University of Colorado Denver | Anschutz Medical Campus

IACUC
Institutional Animal Care and Use Committee

Subject:	**Zebrafish Housing and Euthanasia**
Source:	Institutional Animal Care and Use Committee (IACUC)
Effective Date:	08/11/2025
Replaces:	12/11/2023
Applies to:	Personnel involved in Research or teaching studies involving animals.
Reference:	Animal Welfare Act; PHS Policy on Humane Care & Use of Laboratory Animals. Guide for the Care & Use of Laboratory Animals

The Institutional Animal Care and Use Committee (IACUC) maintains oversight review for federally mandated rules and regulations with regard to animal research, ethics, misconduct and biomedical research for the University of Colorado Denver | Anschutz Medical Campus (CU Denver | Anschutz). Zebrafish are an important research model with unique housing and care requirements compared to terrestrial animals.

Policy Statement

This policy provides information on the housing and care of zebrafish at CU Denver. Per guidance from the National Institutes of Health (NIH), Office of Laboratory Animal Welfare (OLAW), zebrafish are regulated research animals once they have hatched, approximately 3 days post-fertilization (dpf).[1,2,3] Therefore, zebrafish are overseen by the IACUC beginning at 3 dpf. Zebrafish embryos, larvae, and adults are maintained in a centralized facility. Research labs may also house zebrafish embryos and larvae in IACUC-approved satellite facilities. The Office of Laboratory Animal Resources (OLAR) works closely with laboratory staff to ensure excellent care is provided for zebrafish at all life stages.

Zebrafish numbers:
IACUC protocols must describe the use of zebrafish and justify the number of fish needed to achieve research objectives. To provide a complete narrative of animal work, experiments using zebrafish embryos < 3 dpf should be briefly summarized in the IACUC protocol, but should not be factored into the animal numbers calculations. Good faith estimations of fish ≥ 3 dpf used in research activities, including breeding, are reported to the IACUC as requested.

Satellite zebrafish facilities:
Prior to housing ≥ 3 dpf zebrafish outside the central facility for ≥ 24 hours, researchers must submit a satellite facility application to the IACUC Office and arrange inspection of proposed housing space.

- To establish a satellite, labs must:
  - Provide adequate justification, to be reviewed and approved by the IACUC.
  - Provide a temperature-controlled environment with a diurnal light cycle and all needed animal care.
  - Conduct and document daily health and facility checks while ≥ 3 dpf zebrafish are present (including weekends/holidays).
- OLAR veterinarians arrange regular visits to ensure standard of care for fish ≥ 3 dpf in satellites.
- Labs ensure safe transport of zebrafish embryos, larvae, and adults between lab areas and the fish facility:
  - To minimize risk of spills, primary and/or secondary containers must be sealed
  - Primary containers, such as petri dishes, conical tubes, or fish tanks must be contained in a water-proof hard-sided or Styrofoam container consistent with EHS Guidelines for Transportation of Biological Materials.
  - Minimize transit time, time out of incubator, and avoid public areas when possible. Use freight elevators.
  - See the Animal Transportation policy for approved routes between buildings on campus.

Housing density:
Zebrafish are a social species and live in groups of varying sizes in the wild. As per the Guide for the Care and Use of Laboratory Animals, zebrafish should be housed in social groups.[4] Aggression between zebrafish increases with small group sizes, therefore, it is recommended to house zebrafish in groups of at least four fish.[5,6]

- **Individual and pair housing:** Long-term individual or pair housing is discouraged. Companion fish with a different physical appearance (e.g. fin or pigment mutants) are encouraged for maintaining valuable individuals.
    - As an important exception, fish may be separated individually while genotyping (less than 1-2 weeks) or paired overnight for breeding.
    - Singly-housed fish should be in visual contact with other fish in the tank next to them.[7]
    - Female zebrafish can become egg-bound without regular breeding opportunities (every 2-4 weeks is recommended).
    - In-tank enrichment objects (e.g. non-toxic artificial aquarium plants) may be used to provide a visual barrier to reduce fighting at low housing densities.[6]

- **Maximum allowed housing density:** The Guide for the Care and Use of Laboratory Animals recommends a maximum stocking density of 5 adult zebrafish per liter of water, though it notes that this number may change as our knowledge about zebrafish welfare advances.[4,8-10] More recent publications have not found significant differences in growth and/or breeding performance in zebrafish housed in modern systems at higher densities, suggesting these may also be appropriate.[11,12] Hence, the CU Denver | Anschutz IACUC recommends a housing density of 5-7 adult zebrafish per liter, but up to 12 adult zebrafish per liter may be permitted as long as no animal welfare issues are identified.

Tank Size	Maximum number of adult fish
1.4 Liter	16
1.8 Liter	21
3 Liter	36
6 Liter	72
9 Liter	108

Static tank housing: Fish may be held in static tanks for breeding, genotyping, and sperm collection.

- Feeding and water change frequency: Fish should be fed sparingly when housed in static tanks, as water quality will rapidly degrade. Limit provisions to approximately 3-5 grains of food per fish and change the water 2-24 hours after feeding.
- Duration of static housing: Time spent in static housing should be minimized, as rapid water quality changes can stress the fish and lead to declining health. If static housing may be needed for longer than 7 days, researchers should work with the fish care team to find an alternative solution.

Tricaine methanesulfonate (MS-222) preparation, storage, and disposal:

- Tricaine methanesulfonate (MS-222) is available as a pharmaceutical-grade compound for fish anesthesia and euthanasia. As for other species, the use of non-pharmaceutical-grade anesthetics must be justified in the IACUC protocol.
- MS-222 in its powdered form is a respiratory irritant. Researchers working with this agent should take precautions, including use of a certified fume hood, respirator, or other methods recommended by Environmental Health and Safety (EHS).
- Stock solutions of MS-222 must be appropriately labeled with the date created and may be stored frozen for up to six months.
- Working solutions of MS-222 must be buffered to neutral pH to prevent irritation when fish are immersed in the solution. Working solutions should be stored protected from light for a maximum of 30 days. Discard and replace working solutions if precipitate or color change occurs. See the Use of Non-pharmaceutical Grade Chemicals and Compounded Pharmaceutical Grade Drugs Policy for general guidance on labeling and storage.
- MS-222 is considered a hazardous chemical and must not be disposed via sink. MS-222 of all concentrations must be collected in a registered satellite accumulation area and disposed through the EHS chemical waste management system.

Euthanasia:

- Acceptable methods of euthanasia are described in the AVMA Guidelines on Euthanasia[7] and must be included in all IACUC protocols.
- Adult fish euthanized by immersion methods must be monitored for spontaneous movement (e.g. opercular movement, "gilling").
    - Ice water bath duration: At least 10 minutes after the last opercular movement.[13-15]
    - MS-222 immersion duration: At least 30 minutes after the last opercular movement.[13]
    - Carcasses of adult fish should be frozen and disposed through OLAR.
- Fish embryos and larvae are more tolerant of chemical or temperature changes and therefore require longer exposures and a secondary method to ensure euthanasia. Primary methods include 20-30 minute immersion in high-dose buffered MS-222 or ice water bath, followed by freezing or immersion in a 1:5 bleach solution as a secondary method. Euthanized embryos and larvae must be collected for proper disposal through OLAR or EHS red bin waste. Removal of euthanized zebrafish embryos and larvae from immersion baths is facilitated by use of a fine-mesh tea strainer lined with a kimwipe, cheesecloth, or paper towel to be disposed with the carcasses through OLAR or EHS.

Exceptions to this policy will be considered by the IACUC on a case by case basis on presentation of adequate scientific justification.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at UC Denver might be temporarily suspended or even completely revoked.

References:

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12. Rabbane G., Rahman M., and Hossain A. (2016) Effects of stocking density on growth of zebrafish (Danio rerio, Hamilton, 1822). Bangladesh J. of Zool. 44(2): 209-218.

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15. Wallace CK, et al. 2018. Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio). J. A. Assoc Lab Anim Sci, vol 57(1)