Title: Zebrafish (Danio rerio) Colony Care and Maintenance

Author(s): Susan Chapman, Assistant Professor, PI  
Date: ____________

Amy Anderson, Research Assistant, Technician  
Date: ____________

Facility Manager: Jennifer Seda, Lab Manager  
Date: ____________

Attending: Veterinarian  
Date: ____________

1.0 OBJECTIVE
The objective of this Standard Operating Procedure (SOP) is to describe standard operating procedures for the care of zebrafish (Danio rerio). This SOP is based on standard aquarium trade procedures and care protocols from The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (Danio rerio), by Monte Westerfield. Like all SOPs, these procedures are subject to revision as better techniques and products become available.

2.0 HEALTH AND SAFETY
- Attire appropriate to the task must be worn at all times.
- All Clemson University personnel must be enrolled in the Clemson University Medical Surveillance Program.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES
- Personnel familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure.
- All Clemson University personnel must have completed the IACUC on line training.
- Prior to being assigned full responsibility for performing this procedure, personnel must have demonstrated proficiency in the use of the technique in a closely supervised environment.
- Documentation of training should be kept for each person performing the procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Required Materials
- Water reservoir
- Aquaria (3 & 10 liter)
- Aquarium covers (3 & 10 liter)
- Baffles
- Aquarium heater
- Air pump
- UV sterilizer

- Biological and mechanical filtration
- Air stone and air pump
- Buckets
- Small nets
- Thermometer
- Ziplock baggies
- Liquid Test Kits
Aquatic Animal Research Lab Rm 6/SOP AARL_Rm6_001.02

Page 2 of 9

Effective Date: 1/20/09

• TDS meter
• Instant Ocean Salt
• Acid and Alkaline buffers
• RO replacement salts
• Sharpies and label tape
• Metal shelving units
• Lighting units with UBV bulbs
• Measuring spoons & cups
• Exterior cage identification
• Brine shrimp hatcheries
• Air pumps for brine shrimp hatcheries
• Brine shrimp cysts

• Breeding tanks
• Artificial plants for breeding tanks
• Nursery tanks
• Mesh baffles
• Petri dishes: 150mm, 35mm
• Transfer pipettes
• Filters
• 7.5 grain brewers yeast tablets
• Wheat berries
• Paramecium
• Backup reservoir with pre-mixed water

4.2 Recommended Materials
• Replacement pump

4.3 Prepared Materials
• Flake food mixture of powdered Spirulina, and Zeigler Zebrafish Adult diet and larval diet

5.0 GUIDELINES
The following procedures were developed to meet or exceed the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. AVMA (American Veterinary Medical Association). 2007 AVMA Guidelines on Euthanasia.

6.0 PROCEDURE

6.1 Animal acquisition and identification- Embryos are procured from Zebrafish International Resource Center (ZIRC) and bleached to remove any diseases. Cards on each tank include date received (or fertilized), strain, tank number, and sex.

6.2 General Care of Fish
A. **Check water temperature daily** – Optimal temperature is 83.3˚F. Room temperature should be between 70-80˚F.

B. **Check each tank for mortality daily**- Any fish found dead should be removed from the tank immediately. Place the dead fish in a ziplock bag and label the bag with all the names and codes written on the tank and the date. Take the bag to the freezer in room 17 that is approved to house vertebrate mortalities. Record the mortality on the daily mortality log including: the date, strain of the fish, tank number, and sex. Multiple dead fish from a tank can be placed in the same ziplock bag. Sick or wounded fish should not be removed, but the PI or the lab technician should be contacted immediately. If there are no dead fish, record a zero on the daily mortality log.

C. **Check each tank for water flow daily** – Water should be flowing into each tank, except for those with larvae that require reduced or intermittent water flow.

D. **Feed each tank twice daily** – Each tank if fed a Pasteur pipette of diluted brine shrimp per 8 adult fish, twice daily. Dry fish food should also be fed twice weekly. After
opening, the dry food is stored in a plastic container in the freezer that is approved to house vertebrate food located in room 17. If flakes are low, you should notify the PI or lab technician. Tanks that appear very cloudy or have sickly fish should not be fed and should be reported immediately to the PI or lab technician. After feeding, the feeder should sign the daily feed sheet. Larvae are fed paramecium, Zeigler powdered food, and/or brine shrimp according to their age.

E. **Water level checked daily** – The level in the main water reservoir should be checked daily and fresh water added as needed.

F. **Chemical testing in tanks weekly** – Pick one large and one small tank and measure pH, ammonia, nitrite, nitrate, GH, and KH levels using Liquid Test Kits (Aqua. Pharm.). Check total dissolved solids with TDS meter. Record all data on the Water Quality data sheet. Safe ranges for each water quality parameter are listed on the top of the Water Quality data sheet. If levels for ammonia or nitrate are high, 33% of the water in all tanks should be changed and recorded on the Water Change Sheet.

G. **Water changes weekly** – The system should have a 10% water change once a week. The water flow should be turned off and debris siphoned out of each tank with care not to suck up any fish. It is good practice to check the bucket after siphoning each tank and return any fish. If fish are discovered after multiple tanks have been cleaned, they should be euthanized rather than returned to an incorrect tank. After siphoning, any additional water should be removed to total 10%. Water should then be added from the pre-mixed backup reservoir. The cleaning date and amount of water changed should be recorded on the Water Change Sheet.

H. **Mix backup water reservoir when emptied** – The 22-gallon backup reservoir holds prepared water for the aquaria. In filling the barrel, the RO water should be allowed to run for 3-5 minutes to clear any dissolved metals from the water pipes and hose. Add 4.8 grams Instant Ocean, two teaspoons R/O Right, one teaspoon Acid Buffer, and one and a half teaspoons Alkaline buffer. Mix well and test. After water meets the parameters on the Water Quality sheet, cover and make sure the heater is plugged in and under the water level.

### 6.3 Specialized Care of Fish

**A. Mortality or cloudy water**

a. Identify the source of mortality as quickly as possible. First, examine the remaining live fish for signs of distress. Look carefully at their fins, scales, eyes, and gills. White slime, white spots, blood streaks, or ragged fin edges are signs of disease. Look carefully at their swimming posture. Fish that seem to float, sink, or lean to one side are also showing signs of disease. Fish that have an elevated respiration rate, inflamed gills or are breathing from the surface may be suffering from ammonia toxicity. Contact the PI or lab technician immediately if any of these symptoms are observed. See Diseases and Ammonia Toxicity below for more information. An immediate change of water might be necessary.

b. Check tank water chemistry – Any tank with abnormal mortalities or with cloudy water should have a full water chemistry check (temperature, pH, salinity, ammonia, nitrite, and nitrate). If conditions are out of range, perform a 50% water change and replace with pre-mixed water.

**B. Treatments**
a. **Diseased tanks** – Any tank diagnosed with diseased fish should receive their daily dose of medication as noted on the tank. See diagnosis and treatment of diseases below.

C. **Maintenance**
   a. **Clean the room daily** – Coil all hoses and place in designated containers, use the squeegee to wipe any excess water from the floor, put away all pens, Sharpies, tape, nets, and buckets before leaving. NEVER clean floors with any type of soap or disinfectant containing ammonia or an antibacterial agent. Toxic fumes could be transferred to the water in the aquaria.
   b. **Backup reservoir** – To keep a barrel from becoming laden with algae and hard water deposits, rinse it with tap water while scrubbing the sides each time the barrel is empty. Occasionally it is necessary to soak the barrels in vinegar water (to get rid of hard water deposits) or bleach water (for excess algae growth). Two days may be necessary to soften the calcium. After a soak, rinse the barrels well twice.
   c. **Supplies** – Extra water line, nets, etc. are located in the labeled drawers. Ask the PI or lab technician about needed supplies. Please let the PI or lab technician know when supplies are getting LOW so new supplies can be ordered.

D. **Water Quality**
   a. **Ammonia is the most critical water quality issue** – It can kill fish if the concentration in water gets too high. If the fish start to act sluggishly or do not behave as normal, check the ammonia concentration in the tank. Inflammation of the gills, surface breathing and ‘shimming’ are all signs of ammonia stress. Test for ammonia, and if it registers high, then a water change is needed.
   b. **Cloudiness can also be due to good bacteria searching for a home** – The bacteria are the kind that break down ammonia, but can take some days to settle into the biofilter and start converting the ammonia. Why the bacteria take so much time to ‘settle’ is not known.

E. **Disease and Toxicity**
   a. **Diseases and parasites** – While relatively uncommon, since fish are only introduced from bleached embryos, if you suspect disease, consult the online manual, “Diseases of Zebrafish in Research Facilities” available at http://zebrafish.org/zirc/health/diseaseManual.php. Diagnostic services are provided by the Zebrafish International Research Center: http://zebrafish.org/zirc/health. If a fish is to be treated, place it in a clearly marked isolation tank and write the date and dosage given on the tank.
   b. **Ammonia toxicity** – The most common source of fish mortality is ammonia toxicity. Ammonia is colorless and odorless so the tanks may not look unhealthy. Ammonia is the nitrogen waste product that fish urinate and can also be given off by decaying bacteria and uneaten food. When a new tank is set up, the *Nitrosomas* bacteria that break down ammonia are not yet present in sufficient quantities to keep up with ammonia production. As ammonia concentration rises, fish begin to show signs of respiratory stress by breathing faster, their gills becoming red and inflamed, or by attempting to breathe at the surface of the water. They may also appear listless and just lie
on the bottom of the tank or ‘shimmy’. High ammonia levels are easily
detected with an ammonia test kit. Follow the instructions on the kit to
determine the ammonia level. While additives are available to combat
ammonia toxicity, often the best treatment is a 33%-67% water change,
depending on the severity of the case. If half of the fish have died and the
other half are lying on the bottom of the tank, up to a 100% water change is
recommended. During the first seven days after a tank is set up, ammonia
levels will usually rise from 0.25ppm to 6.0ppm. An ammonia level above
3.0 requires some preventative action. Days 7-14 should be the peak of
ammonia. Days 14-21 should be the peak of nitrite. After day 21, the
biological filters should be converting ammonia to nitrite and nitrite to
nitrate. You know when nitrate is being produced as algae will begin to grow
in the tank.

F. Euthanasia – Moribund fish are to be given an overdose of 2g/L buffered tricaine
methanesulfonate (MS-222) applied exogenously by dilution into the water of a tank
reserved for euthanasia. Fish remain in the anesthetic for at least 10 minutes after
cessation of gill cover movement. Fish are then frozen until disposal.

G. Emergency Power Outage – This facility is equipped with a backup generator that
will provide power to one outlet per lab. We have our air blowers plugged into the
generator so that the fish will continue to receive aeration, which is most critical to
their survival. Tanks should be monitored closely for temperature and water quality
in the unlikely event of a long term power outage. If necessary, emergency heat
should be supplied.

7.0 QUALITY CONTROL CHECKS AND ACCEPTANCE CRITERIA
All procedures are subject to periodic review by the Attending Veterinarian(s) and the
Institutional Animal Care and Use Committee.

8.0 LITERATURE CITED
Westerfield, Monte. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (Danio

9.0 APPENDICES
9.1 Water Quality Data Sheet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temp</th>
<th>pH</th>
<th>TDS</th>
<th>Nitrite</th>
<th>Nitrate</th>
<th>Hardness</th>
<th>Alkalinity</th>
<th>Ammonia</th>
<th>Chlorine</th>
<th>Water</th>
</tr>
</thead>
</table>
| Date      | 28.5°C/83.3°F | 6.8 - 7.3 | 720-1250 ppm | <0.1 ppm | <5-10 ppm | ~2-6 GH | ~2-6 KH | 0 ppm | 0 ppm | 1" from top

<table>
<thead>
<tr>
<th>Date</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Water Change Date Sheet

**Water Change Log**

<table>
<thead>
<tr>
<th>Date</th>
<th>Amount Changed</th>
<th>Comments</th>
<th>Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9.3 Daily Feeding Data Sheet

<table>
<thead>
<tr>
<th>Date</th>
<th>Adults Morning</th>
<th>Adults Afternoon</th>
<th>Babies Morning</th>
<th>Babies Afternoon</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9.4 Daily Mortality Data Sheet

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Strain</td>
<td>Tank</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>