Curtin University Standard Operating Procedure

FISH DISSECTION AND BIOPSY COLLECTION

Number: CARL 08
Version: 2
Date: 11/06/2015

Author: Dr Monique Gagnon, Rowan Kleindienst
Date of Approval: 11/06/2015
Reviewed:

DATE	11/10/2016				
REVIEWER	Dr Tara Pike; Rowan Kleindienst				

Aim/Purpose: To provide directions for investigation into adverse or unexpected events in a project approved by the Curtin University Animal Ethics Committee (AEC) and held at the Curtin University Aquatic Research Laboratory (CARL).

Definitions:
AEC: Animal Ethics Committee

Moribund: An animal displaying signs of dying or near death. For example fish swimming erratically or stationary, loss of equilibrium, rapid or slow ventilation, no response to external stimuli.

Necropsy: An examination and dissection of a dead fish to determine cause of death or the changes produced by disease

Ice Slurry: please refer to SOP CARL 05

Diagnostic Investigation:

The procedure to follow will be directed by the approval granted by the AEC. A necropsy may be required to be undertaken by the researchers, or an external necropsy may need to be carried out by the Department of Fisheries.

Initiate when moribund fish are observed, mortality levels are elevated/abnormal, outside of procedures approved by the AEC.

External Necropsy Procedure:

Fish found ill or moribund:

1. Deliver a live sample selection of any ill or moribund fish (2-3 fish is adequate) directly to Department of Fisheries, Fish Health Unit (1 Baron Hay Court, Kensington, Animal Health Lab Reception in C-Block), Monday – Friday 9am – 3pm.

   It is possible that the Fish Health Laboratory will perform blood sampling and bacteriology on the live fish provided. Bacteriology may also be performed on freshly dead specimens.

   If the fish are **smaller than 20g**, they can be transported in culture water within a double plastic bag inside a polystyrene box with the air space filled with pure oxygen.

   If fish are **larger than 20g**, they can be transported in an esky, the water saturated with pure oxygen, or a portable aerator attached to the esky for the duration of the journey.

Fish found dead in tank

1. If one fish is found dead, do not freeze the carcass, as a post mortem / necropsy is required.
2. If more than one fish is found dead, and others are looking ill in the tank, collect the dead fish and put into the fridge (DO NOT FREEZE). Collect some ill/ moribund fish and take directly to Department of Fisheries, Fish Health Unit (1 Baron Hay Court, Kensington)

CARL Necropsy Procedure if Live Fish Cannot be Taken Immediately to Fish Health i.e. weekend or after business hours

1. **Collect 5 freshly dead fish (need to be very fresh i.e. less than 5 minutes dead, as autolytic changes will render samples unsuitable for analyses) OR** anesthetise 5 moribund fish in AQUI-S at a dose rate of 175mg/L for 20 minutes (refer to SOP CARL01 Euthanasia of Fish) and individually tag for later identification.

2. **Visual Assessment** of condition of each fish. If either an ulcer, reddening underneath operculum or of junction between gills and body or tissue necrosis is detected, make a note or take a photo.

3. If necropsy is unable to be performed immediately, **place fish into a plastic bag in an ice slurry**. This is to slow autolytic changes if there is a slight delay in the necropsy.

4. **Morphometric Measurements** of fish to include fork length, width, height and weight of individual fish. Use these measurements to calculate a condition index. Note any physical malformations

5. **Gill Tissue:** Dissect out 1st gill arch and prepare gill scrape and view under a compound microscope. Dissect out 2nd gill arch and initially view under dissecting scope then prepare a slide using a section of this material and view under compound microscope. These methods will detect for presence of hyperplasia, epitheliocystis, gill fluke or blood fluke eggs.

6. **Skin Scraping:** Use the back of a scalpel blade to remove a section of mucus from the skin especially near skin lesions. Place onto a microscope slide with a cover slip and a drop of water, and observe under compound microscope. Look for the movement of live skin parasites. If possible, take a photo of the parasite.

7. **Sampling Organs for Histology:** Dissect out each organ including tissue from the heart, liver, spleen, gonads, kidney, muscle, stomach, hindgut and brain. Look for obvious abnormalities. Take weights of the liver and gonads (if mature) and visually assess their condition. Photos of fresh organs may assist with diagnosis.

8. **Histology Examination:** Preserve tissues extracted from **Step 7** in 10% neutrally buffered formalin and send preserved tissues to Fish Health Unit for histological analyses and subsequent report.

9. **Examine Records of Water Quality and Husbandry Activities:** Fluctuating water quality parameters and handling may be a trigger for mortality, especially if there are underlying factors such as high bacterial loads in the culture water.

10. **Submission of Adverse Event Report** to the AEC within 24 hours of being aware of the events. Depending on the prognosis, symptoms and severity, the population of fish may be required to be culled.