Guidelines for Use of Fishes in Field Research

American Society of Ichthyologists and Herpetologists (ASIH)
American Fisheries Society (AFS)
American Institute of Fishery Research Biologists (AIFRB)

Preface

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The "Guidelines for Use of Fishes in Field Research" that are reprinted here serve two purposes. They remind all scientists and managers conducting field studies that respect for life in all forms is a fundamental characteristic of and obligation for all members of our profession. Secondly, they provide guidance to Institutional Animal Care and Use Committees (IACUCs) in those institutions and agencies where such committees are required.

Conflicts concerning the appropriate use and treatment of fishes, and other animals, have their roots in disparate ethical beliefs. I suggest that our Society's position should be that professional organizations and governmental agencies deal with disputes concerning animal ethics/animal rights/animal welfare as differences among humans with conflicting beliefs, not as human "rights" vs. animal "rights." All animals "use" other forms of life in various ways. "Rights" are human concepts that cannot be applied uniformly and absolutely to all other forms of life. The basic tenets of conservation caution us to use all resources wisely and with respect. The "Guidelines for Use of Fishes in Field Research" suggest procedures for use of fishes that are founded on basic conservation ethics, common sense, and good research design.

The need for field research guidelines resulted from the 1985 amendment to the Animal Care Act that extended the principles of laboratory animal care to field research. All of the regulatory apparatus was already in place, with special emphasis on a few mammals. When the words "Field and" were added to the legislation, coverage became vastly greater (even for mammals). The existing laboratory care regulations were unworkable for field research. The National Science Foundation then requested assistance from professional organizations in developing field guidelines for use by IACUCs.

The role and authority of IACUCs are very familiar to AFS members in academia, but to others they may be unknown. Fear of the unknown, specifically another intrusion into the way researchers and managers do business has stimulated many questions. The material that follows provides a brief review of IACUCs and their role concerning field research on fishes. Research proposals submitted to the National Science Foundation, the National Institutes of Health, and the Public Health Service must be approved by the IACUC of the sponsoring institution prior to submission to the funding agency. The exact composition of IACUCs varies from institution to institution; however, a veterinarian must be included. IACUCs also vary in their philosophical makeup. As a result, standards for animal care and use have varied considerably from one institution to another. Few IACUCs have had members familiar with field research on fishes. The "Guidelines for the Use of Fishes in Field Research" provides IACUCs with general standards and alerts them to many of the special characteristics of fish and field research that require guidelines different from those developed for mammals and birds used in laboratory research. There is no requirement for IACUCs in agencies or institutions that do not receive funding from the National Science Foundation, the National Institutes of Health, or the Public Health Service. I believe the American Fisheries Society should resist strenuously any attempt to require IACUC approval for field activities of state or federal resource management agencies or as a prerequisite for receipt of federal aid funds.

The format and content of the "Guidelines For Use of Fishes in Field Research" provide as much consistency as possible with guidelines for the use of other vertebrate animals in field and laboratory situations. The present version has undergone substantial review and modification by committees of the American Society of Ichthyologists and Herpetologists, the American Institute of Fishery Research Biologists, and a committee representing the AFS Sections most concerned with the issue. The result is a set of guidelines that do not credit fishes with human emotions and value systems, but do provide reasonable guidance for scientists and managers conducting field research on fishes. The comments and suggestions of all AFS members for improvements in future versions of these guidelines are requested.

Introduction

Respect for all forms and systems of life is an inherent characteristic of scientists and managers who conduct field research on fishes. Consistent with our long standing interests in conservation, education, research, and the general well-being of fishes, the ASIH, AFS, and AIFRB support the following guidelines and principles for scientists conducting field research on these animals. As professional scientists specializing in fish biology concerned with the welfare of our study animals, we recognize that guidelines for the laboratory care and use of domesticated stocks of fishes are often not applicable to wild-caught fishes, and in fact may be impossible to apply without endangering the well-being of these fishes. Laboratory guidelines may also preclude techniques or types of investigations known to have minimal adverse effects on individuals or populations (1,2,3), and which are necessary for the acquisition of new knowledge.

The respectful treatment of wild fishes in field research is both an ethical and a scientific necessity. Traumatized animals may exhibit abnormal physiological, behavioral, and ecological responses that defeat the purposes of the investigation. For example, animals that are captured, marked and released must be able to resume their normal activities in an essentially undisturbed habitat if the purposes of the research are to be fulfilled.

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

Because of the very considerable range of adaptive diversity represented by the over 20,000 species of fishes, no concise or specific compendium of approved methods for field research is practical or desirable. Rather, the guidelines presented below build on the most current information to advise the investigator, who will often be an authority on the biology of the species under study, as to techniques that are known to be appropriate and effective in the conduct of field research. Ultimate responsibility for the ethical and scientific validity of an investigation and the methods employed must rest with the investigator. To those who adhere to the principles of careful field research these guidelines will simply be a formal statement of precautions already in place.

General Considerations

Research proposals may require approval of an IACUC (see below). In situations requiring such approval, each investigator must provide written assurance in applications and proposals that field research with fishes will meet the following requirements.

a. The living conditions of animals held in captivity at field sites will be appropriate for fishes and contribute to their health and well-being. The housing, feeding, and nonmedical care of the animals will be directed by a scientist (generally the investigator) trained and experienced in the proper care, handling, and use of the fishes being maintained or studied. Some experiments (e.g., competition studies) will require the housing of mixed species in the same enclosure. Mixed housing is also appropriate for holding or displaying certain species.
b. Procedures with animals must avoid or minimize distress to fishes, consistent with sound research design.
c. Procedures that may cause more than momentary or slight distress to the animals should be performed with appropriate sedation, analgesia, or anesthesia, except when justified for scientific reasons in writing by the investigator.
d. Fishes that would otherwise experience severe or chronic distress that cannot be relieved will be euthanized at the end of the procedure, or, if appropriate, during the procedure.
e. Methods of euthanasia will be consistent with the rationale behind the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (4), but fishes differ sufficiently that their specific techniques do not apply. The method listed by the Royal Society (5) may be followed.

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

f. 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. Investigators must uphold not only the letter but also the spirit of regulations. [Most applicable regulations are referenced in publications of the Association of Systematics Collections (6,7,8)] Researchers working outside the United States should ensure that they comply with all wildlife regulations of the country in which the research is being performed. Work with many species is regulated by the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (see "CITES" references in 6,7). Regulations affecting a single species may vary with country. Local regulations may also apply.
g. Individuals of endangered or threatened taxa should neither be removed from the wild (except in collaboration with conservation efforts), nor imported or exported, except in compliance with applicable regulations.
h. Investigators must be familiar with the fishes to be studied and their response to disturbance, sensitivity to capture and restraint and, if necessary, requirements for captive maintenance to the extent that these factors are known and applicable to a particular study
i. Taxa chosen should be well-suited to answer the research question(s) posed.
j. Every effort should be made prior to removal of fishes (if any) to understand the population status (abundant, threatened, rare, etc.) of the taxa to be studied, and the

numbers of animals removed from the wild must be kept to the minimum the investigator determines is necessary to accomplish the goals of the study. This statement should not be interpreted as proscribing study and/or collection of uncommon species. Indeed, collection for scientific study is crucial to understanding why a species is uncommonly observed.

3. The number of specimens required for an investigation will vary greatly, depending upon the questions being explored. As discussed later in these guidelines, certain kinds of investigations require collection of relatively large numbers of specimens, although the actual percent of any population taken will generally be very small. Studies should use the fewest animals necessary to reliably answer the questions posed. Use of adequate numbers to assure reliability is essential, as studies based on insufficient numbers of fishes will ultimately require repetition, thus wasting any benefit derived from any animal distress necessarily incurred during the study.

Numerous publications exist that will assist investigators and animal care committees in implementing these general guidelines; a number of such journals, monographs, etc. are listed in Appendix A.

Role of the Institutional Animal Care and Use Committee (IACUC)

Field resources for the care and use of fishes are very different from laboratory resources, and the role of the IACUC necessarily is limited to considerations that are practical for implementation at locations where field research is to be conducted. Prevailing conditions may prevent investigators from following these guidelines to the letter at all times. Investigators must, however, make every effort to follow the spirit of these guidelines to every extent possible. The omission from these guidelines of a specific research or husbandry technique must not be interpreted as proscription of the technique.

The IACUC must be aware that while fishes typically used in laboratory research represent a small number of species with well understood husbandry requirements, the classes Agnatha, Chondrichthyes, and Osteichthyes contain at least 20,000 distinct species with very diverse and often poorly known behavioral, physiological, and ecological characteristics. Therefore, ". . . in most cases, it is impossible to generate specific guidelines for groups larger than a few closely related species. Indeed, the premature stipulation of specific guidelines would severely inhibit humane care as well as research" (9). The IACUC must note the frequent use of the word "should" throughout these guidelines, and be aware that this is in deliberate recognition of the diversity of animals and situations covered by the guidelines. Investigators, on the other hand, must be aware that use of the word "should" denotes the ethical obligation to follow these guidelines when realistically possible.

Before approving applications and proposals or proposed significant changes in ongoing activities, the IACUC shall conduct a review of those sections related to the care and use of fishes and determine that the proposed activities are in accord with these guidelines, or that justification for a departure from these guidelines for scientific reasons is presented.

When field studies on wild vertebrates are to be reviewed, the IACUC must include personnel who can provide an understanding of the nature and impact of the proposed field investigation, the housing of the species to be studied, and knowledge concerning the risks associated with maintaining certain species of wild vertebrates in captivity. Each IACUC should therefore include at least one institution-appointed member who is experienced in zoological field investigations. Such personnel may be appointed to the committee on an ad hoc basis to provide necessary expertise. When sufficient personnel with the necessary expertise in this area are not available within an institution, this ad hoc representative may be a qualified member from another institution.

Field research on native fishes usually requires permits from state and/or federal wildlife agencies. These agencies review applications for their scientific merit and their potential impact on native populations, and issue permits that authorize the taking of specified numbers of individuals, the taxa and methods allowed, the period of study, and often other restrictions that are designed to minimize the likelihood that an investigation will have deleterious effects. Permission to conduct field research rests with these agencies by law, and the IACUC should seek to avoid infringement on their authority to control the use of wildlife species.

If manipulation of parameters of the natural environment (daylength, etc.) is not part of the research protocol, field housing for fishes being held for an extended period of time should approximate natural conditions as closely as possible while adhering to appropriate standards of care (10,11). Housing and maintenance should provide for the safety and well-being of the animal, while adequately allowing for the objective of the study.

An increasing body of knowledge (e.g., 12) indicates that pain perception of the many species of vertebrates is not uniform over the various homologous portions of their bodies. Therefore, broad extrapolation of pain perception across taxonomic lines must be avoided. For example, what causes pain and distress to a mammal does not cause an equivalent reaction in a fish (13).

Field Activities With Wild Fishes

1. Collecting

Field research with fishes frequently involves capture of specimens, whether for preservation, data recording, marking, temporary confinement, or relocation. While certain of these activities are treated separately below, they form a continuum of potential field uses of fishes.

The collection of samples for museum preservation from natural populations is critical to: (1) understanding the biology of animals throughout their ranges and over time; (2) the recording of biotic diversity, over time and/or in different habitats; and (3) the establishment and maintenance of taxonomic reference material essential to understanding the evolution and phylogenetic relationships of fishes and for environmental impact studies. The number of specimens collected should be kept at the minimum the investigator determines necessary to accomplish the goal of a study. Some studies, e.g., diversity over geographic range

fish species, particularly with internally implanted transmitters.

Researchers intending to use radiotelemetry on fish species should consider the following guidelines and comments:

a. *Force-fed and Implanted Transmitters:* Force-fed packages should be small enough to pass through the gut without obstructing the passage of food. Force-fed or implanted packages should be coated with an impervious, biologically inert coating. Residence time of up to several days in the gut is generally long enough to provide useful information on movement and body temperature.

Implanted transmitters should not interfere with the function of the organs surrounding them or with the fish's normal behavior. For intracoelomic or subcutaneous implants, the transmitter package may have to be sutured in place to prevent its movement or interference with vital organs.

b. *Externally Attached Transmitters:* Consideration must be given to the effect of an externally attached transmitter package on behavioral interactions between tagged fishes and other individuals. For example, the transmitter should neither conceal nor enhance the appearance of dorsal fins or opercular flaps. Transmitters should be shaped and attached so as to eliminate or minimize the risk of entanglement with underwater vegetation or other obstructions.

Most fishes continue to grow throughout life. External transmitters should be removed or designed to be lost after a time, or they may constrict or irritate the animals. Special consideration must be given to soft-skinned species to prevent abrasion.

Radioisotopes

The use of radioisotopes as markers in natural systems is very valuable, and may be the only means of adequately gathering data on movements of very small species; the technique, however, should be undertaken with caution. Special training and precautions are required of researchers by federal, and frequently state law (8). A license, which specifies safety procedures for laboratory use, is required for release of isotopes into natural systems and for disposal of waste material. The pros and cons of using strong emitters must be assessed in terms of possible deleterious effects on the animal, to predators that might ingest isotope-labelled animals, and potential hazard to the public.

When marking with radioisotopes, the animal does not have to be handled for identification, several individuals can be monitored rather quickly, the label is easy to apply, and it can be useful for a limited time if desired. Strong emitters, however, cause extensive tissue necrosis at the implant site, and even weaker ones carry the chance for induction of mutations that may compromise future genetic studies of these populations.

Housing and Maintenance at Field Sites

Because the biological needs of each species and the nature of individual projects vary widely, only the most general recommendations on housing wild vertebrates in the field can be made. When dealing with unfamiliar species, testing and comparing several methods of housing to find the method most appropriate for the needs of the animal and the purposes of the study may be necessary. *Restraint and ease of maintenance by animal keepers should not be the prime determinants of housing conditions, though these are certainly important considerations.*

Normal field maintenance should incorporate, as far as possible, those aspects of the natural habitat deemed important to the survival and well-being of the animal. Adequacy of maintenance can be judged, relative to the natural environment, by monitoring a combination of factors such as changes in growth and weight, survival rates, breeding success, activity levels, general behavior, and appearance (29). Nutritionally balanced diets should be provided or natural foods should be duplicated as closely as possible. Natural light and temperature conditions should be followed unless alteration of these are factors under investigation.

Frequency of aquarium cleaning should represent a compromise between the level of cleanliness necessary to prevent disease (30,31,32), and the amount of distress imposed by frequent handling and exposure to unfamiliar surroundings. Applied knowledge of animal ethology can assist the investigator in providing optimum care and housing.

Disposition Following Studies

Upon completion of studies, researchers should release wild-caught specimens whenever this is practical and ecologically appropriate. Exceptions are: if national, state, or local laws prohibit release, or if release might be detrimental to the well-being of the existing gene pools of native fishes in a specific geographic area.

As a general rule, field captured fishes should be released only:

a. At the site of the original capture, unless conservation efforts or safety considerations dictate otherwise. Release should never be made beyond the native range of distribution of a fish without prior approval of the appropriate state and/or federal agencies, and approved relocations should be noted in subsequent publication of research results.
b. If their ability to survive in nature has not been irreversibly impaired.
c. Where it can be reasonably expected that the released animal will function normally within the population.
d. When local and seasonal conditions are conducive to survival.
e. When release is not likely to spread pathogens.

Captured animals that cannot be released or are not native to the site of intended release should be properly disposed of, either by distribution to colleagues for further study, or if possible by preservation and deposition as teaching or voucher specimens in research collections.

In both the field and laboratory, the investigator must be careful to ensure that animals subjected to an euthanasia procedure are dead before disposal. In those rare instances where specimens are unacceptable for deposition as vouchers or teaching purposes, disposal of carcasses must be in accordance with acceptable practices as required by appli-

Hazardous Species

Sharks and other large or venomous fishes are potentially dangerous to the investigator, and thus require special methods of restraint that must involve a compromise between potential injury to the handlers and injurious restraint of the animal. The particular method chosen will vary with the species and purpose of the project. Adherence to the following general guidelines is recommended when working with hazardous fishes:

a. Procedures chosen should minimize the amount of handling time required and reduce or eliminate contact between handler and animal.
b. One should never work alone. A second person, knowledgeable in capture and handling techniques and emergency measures, should be present at all times.
c. Prior consultation with workers experienced with these species, as well as a review of the relevant literature, is of particular importance since much of the information on handling dangerous species has not been published, but is simply passed from one investigator to another.

Prolonged distressful restraint should be avoided. In some cases, utilization of general anesthesia for restraint in the field may be advisable. If so, the anesthetic chosen should be a low risk compound that permits rapid return to normal physiological and behavioral status, and the animal must be kept under observation until appropriate recovery occurs. The relatively unpredictable response of some poikilotherms to immobilants or anesthetics under field conditions may contraindicate field use of these chemicals under certain conditions.

Chemical Restraint

Many chemicals used for restraint or immobilization of fishes are controlled by the Federal Bureau of Narcotics and Dangerous Drugs/Drug Enforcement Administration (DEA). A DEA permit is required for purchase or use of these chemicals. Extensive information on these substances and their use is available (22,23), and permit application procedures are available from regional DEA offices. Investigators should choose the chemical for immobilization with consideration of the impacts of that chemical on the target organism.

The potent drugs available for wildlife immobilization when properly used are, with the exception of succinylcholine, safe for target animals but can be extremely dangerous if accidentally administered to humans. The degree of danger varies according to the drug, and users must be aware of the appropriate action to take in the event of accident (9).

3. Animal Marking

Fish marking, by a variety of techniques, provides one of the most important methods of analyzing fish movements, abundance, and population dynamics (cf. 24). It is basic to all field studies. Important considerations in choosing a marking technique are its effect on behavior, physiology and survival of the target species or a close relative. Investigators must consider the nature and duration of restraint, the amount of tissue affected, whether distress is momentary or prolonged, whether the animal, after marking, will be at greater than normal risk, whether the animal's desirability as a mate is reduced, and whether the risk of infection or abscess formation is minimal. Careful testing of markers on preserved or captive animals before use on wild animals may reveal potential problems and is recommended. Marking techniques for fishes have been extensively reviewed (25) and are summarized below.

Fin-clipping is relatively easy, may have minimal impact on survival and social structure of the marked fish, and is a recommended procedure for many studies. Fins used for clipping or removal would depend upon the species selected, i.e., clipping of the anal fin of poeciliid males would be inappropriate, but removal of the adipose fin of a salmonid would have negligible impact. The importance of fins to the survival and well-being of fishes varies so widely that specific guidelines are not possible.

Marking techniques involving tissue removal or modification (branding, etc.) should be preceded by local anesthetic (aerosols containing benzocaine, such as Cetacaine, may be applied) and followed by the application of topical antiseptic. Chilling of fishes prior to marking may be effective for immobilization.

Electrocauterization of a number, letter, or pattern on the skin, in which deep layers of skin are cauterized to prevent regeneration, provides a marking system that, if performed properly, heals rapidly and seldom becomes infected. Brand marks typically, however, are not visible in captive fishes after a few months. Freeze branding is often the preferred branding technique.

Tattooing and acrylic paint injections have been used with success on fishes. Two potential problems that must be resolved prior to marking are: (1) the selection of a dye which will be visible against the pigmentation of the skin, and (2) the loss of legibility due to diffusion or ultraviolet degradation of the dye.

Tagging is perhaps the most widely used and best investigated means of fish marking. Several logical constraints should be considered in planning any tagging program. Tags that cause projections from the body could produce physical impairment and enhance the risk of entanglement in underwater vegetation. Brightly colored tags may compromise a fish's camouflage. The size, shape, and placement of tags should permit normal behavior of the animal to the greatest extent possible.

Radiotelemetry

Radiotelemetry is a specialized form of animal marking, and the same general procedures apply. Underwater telemetry, however, is primarily limited to acoustic rather than radio frequency transmission. Radio transmission is only practical in freshwater and at relatively shallow depths. Radio transmission is regulated by the Federal Communications Commission, and investigators should inquire about availability of frequencies they plan to use. General telemetry techniques are summarized by Mackay (26), Amlaner and MacDonald (27), and Stasko and Pincock (28).

Many fishes are unsuitable for radiotelemetric studies because of their small size and habit of living in confined spaces. Component miniaturization will undoubtedly facilitate the future use of radiotelemetry in studies of small

or delineation of variation of new species, require relatively large samples.

Capture Techniques

Capture techniques should be as environmentally benevolent as possible within the constraints of the sampling design (14,15). Whenever feasible, the potential for return to the natural environment must be incorporated into the sampling design. Current literature should be reviewed to ascertain when and if capture distress has been properly documented. Those capture techniques (seines, traps, etc.) that have minimal impact on the target fishes are not discussed below. Many capture techniques must mimic those of commercial and recreational fishermen in order to obtain reliable data on population trends for the regulation of such fisheries.

Gill netting (15,16) and other forms of entangling nets are an accepted practice in fish collecting. Many studies contrast recent and prior sampling and thus repetition of a prior technique is mandated for sampling reliability. Net sets should be examined at a regular and appropriate schedule, particularly in warm water, to avoid excessive net mortality.

Collecting fish using ichthyocides is often the only and by far the most efficient sampling technique (cf. 17). Use of ichthyocides should be accomplished with maximal consideration of physical factors such as water movement and temperature, so as to avoid extensive mortality of natural populations and nontarget species.

Electrofishing is a suitable sampling technique in water of appropriate conductivity inasmuch as fish mortalities will be minimal. Proper adjustment of current will stun fishes and complete recovery is possible. Fish can be returned with minimal adverse impact. Care must be exercised to avoid excessive electric currents that may injure or harm the operators as well as the fish.

Capture of fishes by hooks or spears is an accepted practice of recreational fishermen. Spearfishing is appropriate to cases in which capture in special environments is necessary, e.g., deep reefs, caves, kelp beds, etc., and to provide comparable data for recreational fishing statistics. Similarly, many fishes are most efficiently captured by hooks.

Museum Specimens and Other Killed Specimens

The collection of live animals and their preparation as museum specimens is necessary for research and teaching activities in systematic zoology and for many other types of studies. Such collections should further our understanding of these animals in their natural state. Descriptions of ichthylogical collecting techniques and accepted practices of collection management have been compiled (18,19), as have references to field techniques. Whenever fishes are collected for museum deposition, specimens should be fixed and preserved so as to assure the maximum utility of each animal and to minimize the need for duplicate collecting. In principle, each animal collected should serve as a source of information on many levels of organization from behavior to DNA-sequencing. Whenever practical, for example, blood and other tissues should be collected for karyotypic and molecular study prior to formalin fixation of the specimen (20).

Formalin fixation of specimens is an acceptable practice; however, fishes that do not die rapidly following immersion in a formalin solution should be killed before preservation by means of a chemical anesthetic such as sodium pentobarbital, hydrous chlorobutanol, MS-222, urethane or similarly acting substances, unless justified in writing by the investigator. When field fixation of formalin resistant fishes without prior introduction of anesthetics is necessary, prior numbing of the specimen in ice water should be considered. Several kinds of anesthetics and their efficacy have been reviewed in the Investigations in Fish Control series (21). Their use requires little additional time and effort and adds little to the bulk or weight of collecting equipment. Urethane has been shown to be carcinogenic; thus, caution should be observed with its use and field disposal.

Live Capture

Investigators should be familiar with the variety of ichthyological capture techniques and should choose a method suited to both the species and the study. Capture techniques should prevent or minimize injury to the animal. Care should be exercised to avoid accidental capture or insure field release of nontarget species. The interval between visits to traps and net sets should be as short as possible, although it may vary with species, weather, objectives of the study, and the type of trap or net.

Habitat and Population Considerations

Whether collecting for future release or for museum preparation, each investigator should observe and pass on to students a strict ethic of habitat conservation. Collecting always should be conducted so as to leave the habitat as undisturbed as possible. The collection of large series of animals from breeding aggregations should be avoided if possible. Systematists should be familiar with extant collections of suitable specimens before conducting field work. If the purpose of an experiment is to alter behavior, reproductive potential, or survivability, the interference should be no more than that determined by the investigator to accurately test the hypothesis.

2. Restraint and Handling

General Principles

Restraint of wild fishes ranges from confinement in an aquarium through various types of physical restrictions or drug-induced immobilization. The decision whether to use physical or chemical restraint should be based upon the design of the experiment, knowledge of behavior of the animals, and the availability of facilities. Investigators must use the least amount of restraint necessary to do the job. When not under study, aggressive species should not be confined with other animals (other than food) which they may injure or may injure them. The well-being of the animal under study is of paramount importance, and we emphasize that improper restraint, especially of traumatized animals, can lead to major physiological disturbances that can result in any of a series of deleterious or even fatal consequences.

Animals should be handled quietly and with the minimum personnel necessary. Darkened conditions tend to alleviate stress and subdue certain species, and are recommended whenever possible and appropriate.

cable regulations. Animals containing toxic substances or drugs (including euthanasia agents like T-61) must not be disposed of in areas where they may become part of the natural food web.

Preparation and Revisions of These Guidelines

The initial draft of these guidelines was prepared by Clark Hubbs (ASIH), John G. Nickum (AFS), and John R. Hunter (AIFRB). The final product represents the collective efforts of over 100 persons and the societies extend sincere thanks to all participants.

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

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