University Animal Care Committee Standard Operating Procedure		
Document No: 15.1	**Subject:** General Husbandry 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 husbandry procedures for receiving, monitoring, cleaning and maintenance of zebrafish colonies and rotifer cultures.

1) Introduction and Definitions: It is important that all materials that come in contact with zebrafish, or the water that will come in contact with zebrafish, is properly cleaned and disinfected, as the use of inappropriate detergents and soaps can be extremely detrimental to the system and could result in significant mortality of the zebrafish. All tanks should be cleaned and disinfected as required depending on the build-up detritus and algae in the tanks.

2) Materials

- Tap water
- Reverse osmosis (RO) water
- 3L, 5L and 10L buckets, 2 of each
- Stand-alone aquarium, minimum of 2
- Commercial net disinfectant: Iodine based, Virkon Aquatic, Clinicide
- Instant Ocean salt
- Sodium bicarbonate
- Conductivity and pH calibration solution
- Small brush/toothbrush
- Stirring utensil
- Personal Protective Equipment (PPE)
- Commercial water quality testing kit
- Small fish tank net(s)
- Siphon tubing
- pH meter (measure pH and temperature)
- Refractometer (measure salinity)

3) Procedures:

Water Quality Monitoring Schedule:

- The following parameters as noted in the following charts are monitored daily for both the zebrafish and rotifers. Temperature, conductivity, and pH readings are part of the automated Zebtec system. Temperature and pH can be confirmed with a pH meter. The other parameters are confirmed with a commercial water quality testing kit. If any parameters fall outside the ranges below the Veterinarian should be notified immediately.

- The parameters alkalinity and ammonia noted in the "Rotifer Water Quality Guidelines" will be affected by the fact that the rotifers are kept in a saline solution. Temperature and pH readings are of higher importance and if these are normal and the rotifers appear healthy, they should be okay.

Zebrafish Water Quality Guidelines			
Parameter	**Range**	**Preferred**	**Confirmed**
Temperature	23-32°C	25-28°C	Daily
PH	6.0-8.0	7.2	Daily
Conductivity	450-1500 mS	700-900 mS	Daily
Alkalinity	50-200 ppm	170 ppm	Weekly
Hardness	4-8 dGH	6 dGH	Weekly
Ammonia	0	0	Weekly
Nitrite	0-25 ppm	0	Weekly
Nitrate	0-40 ppm	10 ppm	Weekly

Rotifer Water Quality Guidelines			
Parameter	**Range**	**Preferred**	**Confirmed**
Temperature	10-30°C	26-27°C	Daily
PH	6.5-9.0	7.0-8.5	Daily
Salinity	3-40 g/L	~15g/L	Daily
Alkalinity	50-200 ppm	170 ppm	Weekly
Ammonia	<1 mg/L	<1 mg/L	Daily

Receiving of Zebrafish:

- Health reports would have been examined prior to authorization to receive the zebrafish and zebrafish embryos.
- Inspect transport bags for signs of damage and shipment accuracy.
- Inspect zebrafish for any signs of stress, trauma, or mortality that may have occurred during transit.
- Any ill or dead zebrafish should be removed from the shipping container. Ill looking zebrafish should be isolated in a stand-alone quarantine aquarium.
- Zebrafish from approved vendors are introduced into an appropriate size tank and isolated from resident zebrafish until they are fully acclimated to the aquatic system. The tanks are labeled with the age, strain and sex of the zebrafish contained in within.

- Zebrafish are acclimated to the aquatic system within 24 hours of arrival by emptying them and their shipping water into the appropriately sized tank. They are then placed on the Zebtec rack with the water inlet valve slightly opened to allow for the fish to slowly acclimate to the system water and conditions.
- Health reports for zebrafish from non-approved vendors (if available) will be reviewed. Zebrafish from unapproved vendors, arriving with health concerns, or found sick, are isolated from healthy zebrafish by placing them in their own stand-alone aquarium and the Veterinarian is notified. These zebrafish will remain in their own tanks off the Zebtec rack for as long as they are kept. The aquarium is labeled with age, strain, and sex of the zebrafish.
- Zebrafish should be housed at a maximum density of 5 adult fish per liter of water, separated by sex. Information such as protocol number and Principal Investigator's name and contact information will be kept in a separate logbook to be kept near the entrance to the room. Any system maintenance, system issues, health issues identified, death losses, procedures, mating, etc. will also be recorded in the room logbook.
- If the embryos are less than five days of age, they will be placed in large petri dishes with some of the water they have been transported in topped-up with Zebtec system water. They are held in an incubator at 28C for the first five days until they are transferred to a Zebtec tank. They are checked daily to make sure the water does not evaporate. If the embryos are five days of age or older, they will be set up in a Zebtec tank as described in SOP 15.2 "Feeding of Zebrafish".

Daily duties:

- **Conduct general health surveillance.**
  - Record the source tank of any zebrafish exhibiting any of the behavioral and/or physical signs below.
  - Alert an animal care technician or Veterinarian that you have identified a moribund zebrafish.
  - If the zebrafish requires immediate attention, and an animal care technician or Veterinarian is not available (zebrafish is at the surface or on the bottom of the tank, exhibiting rapid or minimal opercular movements, or has advanced coelomic distention), remove the zebrafish from home tank with dry, disinfected net and proceed with SOP 15.3 "Anesthesia and Euthanasia of Zebrafish".
  - Alert the Principal Investigator, animal care technician and Veterinarian that a zebrafish was euthanized and find out if the zebrafish need be submitted for any diagnostics. If so, proceed with SOP 15.4 "Fixing Zebrafish" for histopathology. It not, place the zebrafish in a body bag and place in freezer until disposal.
- Check the automated system for power supply presence and any alarm messages. If there are any alarm messages, alert an animal care technician or Veterinarian immediately.
- Inspect the system computer display and record the temperature, pH, and conductivity on the room status sheet kept on the Zebtec system.
- Visually inspect the biofilters to ensure they are circulating in their tank. Stir up as required.
- Inspect the system sodium bicarbonate and sodium chloride reservoirs and top up as required.

Behavioral Abnormalities	Physical Abnormalities
Fish at surface or near water inlet Rapid opercular movements Sluggish movements/lethargy Flashing/rubbing on tank surfaces Circling, twirling, spinning Loss of equilibrium	Color change Weight loss Exophthalmia Distended abdomen Skeletal deformity Mass/swelling Hemorrhage/redness Gas bubbles Protruding scales Fin erosion or lesion Skin ulceration or lesion

- ***Check rotifer culture:***
  - Verify that the rotifers have eaten and that the cultures are grossly healthy. Healthy rotifers will be fast moving with attached eggs (a few drops can be examined under a microscope to confirm this). Grossly, rotifers can be identified in large numbers when drawn up in a 3ml syringe and examined by the naked eye.
  - Examine the color and turbidity of the cultures. Healthy cultures are a relatively clear green/brown color. Turbid green implies overfeeding and possibly a crash in rotifer numbers. Clear brown to red implies inadequate feeding and starving rotifers.
  - Check aeration. The air stone should be bubbling gently. The air stone should be cleaned twice weekly at a minimum.
  - Add 20 ppt saline to the rotifer culture pail as needed to maintain at 14L volume.
  - If there is a rotifer subculture, add 5 ppt saline to the subculture pail to maintain at a 3.5L volume.
- ***Clean rotifer culture:***
  - Clean the rotifer culture bucket once weekly.
  - Disconnect the air hose from the pump.
  - Pour the contents of the rotifer bucket into the spare blue bucket available in the room. When you get to the bottom of the culture bucket, pour the remaining contents into a 5L beaker, and set aside.
  - Clean the interior surface of the culture bucket to remove all adhering detritus. When the bucket is clean, pour the contents of the blue bucket back into the rotifer bucket.
  - Pour the contents of the 5L beaker through a filter floss and into the rotifer bucket.
  - Top the rotifer bucket up to 14L of saline solution from the 20 ppt saline solution.
  - Clean the air stone (to be done twice weekly) and then reattach the air tube to the pump, place the air stone in the bucket and turn on the air pump.
- ***If there is a rotifer subculture, clean the subculture container weekly:***
  - Disconnect the air hose from the pump.

- Pour the contents of the subculture container into a 5L beaker and set aside. Any thick detritus in the bottom of the subculture container should be held back and placed in a separate beaker.
- Clean the inside of the subculture container and pour the subculture that had been poured into the 5L beaker back into the cleaned container. The thicker detritus that had been held back can be filtered through the filter floss and the filtered solution added to the subculture.
- The subculture is then topped up to 3.5L with 5 ppt saline solution.
- The air stone is cleaned and set back into the subculture, hook into the air pump and turn it on.
- Twice weekly 250ml of rotifers are transferred from the main culture pail to the subculture container.

- Feed zebrafish twice daily as outlined in SOP 15.2 "Feeding of Zebrafish". Ensure that rotifers are fed approximately 1 hour before feeding to the zebrafish fry.
- Feed the rotifers twice daily as outlined in the SOP. Check zebrafish nurseries. Remove dead embryos/fry with small pipette. Check temperature, pH and ammonia levels. Replenish ~ one third of the water with system water.
- Check supplies and refill if needed (e.g., soap, paper towels, etc.).
- Clean counter/sink area and wipe down with disinfectant as needed.
- Sweep floor as needed and empty trash.

Weekly duties:

- Mop floor with Microquat or similar disinfectant available in room.
- Siphon debris from bottom of tanks, if needed.
- Replace approximately 25% of the total system water volume with RO water added directly to the sump. This is done automatically by the system. The replacement volume can be adjusted depending on the number of tanks on the Zebtec system.
- Dispose of and replace used net disinfectant.
- **Clean tanks: tanks containing fish and empty tanks on the system that are dirty.**
  - Turn off water to tank to be cleaned.
  - Turn off water to tank that will have zebrafish transferred into it.
  - Transfer zebrafish from tank to be cleaned to a clean tank with a dry, disinfected fish tank net. Be sure to transfer identifying information to new tank.
  - Return tank with zebrafish to the Zebtec rack and re-establish water flow.
  - Disassemble tank to be cleaned.
  - Wash all components separately using reverse osmosis water. *Caution: Use of chemicals and/or detergents can be detrimental to zebrafish.* Diluted iodine solution can be used for cleaning the tank. Use a small wire brush or toothbrush to loosen any detritus and algae.
  - Rinse all components with RO water.
  - Place all tanks and components that have just been washed and place in the dish washer to run on the "Sanitize" cycle.

As needed duties:

- **Disinfect fish tank nets**. Fish tank nets should be disinfected after each use, before being used on a new tank. This may require having multiple nets "on rotation" within a room.
  - Prepare commercially available net disinfectant, as per manufacturer instructions, in a 5-gallon bucket with lid. The bucket should be labelled appropriately. The working solution can be kept for 1 week at a time.
  - Rinse any gross debris from nets with RO water.
  - Soak in net disinfectant solution for 1 hour.
  - Remove from disinfectant solution and rinse thoroughly with RO water.
  - Hang to dry
- **Clean tanks (as described above) any time they are emptied, prior to return of fish.**

	Daily	Weekly	Biweekly	Monthly	Every 3 months	Every 6 months	Yearly
Power supply							
Absence of alarm messages							
Calibrate pH probe							
Calibrate conductivity probe							
Update water exchange rate							
Check UV lamps are functioning from the UV bulb inspection hole							
Clean tanks							
Replace UV bulb							
Inspect, and, if necessary clean UV bulb quartz							
Replace dosage unit pipes							
Check Rotifer culture							
Clean Rotifer culture							

References:

1) Nicole Compo, DVM
2) Zebrafish Husbandry Course 5th International, Leading Advances in Zebrafish Husbandry, September 27-30, 2016 in Bugaggiate (VA), Italy
3) Reed Mariculture Inc., 900 East Hamilton Avenue, Suite 100, Campbell, CA
4) CALAS June 11-14, 2016, Toronto, Ontario
5) Zebrafish International Resource Centre (ZIRC), University of Oregon

Revised: October 22/2020