

**University of Prince Edward Island
Animal Care Committee
Standard Operating Procedure**

SOP #: ACC - CT05

SOP Title: Euthanasia of Fin Fish

SOP Section: Clinical Technique

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1.0 Introduction

- 1.1 Ensure that all individuals responsible for euthanasia are appropriately qualified, trained and adhere to UPEI Animal Care Committee-approved protocols and policies.

2.0 Materials and Equipment

- Plastic Container, e.g. tote to hold fish and anaesthetic solution
- Plastic buckets
- Tricaine Methane Sulphonate (TMS) (MS222); Benzocaine hydrochloride or other appropriate anesthetic
- Electronic mass balance
- Sodium bicarbonate (if required)
- Dip Nets
- Surface disinfectant (appropriate for the organism being studied; as per the UPEI Biosafety Permit)
- PPE Required: Module specific laboratory coat; Waterproof footwear; Disposal gloves
- PPE as needed: Module specific safety glasses/face shield/respiratory mask; Waterproof apron

3.0 General Considerations

- 3.1 The person applying the method of euthanasia is the most important factor in ensuring that an animal's death is humane. Regardless of whether the procedure is applied to an individual animal or to a group, you must always attempt to meet the following criteria:
- a) Death occurs without signs of panic, pain or distress;
 - b) Assurance of a minimum time to loss of consciousness;
 - c) Reliability and reproducibility of euthanization technique;
 - d) Safety for personnel involved;

- e) Minimization of undesirable physiological and psychological effects on the animal;
- f) Compatibility with the requirements and the purpose of the scientific study;
- g) Incur only minimal or preferably no emotional effects on the observer and the operator;
- h) Minimization of perceived environmental or ecological impact;
- i) The use of simple, inexpensive mechanical equipment which is relatively maintenance-free;
- j) Euthanize in a location either remote or separate from the animal rooms

3.2 If euthanasia is scheduled as part of the study protocol, fish should be fasted at least 24 hours prior to euthanasia.

3.3 Clean all required equipment:
Refer to Aquatic Animal Facility (AAF) Unit B SOPs
Buckets - SOPAQ 2035 *Cleaning and Disinfection of Plastic Ware*
Dip Nets - SOPAQ 2041 *Cleaning and Disinfection of Dip Nets*

Rinse **the inside and outside** of all cleaned buckets, at least 3 times with water from the hose in the module immediately prior to use.

Direct rinse water into the floor drain in the module.

4.0 Fish Euthanasia

- 4.1** The appropriate concentration of anesthetic for the species and size of animal should be used.
- 4.2** If using **Freshwater**, add 1-2 tablespoons of sodium bicarbonate into a plastic container. Sodium bicarbonate is added to buffer the pH of the anesthetic solution. Seawater is naturally buffered therefore this step is not needed.
- 4.3** Remove water from the fish holding tanks using the clean buckets. Fill a plastic container with a measured volume of water.
- 4.4** Add the anesthetic and the sodium bicarbonate buffer to the water. Use a clean dip net to stir the bucket.
- 4.5** Remove a few fish at a time from the tank with a dip net and place in the anesthetic solution.

4.6 **Signs of completed euthanasia**

The fish are considered dead when respiration stops and the heart stops, thus ensuring a cessation of blood delivered to the brain. This is established when both gill operculum movement and heart movement cease. No animal should be considered dead until reflex movement as well as cardiac and respiratory movements have ceased. It is prudent to allow extra time for the euthanasia to be complete – at least ten minutes in this euthanizing solution should be sufficient to render the fish dead, although longer may be required for some species.

Remove euthanized fish from the euthanizing solution.

- 4.7 Percussive stunning involves a forceful and accurate blow to the head with a blunt instrument, e.g. fish bonker obtainable from angling retailers. The force required will depend on the size of the fish. Aim the blow just above the eyes to impact the brain. The effectiveness of the stun must be checked and another blow applied if the fish is not fully euthanized.

Note: Anesthesia or sedation must be applied prior to the use of physical techniques unless approved by the University Veterinarian.

4.8 **Decapitation**

Note: Anesthesia or sedation must be applied prior to the use of physical techniques unless approved by the University Veterinarian.

- a) Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is quickly separated from the body rapidly and completely;
- b) Follow decapitation with pithing;
Insert a rigid metal rod into the foramen magnum which is identified by the slight midline skin depression posterior to the eyes. Ensure that both the brain and the proximal end of the spinal cord are destroyed;

4.9 **If the fish are not to be necropsied:**

Unit A: Dispose of the fish by placing in a compostable bag and then into a secondary sealable container labelled with the contents, room number and the Principal Investigator. This is either taken to the refrigerator in Unit A until disposal by the researcher or taken directly to the AVC Post Mortem area for incineration.

Unit B: If fish are in AAF Unit B, place the fish in a compostable bag which is placed inside a leak-proof, impact resistant, sealable container labelled as biohazard waste and labelled appropriately.

Transfer the secondary container to Room 1183N/1184N and place the compost bag containing the fish into the designated

biohazard waste freezer for later incineration by AVC Diagnostic Services.

4.10 If the fish are to be sampled as part of a research protocol:

Unit A: Samples can be collected in the module. Alternatively, the fish may be transported to the researcher's laboratory but prior approval must be obtained from the Animal Care Committee/Biosafety Committee as applicable. Disposal of carcasses must follow guidelines approved by the UPEI Biosafety Committee.

Unit B: Transfer the container holding the fish to the procedure room (Room 1183N/1184N). Waste generated during sampling should be disposed of as per AAF Unit B SOPAQ 1014 *Handling of Test and Laboratory Material*.

4.11 If the fish are to be submitted to Aquatic Diagnostic Services:

Units A& B: Contact the fish pathologist prior to removal of the fish to ensure that the animals can be transferred directly and are not left waiting in the fish post mortem room unattended. Transfer the container holding the fish to the fish post mortem room in the AVC Post Mortem area.

4.12 Immediately prior to removing waste from the module, surface disinfect the outside of the secondary container with disinfectant solution as per AAF Unit B SOPAQ 1014 *Handling of Test and Laboratory Material*.

5.0 Disposal of Anesthetic Water

5.1 Dispose of anesthetic water in the floor drain of the module with an excess of water.

5.2 Clean and disinfect the floor of the module (SOPQAQ 2039 *Cleaning and Disinfection of Modules*) and the equipment (SOPAQ 2035 *Cleaning and Disinfection of Plastic Ware*; SOPAQ 2031 *Cleaning and Disinfection of Dip Nets*).

5.3 If in a remote location where a sewer may not be readily available, further dilute the solution with water and dump wastes on land, in a location away from water.

5.4 Do not discard MS-222 directly into surface water, storm water conveyances or catch basins.

6.0 Safety

- 6.1 Wear protective clothing, gloves and goggles.
- 6.2 Wear gloves to handle animals exposed to anaesthetic.

7.0 Documentation

- 7.1 Document all procedures in the module Log Book.
- 7.2 Report any animal health and welfare concern to the UPEI University Veterinarian or their designate as per ACC-CT06 - Reporting Animal Health & Welfare Concerns to Veterinary Staff ([http://files.upei.ca/research/sop-ct06-reporting animal health welfare concerns to veterinary staff.pdf](http://files.upei.ca/research/sop-ct06-reporting%20animal%20health%20welfare%20concerns%20to%20veterinary%20staff.pdf)).

8.0 References

- 8.1 UPEI Animal Care Committee Terms of Reference:
http://research.upei.ca/animal_care
- 8.2 Canadian Council on Animal Care Guidelines on the Care and Use of Fish in Research, Teaching and Testing:
<http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf>
- 8.3 Canadian Biosafety Standards and Guidelines (CBSG) – 1st Edition:
<http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/assets/pdf/cbsg-nldcb-eng.pdf>
- 8.4 CCAC Guidelines, I. EUTHANASIA .
<http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf>
- 8.5 Cornell University, Institutional Animal Care and Use Committee, ACUP 306.2 Fish and Amphibian Euthanasia
<http://www.research.cornell.edu/care/documents/ACUPs/ACUP306.pdf>
- 8.6 McGill University, Standard Operating Procedure, Fish and Amphibian Euthanasia
<http://www.mcgill.ca/research/researchers/compliance/animal/sop/>