the proposed research at the population level. There is no legal authority for the assessment of population-level impacts by Institutional Animal Care and Use Committees. Neither the Animal Welfare Act nor the Public Health Research Extension Act of 1985, nor the regulations or policies issued pursuant to those statutes, mention population-level impacts. Nonetheless, there is no official recognition or acceptance by government agencies, private organizations, or individual Institutional Animal Care and Use Committees, that there is no need or authority for the assessment of population-level impacts. To the contrary, questions about population impact are routinely asked.

To address this situation, the Ornithological Council has organized meetings and participated in training programs for agency officials and members of Institutional Animal Care and Use Committees to impart information about permit requirements and to assure these oversight entities that the permit systems that implement the Migratory Bird Treaty Act and the Endangered Species Act, as well as state laws, are intended to address population impacts. The issuance of permits by the U.S. Fish and Wildlife Service, the U.S. Bird Banding Laboratory of the U.S. Geological Survey, and state agencies signifies that these agencies – who possess the expertise to make such assessments – have determined that the permitted activity will not affect bird populations or that any such impacts are merited by the need for the scientific information that will be generated. When the Applied Research Ethics National Association (now known as Public Responsibility in Research & Medicine) revised its Institutional Animal Care and Use Committee Guidebook, the editors graciously included submission of text by the Ornithological Council explaining the permit requirements and the significance of the issuance of permits. That guidance specifically states that:

> The investigator should provide information on the population to be studied and a rationale for choosing that particular population. The U.S. Fish and Wildlife Service (USFWS) issues many of the necessary permits. In issuing permits, the USFWS assesses the risk to the animal population and the Institutional Animal Care and Use Committee may rely on that assessment rather than attempt to determine the potential impact to the population. With regard to small or declining populations, many state wildlife or natural resource agencies also issue research permits. In the event that a state permit is required and has been issued, the Institutional Animal Care and Use Committee may assume that the state agency has assessed the risk to the population and found it to be acceptable. An Institutional Animal Care and Use Committee that has additional questions about the selection of species or the impact on the population to be studied may require the investigator to provide additional information or the Committee may consult with biologists with relevant expertise.

It is difficult to determine population-level impacts. On a practical level, it may be impossible to make an assessment because:

- in the wild, it is difficult, if not impossible, to assess the size of a local population, even with time-consuming surveys (even in the unlikely event that the researcher has adequate funding to conduct such surveys)
- in some cases, the ability to detect impacts requires the use of the same methods that are to be used in the study itself; for instance, to determine the impact of capture and marking requires that individuals be captured and marked as it is otherwise impossible to identify individuals in the field. Capturing, marking, and holding in captivity is not an adequate substitute to determine the impact of marking methods in the field unless field conditions can be simulated in captivity – an expensive proposition that would also require additional permits. It is also highly unlikely that field conditions could be adequately simulated in a captive holding facility.

The number of individuals typically involved in a single study is highly unlikely to have a population-level impact, even when the study subjects are removed permanently from the population. A 1975 assessment by the U.S. Fish and Wildlife Service (Banks 1979) estimated that the 15,000 birds taken under scientific collecting permits (the deliberate and permanent removal of individuals from the population) between 1969 and 1972 accounted for less than one percent of overall annual avian mortality from direct causes (the deliberate killing of birds, including hunting, depredation control, and other purposes requiring a permit) and a miniscule fraction of mortality from all causes, including collisions with man-made structures and vehicles, accidental poisoning, and oil spills. In recent years, the numbers taken under scientific collecting permits have been considerably lower. A recent analysis of annual reports submitted by holders of scientific collecting permits revealed that the highest number of individuals of any species taken under a scientific collecting permit totaled 183 individuals in a single year. The number of individuals taken in a single year exceeded 100 for only four species, all of them abundant (unpub. analysis by E. Paul; data obtained from mandatory reports submitted to the U.S. Fish and Wildlife Service). All others were taken in numbers below 100 per species. In a draft 1997 policy on scientific collecting, the USFWS recognized that "The numbers of birds collected in the United States for scientific study are extremely low compared with other categories of human-related activities and apparently have had no obvious or significant impact on species or local populations." Clearly, then, research methods that do not result in the death of an individual or the removal of a live individual from the population have little or no impact on populations. Obviously, there may be some mortality incidental to research methods. As discussed in pertinent sections below, the mortality rates are extremely low. For instance, as noted in the discussion of capture and marking, the U.S. Bird Banding Laboratory estimates a mortality rate of 1% resulting from mist netting and bird banding.

Canada

The Canadian Council on Animal Welfare oversees the basic system of regulation of the welfare of animals used in research which entails involves the inspection of facilities and the development of standards that are implemented by Animal Care and Use Committees at research institutions. The programs of the Council are deemed to be universal in application, meaning that they apply to all animals used by: i) members, ii) individuals, and iii) employees, agents or owners acting on behalf of organizations or businesses registered or operating in Canada for any of the following purposes:

* to investigate or to search carefully for fact or truth in order to produce knowledge about humans and/or animals;

* to transfer or to permit the acquisition of knowledge; or, to develop or improve skills;

* to use an established or legislated procedure to demonstrate, determine or verify a fact or findings. This includes, but is not limited to: the testing of sera, vaccines, diagnostics or medical/veterinary/biological products or conducting biological tests; performing toxicological or pharmacological procedures; identifying or detecting pregnancy, disease or other physiological conditions, or characteristics other than those used in normal and proper veterinary treatment.

* to produce products for the purpose of generating a profit. This includes, but is not limited to: the manufacture of sera, vaccines, diagnostics, or medical/veterinary/biological products; the capture, production or transportation of animals for use in research, teaching, testing or manufacturing; and agricultural quality improvement programs."

Unless an institution holds a valid Canadian Council on Animal Care Certificate of Good Animal Practice®, it cannot receive funding from the federal granting Agencies, and contracts issued by the federal government can only be awarded to institutions holding a Canadian Council on Animal Care Certificate of Good Animal Practice.

Legislation in Canada is not identical to the Animal Welfare Act or the Public Health Service Act. As noted above, the Canadian Council on Animal Care is a nongovernmental organization that has no legislative mandate. However, federal legislation in Canada that applies to the use of animals in research includes Section 446 and 447 of the Criminal Code, which protects animals from cruelty, abuse and neglect. The Health of Animals Act defines conditions for the humane transportation of all animals in Canada by all modes of transport. Each province has legislation in the area of animal welfare. In addition, the federal government imposes conditions related to the care and use of experimental animals on recipients of funding from the federal granting Agencies, and on public works and government services.

D. Additional considerations

Publication

Many journals require that authors provide written assurance that the research project was reviewed and approved by an Institutional Animal Care and Use Committee. They may also require written assurance that required permits were obtained and were current throughout the entire research project. Reviewers of submitted papers and journal editors should look for such assurances and inquire of the authors about omissions of this information. Editors should consider the potential ramifications of publishing papers reporting research that was not conducted in compliance with legal and ethical requirements. Likewise, scientific program committee members reviewing submissions for presentations at society conferences may request that similar evidence accompany requests for a place on the program. However, ultimate responsibility remains with each investigator. Whether or not required to do so, researchers should include such written assurances at the conclusion of each manuscript, report, or oral presentation, to assure not only the editors and readers, but also the general public that the conduct of ornithological research meets ethical and legal requirements.

As a matter of good practice, researchers should also provide a copy of the paper to the permitting agency and to the manager of the land unit where the research was conducted.

The importance of publishing methods papers

No field ornithology course covers all research methods; many cover little more than field identification, capture and marking, and censusing methods. No advisor knows every research method or the impacts of every method. The importance of sharing your experiences with various methods – even commonly used methods – cannot be overemphasized. If a researcher observes an impact on the welfare of the animals you study, or experience a problem, but doesn't share that information, others will not be able to avoid that same problem. Conversely, they may avoid something that need not be avoided. Or they may find that their Institutional Animal Care and Use Committees or regulatory agencies are voicing concerns or even denying permission for a particular method, based on incomplete or erroneous information. For instance, a permit biologist may be concerned about the incidence of mortality and leg injuries resulting from plastic color bands placed on a certain passerine species. A review of the literature would reveal that the injury rate was 2.9% and there was no mortality (Haas and Hargover 2003). Were it not for this paper, the agency might have denied the permit, or the Institutional Animal Care and Use Committee might have refused protocol approval. The researcher would learn that the injuries occurred only when two bands were placed on one leg, and could therefore choose to use dual-color ("striped") bands.

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CHAPTER 2. IMPACTS OF INVESTIGATOR PRESENCE

A. Overview

Research activities of field ornithologists, including the mere presence of researchers, can influence the phenomena and animals they observe. Ornithologists have an obligation to assess their research for potential negative effects on their study subjects, on other animals in the research area, and on the environment in general, and to minimize such effects. Investigators should weigh the potential gain in knowledge against the consequences of disruption. In assessing the consequences of disruption resulting from research activities, it should be recognized that individuals and populations usually recover rapidly from short-term adverse effects and that research often yields long-term positive effects for the affected populations. Nisbet and Paul (2004) discuss the balancing of knowledge of value to be gained against potential impacts, stressing the need for objective measures of impacts.

Investigator presence is a necessary component of a wide variety of observational studies that entail little more than walking through an area or remaining stationary in the vicinity of a bird or nest. Investigator presence is also commonly associated with nest visits, aircraft surveillance, and the use of boats to access observation points. Investigator presence is also a component of studies involving capture, handling, marking, and other forms of manipulation that are discussed in later sections.

Federal Endangered Species Act take permits are required to survey endangered species. Virtually every state requires state permits. See the Ornithological Council Permits guides.

The term “disturbance” is often used to describe the impacts associated with researcher presence. Nisbet (2000) proposed that human disturbance for colonial waterbirds be defined as “any human activity that changes the contemporaneous behavior or physiology of one or more individuals within a colony”. These Guidelines follow that definition because as Nisbet (2000) noted, unless the birds actually respond to the human activity, there is no disturbance. This definition then allows the focus to change to the issues of concern: the nature and extent of the effects. Nisbet (2000) points out that human disturbance is not always adverse, and that what should be minimized are *adverse effects of disturbance.* If the effects are negative and significant in kind and/or duration, more effort is required to avoid or minimize those effects, including potential changes in methodology, provided that the alternate methods are capable of generating the necessary data.

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 affect the status and well-being of the study subjects. Both effects vary along a continuum from obvious to subtle (MacArthur et al. 1982; Jordan and Burghardt 1986).

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. A substantial part of the literature reporting the effects of human disturbance on birds has focused on colonial waterbirds (Nisbet 2000). Fyfe and Olendorff (1976) review observer-caused disturbances primarily with raptors with many suggestions worth reviewing on minimizing impacts to nesting sensitive species.

B. Preliminary studies to assess impacts

It may be possible to conduct a preliminary study to determine the impacts of researcher presence. However, numerous scientific, ethical, and practical concerns arise. For instance, birds may display differing responses at different times of year or at different points in the breeding cycle. Observations made during a preliminary study during the nonbreeding season may not be applicable to the same research protocol during the breeding season, when birds may be more sensitive to human presence. This in turn raises a practical issue in that the investigator may not be able to wait a full year after conducting a preliminary study at the time of year when the primary research is to take place. Additionally, it is difficult to document the effects of investigator presence because the measurement of impacts on wildlife requires investigator intrusion into the breeding habitat or non-breeding territory and virtually always involves capture, handling, and marking. In other words, it is impossible to assess impacts without conducting the very activities that are needed to collect the data to answer primary study questions. Confounding the problem is the phenomenon of habituation.

Nisbet (2000) discusses the development of tolerance to human presence, which he defines as “the intensity of disturbance that an individual bird tolerates without responding in a defined way.” Noting that tolerance can be measured easily, he suggests that demonstrating that disturbed colonies are more tolerant than undisturbed colonies strongly suggests habituation but that only repeated measures of tolerance among the same group of individuals can prove habituation. Whether some level of tolerance or full habituation, the presence of an investigator for a preliminary study of impacts may provoke a stronger response than will subsequent, repeated visits.

Finally, the preliminary study itself raises ethical concerns. While recognizing the importance of exploratory research, non-exploratory studies of wild birds with clear hypotheses and design are ultimately preferred so that any impacts investigators may have on birds will be in pursuit of more rigorous science that answers the question(s) at hand.

C. Impacts associated with investigator presence

Many field studies involve essentially the same activities as birdwatching, which is the act of observing and identifying birds in their native habitat and is often used for citizen science projects such as bird censuses. In a review of some of the observed impacts of birdwatching, Sekercioglu (2002) described some of the practices of birdwatchers that disturb birds, including photography, the use of playback, and flushing birds. The use of playback is considered in the section on Minor Manipulations. Although researcher presence and associated non-manipulative activities may occasionally have severe effects (see reviews by Duffy and Ellison 1979; Anderson and Keith 1980; Fetterolf and Blokpoel 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 the precise point in the breeding cycle (Fyfe and Olendorff 1976; Griere and Fyfe 1987).

Adverse effects of investigator disturbance have been of concern in colonial-nesting birds, where the impacts have been documented for a variety of families (Fetterolf 1983; Boellstorff et al. 1988). In two experiments developed to quantify effects of human disturbance on foraging and parental care in European Oystercatchers (*Haematopus ostralegus*) it was found that disturbance reduced the amount of parental care. However, investigator activities had no impact on the survival of Snowy Egrets (*Egretta thula*) (Davis and Parsons 1991). In addition, it has been reported that nightlighting may minimize investigator disturbance for colonial-nesting birds (Bowman et al. 1994). Tolerance to human intrusion in forest birds can has been found to differ with species and other social factors (Gutzwiller et al. 1998)

Nest visits

The potential for detrimental effects of visits to nests have long been known (Evans and Wolfe 1967). Problems from nest visitation have resulted in potentially biased data and decreased reproductive success in both terrestrial birds (Willis 1973; Mayfield 1975; Howe 1979; Lenington 1979; Westmoreland and Best 1985) and aquatic birds (Hunt 1972; Gillett et al. 1975; Kury and Gochfeld 1975; Robert and Ralph 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). However, there are studies reporting that nest visitation produced no evident adverse effects in a variety of bird species (Götmark 1992; Schreiber 1994; Schreiber 1996; Skagen et al. 1999).

Predators following investigators or their smell to a nest can lead to greater predation rates, particularly when repeated nest visits are needed. Because the types of predators in a study area and habitat structure may differ, it is prudent to consider investigator impacts on predators before assuming that rates of predation on nests are unaffected by human visits (Hendricks and Reinking 1994). Bird eggs and hatchlings in the nest are particularly vulnerable to human disturbance because their survival depends on parental care. The two principal causes for bird nest failure are nest desertion by the parents and predation (Götmark 1992). With the likelihood of nest desertion decreasing with time post-hatch, depending on the species, it is advisable to visit nests after hatching. Low or ground-level nests should be approached tangentially, with a 3- to 4-meter detour to the nest. The investigator should return along the detour to the tangential path and continue in the same direction. Spreading naphthalene crystals along the detour segment can discourage some mammalian (?) ground predators (Redmond 1986). If flagging is used to mark nest sites, care should be taken that the flags neither impede the parents' access to the nest nor draw the attention of predators. Where flagging may result in increased predation, it is recommended that nests not be marked with flagging and that natural objects and GPS coordinates be used to aid in nest relocation (Hein 1996).

Aircraft overflights

Low-flying aircraft may be used in censusing birds. Although such flights may disrupt 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 (1981a) showed that Herring Gulls (*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, American White Pelicans (*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 to minimize the impacts of 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. Guidelines developed for aircraft operations near concentrations of birds such as in Antarctica (Harris 2005) can be used to help design census studies and mitigations for reducing the impacts of aircrafts on birds or from similar studies of the impacts of boats on birds (Bellefleur et al. 2009).

Boats

Boats are used to access study sites on islands or in wetlands and to census waterbirds, seabirds, or other species that use waterside habitat (Gerrard et al.1990; Gaston et al.1987). All manner of watercraft have been used, from canoes to small motorized watercraft and even large ocean-going ships (Tasker et al. 1984). Most studies of the impact of watercraft on birds involve recreational watercraft and are intended to guide management decisions. Burger (1998) examined the effect of watercraft type, speed and route. During the most sensitive time of year – the early part of the reproductive cycle – the type of craft, speed, and route explained 95% of the variation in flight behavior. Personal watercraft (i.e., jet skis and wave runners) had the greatest impact because they moved faster and could came closer to the breeding birds and in some cases, even ran over nests. Larger motorized watercraft moved more slowly and tended to stay in marked channels. Overall, the type, speed, and location of watercraft accounted for 66% of the variation in response by the birds. Some seabird studies have documented boat avoidance behavior by loons and grebes, including flying and diving (Henckel et al. 2007). However, as the alternate method is aircraft overflight, the impact on birds may be less an issue than the accuracy of the count. The effect of avoidance behavior on overwintering fitness is also of concern but has been little studied. Peters and Otis (2006) looked at this issue in the context of recreational boating and determined that flushing responses to watercraft varied among species and did not affect site occupancy. Only Yellow-crowned Night Heron (*Nyctanassa* *violacea*) and Great Egret (*Ardea herodias*) appeared to avoid high-traffic creeks. Boat-based research would, in places where recreational boating occurs, account for a very small fraction of boat traffic, but regardless of the presence or absence of other boats, researchers should consider speed and distance when using boats to study birds and particularly breeding birds.

Approach and nearness to sensitive areas

Individuals not under study, including individuals of other species in the study area, may also be affected by investigator presence. Hockey and Hallinan (1981) found that both near-approach and passage by people had detrimental effects in penguin colonies. Human passage disturbance led to egg loss through predation by Kelp Gulls (*Larus dominicanus*) and frightened nest-prospecting penguins away from the colony. Burger and Gochfeld (1981) demonstrated that Herring Gulls and Great Black-backed Gulls (*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 an investigator's specific behavior may have an effect in creating or minimizing disturbances. Birds are less sensitive to observers if they are shielded from them (Knight and Temple 1995), and it may help to wear inconspicuous clothing (Gutzwiller and Marcum 1993; Riffell and Riffell 2002). Other ways to minimize shielding include blinds, vegetation, or vehicles (Larson 1995).

Researchers' activities may draw the attention of curious persons. Unfortunately, considerable disturbance may result from the innocent attempts of members of the 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 minimize detrimental activities or alleviate their impacts. Research activities should be consistent with the gathering of adequate samples for valid research results, yet be balanced to minimize adverse effects. A general system of nest checking is outlined for colonial birds that minimizes investigator disturbance while maximizing data yield (Mineau and

Weseloh 1981). 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 (Shugart et al. 1981). Other researchers suggest that visits be 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). Consideration must also be given to naïve individuals that have had little previous experience with humans as there may be a more substantial impact (Blackmer et al. 2004).

Interspecific differences in response to disturbance require that field investigators be familiar with their study species such that they can reasonably predict reactions to certain field activities. Personal experience is desirable but familiarity with the literature and consultation with others may suffice. Because some habituation to investigator disturbance is possible (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.

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CHAPTER 3. CAPTURE AND MARKING

A. Overview

Scientific studies of birds often require that birds be captured to gather morphometric data and to collect samples for pathological, genetic, and biogeochemical analysis. These data and samples can be used to understand evolutionary relationships, genetics, population structure and dynamics, comparative anatomy and physiology, adaptation, behavior, parasites and diseases, geographic distributions, migration, and the general ecology of wild populations of birds. This knowledge informs us about avian biology and natural history and is necessary to effect science-based conservation and management policies for game and non-game species, endangered species, economically important species, and bird habitat conservation (White and Garrott 1990).

Capture is generally necessary to mark birds, which allows scientists to investigate demography, migration/movement patterns, or identify specific individuals after release (Day et al. 1980). Many techniques have been developed to capture and mark birds (Nietfeld et al. 1994; Bub 1995). The assumption that marking does not affect the birds is critical because it is the basis for generalizing the data to unmarked birds (Murray and Fuller 2000).

The purpose of this section is not to describe capture and marking techniques, but instead to discuss the effects that different capture and marking techniques have on a bird's short- and long-term physiological well-being and survival. The more commonly used methods are covered and described briefly, but the focus is on the potential impacts of the method. Thus, even if a particular method is not covered, the researcher is alerted to concerns that may arise and questions to be considered in refining methods so as to reduce impacts. Representative literature citations are provided to illustrate each point, but this document is not intended to be an exhaustive critical literature review. The North American Banding Council publishes peer-reviewed, taxon-specific manuals describing capture and marking methods in detail and offers training programs and certification. The standard references for bird capture and marking by Bub (1995) and McClure (1984) are comprehensive.

Training is the key to avoiding avian injury and mortality. Despite the availability of excellent reference materials such as the publications of the North American Banding Council, no one should attempt to capture birds or remove birds from nets or other traps without training. Supervision by the trainer or other experienced researcher may be discontinued once proficiency has been demonstrated. Under U.S. law, "A banding or marking permit is required before any person may capture migratory birds for banding or marking purposes or use official bands issued by the Service for banding or marking any migratory bird." (50 CFR 21.22). Permit applications must be accompanied by references from licensed banders attesting to the proficiency of the applicant. However, 50 CFR 13.25(d) allows permitted banders to teach and supervise others who do not yet have permits: "Except as otherwise stated on the face of the permit, any person who is under the direct control of the permittee, or who is employed by or under contract to the permittee for purposes authorized by the permit, may carry out the activity authorized by the permit."

The North American Banding Council's Bander's Study Guide (North American Banding Council 2001) provides an exhaustive list of the causes of injury and mortality and methods to assure that these are infrequent occurrences. Even experienced banders can benefit from reviewing this material periodically, and those just learning to band or who have little experience should study this manual diligently. Some of the basic good practices are discussed here.

B. General considerations

The chosen method of live trapping birds must minimize the possibility of injury or death to captive individuals and minimize stress. Investigators need to consider the time of day, time of year (moult or breeding status of the birds), weather, number of birds to be captured, number and training of staff required, and the possibility of predation. They must be familiar with the biology and behavior of the species they are capturing, and plan all captures and releases accordingly. For example, some species are flightless during moult and should be captured and released in a way that does not affect their survival during this vulnerable stage. Breeding birds (e.g. incubating females) must be released as soon as possible to avoid prolonged absence from the nest (less than one hour depending on the species). Diurnal birds should never be released after nightfall as they may have difficulty finding a suitable roost for the night and be vulnerable to nocturnal predation. The mesh size of a net or size of a trap should be appropriate to the species targeted so that birds are not able to escape, become entangled or injured. Traps should be have no sharp edges that might injure birds or investigators. The opening of a trap should be positioned to allow the investigator to reach all parts of it to remove birds easily. For units with trap doors or moving parts, all mechanisms should be in good working order and be safe for trapped birds and investigators. Avoid disturbance to vegetation except as needed to place the net or trap, as flattening of vegetation may affect concealment and result in increased predation.

Before trapping begins, investigators must have management plans in place for birds injured or killed during capture. The plan should include information on evaluating the condition of the bird, determining when euthanasia is appropriate, and assuring that persons who will euthanize birds are properly trained, have the appropriate materials on hand, and, when required by law, have the appropriate permits. If a licensed wildlife rehabilitator or veterinarian is nearby, consider taking the bird to that individual for assessment, treatment, or euthanasia. Attempt to donate carcasses to museums or teaching collections. See the Ornithological Council fact sheet (Appendix A) for instructions on preparing carcasses for instructions on how to save a bird for science.

As with all research methods, some injury or mortality will occur no matter how skilled or experienced the researchers and even when great care is taken to prevent harm. It is difficult, if not impossible, to know the actual rate of mortality because some birds that die between the time of capture and release, or shortly thereafter, will die from causes unrelated to the capture and handling. Further, birds are rarely seen after release, except for a short period if banded on breeding territories. Mortality resulting from capture or marking will go undetected. Attempt to determine the causes of injury and mortality and adjust practices accordingly.

Researchers and other banders should record injuries and mortalities and share this information with others, by publishing, presenting at scientific meetings, or through the North American Banding Council or other professional organizations. Problems resulting from the use of particular kinds of markers or capture methods, or in individual species are particularly important.

C. Capture methods

Mist Nets

Modern mist nets are made from nylon and vary in mesh size and length. Mist nets have three to four panels that overlap to form pockets; when a bird strikes the net, it will drop into the pocket and become entangled (Bub 1995).

Bird injuries and death sometimes occur from capture and handling, even when a highly experienced handler or bander is following all good practices, but injuries to birds and/or death resulting from mist netting seem to be infrequent. There is currently no requirement or opportunity for routine reporting of injury and mortality. The U.S. Bird Banding Lab and the Canadian Bird Banding Office do not require that injury and mortality be reported routinely although the Canadian Bird Banding Office will sometimes ask for reports on injury especially for novel capture or marking methods. However, many banding stations and individual researchers maintain records of injury and mortality. In 2009, Spotswood et al. (unpublished data) collected data from 20 banding organizations in the United States and Canada and determined that injury rates ranged between 0.06 and 2.37% while mortality rates ranged between 0.07 and 1.15%. Of the 20 organizations that provided mortality and injury rates, five also provided detailed records of individual injuries and mortalities. These data reveal that 66% of all incidents were net-related injuries and 25% were net-related mortalities. The most common causes of mortality and injury were handling, predation, net trauma, strains and cuts.

Determining mortality rates resulting from capture and marking is difficult because capture, handling, and marking may be proximate in time to the death, but not the cause of the death. A bird that died in the net or in the hand might have had a previous injury, disease, or condition such as parasites severe enough to cause death. Absent evidence of injury or predation, cause of death may not be evident without a necropsy. If practical to do so and if funding and personnel are available, consider performing necropsies under such circumstanes, necropsies will yield information that may identify practices that can be modified to reduce or eliminate the risk of injury and mortality. Conversely, mortality might be under-reported because banded birds more often than not are not seen again, particularly when banded on migration. Due to the difficulty (impossibility) of studying unmarked birds, it is difficult to assess the normal rate of mortality of wild birds and therefore, it is hard to know if mortality associated with capture and marking differs significantly from the background (natural) rate of mortality.

Recher et al. (1985) analyzed the rate and causes of mortality at a woodland banding site and at a heathland banding site in Australia from 1979 to 1981. A total of 53 out of 4184 birds died. Of these, 68% died in the net and 32% died during handling. The disparity between mortality rates at the two banding sites — at the woodland site, 2.8% of the birds died but at the heathland site, only 0.5% of birds died — was attributed this difference to the fact that at the woodland site, there were more nets open and fewer experienced banders. As a result birds were left in the mist nets too long or left open during the hottest part of the day.

Simple, basic measures can prevent most injury and mortality with the use of mist nets. Nets set for diurnal species should be closed or taken down at dusk to avoid accidental capture of nocturnal species and vice versa. When nets are closed, clothespins or other fastener will keep them from unwinding in the wind; loose sections can trap bats and nocturnal birds or birds active in the early morning. When mist nets are set near the ground, it is important to clear away plants and debris so that birds are not accidentally overlooked when nets are checked. It is also more difficult to remove a bird from a net that is entangled in vegetation.

Mist nets must be checked frequently; the number of nets set up should reflect the skilled manpower available to check them (Recher et al. 1985). Birds are susceptible to heat, cold, thirst, or hunger and so should not be left in nets longer than necessary (Recher et al. 1985). If the substrate below a net becomes heated by insolation, temperatures lethal to small birds may be reached quickly. Similarly, extreme cold poses special problems, especially for small species. Nets should be shaded or positioned to avoid full exposure to the sun. Trapping or netting should be avoided if the ambient temperature is below 0^0C or above 35^0C, or in windy or rainy weather. Nets and traps should be watched or checked at least every 20 minutes during the nesting season, during migration, or if it is hotter from direct sun or cooler due to microclimate of the area, and about every 30 minutes (at least once per hour) during the rest of the year.

Put anti-predation measures in place. If predators in the area seem to be observing the nets, the nets should be closed. If a bird is taken by a predator, check nets more frequently or close the nets. Ground-dwelling predators – even frogs – can take birds from the lowest tier of the net so raising the net may be adequate to prevent this problem. Killing predators is not an acceptable option, and killing avian predators is a federal offense under the Migratory Bird Treaty Act (16 U.S.C. 703 et seq.). Before releasing birds, scan for predators in the area and check the condition of the bird. Released birds may be disoriented, slower, or in a weakened state, making it harder to evade predators. Fire ants and other insects can be problematic. Clearing vegetation and raising the nets to avoid contact with vegetation or the ground is necessary in these cases. In addition, it is good to know the possible large mammal species that may be in the area, such as moose, elk and deer that have been known to cause problems with mistnets.

Removal of birds from mist net requires training and skill gained from experience. A small crochet hook can be used to help remove more entangled birds from the net. Small scissors or knife can be used to cut the net for the most difficult birds. Infrequently, a bird will get its tongue entangled in the net and great care must be taken to gently remove the net.

Injuries sometimes occur in-transit between the net and the banding station. Banders employ various methods for transporting birds including nylon bags, cloth or mesh bags, and small buckets. Birds can be safely transported from the net to the banding station in nylon, cloth or mesh bags. Small modified buckets may be useful to transport species such as towhees or other long-legged perching birds that may be prone to leg joint dislocations. Carrying them in a bucket that allows them to stand can help alleviate this risk (Cox, pers. comm.).

Cannon/Rocket Nets

Cannon and rocket nets are fired over a predetermined area, usually to catch shorebirds, waterfowl, or waterbirds. Cannon and rocket nets are dispersed quickly using explosive charges. Phutt nets are fired using compressed air and do not have the range or netting area that cannon or rocket netting can achieve. Cannon/rocket netting can be effective in catching adult waterbirds away from the nest. Birds are lured to the site with bait or decoys (Parrish et al. 1994; Heath and Frederick 2003). Because cannon/rocket netting involves the use of explosive charges, special training and permits are required and an experienced team is needed to coordinate the set and firing and to remove and process birds quickly and efficiently. If nets and explosives are not set up and detonated correctly, birds and or humans can be injured or killed (Bub 1995).

As reported in the few papers published on this subject, injuries and mortalities seem to be rare occurrences. King et al.(1998) reported one avian mortality and a broken wing when rocket netting 142 American White Pelicans (*Pelecanus erythrorhynchos*) due to strikes by the rocket and leading edge of the rocket net. Cox and Afton (1994) reported a 1% mortality rate for 18 firings that captured 1,116 waterfowl. Eleven of the 12 mortalities resulted from drowning when they became trapped between the platforms from which the nets were fired and the stakes holding the nets. Over several years of rocket netting shorebirds, mortality ranged from 0 to 2.1% except for the first net attempt, when a lack of a sufficient number of banders and adequate holding facilities resulted in mortality of 10.7% of the birds caught (Jurek 1974). The longer Ring-billed Gulls spent under the net, the less likely they were to be resighted and it was assumed that they had deserted the colony. Of those that remained, however, time under the net did not affect resumption of breeding. This particular study reported no adult or chick mortality but three nests were damaged by the net (Southern and Southern 1983).

Funnel Traps

Funnel traps consist of a funnel leading into a trap. Birds walk through the funnel into the trap, often lured by bait, where they are most often unable to exit. These traps are used most commonly to trap birds that walk or feed on the ground (Bub 1995). Buck and Craft (1995) reported minor injuries (minor scrapes) associated with capturing Great-horned Owls (*Bubo virginianus*) and Red-tailed Hawks (*Buteo jamaicensis*) in funnel traps, but none were serious or life- threatening. Kearns et al. (1998) reported 48 trap mortalities (1.6% of total captures) using a modified cloverleaf funnel trap; of these, 22 (46%) were due to predatory mammals, 16 (33%) resulted from drownings due to changing tides, 10 (21%) were due to unknown causes.

Trapping at Nest Sites

Trapping at nest sites is a common practice when the investigator hopes to mark nestlings and it is also useful because adult birds are reliably found at nest sites. The method is often employed for long-legged wading birds such as storks or ibis. However, trapping on the nest and repeated visits to wading bird colonies may have adverse effects on nesting success (Jewell and Bancroft 1991) and may bias reproductive and population studies. Additionally, nest-trapping techniques limit researchers to capturing only incubating or brooding birds. As with all species, a variety of capture techniques may be needed, particularly if large sample sizes are needed. King et al. (1998) used padded, modified leghold traps submerged in flooded fields. Of the 52 birds caught, none suffered injury other than a mild abrasion to the leg of one bird. Fuertes et al. (2002) used a modified fish trap called a “single strip Dutch sleeve” to capture rails, crakes, and moorhens. One mortality was reported due to a mammalian predator and four birds exhibited skin abrasions at the base of the bill. Mehl et al. (2003) used leg-hold noose mats to capture Killdeer (*Charadrius vociferus*), Dunlin (*Calidris alpina*) and Piping Plovers (*Charadrius melodus*) in Texas and California. Mehl et al. (2003) reported three leg injuries and one mortality due to an avian predator out of 2410 birds captured. Finally, Herring et al. (2008) used modified flip traps and net guns to capture Great Egrets (*Ardea alba*) and White

Ibis (*Eudocimus albus*). The flip trap caused two birds to receive minor abrasions and a mild hematoma but no mortalities. Four mortalities occurred using the net gun (3 Great Egrets and 1 White Ibis) when weights on the net struck the birds. Anti-predator measures are particularly important when trapping at nest sites, both for the trapped birds and the young at the nest.

Raptors

Raptor banding requires special permission from the U.S. Bird Banding Laboratory of the U.S. Geological Survey and in Canada, from the Bird Banding Office as well as provincial or territorial permits. A comprehensive review of raptor research and management techniques has recently been updated (Bird and Bildstein 2008).

It might seem a matter of common sense to wear heavy gloves to protect against puncture wounds from the talons and beaks of raptors. However, the North American Banding Council cautions against the use of gloves in most raptor handling situations. Gloves can make it difficult to be sure that the bird is not held too tightly, harming the bird, or too loosely, allowing the wings or feet or even the entire bird to slip free (Hull and Bloom 2001). Some banders give the bird an empty glove to bite and grasp while handling the bird with the other (bare) hand. This method is useful in removing raptor nestlings from the nest. In addition, a hood can be used to quiet the bird.

Most raptor banding involves the use of a live lure animal. The animal is harnessed in a protective jacket and tethered and can be placed behind a net, within the bownet, or inside a trap. When a raptor approaches, the tether line is pulled to cause the lure animal to move. The North American Banding Council manual of raptor banding techniques details the appropriate care of the lure animal, stating that, “it is of utmost importance to treat any living animals within your care in a thoughtful, humane manner at all times.” Specifics include providing adequate shelter, clean enclosures, and a diet appropriate in food types and quantity. In the field, lure animals should be given food and water, and sheltered from heat, cold, and rain. The protective jacket must be large enough to allow the bird to breath and flap freely and should have no rough or sharp edges. Use only healthy animals, and use no individual for more than 1-2 hours in good weather. Lure animals should be rotated out of use for one or more days to allow them to recover from the stress. Each bird should be checked for injury when returned to its enclosure, and injuries should be treated immediately (Hull and Bloom 2001). Bal-chatri traps used along roadsides are usually in place for no more than an hour, so lure animals can be given food and water when retrieved from the traps. The lure animals should have some kind of refuge to escape the raptor's talons and beak.

In some limited instances, non-living lures may be used to capture some species of raptors. Eagles and vultures are attracted to carion. Taxidermied owls, alone or in combination with taped vocalizations, may lure some owl species into mist nets. Nero (1980) had success casting a fishing line to drag a stuffed mouse across the ground. This method has been used for both Great Gray Owls (*Stix nebulosa*) and Spotted Owls (*Strix occidentalis*). Generally, though, trapping diurnal raptors requires the use of live lures. The Golden Gate Raptor Observatory devoted 15 years of effort and more than $150,000 trying to develop an effective mechanical lure. Although a professional robotocist succeeded in creating a lure with a reliable power supply and remote control systems as well as realistic appearance and movement, it was difficult to use and proved unreliable in capturing the majority of raptors to which the mechanical lures were presented (Hull, pers.comm. 2010). Others have used mechanical lures entailing a motorized head inside a stuffed owl toy or taxidermied owl with a motorized head with limited success (Jacobs and Proudfoot 2002; Jacobs 1996), but mechanical lures can fail at critical moments. An extensive and detailed review of raptor capture methods by leading raptor biologists each with many decades of experience suggests the possible use of non-living lures in only two methods (Bloom et al. *in* Bird and Bildstein 2008).

Capture myopathy

Most research that requires capture of a wild animal presumes that the individual will be returned to the wild in as close to its original condition as possible. Capture myopathy, also known as cramp or exertional rhabdomyosis, which can result from handling and capture, delays release or may preclude release altogether and may even result in morality. Capture myopathy is a state of muscle tissue degradation that can render a bird incapable of standing, walking, or flying (Purchase and Minton 1982; Rogers et al. 2004). The condition is most common in long-legged birds waterbirds but may frequently occur but not be recognized in other species such as Mallards, Wild Turkeys (*Meleagris gallopavo*), and Northern Bobwhites (*Colinus virginianus*) (Minton 1993). Susceptibility to capture myopathy varies from species to species and from bird to bird because normal levels of creatine kinase and aspartate transaminase vary with nutrition, physical condition, reproductive status, and season (Dabbert and Powell 1993; Hulland 1993; Mueller 1999; Viña et al. 2000). As a cause of mortality, it frequently impedes captive breeding programs (Bailey et al. 1996a; Bailey et al. 1996b) and biases the results of field experiments (Abbott et al. 2005; West et al. 2007).

The underlying effect of capture myopathy is muscle necrosis caused by the inability of the vascular system to remove waste products and re-oxygenate the tissue after contraction (Wight et al. 1979). In critical cases, a bird may suffer dyspnea, hyperthermia, weakness, muscle rigidity, and collapse but less obvious symptoms may last longer, increasing susceptibility to predation or contributing to death weeks or months after capture.

To avoid causing capture myopathy, the method of capture needs to be well-planned so as to trap, process, and release birds quickly. This entails have enough people with enough experience and preparation to reduce handling time. Do not chase birds and minimize pursuit time when using funnel traps. As struggling is thought to be a cause of capture myopathy, reduce struggling by covering the bird’s eyes or placing it into a darkened box or holding cage. Investigators should avoid catching birds in high temperatures. When high temperatures cannot be avoided, ensure that the bird’s temperature is controlled with good ventilation. It may also be necessary to shade birds in the net both before and while they are being extracted. Holding cages should be placed on damp sand or ground, as heat from dry sand considerably increases the temperature in the cages. If only dry sand is available, the hot top layer should bescraped away before the keeping cages are erected. Damp cloths located out of reach of the bird may provide shade and cooling (Clark and Clark 1992).

Some capture methods may be more likely to cause capture myopathy than others. For the capture of Little Bustards (*Tetrax tetrax*), cannon nets (as compared to leg nooses or funnel traps), along with longer handling and restraint times, explained 41% of the variance in the probability of the occurrence of mobility disorders following release (Ponjoan et al. 2008). However, Minton (1993) found that for large wading birds, mist nets resulted in more cases of capture myopathy than did cannon nets. Certain procedures were found effective in reducing the incidence of this condition, including removing susceptible species from nets first and moving them to holding cage, allowing the legs to dangle when carrying birds, and processing susceptible species first.

When planning capture of birds known to be susceptible to capture myopathy, learn to recognize the symptoms. Some of these symptoms, such as shallow panting or open-mouth breathing, can also be signs of the ordinary stress associated with capture. Elevated body temperature, weakness or tremors in a limb, inability to stand or walk, ataxia (lack of muscle coordination), depression, and shock.

Observe birds if capture myopathy is suspected. It may be possible to treat incipient cramp by bathing the birds' legs in water. Banders have observed that Red Knots, released after a catch with signs of slight cramp, went immediately to stand in the water. After a short time they moved away, having apparently lost their symptoms or shown great improvement. Following these observations, banders immediately took birds with incipient cramp to the tide edge and their legs were bathed before release. This treatment seemed to be effective, with all birds on which it was tried recovering, probably because it rapidly reduced the bird’s temperature. This technique was only tried on birds in the very early stages of cramp and may not be effective if the condition is allowed to develop (Clark and Clark 1922). Muscle relaxants have been shown to alleviate cramp. Diazepam given to Red Knots and Eurasian Oystercatchers (*Haematopus ostralegus*) completely abated the clinical symptoms (Piersma et al. 1991). Of course, consult with a veterinarian before treating birds with any form of medication to assure proper dosage. Birds treated with diazepam or similar substances must be kept in safe, quiet, dark places while they recover.

If planning to capture species known to be susceptible, obtain veterinary advice or arrange to have a veterinarian onsite if possible; determine options for veterinary and rehabilitative care in advance of the planned capture. If veterinary and rehabilitative care are not available, and field treatments such as those described above fail, arrange for a humane method of euthanasia to be administered by someone skilled (and if required by law, holding a valid permit to do so). Releasing a disabled bird is not humane.

The course of treatment for capture myopathy potentially involves drugs, nutritional and mineral supplements (vitamin E and selenium), as well as physical therapy and massage. Three Sandhill Cranes were treated 2-8 times/day for 8-12 days until they could stand on their own (Businga et al. 2007); a group of knots (*Calidris spp.*) required 2 weeks of rehabilitation (Rogers et al. 2004); and a Rhea (*Rhea americana*) regained the ability to walk only after 4 weeks of “persistent, aggressive physical therapy, muscle relaxants, and anxiolytics…” (Smith et al. 2005).

With appropriate treatment, survival and release may be possible. In the Sandhill Cranes mentioned above, all three birds survived; both adults were observed with chicks in the subsequent 3-7 years. The juvenile was observed flying with a flock for two days after release (Businga 2007). The knots suffering the effects of capture myopathy were kept in slings and given rehabilitative exercise. Of these birds, 80% survived. When able to walk steadily and flap effectively, the birds were released. Half the released birds were resighted the following year; this compared favorably to the resighting rate of 52% of birds that did not suffer myopathy (Rogers et al. 2003).

Necropsy by a veterinarian is the only way to confirm that capture myopathy was the primary cause of death. If capture myopathy is confirmed, capture and restraint techniques and protocols should be reviewed and modified.

D. Marking

General considerations

Studies that use marking techniques operate under the assumption that the marking technique does not affect the individual or that the negative impacts from the mark are negligible. It is essential to the welfare of the birds and to the integrity of the research that the marking procedures not adversely affect the behavior, physiology, or survival of individuals. Because of the difficulty in providing appropriate controls for the marking method and the difficulty of observing and measuring impacts in the field, where individuals may be seen only once, systematic studies of possible adverse effects of marking procedures are still few and often suffer from small sample sizes that lead to weak statistical inference (Murray and Fuller 2000). However, the number of published studies reporting the effects of marking techniques on survival, reproduction, and behavior of captive and wild populations is increasing and reported here for consideration in the design and implementation of marking studies on birds. Investigators should not assume that marking procedures will have no adverse effects on their subjects and should make efforts to evaluate and report any such influences.

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.

Despite the effects marking techniques can have on birds, marking is a necessary research technique. In selecting a marking method, attempt to meet as many of these criteria as possible, giving special consideration to the adverse effects attributed to the marking technique you choose, the effects it may have for the species you are studying, the effect it may have on the data that will be generated, and its acceptability for the proposed study.

The North American Banding Council's Bander's Code of Ethics outlines the basic standards that constitute ethical banding and marking practices. A particular problem arises with regard to the accuracy of data. Birds vary in appearance among individuals, between sexes, between life stages, and across seasons. Identifying the species and ageing and sexing the bird, along with collecting other morphometric measurements, can be time-consuming. This time can be reduced using tabular format that summarizes the diagnostic criteria (Sakai and Ralph 2002). Covering a wide range of taxa, including raptors, passerines, hummingbirds, and woodpeckers, the publication can be ordered from the Klamath Bird Observatory.

All types of markers require permits from the U.S. Bird Banding Laboratory or the Bird Banding Office of the Canadian Wildlife Service. Special permission must be obtained to place "auxiliary" markers – essentially, anything other than the government-issued, numbered metal bands – on a bird.

In special cases it may be possible to identify individuals of some species 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 and where feasible, these methods should be considered as an alternative to physical marking by the researcher.

Metal Bands

Prior to the use of banding (ringing), most bird life histories were a complete mystery (Bairlein 2001). By the early 20th century, bird banding became organized and banders submitted their data to the American Bird Banding Association, which compiled and stored the information. The U.S. Bird Banding Lab has handled these functions since 1920. Because of the advances banding has contributed to our understanding of birds, banding has been called the greatest advance in the study of birds in the 20th century. Researchers have been successfully using bands to study birds for many decades (Coulson 1993).

In North America, numbered metal (usually aluminum, but various alloys for special purposes) bands are issued by the Bird Banding Laboratory of the U.S. Geological Survey or by the Bird Banding Office of the Canadian Wildlife Service to approved banders and are applied to a variety of bird species. Birds banded with metal bands almost always need to be recaptured in order for the band numbers to be read and for data to be generated. The large bands placed on larger birds such as raptors and some waterbirds are considered field-readable because they can often be read with the use of binoculars or a spotting scope.

It is imperative that bands of the correct size be used. Applying bands that are too small for the species in question, that do not account for growth in juvenile birds, that fail to consider size dimorphism when choosing band size which could result in a large band slipping over the foot, or incorrectly determining how many bands can be safely fitted on one leg may cause serious injury to or even loss of the banded leg (Calvo and Furness 1992; Reed and Oring 1993; Gratto-Trevor 1994; Sedgwick and Klus 1997; Amat 1999). Recommended band sizes for all species of North American birds can be found in the North American Bird Banding Manual (Gustafson et al. 1997) and in the Identification Guide to North American Birds (Pyle 1997, 2008).

When appropriate band sizes are used, the occurrence and rate of adverse effects on the subjects is ordinarily very low. In their 19-year study of Spotted Sandpipers (*Actitis macularia*), Reed and Oring (1993) documented toe or leg loss in eight of 267 birds banded and seen again after banding. During that same time, the researchers observed three unbanded birds missing feet and one missing a toe. Gratto-Trevor (1994) reported similar results: in an eight-year study of Semipalmated Sandpipers (*Calidris pusilla)* no leg injuries were observed in the 278 banded birds seen in subsequent years. In some cases, however, much higher injury rates have been reported. Amat (1999) reported an injury rate of 1.9% in a seven-year study of Snowy Plovers (*Charadrius alexandrinus*). Both Reed and Oring (1993) and Amat (1999) reported that the injuries did not affect reproduction. An unusually high incidence of leg injuries in Willow Flycatchers (*Empidonax traillii*) was reported by Sedgwick and Klus (1997). The overall injury rate of 9.6% of banded birds that returned in subsequent years ranged from relatively minor (irritation) to leg loss in 33.9% of the cases. Survival of these birds was significantly lower than the population at large. The problems seemed largely attributable to the use of two color bands or one aluminum band and one color band on one leg. Two or more aluminum bands should not be applied to the same leg as the edges may flange over and injure the leg (Berggren and Low 2004).

A literature review compiled by Marion and Shamis (1977) covers a wide range of species-specific reports, including report of adverse impacts or band loss. Band injuries include simple irritation or discomfort, entanglement of a toe in the band (Berggren and Low 2004), accumulated mud, ice, or fecal matter between the band and leg (MacDonald 1961; Amat 1999), and loss of the foot (Calvo and Furness 1992; Reed and Oring 1993; Gratto-Trevor 1994; Amat 1999; Pierce et al. 2007). Observations of banded chicks becoming entangled in vegetation led Bart et al. (2001) to study the impact of color bands on Semipalmated Sandpipers banded at hatch; in fact, there was no difference in mass or survivorship of the banded chicks, even when two or three bands were placed on a chick. The birds wearing three bands were actually re-sighted more often that the birds with no bands or one or two bands. Causes of injury often seem to be species-specific. Henckel (1976) reported leg lesions on banded Turkey Vultures (*Cathartes aura*), apparently resulting from the accumulation of fecal matter under the bands; these birds defecate on their legs to take advantage of evaporative cooling. Long-legged birds trying to remove bands or preen legs may catch their bills in the bands; banding above the tarsal joint prevents this problem (Salzert and Schelshorn 1979).

Colored Leg Bands

Color banding (ringing) has proved to be a useful technique in recognizing individual birds without the need for recapture. The ability to track individual birds has increased our understanding of bird movements and behavior (Hole et al. 2002). One or two colored leg bands are often applied to one or both legs of a bird. They are being used increasingly in studies of behavior and ecology, often involving large numbers of individuals (Cresswell et al. 2007; Pierce et al. 2007). The use of color bands requires special permission from the U.S. Bird Banding Lab or the Canadian Bird Banding Office.

When used in combination with aluminum bands, plastic bands must be of the same size or upper bands can slip inside the lower bands, resulting in leg injury or loss (Berggren and Low 2004). Most injuries appear to occur more frequently when metal bands or combinations of metal and colored bands are applied to the same leg (Sedgwick and Klus 1997). Berggren and Low (2004) found that 2.5% of banded North Island Robins (*Petroica longipes*) exhibited adverse effects from colored bands. The most common injury sustained was trapping of the back toe between the band and the tarsus. This particular injury was believed to be caused by the bird perching sideways on upright vegetation. Accumulation of leg scales between the band and the leg resulted in constriction that caused the loss of toe function and swelling above and below the band in Bell Miners (*Malurus cyaneus*) (Splittgerber and Clarke 2006). Armstrong et al.1999 found that 54% of banded Hihi, or Stitchbird (*Notiomystis cincta*) suffered injuries due to split color bands and experienced partial loss of foot function. Foot function and survival increased when split bands were replaced with wrap-a-round plastic bands. Pierce et al. (2007) observed plastic (celluloid or PVC) color band injuries in 13.2 – 35.3% of recaptured flycatcher species that included accumulation of scales, swelling above and below band, and loss of a foot. No further injuries were observed when the plastic bands were replaced with color bands made of anodized aluminum. The authors recommended the use of a single color band of anodized aluminum, which reduces the number of available color combinations. However, Koronkiewicz et al. (2005) developed a method to make striped color bands of anodized aluminum. Alternatively, if only one plastic color band is used, it could be placed above the numbered metal band. The authors also noted that the behavior or morphology of the flycatchers Muscicapidae and closely related Monarchidae families, and possibly Tyrannidae flycatchers of the Americas, might explain why these species seem to suffer injuries from plastic color bands that are not seen in other species.

Particular care must be taken when banding nestlings before they are old enough to be banded with permanent bands. A technique for color banding nestling passerines is given by Harper and Neill (1990). This technique involves the use of bands made from colored plastic straws; removing these temporary markers requires that the birds be recaptured before fledging. When nestlings are banded close to the fledging stage, they may fledge prematurely and may be difficult to recapture. It is therefore important to estimate the age of the nestlings from size, appearance, and behavior if the hatch date is unknown, and to know the duration of the nestling period (from species accounts or prior experience).

Color bands can also affect behavior. 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 in some species. Early investigations focused on the use of bands that matched the color of natural ornamentation, such as throat patches, wing patches (or epaulets), eye ring, or bill color. Not unexpectedly, results were mixed. Mating preference among captive Zebra Finches (*Taeniopygia guttata*) was observed in several experiments (Burley 1981; Burley et al. 1982; Burley 1985, 1986a, b). Beletsky and Orians (1991) found that red color bands had no impact on male mortality or reproductive success of Red-winged Blackbirds (*Agelaius phoeniceus*). Weatherhead et al. (1991) placed red plastic bands or red anodized aluminum bands on Red-winged Blackbirds and found little effect, positive or negative, for either band type, in terms of territory loss or harem size. Mating success was not determined, and it was suggested that genetic analysis is needed to determine the extent of extra-pair paternity before determining reproductive success in polygamous or polyandrous species. Holder and Montgomerie (1993) found that male Rock Ptarmigan (*Lagopus mutus*) banded with red or orange bands did not have greater mating success, in contrast to a study of the same population in earlier years. However, they did observe a higher rate of territorial intrusion. Hannon and Eason (1995) found no evidence that red or orange color bands affected mate choice, reproductive success, or survival in Willow Ptarmigans (*Lagopus lagopus*). Johnsen et al. (1997) found that color bands matching natural ornamentation did not affect pairing success, but males with matching bands spent less time guarding mates and more time displaying and intruding into neighbors' territories. Johnson et al. (1993) found the same result in American Goldfinches (*Carduelis tristis*). Hansen et al. (1999) used color bands to create artificial asymmetry on female Bluethroats (*Luscinia svecica*) to determine if males preferred symmetrical mates; the positive result suggests that asymmetry rather than specific colors might account for observed impacts of color bands on mate choice.

Another concern of banding is that it may increase predation due to the conspicuousness of the band. In a study of Common Redshanks (*Tringa totanus*), Cresswell et al. (2007) did not observe increased predation risk due to the presence of color bands.

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 (Hill 1992; Lindsey et al. 1995). Several suppliers offer UV-stable bands. 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, studies of long-distance migrants, especially shorebirds, have employed plastic flags with unique colors representing different countries and different positions being used to represent points of origin. This system is coordinated by the International Shorebird Banding Project. The flags are larger and more conspicuous than bands, so can be seen over longer distances. To avoid interference with the many ongoing shorebird studies, always consult with the International Shorebird Banding Project before affixing flags to shorebirds.

Loss of color leg bands can also affect estimates of mortality and population size and this should be considered when choosing band types (Nelson et al. 1980). An inexpensive alternative to commercial color bands is described by (Hill 1992).

Dyes and Ultraviolet Markers

Dyes applied to the plumage are used extensively on birds, especially colonial waterbirds and waders. It can be hard to see bands on these birds because their legs are often underwater. 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 (picronitric acid; trinitrophenol; nitroxanthic acid: carbazotic acid; phenoltrinitrate) can pose 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 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). Fingernail polish can be used on nestling toenails to mark individuals prior to being old enough to band. 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 1995).

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 in terms of visibility and retention as well as the elimination of injury (Sherwood 1966), and have little impact on behavior or survival on geese but may not be acceptable for use in ducks, which can get their bills stuck in the collars (Helm 1955) and may interfere with reproductive success in female Black Brant (*Branta bernicla nigricans* (Lensink 1968). Ankney (1975) found that female Lesser Snow Geese (*Chen caerulescens*) that were neck-banded died of starvation at a rate disproportionate to that observed in the population as a whole, which was supported with additional data the following year (Ankney 1976). One unbanded adult Tundra Swan (*Cygnus columbianus*) seized the neck band of another to hold the second bird during an aggressive encounter, thus preventing the second bird from fighting back (Hawkins and Simpson 1985). MacInnes and Dunn (1988) examined the recovery and recapture rates for neck-banded Canada Geese over seven years and found that the rate of recapture was about half that for birds marked with leg bands although they could not determine if the cause was increased mortality, emigration, or both. When both members of a pair were banded, or the male was banded, nest initiation was slightly delayed but there were no significant differences in clutch size or brood size in three of four years. Greater White-Fronted Geese (*Anser albifrons* *frontalis*) spent significantly more time preening on foraging grounds but not at roosting sites, and seemed to compensate by spending less time in alert postures, but otherwise seemed unaffected (Ely 1990). In a rigorous study to address potential concerns of necks collars on Emperor Geese (*Chen canagica*), Schmutz and Morse (2000) found that adult females marked with tarsal bands had a 17% higher mean annual survival rate than those marked with neck collars and they speculated that the negative effects of neck collars principally arise from a chronically increased energetic demand.

Icing is a particular concern. Ballou and Martin (1964) applied neck bands to 1,564 Canada Geese *(Branta canadensis*) over a four-year period. They observed two deaths resulting from severe icing. In another study, all 68 Canada Geese wearing collars experienced some icing; 12 accumulated a half kilogram of ice while the others acquired a thin layer of icing. All birds were able to fly and the ice fell off spontaneously as temperatures warmed. In a second incident, 10 birds experienced heavy icing of nearly 1 kg; 1 died and four were unable to fly. Ice on the collar of the dead bird was 1 cm thick inside the collar and apparently constricted the neck (Greenwood and Bair 1974). Two icing incidents occurred among 164 neck-banded Pink-footed Geese *(Anser branchyrhynchus*). During the first incident, light icing formed on the collars of about 25 birds and heavy icing (5 – 10 cm) on the collars of about 25 birds. In the second incident, five birds (of 123) experienced light icing and 13 experienced heavy icing. In all cases, the birds were observed feeding and there was no significant difference in the abdominal profile indices of the iced birds compared to birds without icing. There was no observed mortality and no statistically significant difference in resighting rates (Madsen et al. 2001). In unpublished research, Hestbeck (pers. comm.) found that cone-shaped rubber collars collected ice but sat lower on the neck such that the body, rather than the neck, carried the weight of the collar and the ice. Aluminum neck collars seem less susceptible to icing than plastic collars (MacInnes et al. 1969) but some species will catch their bills in the bands if the ends are not overlapped to eliminate a gap.

As with all marking techniques, responses differ among species, and investigators should systematically evaluate any possible influences of the marker. Because neck collars affected survival, Schmutz and Morse (2000) suggested that collars are useful for providing information on distribution, but may be undesirable when estimates of demographic parameters are required.

Nasal Discs and Saddles

These numbered and/or colored plastic discs or plates are applied to each side of the bird's bill and fastened together through the nasal opening by various methods (Bartonek and Dane 1964; Sugden and Poston 1968; Doty and Greenwood 1974). 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; Regehr and Rodway 2003). Due to the potential for entanglement with vegetation or submerged fishing nets, nasal discs are better suited for species of birds that do not dive and should not be used to study pairing success of the birds (Alison 1975).

Pelayo and Clark (2000) found no evidence that nasal markers had an adverse influence on nesting patterns of pre-laying and laying female Ruddy Ducks (*Oxyura jamaicensis*). Although bill scratching was more frequent, nasal markers did not appear to influence overall reproductive behavior during nesting and or brood rearing. Regehr and Rodway (2003) also found no impact of nasal discs on Harlequin Ducks (*Histrionicus histrionicus*) on behavior, timing of pairing, or female pairing success. However, they did find that males with nasal discs had lower pairing success, and females with nasal discs were less likely to reunite with previous mates. A comprehensive study on the effects of nasal saddles on dabbling ducks (Anatinae) was completed by (Guillemain et al. 2007) who studied the impacts of these markers on Mallards (*Anas platyrhynchos*), Green-wingedTeal (*Anas crecca*), Northern Pintail (*Anas acuta*), and Eurasion Wigeon (*Anas penelope*) in the field and in the aviary. The found that nasal saddles had no effect on body mass, time budgets or other aspects of behavior, apart from a reduced pairing probability in the Teal and, in the Pintails, a slight reduction in the number of aggressive interactions won after marking.

Patagial (Wing) Markers and Leg Tags

Wing tags are highly visible, may be coded for individual recognition, and are retained by birds for relatively long periods of time (Marion and Shamis 1977). Like other markers used to identify individual birds, patagial markers are useful in studies of social behavior, migration, and natal and winter site fidelity. Descriptions of tag types and evaluations of their effectiveness may be found in Anderson (1963), Hester (1963), Hewitt and Austin-Smith (1966), Southern (1971), Curtis et al. (1983), Stiehl (1983), and Sweeney et al. (1985). Some reports indicate that most birds accept patagial tags readily, and adverse effects seem to be minimal (e.g., Maddock 1994). American Kestrels (*Falco sparverius*) marked with patagial tags actually had higher breeding success than did unmarked control birds (Smallwood and Natale 1998). On the other hand, (Kinkel 1989) reported that the survival and reproductive behavior and abilities of Ring-billed Gulls (*Larus delawarensis*) were adversely affected for up to four years after tagging. The effects disappeared when the tags were replaced with color bands. Howe (1979) reported significant impact on survival, as determined by interannual return rates, when none of 27 Willets (*Catoptrophorsus semipalmatus*) returned in the year after banding, compared to a return rate of 64% of birds banded but not marked with wing markers. Howe surmised that for these very long-distance migrants, the drag that might be caused by the markers, or abnormal replacement of feathers at the time of molt might have impaired flight during migration. Tags sometimes result in some wing callouses (Curtis et al. 1983; Kochert et al. 1983) and feather wear (Southern 1971) and in some species, feathers in the area of the tag may not be replaced at the time of molt (Howe 1980; Kochert et al. 1983).

A Velcro™ leg tag developed for marking gull chicks (Willsteed 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).

Radio/Satellite Transmitters

Radio and satellite transmitters represent a great advance for studying birds in the 21st century. Radio transmitters emit a radio frequency that can be detected by a researcher utilizing specialized hand-held equipment. Satellite transmitters send signals to earth-orbiting satellites that transmit the data to a central computer from which researchers can download the data.

Studies utilizing radio/satellite transmitters make the assumption that data collected from tagged animals reflects the natural state of the organism being studied. This is not always the case. Researchers utilizing radio/satellite transmitters to study birds should consider the effects the transmitters may have on a bird's natural behavior and measure these affects during the study.

Impacts generally

Many studies have examined the impacts of external and implanted radio transmitters on survival, reproductive success, various aspects of behavior, and physiological indicators of stress on a wide variety of species in captivity and in the wild. A small sampling of the extensive literature demonstrates the importance of searching the literature for information specific to the species to be studied and for reports of particular problems and solutions, such as transmitter design and methods for attaching the transmitters. For any particular species, some papers report no effect, some describe behavioral changes of short duration, but others report reduced reproductive success or reduced survival. Casper (2009) devised guidelines for the instrumentation of wild birds and mammals. After reviewing the literature, she concluded that there is a lack of evidence with which to justify the broad application of hard and fast rules for instrumentation across the wide range of avian species, which differ in size and lifestyle. Further, the causes of adverse impacts, when they occur, are multifactorial and are related not only to the mass, size, and shape of the device, but also, capture method, the handling time, the attachment method, food availability and the length of deployment.

A recent meta-analysis by Barron et al. (2010) of 84 papers reporting the use of transmitters found that overall, birds are significantly negatively affected by devices in each of 12 measures except flying ability. For two of these 12, energetic expenditure and nesting propensity were substantial, while the impact on offspring quality, body condition, device-induced behaviors, nest success, and foraging behaviors were less so.

Studies of impacts on passeriformes, an order that includes many small birds and many that migrate between the Northern and Southern hemispheres each year, are relatively few in number, probably because transmitters small and light enough to be used on these species necessitate the use of very small, short-lived batteries. Data can be collected for only a brief period. As transmitter and battery size and weight continue to shrink, however, it is likely that more studies of passerines will involve the use of radio or satellite telemetry. Negative reactions by parents to back-pack transmitters on juvenile Louisiana Waterthrush (*Seiurus motacilla*) may have contributed to the mortality of the marked birds or removal of the devices; success in tracking fledglings marked only with color bands was much greater and less time-consuming than the time needed to find and recover transmitters that had become detached (Mattsson et al. 2006). Two studies found no difference in following-year return rates in Wood Thrushes (*Hylocichla mustelina*) or Swainson's Warbler (*Limnothlypis swainsonii*) (Powell et al. 1998;

Anich et al. 2009). Overwintering Hermit Thrushes (*Catharus guttatus*) carried backpack transmitters for one month. Assessments of hematological indicators of stress (heterophil-lymphocyte [H/L] ratios) did not change and did not differ from birds that did not carry transmitters (Davis et al. 2008). Sykes et al. (1990) compared three attachment methods (harness, velcro, and adhesive) and observed a weight loss in both male and female Common Yellowthroats (*Geothlypis trichas*) though the weight loss was significant as compared to control birds only in the birds wearing harnesses.

A similar device known as a geolocator collects information that is retrieved when the bird is recaptured. Because geolocators do not transmit signals, they are smaller and lighter than transmitters and can be used on smaller birds. However, the ability to collect the data depends on recapturing the bird.

Though some behavioral changes, such as increased preening and/or reducing feeding, can impact survival, reported behavioral changes tend to be of short duration.

Gilmer et al. (1974) observed numerous behavioral changes, particularly as to increased comfort behaviors such as preening, by Mallards and Wood Ducks (*Aix sponsa*) fitted with external transmitters, though the changes abated over time. (Hill and Talent 1990) reported that neither Least Terns (*Sterna antillarum athalassos*) and Western Snowy Plovers (*Charadrius alexandrinus nivosus*) showed behavioral changes related to the radio transmitters and they detected no difference between birds with transmitters and controls in terms of daily nest and egg survival, nest depredation, or nest desertion.

Greenwood and Sargeant (1973) observed significantly greater weight loss in captive Mallards and Blue-winged Teal fitted with transmitters than in the control birds (the extent of the loss was directly related to the weight of the transmitter for the Teal but not for the Mallards. When harnesses were fitted to account for age (based on wing length), sex-based difference in size, and body mass data, as well as general body shape, so as to assure a good fit, survival of Saker Falcons (*Falco cherrug*) as measured by return rates, was not affected (Kenward et al. 2001).

Reports on survival and reproductive success have also been mixed and often contradictory, even within a single study. Sooty Shearwaters (*Puffinus griseus*) fitted with imitation satellite transmitters to test for effects before embarking on an expensive telemetry study experienced significant weight loss compared to birds without transmitters during the pre-breeding period but not during the mid-breeding period. There was no difference in colony attendance during the breeding period, but a significant difference during the mid-breeding period, when birds were also handled; colony attendance also dropped for birds that were handled but not wearing transmitters. However, in contrast to other studies, the transmitters seemed to have no effect on chick-raising. The chicks of birds with transmitters emerged from the burrows at the same time as other chicks and showed no reduction in growth rate (Söhle 2003). Paton et al. (1991) documented significantly lower survival among female Spotted Owls fitted with backpack-mounted transmitters (*Strix occidentalis caurina*) as well as reduced nesting and fledging rates. In contrast, Foster et al. (1992) found no differences in survival or body mass in Spotted Owls carrying transmitters but these birds produced significantly fewer young. Two birds died from entanglement in the harnesses and another died from subcutaneous abrasions caused by the harness. Sixteen birds retrapped to remove or replace harnesses had abrasions; in three cases, these were considered life-threatening. These problems appeared to be caused by poor harness fit. Pietz et al. (1993) found that radio-marked female Mallards preened and rested more and fed less than did birds without transmitters which seemed to result in delayed nest initiation, smaller clutches, and reduce egg volume. Houston and Greenwood (1993), however, found no differences in number of clutches, egg mass, or time between nestings in a study of captive female mallards fitted with transmitters of weights ranging from 4 g to 18 g, whether attached with surgical glue or a harness. Results obtained by Rotella et al. (1993) were similar to those reported by Pietz et al. (1993) in that wild female Mallards fitted with harness backpacks nested fewer times and spent less time incubating than did birds without transmitters or birds whose transmitters were sutured or implanted; further more than half the sutured backpacks became detached within two months.

Increased predation on tagged birds is also a concern. All 38 radio-collared Sharp-tailed Grouse (*Tympanuchus phasianellus columbianus*) died over the course of a year (Marks and Marks 1987). Based on the return rate of unmarked birds, 17 marked birds should have returned the following year. Examination of remains of 22 of 23 recovered carcasses showed that mortality was due to predation. The researchers suggested the tagged birds were more conspicuous or perhaps reluctant to fly due to the slapping of the antenna (Marks and Marks 1987). However, Wheeler (1991) reported that predation of Blue-winged Teal (*Ana discors*) hens marked with transmitters did not seem abnormally high, though there was no control group comparison.

Transmitters can also affect nestlings and fledglings. Adults carrying transmitters may feed at reduced rates and outright nest abandonment has been reported, although these impacts may be reduced if transmitters are fitted when the chicks are older. Three of four female American Woodcocks (*Scolopax minor*) fitted with transmitters when their chicks were one-two days old abandoned their broods, but four other females radiomarked when their broods were four or more days old did not abandon the broods; none of the 22 females banded when their chicks were one or two days old abandoned the broods (Horton and Causey 1984). Transmitters negatively affected survival of Dusky Canada Goose goslings (*Branta canadensis occidentalis*) during the first 28 days of life, but not thereafter, and the affect was greatly reduced for goslings that survived the first two or three days after hatching (Fondell et al. 2008). Reduced growth rate and fledging success of chicks raised by parents carrying transmitters has been reported in Cassin's Auklet (*Ptychoramphus aleuticus*) (Ackerman et al. 2004) and Tufted Puffins (*Fratercula cirrhata*) (Whidden et al. 2007).

Impacts of attachment methods

Impacts can sometimes be attributed to attachment methods. Both radio and satellite transmitters can be attached in a variety of configurations each with its own set of limitations for durability and duration of use (Mong and Sandercock 2007). These include backpack harnesses (Kenward et al. 2001; Taylor et al. 2001; Gervais et al. 2006), necklace transmitters (Haug and Oliphant 1990; Leupin and Low 2001; Sissons et al. 2001; Gervais et al. 2003; Rosier et al. 2006), gluing to skin and/or feathers (Farmer and Parent 1997), tail mounts (Giroux et al. 1990; Irvine et al. 2007), and subcutaneous or abdominal implants (Korschgen et al. 1984; Mauser and Jarvis 1991; Wheeler 1991; Pietz et al. 1995; Korschgen et al. 1996; Hupp et al. 2006; Fondell et al. 2008).

Depending on the type of transmitter implanted, nesting behavior can be negatively affected by the implants such as was found in two species of breeding Murres (Meyers et al.1998). However, transmitters that use internal coiled antennas (Korschgen et al. 1984; Olsen et al. 1992) implanted in the coelom of Mallards (*Anas platyrhynchos*) were found to not affect reproduction (Rotella et al. 1993). Transmitters that are completely implanted suffer transmission path loss, which reduces signal strength and limits detection range. A fabric-covered tube that forms a collar at the base of the antenna may help stabilize the antenna and minimize coelomic contamination, but did not control bacteria migrating along the antenna passage through the body wall. No significant health problems were found up to a year after implantation (Mulcahy et al. 2007). Mulcahy et al. (1999) caution that investigators using implanted radios with percutaneous antennas should be aware of the potential for radio extrusion and should minimize the problem by using transmitters that have no sharp edges and that are wide, rather than narrow.

Concerned about earlier reports of aberrant behavior in terns bearing radio transmitters, (Hill and Talent 1990) avoided placing the transmitters on the interscapular region, which flexes in flight, and instead glued them lower on the birds' backs, which also maintained the birds' center of gravity. Racing pigeons (*Columba liva*) suffered little effect when fitted with tail-mounted transmitters, but birds with sacral-mounted transmitters suffered lesions, flew slower, and lost weight and body condition compared to control birds (Irvine et al. 2007). Reynolds et al. (2004) found that tailmounted transmitters on male Northern Goshawks (*Accipiter gentilis*) significantly reduced apparent annual survival from 0.75 (without transmitters) to 0.29 (with transmitters). However, backpacks that weighed more than tailmounts had no sigificant effect on survival of adults.

Demers et al. (2003) tested neck collars as an alternative to harnesses on Snow Geese and found that behavior indicating discomfort eventually ceased, but that all reproductive parameters were negatively affected, including laying date and clutch size. Females with radio collars separated from their mates at a rate eight times that of females with ordinary neck collars. Gervais et al. (2006) on Burrowing Owls (*Athene cunicularia*) found that harnesses for radio transmitters had a much greater survival effect than the necklaces (another term for collars), which were not very different in mass.

Implantation averts impacts on flight but involves surgery, which can have other consequences. As Barron et al. (2010) found in a meta-analysis of 84 papers reporting the use of transmitters, anchoring or implantation, which both require anesthesia, had the highest reported transmitter-induced mortality rates. However, abdominal implantation of transmitters with percutaneous antennae can avert negative impacts such as diminished survival, increased predation, reduced physiological condition, and reduced fecundity; in a study of female Canada Geese, the implanted transmitters had no impact on migration dates and at most, a small effect on nesting propensity, but no effect on other measures of reproductive success and no impact on survival over one year in this particular species. Data on survival rates from years two to four were slightly lower, but not statistically significant, among birds carrying larger transmitters (Hupp et al. 2006). Subcutaneous implantation in female Wood Ducks did not affect reproduction, incubation, or survival as measured by interannual return rates over two years (Hepp et al.

2002). Interannual return rates of female Harlequin Ducks over two years with abdominal implants did not differ from that of birds without implants, despite a short-term reduction in mass (Esler et al. 2000). Captive Florida Sandhill Cranes (*Grus canadensis pratensis*) implanted with biotelemetry devices to measure heart rate and temperature showed no behavioral changes attributable to the transmitters or the surgery compared to control birds that had not undergone surgery or implantation (Klugman and Fuller 1990). Internally placed transmitters (i.e. in the abdominal cavity) appear to have fewer effects on reproduction (Rotella et al. 1993; Garrettson and Rohwer 1998), survival (Dzus and Clark 1996; Paquette et al. 1997), and behavior (Garrettson et al. 2000) than do external transmitters. A drawback to this technique is that the antenna is also contained in the body cavity, reducing reception range. Recent work by (Hupp et al. 2006) demonstrates that if an implanted transmitter is used with an antenna that exits the body, transmitting range increases.

Transmitter weight

Researchers should think carefully about how long data needs to be collected in determining the size of the transmitter needed. Briefly, transmitters need larger batteries to enable a longer period of data collection.

A typical guideline for the use of radio/satellite transmitters is that the transmitter not be more than 5% of a bird's mass (Cochran 1980). Caccamise and Hedin (1985) suggested that percentage of body mass is not the best determinant of the upper limit. Instead, they proposed a formula based on power requirements for flight to estimate the added cost of transportation due to the transmitter. In doing so, they illustrated that small birds can carry much larger loads relative to body mass than can large birds**.** Caccamise and Hedin (1985) also provided a general method based solely on body mass, with a process to refine that estimate for individual species by taking simple measurements of wing morphology and wing beat frequencies**.** Naefdaenzer (1993) tested the effects of radio transmitters on a various small passerine species in the 1990s. He concluded that even the smallest tits could carry up to 5% of their weight without impacts on behavior or survival. Wikelski et al. (2007) noted that the smallest commercially available satellite transmitters available in 2006 (9.5 g) were too large for ~81% of all bird species for which body weights are available following the 5% of body mass guideline. Warner and Etter (1983) found that both reproductive success and survival of female Ring-necked Pheasant (*Phasianus colchicus)* were inversely related to transmitter weight (including harness, battery, and antenna) with transmitters of between 1.98% and 3.22% of body mass. In contrast, Hines and Zwickel (1985) failed to find an effect of transmitters on survival of Ring-necked Pheasants with transmitter weights (including harnesses, antennae, and solar-power devices) ranging from 1.46% to 2.58% of body mass. Casper (2009) concluded that the 5% guideline is essentially arbitary and that the less-commonly cited guideline of 3% appears to have been extrapolated from a review of albatross and petrel studies correlating device loads with foraging trip durations and nest desertions.

In an meta-analysis of 84 papers assessing the impacts of radio transmitters Barron et al. (2010) found no relationship between proportional device mass and the magnitude of the effect when all studies were included in a regression. This suggests that relatively small devices had a similar effect as larger devices. Few studies used transmitters exceeding 5% of body mass and the meta-analysis did not compare the magnitude of effects between studies using devices above and below 5%. That there is little evidence that proportionally larger devices have greater effects suggests that increased attention to design, attachment method, and attachment site is warranted.

Weight may be less important than transmitter design. In a study of harness types, Steenhof et al. (2006) found that female Prairie Falcons (*Falco mexicanus*) that shed their harnesses and transmitters were far more likely to survive than birds that retained the equipment. The authors noted that the transmitters seemed to have no impact during the year the birds were marked; mortality apparently occurred after the birds left the breeding range, suggesting that the transmitters and harnesses may have hampered long-distance migration. The combined weight of the equipment was less than 5% of mass, leading the authors to suggest that a streamlined transmitter design that reduces drag may be more important than weight and that wing morphology and flight characteristics may be more important than body mass. Obrecht et al. (1988) suggested much the same based on the results of a wind tunnel study of the drag caused by dummy transmitters of various shapes and sizes. They found that the drag was sufficient to reduce flight range, which is an important factor in successful migration, and that drag was reduced if the transmitter was elongated with faired endings.

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CHAPTER 4. TRANSPORT OF WILD BIRDS

A. Overview

Research protocols and objectives often call for the transport of birds from the point of capture to a holding facility or laboratory or to release areas removed from the capture site. Transport should not be undertaken lightly because even minor dislocations may disrupt territoriality, nesting and foraging behavior, or social grouping. Transportation also generates stress and may contribute to capture myopathy (see section on Capture and Marking). It is both an ethical obligation and a practical requirement that birds used for scientific purposes remain as close as possible to their original condition on release. Birds subjected to stressful conditions or suffering the effects of mishandling may seriously prejudice the value of the project. Similar considerations apply to the eggs of wild birds because lost eggs may reduce reproductive success. Most importantly, birds are a diverse group and procedures for one species may not be suitable for another even if they are of similar size and behavior.

Unfortunately, as the Institute for Laboratory Animal Research noted in 2006, “there is sparse scientific literature on the effects of transportation on most common research animals, but good practices for all research animals can be established by drawing some universal concepts from the available scientific literature and by understanding species-specific needs. Although precise engineering standards are often preferred by human assessors, the scientific literature supports few engineering standards. This report emphasizes *science-based performance standards*, which define an outcome (such as animal well-being or safety) and provide criteria for assessing that outcome without limiting the methods by which to achieve that outcome (ILAR 2006). Most of the references cited in that publication pertain to traditional laboratory animals, cats, dogs, and other mammals. The few papers about birds pertain to poultry. Nonetheless, this is a worthwhile overview of animal welfare concerns that arise in the context of transportation; the discussions on ambient temperature range and on regulations are particularly useful.

Seek guidance, and especially species-specific information regarding transportation techniques and signs of stress, from experienced personnel, such as zoo personnel, licensed wildlife rehabilitators, or other ornithologists, when possible.

B. Regulatory guidelines

The transportation of wild birds is subject to a variety of international, federal, and state or provincial regulations. Permits and health certificates may be required, particularly for international movement. For detailed information on these regulations see the Ornithological Council's Permit Guide for Importing Live Birds. Container design and other requirements for international air transport are specified by the International Air Transport Association's Live Animal Regulations. Although these regulations are intended for air transport, they are useful guidelines for all kinds of transport. Be sure to obtain the most recent edition before shipping birds. These standards have been adopted by the Convention on International Trade in Endangered Species and are recommended by the World Organization for Animal Health (formerly known as the "OIE"). At the moment, the U.S. Department of Agriculture Animal and Plant Health Inspection Service has no regulations specific to birds. The agency is planning to issue regulations some time in 2010. When that occurs, this section will be revised accordingly. Regulations issued by the U.S. Fish and Wildlife Service (50 CFR 14.101 et seq.) establish standards for the transport of live mammals and birds to the United States. These regulations are largely consistent with the Live Animal Regulations of the International Air Transport Association. Each country has its own regulations that may vary slightly from these international standards.

Some states require a state permit before some (or all) species of birds can be moved into or across the state, whether to be kept in captivity or released to the wild. Before moving live birds across state lines, check to be sure that there are no extant quarantine restrictions imposed by the U.S. Department of Agriculture or by the state to which the birds are to be moved. Quarantine restriction imposed by the U.S. Department of Agriculture are announced in the Federal Register and on the website of the Animal and Plant Health Inspection Service.

C. Considerations for all types of transportation

Containers

Generally: The key to successful transportation is a carefully designed and constructed container to minimize stress and prevent injury and escape. For trips of less than 30 minutes it may be safe to move small birds in simple containers such as bird bags (Redfern and Clark 2001) but large species and longer trips need appropriately designed and constructed containers. A container must be clean and free of protrusions that might cause injury. It should offer easy access for care and removal of the animals in an emergency but must be designed to prevent escape.

Size: There should be enough headroom in any container that the bird can adopt a normal posture and carry out comfort and maintenance activities.Some small birds may be given enough room to fly up and down from perches but flight of larger birds is undesirable. If it is necessary to restrain the wings, the technique must not impair the bird's ability to breath or regulate its body temperature.

Temperature and ventilation: Temperature and ventilation are likely to be the single most important issues in the design of shipping containers. Birds collapse quickly if they cannot keep their body temperature between narrow parameters and will suffer from stress if temperatures are above normal for extended periods. When transporting precoccial chicks or altricial nestlings (see discussions on duration of travel and on food and water, below, with regard to altricial nestlings) provide a protected heat source. It must be of a design that cannot burn the birds, and there must be room for the birds to move away from it. Even where it is otherwise acceptable to put more than one bird into a container or compartment, crowding in the container will increase the risk from poor ventilation or excess heat.

Perches: Perches and other contents in carriers should be securely fastened to avoid bouncing and tipping over during transport. Non-slip perch coverings can help prevent injury and help the bird feel more secure.

Padding: Bird feet, beaks, and eyes (or other parts of the body not protected by plumage) are susceptible to damage and subsequent infection. Padding the floor and other internal surfaces of the container may reduce that hazard. Disposable diapers or other materials that trap moisture might provide protective padding and reduce fouling of plumage by fecal material. Anchored clean carpeting or astro-turf may be more appropriate for raptors and other species capable of shredding less robust materials.

Multiple birds: It may be safe to ship more than one bird per compartment if they will tolerate each other and not fight. Otherwise, provide separate containers or separate compartments when transporting more than one bird. Birds of some species will attack unfamiliar birds of the same species. As a general rule, birds of different species and raptors (except eyases) should be transported in different containers or compartments.

Behavioral considerations: Containers should protect the birds from external auditory and visual stimuli as much as possible. Cover openings in the container with fine, non-fraying wire mesh (such as hardware cloth) to limit visibility in and out without compromising ventilation. Avoid galvanized products because these contain zinc, which is toxic to birds if ingested. Even poultry suffer from the effects of transport in spite of the fact that they have been raised in an industrial environment and are relatively familiar with handling by people (Cashman et al. 1989).

Food and water

It is probably not necessary to provide either food or water to birds for trips that last less than an hour, but for longer trips both may be essential. For some species, a wet sponge may provide water or pieces of apple, cucumber, or other fruit may serve as a convenient source of both food and water. The birds may need to be exposed to these items prior to the trip to accept them as sources of food and water. It may be necessary to design and build suitable dispensers that the birds recognize as a source of food or water or interrupt the journey so that fresh supplies can be provided. As altricial nestlings will almost certainly need to be hand-fed, which precludes transport by methods that do not allow frequent access to the container.

Timing and duration

Transport birds from the site of capture to the research facility as quickly as possible, subject to the need to wait until all birds have been captured. It may also be advisable to evaluate the condition of the bird prior to transport. Bocetti (1994) developed techniques for confining and transporting small insectivorous passerines that entailed evaluating the bird prior to transport. Birds that were lethargic, crouched, or fluffed after 20 min of captivity were released. Birds that did not eat or drink during the first hour of captivity, as determined by the color and texture of the feces, were also released. These precautions resulted in the survival of all birds over a 612 km car trip.

If birds are to be shipped to a distant site, it may be advisable to transport birds to a holding facility to provide a period of acclimation to captivity prior to shipping. Recently captured birds may experience difficulty in adjusting to conditions of captivity. They may fail to eat or may injure themselves thrashing and flapping in the cage or transport container. 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.

It may benefit diurnal birds to transport them at night when they are less active and when both ambient temperatures and their own body temperatures are likely to be low.

The trip should be planned well in advance to minimize the number of transfers and delays and to ensure that a person competent to provide appropriate care is available to meet the shipment upon its arrival. Shipment dates should avoid holidays, and arrivals and departures should occur during normal working hours. Multiday shipping may require a qualified person to accompany the shipment to resolve unexpected problems in ways that protect the welfare of the birds (CCAC 2003, Hawkins 2001). To avoid delays, all permits, health certificates, and other documents should be obtained and completed before shipping. There must be a contingency plan to assure the birds' safety and comfort should unforeseen delays arise.

D. Specific modes of transportation

Air transport

Commercial airlines may ship wild birds. Generally, airlines adhere to the International Air Transport Regulations, even for domestic travel, but each airline has its own rules about carrying live animals, and typically the rules for birds differs from the rules for cats and dogs. Therefore, in addition to consulting the latest edition of the International Air Transport Association's Live Animal Regulations, consult the airline on which you plan to travel on or have ship the birds. Some allow live animals on board in limited numbers and under limited conditions, subject to advance reservations. At the time of boarding, however, they may refuse to allow the animals on board. Many carriers no longer accept live animals as "accompanying baggage" and require shipment through a designated third-party specialist shipper such as the International Animal Exchange, which handles many zoo animals, or Global Animal Transport. Such a specialist shipper will be familiar with the International Air Transport Association's Live Animal Regulations. Airlines that accept birds as accompanying baggage have restrictions based on ground temperatures and other considerations.

Ground transport

Birds moved by ground transport should not be exposed to direct sunlight or subjected to external visual stimuli. Long trips should be broken up by uninterrupted rest periods during which the birds may feed and drink. Redig et al (2007) and Arent (2005) describe techniques and considerations for transport of raptors

E. Health and safety during and after transport

Stress and physiological considerations

Birds may continue to struggle or attempt to flee long after initial capture. This may cause cramp, also known as “capture myopathy” or “exertional rhabdomyosis” (see Capture and Marking section). This and other less obvious physiological responses to stress, such as impairment of the immune system, may be exacerbated by transport (Kannan et al. 1997). An understanding of the species’ behavioral signs of stress is also a helpful in assessing the transport method and journey. Birds that have received general anesthesia should be fully recovered prior to transport (CCAC 2003).

Disease

Wild birds are exposed to a variety of diseases, parasites, and epizootic organisms. Transported birds might carry undesirable strains of microorganisms into new habitats or be a source of *Salmonella* contamination (Proctor and Malone 1965). In addition, the stress of transportation is likely to make birds more susceptible to disease and infection (Elrom 2000). It is extremely important that shipping containers be sterilized before and after use and that sick birds be isolated from the remainder of the shipment as soon as possible.

Quarantine

A quarantine and observation period before research begins, or before birds already in captivity are exposed to the new birds, or before release can help to rule out many health concerns. Public health or agricultural authorities may require quarantine and medical testing. The duration of the quarantine should be as required by law or if not specified, then quarantine should reflect the incubation period of known diseases (usually 15 to 60 days). If birds were imported into the United States, they will be subject to 30-day quarantine by the U.S. Department of Agriculture in its facilities at the importer's expense. Arranging for this limited space is a complicated procedure involving careful timing. Consult the Ornithological Council's Guide to Importing Live Birds into the United States for details. Most countries have similar procedures.

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CHAPTER 5. CAPTIVE MANAGEMENT

A. Overview

Maintaining wild birds in captivity is expensive, time-consuming, and requires expertise. That expertise can often be found outside the community of ornithologists. The captive living conditions should be appropriate for each species of bird and should contribute to both health and comfort. Investigators maintaining captive flocks of wild birds may refer to King et al. (1977) and Ritchie et al. (1994), and especially Ritchie et al. (2008) for a thorough discussion of topics related to captive maintenance, health issues, and veterinary care of birds. Zoos are a potential source of help and information. The American Zoo and Aquarium Association (AZA) publishes a series of species- or taxon-specific captive management guidelines: Animal Care manuals (which will replace the Husbandry Manuals) and Feeding Program Guidelines) that will be very helpful.

Choosing appropriate veterinary assistance is critical. A veterinarian with experience in avian medicine is preferred. The American Board of Veterinary Practitioners offers board certification in avian medicine and offers a list of boarded veterinarians. Attending veterinarians on the staff of a research institution with vertebrate animal facilities can help a researcher find a veterinarian knowledgeable about birds, if s/he is not. Often, a suitable veterinarian must be identified as part of a vertebrate animal protocol. Other sources of information about wildlife veterinarians include your local zoo, the American Association of Wildlife Veterinarians, the American Association of Zoo Veterinarians, and the American Association of Avian Veterinarians. For routine care and maintenance, it is advantageous to have an animal health technician who has obtained a degree from a program accredited by the American Veterinary Medical Association or the Canadian Veterinary Medical Association (or such other veterinary associations as exist in your country) and who has been licensed in your state on staff. The American Association for Laboratory Animal Science offers certification at three levels of competence: assistant technician, technician, and technologist. Each requires a certain amount of experience and an examination.

B. Regulatory requirements and oversight

Federal and state agencies require permits to remove birds from the wild. See the scientific collecting permit information (U.S. Fish and Wildlife Service), state permit information, and

Canadian provincial information provided by the Ornithological Council. Outside the United States or Canada, check national and provincial or state laws.

Under the Animal Welfare Act regulations, Institutional Animal Care and Use Committees "must inspect at least once every six months, all of the research facility's animal facilities, including animal study areas, using title 9, chapter I, subchapter A-Animal Welfare, as a basis for evaluation; Provided, however, That animal areas containing free-living wild animals in their natural habitat need not be included in such inspection" [9 CFR 2.31(c)(2)]. A study area is any building room, area, enclosure, or other containment outside of a core facility or centrally designated or managed area in which animals are housed for more than 12 hours (9 CFR 1.1). To the extent that a research facility or study area is subject to inspection, the housing standards established by the Animal Welfare Act regulations are applicable. As of January 2010, however, the Animal Care program of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service had not yet promulgated regulations pertaining to birds. It is anticipated that the regulations will be proposed in 2010 and will become final by 2011. When this occurs, these Guidelines will be updated accordingly.

C. Quarantine of animals

Introducing new birds to a facility, whether from the wild or from another captive setting, carries the risk of disease transmission between new and established birds. The increased stress of capture stress and acclimation to captivity for wild birds or a new environment for birds already in captivity, as well as shipping, has the potential to reduce immunity, making them more susceptible to new infections or resulting in subclinical infections becoming life threatening (Ferrell et al. 2007). Thus, quarantine procedures should protect both incoming birds and any established birds. Quarantine assumes that new arrivals may have been exposed to a contagious pathogen and are therefore kept apart from others to prevent disease spread. Though in practice quarantine and isolation entail the same procedures, the term isolation is used when the individual is known to have contracted an infectious disease.

Research institutions with vertebrate animal facilities will have standing quarantine procedures covering all animals. The avian researcher may, however, want to consult with the avian veterinarian to make sure that these procedures adequately protect birds from specific avian diseases and from additional stress during quarantine, e.g. from social isolation or inadequate space.

Generally, all newly acquired birds must be strictly quarantined from other captive birds for a minimum of 30 days. If the birds were imported from outside the United States, they must, by law, be quarantined for 30 days in a U.S. Department of Agriculture quarantine facility. Special regulations [9 CFR 93.106(b)] apply to psittacines and ratites. Quarantine times (pre-shipping and post-arrival) vary from one country, as will import permit requirements. As government quarantine facilities test only for diseases of concern to the government agency (primarily non-native diseases that can impact poultry) it is advisable to re-quarantine the birds when they arrive at your facility. For more information about the complex process of importing live birds, see the Ornithological Council's Guide to Importing Live Birds.

During daily care, caretakers should deal with quarantined birds last and not return to other housing areas. If you must return to other areas, wear disposable outer garments and wash hands and other exposed areas before moving between quarantined and post-quarantine birds. The quarantined birds should be observed for symptoms of disease by personnel familiar with birds. Fecal examinations for intestinal parasites and visual examination for external parasites should always be performed on arrival and when the quarantine period ends, before exposing quarantined birds to other captive birds. Diagnostic procedures for *Salmonella, Chlamidia,* tuberculosis, and other significant diseases of concern should be considered; an avian veterinarian should be consulted to determine which tests should be performed. For West Nile virus-susceptible species (e.g. corvids, vultures, and raptors) maintained in outside aviaries, an assessment of serologic status with respect to West Nile virus or other vector-borne disease should be considered if there is evidence of recent and localized increase in West Nile virus activity. Outdoor housing, which is often needed for large flight areas and normal social groups, remains appropriate except in case of outbreaks. In that case, indoor housing may be appropriate on a temporary basis. At present, vaccines for birds are not yet considered effective and are not commercially available (Davis et al. 2008; Jarvi et al. 2008). A wildlife health professional should be consulted for assessment and testing.

Plan ahead for post-quarantine disease protection. Some infectious agents (e.g. *Coccidia* and *Mycoplasma spp.*) are shed in feces by infected birds and may contaminate the housing or substrate (Dhondt et al. 2007a; Dhondt et al. 2007b). Replace or sterilize perches, substrates, and especially soil substrate, in outdoor aviaries where new birds are to be quarantined or released. If infected, these birds should be treated and when cleared of infection moved to enclosures with clean substrates. The substrate in the original enclosure should be disinfected or replaced. Any potentially contaminated soil from the quarantine aviary should be disposed of off-site and replaced before that space is again used to house birds. Check local and state regulations for disposal.

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

D. Prevention and control of animal disease

The investigator and all animal care staff should observe all laboratory birds closely at least once a day for clinical signs of illness, injury, or abnormal behavior. Investigators and animal care staff should familiarize themselves with common problems and signs of illness. All deviations from normal conditions and deaths from unknown causes should be reported at once to the investigator and person responsible for veterinary care. Common signs of illness include:

a. 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;

h. obstructed nares or matted feathers around the nares;

i. decreased consumption of food, increased consumption of water;

j. open-mouthed breathing, panting

Consult a veterinarian immediately if any of these signs are observed.

Generally speaking, by the time a bird looks ill, the illness is usually well advanced. Therefore, an immediate response to apparent illness is required. The potentially ill bird should be isolated immediately. A sick bird should be moved to a room designated at least temporarily as a treatment room. Small incubators or commercial brooder units are ideal to hold ailing birds, but be sure that the unit provides adequate ventilation and can be disinfected later. Food and water may need to be placed on floor if bird is too weak to continuously perch. Do not come into contact with the sick bird before caring for healthy birds without taking precautions (such as disposable outer garments and hand-washing) to prevent transmission of illness.

If the illness is contagious, by the time it is detected, other birds have probably been exposed, and treatment of additional birds may be necessary. Regular monitoring of feces for both macro and micro-parasites is prudent and non-invasive, especially for birds in outdoor flight cages, exposed to local wild birds.

All laboratory or aviary birds that die from causes other than a planned portion of the experimental design should be submitted for necropsy. A diagnostic facility that deals with diseases of wild birds will be the best choice if birds were brought in from the wild. Some avian diseases are zoonotic, so determining cause is both a research care issue and a human safety issue. The USGS National Wildlife Health Center provides a wealth of information, including a Field Manual of Wildlife Disease: General Field Procedures and Diseases of Birds (Friend and Franson 1999).

E. First aid

Bleeding is the most common emergency. Bleeding from the mouth or nares usually indicates serious internal injury that requires immediate veterinary attention.

Newly forming feather that still have a blood supply in the feather shaft may break. These broken blood feathers may clot on their own, but if they do not, one of several actions may need to be taken. If the damage to the feather shaft is minimal, then the blood flow can be stopped by applying corn starch or a styptic to the site. If the break is close to the skin, corn starch should be used as the coagulant since styptic may irritate soft tissue. Some use needle-nosed pliers to pull the broken feather. This is rarely necessary and should be performed only by experienced personnel. Broken blood feathers and small flesh wounds can be closed using new, fast-drying versions of cyanoacrylate surgical glues such as Dermabond®, LiquiBand®, SurgiSeal®, and Nexaband® which take under a minute to set and are far less toxic than earlier surgical glues. Older cyanoacrylic tissue glues (e.g., Tissu-Glu®, Ellman International, or Vetbond®, 3M Corp. took several minutes to dry, which markedly increased handling time. Household super glues are toxic to tissues It may be advisable to flush the wound with sterile saline solution and apply antibiotic ointment on the wound.

Overgrown toenails and beaks are also common problems. Beaks can be trimmed using a small dremel tool. Toenails can be cut with blunt scissors or a toenail clipper, avoiding the quick (which can be impossible to see in birds with dark nails). Veterinarians often have an assistant hold the bird in a towel but this can be difficult for large or strong birds as they are often able to free their wings. A stockinette may be useful in restraining these birds. Kwik-Stop styptic powder or gel should be available to stop bleeding in case the quick is cut. Never use styptics on skin or soft tissue. Natural methods of beak growth regulation can be achieved by providing birds with hard surfaces upon which they can rub their beaks.

F. Separation by species

Several species may be routinely held in a single area or facility, provided the requirements or habits of the species are not in conflict and social factors such as interspecific dominance over food or nervous responses of one species to another's calls does not result in additional stress (Hahn and Silverman 2007). 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 easily transmitted or fatal in the other.

G. Daily care

Staple food

Animals should be fed palatable, uncontaminated, and nutritionally balanced food daily or according to their particular requirements, unless the experimental protocol requires otherwise. Birds need to have food readily available to them in the morning, due to their high metabolism and energy expenditure throughout the night to perch and maintain body heat. Feeding *ad lib* can be problematic with some species, such as psittacines, which may become obese due to the constant food supply and the relative lack of activity in confinement. Diet should take into consideration the natural diet, including micronutrients, such as carotenoids that are involved in immune function as well as normal mate choice (Blount et al. 2003). One major problem with formulating diets is the greater diversity of foods available in the wild, even to specialist species (Koutsos et al. 2001). For instance, seed eaters may eat dozens of different seed types and a mix of only two or three seed types will greatly reduce the total nutritional breadth (Pruitt et al. 2008). Consider time of year, ambient temperature and breeding activities, all of which may alter the optimal diet even within species (Harper 2000).

Because diets can be highly specialized, they must be tailored to the species in question, with attention paid to role of the natural diet in beak maintenance such chewing materials and the role in social behavior. Where possible, food should be presented in ways that foster natural foraging behaviors. A zoo nutritionist, avian veterinarian, or other expert should be consulted before formulating a diet or adding grit, vitamins, or other supplements to an existing diet that is specific to the species, feeding behavior and possible formulas for cleanliness versus nutrition.

The form of food and its presentation are important to many species. Wild birds may prefer more caloric food items when offered beside less caloric food items, and it would be unwise to assume a bird would select a balanced diet (but see Boa-Amponsem et al. 1991; Steinruck and Kirchgessner 1992, 1993; Denbow 1994; Lee 2000 for evidence that poultry can seek specific nutrients). Some species may become "addicted" to certain preferred or easily eaten foods, e.g., sunflower seeds, and will refuse anything else, even to the point of severe malnutrition. Placement of the food in the cage may alter its appeal, e.g., vigilant, predator-phobic birds may be unwilling to feed on the floor if newly placed in a large cage. Alternatively, feeding on the floor or other location that requires flight to and from perches may increase energetic expenditure and help maintain fitness (Schnegg et al. 2007).

If hand-rearing birds, consider that experience with a varied diet early in life may help prepare them to accept a broad healthy diet as adults and will be especially important if they are released (Liukkonen-Anttila et al. 1999; van Heezik et al. 2005; Moore and Battley 2006).

Grit

Many birds may require grit in their gizzards to process their food or as a source of minerals. The need for grit differs based on both diet and taxon; not every species needs or will benefit from the addition of grit to the diet (Amat and Varo 2008). While some birds may require grit to digest their food, there is concern among some bird researchers that improper grit in the diet could lead to an increased risk of impaction (Gionfriddo and Best 1999). López-Calleja et al. (2000) found that grit consumption varied greatly with season in the Rufous-collared Sparrow (*Zonitrichia capensis*) and laboratory experiments suggested that grit consumption is voluntary behavior rather than an incidental ingestion. However, grit use is less common in insectivores and frugivores (Gionfriddo and Best 1996) and while it may reflect a need for minerals such as calcium, it is best to avoid offering grit *ad lib* to avoid impaction.

If grit is needed, 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. Some investigators may prefer incorporating calcium and minerals directly in the staple diet. Calcium and other minerals can also be offered in other forms. See Dhondt and Hochachka (2001) and Dawson and Bidwell (2005) for information on calcium requirements of breeding birds.

Vitamins

Supplemental vitamins might be needed, depending on the quality of the bird’s rations. Commercial diets, such as the pellets available for psittacines, already contain vitamin and mineral supplements; additional vitamins and minerals should be provided only after veterinary consultation. Overdoses of some vitamins can be toxic (e.g., Vitamin A, Allen and Ullrey 2004), and can produce symptoms similar to vitamin deficiency (Koutsos et al. 2001). Vitamin deficiencies may show up in varied ways. Physical symptoms of vitamin A deficiency include thickening of the skin, especially around mucous membranes, and poor body condition (Koutsos et al. 2001). Vitamin deficiencies in psittacine species can manifest themselves in behavioral problems; for example, vitamin A deficiency can result in feather picking (Torregrossa et al. 2005) and alter production of vocalizations (Koutsos et al. 2003).

Vitamins may be given in food or water. Most pet stores sell water-soluble vitamin powders. Some supplements are meant to be placed in bathing water or misted on feathers and ingested during preening. This is a handy technique for finicky eaters. Drinking water supplements should be avoided in species that do not drink large amounts of water (e.g. arid zone species), because the birds may ingest very little. Conversely, in species that dunk their food (e.g., many corvids), vitamins in the water could increase the risk of vitamin toxicity (Allen and Ullrey 2004). Researchers should consider the lack of control over the amount of vitamins taken by individuals when vitamins are added to a communal water dish.

Water

Give fresh water daily. For species that normally bathe in water, water should be provided in open, shallow containers to allow bathing. Some birds may be misted for feather maintenance. Containers should be made of non-porous materials such as heavy, tempered glass, glazed porcelain, or stainless steel.

Perches should not be placed directly over open water containers. Drinking water may also be provided in commercial bird-drinking tubes. Drinking tubes for small mammals (nipple waterers) may be used if birds will adapt to use them, but some birds will refuse to drink from these. Automatic tube watering systems reduce spillage onto cage liner material, thus reducing the growth of fungus and allowing the main water source to be cleaned without opening the cage. Open water containers should be washed daily with soap and water and at least twice weekly with diluted household bleach. Rinse thoroughly with clean water. Prepare a fresh dilution for each use, as bleach breaks down in water after 24 hours and loses its disinfecting properties. Other options include A-33® or Simple Green®. Simple Green® is non-toxic and biodegradable and has even been used to clean oiled wildlife. It does not pose the risk of soil contamination if water tubs for large birds need to be cleaned on site. Closed water bottles may not need daily cleaning.

Cleaning

Cages should be thoroughly cleaned at appropriate intervals determined by how quickly they are soiled as well as problems with mites or other pests. Cages should always be cleaned with disinfectant and/or in commercial cage washers after use by one bird is completed and before another is introduced.

Change cage liners often enough to maintain good hygiene. Seed-eaters usually have relatively dry feces and, for such species, cage bottoms may be lined with newspaper and changed less frequently than other species. Insect and fruit eaters tend to have messier (and smellier) droppings and should have the cage trays (bottoms) cleaned as often as necessary to maintain a clean landing and feeding area. Species-appropriate cleaners should be used on cage trays and cage wires. Cage liner materials range from wood particles or pelletized paper to newspaper or commercially available cage liner paper. Newspaper is now generally printed with soy-based inks, but some inks may be toxic to birds that chew or shred cage paper. Water absorbency and the ease of thoroughly cleaning out and replacing used lining material are considerations. Cage lining material should be stored in an area secure from rodent or other contamination.

When choosing cleaners, it is wise to consult the Material Safety Data Sheets to make sure the chemicals involved are safe for birds. The amount of ventilation should also figure in the choice of cleaning agents. Other considerations include the effectiveness of cleaners in reducing or killing specific disease organisms or whether a bird might contact or even ingest cleaning agents through food being dropped on the floor and then eaten or through chewing on washed perches or hanging on cage wire by their beaks (as do psittacines). For species that dunk food in their water dishes, researchers should take extra care to rinse water dishes thoroughly after washing.

Wash seed dishes twice weekly using a safe and effective disinfectant such as sodium hypochlorite (household bleach) diluted 1/10. Prepare a fresh dilution for each use. Rinse thoroughly with clean water. The amount of ventilation may also influence in the choice of cleaning agents. Other considerations include: the effectiveness of cleaners in reducing or killing specific disease organisms; whether a bird might contact or even ingest cleaning agents such as through food being dropped on the floor and then eaten or through chewing on washed perches or hanging on cage wire by their beaks (as do psittacines). See Patnayak et al. (2008) on the effectiveness of different disinfectants and hand sanitizers. 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.

H. Caging and housing

Birds in captivity can be held in cages, aviaries, and outdoor pens. Depending on the species, one or another might be more appropriate, and the maintenance of each differs. The size, shape, and design of the enclosure shall be appropriate for the species being housed and allow space, without overcrowding, for the normal movements of each bird. If experimental design requires that birds be housing individually, it may not be possible to provide enclosures large enough to permit flight.

Cages

Stainless steel, galvanized steel, fiberglass, or plastic cages permit easy cleaning as they may be steam-cleaned 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). Soldered joints should have a protective coating to prevent lead poisoning or have lead-free solder, though the content of lead-free solder should be investigated to rule out other possible toxicity. Painting metal surfaces with a durable, moisture-proof substance such as epoxy paint or spar-varnish can protect against rust. These paints should resist cleaning agents, disinfectants, and scrubbing. Wood cages be more difficult to clean and maintain and thus, may not appropriate. Cages, runs, and pens should be in good repair and devoid of sharp protrusions that might injure the birds or hook on bands.

If experimental designs require the use of wood-and-wire cages, these should be checked frequently for mites. Pyrethrin sprayed into cracks and corners will kill these pests. Always consider the toxicity of pesticides to birds that may inhale or ingest them. Post (2007) provides information on low risk pest management. Cages that have been infested with mites must be free of them before being reused.

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 ultimate responsibility in determining adequate cage size, but there are published minima for some species (e.g., Hawkins 2001). Cage shape is also important in allowing normal movement; for instance, a cage with a greater length supports flight better than a tall cage of the same volume. Conversely, a tall cage can allow birds to get above care personnel and feel safer. Zoo publications (e.g. Association of Zoos and Aquariums Animal Care Manuals) can provide information on specific species.

Cage Bottom

Paper, fine sand, wood-shavings, or newspaper are among the materials that may be used on cage bottoms. Newspaper is now generally printed with soy-based inks, but some inks may be toxic to birds that chew or shred cage paper. In choosing material, consider the need for water absorbency and the ease of thoroughly cleaning out and replacing used lining material. 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 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. Wire bottom cages may be appropriate for some species (e.g., some galliforms), but effects on birds' feet should be considered. They should be avoided for seed-eating song birds as some individuals may knock their entire seed allotment through the wire. Cage lining material should be stored in an area secure from rodents or other sources of contamination.

Perches

Perch type should be appropriate to the species. Perches should provide good footing. 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 may increase the incidence of foot sores due to slippage, it may be necessary to wrap the perch with a non-abrasive, non-slip surface. For example, raptor keepers often wrap rope around a perch. Perches should not be covered with sandpaper. Inappropriately sized perches will lead to leg swelling. A variety of perch sizes provides more foot exercise and relieves repeated pressure on the areas of the feet and toes that come into contact with the perch. This pressure can lead to bumblefoot, a common affliction in captive birds. The initial inflammation can lead to infection, degeneration of the bone, and ultimately the loss of the foot or entire leg. Concrete perches may be good for toenail and beak maintenance.

Aviaries

The natural behavior of some species entails social groupings. In such cases, housing of groups may enhance well-being. Naturalistic social groupings may also be desirable in behavioral studies. Where space is available, aviaries can accommodate groups and allow birds to fly and maintain their flight musculature. It may be harder to catch individuals flying free in an aviary. Double entry doors are essential to prevent escape. Ideally, the doors will interlock to prevent the exterior door from being opened before the interior door is closed.

Every substrate has drawbacks. Concrete, for example, can lead to foot ailments in ground-dwelling birds (Martrenchar et al. 2002). The floors of indoor aviaries may be covered with newspaper, washed and sterilized sand (commercially available, often as playbox sand), 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 the use of 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).

Surfaces 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.

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. Shrubs and trees in pots or planter boxes, or planted on the ground in the aviary, enables birds to hide when potential predators or unfamiliar human 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 ground. Plantings may attract insects relished by many birds. However, vegetation may make it difficult to detect and exclude pests and predators and to clean the enclosure. 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 and snakes are especially dangerous. Single raccoons are known to kill confined birds as large as cranes and pull sleeping birds through chain link fencing; additional protective barriers should be placed near perimeter perching sites. Further, raccoon droppings may carry parasites *(Baylissascaris procyonotis*) capable of attacking the avian central nervous system (Ritchie et al. 1994). Electrified fencing outside the enclosure fencing but out of reach of the birds' beaks can be effective in deterring some predators as well as enhancing security.

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 also breed in wooden boxes into which a layer of wood shavings may be introduced. Nest boxes should be made of materials that don't allow buildup of heat and moisture. Some birds may build nests in bushy boughs tied together in a bunch to simulate a bush, or in a potted Boston fern or ornamental bunch grass. Consult the literature and zoo experts for species-specific information about successful captive breeding of individuals. Consider cleaning problems during nesting (e.g. parrots do not remove feces from the nest box).

Lighting

Many bird species see into the ultraviolet range (Cuthill and Partridge 2000; Rajchard 2009) and use ultraviolet cues in various visual behaviors such as mate choice and foraging (Maddocks et al. 2001) so it may be advantageous to use full spectrum light sources in indoor facilities. Young birds may also benefit from full spectrum light (Maddocks et al. 2001) and is important for health and avoiding diseases such as rickets (Edwards et al. 1994). A small night light placed near the food source is desirable in outdoor aviaries during cold weather, to allow late evening feeding. A night light may also alleviate stress in recently captured birds and in certain experimental protocols but it is important that night lighting be minimal so as not to interfere with natural photoperiodicity (except, of course, where manipulation of photoperiodicity is part of the experimental design).

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. Breeding and molting may be facilitated or suppressed by photoperiod and thus affect research goals inadvertently if not properly planned. Some birds will produce eggs continually, which can deplete calcium levels; egg-binding is also a potential problem. Bird breeders commonly reduce the incidence of egg production by increasing the duration of the periods of darkness. Behavioral problems such as aggression resulting from increasing hormone levels may also be managed by increasing the duration of the periods of darkness.

Temperature

Maintain a temperature range appropriate to the species with a thermostat-controlled heating source. The recommendations for several common species offered by the Guide for the Care and Use of Laboratory Animals (ILAR 1996) are based on professional judgment and experience. Although the Guide also recommends that daily temperature fluctuations be kept to a minimum to avoid repeated large demands on the animals' metabolic and behavioral processes to compensate for changes in the thermal environment, it also recognizes that the recommended temperature ranges might not apply to captive wild animals, wild animals maintained in their natural environment, or animals in outdoor enclosures that are given the opportunity to adapt by being exposed to seasonal changes in ambient conditions. The same would be true of daily fluctations. However, extreme temperature changes may be stressful to the immune system or even lethal, 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 are available in pet stores. Portable heaters can pose a fire hazard. The Consumer Product Safety Commission recommends that portable heaters be turned off when no one is in the area to monitor them. They may be necessary for emergency use, but be sure that the units meet current safety standards, that they are kept at least three feet from combustible materials, and be sure someone is present at all times.

Humidity*, *ventilation, and air exchange

Standards for humidity, ventilation, and air exchange have not been established for birds other than poultry species. Keep humidity within the normal range of the natural environment of the species, particularly if normal behavior and reproductive success are expected. Tropical species can incur health problems such as skin flaking and feather plucking if housed in an environment that is too dry. Hatching success of eggs of some species is sensitive to humidity. The ILAR Guide (1996) stipulates that 10-15 fresh air exchanges per hour is an acceptable general standard.

I. Enrichment for birds in captivity

The concept of behavioral enrichment for captive animals as a welfare issue extends to birds in captivity (Dawkins 2006). The goals of enrichment range from providing opportunities for natural behaviors such as exercise, foraging, or social interaction to providing new challenges that engage birds cognitively, relieve “boredom” and offset the development of abnormal repetitive behaviors (Meehan et al. 2003). The latter may be particularly important for specific groups (e.g., Psittacidae and Corvidae) but the concept is relevant to all species. The need for and nature of enrichment will vary depending on the research protocol, time in captivity, space and numbers of individual birds housed, and other factors. There are numerous treatments of enrichment techniques in the aviculture literature, zoo publications, and increasingly in academic literature. A nonprofit organization known as The Shape of Enrichment organizes workshops and regional and international annual conferences on environmental enrichment, publishes a quarterly journal, and maintains a lending library of training videotapes.

J. General maintenance

Storage of feed and supplies

Store supplies and equipment in cabinets or rooms that can be fumigated, i.e., that are not used to house animals. Store feed and bedding supplies in rodent-resistant, covered, labeled containers that are easily cleaned and disinfected. These may be housed in close proximity to the bird colonies or aviaries. Post (2007) provides information on low risk pest management.

Keep food at appropriate temperatures to maintain freshness and avoid bacterial or fungal growth or deterioration of fats. The shelf life recommended by the manufacturer should be noted and containers marked with expiration (discard) dates.

Disposal of waste material

Keep garbage cans holding waste material outside the immediate area of the laboratory or away from the aviary. Use of garbage liner bags and daily removal of garbage is encouraged. It is a good idea to label the contents of garbage cans as they may be used for either garbage or storage of food or bedding.

Cleaning laboratory floors

Sweep or mop laboratory floors regularly and maintain in a clean condition. Choose floor cleaners both for their ability to disinfect and inhibit the growth of harmful organisms and also for safety for inhalation, ingestion, or contact exposure.

Provisions for emergency care

Post the names, addresses, and phone numbers (including emergency numbers) of consulting veterinarians, physical facility personnel, and individuals responsible for the animals in a prominent place. Animals should be observed and cared for every day, including weekends and holidays, to safeguard their well-being and to satisfy research requirements.

Dead animals

Wash and disinfect the cage after the carcass has been removed. It is advisable for all dead animals to be necropsied by a veterinarian familiar with wildlife diseases. "Fresh" necropsies are preferable, but if that is not possible, dead birds should be refrigerated in a sealed plastic bag and taken to the veterinarian as soon as possible. If a delay of longer than 24 hours is likely, the carcass may be frozen and sent to the National Wildlife Health Center. See the National Wildlife Health Center video for shipping guidelines.

Depending on the condition of the carcass, dead birds may be valuable to museums, teaching collections, or other researchers. However, if the bird is not properly preserved and the data needed by scientists is not recorded, the time and energy it takes to bring the specimen to a museum or other research institution may be wasted. These instructions will help to ensure that your donation will be useful.

Prepare a label with the following information written in waterproof ink or pencil: date bird taken from the wild; date bird brought to you; date bird died; your name and contact information; (optional) cause of injury, if known; medical reports, including lab results (especially toxicology), medications, necropsy. Place bird and its associated label in a separate clear plastic bag. Using clear plastic bags is helpful when possible because then the receiving party can immediately see the specimen and determine its identity, quality, and preparation or sampling future. Close the bag and squeeze out as much air as possible. Ziploc bags or heat-sealed bags are best. It is helpful to place this bag in a second closed bag, particularly if the specimen is going to be stored in a freezer for some time before it is donated. For large birds, kitchen trash bags or larger trash bags are acceptable, but be sure to close the bag tightly. If you really want to do a professional job, put a wad of absorbent cotton or tissue down the bird's throat to prevent fluids from seeping out onto the plumage, then arrange the bird in the bag so the feathers (especially the tail) aren't bent and the head, neck, wings, or legs aren't projecting at awkward angles (they are easily broken when frozen).

If you need assistance finding an institution to accept your salvage specimens, the Ornithological Council can provide a list of museums that are willing to accept specimens. It is helpful to contact museum staff in advance, to determine if they have specific requirements, and to work out arrangements for shipping and shipping costs. Not every museum will accept every bird. There is a cost associated with this process, and the specimen may not be of sufficient interest to warrant the cost.

K. Special considerations for aquatic birds

Aquatic species have special needs mainly 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. Researchers should consult species-specific information such Animal Care manuals of the American Zoo and Aquarium Association.

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 enclosures that allow swimming and an easy exit from the water. In general, a pan of water in the cage is insufficient unless it is large enough to allow bathing *and* water is changed frequently. How frequently will depend on how rapidly a dirt, feces, or dropped/dunked food accumulates. If a film appears on the surface, the water should be changed. 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 less disturbing to the birds. 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 (unreported pers. obs.) report excellent results keeping shorebirds in a filtered system that employs activated charcoal and a UV sterilizer, and filters particles larger than two micrometers. 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 that result primarily from pressure sores developed when the bird is forced to stand for long periods on hard flooring. These sores 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 can lead to slow and painful death and always lead to some loss of function. 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. Contact a veterinarian immediately.

For birds that will be held for more than two to three days, cage and, in some cases, wading pool bottoms must be lined with some resilient material. Possibilities range from natural materials such as sand or small gravel to plastic or rubber mats. All have their pros and cons, including risk of ingestion and impaction (sand), and buildup of bacteria or fungi and hence frequent replacement (sand, wood shavings) to slipperiness and the risk that taller wading birds or keepers will fall. Rubber or plastic mats (e.g. carpet padding) and a commercially available, slip-proof, rubberized waterproofing system called Tufflex☐ are among the possible flooring materials. Flushing these surfaces with running water is reported to give good results (Rubega and Oring, unpublished pers. obs).

L. Raptors

Standards applicable specifically to raptors are discussed in several books (Carpenter et al. 1987; Redig et al. 1993; Naisbitt and Holz 2004; Arent 2007; Bird and Bildstein 2008). As raptors comprise a large percentage of rehabilitated wild birds, rehabilitation guidelines are useful resources for those holding raptors in captivity. The U.S. Fish and Wildlife Service in 2003 published a new regulation establishing rehabilitation housing standards using as a guideline the standards developed by the National Wildlife Rehabilitators Association and the

International Wildlife Rehabilitation Council (Miller 2000). Copies may be obtained by contacting either the National Wildlife Rehabilitators Association or The International Wildlife Rehabilitation Council. These guidelines address housing for both waterbirds and raptors, including enclosure size.

M. Identification and records

A durable label attached to each experimental cage should contain the following information:

a. Species; number of animals and individual identifying information

b. Date experiment started, and projected end (approximate);

c. Feeding instructions;

d. Name of responsible investigator and contact information, including emergency contact numbers if the investigator is unavailable.

Metal label holders will be helpful if the birds chew readily (e.g. psittacines).

Records should include source and eventual disposition of each animal. 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.

It is recommended that birds be leg-banded with plastic or metal bands to facilitate identification of individuals. Open bands can catch on caging but it is difficult, if not impossible, to put a closed band on an adult bird. See the chapter on Capture and Marking for detailed information about methods of marking individual birds. Implanted microchips are a good option if birds can be approached with a microchip reader or if microchip readers can be incorporated into perches or food stands.

N. Disposition of birds after studies

Upon completion of studies, researchers should release field-trapped animals whenever this is practical and allowed under national, state, or local laws and under permit conditions. Even then, do not release animals if release might be detrimental to the existing gene pools in a specific geographic area or if the animal has been exposed to potential pathogens that could be released into wild populations. Never release animals if their ability to survive in nature has been irreversibly impaired by major structural or physiological damage, e.g., surgical deafening. Birds that have been so impaired, but are otherwise healthy may be donated to zoos or other appropriate organizations that hold permits to maintain such birds. Animal should also be assessed for the presence of stereotypic or otherwise detrimental behaviors that may have been acquired in captivity. Pre-release conditioning, such as housing in a large flight cage or aviary to improve flight musculature, is essential. Be sure they recognize natural food and gradually remove any supplemental foods that they will not encounter in the wild. Post-release supplemental feeding may increase the chance of survival by giving the bird adequate nutrition while it learns where to find natural food sources. Each bird should be examined for signs of injury.

Release birds at or near the site of the original capture, unless conservation efforts or safety considerations dictate otherwise. Otherwise, release them into areas where conspecifics exist. Some states prohibit the release of animals or require a permit, so always consult the state wildlife agency in the state where birds are to be released. Birds should be released early in the day so that they will be able to feed and locate suitable roosting sites before dark. Wait for favorable weather conditions and release birds when seasonal conditions are conducive to survival. If birds are wearing color bands, remove them prior to release or check with the U.S. Bird Banding Laboratory to determine who is using color bands in your area on that particular species. Contact those researchers to avoid color combination overlap.

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 as permitted by law, or by preservation and deposition as teaching or voucher specimens in research collections.

In both field and laboratory, the investigator must be careful to ensure that euthanized animals really are dead before disposal. When carcasses are unacceptable for deposition as museum specimens, other research purposes, or teaching purposes (as may be the case if necropsy has been performed), disposal of carcasses must be in accordance with applicable regulations. Animals containing toxic substances or drugs (including euthanasia agents such as barbiturates) or that have died from transmissible diseases such as West Nile virus must not be disposed of in areas where they may become part of the natural food web. Incineration is a better option.

O. Variations on standard procedure

In most experimental protocols it is desirable to keep disturbance resulting from 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, suspend routine daily inspection, and establish a schedule 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 less often and birds checked with video monitors or through one-way glass. For some species, 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, it may be possible to reduce intrusion even further by providing food and water mechanically, 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. Facility inspections should be performed with sensitivity to the possibility of disturbance by unfamiliar individuals.

P. Zoonoses and other risks to humans

The routine handling of animals entails certain personal risks. Steps must be taken to protect the handler. Training is the best way to learn how to handle a bird without injuring the bird or running the risk of a bite. Any wild animal, even if not aggressive, may attack with painful if not serious results.

A variety of diseases are transmittable from birds to humans (Evans and Carey 1986; Abulreesh et al. 2006). Common among these are campylobacteriosis, histoplasmosis, ornithosis, tuberculosis, salmonellosis, and *Yersinia spp.* (enterocolitia and pseudotuberculosis) as well as tick-borne diseases. The most well-known of these is a form of chlamydiosis known as ornithosis, and often, but inaccurately, termed psittacosis or parrot fever. In fact, this highly contagious agent (*Chlamydia psittaci*) is known from more than 120 nonpsittacines and several domesticated mammals (Gerlach 1994). 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. The standard antibody test is subject to a cross-reaction with *Chlamydia trachomatis,* a human venereal disease.

Depending on the source of the birds and the time of year, they could also carry West Nile virus. Some species, particularly corvids, will become ill or die while others may have subclinical infections for a short time (Komar et al. 2003). Both saliva and feces may contain infectious virus (Komar et al. 2002; Kipp et al. 2006). Healthy adults may experience mild flu-like symptoms; the disease has been dangerous only for the elderly and the immune-compromised. Avian influenza variants such as H5N1 pose new threats for handling many species including but not limited to gallinaceous birds and waterfowl (Redrobe 2007; Siembieda et al. 2008). As of 2009, extensive monitoring of wild birds in North America has detected no highly pathogenic H5N1-positive birds. For recent information, see the American Veterinary Medical Association publication *Zoonoses Updates.* See also the Ornithological Council's peer-reviewed fact sheets on precautions researchers can take to avoid contracting West Nile virus or H5N1 highly pathogenic avian influenza (should the latter occur in North America; live birds cannot be imported from countries and regions where H5N1 highly pathogenic avian influenza occurs) and other zoonotic disease.

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CHAPTER 6. MINOR MANIPULATIVE PROCEDURES

A. Overview

In the past two decades, the emergence in ornithology of immunology, endocrinology, and genetics have increased the need for the collection of blood and other fluids and feathers and tissues from wild birds. New technologies and methods make it easier to answer questions about every aspect of bird life using information obtained from samples taken from wild birds. Most of these procedures, such as experimental injections and implants of hormones and drugs, playbacks of tape-recorded vocalizations, and presentation of decoys, have long been fundamental tools for ornithologists and are considered minor manipulations to an individual bird. Minor manipulations are procedures that are considered less invasive than surgery and administering anesthesia. However, the term “minor” refers to the nature of the manipulation and not to potential impacts. The procedures as discussed in this section all have the potential to have serious impacts, including mortality.

There are a number of underlying themes for each of the procedures discussed. First, each technique or procedure should be appropriate to the scientific question and goals. Second, there is the need for training and/or practice with these techniques and training should be documented. Third, in many instances, veterinary oversight may be needed, and lastly, appropriate permits and approvals must be obtained prior to beginning research.

If these activities necessitate the handling of a bird, federal (and usually state) permits are required; for endangered species, permits are needed even if birds will not be handled (see Introduction and the Ornithological Council’s Permits website). Submission of a research protocol for approval by an Institutional Animal Care and Use Committee is required for all of the procedures discussed here.

For all procedures covered here, handling time should 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 and be appropriate for the scientific objectives. In addition, researchers should be prepared to euthanize birds if they become injured during the handling and sampling process and the bird cannot be taken immediately to a veterinarian (or reputable animal rehabilitation center) and has a severe injury that, in a comparable injury, would likely induce considerable pain and distress to a person (see section on Major Manipulations regarding appropriate euthanasia methods). The IACUC protocol should describe euthanasia methods that will be used.

B. Wild birds studied in captivity

Many studies entailing minor manipulations can be done in the field but others may require wild birds to be held in captivity. Wild birds used in captive studies should be as healthy and free of trauma as possible when initially brought in. Researchers should determine the appropriate housing and feeding conditions and should accommodate the social and behavioral needs of the birds (see section on Housing and Captivity). Consulting other researchers or zoos that have kept the same or similar species in captivity is advisable. Consult the American Zoo and Aquarium Association's taxon-specific housing and husbandry standards. Keeping a few birds in captivity before capturing all birds needed for the study can help in determining optimal conditions. A period of acclimation to captivity allows the birds to adapt to the new environment, ensuring that study results are not affected by stress responses to capture and captivity. It also gives the researcher time to identify and address health issues that emerge during the acclimation period. Studies show that passerines require three to four weeks to acclimate to captivity before experiments begin (Cockren, et al. 2008; Hull et al. 2007; Wingfield et al. 1982). Body mass usually declines after capture and plasma levels of metabolic and reproductive hormones are often abnormal. Wingfield et al. (1982) demonstrated that corticosterone decreased after an acclimation period of two to three weeks for White-crowned Sparrows (*Zonotrichia leucophrys*) kept in small cages with other individuals. However, Marra et al. (1995) found that corticosterone levels in birds acclimated for 35 days remained high compared to newly caught wild *Zonotrichia* sparrows. After three to four weeks, body mass returns to that of capture and hormone levels stabilize. It is known that handling stress raises the stress hormone corticosterone (Hull et al. 2007; Cockrem et al. 2008); studies have also shown that handling stress can affect the immune system in captive Red Knots (*Calidris canutus*) for a short period (Bueler et al. 2008) mostly leading to a redistribution of white blood cells in the body (Dhabher 2009).

Birds kept in captivity for a prolonged period of time may not be releasable. In many cases, federal or state permits or regulations prohibit release. In addition, the birds' survival may be affected by changes in body condition and behavior that occurred during the study. If permits allow release, birds should be conditioned for release as needed. For instance, they should be maintained in a flight cage to allow the flight muscles to strengthen and should be given natural foods in a manner that resembles natural foraging conditions. Birds should be released into the same kind of habitat from which they were captured. The release of birds studied in captivity could pose a risk to wild populations. All birds must be checked for disease prior to release, particularly if they have been held in a facility where other species are present. If permits do not allow release, or if birds are otherwise not suited for release, they can be held for additional research if circumstances permit. Otherwise, euthanasia is necessary. In that case, contact a museum or teaching collection to make arrangements to donate the remains for future study. See the appendix on preparing birds for donation to museums.

C. Collection of blood samples

Effects of collection on survival and behavior

Specific methods for collecting blood samples from birds have been reviewed by Morton et al. (1993) and Campbell (1994). There are numerous resources available on proper blood sampling techniques, including an instructional video demonstrating blood collection that is available from the National Wildlife Health Center
http://wildlife1.wildlifeinformation.org/000VIDEOS/V2_BloodCollBirds/VideoBirdBloodCollectCDROM.htm
However, blood collection techniques, including the proper way to hold the bird, stop bleeding, and reduce stress, should be learned by working directly with an experienced mentor, such as a veterinarian or experienced avian researcher. Only after sufficient practice under the guidance of an experienced teacher should a researcher use these techniques without supervision. Sheldon et al. (2008) provides the most thorough literature review to date on the potential effects of blood sampling. There are considerable data gaps on the impacts of blood collection, such as the impacts on developing birds or birds of the tropics. In semi-captive or free-living species, collection of blood has been shown to not affect survival (Raveling 1970; Wingfield and Farner 1976; Bigler et al. 1977; Gowaty and Karlin 1984; Frederick 1986; Dufty 1988; Hoysak and Weatherhead 1991). Brown (1995) found that collection of blood samples from the jugular vein of 9-day-old Ring-billed Gull (*Larus delawarensis*) chicks had no effect on survival rates to 21 days of age and the rate of nest desertion by adults was also unaffected. Lanctot (1994) determined that withdrawal of up to 0.05 ml of blood from the jugular vein of Buff-breasted Sandpipers (*Tryngites subruficollis*) chicks within 24 hr of hatching had no effect on growth or

survival to fledging. Further, the occurrence of hematomas on the jugular in some chicks did not impair survival. In addition, Schmoll et al. (2004) found that blood sampling does not affect fledging success in Coal Tits (*Parus ater*). However, with chicks the risk of dehydration exceeds that of blood loss Schmoll et al. (2004), so investigators should provide birds with fluids, if possible. However, fluids that are too warm or cold could lead to additional problems, such as heat or cold stress. Too much fluid given intravenously can result in too great a reduction in packed cell volume (the portion of the blood comprised of red blood cells) or plasma total solids (Wicks and Schultz 2008).

However, in a statistically rigorous study using controlled comparisons of bled and non-bled Cliff Swallows (*Petrochelidon pyrrhonota*), Brown and Brown (2009) found that blood sampling reduced annual survival in the first year after sampling. Apparent survival of the 2,945 birds bled over the 20 years over study was reduced between 21% and 33%, depending on the amount of blood taken. The blood samples ranged from 0.3% to 1.2% of the birds' body mass. Non-bled birds included in the survival analysis were captured and handled at the same sites, at the same time, and in the same manner as the bled birds except that no sham non-bleeding was done, i.e., birds were not held in the posture used to bleed birds. The same four investigators undertook the blood sampling throughout the study period. Survival was also affected by the fumigation of colonies to control nest parasites; although survival rates were higher for birds in fumigated colonies, the effect of bleeding was greater among birds in the non-fumigated colonies.

Applicability of these results to other species may be limited by the fact that the blood samples in this study were taken with brachial venipuncture on a species that feeds on the wing and is in flight most of the day. Brown and Brown (2009) acknowledge the possibility that hematomas resulting from brachical venipuncture could be a problem for this very reason. Voss et al. (2010) also suggest that the birds in a less arid environment than that where the Bomberger and Brown study took place might recover more quickly from the fluid loss resulting from bleeding. A thorough commentary (Voss et al. 2010) also points out that the Bomberger and Brown (2009) study was the first on an aerial species, with higher mass-specific metabolic rates and a higher demand for oxygen from circulating red blood cells.

There may be sub-lethal or short-tem effects that alter forms of behavior or physiology that are not ordinarily assessed, particularly if multiple procedures of different types are being done on individuals. Repeated handling and blood sampling of birds has been shown to have sub-lethal long-term effects (van Oers and Carere 2007). Captive Great Tits (*Parus major*) handled seven times and bleed give times had a higher rate of respiration and were more docile when handled thirty days later than were tits handled three times and bled once, suggesting that repeated manipulations can cause long-term behavioral and physiological changes.

Normal feeding and brooding activities, molt, and ability to migrate also have been shown to not be affected by blood collection (Wingfield and Farner 1976; Frederick 1986). Overall, the appropriate collection of blood samples from wild birds has not been shown to impair behavioral patterns or reproduction of wild birds.

Amount of blood

Earlier editions of these *Guidelines* specified that “a general rule of thumb has been 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 100μlof whole blood or one to two 70-μl capillary tubes; for a 50-g bird the maximum would be about 500μlof whole blood or 8 70-μl capillary tubes.” A closer reading of McGuill and Rowan (1989) suggests that a single blood sample should comprise no more than 15% of total blood volume, in lieu of the rule-of-thumb then widely used in laboratory-based studies, which assumed that total blood volume comprised 10% of body mass and that 10% of the blood volume could be taken without harm to the animal. In fact, total blood volume may be less than 10% of body mass. Voss et al. (2010), noting the results of the Bomberger and Brown (2009) study of the impact of blood sampling on Cliff Swallows, suggest a more nuanced approach. They suggest, as did McGuill and Rowan (1989) that, “A more conservative method of determining safe blood sample volumes would involve calculating average blood volume for species specific lean body mass and limiting sample volume to less than 10% of total blood volume.” Voss et al. (2009) acknowledge that 1% of body mass is an easily adopted metric for field use, but urge that with additional research similar metrics could be developed that are more conservative and appropriate to account for variation in seasonal and individual body composition. This more conservative approach may also provide a measure of safety for animals that are studied in the wild shortly after blood is drawn for, unlike laboratory animals, they will need to forage for food and escape predators shortly after release. Additionally, the more conservative approach may address the fact that some individuals will have lower blood volumes than others. A further consideration might be the foraging behavior of the species and the resultant demand oxygen from circulating red blood cells. Species with high metabolic rates, such as hummingbirds, may be more susceptible to the effects of blood loss. Voss et al. (2010) also suggest that researchers take into account environmental variables that may affect survival after blood draws. Specifically, they suggest consideration of temperature and humidity which may make it more difficult for birds to compensate for the physiological effects of sampling. Finally, they also suggest decreasing the volume of blood taken when birds are facing additional stressors, such as heavy parasite loads or during energetically demanding periods such as migration or reproduction. In addition, they suggest adjusting the volume of blood taken to reflect the lean body mass of the individual rather than a species' average, which should be helpful where the average total blood volume of a species is not known.

Choice of methods

One of the most important considerations for blood sampling is the site from where the blood is collected (Sheldon et al. 2008). There are numerous things to consider first when choosing the method of blood collection, that includes: (1) how the blood be used for and the amount needed, (2) the need for whole blood, serum, or plasma needed, (3) the need for core blood rather than peripherally collected blood, (4) the proficiency of the collector for the various blood draw techniques, and (5) any structural, physiological, behavioral, or ecological considerations for the birds species to be sampled. These are all very important considerations that must be made before choosing an appropriate site for blood collection. The site of the blood collection is important not only for the different impacts to the birds; it may also impact the biological endpoints being measured. Additionally, as Sheldon et al. (2008) point out, different handling techniques are required depending on the site of blood collection. Blood collection techniques include the use of a syringe for obtaining blood from the jugular vein, occipital venous sinus, from the ulnar vein in the wing, or heart puncture (see also Dorrestein 1978; Vuillaume 1983). 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).

There may also be a scientific reason where core blood is necessary. Core blood is obtained through heart puncture by the furcular route or through the sternum. The scientific justification for employing this method should be very compelling because these techniques may result in severe debilitation or death, especially among smaller species, though the sternal approach resulted in no mortality in a number of taxa, including passerines (Utter at al. 1971). This may be the result of the structure of the heart; from the furcular region a needle may inadvertently pass through the aorta or other major blood vessels before entering the heart and thus may be more likely to cause irreparable damage. It is difficult to judge where the needle enters the heart and to determine what kind of bleeding or damage may occur as a result of employing this technique. In terminal studies where birds are under anesthesia, exsanguination can be used both to draw blood and as euthanasia when used as an adjunct to other agents or methods (AVMA Guidelines 2007). This method should be performed only by highly experienced researchers or veterinarians.

When larger amounts of blood are required, the jugular vein is commonly preferred. Hematomas do not occur as readily there as with the brachial vein. Hematomas that occur as the result of taking blood represent an additional immediate loss of blood that is an important consideration if maximal blood samples are being taken. Also, a hematoma can be painful, especially if it over or adjacent to a joint.

For jugular bleeding in general, it is important the correct technique is applied and following the blood draw pressure is applied to the vein to prevent continual bleeding. To somebody proficient in this technique there are rarely problems. For a jugular blood draw, the collector must be very proficient. No beginners should attempt to do this. Training in the field on research subjects is inadvisable; it is better to learn in a controlled environment, perhaps obtaining birds from a pet shop (see section on quarantine in captive housing before bringing these birds into the lab if research subject are housed or will be housed in the lab). An appropriate size of needle and syringe should be used. For example, for small species weighing less than 10 g, a 30 gauge needle with a 0.3 cc total volume tuburculin syringe allows for the measuring of blood drawn to in increments of 0.01 ml. Blood samples where more than a drop or two of blood is required are much more quickly obtained from the jugular vein than from the brachial or femoral vein and likely result in much less stress to the bird (when performed by an appropriately trained and experienced individual). Hands-on training and initial supervision from someone experienced in this technique should be a requirement for this technique.

Given the small amount of blood required for many studies, investigators may prefer to obtain small amounts of blood from the ulnar (wing) vein or from vessels in the tibio-tarsi. Either one will yield a suitable blood sample. In larger species a syringe and needle is appropriate. For smaller species (i.e., less than 100 g) it is recommended that the vein be punctured with a 26 gauge or smaller needle and blood collected directly into microhematocrit capillary tubes. Using the microhematocrit tube for blood collection, it is recommended that quantities of one-third to one-half capillary tube (70 μl for birds less than 7 g, one tube for birds seven7 to 15 g, and two tubes for larger birds. However, this may be too much blood collected if repeated sampling is completed or the birds are stressed in other ways. Proper training for bleeding from the ulnar vein is important. It is easy to hit an artery and get excessive bleeding. If the needle is not used properly a hematoma can occur. New researchers should obtain training from someone with extensive experience to ensure proper collection of venous blood with minimal stress. Nestlings may be more susceptible to hematomas and the tibio-tarsal.

Toe-clipping is acceptable in the field only for very small birds such as hummingbirds if only a small amount of blood is required. It is generally necessary to clip only the toenail (Leonard 1969). Although toe-clipping may have the added benefit of identifying previously sampled birds, it is not an accepted procedure for marking birds.

Stopping bleeding

The normal clotting time for most bird species has not been determined, but can be considered to be about 5 min. Therefore it is imperative not to prematurely remove the pressure being applied to the incision site.

Investigators should always be prepared to stop the bleeding if clotting does not occur spontaneously and quickly. On occasion, bleeding will stop on its own but this should not be the assumption. Soft, direct pressure sort should be applied to the puncture site. A cotton ball may be used but the cotton may stick to the clot and pull the clot away when the cotton is removed, causing bleeding to resume. A non-adhesive bandage pad can be applied in lieu of cotton.These pads are slightly stiff, preventing good contact with the site, especially for smaller birds. In addition, the area can be tilted to be raised to be above the heart. Corn starch or flour can aid in blood clotting. Veterinarians sometimes use a styptic powder such as Kwik-Stop® which is useful for stopping bleeding from toenail clips but aviculturists have raised concerns about using styptics on soft tissue or bleeding feather shafts. It is unclear if this concern emanates from the minor stinging sensation caused by stypic; no literature suggesting toxicity or other specific adverse reaction has been found.

It is better to prevent hemorrhage from the brachial vein than to try to control it. Before a needle is withdrawn from the vein, proximal occlusion of the vein must be released and pressure must be provided over the insertion site before the needle is withdrawn. Pressure is increased as the needle is withdrawn. Too much pressure may completely empty the vein of blood, which will prevent clotting. The proper amount of pressure can only be learned with experience.

Blood samples

Once taken, the blood (and other tissue) samples should be properly preserved for survival under field conditions and protocols should be established for handling of the blood sampling prior to collecting blood to ensure that blood is not wasted. Because avian erythrocytes are nucleated and better DNA techniques have been developed, one drop of blood is now sufficient for most genetic studies. New techniques are being developed every year to make DNA analysis cheaper and more field-friendly (Quintana et al. 2008). For instance, until 2009, genetic sex identification analyses relied on between-sex variation in intron size in two genes on the sex chromosomes, but in about 50% of bird species, intron size does not vary between the sexes (Griffiths et al. 1998). In 2009, a new technique was developed that uses multiplexed PCR and that should work for all avian species (Han et al. 2009). If only DNA is needed, researchers should consider feather sampling as discussed below.

Repeated sampling and time of sampling

Measuring stress hormones, immune function, or other physiological endpoints has become an important tool for assessing health and for measuring the effects of various environmental and anthropogenic stressors in wild birds. Such studies often require repeated sampling and may cause lasting effects on the bird or may affect the value of the biochemical indicator being measured (Davis 2005, Pérez-Rodríguez et al. 2007, van Oers and Carere 2007).

Repeated sampling over a short time has been used successfully in smaller species (Marra and Holberton 1998; Wilson and Holberton 2004) and has not been shown to cause negative impacts. The capture, handling, and restraint comprise the baseline stressors; it is assumed that all birds 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, safe place in the shade and sheltered from direct negative effects of weather. Paper lunch bags can also be used as a disposable alternative that can help to reduce the possibility of disease transmission between individuals and populations. Bags are not an appropriate form of confinement or restraint for species with long necks, long bills, log legs, or towhees that may dislocate their legs in the bags [see discussion in Capture and Marking]. In captivity, wild birds survive well after repeated blood sampling (even at three to five-day intervals), and body mass remains normal (Wingfield et al. 1982; Stangel 1986). However, hematocrits can be reduced by repeated blood sampling (Aramaki and Weiss 1961; Fair et al. 2007) and the combined volume of blood taken during a stress series should not exceed the equivalent of 1% of body mass per sample. With care, serial 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. Care should also be taken to ensure that breeding birds are not withheld from their nests for too long. A 30- to 60-minute period before or after bleeding is not a problem, unless the individual becomes separated from a flock, or could potentially lose a territory. Investigator discretion is advised if environmental conditions, daylight, or weather have changed during the holding period.

Time of sampling can affect the value of the hormone being sampled. To ensure that blood samples are reliable estimates of precapture corticosterone concentrations (Romero and Romero 2002), a small initial blood sample should be collected within two to three minutes of disturbance, including time spent capturing and handling the bird prior to blood collection (Chloupek et al. 2009, Lynn and Porter 2008, Romero and Reed 2005, and Romero and Romero 2002). Comparing the rate of corticosterone secretion over a predetermined period can be an indicator of the strength of the acute adrenocortical response (Wingfield 2005).

Alternate means to obtain blood or material for genetic studies, stable isotope analysis, and contaminants

Other means to obtain DNA from birds include feathers (Smith et al. 2003, Quintana et al. 2008) and eggshells for maternal DNA (Egloff et al. 2009). Feather pulp is commonly collected for genetic investigations (De Volo et al. 2008; Freedman et al. 2008; Hogan et al. 2008

Recently, a less invasive technique has been developed that utilizes blood-feeding insects (Heteroptera, Triatominae) obtained from incubating birds (Becker et al. 2006); blood collected using this method was found to contain similar corticosterone levels as other methods (Arnold et al. 2008). Any technique that involves the movement and possible introduction of non-native species into a geographic area should be carefully evaluated for unintended consequences.

Fecal sampling provides a non-invasive alternative to blood for measuring stress hormone levels but lack the time precision of blood hormones and require extensive validation to correct for feeding rates, gut passage time and other factors (Romero and Romero 2005).

D. Collection of other tissues

Research in physiology and genetics often requires tissue biopsy. A biopsy involves the removal of cells or tissues for examination and the most commonly sampled (other than blood) are adipose tissue, muscle, liver, and gonad. Many of the procedures used could be considered surgery and a veterinarian should be consulted in the planning of these procedures. Samples taken should involve the minimal amount of tissue necessary for scientific validity. Depending on the tissue to be sampled, analgesia or anaesthesia may be required to effectively and humanely obtain the necessary sample and the researcher may need to consider incision closure options (e.g., surgical glue or sutures). In addition, care should be taken during the procedure to keep any invasive techniques sterile. Refer to relevant sections within Major Manipulations for details on aseptic technique, pain management, and incision closure . The survival ability of birds that are released following a biopsy procedure should not be compromised.

Various studies (Baker 1981Westneat et al. 1986; Westneat 1986b) show that muscle biopsy has little effect on body condition or survival in either wintering or breeding birds. After prompt handling and release, the birds often returned 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. Even biopsy of the pectoralis major muscle was found not to hinder flight (Westneat 1986b). However, Frederick (1986) determined that incubating adult White Ibis (*Eudocimus albus)* subjected to biopsy of the pectoral muscle abandoned their nests, with the resulting loss of their nestlings. Other ibises subjected only to blood sampling did not abandon their nests. Taking muscle biopsies may not cause mortality of birds, but may affect reproduction, dispersion, recapture rates, and short-term changes in body mass (Westneat 1986, Westneat et al. 1986).

Surgical liver biopsies are taken from birds for studies of contaminants and for diagnosis of disease. Degernes et al. (2002) give a thorough description of anesthetic and surgical techniques for obtaining liver biopsies in the field but observed that biopsied adult birds abandoned their nests.

Feathers are also increasingly used for stable isotope analysis (Hobson and Wassanaar 2008). Plucking a few remiges or retrices is usually an 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. The removal of growing feathers can result in bleeding, and release of the bird should be delayed until bleeding has stopped. Additionally, the energy requirements and consequences of replacing plucked feathers during different time period is not completely understood. Down feathers can be successfully removed from nestlings without serious effect (Stangel et al. 1988, Evans et al. 2009).

Pathogens such as avian influenza may persist in the cloaca or oropharyngeal tract, which can be swabbed to obtain samples. Collecting swab samples from birds is not considered to have a major impact on birds but care must be taken in handling the bird and completing the technique quickly, especially for the orophyaryngeal swabs which can cause discomfort. Due to the large number of researchers now swabbing wild birds, there are several online training videos for collecting swab samples. See
<http://wildlifedisease.nbii.gov/media/Sample Collection04010301_256k.zip>.
However, videos are no substitute for training by experienced researchers. Lastly, handling of birds should include proper human health and safety precautions that may be recommended when pathogens that have the potential to cause serious disease in humans may be present (Ornithological Council 2006).

E. Collection of food samples

Obtaining information on a species' diet in the field is often an important component of ecological and nutritional studies. Historically, dietary analysis involved sacrificing birds to enable direct observation of stomach contents (Duffy and Jackson 1986; Barrett et al. 2007). In some cases 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. If birds are to be sacrificed to obtain stomach contents, investigators must obtain scientific collecting permits and select the most appropriate euthanasia technique (see Section on Major Manipulations). In addition, arrangements should be made to assure that the specimen is donated to a teaching institution or a museum collection or otherwise made use of for scientific study.

Several techniques available for dietary analyses do not require sacrificing birds: neck ligatures, fecal analysis, pellet analysis, stomach pumping or flushing, emetics, and biochemical methods (Rosenberg and Cooper 1990). Some seabirds may regurgitate stomach contents soon after capture. However, others may not, and the use of other techniques may be necessary.

Neck ligatures on nestlings

Use of neck ligatures to obtain food samples from nestlings may occasionally be justified. Neck ligatures can be used in nestlings but not adults due to the need for easy recapture. In such cases, the investigator should be careful to ensure normal blood circulation and tracheal function. Different ligature materials can have different levels of efficacy and. For example, plastic cable-ties are more effective and less dangerous (50% less fatalities) than are pipe cleaners (Mellott and Woods 1993). An additional variable to consider is nestling age (Johnson et al. 1980; Poulsen and Aebischer 1995). If the nestlings are too young, researchers risk damage as a result of physical handling; if the nestlings are too old, researchers run the risk of having the nestling fledge with the ligatures still in place. In many cases, other, less-constrained methods might provide similar results (e.g., fecal analysis, Poulsen and Aebischer 1995). Consideration also should be given as to whether the procedure will result in food deprivation such as will jeopardize survival. Not only are nestlings deprived of their meal, but parents can alter their feeding behavior in response to ligature presence (e.g., Robertson 1973) and have found to aggressively pull on the ligatures on nestlings (Little et al. 2009). McCarty and Winkler (1991) used artificial nestling puppets constructed of the skins from salvaged nestlings to entice adult Tree Swallow (*Tachycineta bicolor*) to provide food items; this technique also worked with nestling puppets made of clay.

Fecal analysis and pellet analysis

Fecal analysis, either of opportunistically collected droppings or of droppings of held birds (Parrish et al. 1994), has the advantage of being non-invasive but is limited to assessing components of bird diets that survive the digestive process, such as insect exoskeletons. In some bird groups, fecal analysis can yield comparable results to more invasive techniques, such as stomach pumping (e.g., shorebirds). In others, however, fecal analysis may not be useful because feces either may not contain identifiable material (e.g., frugivores) or feces may not be deposited in an accessible location (e.g., birds at sea).

Pellet analysis has been used in a wide range of species, including seabirds (reviewed in Barrett et al. 2007) and raptors (Redpath et al. 2001). Like fecal analysis, pellet analysis is non-invasive. However, it is a largely opportunistic approach to dietary analysis that might not lend itself to statistical or experimental design rigor (Rosenberg and Cooper 1990).

Stomach and crop flush

Wilson (1984) and Ryan and Jackson (1986) have developed a pump to flush stomach contents. A bird's stomach is filled with warm water administered via a plastic tube. The bird is then inverted over a bucket and its stomach palpated to induce regurgitation. The pump gave qualitative and quantitative results (at least in larger birds) comparable to those obtained from sacrificed birds and had no apparent ill effects in several studies (e.g., Robertson et al. 1994). Jahncke et al. (1999) used a stomach pump based on Wilson's (Wilson 1984) design to sample diets of Peruvian Diving-Petrels (*Pelecanoides garnotii*); five of 220 birds (2.3%) died during handling although the precise cause of death was not reported.

Hess (1997) used stomach flushing with a saline solution on Red-cockaded Woodpeckers (*Picoides borealis*) with no significant effect on adult survival. Gionfriddo et al. (1995) had similar success with House Sparrows (*Passer domesticus*), implying that stomach flushing may be a viable technique for granivores and other birds with muscular gizzards. A key to success for both of these studies was the careful usage of plastic tubing to deliver the flushing solution and not puncture the airsac or digestive lining. Knowledge of the species to be studied is important in assessing whether it may respond adversely to any regurgitation technique. Ryan and Jackson (1986) found in several seabird species that the percent of food items recovered in a single pumping was correlated with how full the stomach was ranging from approximately 70%

to 100% obtained. Grebes of any species should not be subjected to any regurgitative techniques because of the feathers in their crops; however, the pellets from grebes can be used for diet analysis (Jordan 2005).

Emetics

When fecal analysis and stomach flushing/pumping are either not practical or advisable, emetics may be administered to obtain crop contents. For the purposes of this discussion, we consider emetics to be any chemicals other than sodium chloride or saline solution used to induce regurgitation. There are three major considerations in the application of emetics: type, dosage, and method of delivery (e.g., direct or via plastic tubing). Investigators have a wide range of chemicals to choose from, including tartar emetic (antimony potassium tartrate), apomorphine, and ipecac. Due to the potential effects of some emetics, this technique should be considered with only the most rigorous scientific justifications.

Tarter emetic is the most widely used of these options, despite reports of high mortality (Zach and Falls 1976; Lederer and Crane 1978). There are two problems with the use of tartar emetic: it is toxic if absorbed into the bloodstream and it is difficult to determine the correct dose (Prŷs-Jones et al. 1974). Too low a dose may fail to induce vomiting, thereby allowing the emetic to be absorbed into the bloodstream (Herrera and Hiraldo 1976, cited in Diamond et al. 2007); too high a dose may result in severe trauma or shock (Prŷs-Jones et al. 1974).

Poulin et al. (1994) tested the effectiveness of tartar emetic on 3,419 birds from 137 species in 25 families. The base dosage was 0.8 ml of a 1.5% solution of antimony potassium tartrate per 100 g of body mass delivered through a flexible plastic tube. Analyzable samples were obtained from 79% of tested birds and 2% of tested birds died. Mortality of sensitive species was lessened by dosage reductions. Mortality rates did not differ between birds that did regurgitate and those that did not. Poulin and Lefebvre (1995) tested 2,656 birds of an additional 137 species. Unlike Poulin et al. (1994), base dosages were adjusted downwards for birds weighing less than 10 g and upwards for birds over 40 g. Analyzable samples were obtained from 73% of tested birds and 2.6% of birds tested died.

Durães and Marini (2003) tested the effectiveness of tartar emetic on 369 individuals from 44 species. Analyzable samples were obtained from 70% of tested birds and 10% of tested birds died prior to release. The birds were maintained in a dark, ventilated box until regurgitation, were checked periodically, and were kept up to a maximum of one hour. In order to evaluate more precisely the effects of tartar emetic, birds were released only when they showed no signs of side effects (drowsiness, disorientation) and could fly normally. Mortality was significantly higher during the early morning hours, leading the authors to suggest that emetic sampling should occur later in the day after most birds have had sufficient time to rebuild reserves expended during the previous night. Durães and Marini (2003) also noted a significant difference in the mortality rates of birds that did not regurgitate (85% of fatalities) and those that did (15% of fatalities). This latter finding highlights the critical nature of dosage accuracy when using tartar emetic.

Carlisle and Holberton (2006) tested the relative effectiveness of fecal analysis versus tartar emetic administration in assessing diets of migratory songbirds. Regurgitated samples allowed for a faster assessment of diet than fecal samples did (i.e., fewer samples were required). However, if enough samples were collected, fecal analysis eventually produced a similar assessment of diet. Recapture rates of birds treated with tartar emetic were less than half that of untreated birds.

It is important to remember that the administration of emetics can have indirect effects that may not be immediately apparent during sampling. For example, Carlisle and Holberton (2006) report that all recaptured tartar-treated birds had lost mass. They went on to perform a dosage experiment on captive Dark-eyed Juncos (*Junco hyemalis*). Eighteen individuals were included in this experiment: six received the full mass-specific dose (0.8 ml of a 1.5% solution of antimony potassium tartrate per 100 g of body mass), six received one-half the recommended dose, and six received one-quarter the recommended dose. All 18 individuals were alive 15 to 20 min post-treatment (the standard pre-release holding time); 17 of the individuals were dead within 30 min.

Johnson et al. (2002) tested for another indirect effect of emetics using resighting rates of migratory songbirds during the non-breeding season. Johnson et al. (2002) captured 18 Black-throated Blue Warblers (*Dendroica caerulescens*), nine of which were administered tartar emetic (0.8 ml of a 1.5% solution of antimony potassium tartrate per 100 g of body mass delivered orally through a 1.5-mm-diameter flexible plastic tube) and released. Of the nine treated birds, only one was resighted within the following week; seven of the nine control birds were resighted over the same time period. Johnson et al. (2002) also captured 74 other warblers from different species, 61 of which were treated with tartar emetic. Even with the lower sample size for untreated birds, the resighting rate of untreated birds was statistically higher than the resighting rate of treated birds and although the direct conclusions remain unknown, the comparative resighting rates do suggest an impact.

Valera et al. (1997) tested the emetic effectiveness of apomorphine and reported effectiveness and mortality rates similar to those reported in tartar emetic studies. In this study, only nestlings suffered mortality; however, nestling mortality rates resulting from apomorphine application were lower than those reported as a result of ligature usage (Johnson et al. 1980). One advantage that apomorphine may have over tartar emetic is that it can be applied repeatedly to the same individual, seemingly without negative effects. This is not the case for tartar emetic (Zach and Falls 1976).

Diamond et al. (2007) reintroduced the use of ipecac as an emetic. Initially recommended by Kadochnikov (1967, cited in Diamond et al. 2007), ipecac has not been used to good effect, likely due to inadequate dosage. In the Diamond et al. study (2007), ipecac and tartar emetic were used in assessing diets of Kenyan birds; the tartar emetic dosage used was 0.025 ml per g body weight of a 1% solution in water and the ipecac dosage used was 0.1 ml per g body mass of a 1:20 solution by volume of an ipecac tincture in water. There was no difference in emetic efficacy. Three of the 63 birds (4.8%) treated with tartar emetic died; none of the 93 birds treated with ipecac died. Diamond et al. (2007) reported on an additional 44 wood-warblers in New Brunswick treated with ipecac; all individuals successfully regurgitated and there was no evidence of negative effects on the birds. In addition to being less toxic than tartar emetic, ipecac's effectiveness is less reliant on the amount of emetic delivered to the birds. Given that ipecac appears to be at least as effective as tartar emetic, it should be considered the emetic of choice for diet analysis.

Biochemical methods of dietary analysis include stable isotope analysis and quantitative fatty acid signature analysis. Quantitative fatty acid signature analysis is primarily suitable for assessing the diets of marine organisms and relies on the fact that predators tend to store the fatty acid signatures of their prey items in their adipose tissues (reviewed in Barrett et al. 2007). These tissues can be non-destructively sampled using biopsy (discussed below). Fatty acid analysis allows for a more precise determination of diet than stable isotope analysis but using the two in concert could provide a powerful dietary assessment tool.

F. Force feeding

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). Holding the bird upright will help to prevent regurgitation. Intubation may also injure the esophageal wall. If done improperly, the tube can damage the choana (slit in the roof of the mouth) or the trachea. Food in the trachea will be aspirated into the lungs and can cause pneumonia or death from suffociation. As an alternative, Murphy and King (1986) suggested 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; however, the use of pellets takes much longer than tubal feeding. Researchers may seek training from aviculturalists who have extensive experience tube feeding young parrots and other birds.

G. 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 1985, 1986, 1987). However, Immler and Birkhead (2005) also found that sperm can also be found in fecal samples appropriate for answering many questions. Cloacal lavage is also used to investigate pathogens in lower digestive system of birds (Brown et al. 1993; Yashkulov et al. 2008). Cloacal lavage uses distilled water or saline in a quantity appropriate for the size of the species. The water is administered via a disposable plastic pipette that is introduced into the cloaca. As much as possible of the lavage is sucked back into the pipette and transferred into a specimen tube. The technique is sometimes extended by the implantation of cloacal microspheres (Quay 1988). Considerations should be made for the potential pain and discomfort for this procedure and should be performed by a properly practiced person.

H. Injections and insertion of implants

Injections of appropriate solutions or implants, whether subcutaneous, intramuscular, intracoelomic, or intravascular, may usually be made with very little effect on survival or normal bird behavior. Some solutions may be irritating or dangerous to the subject if they are not properly injected. Implants may migrate or become inactive if they are not properly inserted. It is strongly recommended that new techniques are evaluated on captive individuals before used with wild birds. The personnel performing the procedures of injections or implants, whether subcutaneous, intramuscular, intraperitoneal, or intravascular should be properly trained. In the United States, the Federal Food, Drug, and Cosmetic Act provides that drugs administered legally to animals must be approved by the Food and Drug Administration or recognized by experts (e.g., the agency) to be generally safe and effective. The Animal Medicinal Drug Use Clarification Act provides that an approved drug must be used if available, but there are few drugs approved for use in birds. Veterinarians under certain conditions may legally use approved human and animal drugs in an extra-label fashion. Therefore, extralabel use should take place under the supervision of a veterinarian and adequate records of extralabel drug useage must be maintained (http://www.avma.org/reference/amduca/extralabel_brochure.pdf).

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. Intracoelomic injections require more stringent justification because some drugs may irritate the viscera or the possibility of mechanical or chemical damage of the viscera. Also, drugs intended for injection into the coelomic space are easily deposited into the respiratory sytem of abird, given the unique airsac anatomy of birds.

No study has directly investigated the impacts of injections on individual survival. However, hundreds of published field studies involving injections, especially subcutaneous injections, support the conclusion that these injections appear to have little effect on survival. This undoubtedly varies for the type and amount of injection given. Due to number of types of injections that could be given, a review of each type is not given here. For longer-term studies, repeated injections are sometimes necessary, requiring multiple captures at frequent intervals. This in itself may cause serious disruption of normal activities. For these reasons, implants in silicone rubber tubes, pellets, or mini-osmotic pumps may be used to provide long-term administration of the experimental substance (up to several weeks). Whenever possible, such implants should be made subcutaneously because intracoelomic implants are often encapsulated by connective tissue. Implants inserted under the skin of the flank or sides of the thorax are most effective and are easy to remove after the experiment is terminated. Implants placed under the skin on the back may rupture the skin, allowing infection. It is important that the size of the implant should be such that it does not place pressure on the skin, regardless of location. Implants under the skin of the neck are also not advised as 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 from companies that also provide free training videos for the use of these pumps in rodents that can be applicable to birds. As with all invasive procedures, the area of operations and instruments should be as sterile, with separate sets of sterile instruments used for each implant surgery. See the section on Major Manipulations for a thorough discussion of sterile procedures.

Timing of implant placement 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. The impacts of additional sex steroids on birds can be varied and includes suppression of immune function (Duffy et al. 2000; Castro et al. 2001). Hence, every effort should be made to remove the implant after the experiment. In 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. If these outcomes are likely under the conditions of the particular study then the investigator should remove the implanted devices from controls and experimental birds either by removal of the implant or removal of the bird from the wild. However, experimental subjects with control implants, or implants from which all hormone has diffused, do survive over winter at the same rate as individuals without implants (Wingfield 1984). Further, the stress of recapture may cause problems that interfere with results and cause impacts to the birds. 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.

The field of immunology in wild birds has grown significantly in the last decade, requiring injections of immunizations or mitogenic substances to measure immune response. Similarly, doubly-labeled water is a common injection in energy studies in birds. Wilson and Culik (1995) found that seabirds injected with doubly-labeled water differed in their foraging parameters (e.g., dive depths, dive angles and foraging ranges) from the non-injected birds. The authors suggest that the relatively large volume of liquid injected intramuscularly causes discomfort which lasts for at least two days, which dissuades birds from engaging in normal foraging behavior and they suggest this problem may be alleviated by multiple small intramuscular injections or intra-peritoneal injections. Other implants include radio or satellite transmitters placed subcutaneously (Berdeen and Otis 2006) or in the coelom of birds. These are discussed at length in the section on Capture and Marking.

I. 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. Breaking eggs has obvious deleterious effects on reproductive success but can be scientifically justified in some cases. Alternative means should be pursued when available. Two alternative, non-invasive, and inexpensive techniques are candling and flotation.

Candling, or transillumination, involves placing an egg in front of a light source and assessing or measuring the amount of dark space within the field of view (Westerskov 1950; Weller 1956). More modern approaches to field candling and photography allow for clear delineation of embryonic development and are designed to minimize the amount of egg handling time and how long the adult is away from the eggs, an animal welfare consideration with this technique (Young 1988; Lokemoen and Koford 1996).

Floating involves placing eggs in water or other aqueous solution at ambient temperature; egg viability and embryo age determinations are based on whether or not the eggs float and the orientation of floating eggs (Westerskov 1950; Fisher and Swengel 1991; Walter and Rusch 1997). For example, if the egg is more than 10 days old and does not float, there is no viable embryo. Devney et al. (2009) present results that suggest that floatation in salt- rather than freshwater may provide more accurate results, at least for colonially nesting seabirds. Egg flotation is useful for eggs with shells too thick or too heavily pigmented for candling. The primary concern with the use of floating techniques is that they may decrease hatchability by allowing excess water to permeate the egg. However, Alberico (1995) tested the effect of egg flotation on hatchability of clutching in 131 American Avocet (*Recurvirostra americana*) and Black-necked Stilt (*Himantopus mexicanus*) nests and detected no significant effect.

Recently, Reiter and Anderson (2008) tested the relative efficacy of egg floatation and egg candling in estimating incubation day of Canada Goose (*Branta canadensis*) nests. Both techniques overestimated incubation day early in incubation and underestimated incubation day late in incubation. Although egg flotation provided less biased results, both techniques provide a level of precision required for robust estimation of nesting parameters.

Electronic devices, such as doppler stethoscopes and audiocartridges, that can detect the embryonic heart beat or movements of the embryo within the shell may also be useful but have limited field potential (Mineau and Pedrosa 1986; Tazawa et al. 1991).

J. Playback of recorded vocalization and the use of decoys

Playback of conspecifics or a recording of the study subject's own voice is a common research technique. Predator calls, particularly those of small nest predators such as pygmy owls, are often used to attract passerines. Studies of the impact of playback are sparse, but inferences about the nature of possible impacts can be made from the fact that birds are enticed to move in response of the recorded sound. For instance, nesting birds may leave eggs or hatchlings unattended. Documented physiological responses include increased plasma testosterone levels in Spotted Antbirds (*Hylophylax n. naevioides*) (Wikelski et al. 1999). Mennill et al. (2002) determined that male Black-capped Chickadees (*Poecile atricapillus*) enticed to engage in song contests with playback lost paternity in their nests when songs simulating aggressive males were played for six minutes.

Playback has long been used as a tool to assess species presence and abundance to assess species presence and abundance (Johnson 1981; Proudfoot and Beasom 1996; Turcotte and Desrochers 2002) and to manipulate behavior in ethological and behavioral ecology research. More recently, it has been used to study reproductive activity (Gunn et al. 2000; Doran et al. 2005). Unfortunately, these studies rarely assess the potential negative impacts of the use of playback. Playback of recorded vocalizations to free-living birds causes little disturbance if the duration of the playback is kept within reasonable bounds (normally less than 30 min) (Turcotte and Desrochers 2002; Hahn and Silverman 2007; Celis-Murillo et al. 2009). Playback may distract subjects from activities that are essential to reproductive success. Playback (both conspecific and predator vocalizations) during the breeding season has been shown to negatively affect pair bond status and breeding success in some birds, such as owls (Springer

1969; McNicholl 1978) cited in (Proudfoot and Beasom 1996) and songbirds (Baptista and Gaunt 1997). Unless required for an experiment, speakers should not be placed close to a known nest location. Overuse of both conspecific and predator playback during the non-breeding season, especially on cold winter days, can result in birds wasting valuable foraging time responding to playback.

Live decoys are frequently used in conjunction with playback and require particular attention in the field. Animal welfare concerns are just as important to the decoys as to the wild birds. This topic is discussed thoroughly in the section on Capture and Marking. Generally, though, the use of live decoys to lure hawks to a mist net or trap should be carefully monitored. Avoid stressing the decoy by providing shelter from direct sun and by providing a refuge to escape from the raptor. A live decoy should be observed constantly by the investigator. Except for very short periods of use, live decoys must be provided with food and water (Evrard and Bacon 1998). Birds used as live decoys should be habituated to housing in a cage under field conditions for at least a day prior to onset of the experiment.

Decoy traps can be readily designed for both terrestrial (e.g., Burtt 1980) and aquatic systems (e.g., Anderson et al. 1980). Evrard and Bacon (1998) tested the efficacy in capturing ducks of four different trap designs: swim-in bait traps, swim-in bait traps with live decoys, floating bait traps, and decoy traps. The two trap types with decoys were more effective than traps without live decoys. Evrard and Bacon (1998) checked on and fed the decoy ducks daily. Despite daily checking, several decoy (exact number not given) and trapped ducks fell victim to mink and raccoons. The mortality rate was higher than previously reported for other studies using decoy traps (e.g., Anderson et al. 1980; Sharp and Lokemoen 1987), perhaps due to difference in predator communities. In addition to losses to predators, Sharp and Lokemoen (1987) reported one decoy fatality in which a decoy got its bill wedged in the side of the trap and drowned. Sharp and Lokemoen (1987) also make the important observation that farm-raised ducks fared much better during their time as decoys than did wild-caught decoys.

K. 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 various materials, including wood, paper maché, 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 (e.g., Antonov et al. 2006; Prather et al. 2007). For others, egg recognition mechanisms may be very general (Moskát et al. 2003). When artificial eggs are used briefly, such as during trapping, a general approximation of real eggs should 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 (e.g., Marchetti 2000). Birds that are uncomfortable sitting on surrogate or artificial eggs may desert the nest, resulting in a loss of data.

L. Experimental manipulation of plumage

Experimental approaches to altering the external appearance of a bird fall into two broad categories: alteration of size and shape of feathers and other body parts, and the alteration of plumage coloration. A common experimental approach to the manipulation of feather size and shape has been the alteration of tail structure in the context of testing hypotheses and predictions arising from sexual selection theory. Perhaps most famously, Andersson (1982) experimentally shortened and elongated the tails of Long-tailed Widowbirds (*Euplectes progne*) to test female preferences for male secondary sexual characteristics. 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. Certain types of long tails, primarily those that appear to exist as a result of sexual selection pressures (e.g., widowbirds), compromise a bird's flight ability (Balmford et al. 1993; Norberg 1995). Experimentally increasing already unwieldy tails may compromise an individual's health and fitness. Tail manipulations can also affect a bird's ability to move in complex habitats; Romero-Pujante et al. (2005) reported that experimental manipulations of tail length compromised the ability of Bearded Tits (*Panurus biarmicus*) to move about in reedy marshes, its primary habitat.

The most common contemporary approaches to plumage color manipulation of feathers on birds are the hiding or removal of an obvious trait (e.g., using black dye to hide the epaulets of Red-winged Blackbirds [*Agelaius phoeniceus*]) and the manipulation of the plumage coloration gradients towards the extremes of natural trait expression (e.g., using marking pens to alter

House Finch [*Carpodacus mexicanus*] plumage) (see Hill 2006a,b for a complete treatment of bird coloration). More recently, researchers have begun raising captive birds on pigment-free diets (e.g., carotenoid-free diets; McGraw and Hill 2001), thereby creating a cohort of birds with minimal coloration that can be colored to suit experimental needs. The advantage to this approach is that researchers can initially manipulate bird plumage color without resorting to external applications while maintaining subject health (e.g., McGraw and Hill 2000). Changes in plumage coloration do not appear to influence predations rates on altered birds (Stutchbury and Howlett 1995) .The primary animal welfare concern with regard to plumage color manipulation is to avoid the use of marking or coloration chemicals that may be toxic to birds. In this respect, the use of marking pens is preferable to shoe polish, hair dyes, or colored oils. Given that birds use their bills to self-preen, simply avoiding the mouth, nostrils, and eyes while applying dyes known to be toxic (as recommended in Rodgers 1986) is not sufficient.

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CHAPTER 7. MAJOR MANIPULATIVE PROCEDURES

A. Overview

This section concerns procedures most commonly used in conjunction with surgery (i.e., entry into the body cavity), such as restraint and the use of pharmaceuticals to alleviate pain, as well as recent innovations in surgical techniques. We also discuss euthanasia as an endpoint for manipulations that result in inadvertent or unavoidable pain that cannot be remedied.

Avian veterinary medicine has become highly advanced in recent years. Modern techniques are well presented in several texts and review documents: Harrison and Harrison 1986, Ritchie et al. 1994, 1997, Altman et al. 1997, Tulley et al. 2000, Hawkins et al. 2001, and Samour 2008. No researcher conducting invasive studies of birds should be without one or more of these books. However, no text, video, or anything that follows in these Guidelines suffices as a self-training manual. As is the case for all complex procedures, surgery should not be undertaken by novices. Training is essential and even those with adequate training should seek the guidance of a veterinarian prior to undertaking an invasive procedure. Any invasive technique is potentially 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 and may not be well communicated in text books, instruction manuals, or videos. An investigator should always seek direct instruction from an expert and should practice on appropriate models and under the supervision of a skilled practitioner or veterinarian.

These Guidelines present detailed material concerning frequently used procedures and commonly encountered problems in order to facilitate communication between investigators and the members of their Institutional Animal Care and Use Committees (IACUC), who may be more familiar with mammals than birds and with laboratory conditions than field conditions. This summary is not an attempt to catalogue all acceptable techniques. Rather, it is an attempt to establish a philosophy that will help all involved to determine the appropriateness of a given approach. The techniques discussed should be considered as examples.

The nature of these procedures and the potential for impairment of function, pain, infection, and death warrant a repetition of the fundamental “alternatives” principles. Though replacement with a non-animal model is rarely a viable alternative in wildlife research, the investigator should consider reduction in the number of animals through careful planning of the statistical design of the investigation. The most pertinent consideration is the refinement of procedures. A non-invasive or less invasive procedure should always be considered, assuming that the alternate procedure can yield equally useful results.

B. Intended fate of subject

The conditions governing the adoption of procedures may depend on the intended fate of the bird. There are four categories of subjects: wild birds in the field that are to be released immediately upon recovery; wild birds that have been brought into a laboratory and will be released after recovery in a holding facility; wild or captive bred birds that are to remain captive permanently or for an indefinitely long period after the procedure; and birds that will be euthanized.

For any animal that is to be released to the wild either immediately or following a holding period, the prime consideration should be that the procedure will have a minimal effect on the subsequent survival of the subject. It is important to consider the age and sex of the subjects as nestling and juveniles may respond differently than adults, and females may respond differently than males (e.g., Mulcahy et al. 2003).

For wild birds that are to remain captive permanently or for an indefinitely long period after the procedure or for birds that will be euthanized, less emphasis can be placed on survivability, at least in the short term. However, there should be no compromises on antisepsis and surgical standards or on fear and pain management.

C. Pre-surgery

General considerations

Avian surgery is considerably different from mammalian surgery (Ritchie et al. 1994; Altman et al. 1997). In part, the differences are due to avian anatomy, especially the air sacs, respiratory system, and physiological differences, such as blood-pH and proclivity to fall into hypothermia. Birds tend to have high metabolic rates; as a consequence, 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 to 6 hrs at most for small birds such as passerines; Curro 1998).

Any invasive procedure more complicated than a simple injection should be rehearsed with an appropriate model (mock-up, cadaver, or anesthetized subject), and the conservative limitations on techniques should be maintained until they can be performed quickly and smoothly. In addition to supervised practice of the invasive procedure, all other aspects of the procedure (e.g., capture, restraint, intubation, use of equipment) must also be practiced and specifics researched prior to performing the procedure. For example, the proper size of intubation tube is very important and will vary widely among birds.

Aseptic technique

High standards of antisepsis, which is the prevention of infection through the elimination of microbes, should be practiced routinely during invasive procedures. Proper training in and a need for "sterile technique" is key to any surgery or invasive procedure. The Guide for the Care and Use of Laboratory Animals (ILAR 1996) provides a broad discussion of the maintenance of antisepsis. Note the difference between disinfectants, which are agents used to reduce the number of microbes, and sterilization, which means the complete absence of all disease-causing organisms. A strong-enough disinfectant could sterilize a surface or an instrument if left in contact for a sufficient period of time. While no single procedure for sterilization is appropriate to all materials and all situations, precautions should always be taken to reduce the possibility of transmission of microbes. Sterile conditions are not required in the entire laboratory but commonly accepted practices that reduce the presence of microbes should be adhered to, including the use of a disinfectant on all surfaces. The surgical area should be specifically designated and set aside for that sole purpose; it should be scrubbed with a strong disinfectant, such as dilute sodium hypochlorite (household bleach, dilute 1/10), a quarternary 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 prior to disinfection or sterilization. Special precautions, such as color coding and separate storage areas, must be taken to ensure that surgical instruments are used for that purpose only. They must not be mixed with necropsy, dissection, or skinning instruments. All non-disposable equipment should be sterilized by autoclave between uses.

The surgical site itself must be sterile, which is achieved through the use of a sterile drape and the use of sterile instruments. Aseptic technique in the laboratory setting requires that the surgeon thoroughly scrub and rinse hands and arms with proper agent (e.g., betadine, chlorhexidine, inactivated alcohol) allowing an appropriate amount of contact time prior to rinsing, wear sterile gloves, a face mask, and hair cover, as well as scrubs and a sterile surgical gown or apron. The surgical site should be free of feathers (may need to be gently pulled from where the feather shaft meets the skin in the vicinity of the planned procedure), disinfected with several cycles of gauze soaked with chlorhexidine or betadine, followed by alcohol, beginning with the area where the incision will be made and circling outward each time. Sterile drapes available from surgical supply houses should cover the surgical site (disposable drapes are available, versus reusable drapes that must be washed and autoclaved between procedures).

Though strict adherence to these aseptic protocols may be impossible in the field, certain basic practices must be observed. Specifically, the surgical site must be sterile and sterile gloves and instruments must be used. Surgical instruments are now sufficiently inexpensive that cost should not be a barrier to single-use where sterilization of instruments is not feasible.

The Guide for the Care and Use of Laboratory Animals acknowledges that modification of standard techniques might be desirable or even required in field surgery, but these modifications should not compromise the well-being of the animals. However, a sterile field around the surgical site is always necessary, as are sterile gloves and sterile instruments. It is obviously impossible to sterilize, or even disinfect, natural surroundings, but the general area, such as the surface upon which the bird rests, can be disinfected. For instance, unscarred, monolithic high-density polyethylene or polypropylene plastic boards (Oliver, pers.comm.) are suitable for surgical procedures because they can be disinfected (Ak 1994) by soaking in a 1/10 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 surgery drapes, disposable surgery drapes or paper covers, or plastic-backed absorbent cage liners that can be changed as soiled or between surgeries. A foam pad can also be used as a surface, and the pad or the plastic board can be nested inside a plastic garbage bag to further reduce the potential for airborne or other environmental contaminants. If possible, procedures should be carried out in some sort of shelter that reduces the possibility of wind-borne contaminants.

Sterilizing instruments under field conditions can be difficult so it is best not to re-use instruments that enter or touch the surgical site. Although a variety of chemical sterilizing solutions exists, the instruments must remain in the solution until needed. After removal from the solution, they must be handled aseptically and must be rinsed in sterile water and dried with a sterile towel, then used immediately. They cannot touch any non-sterile surface. For these reasons, field sterilization of instruments is impractical and therefore it is best is to use disposable instruments and blades. Use of an assistant – who can access necessary items that may be outside the sterile zone – is also recommended.

Physical restraint

Invasive procedures require restraint and sometimes immobilization to minimize stress and the chance of unintended injury to the subject. The precise nature of the restraint depends on the procedure and the species (Fowler 1978, 1995) and can be achieved using either physical or chemical means. Chemical restraint will be discussed below under *Pain Management*.

For smaller birds, variations of handling techniques used in banding are adequate (Donovan 1958) to provide physical restraint of individuals prior to administration of pharmaceuticals. To minimize potentially damaging movement during recovery from invasive procedures, small- to medium-sized birds can be enclosed in cardboard or fabric tubes or comparable devices. See the section on Capture and Marking for precautions to take when holding birds in this manner. Large species can often be calmed by enclosing the head in an opaque hood (Maechtle 1998) or the body in a fabric sleeve (Bolen et al. 1977). Dark hoods are also useful for reducing struggling and stress during pre-surgical evaluation and post-operative recovery. Care should be taken that the restraint does not interfere with ventilatory movements of the abdomen and thorax or impede respiratory air flow. In addition, hoods or other external coverings should not be adhered or attached to the bird so strongly that in an outdoor procedure, the bird may awake and flee with the apparatus still attached.

Physical restraint must not create a situation that may induce hyperthermia or hypothermia. Temperature-controlling equipment such as ice, fans, or warming pads may be needed (e.g., Rembert et al. 2001). Minimizing external stimuli such as vocalizations or other noise or rapid changes of light or temperature helps ensure successful restraint and recovery. All handing and restraint equipment must be cleaned and disinfected between every animal and procedure to minimize the potential spread of disease.

One effect of handling and physical restraint that often goes unnoticed or unmeasured is a physiological response to the activity. For example, Hood et al. (1998) measured corticosterone levels in Magellanic Penguins (*Spheniscus magellanicus*) following capture and subsequent restraint and found that corticosterone levels were higher in birds that had been held and restrained for longer periods. Given the wide-ranging effects of stress hormones on bird behavior, physiology, and reproductive success, additional care must be taken to minimize the amount of time a bird is handled and restrained during major manipulations. In another example, Greenacre and Lusby (2004) examined the effects of handling and restraint on body temperature and respiratory rate in 17 Hispaniolan (*Amazona ventralis*) and Blue-fronted Amazons (*A. aestiva*). Body temperatures rose by 2.3 ^{o}C within 4 min, and respiratory rates almost doubled within 15 min. The authors concluded that birds restrained for more than 4 min must be monitored for signs of overheating, such as open-mouthed breathing and tachypnea (increased breathing rate).

As some species may be dangerous to the handler, proper restraint should include protection for the handler as well as the bird to prevent accidental injury to the bird during defensive maneuvers. Heavy gloves are appropriate for handling raptors, psittacines, and other birds with strong and sharp talons or beaks. Safety goggles should be worn when handling birds with long beaks and ear protectors or plugs when working near species capable of loud calls.

D. Pain management

Many birds show little to no behavioral evidence of pain or discomfort from punctures or incisions over much of the body, especially in the bare skin areas between the feather tracts (Green 1979; Steiner and Davis 1981); the head and bill, scaled portions of the legs, and vent area are exceptions. For discussions of the complex topic of pain in animals, see Bateson 1991, Elzanowski and Abs 1991, Gentle 1992, and Andrews et al. 1993. The evident psychological component of pain can be aggravated or suppressed by fear. In addition, various species respond to traumatic experiences differently, and either restraint or disorientation may elicit a more evident response or a response of greater magnitude than that provoked by such physical injuries as punctures or small incisions. Unfortunately, an animal's fear of the unknown cannot be lessened by assurances. For this reason, analgesics and anesthetics may be used reduce the total stress of a procedure, as long as their application does not decrease a subject's chances of survival.

The lack of an apparent pain response and the potentially stressful affects of anesthesia and prolonged handling have led past investigators to perform some surgical procedures with little or no anesthesia and to close incisions without sutures (Gandal 1969; Risser 1971; Wingfield and Farner 1976; Baker 1981). Such procedures may not affect the overall 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 gaseous anesthetics such as isoflurane (Degernes et al. 2002), the practice of invasive techniques without the use of analgesics or anesthetics requires special justification. If anesthesia is used, the bird should not be released until the effects of anesthesia have completely disappeared (e.g., bird is alert and able to perch or stand without assistance). The decision to use or not to use analgesic and/or anesthetic agents must be based on the best interests of the animal and not on the convenience of the investigator.

Access to controlled substances

Non-veterinarians may be allowed to register with the Drug Enforcement Administration (DEA) to legally obtain substances on the Schedules (Class) II–V of the controlled substances list. 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 if permitted by state law. Most states will issue licenses to researchers. Investigators outside the United States should consult with their own law enforcement agencies about access to these substances.

In the United States, the Federal Food, Drug, and Cosmetic Act provides that drugs administered legally to animals must be approved by the Food and Drug Administration or recognized by experts (e.g., the agency) to be generally safe and effective. The Animal Medicinal Drug Use Clarification Act provides that an approved drug must be used if available, but there are few drugs approved for use in birds. Veterinarians under certain conditions may legally use approved human and animal drugs in an extra-label fashion. Therefore, extralabel use should take place under the supervision of a veterinarian and adequate records must be maintained (see http://www.avma.org/reference/amduca/extralabel_brochure.pdf).

Analgesia

Analgesia is the reduction of pain. Analgesics are used to: alleviate pain in an animal prior to treatment or to ease pain in an individual recovering from an injury or surgery; sedate an animal prior to a minimally invasive but painful procedure; sedate an animal prior to induction of anesthesia for surgery. Injection is the most common delivery method of an analgesic. Chemical compounds commonly used as analgesics include opioids, steroidal and non-steroidal anti-inflammatory drugs, ketamine (discussed under Anesthesia), and α_2-agonists (Machin 2005).

Opioids have been used effectively as analgesics in birds but the literature contains conflicting reports on species-specific efficacy and dose-specific responses (Machin 2005, Myers 2005). The primary concern surrounding the preemptive administration of an opioid analgesic prior to anesthesia is the potential for the opioid to critically depress cardiopulmonary function during the procedure. Klaphake et al. (2006) tested the effectiveness of butorphanol tartrate as an analgesic prior to anesthesia with sevoflurane on Hispaniolan Amazons. They reported no adverse effects in cardiopulmonary function of birds that received butorphanol prior to anesthesia compared to those who did not. However, as evidenced by the results of studies by Hoppes et al. (2003), Paul-Murphy et al. (2004), Sladky et al. (2006), and Riggs et al. (2008), great care must be taken (e.g., pilot studies or cage tests) to determine the appropriate dosage of an analgesic for a particular species..

The suppression of inflammatory responses can contribute to pain reduction (Hockin et al. 2001, Machin 2005). Although steroid-based anti-inflammatory compounds (e.g., corticosteroids) are attractive due to their strength and rapid action, they carry a risk of immunosuppression and potential antagonism with anesthetics. In contrast, non-steroidal anti-inflammatory drugs (NSAIDs) appear to be as effective as analgesics with fewer negative side effects. Preoperative administration of NSAIDs can reduce postoperative opioid requirements (Machin 2005); they can also be used effectively during surgical recovery. Commonly used NSAIDs include ibuprofen, carprofen, flunixin, and ketoprofen. Side effects of NSAIDs include gastric ulcers, regurgitation, tenesmus and,nephrotoxicity and, with repeated and/or prolonged use, muscular necrosis at the injection site (Machin et al. 2001). Oxicams are a family of non-steroidal anti-inflammatory agents (such as Meloxicam) that have been recommended for use in birds, including passerines.

Alpha$_2$-agonists, such as xylazine and metatomidine, are useful as precursors to the administration of anesthetic compounds (see discussion below). However, they have limited utility as the sole analgesic or sedative agent as they can cause respiratory and cardiac depression, muscle tremors, and auditory sensitivity (see references in Machin 2005).

Chilling has been used for analgesia (Mueller 1982). Ethyl chloride can temporarily numb a small area for quick incision, such as in laparotomy (Risser 1971). Refrigerants such as dichlorodiflurormethane may also be used for cryosurgery. However, it is difficult to control the degree of chilling, and frozen tissue may be permanently damaged or rendered inoperable. As the relationship between hypothermically induced immobility and analgesia has not been clearly established, the use of chilling as an analgesic is strongly discouraged.

General anesthesia

An anesthetic is an agent that produces analgesia (loss of pain sensation) and, in the case of general anesthetics, immobilization and loss of consciousness so that the individual is unresponsive to stimulation. Anesthesia ideally minimizes stress and eliminates pain during a research procedure. It also provides adequate restraint during the procedure. Every anesthetic agent has specific advantages and disadvantages. The investigator must be fully knowledgeable about the physiologic and pharmacologic sensitivities of the avian species to be studied (Ludders 1998) as well as the pharmacologic characteristics of the drug(s) to be used in the research project. The investigator should be aware of the potential synergistic or antagonistic effects of the drugs to be used. Regulations that implement the Animal Welfare Act mandate the requirement for training and instruction of personnel who administer analgesic and anesthetics [9 CFR 2.32(c)(3)].

The most important message on the subject of anesthesia is that there are no easy answers and no single agent that is ideal for all situations. The investigator, in consultation with an avian veterinarian or veterinary anesthesiologist, must take the time to determine which agent or combination of agents is appropriate for the species being studied, the procedure to be performed and be able to justify that decision. When information concerning the effect of a drug on the species under consideration is unavailable, pre-experimental testing to assess dosages is strongly advised (Mercado et al. 2008). Curro (1998), Machin (2004), and Gunkel and Lafortune (2005) provide excellent reviews on avian anesthesia. However, the state of knowledge about avian medicine and anethesiology is developing rapidly, so even the most experienced investigator should take the time to review recent literature before selecting a specific drug.

General anesthetics are administered either as a gas or as an injection. Inhalation anesthetics have the advantage that dosage is easily adjusted during the procedure. In addition, due to the rapid clearing that occurs in the avian respiratory system, recovery usually is extremely rapid. If birds are to be released soon after a procedure, inhalant anesthetics may be the better choice. Injectable anesthetics have the advantages of rapid administration and require less equipment in the field. However, the injectable anesthetics usually result in prolonged recovery periods.

Some inhalants require little equipment. Methoxyflurane (Metofane) has been used successfully for recovery surgery in the field, by placing the drug on a cotton ball in a small jar, and placing this over the bird's face until anesthesia is induced (e.g., MacDougall-Shackleton et al. 2001, 2006). Unfortunately, methoxyflurane is no longer commercially available in many countries, including the United States. Isoflurane is a common choice of inhalant anesthetic for birds (e.g., Redig 1998). However, this compound is much more volatile than methoxyflurane and maintenance of proper anesthesia requires the use of a vaporizer. Light-weight portable systems are available for field use, and portability is primarily limited by the size of oxygen tank used (e.g., Lewis 2004, Small et al. 2004, Boedeker et al. 2005). Using isoflurane, anesthesia can be rapidly induced with a dose of 2 to 4% with a flow rate of 2 L/min oxygen. Recovery occurs very rapidly, within a minute or two of removing anesthesia. Placing birds in an induction chamber designed for rodents can lead to injury as birds flail about prior to becoming unconscious.

Injectable anesthetics may be administered in a muscle mass (IM), in a vein (IV), or intra-osseous (Heard 1997). Intravenous administration provides more predictable reactions, faster induction and usually results in a faster recovery. These methods of administration require some skill, even with large species and can be inappropriate for small species. The dosage for most injectable anesthetics varies inversely with weight (Boever and Wright 1975); that is, small birds may require relatively higher dosages. Hence, the weight of the subject must be accurately measured prior to administration of any drug. However, dosage information for specific anesthetics should be researched for each species prior to administration.

Machin and Caulkett (2000) compared the effects of the anesthetics isoflurane (an inhalant) and propofol (an injectable) on female Canvasbacks (*Aythya valisineria*) in preparation for implanting intraabdominal transmitters. Ducks that received propofol experienced a smoother, more rapid induction and recovery than those given isoflurane. While some females in both treatment groups abandoned their nests following the surgical implantation procedure, the abandonment rate of the group that received propofol was approximately half of the group that received isoflurane.

Investigators cannot assume that what works for one species will work with another (Samour et al. 1984; Kabat et al. 2008). Langlois et al. (2003) tested the same two anesthetics as Machin and Caulkett (2000) on Hispaniolan Amazons but used a lower dose of propofol (5 mg/kg). Propofol recovery times, even at this lower dosage, were prolonged relative to recovery from isoflurane. In addition, six of 10 propofol-tested birds had agitated recoveries. The contrasting results of these two studies highlight the often dosage- and taxon-specific effects of anesthetic agents.

Drug combinations

Anesthetics and analgesics may be combined with each other or other drugs for synergistic or antagonistic effects (e.g., Vesal and Eskandari 2006, Vesal and Zare 2006). Muscle relaxants such as diazepam or midazolam may be used in birds, but only in conjunction with an analgesic agent. Currently, ketamine, which was once the injectable anesthetic of choice, is rarely used as the sole chemical anesthetic agent because muscle relaxation is poor, analgesia is inadequate, and recovery is often violent. Due to issues with its activity and effectiveness, ketamine is commonly mixed with other pharmaceuticals (Kilander and Williams 1992; Muir et al. 1995; Heard 1997; Mostachio et al. 2008; Rahal et al. 2008). Understanding the complexities of drug interactions requires specialized knowledge. Investigators wishing to use pharmaceuticals should consult, and practice with, a veterinarian experienced in working with avian species.

Example 1 - Durrani et al. (2008) tested the relative utility of detomidine and ketamine on the induction, maintenance, and recovery from anesthesia in Rock Pigeons (*Columba livia*). Birds given either detomidine or ketamine exhibited light anesthesia and superficial analgesia; birds given both drugs exhibited deep anesthesia and analgesia (assessed by testing of body reflexes). During recovery, birds given detomindine or both drugs exhibited hypothermia, depressed respiration, and brachycardia, but a generally smooth recovery; birds given only ketamine exhibited hyperthermia, rapid respiration, tachycardia, and a rough recovery. The authors concluded that detomidine is safe for use in bird handling and minimally invasive procedures whereas a combination of detomidine and ketamine is suitable for major surgery; ketamine alone is not a suitable option as an anesthetic agent.

Example 2 - Atalan et al. (2002) assessed the sedative-anesthetic effects of a combination of medetomidine, butorphanol, and ketamine on domestic pigeons. The authors concluded that this combination was a reliable option. The authors applied atipamezole as an antagonist following ketamine and were somewhat dissatisfied with the length of time that atipamezole took to reverse the sedative effects. In this particular study, the pigeons tended to wildly flap their wings during recovery and had to be restrained to prevent self-injury. Other experiments using medetomidine on pigeons and parrots have met with mixed success (Sandmeier 2000; Pollock et al. 2001; Lumeij and Deenik 2003). In contrast, Langan et al. (2000) used a combination of medetomidine and ketamine followed by propofol on captive Ostriches (*Struthio camelus*) and found the combination to be very effective in achieving profound sedation and anesthesia. Administration of atipamezole resulted in smooth anesthetic reversal. Taken together, these studies illustrate: the taxon-specific nature of anesthetic effectiveness; the importance of background research and consultation with veterinary anesthesiologists; and, the importance of recognizing the fundamental differences between working with birds and with mammals.

Example 3 - Teare (1987) examined the effectiveness of yohimbine hydrochloride (an α_2-antagonist) in easing the recovery of Helmeted Guineafowl (*Numida meleagris*) from anesthesia induced by a xylazine-ketamine combination. Administration of yohimbine 40 min after induction of anesthesia shortened all facets of the recovery process relative to a saline control with no apparent side effects.

Example 4 - Thil and Groscolas (2002) tested the efficacy of tiletamine-zolazepam on King Penguins (*Aptenodytes patagonicus*) under field conditions. Tiletamine is a dissociative anesthetic and zolazepam is a muscle relaxant with anti-convulsive properties. All treated individuals were immobilized within 5 min and remained so for approximately 1 hr. While three of the eight incubating adults did not resume incubation following treatment, the authors reported no adverse physiological effects. They did state, however, that future experiments with this treatment should examine the utility of various antagonists to reduce the recovery time.

Example 5 - Mulcahy et al. (2003) treated 20 Spectacled Eiders (*Somateria fischeri*), 11 King Eiders (*S. spectabilis*), and 20 Common Eiders (*S. mollissima*) with propofol, bupivacaine, and ketoprofen for the surgical implantation of transmitters. Propofol was administered to maintain anesthesia, bupivacaine was injected subcutaneously along the incision inducing local analgesia, and ketoprofen was injected IM at the time of surgery for analgesia during recovery. Of the 16 males operated upon, nine (56%) died within 4 days; one of the 35 (2%) females died. Necropsy revealed severe renal damage and visceral gout. The authors concluded that the ketoprofen caused the lethal renal damage and that male eiders may be more susceptible to renal damage than females due to the relatively short period of time they spent on land. Mulcahy et al. (2003) expanded their conclusions to suggest that non-steroidal anti-inflammatory drugs should not be used on any species prone to renal insufficiency. Alternative options for analgesia should be considered.

Local anesthesia

Given the difficulties of administering some of the common general anesthetics and the potential adverse effects, the use of local anesthetics is attractive, especially if the procedure is simple and the bird is to be released quickly. As with general anesthesia, however, the dosages for local anesthesia are uncertain and the effects may be prolonged and unpredictable (Graham-Jones 1965). To some extent, the problem is one of size (Gandal 1969; Klide 1973), with small species more susceptible to overdose. Extreme care must be taken in calculating dosages. A dose as small as 0.1 ml of 2% lidocaine is a lethal overdose for a 30-g bird. Studies on mammals indicate that several common local anesthetics, including 1% procaine, 0.2% tetracaine. 0.5% lidocaine (with and without epinephrine), 2% chloroprocaine, 0.25% dibucaine, 2% mepivacaine, and 2% piprocaine, have temporary but severe myotoxic effects (Basson and Carlson 1980; Foster and Carlson 1980). Diluting local anesthestics (with sterile, preservative-free normal saline solution or sterile water) would increase their margin of safety. The intramuscular use of local anesthetics should be undertaken with caution. Currently, the preferred short-acting drug is lidocaine and the long-acting drug is bupivicaine (Machin 2005). However, the most appropriate route of administration should be investigated for each species and drug.

Anesthetic compounds have also been employed in two areas unrelated to use in surgical procedures. Anesthesia as an agent for capture technique is discussed in the section on capture and marking. It is also used to minimize nest desertion by adults following capture and handling. Smith et al. (1980) developed an anesthetic technique using methoxyflurane (an inhalant anesthetic) to reduce nest abandonment by Gray Partridge (*Perdix perdix*) following handling and transmitter attachment. Of the six birds anesthetized with methoxyflurane following handling but prior to replacement on the nest, none abandoned and all but one successfully fledged young. No mortality was reported. Rotella and Ratti (1990) used the same technique to reduce nest abandonment in Mallards (*Anas platyrhynchos*). Following handling, transmitter attachment, and marking, female Mallards were anesthetized and placed back on their nest to recover. Only two of 80 treated females abandoned their nest following treatment, a rate significantly lower than previous studies. Individuals should always be monitored until fully recovered. The authors caution against placing anesthetized individuals back on nests in areas with predators or during inclement weather.

E. Surgery

Positioning

The subject should be positioned in as natural a position as possible but appropriate for exposure of the surgery site. If the procedure is relatively long, consider regularly rotating the animal to avoid blood pooling.

Monitoring

Nevarez (2005) provides an excellent review of considerations for monitoring animals while under anesthesia. Navarez (2005) stresses two critical points at the start of the review. First, planning how vital signs will be monitored is a critical component of pre-operative decision-making. Second, equipment cannot replace knowledge of and experience with the anesthetic agents and the species involved. Basic vital signs, such as reflexes or respiration, can be monitored without equipment and an investigator must be ready to perform continuous observations should equipment fail. Second, planning how vital signs will be monitored is a critical component of pre-operative decision-making.

Testing an animal's reflexes is perhaps the simplest method for assessing the depth of anesthesia. In birds, testing corneal reflexes using a lubricated cotton swab can be an effective reflex test. Another simple test is the toe pinch; foot withdrawal following the pinch can be interpreted as a pain reflex, which indicates an insufficient depth of anesthesia (Nevarez 2005). In addition to monitoring reflexes, investigators also need to monitor heart rate and respiration, both as indicators of depth of anesthesia and adequate tissue perfusion. Methods for monitoring heart rate include stethoscopes, Doppler monitoring, electrocardiograms (ECG), and direct blood pressure monitoring using arterial catheters and pressure transducers. Methods for monitoring respiration include respiratory rate monitors, pulse oximetry, capnography, and blood gas analysis. Stethoscopes can also be used to monitor respiration. Of these monitoring options, only respiratory rate monitors are suitable for deployment in most field situations. Some anesthetics may not induce eye closure. In such cases, the open eyes should be bathed with an optical wetting agent every few minutes or should be protected by application of an ophthalmic ointment which should be placed on to the eye as a strip across the eye along the upper or lower lid. Drops and ointments containing steroids should be avoided unless specifically prescribed by a veterinarian.

Hypothermia is one of the most common complications of anesthesia (Boedecker et al.2005, Nevarez 2005). Normal bird body temperature ranges from 40 to 44^{o}C. Heat loss can occur via convection (i.e., air exchange at the body surface), radiation (i.e., heat loss due to heat differential between body and surroundings), conduction (i.e., heat loss due to contact with a colder surface), and evaporation from lungs and skin. All four forms of heat loss must be accounted for during a surgical procedure and subsequent recovery. Heating pads and forced air warmers are commonly used to maintain both the body temperature of the subject and the temperature of the subject's surroundings. Even if steps have been taken to maintain temperatures during the procedure, investigators should also monitor their subject's core temperature. Heating pads should be avoided or used with extreme caution, as they have been known to cause severe skin burns even if used at low temperatures.

Other potential complications with anesthesia include cardiac arrhythmias or arrest, and severe respiratory depression leading to respiratory arrest. A detailed discussion of these issues with recommendations for their avoidance and management can be found in Gunkel and Lafortune (2005).

Incision closure and treatment

Closure of surgical incisions can be difficult and proper closure techniques require appropriate skills and materials. Consultation with a veterinarian is recommended to learn an appropriate closure technique for the incision and surgical procedure to be used, particularly if sutures are necessary. Older cyanoacrylic tissue glues (e.g., Tissu-Glu®, Ellman International, or Vetbond®, 3M Corp.; N.B. household super glues are toxic to tissues) took several minutes to dry, which markedly increased handling time. New, fast-drying versions of cyanoacrylate surgical glues such as Dermabond®, LiquiBand®, SurgiSeal®, and Nexaband® take under a minute to set and are far less toxic than earlier surgical glues. The need to close incisions to avoid infection justifies the cost (~$20/vial). It may be advisable to flush the incision with sterile saline solution as well as place triple (or other) antibiotic ointment on the incision. Inadvertent wounds caused during the procedure should also be treated. Surgical staples are an effective and rapid means of closing large incisions in medium-sized and large birds that are maintained in captivity or that can be held in captivity until the incisions heal and the staples are removed. Staples are not advised for field use. If multiple tissue layers have been incised, it is important to use the appropriate incision closure technique for each. Some small incisions heal faster if no surgical glue is used and in some cases of small skin incisions, the best decision may be to not use sutures or glue. Repeated surgeries on a single subject are discouraged unless they are part of a single experiment and have scientific justification.

Specific field surgeries

Laparotomy: Laparotomy penetrates a body cavity and, thus, is considered a major surgical procedure. Exploratory laparotomy has several uses. Since sex cannot be determined by external appearance in juvenile birds and adults of many species, laparotomy provide information on sex in monomorphic species (e.g., Lawson and Kittle 1973) and stage of gonadal development (e.g., Wingfield and Farner 1976; Schwab 1978), it can indicate presence of parasites, respiratory disease, gross condition, and activity of other organs, and it can be used for placement of transmitters into the body cavity.

Many experts perform laparotomies with only a topical anesthetic or no anesthetic at all (Risser 1971; Piper and Wiley 1991), 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. 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.

Isoflurane and methoxyflurane are both used as anesthetics for this procedure. Recently, MacDougall-Shackleton et al. (2001, 2006) used methoxyflurane prior to field laparotomies for their studies on gonadal development in sparrows and cardueline finches. Unfortunately, methoxyflurane is no longer commercially available. Isoflurane is a superior choice, but requires a portable vaporizer for field use. For the studies cited above, McDougall-Shackleton et al. (2001,2006) placed a few mLs of metofane (methoxyflurane) on a cotton ball in a jar and held that over the birds face until it didn't respond to toe pinch, then remove the jar. The laparotomies took about 2 minutes, and the birds recovered within 5 minutes. Recovery from isoflurane is even more rapid. Topical application of xylocaine cream or ethyl chloride to the incision site may reduce discomfort of laparotomized birds (Risser 1971; Ritchie et al. 1994).

Several reports have shown that laparotomy has no effect on survival and does not disrupt breeding activity or winter foraging (Bailey 1953; Miller 1958, 1968 cited in Risser 1971; Wingfield and Farner 1976; Marion and Myers 1984; Ketterson and Nolan 1986; Piper and Wiley 1991). Piper and Wiley (1991) examined the effects of laparotomies on White-throated Sparrows. They used no anesthetic, restrained birds using a modified bander's grip (see Bailey 1953 for diagram), and allowed incisions to close naturally. Birds were held in cages post-procedure for 5 to 30 min before being checked for alertness and then released. Laparotomies appeared to have no significant effects on short-term fat storage, long-term survival, dominance status, or range size. The only significant effect reported was the observation that laparotomized birds were more likely to remain on the study site as winter residents. The consequences for this last observation are unclear. One explanation is that the occasional rupturing of an air sac during the operation might have hindered an individual's ability to fly long distances. Another possibility is that becoming more sedentary might be a generalized response to injury, as previously suggested by Westneat (1986).

Laparotomy has been used successfully on hatchlings (Fiala 1979). Fiala (1979) performed laparotomies on nestling Red-winged Blackbirds (*Agelaius phoenicus*) using methoxyflurane (Metofane) vapors as an anesthetic, restraining birds on plexiglass sheets with adhesive tape, and closing the incisions with liquid skin adhesive. The only serious problem reported was occasional renal hemorrhage. Fiala (1979) reported minimal long-term effects. Plastic bands are an excellent alternative to adhesive tape. This study highlights the fact that the most appropriate surgical approach should be researched and selected. It is important to avoid vital structures (like kidneys) and air sacs to the extent possible. Repeated laparotomies on birds at intervals of about a month to trace gonadal growth and regression can be done with the caveat that scar tissue at the incision site can make successive surgeries more difficult.

Any unsealed wound can be a route for infection or for herniation of abdominal tissues and organs. Therefore, laparotomy wounds should be sealed. Surgical glues serve this purpose well. Wounds in waterbirds should be sutured to reduce the potential for infection. In diving species, the wound must be sealed to avoid penetration of water into the body cavity as pressure increases with depth.

Over the last decade, several less invasive techniques have become available for sexing birds, including flow cytometry (Tiersch et al. 1991), fecal sex steroid (Goymann 2005; Palme 2005), and polymerase chain reaction-based techniques using feathers, blood, or tissue (Han et al. 2009; Griffiths and Tiwari 1993; Ellegren 1996; Griffiths et al. 1998; Fridolfsson and Ellegren 1999, Underwood et al. 2002). A major limitation to using PCR to sex birds has been resolved by the development of a multiplexed PCR that works in all avian species, even the many species for which there is no difference in between-sex intron length, which is the basis of traditional DNA-based sex determination (Han et al. 2009). All of these less invasive techniques require the return of samples to the laboratory and some delay during processing. Nevertheless, investigators are encouraged to explore the appropriateness of these techniques to their studies. Unless the research question requires immediate knowledge of an individual's sex or gonadal development, the less invasive techniques should be given primacy over laparotomies.

<u>Implantation of transmitters</u>: One of the most common field surgical techniques involves the implantation of a transmitter or biomonitor (e.g., Korschgen et al. 1984, 1996; Olsen et al. 1992; Harms et al. 1997; Hatch et al. 2000; Machin and Caulkett 2000). Most current studies follow the techniques of Korchgen et al. (1984, 1996) and Olsen et al. (1992). Korschgen et al. (1984) were among the first to explore the feasibility and utility of placing radio transmitters inside the body cavities of birds. Using several species of captive and wild ducks, the authors first evaluated the effectiveness of several anesthetics, including pentabarbitol, ketamine, and xylazine, and discovered that the use of local anesthetic (2% lidocaine hydrochloride) was sufficient for the procedure. Although conditions were not aseptic, the authors wore gloves and kept surgical equipment in a cold sterilization tray containing zepharin chloride prior to use. The

authors made 2 cm incisions, inserted the transmitters, closed the incisions with surgical sutures, and treated all birds with antibiotics. Including time for anesthesia, the operations took 25 min per subject. Of the 31 subjects, the only complications reported were a systemic infection in a single female Canvasback and a local infection around the internally coiled antenna in a male Mallard. Later, Korschgen et al. (1996) modified their earlier technique by switching to isoflurane. They reported minor physiological responses to the surgery but no major adverse effects.

Olsen et al. (1992) used a similar technique to implant transmitters in Canvasbacks but used isoflurane as the anesthetic. Investigators wore gloves and equipment and transmitters were maintained in cold sterilization; incisions were closed with absorbable sutures. Over the three years of the study, 253 birds received implanted transmitters. Five individuals (2%) died during or immediately following surgery: one from hemorrhage in the airways, one from lung congestion, and three from respiratory complications due to lung flukes. The authors reported no post-operative complications for the remaining 248 individuals. Korschgen et al. (1996) modified their earlier technique by switching to isoflurane. They reported minor physiological responses to the surgery but no major adverse effects.

Mulcahy and Esler (1999) implanted transmitters into 307 Harlequin Ducks (*Histrionicus histrionicus*). Initially, birds were anesthetized using isoflurane. Surgeries were performed in a covered but unheated workspace on a motor vessel and birds were allowed to recover for a least one hour before release. In the first year of the study, 10.7% (11 of 103) of individuals died during surgery or within 14 days of release. As a result, the authors made the following alterations to their surgical procedures: birds were intubated, birds were placed on foam pads so that their heads rested below their body during the surgical procedures, and researchers monitored and maintained patient body temperature using temperature sensors, electrocardiograms, and hot water pads. Following the implementation of these alterations in procedures, mortality dropped to 2.9% (six of 204). Additional examples of surgical procedures for transmitter implantation can be found in Schultz et al. (1998, 2001).

F. Post-surgery

Most birds will experience emergence delirium during recovery from general anesthesia, regardless of the anesthetic agent used (Curro 1998). If a subject was intubated during surgery, extubation should occur when bird is awake enough to exhibit signs of objecting to the tube's presence (e.g., has swallowing reflex); the glottis should be examined for damage or obstructions following extubation (Curro 1998). During emergence delirium, subjects should be restrained with sufficient force to prevent self-injury (e.g., due to uncontrolled wing flapping) but the restraint should not interfere with ventilation and thermoregulation. It was once thought that rocking a bird from side to side during recovery from general anesthesia minimized emergence delirium; however, this procedure is now discouraged due to potential interference with normal blood flow and respiration during recovery (Gunkel and Lafortune 2005). Subjects should be provided with a warm and dimly lit (or dark) place in which to recover and should not be released into the wild until fully alert and are able to perch or stand on their own. One technique for passerines is to **loosely** roll them in a clean paper towel following surgery, and place them on their side in a recovery cage. This gently restrains them until they are sufficiently recovered to wriggle out.

G. Euthanasia

The question of euthanasia primarily arises when an animal experiences pain and suffering that cannot be relieved or that will not abate over time or has an impairment that will affect its probability of survival. Euthanasia is also a necessary component of scientific collection (see Scientific Collecting). Irrespective of the purpose of the euthanasia, the technique used first and foremost, should produce unconsciousness swiftly and painlessly. In addition, the euthanasia technique should not interfere with post-mortem analysis and will be considerably influenced by what an investigator wishes to do with the cadaver (i.e., use it for a museum specimen or for tissue chemistry). Many techniques for euthanasia have been reviewed by the American Veterinary Medical Association (2007); these euthanasia guidelines were under review in 2009 and the American Veterinary Medical Association expects to issue an update in 2010. The American Veterinary Medical Association recognizes that recommended modes of euthanasia for captive animals are not always feasible in field situations. However, the challenges presented by field conditions do not release investigators from the responsibility of minimizing the pain and distress of animals to be euthanized.

The American Veterinary Medical Association (2007) considers the following techniques to be acceptable for use with wild birds: barbiturates (injected, ideally intravenously), inhalant anesthetics, carbon dioxide inhalation, carbon monoxide inhalation. Gunshot to the head is considered conditionally acceptable when other methods cannot be used. However, the carcass will have no value as a specimen if the head is destroyed by gunshot and so this method is inappropriate unless there is no intent to retain the carcass as a specimen.The following techniques are considered conditionally acceptable: nitrogen and argon inhalation, cervical dislocation, and thoracic compression. The use of injectable agents, such as barbiturates, is a rapid and dependable method of euthanasia. The use of barbiturates as a euthanasia agent in the field may influence carcass disposal due to possible detrimental effects on scavengers. The fastest and most humane result occurs if the drug is injected directly into the vein. Therefore, the needle gauge should be carefully considered so that it is not so large that it will damage the vein upon entry and make injection into the vein difficult. When it is believed that the needle has entered the vein, prior to injecting the drug into the vein, the plunger of the syringe should be gently and slightly pulled back to confirm venipuncture by a red flash of blood into the syringe. Generally acceptable techniques involve overdose with an anesthetic, either injected or inhaled, followed by the administration of a specific euthanasia compound. 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 small bottles of anesthetic or euthanasia compound add little burden. Investigators who wish to consider this option must consult with a veterinarian to determine the appropriateness – especially the required dosage - and legality of the use of certain chemicals in the field. If pre-euthanasia chemicals are unavailable, the American Veterinary Medical Association (2007) states that an intraperitoneal injection of sodium pentobarbital is acceptable if an intravenous injection is impractical or impossible. Although intraperitoneal injections are slower to produce loss of consciousness, they do not require the same level of handling and restraint of subjects and can minimize an animal's distress. Intramuscular, subcutaneous, intrathoracic, intrapulmonary, intrahepatic, intrarenal, intrasplenic, intrathecal, and other nonvascular injections are not acceptable methods of administering injectable euthanasia agents. Euthanasia via inhalant (e.g., carbon dioxide or monoxide) is also a generally acceptable method but it can be cumbersome for field use and deployment. A portable solution is to carry a small tightly sealed vial with a cotton ball in it that has been soaked in isoflurane. Euthanasia is very rapid and is done by placing the bird's face in the jar. The realities of field situations often require mechanical means of dispatch. This technique, known as thoracic compression, results in a very rapid loss of consciousness, with death following soon after. It is easy to learn, thereby minimizing the probability of mistakes and the potential for subject distress. The technique also maximizes the scientific utility of specimens, thereby minimizing the number of individuals collected for scientific research. The AVMA (2007) considers thoracic compression to be conditionally acceptable. The Ornithological Council has prepared a peer-reviewed fact sheet about thoracic compression that is available upon request.

The American Veterinary Medical Association (2007) considers the following chemical methods to be unacceptable under any conditions: chloroform, cyanide, formalin, household solvents (e.g., acetone), neuromuscular blocking agents, and strychnine. Chloroform, cyanide, and formalin are unacceptable both for the danger they pose to personnel and for the distressing aspects of their chemical actions in the body of research subjects. Neuromuscular blocking agents (e.g., nicotine, magnesium and potassium salts, all curariform agents) produce muscle paralysis in conscious animals, causing death through 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 deeply anesthetized animals. Strychnine causes painful and protracted convulsions, prior to death by asphyxiation. The American Veterinary Medical Association (2007) considers the following physical methods to be unacceptable under any conditions: air embolism, blow to the head, burning, decompression, drowning, exsanguination, hypothermia, rapid freezing, and smothering.

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CHAPTER 8. SCIENTIFIC COLLECTING

A. Overview

Scientists use the term scientific collecting to mean permanent removal of individual birds from the wild. That is, scientific collecting entails the capture and sacrifice of a living bird to address myriad scientific questions. The specimens —preserved corporal parts and associated data — are retained, managed and conserved permanently in scientific collections**.**

Bird specimens have been used to answer questions that few would have anticipated when the specimens were collected. For example, environmental change and its effects on bird populations have been detected from museum specimens. Seabird specimens collected over 14 years yielded the only evidence of increased consumption of plastic pollutants (Moser and Lee 1992) and later provided critical support for restriction of offshore oil extraction (Lee and Socci 1989). Bird specimens have also been instrumental in forecasting conservation implications of climate change for biodiversity (Gardner et al. 2009). Museum specimens collected over a span of 100 years showed that body size of four passerine species decreased over time, causing a seven degree shift in a latitudinal cline over a period of 60 years. By studying feathers on the specimens to rule out nutritional causes, the researchers determined that the differences were most likely due to global warming. The specimens studied were taken decades ago for an entirely distinct purpose. Were it not for collection and careful preservation of specimens, this information would not be available (Remsen 1995). No one can foresee what valuable information specimens collected today will offer, or to what fascinating uses they will be put, 100 years from now, any more than one could have predicted 100 years ago how then-new specimens would answer intriguing questions today. For instance, many specimens used today to identify birds that have collided with aircraft were collected before airplanes were invented.

No other aspect of ornithological research generates so much controversy, yet the debate is disproportionate to the magnitude and impact of the activity. Most objections stem from personal value judgments and emotional responses rather than scientific considerations. Some oppose any collecting whatsoever, for any purpose, even of single individuals and even of abundant and widespread species, based on personal views and concern for the well-being of individual animals. Some who oppose collecting assume, wrongfully, that there are scientifically acceptable alternatives. Others do not oppose scientific collecting *per se*, but worry instead about impacts on declining or rare species, or on particularly sensitive populations. In fact, as discussed below, levels of collecting are extremely conservative, with negligible effects on populations, while engendering long-term benefits for bird conservation and science. Winker et al. (2010) offers a comprehensive review of the importance, effects, and ethics of scientific collecting.

Other definitions

The term “scientific collecting” has different meanings in other contexts. The regulatory (U.S. Fish and Wildlife Service) meaning of scientific collecting and the permits required for collecting encompasses the collection of various tissues and fluids, including whole birds, blood, feathers, or toenail clippings. It can also include tracheal or cloacal swabs or samples of crop or stomach contents. Scientists may also collect fecal sacs and nests. State permit agencies often use the term “scientific collecting” to refer to any research method that involves the capture of a live bird, whether for temporary holding or permanent removal from the wild, and even without the collection of samples.

In some cases, individuals are removed from the wild for study in captivity, in a laboratory, aviary, or specialized enclosure. This activity also requires federal scientific collecting permits and usually state permits. The disposition of wild birds studied in captivity varies. Federal and/or state permits may require that the birds be euthanized. Others require release, usually in situations involving very short periods of captivity. Institutional Animal Care and Use Committees may require euthanization out of concern that the individuals will not successfully re-adapt to the wild. Zoos or aviaries may be willing to accept wild birds that were taken into captivity for research projects. In the event that a bird can not be released and no zoo or aviary can accept the bird, and the bird must be euthanized, the carcass should be offered to a museum where it has can serve as a voucher specimen for studies for which it was used or for other research purposes. If no museum can accept the carcass, it should be offered to a teaching collection.

B. Purpose of scientific collecting

Scientific collecting is a method of obtaining scientific information. Some questions can be answered with observation, some require some kind of manipulation, and others require capture and marking. Other questions can be answered with blood, feather, or tissue samples. An entire range of questions require the collection of an entire bird.

Scientific collections document the world's biodiversity. Each animal collected serves as a voucher for the existence of that species in its place and time, providing scientifically rigorous documentation that can be reexamined visually, structurally, or biochemically for centuries into the future. Each specimen also holds staggering amounts of information in the tissues of its body. Information about the ecological placement (what an individual is eating and what is eating it), reproductive status, migratory routes, exposure to pollutants, demographic patterns, genetic distinctiveness, and much more is represented in the various tissues and organs of an individual, and can be used to infer important facts about whole species. These data can address ecological or evolutionary questions, many of which are critical to species conservation. For instance, provenance and genetic data taken from 238 museum specimens collected from 1879-1935 document the range expansion of Greater Prairie Chickens (*Tympanuchus cupido*) did not result from human alteration of habitat (Ross et al. 2006). Conservation policy and practice often exclude populations or regions that are thought to exist due only to human activity, such as the deliberate introduction of non-native species. On this basis, legal protection and recovery efforts for the species excluded populations on the northern prairies of the United States and the central plains of Canada. The specimen-based analysis showed that the supposed cause of range expansion was not feasible and that the historical range of the species in fact included these areas. Recognition of the species severe decline (and extirpation in Canada as of 1987) and conservation efforts began about five decades after the specimens used in this study were collected. Scientists may not know at any given moment what is important to study. Some of the most important questions involve how organisms change over time. Preserving information over time through scientific collecting allows us to increase knowledge today and to answer the unanticipated questions of the future.

Scientific collecting generally entails collection of a wide range of species throughout the species' ranges and of enough individuals to permit scientifically valid inferences. Typically, the collector will not know in advance of the expedition exactly what species will be collected; it is, to some extent a matter of chance. Some species that are sought-after may not be found, whereas others not anticipated may be encountered. It is difficult, therefore, for the ornithologist to identify all species and the numbers to be collected when submitting a protocol for approval. This may pose a dilemma for Institutional Animal Care and Use Committees, who often ask the researcher to state in the protocol how many individuals of each species will be collected. As it is impossible to make this determination in advance, the best answer is to state that collecting will not exceed permit limits.

Some studies that entail scientific collecting focus on specific, immediate questions. In these cases, the study design determines the number of individuals of each species to be collected. An adequate sample is the minimal number of specimens 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 often require larger samples than do laboratory studies, because field investigators have less control over the conditions that produce variation. The precise number of individuals required for statistical inference can be difficult to predict at the outset of a study because the extent of natural variation may not be known. Many studies requiring specimens are studies of variation *per se*, and thus require large sample sizes. For example, empirical results demonstrate that at least 20 and preferably 30 individuals per locality would be appropriate for accurate estimation of population genetic parameters in microsatellite studies that assess genetic diversity when working in a population that has an unknown level of diversity (Pruett and Winker 2008). In general, large data sets allow a wider variety of scientific questions to be addressed and therefore have a greater ability to aid conservation and management decisions and address unanticipated future questions. Even in the case of focused studies, however, the ornithologist, who may have devoted considerable time and resources to travel to a field site, and to obtain permits, may choose to collect other birds while in the field to maximize the contribution of the scientific effort.

Some assume that because museums already hold very large numbers of specimens, no additional collecting is necessary or warranted, or question the need to collect the number of species or number of individuals typical of a general collecting effort. In fact, as detailed below, museum holdings are inadequate in myriad ways. Furthermore, the growth of museum specimen holdings is a by-product and not the purpose of scientific collecting. Recognizing that because it is impossible to predict what questions will be asked about any particular species, it becomes evident that collecting as many species as possible is not only justified, but necessary to document populations and archive materials for future studies of environmental change. For instance, if a species that is common today suffers a sudden decline 30 years from now, the specimens collected decades earlier, across place and time, can be used to determine when problems started, where, and what form they took (pollutants in body, new parasite introduced, genetic variation within populations, demographic shift such that suddenly young animals became rare, reduction in number of individuals migrating from a given breeding site, etc.). Comparisons can be made to other populations of the same species, or other species collected at the same sites. A classic case of this principle in action was the use of nearly 1800 eggs accumulated over 100 years of collecting from 39 different museum collections to document sudden changes in eggshell thickness as a result of the accumulation of DDT in the bodies of fish-eating birds. This scientific finding resulted in the end of the use of DDT in the United States.

The number of individuals collected is a simple matter of statistically valid inferences. Differences among a few individuals are meaningless as they could be due to chance. For scientists to draw reliable conclusions, series are necessary allow to allow the researcher to distinguish between real differences as opposed to normal variation among individuals.

C. Alternatives

Availability of specimens from other museums or institutions

A common misperception is that scientific collections worldwide hold ample representations of the world's avifauna, so additional scientific collecting is not needed. This notion belies misunderstandings both of the scientific uses of specimens and the composition and condition of existing collections.

<u>Underrepresented species</u>: Representation of some species in collections is simply not adequate. The avifaunas of many geographical areas remain poorly documented by specimens. Peterson et al. (1998) examined data pertaining to 221,757 specimens from 26 museum collections, among them the four largest collections in the world and largest collection in Mexico. This sample represented an estimated 70% of all bird specimens from Mexico. The origin of the specimens was mapped, leading to the determination that most regions were severely unrepresented in museum collections, even for the best-sampled species. Basic taxonomic information for many species is still not adequately represented in museum collections in the form of representatives of both males, females, immatures, juveniles, basic and alternative plumages, and geographic and individual variation.

Insufficient number of individuals: It is often mistakenly believed that museums specimens serve only to document identifications, so if a scientific collection holds a specimen of a given species, no additional collecting would be warranted. It is true that a single specimen, known as a holotype, documents the first, or formal, description of a species. However, even to identify species, single specimens are vastly insufficient. Frequently, species or subspecies are distinguishable only via careful comparison of series of individuals, to be able to account for individual variation. In addition, at times, what might seem to be a distinct species proves to be an aberrant individual or different color morph of the same species. Further, documenting basic information about that species, such as differences between sexes, seasonal variation, developmental stages, and geographic variation all depend on series of specimens to document individual variation, which itself is frequently considerable.

Availability to researchers: Access for all is critical for maintaining a worldwide network of knowledgeable professionals. For existing specimens to serve the purpose of education and guiding identification, they must be available at least regionally. Frequently, species are represented in a single or very few institutions, impeding efforts to make use of those specimens. International movement of specimens and associated materials is becoming increasingly difficult and costly due to permit restrictions and biosecurity concerns.

Inadequate information: Existing specimen resources are also inadequate in terms of information content. The vast majority of bird specimens were collected prior to 1960 (Winker 2004), which means that they are not completely adequate from a number of perspectives. Older specimens often consist only of the skin and feathers and bear minimal data documenting provenance. Retention of soft tissue, stomach contents, and related material developed as the standard practice in most major museums approximately 30 years ago; some museums still preserve and retain only the skin and part of the skeleton. Anatomical material, tissue samples, soft-part coloration, ecological notes, and precise locality references are frequently lacking. Given that most specimens are older, samples sizes of recent, data-rich specimens are quite inadequate, and certainly not sufficient for every (or even most) species on Earth. Stoeckle and Winker (2009) found that of the world's 9,933 avian species, fully 2,705 (27%) were undocumented in tissue collections in the 32 institutions surveyed.

Age of collections: Species and populations are changing constantly, so series need to be continually updated to be maximally informative. Even if the world's collections held complete series of males, females, different age classes in sufficient numbers to document variation, the dimensions of time and place require continual reassessment. Species, populations, and their environments are continually changing, so continual collection is necessary. Winker (2004) examined the date range represented in bird collections. He found that they “suffer from temporal inadequacy, poorly representing the present, especially in developed regions” including the United States, the United Kingdom, and Canada. This situation may make these collections less useful for answering questions about changes in avian biodiversity and the environment causes of those changes.

Genetic material, photographs, and recordings

Some consider blood samples and photographs adequate replacements for physical specimens. This view presumes that the primary or sole purpose use of a specimen is identification. That is simply not true, as detailed above. And, in fact, even for identification purposes, photos and genetic material are insufficient. The scientific community considers a physical specimen the best evidence and documentation of biological material. Specimens provide a definitive picture of the individual organism, in terms of its genotype, phenotype, and context. Genetic samples without a full specimen provide only a genotype, which lacks key information such as the phenotypical characteristics that form the basis of much of taxonomy and identifications (particularly of genetically distinct individuals) may remain forever in doubt. If genetic samples are lost, destroyed, or contaminated, or improperly processed before analysis, no information is available. It is highly unlikely that the individual can be recaptured. A specimen makes it possible to obtain and study genetic material from that individual hundreds of years into the future.

Photographs can be of poor quality or subject to digital alteration, and key characteristics of the phenotype distinguishing species may be too subtle or missed in photography. A photograph of course provides no information about genotype. Recordings similarly may be of poor quality or altered. Birds of the same species can have different local dialects that reflect phenotypic, not genotypic, variation (Marler and Tamura 1962). Sound can be affected by habitat and climactic conditions, and even the most skilled recordists may find it difficult to obtain a recording when conditions are very noisy. Even high quality recordings and photos provide limited information and are no substitute for physical specimens.

For formal taxonomic descriptions, the role of scientific specimens is nothing short of fundamental. Recent years have seen descriptions of small numbers of new bird taxa based on photographic evidence, gene sequences, living individuals, or feathers taken from “catch and release” studies (Smith et al.1991, Sangster and Rozendaal 2004, Athreya 2006). Each of these cases provoked strong statements by the community of scholars and curators whose expertise is in systematics and who consider specimen evidence as *sine qua non* for formal documentation of bird taxa (LeCroy and Vuilleumier 1992, Bates et al. 2004). The International Commission on Zoological Nomenclature recommends strongly that taxon descriptions be based on specimens (Wakeham-Dawson et al. 2002, Polaskek et al. 2005). Most of these descriptions met the requirements of the International Commission on Zoological Nomenclature; the babbler description was based, in part, on collected feathers and Athreya planned to obtain a full specimen if census work indicated a larger population than was known at the time of the capture of the individual upon which the description was published (Athreya 2006). In the case of the Bulo Burti Bush Shrike (*Laniarius liberatus;* Smith et al. 1991), genetic analysis led to the determination that the individual upon which the description had been based was in fact a color morph of another species (Smith et al. 2008). It is also worth noting that none of these descriptions could have been made without comparison to the dozens of specimens collected over many decades.

D. Impact on Populations

Generally

The American Ornithologists’ Union has issued a clear statement that addresses the question of population impact:

> *The AOU regards responsible collecting of birds as an essential research method for studying the biology, ecology, systematics, and genetics of wild birds. As in laboratory research, methods of collecting used by field workers follow humane guidelines. Specimen collection plays an essential role in documenting the biodiversity of poorly known regions. Collecting specimens from populations known to be endangered or in precipitous decline also has important scientific value, but should be exercised with extreme caution and careful documentation that removal of the proposed number of animals will not adversely affect the*

population's projected trajectory in size and genetic diversity. The AOU recognizes the difficulty of making these judgments. The AOU is working to develop explicit procedures and criteria for projecting population effects of collecting and evaluating them relative to its benefits.

AOU Council, Laramie, Wyoming, 8-11 Aug 2007

Many decades of experience with scientific collections in many regions shows that the collection of scientific specimens typically has no lasting effect on avian populations. By looking at all birds taken under permits issued by the U.S. Fish and Wildlife Service for research and captive breeding, and deducting those taken for depredation control research and research involving hunted species or for propagation, and those taken under salvage permits (birds found dead), Banks (1979) estimated that at most, 15,000 birds per year were collected for scientific research per year, or one hundredth of one percent of overall direct annual mortality (defined in this paper as deaths resulting from the deliberate actions of man) and seven thousandths of a percent of annual avian mortality related to human activity.

More recently, the Ornithological Council analyzed data provided by the U.S. Fish and Wildlife Service for the years 1998-2002. Between 51 and 63 permits were issued per year. Cumulatively, the highest number of individual taken of any one species was 260 American Tree Sparrows, (*Spizella arborea*), an abundant, widespread species that numbers ~30 million individuals in North America (Partners in Flight Landbird 2009). In two of the five years, the highest number of individuals taken of any one species was the Steller's Jay (*Cyanocitta stelleri*): 135 taken in 1998 and 183 taken in 1999. Though restricted in range to the West Coast and the intermountain west, the species, with an estimated population of 4.4 million birds (BirdLife International), is considered stable. In the other two years, the Song Sparrow (*Melospiza melodia*) was the most-often taken (143 in 2000 and 159 in 2002). Birdlife International estimates the population of this species at 43 million birds. Only these three species and the White-winged Dove were collected in numbers exceeding 100 in any of the five years. Of the next eight most commonly collected species in each of those five years, the highest number was 89 Spotted Towhees (*Pipio maculatus*) with an estimated population of 14 million, and the lowest was 18 Snow Buntings (*Plectrophenax nivalis*) with an estimated population of 39 million. In sum, the scientific collecting enterprise removes individual birds in numbers that are nothing more than trivial in terms of impact on the population. For perspective, the U.S. Fish and Wildlife Service in 2008-2009 allowed the hunting 'take' of approximately

275,000 American Woodcock (*Scolopax minor*), a species that has been in steep, long-term decline (Cooper and Parker 2009). The species has been designated by the U.S. Fish and Wildlife Service as one of the top nine species for concerted conservation activity. The National Audubon Society estimates the population estimated at 5 million individuals. So, it is clear that no North American species is collected intensively enough even to begin to impact its numbers.

Even in the absence of population-level impact, ornithologists take measures to reduce the number of individuals collected. First, and most fundamentally, every effort is made to assure that the specimen and associated tissues and fluids are properly preserved and deposited in an institution that intends to keep the material permanently and make it available for future research (AOU Committee on Bird Collections 2009). Where biologically appropriate, existing specimens are studied. Ornithologists avoid collecting in conditions where retrieval of the carcass might not be possible. Shooting is often the most effective and practical means of collecting most birds species. Using the appropriate ammunition and avoiding difficult shots reduces the chance that the bird is wounded but able to escape. Wounded birds should be retrieved and sacrificed humanely. It is also standard practice to avoid concentrating the collecting effort in a small area or in a single breeding or roosting aggregation and to avoid collecting large numbers or gravid females, except as required for the purposes of the scientific questions being asked. Both to reduce the number of individuals taken and for humane reasons, ornithologists avoid collecting nesting birds unless the young will also be collected. To avoid taking gravid females or adults with dependent young, adults taken from breeding sites should be taken as soon after they arrive on the breeding grounds as possible or after the young have fledged. Where collecting is undertaken for a specific study, as opposed to general collecting, as in experiments intended to alter behavior, reproduction, or survivability, adequate sample size should be estimated before collecting begins.

Small Populations and Endangered Species

The American Ornithologists' Union stated,

> *Collecting specimens from populations known to be endangered or in precipitous decline also has important scientific value, but should be exercised with extreme caution and careful documentation that removal of the proposed number of*

animals will not adversely affect the population's projected trajectory in size and genetic diversity.

AOU Council, Laramie, Wyoming, 8-11 Aug 2007.

Assuring this outcome entails several considerations.

Permit limits: Most countries require permits for all scientific research, and certainly for scientific collecting. Some of these agencies are considered overly restrictive in that they set limits far below the level that would be biologically warranted. In the United States, for instance, collecting of species listed on the 2008 list of Birds of Conservation Concern – a designation that confers no legal protection that is not already afforded under the Migratory Bird Treaty Act - is limited to either five or ten individuals of each species per year, regardless of the actual size of the population, unless the researcher can justify the need to exceed the limit. Management authorities typically do not allow collection (other than salvage) for species that are truly rare, such as those listed as endangered or threatened under the Endangered Species Act, or even for "candidate species" under consideration for listing. As a rule, population impacts of collecting are limited by the fact that researchers work within the constraints of conservative permit limits that cannot be exceeded. In other countries, permit limits are likely to reflect the legal protection afforded to a given species, or if there is no system of legal protection, the status classifications of BirdLife International or the World Conservation Union (IUCN) Red List for Birds.

Personal responsibility and preliminary research: In some cases, permit agencies may lack adequate information about the status of a species or a population. Therefore, regardless of permit limits, when planning to collect species for which there are serious conservation concerns, researchers should seek robust recent information regarding the status of the population from which collection is planned. Indeed, in the absence of recent information, it may be reasonable to conduct preliminary field surveys to determine the status of the population. The natural history of the species of interest should be also taken into account. For instance, most passerines and other small- bodied birds have high reproductive rates and density-dependent mechanisms of population regulation such that populations can recover quickly from even large mortality events. In contrast, populations of a long-lived, slower-reproducing species such as condors or albatross may be affected more profoundly by removal of even small numbers of individuals. In most cases, population sizes will often prove a function simply of the extent of available resources, and will depend little on individual mortality (natural or science-related). If this preliminary research is undertaken in a thorough manner, the ornithologist may be the most knowledgeable person about the status of the population. With this knowledge and the exercise good scientific judgment, the researcher should be able to make science-based decisions about the extent to which a population can sustain collecting, again subject to permit limits.

Caveat: Official or authoritative conservation status assessments may not always provide reliable indicators of true population status. Over much of the world, so little is known about many bird populations that official listings such as the authoritative IUCN Red List err in both directions. This includes species as extinct or endangered that are neither, and failing to classify species that are probably extinct (Diamond 1987). These official classifications are thus insufficient to determine if (and how much) scientific collecting can be tolerated by a species or a population. For these understudied species, Diamond (1987) proposes a precautionary approach: that they be "extinct or endangered until shown to be extant and secure. In practical terms, this suggests that relatively more field survey work is needed to determine population status in the case of apparently rare species before collecting is undertaken, regardless of permit limits. Conversely, once it is known that official classifications are overly pessimistic, collecting (within permit limits) should be restricted only by the life history and population status of the species or population, subject to permit limits. Even in the face of unambiguous information, the process of changing conservation status is inherently slow. For instance, ornithologists have been recommending since 1980 that the California Brown Pelican (*Pelecanus occidentalis californicus*) be removed from the list of threatened or endangered species under the U.S. Endangered Species Act, and despite petitions to the U.S. Fish and Wildlife Service and an agency proposal for delisting in February 2008, the decision was still pending at the end of 2009. Appropriate permit limits and collecting levels should be based on the latest available data on population status, rather than potentially antiquated lists.

E. Methods

Researchers involved in scientific collecting should assure that the manner of collection is humane and conforms to established ethical standards of treatment for animals in research. Collecting commonly entails trapping birds and employing a humane method of euthanasia to bring about a rapid loss of consciousness and to minimize pain and suffering prior to death. Humane methods of euthanasia are discussed in the section on major manipulative procedures.

Any method chosen must avoid unnecessary damage to the specimen or injury to the body parts required for the investigation. Practical limits inherent in field work often precludes the use of some methods. For instance, it may not be possible to carry and use isoflurane (or other inhalant agents) in the field. Chemical methods are generally unacceptable, unless it can be shown that an agent will not compromise or bias potential tissue analysis. Further, access to many chemical agents is legally restricted (see discussion in section on anesthesia). It is often necessary to use a shotgun, particularly when collecting large birds or birds that will not enter nets or traps. Small bore shotguns minimize damage to the carcass. If attempting to collect a single individual or particular bird, the small bore shotgun will also minimize spread and reduce the likelihood of hitting other birds or animals in the vicinity of the targeted individual. Otherwise, it may be necessary to wait until the targeted individual moves away from other animals. Ornithologists who collect birds with a shotgun or other firearm should be experienced in their proper and safe use and must comply with laws and regulations governing their use. The firearm and ammunition load should be appropriate for the species to be collected to avoid nonfatal wounding. 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 using a humane method of euthanasia.

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APPENDIX A: TO SAVE A BIRD CARCASS FOR SCIENCE

Introduction: If your permit authorizes you to salvage birds (in the United States and in Canada, banding permits authorize the permit holder to "...salvage, for the purpose of donating to a public scientific or education institution, birds killed or found dead as a result of the permittee's normal banding operations, and casualties from other causes..."), these instructions will help you to assure that the carcass is preserved in a manner that will allow researchers to make use of it. In some cases, salvaged bird carcasses can help reduce the number of birds that scientists need to collect. In nearly all cases, the carcass can yield valuable information for research and teaching purposes.

Museums and researchers greatly appreciate these donations, which are an incredibly valuable source of scientific information. Information derived from museum collections includes identification of species and subspecies, geographic distribution, understanding environmental changes - such as the effect of pesticides - and even about behaviors that may be difficult to observe in the wild, such as hybridization or mating patterns. Sometimes, museum collections are the basis for something so basic as identification. Law enforcement agents may need to identify seized wildlife, and may turn to museums for assistance. Donated birds may be useful in the study of wildlife diseases.

However, if the bird is not properly preserved and the data needed by scientists is not recorded, the time and energy it takes to bring the specimen to a museum or other research institution may be wasted. These instructions will help to ensure that your donation will be useful.

On the label, write (in waterproof ink or pencil):

- Date bird taken from the wild
- Date bird brought to you
- Date bird died

Please write dates as date-month-year (“12 December 2004”). Please write the month in letters.

- Where the bird was found. Be as specific as possible.

- Your name and contact information. The museums are required to obtain and maintain this information, and your name and contact information enables the museum to contact you if more data about the specimen are required (for example, sometimes the ink runs or the writing is illegible). For permanently preserved specimens you can receive credit on the permanent museum label for obtaining the specimen.

- Optional: Cause of injury, if known; medical reports, including lab results (especially toxicology), medications, necropsy.

The museum may be able to provide forms or labels for you.

Place each bird and its associated tag or label in a separate clear plastic bag. Using clear plastic bags is helpful when possible because then the receiving party can immediately see the specimen and determine its identity, quality, and preparation or sampling future.The bag should be closed and most of the air squeezed out to minimize freeze drying. Ziploc bags or bags that are heat sealed are best. It is helpful to place this bag in a second closed bag, particularly if the specimen is going to be stored in a freezer for some time before it is donated to the museum. For large birds, kitchen trash bags or larger trash bags are acceptable, but please be sure to close the bag tightly.

Optional: If you really want to do a professional job, put a wad of absorbent cotton or tissue down the bird's throat to prevent fluids from seeping out onto the plumage, then arrange the bird in the bag so the feathers (especially the tail) aren't bent and the head, neck, wings, or legs aren't projecting at awkward angles (they are easily broken when frozen).

Finally, make those arrangements for donation. If you need assistance finding an institution to accept your salvage specimens, the Ornithological Council can provice a list of museums that are willing to accept specimens (contact Ellen Paul at ellen.paul@verizon.net) or contact the U.S. Fish and Wildlife Service Regional Migratory Bird permitting officials, who will also have a current copy of this list.

It is helpful to contact museum staff in advance, to determine if they have specific requirements, and to work out arrangements for shipping and shipping costs. Please understand that not every museum will accept every bird. There is a cost associated with this process, and the specimen may not be of sufficient interest to warrant the cost. This does not mean that you shouldn't contact the museum again. You should!