**SOP for Maintenance and Husbandry of Fish in Experimental Rack System and Quarantine Housing Rack**

**Purpose:**  To describe routine husbandry and maintenance of fish in the experimental rack system.

**Person Responsible:** PI, DVR backup

**Frequency:** Feeding- once to twice daily

Water Quality Testing- weekly

Probe Calibration- monthly

YSI 5200 Calibration- annually

Tank wall cleaning- as needed

**Documentation:** Water quality and calibration records, daily observation log.

**Materials:** Quarantine housing rack and Experimental Housing Rack (Aquatic Ecosystems); Fish feed (adult and fry); *Artemia*; MS-222; NaHCO3, Embryo media, Filtered facility water, Hach water quality test kit, YSI 5200 and probes, algae scrubbing pads, R/O water, Instant Ocean.

**Filtration:** All tanks are maintained with a re-circulating system capable of biological and mechanical filtration. Aeration is achieved via flow through the filtration system and open tops of tanks.

**Water Quality:**

1. Continuous monitoring of pH, conductivity, temperature, total dissolved gasses is provided by the YSI 5200 unit and monitored by PI and DVR staff. 5200 probes are calibrated monthly and the 5200 unit calibrated annually.
2. Weekly monitoring of ammonia, nitrate, nitrite, and total dissolved oxygen is provided by testing with the Hach water quality test kit.
3. Any unexpected mortalities of abnormalities in the fish or eggs can prompt immediate water quality testing.

**Water Conditioning**:

1. Filtered Facility Water: Water is passed through a 2 micron filter prior to addition to any system.
2. R/O water can be used and is conditioned by adding 1 kg of Instant Ocean to each 40L of R/O water to stabilize conductivity at 500 microsiemens. NaHCO3 can be added for pH control.
3. Fish Embryo Medium is prepared as per SOP XXX.

**Tank cleaning:**

1. If the tanks walls become covered with algae, it is necessary to scrape them even when fish are in the tank. This is done with an algae scrubber that is designated for that rack.

**Feeding:**

1. Fish will be fed at least once or twice daily with feed based upon the age and life stage of the fish.
2. For fry, newly hatched *Artemia* will be fed. The *Artemia* will be fed and hatched as per SOP XXX.
3. Fish Food: To make dry fish food, wear gloves and mix the components in a clean 9 liter tank. Once mixed, divide between two large ziplock bags, label, and store at 4 °C.
4. For Juvenile food:
5. grams Larval AP 100 (100–150 µm size)
6. grams crushed spirulina flakes
7. grams ArteMac #2 (100–200 µm size)
8. For Adult food:
9. grams Larval AP 100 (250–450 µm size)

200 grams crushed spirulina flakes

1000 grams ArteMac #4 (300–500 µm size

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**Anesthesia:**

1. Anesthesia is required for some handling procedures such as caudal fin clips for genotyping.
2. Anesthesia will be performed with buffered MS-222 at ~40 ppm.
3. Fish will be monitored during recovery in a clean tank without MS-222 and returned to home tank once recovered. Estimated survival is 98%.

**Sentinel Program:**

1. Since all fish in each rack are on the same system, the retiring adult fish can serve as the sentinels for the system.
2. 5 fish per system every 4 months will be sacrificed and examined for health abnormalities, parasites and other tests as needed.
3. Additional fish may be sacrificed and examined in the event of unexpected morbidity and mortality.

**Euthanasia:**

1. All fish are euthanized with an overdose of buffered MS-222 (≥100 ppm).
2. The buffer is 1:1 NaHCO3 and MS-222.