



STANDARD OPERATING PROCEDURE No. 9

AQUATIC VERTEBRATE HOUSING AND HUSBANDRY

Aquatic vertebrates are housed in the following laboratories within the Toll Science Center:

- SG13 and SG13A - Dr. Martin Connaughton Aquatic Research Lab
- SG21- Dr. Martin Connaughton Research Lab
- S105C - Dr. Mindy Reynolds Research Lab

Note - "daily" below refers to M-F, not M-Su

SG13 - Marine Biosystem X-Rack system (recirculating system, fresh or salt water, warm or cold conditions)

Fresh, warm water species (Tilapia, Zebrafish)

1. Water quality
 - a. fresh water - obtained through filtered water system in SG13A
 - b. temperature range
 - Tilapia 22-30°C (72-86°F)
 - Zebrafish 25-30°C (77-86°F)
 - checked daily & record in daily log
 - c. test ammonia and nitrate levels weekly (TH or F)
 - ammonia levels should remain < 1.0 ppm
 - if levels are > 1.0 ppm
 - test daily & record in log
 - do ½ water change and add StressZyme™ (150 ml)
 - nitrate levels should remain < 50 ppm
 - if levels are > 50 ppm
 - test daily & record in log
 - do ½ water change
 - d. MWF - do a 30% water change by draining the sump to within 6 inches of the bottom, then filling with filtered water (green hose from SG13A).
 - e. MWF - pull out and rinse filter pad - rinse with green hose into the floor drain after removing cover to floor drain
2. Feeding

- a. Tilapia
 - i. trout-chow pellets (approx 15 per large fish if housed individually) & record in daily log
 - ii. daily unless nitrate levels are difficult to control, then MWF only
- b. Zebrafish
 - i. Tetramin™ flake food (a small pinch ground slightly between the fingers to produce smaller flakes) & record in daily log.
 - ii. daily unless nitrate levels are difficult to control, then MWF only
3. Cleaning tanks - Tilapia only
 - a. use siphon to remove feces from tanks - MWF & record in daily log
4. Care of the system
 - a. 1/3 to 1/2 water change - weekly (refill with water from filter system in SG13A) & record in daily log
 - b. rinse filter pad - weekly
 - c. change barrel filter - once a semester unless under heavy use or unless pressure in filter rises above 5 lbs & record in daily log
 - d. change charcoal in barrel filter - once a semester unless under heavy use or unless pressure in filter rises above 5 lbs & record in daily log

SG13A - Fresh, warm water species (Tilapia, Zebrafish) - flow-through rack system

1. Water quality
 - a. fresh water - obtained through filtered water system in SG13A
 - b. temperature range
 - Tilapia 22-30°C (72-86°F)
 - Zebrafish 25-30°C (77-86°F)
 - checked daily
 - Check temperatures in each tank and collect data on spreadsheet of tanks, then average the temperatures for each row and enter these averages and the room temp in the daily log for the system. Note any outliers and make (and note) corrections in temperature.
 - c. since this is a flow through system it is not necessary to track ammonia or nitrate levels because the water is constantly being replaced.
2. Feeding
 - a. Tilapia
 - i. trout-chow pellets (approx 15 per large fish if housed individually) & record in daily log
 - b. Zebrafish
 - i. Tetramin™ flake food (a small pinch ground slightly between the fingers to produce smaller flakes) & record in daily log.
3. Cleaning tanks - tilapia only
 - a. use siphon to remove feces from tanks - MWF & record in daily log
4. Care of the system

- a. top off water in the 4 chiller/heaters responsible for maintaining temperature - MWF (this is VERY important, if water level drops, the heater turns off and the tanks will chill to the temperature of the water entering the system, which is too low for the comfort of the tilapia).

SG13A - Temperate marine species (Atlantic croaker) - three large round tanks

1. Water quality

- a. salt water (30 ppt salinity for current experiment)
- b. temperature range
 - 17-20°C (63-68°F for current experiment)
 - check temperatures in each tank & record in daily log. Note any corrections in temperature.
- c. test ammonia and nitrate levels daily unless levels are low and stable, then MWF & record in daily log
 - ammonia levels should remain < 1.0 ppm
 - if > 1.0 ppm, do ¼ to 1/3 water change and add StressZyme™ (150 ml)
 - nitrate levels should remain < 50 ppm
 - If > 50 ppm, do ¼ to 1/3 water change
- d. water change in this tank requires making salt water in a garbage can or large (150 gallon) tub to match the salinity of the tank, then pumping it from the tub to the tank with submersible pumps
 - ¼ to 1/3 water change = 7-8 inches from water surface to tank edge, this is approximately 100 to 150 gallons of water (from a 500 gallon tank)
 - water can be drained with a submersible pump or by opening the settling tray valve without breaking the siphon from the tank.

2. Feeding

- a. squid or shrimp - thawed and cut to small (1 cm) pieces, quantity depends on number of fish and feeding habits, record amount served and amount consumed in daily log if counting (rather than feeding by weight)
- b. daily M-F unless otherwise noted
- c. net uneaten food out of tank before end of day and note amount/approx % not eaten in daily log.

3. Cleaning tanks

- a. clean out settling tray - daily or MWF depending on feeding schedule and number of fish
 - i. break siphon from tank
 - ii. open valve from settling tray & allow to drain
 - iii. squeegee the tray and rinse material down the drain with approx 1 gallon of fresh water
 - iv. close settling tray valve
 - v. reestablish siphon and attach to T-tube in settling tray
 - vi. confirm that water level is high enough for filter to function and that water is flowing into the tank

- vii. several days of this will lower water level in the tank enough to require topping off in order for the siphon and hence filter to function.

SG13A, SG21, S105C - Fresh, warm water species (Zebrafish) - individually filtered glass aquaria (2.5 to 20 gallons)

1. Water quality
 - a. fresh water - obtained through filtered water system in SG13A
 - b. temperature range 25-30°C (77-86°F) - checked daily, controlled by individual electric heater elements in each tank.
 - i. Check temperatures in each tank and enter average temp if individual logs are not available for each tank. Note any outliers and make (and note) corrections in temperature.
 - c. test ammonia and nitrate levels weekly (TH or F)
 - ammonia levels should remain < 1.0 ppm
 - if levels are > 1.0 ppm
 - test daily & record in log
 - do ½ water change and add StressZyme™ (10 ml/gallon of tank water)
 - nitrate levels should remain < 50 ppm
 - if levels are > 50 ppm
 - test daily & record in log
 - do ½ water change and add StressZyme™ (10 ml/gallon of tank water)
2. Feeding
 - a. Tetramin™ flake food (a small pinch ground slightly between the fingers to produce smaller flakes) & record in daily log.
 - b. if breeding zebrafish (see below) feed twice daily, once in morning and once in late afternoon/early evening.
3. Breeding zebrafish - for collection of larvae
 - a. physical conditions: for breeding it is best to have the fish on a 14 hour light:10 hour dark photo period and a holding temperature of 28-30°C.
 - b. feeding: feeding twice a day can increase the likelihood of egg production by the female
 - c. spawning substratum - place a shallow dish (petri dish or shallow tupperware container) full of marbles in the tank before the lights go out in the late afternoon or early evening. Remove the dish from the tank in the morning, remove the marbles from the dish and pipette (with a wide mouth/cut down disposable plastic pipette or decant the embryos).