

Algoma University Animal Care Committee	
#AU 0037 ZEBRAFISH HOLDING	
Issue date: May 8, 2025	Last revised: May/2025

1. Purpose

This Standard Operating Procedure (SOP) describes specific procedures for rearing zebrafish of different developmental stages, from embryo to adult. See SOP 0032 FISH HUSBANDRY for additional details related to holding fish such as water chemistry and what is required during daily observations.

There are 5 different developmental stages of the zebrafish life cycle:

- Embryo: 0 – 72 hours post-fertilization
- Early larvae: 3 – 12 days post-fertilization (dpf)
- Mid larvae: 14 – 29 days dpf
- Juveniles: 30 dpf to 3 or 4 months of age
- Adults: 3 or 4 months and older

Fish at each stage of development have different rearing requirements. Please note that these time frames are dependent upon their holding temperature. The timeframes given above are for rearing at 28°C.

NOTE: as per the [CCAC guidelines on: the care and use of fish in research, teaching and testing](#); states that “Fish embryos or larvae that have not developed beyond exclusive reliance on their own yolk nutrients are not covered by the guidelines.” Therefore, animal numbers are only counted once the animals have begun to exogenously feed in the early larvae stage. For zebrafish, larvae can feed 5 dpf when held at 28°C as per CCAC Guidelines on: Zebrafish and other small, warm-water laboratory fish. Zebrafish larvae can obtain nutrition from their yolk sac until 7 dpf, meaning there is an approximately 2-day period in which to get the fish on feed before mortalities occur.

NOTE: The highest mortality rate for zebrafish is during the transition from relying on their yolk sac to exogenously feeding, and can be 15-20% (Hernandez, 2018). It is essential to ensure animals are fed and monitored multiple times a day during this period.

2. Policy

CCAC guidelines and regulations

CCAC guidelines on: Zebrafish and other small, warm-water laboratory fish

CCAC guidelines on: the care and use of fish in research, teaching and testing

SOP 0032 Fish Husbandry

3. Responsibility

Principal investigator, their research staff, and their student investigators

4. Training Required

Animal Research Ethics Modules & Completion of Core Stream Quiz

Handling/training in the relevant species

Bio-methodology training in the relevant technique and species

[AU SOP 0032 FISH HUSBANDRY](#)

5. Materials Required

Commercial fish food

- Different developmental stages require different sized pellets
- This SOP uses GEMMA fish food; however, other suppliers are acceptable provided they are sized correctly and are for zebrafish specifically

Artemia nauplii, hatched from commercially available eggs

Nets appropriate to the size of fish

Petri dishes (100 x 20 mm), small beakers, or other vessels for embryonic zebrafish

3-5 L tanks for larval fish

Tanks for adult fish

6. Procedures (See SOP 0032 Fish Husbandry for general considerations, below are conditions specific details for zebrafish)

6.1. Fish density

- Density is dependent upon life stage and holding conditions, embryos can be held at 30 embryos per 100 x 20 mm petri dish (or other shallow vessel) and adult zebrafish can be held at 5-8 fish/L

6.2. Tank selection

- Holding vessels are dependent upon the life stage
 - Embryos are held in a petri dish or another shallow vessel, which can be then placed into a larger tank that is heated to maintain 28°C
 - At 5 dpf, fish are transferred to nursery tanks, which are small 3-5 L tanks held static (i.e., no recirculation)
 - Fish of all ages are held at 28°C and on a natural light cycle

6.3. Housing and Feeding

- Embryos
 - A maximum of 30 embryos per 100 x 20 mm petri dish is recommended to prevent overcrowding. Embryos can also be held in beakers held within heated tanks to ensure embryos are maintained at 28°C
 - Zebrafish will hatch out at 2-3 dpf; however, they hatch with a yolk sac which will sustain them 2-5 days, during this time they do not need to exogenously feed
- Larval fish (5 dpf to 1 month)
 - Zebrafish will start to exogenously feed at day 5, meaning at this point the animal numbers are to be tracked
 - At 5 dpf fish are moved to static holding in a small volume tank (3-5 L). Each tank needs to be carefully monitored with 3-4 water changes being done each week to ensure a healthy environment. See SOP 0032 FISH HUSBANDRY for details on water quality measurement.

- Fish are fed small amounts of Gemma 75 twice daily for a 5-minute satiation (i.e., fish are allowed to consume as much food as they want in 5 minutes), after which any excess food is removed to avoid fouling of the tank
- At 15 dpf, fish are transferred to a Gemma 150 diet in the morning and brine shrimp, *Artemia nauplii*, once in the afternoon
- Juvenile fish (1 month of age)
 - Fish are transitioned into a Gemma 300 diet once in the morning and *Artemia nauplii* in the afternoon until the adult stage
 - At this stage fish can be transferred to holding with a recirculating system (i.e., a tank with a filter unit). Fish can also be held in static holding provided water chemistry can be maintained
- Adult fish (3 – 4 months of age)
 - At this stage fish can be held at a density of 5-8 fish/L in a tank with a filter unit
 - Fish are fed twice with a combination of Gemma 300 and frozen bloodworms or artemia

7. References (if applicable)

Hernandez, RE, Galitan, L, Cameron, J, Goodwin, N, Ramakrishnan, L (2018). Delay of Initial Feeding of Zebrafish Larvae Until 8 Days Postfertilization Has No Impact on Survival or Growth Through the Juvenile Stage. *Zebrafish*. 15(5), 515-518.

This SOP is based partly on the FAR-09 Zebrafish Husbandry SOP from the University of Ottawa.

REVISION HISTORY		
Revision #	Revision Date	Summary of Changes
1	May 8, 2025	Overall SOP is complete with just some minor edits and clarification around training modules.
2		
3		
4		