



University Animal Care Committee Standard Operating Procedure

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15.2

Subject:
Feeding of Zebrafish

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Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for the feeding of various life stages of zebrafish at Queen's University.

1) Introduction and Definitions:

- Dpf: days post fertilization
- RO water: Reverse osmosis water
- Zebrafish embryo (fry) ≤ 5 dpf
 - Feeding of zebrafish < 5 dpf is not required as they subsist entirely on their yolk sac
- Zebrafish larvae: early larvae 6-13 dpf, late larvae 14-29 dpf
 - Zebrafish in these life stages require a nearly continuous source or high volume of diet rich in protein, adequate portions of lipids, with minimal carbohydrates, to help meet growth and energy requirements.
 - This is best achieved by live feed or a combination of a live food source and a commercially available zebrafish food.
- Zebrafish juvenile 30-90 dpf and zebrafish adult > 90 dpf
 - Although not ideal for growth, adult zebrafish can subsist entirely on a commercially available zebrafish diet.

2) Materials:

- Commercial larval/juvenile stage zebrafish feed (GEMMA 75 and GEMMA 150)
- Commercial zebrafish adult feed (GEMMA 300)
- Compact Culture System (Reed Mariculture, Campbell, CA)
- Live rotifers
- Source of RO water used to make salt-water solution
- Salt water (made with Instant Ocean, as per manufacturer instructions)
- Air source (Whisper Aquarium Air Pump, Tetra, Blacksburg, VA)
- Rotifer food (RG Complete, Reed Mariculture, Campbell, CA)
- 40 μ m screen
- 60 ml syringe
- Techniplast Zebrafish Aquatic system

3) Procedures:

Setting up and maintaining the rotifer cultures used to feed all life stages of zebrafish

- Assemble the Compact Culture System, as per manufacturer instructions.
 - Add 14L salt water, with a salinity of approximately 20 ppt and temperature 25-28 °C.
 - Place the purchased bag of live rotifers (still inside the bag) into the bucket, to slowly acclimate the rotifers to the temperature of the water. After about five minutes, open and empty the bag into the bucket.
 - Add 11ml RG Complete to the bucket. After initial set up, do not harvest rotifers for 2-3 days.
 - After the rotifer culture has acclimated over the 2-3 days feed them 7 ml of RG Complete twice daily (0.5 ml/L of rotifer culture).
 - If a large volume of rotifers is needed to feed the zebrafish, set up a rotifer subculture. The subculture will be a 3.5L jug of a 5ppt saline solution of RO water and Instant Ocean Salt with its own air pump. Feed the subculture population 3ml of RG Complete twice daily.
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Feeding of zebrafish larvae (2x/day):

- At 5 dpf, transfer the desired number of viable early larvae to 3.5L tanks that contain 1L of system water. These tanks are marked to indicate there are larvae present in them. They are not hooked into the recirculating system at this point. Begin to feed 80ml of rotifer solution to each tank twice daily making sure to disperse the volume of rotifers across the tank. During each feeding add additional system water to each tank at 2-3 drops/second. There will be a grid on the side of the tank indicating the amount system water to add.
- At 10 dpf, GEMMA 75 commercial zebrafish dry diet is fed at noon and rotifers continue to be fed in the am and pm, 80ml each time. Water is now added once daily at 2-3 drops/second to the mark indicated on the tank.
- At 15 dpf, the GEMMA 75 is increased to two feedings per day and the rotifer feedings are decreased to once a day. The volume of rotifer solution fed is maintained at 80ml once daily at noon. The tanks are now placed on the recirculating system at a flow rate of 1 drop/second.
- At 20-30 dpf, the dry zebrafish diet is changed to GEMMA 150 twice daily and 70ml of rotifers once daily. The flow rate on the re-circulating system is increased to 2 drops/second.
- At 30-89 dpf GEMMA 150 is fed three times daily with 30ml of rotifers added at the mid-day feeding. The flow rate of the water is increased to a steady drip.

Feeding of zebrafish adults:

- At 90 dpf change the dry zebrafish diet to GEMMA 300 twice daily. Rotifers can still comprise one feeding a day but at a lower volume of 25-30 ml.
- Move from tank to tank remembering to observe the previously fed tanks to ensure they are eating.
- Repeat until all adult fish tanks have been fed.
- Wait ~ 5 minutes, if fish have already finished eating, an additional amount (~ half of what was given previously) can be given.
- The water flow rate in the tank is at a steady stream.

References:

- 1) CALAS Symposium, June 11-14th 2016, Toronto, Ontario
- 2) Reed Mariculture, Inc. 900 East Hamilton Avenue, Suite 100, Campbell, CA
- 3) Zebrafish Husbandry Course 5th International, Leading Advances in Zebrafish Husbandry, September 27th-September 30, 2016, Buguggiate (VA) Italy
- 4) Zebrafish Husbandry Association, Windemere, FL

Revised: October 22/2020
