Table of Contents

A. Background

The IACUC is responsible for the oversight of all research, testing, and teaching activities involving live vertebrate animals, including those taking place off-campus or at field sites (either local or remote) where the activity alters or influences (directly or indirectly) the activities of the animals being studied. In the case of field work or wildlife studies, protocols must provide sufficient information such that the IACUC can evaluate the location and nature of the field site(s), impact on study animals, risks to other wildlife or to the surrounding environment, as well as the occupational health considerations for the individuals participating in the research or teaching activity.

Excerpts of this guidance document were obtained with permission from the West Virginia University Office of Animal Welfare and the University of Maine Office of Research Compliance.

For questions related to regulatory or ethical considerations of your proposed research or teaching activity, please e-mail iacuc@une.edu for assistance.

B. Standards

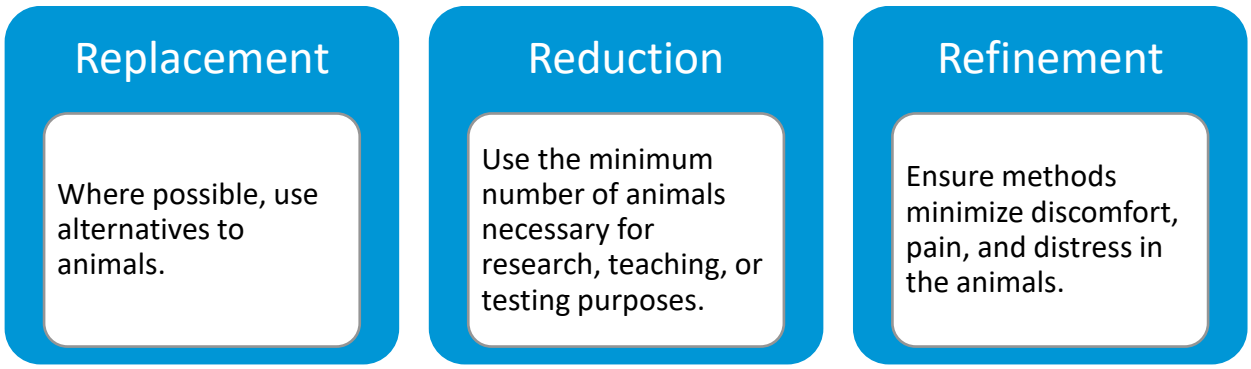
In some cases, professional societies have published guidelines for field work. *The Guide for Care and Use of Laboratory Animals* suggests these standards be used to assist in evaluation of animal use projects involving wildlife. Standards that may be used for the evaluation of field work or wildlife protocols include, but are not necessarily limited to, the following professional society guidelines:

- The Wildlife Techniques Manual (Wildlife Society)
- Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)
- Guidelines for the Use of Live Amphibians and Reptiles in Field Research (Herpetological Animal Care and Use Committee [HACC] of the American Society of Ichthyologists and Herpetologists, 2004)
- Guidelines to the Use of Wild Birds in Research (The Ornithological Council, 2010)
- Guidelines of the American Society of Mammologists for the use of wild mammals in research (American Society of Mammologists, 2011)
- AVMA Guidelines for the Euthanasia of Animals (2020 Edition)

C. IACUC Approval

In general, any wildlife protocol that involves activities that could affect the animals' well-being, environment, or behavior require IACUC review and approval.

The mitigation of pain and distress and the consideration of the 3Rs (reduction, refinement, replacement) are applicable to field work and wildlife studies as with all use of live vertebrate animals in research and teaching activities. Therefore, all activities should be developed with these considerations in mind.



Observational wildlife protocols may not require review and approval by the IACUC when certain conditions are met (see examples below). Requirements of specific funding agencies may supersede this exemption. Researchers/instructors should contact the IACUC (iacuc@une.edu) for assistance in making this determination.

- **No Direct Interaction with Animals**: If the project involves purely observation data collection where there is no physical contact, handling, or manipulation of the wildlife (e.g., no trapping, tagging, or otherwise interfering with the animals), the protocol might not require IACUC review.
- **Non-invasive Observations**: If the project only involves non-invasive methods, such as observing wildlife from a distance without causing harm or disturbance to the animals, and does not interfere with their natural behavior, it may not fall under IACUC jurisdiction.
- **No Risk to Animal Welfare**: If the project poses no risk to the welfare of animals, and the activities are unlikely to cause harm or stress (e.g., watching animals in their natural habitat without disturbing them), IACUC approval might not be necessary.

Additionally, not all activities involving the use of warm-blooded wild animals (e.g., birds, mammals) in their natural habitat are exempt from the mandates of the USDA Animal Welfare Act. Even if a field study is exempt from USDA oversight, an IACUC application may still be required for review and approval. Researchers/instructors should contact the IACUC (iacuc@une.edu) for assistance in making this determination.

Examples of activities that are **NOT** exempt from USDA oversight include:

1. A study that involves an invasive procedure, such as but not limited to:
    - Major operative procedures
    - Intra-cardiac blood collections
    - Arterial/venous cut downs for catheter placement
    - Surgical implantation of devices

2. A study that harms an animal such as instances where:

- The animal experiences pain/distress above minimal and slight (e.g., use of USDA pain category D or E procedures)
- The animal experiences trauma, overheating, excessive cooling, behavioral stress, physical harm, or unnecessary discomfort as a result of handling
- The animal experiences death as a result of the work, in a manner that does not meet the regulatory definition of "euthanasia"
- The animal experiences impaired function such as amputation of a tail/digit used for digging or climbing

3. Procedures that materially alter animal behavior include but are not limited to:
    - Use of hormones or pheromones to change mating or migration patterns for research purposes
    - Repeated nest/den disturbance during breeding and rearing of young
    - Relocation of migratory animals beyond natural migration routes

D. Occupational Health & Safety

Regardless of the requirement for an IACUC protocol, the researcher/instructor should be aware that they are responsible for the occupational health and safety of all individuals involved in the research or teaching activity, and for the assessment and communication of risks/hazards that may impact human health and safety. Please see Appendix A for an example risk assessment matrix.

Researchers/instructors should consider the following safety precautions when conducting field work or wildlife studies:

- Protect your skin by wearing appropriate clothing for the field activity
- Wear gloves appropriate for handling wild animals, tissues, animal waste, bodily fluids, or equipment that may be contaminated
- Refrain from eating, drinking, or applying makeup in the field unless you are able to thoroughly wash your hands prior to conducting these activities
- Use extra care when handling sharps and discard used sharps directly into an approved "sharps container" without recapping
- Thoroughly wash your hands as soon as possible after completing field activities
- Ensure all individuals are up-to-date on relevant vaccinations, such as those for tetanus and rabies, depending on the species and location of the field activity
- Have a first aid kit on hand and ensure that individuals are trained in basic first aid
- Have an emergency plan in place, including contact information for local medical facilities and

wildlife authorities

- Be aware of and prepared for potential hazards associated with field activities, such as venomous animals, pathogens from mosquitos and ticks, aggressive behavior, or weather conditions

E. Areas of Discussion

Location

A description of the location of the animal use activities should be given and include identification of any hazards associated with the area that individuals involved with the research or teaching activity should be aware of. The location may encompass areas used for capture or transport activities.

Capture and Restraint

Procedures for capture and restraint of wild animals should discuss the methods and equipment to be used, and any possible outcomes affecting the welfare of animals in the process. When addressing non-target effects of project activities, consider the types of other species that may also become entrapped, and how they might be dealt with. Researchers/instructors are responsible for maintaining detailed records of any non-target species that are captured or handled and reporting the numbers of these animals in the annual progress report to the IACUC.

In cases where animals are captured, great care should be used to ensure the animals are not injured (e.g., damage to plumage in birds) and are released in optimal condition (e.g., capable of exhibiting normal behaviors) under favorable environmental conditions, and at their original site of capture when possible.

Please also see the 'Minimization of Pain and Distress during Capture' section below for more information.

Special considerations for handling and restraining fish

Source: Adapted from the Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)

1. The choice of sampling method should be dictated by worker safety, research objectives, seasonal considerations, and the habitat to be sampled. Capture techniques should prevent or minimize injury and stress. Appropriately equipped live wells or tanks should be provided if fishes are to be kept for more than the time needed to collect essential metrics. Care should be taken to avoid accidental capture of nontarget species and to ensure release of incidentally collected individuals with minimal or no injury. Species that may be dangerous to workers due to size, anatomy, species-characteristic behavior, or capabilities require additional precautions.
2. Fish will exhibit some degree of stress response when handled. The method of handling fish vary with the species, the environment in which they are found, and the tradition and resources of a particular region or country. Stress responses can be reduced, however, by eliminating rough

handling, reducing the amount of time animals are handled, rapid temperature changes, sudden water quality changes, abrasion, and excessively tight confinement.

3. Inappropriate handling procedures can contribute to changes in blood profiles and substantial mortalities. Handling procedures must be designed to minimize the effects of stress and thereby reduce immediate and delayed losses.
4. Environmental conditions from which fish originated, or are held, should not be changed rapidly. This is especially true for temperature conditions. An instantaneous change of 2°C in water temperature generally is not lethal, but it can cause detectable stress responses. Tolerable changes depend on the species, the life history stage, previous thermal history, and the initial holding conditions. Effects due to previous thermal history have been detected for as long as a month post-treatment. Rapid, substantial changes in water quality also should be avoided as they can have rapid deleterious effects on health and survival, depending on the water quality parameter.
5. Prolong, stressful restraint should be avoided. In some case, general sedation for restraint may be advisable; however, the benefits of sedation and potential effects on data derived from sedated fishes might need to be compared to results obtained from fishes that have not been sedated. The full range of potential effects on the subject fish, not just the sedative qualities, must be considered.
6. The sedative chosen should be one that permits a rapid return to normal physiological and behavioral status and is a low-risk compound for humans, as well as fishes. The compound should be tested on a small sample of fish prior to widespread use. Sedated animals must be kept under observation until appropriate recovery occurs. Researchers should determine appropriate recovery procedures prior to administering sedation.
7. The following substances have been used by various investigators (some are controlled substances available only through appropriately licensed sources, such as veterinarians): benzocaine, clove oil, diazepam (valium), sodium pentobarbital, and tricaine methanesulfonate (MS-222). Hypothermia and exposure to sublethal levels of CO_2 have been used in situations where other sedatives were contraindicated. The only sedative approved by the FDA for general use on fishes is MS-222, but a 21-day withdrawal period is required before the treated fish may be released or consumed by humans. The AVMA guidelines should be consulted. Substance dosages should be carefully researched before beginning any procedure.

Release

Capture procedures should indicate how animals will be assessed for their ability to be re-released into their habitat and include a discussion of any long-term effects associated with the objective of capture. A justification should be given if the animals are to be released in a location different from where they were captured.

Special considerations for releasing wild fish held in the laboratory back into the wild

Source: Adapted from the Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)

1. Fish collected in the wild and brought into the laboratory for experimental or teaching purposes should never be returned to the environment according to the general consensus agencies granting scientific collecting permits.
2. After proper euthanization and preservation, study animals may be again useful as teaching or voucher specimens in research collections.
3. Federal, provincial, state, or local laws may prohibit release of study animals under any circumstance. Non-native fishes should never be released. In some cases, transfer to another research project or educational exercise may be an appropriate disposition, and permitting may be required in the case of federally listed specimens.

Tagging and Marking

The effect of any tagging or marking methods on the ability of the associated animals to maintain normal behavior in their environment should be addressed. It should also be determined if animals will be at greater than normal risk to predation, if their desirability as mate will be reduced, and if a risk of infection is increased substantially. Researchers/instructors should discuss the size, weight, and orientation of any type of attached equipment in relation to the natural movement and positions of the animal.

When tagging devices are used, researchers/instructors should provide information on the expected lifespan of the device. Understanding how long the device will function helps the IACUC to assess its potential impact on animal health, behavior, and welfare.

Special considerations for marking and tagging fish

Source: Adapted from the Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)

1. Identification of fishes by using naturally occurring or artificial tags or marks is often required for studies on age and growth, mortality rates (including natural and fishing-induced mortality), abundance, angler catch or harvest rates, habitat use and movement/migration, stock recognition, or stocking success.
2. Researchers/instructors can use both intrinsic and extrinsic identification systems, allowing the nature of the research or teaching activity to dictate the type of tag or mark employed. Integrated use of more than one tagging or marking technique helps ensure fish identification and is helpful in estimating tag loss rates.

3. Because techniques for tagging and marking fishes have been extensively reviewed and are constantly evolving, literature reviews should inform the researcher/instructor.
4. Multiple methods are available for generating artificial external marks on fishes. Alteration of fins or other body parts, in practice for over 100 years, can be accomplished by clipping or hole punching. The selection of fins for clipping or removal is dependent upon the species under study.
5. Hot or cold branding, the process of marking by placing an apparatus (e.g., liquid nitrogen brand) against the body for a few seconds, may be an effective marking technique in specific situations and does not cause substantial injury to underlying tissues. Fishes should be anesthetized prior to branding.
6. External tags commonly applied to fishes include dart and t-bar anchor tags, disc tags, Carlin tags, and spaghetti or loop tags. Dart and anchor tags are the most frequently used external tags, but a high loss rate has been reported in some species. Proper insertion and use of small tags relative to fish size can reduce the potential for fish injury and tag loss.
7. External colorants for marking fishes include dyes, stains, inks, paints, liquid latex, visible implant elastomers, and plastics that are administered by immersion, spraying, injection, or tattooing. Care is needed for distinguishing external colorant marks of similar tones.
8. Implanted coded wire tags, radio and acoustic telemetry transmitters, archival biologgers, passive integrated transponder (PIT) tags, visible implanted alpha numeric tags, otolith marks, and natural parasites are internal marking systems used to identify fish.
9. The selection of a tag or transmitter and the method and site of attachment or implantation is to be appropriate for the species and size of fish and performed by trained personnel. Surgical implantation of transmitters into the coelom is common with free-ranging fishes.
10. The effects of marking on fishes depend on the physical condition of the fish at the time of release. Occurrence of injury is species and size specific, and smaller fishes may be more susceptible. Minor wounds caused by most tagging and marking procedures typically heal satisfactorily without treatment with antibiotics.
11. Researchers should consider using external tags on fish to inform and/or deter fisherman (recreational or scientific) from collecting fish, depending on the goal of the research study. This is especially important for fish with internal tags, as it may not be obvious they are part of a research study.

Transportation

Protection and environmental considerations for transport of any animals should be indicated. Any zoonotic pathogens or other hazards for animals and individuals associated with transportation activity should also be discussed.

Special considerations for transporting fish

Source: Adapted from the Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)

1. Fish will exhibit some degree of stress response when transported. Stress responses can be reduced, however, by eliminating rough handling, reducing the length of time fish are handled/captured/transported, rapid temperature changes, sudden water quality changes, abrasion, and excessively tight confinement. Transport procedures must be designed to minimize the effects of stress and thereby reduce immediate and delayed losses.
2. Proper equipment for transport should be used. Transport tanks should be well constructed and should be disinfected before use.
3. Actively monitoring and maintaining acceptable ranges of dissolved oxygen, carbon dioxide, temperature, ammonia, and pH during transport is essential.
4. Fish can be transferred between capture and transport units, or between transport units and holding units, by wet or dry transfer methods. Wet transfer usually results in less stress than dry transfer, where the net is used alone. Ideally, fishes should be allowed to recover in the same or similar medium used for transport.
5. The length of recovery may vary depending upon conditions, the amount of handling, and research objectives, but 72 hours typically is considered a minimum following extensive handling. The species and life stage of the fish will also affect recovery periods.

Housing

Any field work or wildlife study that requires animals to be held for greater than 12 hours requires a discussion of husbandry considerations in the protocol. The USDA considers warm-blooded wild animals used in category D or E procedures to be subject to the requirements of the USDA Animal Welfare Act. For these animals the IACUC must review husbandry information when they are held for longer than 12 hours. Researchers/instructors are encouraged to contact the IACUC prior to submission of protocols involving husbandry activities or category D or E procedures.

Special considerations for housing fish

Source: Adapted from the Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)

1. Testing and comparing several methods of housing may be necessary in order to find the most appropriate for the needs of the species and the purpose(s) of the study. Ease of maintenance by animal keepers, though important, should not be the prime determinants of housing conditions; however, such ease generally ensures greater compliance with established maintenance protocols. Researchers should aim to use housing environments or tanks with round edges, as

sharp corners may lead to changes in behavior or cause injury.

2. Normal field maintenance facilities should incorporate those aspects of the natural habitat deemed important to the survival and well-being of the animal. Adequacy of the maintenance facility can be monitored by observing changes in animal growth and weight, survival rates, activity levels, general behavior, and appearance.
3. Nutritionally balanced diets should be provided, or natural foods should be duplicated as closely as possible.
4. Natural light and temperature conditions should be followed unless alteration of these factors is under investigation for achieving a desired effect (e.g., spawning cycle manipulation). Fish species have optimal thermal regimes, and the immune system functions best within such ranges.
5. Frequency of tank cleaning should represent a compromise between the levels of cleanliness necessary to prevent disease and the amount of stress imposed by frequent handling.
6. As with other containment systems, the holding tank needs to allow for the stocking density or the relation of fish biomass to available water volume. Water inflow and turnover rate must be considered because sufficient water exchanges are needed for good water quality. Oxygen available in the incoming water needs to exceed the metabolic oxygen consumption by fishes in the tank. Sufficient aeration can be supplied by compressed air, injected or bottled oxygen, or agitation.
7. Providing water of appropriate physical and chemical quality is probably the single most important factor for the care and maintenance of captive fishes. Inasmuch as each of the 25,000+ species of fishes has its own optimum conditions and limits of tolerance; the investigator is responsible for determining the preferred conditions for the species under study. Transferring fishes into water having a temperature outside their limits of tolerance, or in excess of their capacity to adapt, can lead to death, either immediately or delayed, usually within 72 hours. Sudden changes in water temperatures as small as 2°C can cause stress responses in fishes that are otherwise healthy. Experienced investigators do not routinely expose fishes to temperature changes greater than 2°C per day. Limits of tolerance and ability to tolerate temperature changes are influenced by the previous thermal histories of individual fish as well as species characteristics.
8. Excess noise and vibrations should be avoided because such factors can produce acute or chronic stress response in fish.
9. Before introducing fish into a laboratory, appropriate facilities and plans should be established to prevent the fish from escaping, particularly species that are not native to the watershed, and to ensure that the introduced fish can be physically separated from those already present. Each holding unit should have its own set of nets and other necessary equipment. Facilities and equipment used for previous studies should be disinfected prior to use in new studies, typically with a chlorinated disinfectant or another disinfectant such as Virkon®Aquatic. If the introduced fishes may carry disease agents, especially pathogens or parasites that are not endemic to the

area, quarantine-level facilities should be used. The level of quarantine required will vary with the seriousness of the known or suspected disease agent. Individual fish with suspected ill health should be quarantined from the others so as to negate the potential spread of disease agents. Health screening reports may be required from the source of the animals, depending on the species and procurement method.

10. Before studies begin, fishes should be given time to acclimate to new environments, feeds, and routine activities. Slow acclimation is often critical. It is not uncommon for fishes to exhibit acute health problems 48-72 hours following transfer. The time used for acclimation within and between experiments should be standard and specific for a species. Preliminary studies may be needed to establish the most appropriate time to be used during individual studies. A commonly used acclimation period is 1-2 weeks.

Habitat Manipulation

Manipulation of wildlife habitats can have significant effects on the population of the species of interest, as well as other associated species living in that habitat. Researchers/instructors should consider both when describing the expected impact of such manipulations. This would include impact to mating and breeding behaviors, changes in food supply or nesting sites, and the predator-prey relationships established in these areas.

Field Pathogen Reduction

Researchers/instructors should recognize that research or teaching activities can create an increased possibility of spreading pathogens to humans, between wild animals, and between wild animal populations which might otherwise not come into contact. Researchers/instructors should indicate the materials and methods used to mitigate the spread of disease including the decontamination of equipment. Researchers/instructors should sterilize surgical equipment in accordance with professional society standards and employ decontamination procedures between individual animals in processing.

Employing common sense and using appropriate personal protective equipment (PPE) when working with wild animals will reduce the potential for exposure to zoonotic pathogens that the animals might carry.

Post Approval Monitoring

Researchers/instructors must report any animal welfare concerns associated with their approved work, including unanticipated mortality (this applies to both target and non-target species).

Researchers/instructors should document and maintain records of surgical procedures, as well as assess the animals' abilities both before they are released and after they are captured. These records should be made available to the IACUC when requested.

Research and teaching activities that involve live vertebrate animals may be subject to post-approval monitoring by the IACUC to ensure activities are conducted ethically and in accordance with approved

protocols. Post-approval monitoring activities for a field work or wildlife studies may include:

- Conduct scheduled visits to field sites to observe research or teaching activities, habitat conditions, and ensure compliance with the approved protocol (e.g., observe animal capture, handling, tagging, release, sampling, or surgical procedures)

  Note: The IACUC may ask researchers/instructors to submit videos, photographs, or written descriptions to facilitate post-approval monitoring when field work or wildlife studies are conducted in remote areas.

- Inspect traps, nets, and other capture devices to ensure they are appropriate, safe, and used correctly, minimizing stress and injury to animals
- Examine field logs, capture records, and animal handling notes to ensure detailed and accurate documentation of activities, including any animal health or welfare issues encountered
- Verify that all necessary wildlife permits and licenses are up-to-date and align with local, state, and federal regulations
- Ensure that all field personnel are properly trained and understand protocol requirements, safety protocols, and techniques to minimize stress and harm to animals

Licenses & Permits

Researchers/instructors are responsible for understanding and complying with all local, state, and federal legal restrictions related to their projects. They must inform the IACUC that they have secured all necessary permissions before beginning the activity and submit copies of all licenses/permits, including any future updates, amendments or renewals. It is important to consider not only the species directly involved in the project but also any related species that may be impacted.

Researchers/instructors must identify all required permits or other forms of written authorization including protected species permits at the national and state or provincial levels *(in the U.S.: Migratory Bird Treaty Act, Endangered Species Act, CITES, Marine Mammal Protection Act, and Wild Bird Conservation Act; Lacey Act; state permits for state-listed species)*; national and state/provincial protected areas permits *(in the U.S.: National Wildlife Refuge System, National Parks, National Forest System, Bureau of Land Management; state permits for wildlife management areas, parks, or other protected areas)*.

Researchers/instructors are required to maintain a valid license/permit for the duration of their research or teaching activity when working with wild animals. If the license/permit lapses or is revoked, the researcher/instructor is not legally authorized to engage in the activities covered by that license/permit. The research or teaching activity involving wild animals must cease immediately if the license/permit is no longer valid, unless an extension or renewal is obtained. Continuing activities without a valid license/permit can result in serious consequences, including:

- Violations of federal, state, or local laws governing wildlife protection and conservation

- Legal repercussions such as fines, penalties, or suspension of future licenses or permits
- Institutional consequences, including potential sanctions or loss of institutional support for future research or teaching activities
- Damage to the reputation of the institution or the researcher/instructor's professional standing

Selection of Drugs and Substances

Beyond the specific and prolonged effects of the drugs used as part of research or teaching activities, researchers/instructors need to also consider the possibility of drug effects when animals fall victim to predators or become carrion for scavengers. Use of chemicals in fish, deer, and other species of interest to sportsman, needs to be carefully planned.

Minimization of Pain and Distress during Capture

1. When animals are held in traps, they should not be held any longer than necessary, but not to exceed 12 hours. During trap placements and processing, if the traps are not directly observed at all times, adequate provisioning for high radiant exposures (out of the sun; work during the cool periods of the day); flooding (proper location or floatation use) and freezing temperatures (adequate bedding and food if below 0°C), should be ensured.
2. Leg-hold traps should only be used under special circumstances. Leg-hold traps should ideally be monitored continuously, have padded jaws to prevent unnecessary injury, and feature chains with multiple swivels. There may be circumstances that necessitate the use of leg-hold traps where direct observation is not practical or feasible.
3. Mist nets carry the same concerns as leg hold traps, and should be attended continuously to avoid excessive trauma, excessive environmental exposure, and predation.
4. Fish capture methods should minimize by-catch. Adequate training and personnel safety should be observed with electrofishing or chemical agent use. Federal and state regulations may apply to the use of chemical agents in certain water bodies. These laws should be carefully observed.
5. Whenever baits, oral agents (e.g., alpha-chloralose; oral poisons*, etc.) or generic capture methods such as electrofishing, use of nets (e.g., trammel, hoop, gill nets) or mist nets are used, a description of how to minimize by-catch and unintended losses of animals not under study is expected. Adequate experience or prior training is of considerable importance before the use of nonspecific methods.

   **For example, for terminal fish collections with rotenone, as might be used during population studies, rotenone application should follow standard protocols, and should only be used only in relatively still water or in blocked off coves where rotenone exposure can be segregated.*

USDA pain classifications as applied to animal capture and noninvasive field procedures

USDA Pain Category	Definition
C	Procedures involving no more than momentary or slight pain or distress and no use of pain-relieving drugs, or procedures involving no pain or distress to animals
D	Procedures involving pain or distress for which appropriate anesthetic, analgesic, or sedative drugs were used
E	Procedures that involve pain or distress for which the use of anesthetics, analgesics, or sedatives would have adversely affected the procedure, results, or interpretation of the results

1. The IACUC uses USDA pain categorization for all species regardless of coverage under the USDA Animal Welfare Act.
2. Capture devices are designed either to hold the animal unharmed (live traps) or to kill the animal outright upon capture. Barring mechanical malfunctions and with appropriate placement and trap checking frequency, animals captured in live traps or nets are simply held without injury until removal. Pain or distress, as described in a position statement issued by the American Society of Mammologists and the Ornithological Council (June 2010), is unlikely to result from the simple capture of free-ranging mammals or birds using most live traps or capture techniques approved by the American Society of Mammologists or Ornithological Council Guidelines, so animal usage in these instances **is consistent with USDA pain Category C**. Capture of free-ranging mammals in properly functioning kill traps is also **usually consistent with USDA pain Category C**. Although scientific collecting of birds may sometimes entail capture of a live bird followed by euthanasia, the capture methods themselves are not intended to be lethal and in fact do not kill birds.

   Note: Animal injury from malfunctioning live traps, or animal pain and distress caused by malfunctioning kill traps should be reported to the IACUC as an unexpected outcome via the '**IACUC Unexpected/Adverse Event Reporting Form**' available on the UNE IACUC website.
3. Most tissue sampling and marking techniques in the field also are consistent with **USDA pain Category C** provided that procedures are not more invasive than peripheral blood sampling.
4. **USDA pain Category C** is also appropriate in instances where protocols requiring peripheral tissue sampling or tagging and release of free-ranging animals necessitate chemical immobilization to conduct the procedures, provided that immobilization is performed only to facilitate the procedure and protect the animal and the researcher from injury rather than to alleviate pain or distress induced by the procedure.

5. Any method recognized as an approved method of euthanasia by the American Veterinary Medical Association (AVMA) is consistent with **USDA pain Category C**. Because neither the USDA Animal Welfare Act nor its implementing regulations reference the AVMA's Guidelines for the Euthanasia of Animals, for those methods not approved by the AVMA for euthanasia, the IACUC is the deciding body as to whether the method of death meets the regulatory definition of euthanasia as defined by the USDA Animal Welfare Act.
6. Examples of **USDA pain Category C** procedures in field work and wildlife studies include:
    - Individual or small numbers of animals being confined and maintained in natural habitat that affords an appropriate quantity and quality of food, cover, and water
    - The short-term and skillful restraint of animals for purposes of observation or physical examination
    - Injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but not intrathoracic or intracardiac
    - Acute non-survival studies in which the animals are completely anesthetized and do no regain consciousness
    - Approved methods of euthanasia or humane killing
    - Short periods of food and/or water deprivation equivalent to periods of abstinence in nature
    - Collection of feathers, small skin punches, urine, feces, tracheal swabs, cloacal swabs
    - Application of tagging or marking devices, except implantations into body cavities
    - Most blood collection procedures
    - Administration of an anesthetic, analgesic or sedative drug to an animal for restraint purposes to perform a procedure that involves pain or distress
7. Examples of **USDA pain Category D** procedures in field work and wildlife studies include:
    - Surgical implantation of telemetry devices or identification devices that require anesthesia, analgesia, or sedatives
    - Invasive tissue sampling, such as intracardial blood draws or invasive biopsies
8. Examples of **USDA pain Category E** procedures in field work and wildlife studies include:
    - Experimental increase of litter or clutch size that results in a statistically significant depression in growth rates, excessive loss of parental mass, or death of young or adults
    - Diets that cause a statistically significant reduction in growth or cause excessive loss of body mass

Surgical Considerations

- If surgery is required, the use of non-sedating (e.g., non-steroidal anti-inflammatory drugs, local anesthetics, etc.) at the surgery site may be preferred over more debilitating systemic drugs.
- All surgery procedures should employ an intradermal pattern and absorbable suture and/or tissue glue (small incisions < 1 cm, or when combined with absorbable suture) remove the need for recapture for suture removal.
- Surgical procedures should only be performed by trained or experienced individuals using appropriate anesthetics, and in consideration of applicable withdrawal times where relevant. Recovery should be complete before anesthetized animals are released.
- Muscle relaxants are not adequate for painful procedures during wildlife immobilization.

Special considerations for conducting surgical procedures on fish

Source: Adapted from the Guidelines for the Use of Fishes in Research (American Fisheries Society, 2014)

1. Surgical procedures include such processes as implanting devices, including tags and transmitters, examining internal organs (e.g., gonads), and removing organs.
2. Successful surgery depends on the complexity of the procedure, the expertise of the individual, and the environment in which the procedure is conducted. To perform surgeries on fish, individuals require training and practical experience. Generally, fisheries biologists lack formal educational training in surgery, and as such, training should be provided by individuals with long-standing experience, extensive surgical expertise, and a record of surgical success with fishes. The training should cover the principles of surgery and guidance in performing the specific procedure. At the end of the training, the trainee must demonstrate to the instructor that they are capable of performing timely and effective surgeries similar to the procedures proposed for their research or teaching activities.
3. Written protocols for proposed surgical procedures should clarify whether the surgery is considered a major surgery or a minor surgery. Major surgery is a procedure that penetrate or exposes a body cavity, or a surgery that produces a substantial physical or physiological impairment. Examples of major surgery are implantation of a radio transmitter in the coelomic cavity, or a splenectomy. Minor surgery is less invasive, not penetrating or exposing a body cavity or producing substantial or physiological impairment. Examples of minor surgery include placement of catheters in blood vessels or implanting PIT tags in the muscle.
4. The aquatic nature of fish necessitates surgical procedures not considered customarily with non-aquatic animals. Body surfaces of fish are not covered by a keratinized integument but rather by a variety of living cells and mucus that provide a physical and immunological external barrier. Because many disinfectants can damage fish skin, the complete disinfection of a surgical site is likely not possible. The preparation of the surgical site generally consists of

gently cleaning the area of excess mucus. Efforts to avoid contaminating the surgical field and postsurgical infections are very important.

5. Realistic, pragmatic issues of the logistics for asepsis must be considered in the development of surgical protocols that are to be performed in the field in contrast to the well-equipped and designed laboratory or surgical suite. The ability to employ all of the aseptic techniques of surgery (e.g., use of gowns, mask, drape) is influenced by the environment in which the surgery is performed. Whether the type of surgery is considered a major or minor procedure is especially relevant when high numbers of fish will undergo surgery that must be performed at remote field locations. Modification of standard techniques may be required (for instance, in aquatic or field surgery), but should not compromise the well-being of the animals.
6. One area where a deviation from standard surgical procedures may be appropriate is that of sterilization of instruments between fish. In remote field locations, where high numbers of fish are to be processed, it may not be possible to access and autoclave or other standard sterilization equipment. In such cases, instrument disinfection between fishes might be performed by immersing the instruments in a 1:1,000 solution of benzalkonium chloride or other quaternary ammonia compound and 70% ethanol and then rinsing the instruments in sterile water before use. Other options for processing surgical instruments between surgeries are hot bead sterilizers or ultraviolet systems. In all cases, the surgical team should strive to the best of their ability to avoid contamination of the surgical field.
7. Depending on the anesthetic used (e.g., MS-222) and whether the fish will be stressed during a post-surgery holding period, temporary confinement may be necessary for a withdrawal time prior to their release into the environment, as they may be caught and consumed by the sport fishing community.
8. Generally, listed, imperiled species must be handled noninvasively, and if surgery is to be done, a permit is needed.

Appendix A: Example Risk Assessment Matrix

The following table depicts an example risk assessment matrix involving a field study with small mammals.

Task No.	Description of the Required Task(s) Involved with the Field Work or Wildlife Study	Description of the Hazard(s) Associated with the Required Task	Planned Approach for Managing the Hazard(s) Associated with the Required Task
1.	Setting and baiting traps	**a.** Potential for cuts/abrasions from metal traps **b.** Exposure to infectious agents left from animals (e.g., urine, feces, etc.)	**a.** Traps will be maintained to minimize risk of injury. A first aid kit will be available in the vehicle. Soap and water will be available in the vehicle to clean wounds. In the event of a wound, the area will be washed thoroughly with soap/water and proper first aid measures will be applied. **b.** After capturing and processing animals, all material will be removed from the trap. The trap will be wiped down with a bleach solution to disinfect. Nitrile gloves and masks will be worn at all times.
2.	Anesthetizing/euthanizing small mammals	**a.** Direct contact with isoflurane and/or inhalation of isoflurane **b.** Transport and disposal of isoflurane	**a.** Use of isoflurane will be done outside to minimize risk of inhalation. A second person will be on-hand in the event of a spill/contact. Nitrile gloves, goggles, and a mask will be worn while handling isoflurane. In the event of direct contact with isoflurane, skin/eyes will be immediately flushed with water and

Task No.	Description of the Required Task(s) Involved with the Field Work or Wildlife Study	Description of the Hazard(s) Associated with the Required Task	Planned Approach for Managing the Hazard(s) Associated with the Required Task
			medical attention will be sought. **b.** Isoflurane will be kept in a sealed container in a locked box in the trunk/trunk-bed of the vehicle.
3.	Handling small mammals	**a.** Biting and scratching from animals **b.** Exposure to zoonotic pathogens	**a.** Nitrile gloves will be worn while handling the animals. Kevlar gloves will also be available to personnel. In the event of an animal bite/scratch, the animal will be placed back into the trap, the bite/scratch site will be scrubbed with soap/water for 5 minutes and assessed. For minor wounds an antibiotic cream will be applied and the bite will be bandaged. For deeper punctures with bleeding, pressure will be applied and medical attention sought. **b.** Nitrile gloves will be worn while handling animals/traps. Equipment (e.g., forceps, scissors, etc.) will be wiped down with ethanol or a bleach solution between traps. All personnel involved with this project will be up-to-date on tetanus vaccines.
4.	Walking through tick and mosquito habitat	**a.** Exposure to tick and/or mosquito	**a.** Personnel will be instructed to wear long

Task No.	Description of the Required Task(s) Involved with the Field Work or Wildlife Study	Description of the Hazard(s) Associated with the Required Task	Planned Approach for Managing the Hazard(s) Associated with the Required Task
		pathogens	pants tucked into their socks and check daily for ticks and tick bites. Insect spray will be made available for use on the face and exposed skin. Personnel will be encouraged to wear permethrin-infused clothing. Personnel will be given a brief training on the risks of mosquito and tick-borne diseases and the typical signs of the diseases. Personnel will also be directed to the Maine CDC website to obtain additional information.
5.	Travel to and from field study site	**a.** Unable to get prompt emergency services at field study site; Limited road access **b.** Nearest medical facility over 1 hour away	**a.** Establish a communication plan with the emergency contacts listed in the IACUC application using radio/cell phone/satellite phone. **b.** Training in first aid/CPR for all personnel. First aid kit and/or wilderness first aid kit included in equipment inventory.