Policies and Procedures

SECTION: IACUC	NUMBER: 7.34			
CHAPTER: Animal Use	ISSUED: 6/2019	REV. A:	REV. B:	REV. C:
POLICY: Use of Zebrafish	REV. D:	REV. E:	PAGE 1 OF 4	

Purpose

The purpose of this policy is to define the acceptable use of zebrafish at Creighton University. This procedure is approved by the Creighton University Institutional Animal Care and Use Committee (IACUC). All investigators will follow this policy unless scientific justification is provided and approved by the IACUC.

Background

Current OLAW interpretation of PHS policy considers aquatic species as “live, vertebrate animals” at hatching. This is approximately 72 hours post fertilization (3 days post fertilization (pdf)). All stages of development greater than 3 dpf must be described in an approved Animal Protocol.

Policy

To account for the possibility of early hatching, embryos at 2 dpf will be monitored for signs of early hatching. Investigators will account for an approximate percent of embryos that hatch early using a specific log sheet for an incubator housing larvae.

Because hatching before 3dpf might be a sign of accelerated development which might hasten the sensation of pain or distress that typically begins at 8dpf, embryos that hatch early will not be used for experimental protocols at 7dpf without anesthesia.

Zebrafish that are greater than 8dpf will have pain and distress category determined and listed for specific procedures in an animal use protocol.

Embryo experiments that include microinjections, cell transplantations, or small molecule treatments will be strictly limited to early embryos and ALL will be euthanized by 3dpf unless the larvae are included in IACUC-approved protocol.

Embryos for classroom-based research projects will be transported to the school and adopted or euthanized by 3dpf. (See Fish Transfer Agreement)

Daily Health Checks

ARF staff and the attending veterinarian will begin daily health checks once animals are housed on system, generally greater than 10dpf. Principal Investigators and their staff will perform health checks a minimum of once daily on all fish and will inform ARF staff and the attending veterinarian of any health concerns of animals greater than 3dpf house off system.

Census

Due to a large variation in survivability only fry greater than 28 dpf will be counted against the approved animal numbers on a protocol. In addition, determination of an exact number of fish is difficult without handling them and causing undue stress that affects the health and welfare of the fish. An average census count will be determined based on tank volume and recommended densities

	Maximum recommended density for adult fish, non-breeding Fry≥ 28dpf (The Guide pg. 83)	Average census Fry≥28dpf
*1L tank	5	2
2L	10	5
*3L	15	12
6L	30	25
*10 L	50	40

*Current ARF System tanks

Genotyping

Genotyping via fin clipping should be performed in a manner that prevents injury. Animals must be anesthetized using an appropriate approved method. A maximum of 2 mm of fin tissue can be removed.

Euthanasia

Recent observations indicate that zebrafish up to at least 15 dpf can survive anesthetic overdose and rapid chilling even after prolonged absence of heartbeat. They can revive if returned to water that is within their normal environmental parameters. An adjunct method such as sodium hypochlorite treatment should be used to ensure death in embryos less than15 dpf.

Similarly, embryos less than 3 dpf that are being disposed should be treated with sodium hypochlorite to prevent further development.

Euthanasia of zebrafish must be carried out by the following methods.

1. For zebrafish greater than15 dpf the following methods are acceptable for euthanasia:

   • Immobilization by submersion in ice water (5 parts ice/1 part water, 0-4º C) for at least 10 minutes following cessation of opercular (i.e., gill) movement. In any fish where it is difficult to visualize opercular movement, fish should be left in the ice water for at least 20 minutes after cessation of all movement to ensure death by hypoxia. **Fish must not come in direct contact with the ice.**

   • Overdose using pharmaceutical grade Tricaine Methane Sulfonate (MS222, 200-300 mg/l) by prolonged immersion. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement. MS-222 solution should be buffered with sodium bicarbonate to a neutral pH before immersing fish. Non-buffered MS-222 is acidic and causes an aversive reaction in unanesthetized fish.

• Anesthesia with Tricaine Methane Sulfonate (MS222, 168 mg/l) followed by rapid freezing in liquid nitrogen.

2. For zebrafish larvae up to 8-15 dpf: a secondary method must be used in order to ensure death. Use of the ice water or MS-222 method as above should be used as a method of anesthesia/immobilization. An acceptable secondary method is the addition of bleach solution (sodium hypochlorite 6.15%) to the culture system water at 1 part bleach to 5 parts water. The larvae should remain in this solution at least five minutes prior to disposal to ensure death.

3. For embryos less than 7 dpf, development should be terminated using bleach as described above. Pain perception has not developed at these earlier stages so this is not considered a painful procedure.

4. Additional methods can be used if approved by the IACUC:

- Clove Oil (Eugenol, Isoeugenol) as an alternative to MS-222. AVMA Guidelines recommend products with standardized known concentrations of essential oils (eugenol, isoeugenol) be used so accurate dosing can occur. Clove oil and eugenol products are described in the AVMA Guidelines as "acceptable agents of euthanasia for finfish." They are not available in an FDA approved form but there is at least one commercial form available in the U.S. (Aqui-S) as an Investigational New Animal Drug.
- Decapitation with a sharp blade by a trained individual. **This must be followed by pithing.**
- Anesthetic overdose or rapid chilling by submersion in ice water followed by fixation in paraformaldehyde or other fixative
- For embryos greater than 8 dpf: immersion in paraformaldehyde or other fixative.
- For embryos greater than 8 dpf: rapid freezing in -70 freezer. Embryos should be contained in a minimum amount of water to ensure rapid freezing and death.
- Maceration using a well-maintained macerator designed for the size of the fish being euthanized.

Zebrafish carcasses from any of these methods should be disposed of as Medical Pathological Waste according to Creighton University Policies. Zebrafish tissues, whole fish or embryos are never disposed of through the municipal water system.

These methods ensure death provided the timeframes above are followed. The ice water method should not be extrapolated to other aquatic species without first confirming the effectiveness for that species. Aquatic species, native to a colder environment than zebrafish, may be more resistant to hypothermic shock and may recover subsequently.

References

1. National Institutes of Health (2009) Final Report to OLAW on Euthanasia of Zebrafish.
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.
3. Guide of the Care and Use of Laboratory Animals, Eighth Edition

4. Borksi RJ, Hodson RG. 2003 Fish research and the institutional animal care and use committee. Institute for Animal Laboratory Research Journal 44(4):286-94
5. PHS Policy on Humane Care and Use of Laboratory Animals Retrieved from http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf
6. UAB Institutional Animal Care and Use Committee Standard Operating Procedure Zebrafish