The American Veterinary Medical Association (AVMA) Guidelines for Euthanasia \(^1\) state that rapid chilling (hypothermic shock) in water of 2\(^\circ\) to 4\(^\circ\) C for small bodied (3.8 cm long and smaller) tropical and sub-tropical species is an acceptable method of euthanasia for these species.

The Principal Investigators, in consultation with a ULAR Veterinarian, have devised a strategy to best accomplish euthanasia for this species that meets their needs.

This strategy **must** include the following considerations:

1. Ice that is used to cool the euthanasia tank must not come in direct contact with the zebrafish. Direct contact may induce pain or distress. **There must be a physical barrier between the zebrafish and the ice.**
2. The water temperature in the euthanasia tank must range between 2\(^\circ\)C to 4\(^\circ\)C.\(^3\)
3. Zebrafish must be transferred between the holding tank and the euthanasia tank by net or other device to minimize the amount of “warm” water transferred to the chilled tank.
4. Stocking density of zebrafish in the chilled euthanasia tank may not exceed a total of 40 zebrafish per 1 L of chilled water.
5. Death must be confirmed by decapitation or by maintaining the zebrafish in the chilled water for at least 5 minutes.
6. Euthanasia of zebrafish fry (4-7 days post fertilization) **requires** a two-step method: (1) placement in chilled water as described above, with an additional 5 minutes after cessation of movement; **followed by** (2) addition of a chemical agent (5.75% Sodium Hypochlorite at a ratio of 1 part Sodium Hypochlorite to 5 parts water) to ensure death.\(^2\)

The University of Pennsylvania IACUC approves rapid cooling as a form of euthanasia for zebrafish (*Danio spp.*). The IACUC also continues to acknowledge and accept other methods to euthanize fish as described in the AVMA Guidelines on Euthanasia (2013).

**References**


**APPROVED: May 2013**