

 <p><b>Policies &amp; Procedures Manual</b></p>	<b># Pages:</b> <b>10</b>	<b>Policy Number:</b> <b>AN-02-05</b>
	<b>Approved by:</b> <b>UCN Animal Care Committee</b>	
<b>Section:</b> <b>Standard Operating Procedure</b>	<b>Effective Date:</b> <b>August 27, 2021</b>	
	<b>Title:</b> <b>Assessing Appropriate Endpoints</b>	
	<b>Replaces:</b> <b>New</b>	

## 1 PURPOSE

The purpose of this SOP is to help determine the appropriate endpoints of animals used in field studies at University College of the North (UCN). These endpoints are used to decide whether an animal should be removed from a study or euthanized.

## 2 RELATED DOCUMENTS

- Canadian Council on Animal Care Guidelines on: Choosing an appropriate endpoint in experiments using animals for research, teaching and testing (CCAC, 1998)
- SOP AN-02-04 Euthanasia

## 3 RESPONSIBILITIES

All persons responsible for assessing animals and determining endpoints, must be competent in evaluating the normal physiological, and behavioural state, as well as the body condition of the species under observation.

### 3.1 Principle Investigator (PI)

The PI is responsible for selecting an appropriate end-point following consultation with the designated Veterinarian and the Animal Care Committee (ACC) at UCN.

The PI has the authority to euthanize animals where selected end-points have been met.

The PI is responsible for directly supervising participants performing euthanasia.

### 3.2 Participants

Participants are responsible for attending instructional activities on end-point selection in small mammals and fish prior to participating in any field work.

Participants **MUST** notify the PI/technician that an animal is being assessed for end-points.

Participants are required to assess ill or injured animals for appropriate end-points and complete the appropriate forms.

Only under direct supervision of the PI, participants may be responsible for euthanizing animals where selected end-points have been met.

### 3.3 Animal Care Committee

UCNs ACC is responsible for providing consultation in end-point selection.

### 3.4 Veterinarian

The Veterinarian has the authority to euthanize animals where selected end-points have been met.

## 4 RECOMMENDED PROCEDURES FOR SELECTING AN APPROPRIATE ENDPOINT

As recommended by the Canadian Council on Animal Care (CCAC), pain, distress or discomfort of any animal involved in experimental or field research **must be minimized or alleviated** by choosing the earliest endpoint compatible with the scientific objectives of the Animal Use Protocol (AUP).

Objective determination of changes from an animal's normal state should be considered when defining appropriate endpoints. Some of these considerations include:

- Appropriate assessments of animals, particularly their behaviour, physical and physiological changes from expected (the norm);
- Identifying significant indicators of pain and/or distress in specific situations;
- Identifying significant indicators for further deterioration of an animal's condition and establishing the earliest point of onset;
- Meeting scientific rigour for a significant end-point

By monitoring animals and identifying clinical signs of pain, distress and discomfort, it will help to eliminate, mitigate or minimize pain and distress.

### 4.1 Scoring for end-point selection

Animals used in experimental or field protocols must be observed for changes in their physiological, behavioural, and physical states prior to setting endpoints. An understanding of characteristic behaviours in non-distressed animals is key to assessing abnormalities effectively.

During observation, the following should be assessed using the Distress Scoring Systems for small mammals and fish described in sections 4.1.1 and 4.1.2, respectively:

- Animal appearance and posture,
- Reaction to external stimulus,
- Clinical state including, but not limited to, weight, presence of any lesions or abnormalities such as tumours, provoked behaviour

Using the guidelines provided in the tables below for normal behaviour (Table 1), acute pain (Table 1) and chronic pain, any observation of unusual behaviour or signs of pain, distress and/or discomfort **MUST** be recorded on the appropriate animal-specific Distress Scoring Form and reported immediately to the PI and/or Veterinarian.

If necessary, the PI or Veterinarian have the authority to euthanize (see SOP AN-02-04 Euthanasia) any animal that is in severe pain, distress and/or discomfort.

**Table 1 Guidelines for Normal Behaviours in Small Mammals and Fish**

Expected Normal Behaviours	Animal	
	Small mammals	Fish
Smooth and uninhibited movement	Yes	Yes
Curiosity and exploration of environment	Yes	Yes
Maintenance of coat/grooming behaviours	Yes	n/a
Foraging <sup>2</sup>	Yes	n/a
Normal consumption of food and/or water	Yes	Yes
Normal growth rate	Yes	Yes
Nest Building <sup>2</sup>	Yes	n/a

<sup>1</sup>Normal behaviours are generally indicative of good health and absence of pain/suffering; <sup>2</sup>Species specific behaviours

**Table 2 Guidelines for Behaviours Associated with Acute Pain in Small Mammals and Fish**

Behaviours Experienced with Acute Pain	Animal	
	Small mammals	Fish
Lethargy <ul style="list-style-type: none"> <li>• reluctance or difficulty moving</li> </ul>	Yes	Yes
Hunching or unusual posture	Yes	n/a
Unusual positioning of the body	Yes	Yes
Restlessness, pacing, shifting weight	Yes	n/a
Recumbency <ul style="list-style-type: none"> <li>• lying down for unusual length(s) of time</li> </ul>	Yes	n/a

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Stiff gait, limping	Yes	n/a
Self mutilation • licking, biting, and/or scratching an area of the body	Yes	n/a
Irregular, elevated or depressed respiration	Yes	n/a

**Table 3 Guidelines for Behaviours Associated with Chronic Pain Small Mammals and Fish**

Behaviours <sup>1</sup> Experienced with Chronic Pain	Animal	
	Small mammals	Fish
Decreased appetite	Yes	Yes
Weight loss	Yes	Yes
Reduced activity	Yes	Yes
Irritability	Yes	n/a
Decrease in reproduction	Yes	Yes

<sup>1</sup>Behaviours may be associated with chronic pain (note the behaviours may be subtle and require careful observation)

#### 4.1.1 Distress scoring for small mammals

The *Distress Scoring System* (Appendix I) provided, **must** be used to identify the signs and symptoms of pain, distress and/or discomfort in **small mammals** for objective, humane endpoint selection. This ensures that no animal suffers unnecessarily.

When a small mammal is encountered that appears to be in pain, distress and/or discomfort, proceed as follows:

- monitor the animals identified as ill/sick for changes in health status
- complete the Distress Scoring System form
- for greater objectivity, a second person is required to complete the form
- add all scores to reach a total score using the system
- assess total scores from both individuals to determine the most objective and humane endpoint as follows:
  - i. Animals scoring 4 or less are considered normal and are to be monitored until the issues are resolved.
  - ii. Animals scoring between 5 -14 must be brought to the immediate attention of the principle investigator and/or Veterinarian.
    - a) Carefully monitor animals with scores between 5 and 9; provide treatments as directed by the veterinarian (e.g. for dehydration, administer fluids subcutaneously)

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- b) For animals scoring between 10 and 14, provide immediate relief and consult with a veterinarian; observe every 15 minutes  
     If pain and symptoms cannot be reduced, euthanasia must be considered
- iii. Animals scoring 15 or higher must be euthanized immediately according to SOP AN-02-04 Euthanasia.

**4.1.2 Distress scoring in fish**

The Distress Scoring System for Fish must be used to identify the signs and symptoms of pain, distress and/or discomfort in fish for objective, humane endpoint selection.

This scoring system ensures that no animal suffers unnecessarily, and provides the PI, technician and participants a consistent approach to determining fish fate.

When a fish is encountered that appears to be in pain, distress and/or discomfort, proceed as follows:

- a) measure fish vitality using the modified Natural Resources Management Technology (NRMT) Program Reflex Action Mortality Predictor (RAMP) scoring procedure before release (Appendix 2);
- b) Immediately prior to release, test fish for the presence or absence of the following five reflexes.
  - Tail Grab Response
  - Body Flex
  - Head Complex
  - Vestibular Ocular Response (VOR)
  - Orientation

A description for each reflex is provided in Table 4.

**Table 4 Procedures and expected responses for reflexes tested in fish.**

<b>Reflex</b>	<b>Procedure</b>	<b>Response</b>
Tail Grab Response	Attempt to 'grab the tail' of the fish with the fish submerged in water (e.g., holding tub).	A positive response is characterized by the fish attempting to burst-swim immediately upon contact. Categorize the response as unimpaired.
Body Flex	Hold the fish out of the water using two gloved hands wrapped around the middle of the body over a foamed platform.	Fish actively attempting to struggle free is characterized as a positive response. Categorize the response as unimpaired.

Head Complex	Hold the fish out of the water. Observe the opening and closing of the lower jaw.	A response is considered positive if the fish exhibits a regular patten of ventilation for approximately 5 minutes. Categorize the response as unimpaired.
Vestibular Ocular Response (VOR)	When out of the water, turn the fish on its side (i.e., on a lengthwise axis). Observe eye movement.	A positive VOR is characterized by the fish's eye rolling to track the handler. Categorize the response as unimpaired.
Orientation	Place fish upside-down in the lake just below the surface. Observe movement of the fish for establishment of equilibrium.	A positive Orientation reflex is noted if the fish rights itself within 3-seconds.  Categorize the response as unimpaired

- c) Assess each reflex categorically (Table 4) using a conservative approach and record results in as follows:
- i. Unimpaired
    - Assigned a zero (0) value if:
      - after testing an expected normal reflex is observed
      - the fish is too vigorous to allow student handling and assessment
  - ii. Impaired
    - assign a value of one (1) if:
      - after testing there is no indication of a reflex
      - the handling participant is unsure whether the reflex is present
- d) Based on the assessments performed in 4.1.2c, calculate a RAMP score.
- e) Using the RAMP score, determine the end-point. If euthanasia is indicated, follow SOP AN-02-04.

## 4.2 PILOT STUDIES

For studies where the effects of the treatment on the animals are unknown, investigators are encouraged to use a pilot study. A small number of animals are used to determine morbidity, frequency of observations required and how long after the treatment symptoms / signs could potentially develop.

By utilizing a pilot studies, PIs can compile data necessary to determine appropriate end points for the proposed study.

## **APPENDIX I     DISTRESS SCORING SYSTEM – SMALL MAMMALS**

In the event that a small mammal is exhibiting pain, distress and/or discomfort, the following form must be completed to determine the most objective and humane end-point for the animal.

The form is completed by scoring specific parameters related to the physiological, physical and behavioral state of the animal. Parameters include appearance, food and water intake, hydration status, specific clinical signs, natural and provoked behavior. The following must be taken into consideration when scoring:

### **Normal behaviours**

Normal behaviours are generally indicative of good health and absence of pain/suffering; these behaviours include:

- a) Smooth and uninhibited movement,
- b) curiosity and exploration of environment,
- c) maintenance of coat / grooming behaviours,
- d) normal consumption of food / water and normal growth rate,
- e) interacting with cage mates (if present),
- f) and species-specific behavior (e.g. for mice: nest building, foraging)

### **Acute pain**

The following behaviours are associated with acute pain:

- a) Lethargy – reluctance or difficulty moving
- b) Hunching, unusual posture or positioning of body
- c) Restlessness, pacing, shifting weight
- d) Recumbency - lying down for unusual length(s) of time.
- e) Stiff gait, limping
- f) Self-mutilation – licking, biting, scratching an area of the body
- g) Irregular, elevated or depressed respiration

### **Chronic pain**

The following behaviours may be associated with chronic pain (note the behaviours may be subtle and require careful observation):

- a) Decreased appetite
- b) Weight loss
- c) Reduced activity
- d) Irritability
- e) Decrease in reproduction

DISTRESS SCORING FORM – Small Mammals

Parameter	Variables	Score
Appearance	0 – Normal 1 - General lack of grooming 2 - Coat staring, ocular and nasal discharge 3 - Piloerection, hunched up 4 - Above and eyes half closed	
Food and Water Intake	0 - Normal 1 - Uncertain intake: weight loss <5% of BW 2 - Uncertain intake; weight loss 5-15% of BW 3 - No food or water intake (check for malocclusion)	
Hydration Status	0 - Normal 5 - Abnormal skin pinch test	
Clinical Signs	0 - Normal respiratory rate and pattern 1 - Slight changes, increased rate only 2 - Increased rate with abdominal breathing 3 - Decreased rate with abdominal breathing 4 - Marked abdominal breathing and cyanosis	
Natural behaviour	0 - Normal 1 - Minor changes 2 - Less mobile and alert, isolated 3 - Restless, not alert, vocal, self-mutilating or still	
Provoked behaviour	0 - Normal 1 - Minor depression or exaggerated responses 2 - Moderate change in expected behaviour 3 - Reacts violently, or very weak and pre-comatose	
<b>SCORE TOTAL =</b>		

Using the table below, assess what actions are to be taken based on total score using the system.

Total Score	Actions to be Taken
0 - 4	Normal
5 - 9	Monitor carefully. Consult Veterinarian for treatments (e.g. for dehydration, administer fluids subcutaneously); consider analgesics.
10 - 14	The animal is considered to be suffering. Consult Veterinarian



	Provide relief, and observe every 15 minutes. C If pain/symptoms cannot be reduced, consider euthanasia.
15 - 22	The animal is assessed to be in severe pain. Immediately euthanize animal according to SOP 3 Euthanasia. Consult with the Veterinarian and UCNs ACC chair for reconsideration of the protocol.

### Distress scoring system – Fish

In the event a fish is exhibiting pain, distress and/or discomfort (i.e., via stressors) the Natural Resources Management Technology (NRMT) Program ‘Reflex Action Mortality Predictor’ (RAMP) Form must be completed to determine the most objective and humane end-point for the animal.

Reflex Action Mortality Prediction (RAMP) is an easy-to-use and inexpensive field-based assessment tool that measures fish vitality before release.

Fish are assessed by checking for the presence and absence of stressors using multiple (n=5) reflexes identified to be consistently present in vigorous fish. The five (5) reflexes examined include the:

- Tail Grab Response
- Body Flex
- Head Complex
- Vestibular Ocular Response (VOR)
- Orientation

The Tail Grab and Body Flex both involve use of white myotomal musculature innervated by local motorneurons. These reflexes are likely to be impaired as a result of white muscle exhaustion (e.g., high lactate loading rather than neurological dysfunction).

The Head Complex (or the pattern of regular ventilation) and VOR are functions of the autonomic nervous system (ANS) but are included in this modified RAMP index to help research fish handling variation in students. Neurological control of respiration in fish is complex and likely originates in the brain where neurons discharge rhythmically in time with respiration and the proximate motor nerve (i.e., trigeminal fifth) controlling the opening and closing of the lower jaw in fish. The VOR reflex occurs when static signals in the ‘otolith’ reach the extraocular motoneurons; and is analogous to ‘ocular counter-rolling’ in humans. Note that VOR impairment has been observed in fish exposed for more than 3 minutes.

Equilibrium loss in fish is caused by a combination of broader neural and muscle function breakdown, but more specifically at the fins involved in controlling balance (i.e., pectoral fins).

Complete Table 5 by using a modified reflex impairment index (pers. com. Stepaniuk, 2021) to score specific reflex actions involving different neurological pathways and/or muscle

groups. Table 5: Species, Index Reflex, RAMP Score and teleost fate, Naosap Lake, September, 2021.

**Table 5 Index Reflex Assessment Tool (modified RAMP)**

		INDEX REFLEX										
Fish Number	Species	Tail Grab	Body Flex	Head Complex	VOR	Orientation (seconds)	RAMP Score	Total Air Exposure (seconds)	Unimpaired	Impaired	Released	Euthanized
1												
2												

\*NB: Length, weight, gender, stomach contents and cyst count are documented elsewhere.