Aquatic Toxicology Research Lab

SOP-005 Moribund Description and Euthanasia of Fish

PURPOSE
To identify behaviours or clinical signs indicative of impending death requiring euthanasia.

POLICY
UOIT follows the current guidelines of the CCAC (Canadian Council on Animal Care) in order to work with aquatic animals using the most humane methods, while still allowing the appropriate research to be conducted.

DESCRIPTION
If fish develop symptoms of illness from natural or induced diseases, it is more humane to euthanize them than allow them to suffer until death. Moribund fish requiring euthanasia are identified according to established criteria. Euthanasia of moribund fish is to be carried out in a safe and humane manner.

RESPONSIBILITY
Aquatic Toxicology Lab Research staff and students.

IDENTIFICATION OF MORIBUND FISH
When fish are showing signs of illness, except when not purposely induced for research purposes, every effort should be made to determine the causative agent and treat them with the appropriate measures. However, fish may be ill for a period of time before illness is recognized or they exhibit moribund behaviour. It may not be possible to initiate treatment and because there are limited therapeutic options for fish, therefore the best alternative maybe euthanasia.

As it is difficult for people to visually assess pain and suffering in a fish before it is terminally ill, Aquatic Toxicology Lab Research staff and students and possibly external professionals as required, assess whether or not intervention is required and if that intervention is treatment or euthanasia.

A terminally ill fish may be identified by behaviour or certain clinical signs. For a fish to be considered moribund it should meet the following criteria:
1. The fish is unable to move through the water and/or use whole body movement (except in anguilliform-like swimmers) rather than primarily using the fins (usually pectoral or caudal) to move.

2. Fish is unable to maintain normal body orientation in the tank;

3. Fish is segregated from other members of the group (for schooling fish);

4. Fish has prolonged lack of interest in food and/or is lethargic;

5. Fish has lost normal ability to evade capture;

6. Fish is showing external clinical signs of bacterial or fungal infection despite normal activity. i.e. Saddleback disease, tail rot, fungus.

MATERIALS FOR EUTHANASIA BY ANESTHETIC

- Containers – Anesthetic bath tank; should be adequately sized for the fish to be euthanized.
- Anesthetic. Tricainemethanesulfonate, Marinil, or Eugenol.
- Sodium Bicarbonate
- Handling equipment (dip nets)
- Gloves and eye protection
- Plastic disposal bags
Dosages Guide

Tricainemethanesulfonate (TMS)- To be held in anesthetic until medullary collapse is complete. See Anesthesia Levels Chart below.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Temperature</th>
<th>Concentration for Euthanasia (mg./litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonidae (All Salmonids)</td>
<td>7-17º C</td>
<td>135</td>
</tr>
<tr>
<td>Cyprinidae (eg. Goldfish, carp)</td>
<td>16º C</td>
<td>200</td>
</tr>
<tr>
<td>Ictaluridae (eg. Catfish)</td>
<td>7-27º C</td>
<td>270</td>
</tr>
<tr>
<td>Tropical live bearers (eg. Guppies, Cichlids)</td>
<td>24 – 27º C</td>
<td>100</td>
</tr>
<tr>
<td>Tropical egg layers (eg. Zebrafish)</td>
<td>24 – 27º C</td>
<td>100</td>
</tr>
</tbody>
</table>

Marinil - All Fish (Use With Anesthesia Levels Table)

<table>
<thead>
<tr>
<th>Euthanasia Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg/l</td>
</tr>
</tbody>
</table>

Clove Oil (Eugenol) 1 ml = 1.07gm (Use With Anesthesia Levels Table)

Mix with ethanol 95% to dissolve.

<table>
<thead>
<tr>
<th>Anesthesia Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/l</td>
</tr>
</tbody>
</table>

Ethanol 95% : Eugenol – 10:1
Sol'n 0.47 – 0.71 ml/l
Anesthesia Levels

<table>
<thead>
<tr>
<th>Increasing depth of anesthesia</th>
<th>Stage</th>
<th>Indicator / Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Light sedation - Slight loss of reactivity to external visual and tactile stimuli; opercular rate slightly decreased; equilibrium normal.</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Deep sedation – Total loss of reactivity to external stimuli except strong pressure; slight decrease in opercular rate; equilibrium normal</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Partial loss of equilibrium – Partial loss of muscle tone; swimming erratic; increased opercular rate; reactive only to strong tactile and vibrational stimuli.</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Total loss of equilibrium – Fish turns over, locomotion ceases, fish swims or extends fins in response to pressure on caudal fin or peduncle</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Total loss of reflex – No response to pressure on caudal fin or peduncle; opercular rate slow and erratic.</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Medullary collapse – Opercular activity ceases; further exposure to anesthetic will lead to mortality.</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Death – No movement, respiration, cardiac activity and cessation of brain function</td>
</tr>
</tbody>
</table>

**METHOD**

- Handling equipment, treatment and recovery tanks, and equipment for procedures should all be clean and ready to use before the procedure.
- All equipment and tanks should be placed as close as possible to the holding tank to minimize handling.
- Set up the anesthetic tank with a known volume of water.
- Measure out the appropriate amount of anesthetic, add it to the anesthetic tank and mix well. The amount of anesthetic required will be determined by the species of fish. See Dosages Guide above.
- For each measure of TMS (if used), add double the amount of sodium bicarbonate to the water. (E.g. 5 grams TMS : 10 grams sodium bicarbonate)
- Using a net, remove fish from the holding tank and place in the anesthetic bath.
• Animals must be closely monitored throughout the procedure and removed only after the fish has died. Each species and even fish within the same species will take varying lengths of time to succumb completely so care must be taken to ensure that death has been accomplished.

• Once the fish is determined to be dead, unless it is required for analytical purposes, it must be bagged and disposed of in the appropriate way.

• All equipment and materials used must be disinfected, especially if infectious diseases are suspected.

REFER TO SOPs

Anesthesia - Fish

REFERENCES


Department of Fisheries and Oceans Animal-User Training Template, September 2004, 2.0 Anaesthesia of Finfish, pages 1-31

Department of Fisheries and Oceans Animal-User Training Template, September 2004, 3.0 Euthanasia of Finfish, pages 1-11


Syndel Laboratories, Ltd., 9211 Shaughnessy Street, Vancouver, BC. Marinil Instruction Sheet

TRICAIN S™, Product Documentation, Western Chemical, Inc, 1269 Lattimore Road, Ferndale, WA. 98248, (800) 283-5292, (360)384-5898, FAX (360)384-0270

University of Victoria, Office of Research Services, PO Box 1700 Stn CSC, 3800 Finnerty Road, Victoria, BC V8W 2Y2, (250) 721-7968, Fax: (250) 721-8960, November 2, 2005, http://www.research.uvic.ca/ethics/SOPs/