

## 10.1 PROTOCOL FOR IN-SITU SALMONID EGG BIOASSAYS

### Overview

Developing embryos have periods of extreme sensitivity when their susceptibility to foreign toxicants is maximal. Fish embryos are no different with extensive data from life cycle toxicity testing to support this. The method described below is based upon starting the bioassay with Pacific Salmonid eggs that have just come into the "eyed stage" of development (the stage between the time the eyes become visible and hatching occurs). This stage of development guarantees that the eggs have been fertilized and that the embryos are hardy enough to be handled and transported. Developmental stages prior to this are extremely sensitive to handling and transportation; mortality rates associated with these factors are very high.

### Sources

British Columbia MWLAP (2003)

### At a glance

*100 eggs  
per box*

*computer  
program*

**1** Fill egg boxes in the laboratory prior to traveling to the field location; 100 eggs/box. Use a beverage cooler to transport charged boxes to the deployment site. Keep eggs out of direct sunlight since the duration (exposure period) and development of eggs are subject to temperature.

**2** Different salmonid species have different rates of development. A computer program, "SALMONID INCUBATION PROGRAMME, VERSION 1.3, DFO, PBS NANAIMO, BY J.O.T. JENSEN", is an excellent way to estimate the study exposure period. The bioassay can have several end-points. The most common end-point is to terminate the study after controls hatch or, the study can be extended with continuing the exposure until the control alevins "button up". The latter will provide a longer exposure period; however, maximum exposure should be to the development stage of "yolk sac absorption or buttoning up" (terminate the bioassay).

**3** Excavate the site location to a level and position that ensures the wire cage is slightly angled up current. Ensure that when the egg box is positioned inside the wire cage, it is level with the stream bed-water interface. Ensure that the location will always have water flowing over the eggs.

**4** Place the egg box in the middle of the wire cage and add clean spawning size gravel. (Customized neoprene divers gloves work well for holding the egg box in place while gravel is poured into the cage.)

**5** Close the wire cage and seal it with wire or nylon zap straps once the egg box is totally covered with gravel. Use a minimum of 3 boxes per site.

**6** Measure field parameters such as temperature, dissolved oxygen and pH each time the eggs are checked. Record water

flow rates and climatological factors. Place control boxes in conditions (temperature, depth, light) as similar to test boxes as possible.

**7** Designate one cage at each site as the "check cage". This cage should be the only one observed during the weekly monitoring of the study. When checking these eggs, shield them from direct sunlight. The checking should be conducted as quickly as possible. If a longer time (10+ minutes) is required, keep eggs moist.

**8** Record mortality and any other pertinent observations in field note book.