3.0 Euthanasia of Finfish

3.1 Introduction:

This template is intended for use by instructors to train the Department of Fisheries and Oceans (DFO) staff and students in the humane euthanasia of research fish. The drug dosages described in this template are for salmonids; instructors may have to modify the dosages and techniques described when different species are euthanized. Templates are used to provide the minimum requirements necessary in a training exercise, but the instructor may add additional material.

An experienced instructor must demonstrate the methods outlined in this template, and trainees must be deemed qualified in carrying out one or more of the procedures, before they can be allowed to euthanize fish without an instructor present. Hands-on training of staff is a requirement for facility approval by the Canadian Council on Animal Care, of which DFO is a member. This template is part of a comprehensive DFO Science Branch series on training for users of aquatic research animals.

3.2 Rationale:

Scientific studies often require that fish be sacrificed for reasons of sampling, respect of humane endpoints and at the termination of experimental research protocols. Investigators have an ethical obligation to minimize the pain and/or distress of all laboratory animals. Only those euthanasia methods deemed acceptable by the Canadian Council of Animal Care (CCAC) should be employed.

3.3 Authority:

The staff or consultant Veterinarian or Animal Care Committee is responsible for the distribution of information and training in the methodologies acceptable for euthanasia. Animal Care Committees may delegate training of this procedure to a person who has demonstrated experience in humane euthanasia. Staff must be trained in euthanasia methodology prior to performing the procedure(s).

3.4 Goal of this training exercise:

1. Learn methods to humanely kill finfish.
2. Understand the consequences of each method of euthanasia on the fish and thus potential impact on tissue selection and collection.
3. Identify acceptable location(s) within the facility for euthanasia to be performed.
4. Identify the proper location(s) within the facility for sample collection.
5. Understand where and how to dispose of blood, carcasses and other by-products of the procedure.
6. Understand proper disinfection techniques to use after completing the procedure (provide site biosecurity SOPs).

3.5 Theoretical training – to be completed before hands on session

1. Completed 'The Experimental Fish'
2. CCAC guidelines for fish euthanasia
3. Completed anatomy and anaesthesia modules or has experience or training in both areas.
4. WHMIS training.
5. Review theory material provided with this training template (Appendix A).

3.6 Details of the Procedure:

Methods of euthanasia, that may be included in this training session, are:

1. Anaesthetic overdose with TMS™ (buffered if used in freshwater)
2. Anaesthesia followed by exsanguination.
3. Anaesthesia followed by pithing, cervical dislocation or decapitation
4. Blunt force trauma followed by pithing or exsanguination
5. Discussion of techniques for euthanizing an entire population of fish

3.6.1 Time Estimate:

Set up: 1 hour
Instruction and training: 3 hours

3.6.2 Equipment Required:

- Fish: number will depend on number of trainees in a session; species used will depend on availability and requirements of the facility.
- TMS™ (plus NaHCO3 buffer if freshwater) – dosage in this example is 200 ppm but instructors will have to use their experience with different species to alter this dosage if necessary.
- Assorted dipnets and buckets; a clear container is preferable for demonstrating anaesthetic overdoses so that trainees can monitor fish more easily.
- Air stones and compressed air or oxygen
- Fish bonker (priest) if larger fish are to be euthanized
- Probes appropriate to fish size for pithing, scalpel for cervical dislocation
- Sharp scissors or scalpel for exsanguination
- Sharp knife or scalpel for decapitation
- Paper towel or bucket for containment of blood from exsanguination
- Stopwatch
- Gloves and splash glasses to be worn when handling fish or anaesthesia water
- Disinfectant for clean up after euthanasia
- Thermometer to monitor temperature of water in anaesthetic bath.

3.6.3 Procedure:

The instructor should demonstrate all procedures prior to letting trainees attempt them. Fish euthanized during this module can be used for training in other modules for example anatomy training, length weight sampling, biopsy, blood sampling or tagging.

Instructors will need to use their own best judgement in choosing which procedures are appropriate with the common species used at their research facility. It is recommended that all procedures below be taught to trainees so that they have multiple options at their disposal when faced with the need to euthanize fish of different species. Trainees must be comfortable with performing at least one method of euthanasia.

3.6.3.1. Anaesthetic Overdosage:

- Prepare a TMS™ bath for euthanasia; a drug concentration of 200 ppm is usually sufficient (species dependent). The euthanasia bath should be buffered with $NaHCO_3$ if it is prepared with fresh water. The bath should be aerated and temperature of the bath should be identical to the fish's home tank. Attention to water quality during euthanasia helps to minimize the initial stress of introduction to the highly concentrated euthanasia bath.

- Transfer a fish to the euthanasia bath and start timing with stopwatch.

- Monitor the fish continually throughout the procedure.

- Note the time when the fish's opercular movements stop; this should provide trainees with a guideline for how long cessation of respiration takes at the demonstrated drug dosage and temperature.

- The air stones can be removed from the water bath after a surgical plane of anaesthesia has been reached.

- If the fish is to be euthanized without any physical methods being employed then leave the animal in the water bath for a minimum of 5 minutes after cessation of respiration.

- Ensure that the trainees are aware that some species of fish continue to have brain activity in the face of advanced central nervous system and systemic hypoxia.

- Inform the trainees that the fish's heart may continue to beat even after anaesthetic overdose or physical methods of euthanasia. Muscle twitching is also common after death.

Use the overdosed fish for demonstration purposes:

- Once the instructor is confident that the fish has succumbed to the overdose, the animal can be used to identify anatomical landmarks for subsequent procedures to be performed by students.

- Identify external anatomical features that are important in physical euthanasia methods:
    - Location of brain and start of vertebral column for pithing, decapitation and cervical dislocation techniques.
    - Gill arches and peduncle for exsanguination techniques

- On smaller fish use a scalpel to remove the tissue from the top of the skull to expose the brain and identify the junction between the skull and the first vertebrae.

- In larger fish it will be important to stress how deep an incision must be made in order to successfully transect the spinal column. Note: In many cases it will be easier to exsanguinate larger fish rather than try to perform cervical dislocation on them.

- Drawbacks of using anaesthetic overdose as a method of euthanasia:

    - Drug residue is present.
    - Difficulty in determining when fish is actually dead.

- Advantages:

    - Least traumatic method of euthanasia.
    - Less stressful on fish and staff.
    - Less labour intensive.

3.6.3.2. Anaesthesia followed by exsanguination:

- Anaesthetize a fish to the point of cessation of respiration and remove it from the water.

- Smaller fish (up to several hundred grams): Cut the tail off at the peduncle and allowed it to bleed into a paper towel.

- Larger fish: Hang the fish by the tail and cut the gill arches with a scalpel or scissors. For very large fish there may be a considerable amount of blood. The fish may be suspended over a bucket or the gill arch stuffed with paper towel to help contain the blood.

- Drawbacks to this method of euthanasia:

- Drug residue is present.
- Blood must be contained for biosecurity purposes.
- Trauma to gill tissue or peduncle makes these tissues less desirable for tissue selection.

- Benefits to this method of euthanasia
  - Histopathology samples do not appear congested if animal is bled prior to sample collection.
  - With drug overdose alone it can be difficult to determine when the fish is actually dead; with addition of a physical method of euthanasia you can be sure that the animal is euthanized rapidly.

3.6.3.3. Cervical dislocation, pithing or decapitation subsequent to anaesthesia:

- The instructor will have to decide whether pithing, cervical dislocation or decapitation is the best technique based on the species and size of fish being euthanized.

- All three of these techniques can be demonstrated on a single fish prior to students being allowed to try the techniques.

- Anaesthetize a fish as above and remove it from the euthanasia bath once gilling has stopped. Alternately, if dead fish are available from another source, they can be used for training purposes.

3.6.3.3.1 Cervical dislocation:

- Externally identify the location of the junction of the skull and the first cervical vertebra.

- Properly support the fish and demonstrate the use of a scalpel or knife to incise between the skull and first vertebra.

3.6.3.3.2 Pithing:

- Pithing a fish is easier if the spinal cord has already been exposed by cervical dislocation as above

- Insert a sharp probe into the brain and move it around to destroy the brain tissue (single pithed).

- Push the sharp probe down the spinal column to destroy the nerve tissue (double pithed).

3.6.3.3.3 Decapitation:

- Sever the fish's head at the junction of the skull and first vertebra with a scalpel or knife.

- Drawbacks of these physical methods of euthanasia:
  - Damage may occur to tissues required for tissue sampling such as brain, spinal cord, gill, thyroid, or head kidney.
  - Decapitation without prior exsanguination may result in blood contamination of work area if blood is not properly contained.
  - Without prior exsanguination, the collection of tissue samples can be more difficult due to bleeding when tissues are cut.

- Benefits of these methods
  - With drug overdose alone it can be difficult to determine when the fish is actually dead; with addition of a physical method of euthanasia you can be sure that the animal is euthanized rapidly.
  - Rapid method minimizes the wait for the animal to bleed out.

3.6.3.4. Blunt force trauma followed by pithing or exsanguination

- Instructor must demonstrate this method prior to the trainee performing this procedure. Since the fish is conscious at the time of the trauma it is **essential** to place the first blow correctly.

- The trainee must be thoroughly familiar with the anatomy of the species being handled prior to performing this procedure.

- Fish may vigorously fight being handled. It is important to stress the importance of protecting human safety; if there is any question about the trainee's abilities to restrain the fish for this procedure then the animal must be anaesthetized first.

- Remove a fish from its home tank and restrain it in the net or by the tail.

- Deliver a forceful blow to the head with a fish bonker; the blow should be placed on the top of the head just behind the eyes.

- The fish is then pithed or exsanguinated as described above.

- Drawbacks of this method of euthanasia:
    - Risk of injury to personnel performing procedure.
    - Blow must be accurately placed or animal will suffer prior to becoming unconscious.
    - Trauma can result in gill aneurysms that interfere with interpretation of histopathology.
    - Trauma to the brain makes this organ less desirable for tissue sampling.
- Benefits of this method:
    - No drug residue in tissues.
    - No need to dispose of anaesthetic baths.
    - Decreases need to transport and use drugs in the field.

3.6.3.5. Discussion of euthanasia of large groups of fish:

- For obvious reasons the demonstration of the euthanasia of large groups of fish is generally not possible. However this topic should be discussed with trainees, as they may need to euthanize large groups of fish at the end of research experiments or due to disease outbreaks.
- The flow into the holding tank of fish to be euthanized is stopped.
- The water level is dropped and air stones with supplementary oxygen added.
- The fish are sedated with 1 ppm of Aquacalm™ (1 gram per 1000 litres of water).
- Allow 10 – 15 minutes for the Aquacalm™ to be effective.
- Transport the fish into a euthanasia bath of TMS™ (200 ppm) until death or additional physical euthanasia methodology can be employed (i.e. blunt trauma, exsanguination or pithing).
- Tank disinfection should follow site Biosecurity SOPs.

3.6.4 After Euthanasia:

- Trainees should have clear instructions for carcass disposal.
- Anaesthesia baths must be disposed of in accordance with local waste management regulations.
- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).
- Trainees must wash hands with disinfectant soap.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

3.7 ACC Notes:

- Regional specifics
- Authorization to teach the procedure
- Any other requirements for your region

APPENDIX A: Review Theory for Euthanasia of Finfish.

References:

Bowser, P.R. 2001. Anaesthetic options for fish in Recent Advances in Veterinary Anaesthesia and Analgesia: Companion animals, R.D. Gleed and J.W. Ludders (Eds.) International Veterinary Information Service (www.ivis.org) Ithaca, New York, USA.

CCAC guidelines on: the care and use of fishes in research teaching and testing: Euthanasia Guidelines.

Definitions:

Euthanasia: An easy, painless or stress free death

Pithing: Destruction of the brain and spinal cord by physical disruption; often a needle or other object is pushed into the brain via the foramen magnum.

Exsanguination: Excessive blood loss by internal or external haemorrhage

Cervical dislocation: The displacement of the bones of the neck; the bone surfaces should ideally be entirely separated.

Hypoxia: Diminished availability of oxygen to the body tissues.

- Euthanasia must be performed in a humane manner. Methods used for euthanasia should result in a rapid and complete loss of consciousness that lasts until death.

- If the reason for euthanasia is sampling of healthy fish then it is recommended that the fish be fasted for 18 – 72 hours prior to euthanasia to prevent contamination of gills and skin samples with feces or as a result of vomiting. Consultation with principle investigator is important to determine if experimental protocols should include fasting.

- If fish are in distress or are being euthanized to protect their welfare then fasting should not be carried out.

- Overdosage with anaesthesia will result in a rapid progression through the stages of anaesthesia (review stages as described in Table 1).

Table 1. Stages of Anaesthesia (Bowser, 1991)		
Stage	**Descriptor**	**Behavioural Response of Fish**
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
1	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
2	Deep sedation	Total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate; equilibrium normal
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
6	Medullary collapse (stage of asphyxia)	Opercular movements cease; cardiac arrest usually follows quickly

- The preferred method of euthanasia, as recommended by the CCAC is a two-step process, with initial anaesthesia to the point of loss of equilibrium followed by a physical or chemical method to cause brain death and cessation of cardio respiratory function (CCAC guideline 112).

- Acceptable methods of euthanasia:
    - Anaesthesia followed by pithing, cervical dislocation, decapitation or exsanguination
    - Anaesthetic overdose alone
    - Blunt trauma to the head followed by other physical methods as listed above. Only trained individuals must use this method. The use of anaesthesia prior to trauma is preferable.

- The following are unacceptable methods of euthanasia because many species of fish continue to have brain activity in the face of advanced central nervous system and systemic hypoxia:

    - Hypothermia: the formation of ice crystals during freezing may cause pain and suffering.
    - Carbon dioxide
    - Asphyxiation by removing fish from the water or draining the tank

- Electrocution is also an unacceptable method of euthanasia as it may result in spinal fractures and muscle trauma