Title: Mummichog Maintenance and Care

Author(s):

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Attending:

Veterinarian John Parrish

1.0 OBJECTIVE

The objective of this Standard Operating Procedure (SOP) is to establish a description of the procedures for the maintenance and care of mummichogs (*Fundulus heteroclitus*), which can be applied to specific research protocols. As with any 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.
- Only Josephine Wojdylo and Charlie Rice, who are familiar with the equipment and laboratory techniques, will perform procedures outlined in this SOP.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Required Materials
- Water reservoir
- Aquaria (3 & 10 liter)
- Biological and mechanical filtration
- Air stone and air pump
- Buckets
- Small nets
- Thermometer
- Ziplock baggies
- Liquid Test Kits
- Instant Ocean Salt
- Refractometer
- Sharpies and label tape
- Metal shelving units
- Measuring spoons & cups
- Exterior cage identification
- Brine shrimp hatcheries
- Air pumps for brine shrimp hatcheries
- Brine shrimp eggs

- Breeding tanks
- Nursery tanks
- Petri dishes: 150mm, 35mm
- Transfer pipettes
- Filters
- Small nets
- Instant Ocean
- Tetra flakes

4.2 Recommended Materials

- UV sterilizer
- ShopVac
- StressZyme
- Ammo-Lock 2
4.3 Prepared Materials

- Storage container filled with fresh saline water (15-18ppt).

5.0 GUIDELINES

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

6.0 PROCEDURE

6.1 Animal Room Climate

A. Room temperature will be maintained at 25°C(+/-) 4 ºC

B. The optimal light cycle for fish maintenance shall be 14 hours light (6am EST to 8pm EST) and will not exceed 30ft-candles. Failures or changes in the light cycle will be recorded in Form No. 129.

C. Humidity will be equal to ambient air humidity and air changes will be 10-15 per hours unless otherwise dictated by protocol.

6.2 General Care of Fish

A. **Check water temperature daily**- Water temperature will be monitored in the aquaria using a thermometer and will be maintained between 21-28 ºC.

B. **Check each tank for mortality daily**- All animals will be observed daily for signs of disease. Moribund animals will be euthanized with a buffered solution of MS-222 via gills. 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, 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 (manager of the lab) 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** – Adult fish will be fed Tetra flakes and a supplement of either frozen brine shrimp or bloodworms every other day. Fish will be fed approximately 3% of their body weight daily. After opening, flakes will have an open date displayed on the container and will be stored 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 (lab manager). 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 brine shrimp till they are large enough to eat flake food.

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.). 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, 50% 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 every 7-8 days depending on the water parameters. 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. After siphoning, any additional water should be removed to total 10%. Water should then be added from the pre-mixed backup reservoir. Water shall be monitored daily for salinity and dissolved oxygen levels using a refractometer and dissolved oxygen meter. Optimal values are between 10-15ppt for salinity and 5.0mg/L and greater for dissolved oxygen. The cleaning date and amount of water changed should be recorded on the Water Change Sheet.

H. **Mix backup water reservoir when emptied** – The backup reservoir holds prepared water for the aquaria. In filling the barrel, the tap water should be allowed to run for 3-5 minutes to clear any dissolved metals from the water pipes and hose. Add two tablespoons NovAqua, two tablespoons of AmQuel, use a refractometer to add the appropriate amount of salt to reach a salinity level of 15-18ppt.

I. **Record Keeping**

Door sheets (Form No. 129) will be placed on animal room doors on a monthly schedule. Appropriate boxes for daily animal care and maintenance will be checked off and initialed by the person completing the tasks. The number of animals maintained in the culture will be recorded on an Inventory Sheet (Form No. 130).

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. 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.

C. **Maintenance**

a. **Clean the room daily** – Floors will be swept daily. Animal rooms will be damp mopped weekly with a disinfection solution. The entire room will be wiped down no less than quarterly or at end of a project with a disinfecting solution. 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** – Ask the PI or lab manager about needed supplies. Please let the lab manager 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.

E. **Disease and Toxicity**

**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**

9.0 **APPENDICES**
9.1 Water Quality Data Sheet

**Fundulus heteroclitus Water Chemistry Log**
Normal parameters: NH3 (<.25ppm), Nitrite (<.01mg/L), Nitrate (<.1ppm), pH (8.3-9), salinity (15-24ppt), Room Temp. 25ºC(+-) 4 ºC

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<th>C°</th>
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### 9.3 Daily Feeding Data Sheet

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### 9.4 Mortality Log

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