



SUBJECT: Use of Fish in Research	Effective Date: 8/28/2020	Policy Number: 10.4.24				
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Responsible Authorities: Principal Investigator Vice President, Research Institutional Animal Care and Use Committee Assistant Vice President for Research, Research Integrity Assistant Vice President for Research, Comparative Medicine						

- I. Background
 Florida Atlantic University (FAU) currently has institution-wide animal care standards in place to ensure that all activities involving vertebrate animals and cephalopods are conducted in a humane and ethical manner; that housing and care of animals is appropriate for the species; and that all persons working with animals are safe and protected ([Institutional Policy 10.4.1a](#)). While these standards are intended to cover all animals involved in research, it is sometimes difficult to identify best practices in the application of these standards to fish used in research, due to the variety of species involved, the different types of research conducted, and the unique aspects of fish care and handling.

- II. Purpose
 To establish a policy that will enhance existing institution-wide animal care standards by providing improved guidance to Research Personnel and Animal Care Staff to accommodate the unique and diverse aspects of fish research, while still ensuring that the health and welfare of the research animals and safety of personnel are maintained.

- III. Scope
 This policy applies to all fish included on an approved FAU IACUC protocol that are housed in a Comparative Medicine (CM) or PI managed satellite facility, or in an approved non-FAU facility. Fish uses covered by this policy include, but are not necessarily limited to, research involving fish as experimental models, aquaculture studies, wild caught fish once they are brought into a housing facility, and fish used for teaching or testing.

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Consistent with the Public Health Service's (PHS) definition, and for purposes of this policy, the term "fish" includes all stages of development post-hatching. However, since many species do not feel pain or distress during very early life stages, their numbers may be separated from later stages, and the number can be listed as Column C in the Pain Category column. The pain and distress categorization of later life stages should be determined by the investigator based on the specific procedures described in the protocol. However, procedures performed on fish of any age need to be described in the IACUC protocol independent of pain category.

For policies regarding field studies of wild fish, including capture, handling, tagging, transporting, *etc.*, PI's and their staff should consult FAU's IACUC policies on Wildlife Research ([Institutional Policy 10.4.26](#)) and Animal Transport ([Institutional Policy 10.4.10](#)).

IV. General Statement

1. The IACUC acknowledges that there is considerable variability among taxa of fish in terms of their basic needs and how they should be housed and handled, and the PI is often the most qualified institutional authority on the biology of the species under study, and the techniques appropriate for the conduct of the proposed study.
2. Despite this acknowledgement, the FAU IACUC affirms that the respectful use of animals, which includes the consideration for the well-being of the animal as well as the avoidance/minimization of pain and distress of animals, including fish, remains an ethical and scientific necessity.

V. Policy

1. Primary Documents: The primary documents for guidance on the appropriate use of fish in research will be *Guidelines for the Use of Fishes in Research*, American Fisheries Society, 2014 ([AFS Guidelines](#)); the Public Health Service Policy ([PHS](#)); and the *Guide for the Care and Use of Laboratory Animals* ([The Guide](#)).
2. PI Assurance: The PI shall assure the IACUC in their application that their handling, housing, and use of fish will be in accordance with the *AFS Guidelines*, the PHS Policy, and *the Guide*. The PI and associated research staff should be familiar with the taxon to be studied and their response to disturbance, sensitivity to handling and restraint, and requirements for captive maintenance to the extent that these factors are known or applicable to the study. The PI and associated staff should have adequate experience, training, and knowledge regarding the housing, feeding, and care requirements of the taxon to be studied, to the extent that these factors are known or applicable. The PI acknowledges that it is ultimately his/her responsibility to direct these activities.
3. Collection of Biological Samples: Blood and other biological samples should be collected using sterile or aseptic technique (depending on the procedure), and

appropriate analgesia/anesthesia should be provided for invasive or prolonged sampling procedures.

4. Tissue Sampling for Genotyping: An adequate sample may be acquired by collection of tissue via fin clipping or skin mucus.
 - A. Fin Clip: A tissue sample can be collected by removing a small portion of one fin. The location of the fin clip will vary depending on the species, e.g. caudal fin for zebrafish or adipose fin in salmonids. Aseptic technique should be used. The latter should include the usage of a sterile Petri dish (or other sterile surface) and sterile razor blade, scalpel, or sharp surgical scissors. Fish should be recovered in fresh system water until the small wound is healed. Post-operative care should include monitoring of the animal(s) for health concerns. Individual identification or single housing may be required until test results are obtained.
 - B. Skin Swab: Skin mucus collection with a sterile swab can be used as a non-invasive method to obtain DNA samples from some species of fish and has been validated for zebrafish (Breacker et al., 2017).
5. Surgical Procedures: Surgical procedures should be performed using sterile or aseptic technique as appropriate to the procedure (including the use of sterile instruments), appropriate analgesia/anesthesia, and atraumatic tissue handling. All personnel performing or assisting in surgical procedures should be trained in the techniques applied.
6. Pain and Distress: Whenever possible, the handling of fish, their removal from water, or their restraint under conditions that may cause physiological stress should be avoided or minimized. Changing environmental conditions under which fish are held, especially water temperature and salinity, should be performed gradually. Utilization of general or local anesthetics may be recommended in cases where prolonged restraint or invasive procedures are a necessary part of the research protocol.

Many species of fish are thought not to feel pain or distress during very early life stages, therefore their numbers may be separated from later stages, and the number can be listed as Column C in the Pain Category column. The pain and distress categorization of later life stages should be determined by the investigator in consultation with the Attending Veterinarian (AV) and the IACUC based on the specific procedures described in the protocol.

The appropriate use of neuromuscular blocking agents for fish should be consistent with the [PHS](#) Policy and [The Guide](#). *The Guide* states that, "if paralyzing agents are to be used, the appropriate amount of anesthetic should first be defined on the basis of results of a similar procedure using the anesthetic without a blocking agent (NRC 2003, 2008, 2009a)." The level of anesthesia should be sufficient to induce unconsciousness and analgesia corresponding

with the painful stimulus, and should be monitored before, during, and after paralysis. Methods should include the monitoring of autonomic nervous system changes (e.g. heart rate), since paralysis eliminates many signs and reflexes that might otherwise be used to measure anesthetic depth.

Tonic immobility may be used as a form of anesthesia in some scenarios, however use of this technique should be limited to immobilization for non-invasive procedures in captive research settings. If tonic immobility is used, adequate gill irrigation should be maintained to ensure adequate respiration and to prevent metabolic acidosis, and all efforts should be made to minimize the procedure time necessitating this technique.

7. Identification: Tagging methodology should include use of general or local anesthetics as appropriate to minimize pain and distress, and should aim to minimize the possibility of infection at the tagging site.
8. Animal Numbers: When breeding fish for purposes of maintaining an experimental population, or as part of aquaculture studies, the numbers are often too large to count accurately without causing stress on the animals and / or interference with the study. For early stages, the number of animals used may be provided as an estimate. Estimated numbers may also be used after they have matured to adults if they are group housed. An exception will be made for individuals subjected to pain category D or E, in which case exact number of animals involved will be provided.
9. Transport: Fish transport protocols should comply with the IACUC Policy on Animal Transport ([Institutional Policy 10.4.10](#)) and the *AFS Guidelines*. Adequate recovery time (minimum 72 hours is suggested) in the same or similar medium used for transport should be allowed for transported fish in order to minimize stress, including allowing for gradual acclimation to water parameters such as temperature and pH.
10. Housing: Vendor supplied or wild-caught fish should be maintained under conditions that comply with *AFS Guidelines* and should incorporate, as much as possible, aspects deemed important to the survival and well-being of the fish. Considerations include appropriate acclimation, light level and photoperiod, water quality, animal stocking density, minimization of noise and vibration, and appropriate enrichment, unless these are factors under investigation. Nutritionally balanced diets should be provided, or natural foods should be duplicated as closely as possible. Adequacy of maintenance should be judged by monitoring factors such as animal appearance, activity level, general behavior, and rate of survival.
11. Final Disposition of Fish:
 - A. Release of Wild-Caught Animals: Whenever practical and ecologically appropriate, as soon as possible after capture or upon completion of the study, wild-caught animals should be released at the site of the original

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capture. Release of wild-caught fish should be conducted under the provisions that their ability to survive has not been impaired; they can be expected to function normally under the environmental and habitat conditions at the location and time of release; their release is not likely to spread pathogens to a naïve population; no laws or regulations prohibit their release; their release is in accordance with applicable permits; and their release is not detrimental to the well-being of the existing native animals.

- B. Euthanasia: Methods of euthanasia should be consistent with recommendations found in the American Veterinary Medical Association ([AVMA](#)) Guidelines for the Euthanasia of Animals, 2020 Edition, those described in the *AFS Guidelines*, and those described in the FAU IACUC Policy on Euthanasia Methods ([Institutional Policy 10.4.11](#)). Consistent with these guidelines, method of euthanasia will differ depending on number of animals to be euthanized, size of animal to be euthanized, and whether the euthanized animal is intended for consumption by humans or other animals (see section 9C, “Humane Slaughter”, below). Disposal of euthanized fish should also conform to AVMA and / or the *AFS Guidelines* recommendations, as well as any other governing regulations.

As specified in the AVMA Guidelines for Euthanasia, rapid chilling via immersion in an ice bath is an acceptable euthanasia method for small-bodied (3.8 cm long or smaller) tropical and subtropical stenothermic finfish, for which the lower lethal temperature range is above 4°C. This method may also be acceptable for certain small to medium-sized fish as supported by published peer-reviewed scientific data, provided secondary euthanasia methods are applied after fish are rendered nonresponsive. Rapid chilling is not appropriate in other medium to large-bodied finfish unless supported by published peer-reviewed scientific data.

Training of appropriate methods of euthanasia is the responsibility of the PI. The training must be documented appropriately by the PI as described in IACUC policy Researcher Training ([Institutional Policy 10.4.22](#)).

- C. Humane slaughter is distinguishable from euthanasia and primarily describes the killing of animals intended for human consumption. Whenever slaughter of fish intended for human consumption is a planned endpoint for fish covered under an FAU IACUC protocol, the procedures, animal numbers, training, *etc.* should be described in a separate section, and the methods should be consistent with recommendations found in the AVMA Guidelines for the Humane Slaughter of Animals, 2016 Edition.
- D. Preserved Specimens: The preparation of animals as museum specimens is necessary for research and teaching activities in systematic zoology. However, each animal collected should serve as a source of information on as many levels as practical (*e.g.* behavior, morphology, genetics, *etc.*) to

assure the maximum utility of each animal, and to minimize instances of duplicate collecting.

Note: Formalin fixation of dead specimens is acceptable, however euthanasia of un-anesthetized specimens by immersion in a formalin solution is unacceptable.

12. Standard Operating Procedures (SOPs): SOPs should be based on the husbandry and care of fish species housed within Comparative Medicine or PI managed facilities and should be reviewed by the IACUC. SOPs should outline, at a minimum, methods for observing and documenting animal health and well-being, quarantine, housing, environmental enrichment, sanitation, filtration, feeding, water quality analysis, personnel safety, and recordkeeping. Recommendations and guidance can be found in [the Guide](#) and [AFS Guidelines](#).

VI. Accountability

The Principal Investigator (PI) will be responsible for:

- § Assuring that procedures are performed as described in the corresponding IACUC protocol and, if necessary, submitting an amendment to the protocol and awaiting approval before new method(s) is (are) introduced.
- § Assuring that personnel complete IACUC required training per FAU IACUC policy Researcher Training ([Institutional Policy 10.4.22](#)) and providing personnel with species appropriate training for all research procedures.
- § Following all IACUC approved SOPs for PI managed satellite facilities as applicable.

The IACUC will be responsible for:

- § Reviewing and approving, requiring modifications (to secure approval), or withholding approval of IACUC protocols and/or amendments including addition of personnel.
- § Providing continued guidance for active protocols.

The Research Integrity office will be responsible for:

- § Administrative support of the IACUC members to facilitate their regulatory function.
- § Maintaining policy and assure regular review and update as necessary by the IACUC.

The Office of Comparative Medicine (CM) and Attending Veterinarian (AV) will be responsible for:

- § Providing consultation to the PI and to the IACUC regarding specific procedures impacting the health and welfare of the animal(s), especially as it relates to anesthesia/analgesia, aseptic techniques of sampling and surgical procedures and use of antimicrobials.
- § Contributing to the review of fish protocols and to the determination of specific requirements for training of personnel.

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§ Contributing to review of procedures for transporting animals onto any FAU campus and for procedures for monitoring the health and well-being of these animals.

VII. Policy Renewal Date
8/28/2023

VIII. References

1. Office of Laboratory Animal Welfare. 2015. Public Health Service Policy on Humane Care and Use of Laboratory Animals. <https://grants.nih.gov/grants/olaw/references/phspol.htm#RecordkeepingRequirements>
2. Frequently Asked Questions. PHS Policy on Humane Care and Use of Laboratory Animals. 2016. <https://grants.nih.gov/grants/olaw/faqs.htm#590>
3. Guide for the Care and Use of Laboratory Animals, 8th edition, 2011, p.122-123. <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>
4. Guideline for the Use of Fishes in Research, 2014 <https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf>
5. Breacker, C, et al. "A low-cost method of skin swabbing for the collection of DNA samples from small laboratory fish." Zebrafish 14.1 (2017): 35-41
6. "Guidelines for Use of Zebrafish in the NIH Intramural Research Program"

POLICY APPROVAL

Initiating Authority

Signature: _____

Date: _____

Name: Daniel C. Flynn, Ph.D., Vice President for Research

Executed signature pages are available in the Initiating Authority Office(s)