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**1. PURPOSE**

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This Standard Operating Procedure (SOP) describes routine care and husbandry practices for fish species.

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**2. RESPONSIBILITY**

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Principal investigator (PI) and their research staff, animal care staff.

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**3. MATERIALS**

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- 3.1. Aquaria
- 3.2. Feed
- 3.3. Water monitoring kit
- 3.4. Sanitation kit
- 3.5. Records (water quality, feeding, experimental)

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**4. PROCEDURES**

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- 4.1. Aquaria:
  - 4.1.1. Use preferably glass or Plexiglas tanks for easy observation of fish.
  - 4.1.2. Adapt the system to species-specific and research needs.
  - 4.1.3. Ideally, select systems with water quality monitors that can continually assess temperature, pH, conductivity, dissolved oxygen, etc. If not possible, use water quality meters to check these parameters periodically.
  - 4.1.4. Use preferably tanks that are easy to remove individually for cleaning or access to fish.
  - 4.1.5. Set up breeding tanks with drop-through mesh, marbles or grating to prevent fish from eating their eggs, or apply other techniques to prevent cannibalism. For juvenile fish, provide special screened baffles to avoid them being swept out of the tanks with the water flow.
- 4.2. Space Requirements:
  - 4.2.1. A standard recommendation for space requirements of fish is one inch of fish per gallon of water.
  - 4.2.2. How well the fish survive, grow and reproduce can be used to assess adequacy of space as well as successful care.
  - 4.2.3. The housing density is also dependent on the capacity of the biofilter.
  - 4.2.4. To estimate the capacity, use the following formula:
    - 4.2.4.1. Calculate the surface area of the aquarium (surface = width x length)
    - 4.2.4.2. Divide the calculated surface area to calculate the number of inches of fish the aquarium can house:
      - For fresh water, tropical fish:  $(W \times L) / 12 = \text{Number of inches of fish}$
      - For fresh water, cold water fish:  $(W \times L) / 30 = \text{Number of inches of fish}$
      - For marine tropical:  $(W \times L) / 48 = \text{Number of inches of fish}$
  - 4.2.5. There is a wide range of recommendations regarding space requirements for zebra fish. Ranges of six to twenty five fish per two and a half liters of water are common.
  - 4.2.6. Generally, growing fish and breeding adults are thought to require more space than non-breeders.

- 4.2.7. Schooling fish can be kept at fairly high densities; for aggressive fish, densities and group composition should be carefully monitored.
- 4.3. Environment and Environmental Control:
  - 4.3.1. Temperature of the water (or indirectly, the room for closed systems) should be recorded at least once daily; continuous monitoring is recommended.
  - 4.3.2. Each species of fish has its specific optimal temperature range. Temperatures should be kept stable, unless variation is inherent in the experimental protocol.
  - 4.3.3. Automatic temperature monitoring is preferable as problems can be detected immediately. The water temperature or room air is controlled with thermostats. The room air must be heated a degree or two warmer to allow for evaporative cooling. Ultraviolet light filters will also raise the water temperature a degree.
- 4.4. Dissolved Oxygen Concentration:
  - 4.4.1. Monitor dissolved oxygen periodically.
  - 4.4.2. An adequate reading is 6.0 ppm (mg/l), although tolerance to hypoxia varies widely among species and experiments may require specific dissolved oxygen levels.
  - 4.4.3. Fish remaining at the surface of the water or gulping are probably not getting enough oxygen. Over oxygenation of the water should also be avoided.
- 4.5. Lighting:
  - 4.5.1. Lighting differs a lot for different species. Weakly electric fish are nocturnal and hide during the day. Many species live in murky water and never encounter bright light. Strong light during the day is not required.
  - 4.5.2. Circadian light cycles should be adapted to the species or breeding requirement.
  - 4.5.3. Lights over tanks must have protective covering.
- 4.6. Behavioral Social Environment:
  - 4.6.1. Environment should allow species typical behavior as much as possible without interfering with research objectives.
  - 4.6.2. Most fish will react to loud, sudden sounds, vibration and movement. These stimuli are best avoided when possible as it has not been well investigated if fish are stressed by these stimuli.
  - 4.6.3. Many aggressive species will do better with cover provided within the tank to buffer interactions.
  - 4.6.4. Human interaction can be stressful and care should be taken when moving tanks and netting any type of fish to avoid sudden noise, movements or chasing the fish to net them.
  - 4.6.5. Some fish, such as salmonids, prefer a dark base to the holding tank to mimic the natural environment's dark sea or lake bed.
- 4.7. Feeding:
  - 4.7.1. As a general rule, feed an amount of food that the fish will consume within a few minutes, and only stock food that will be used within one month.
  - 4.7.2. Different feeding regimes may be appropriate depending on the fish species.
  - 4.7.3. Fish should be fed daily, but feeding schedules should be modified to fit the species and life-history stage.
  - 4.7.4. Adapt the food to the species under study.
  - 4.7.5. Reduced appetite and activity during feeding is usually a clear sign that fish are not well.
  - 4.7.6. Many fish foods have no expiration date therefore shipment dates and "in use" dates should be noted. Write an arbitrary expiry date on the food container based on the type of food and the mode of conservation. In general, dry food can be kept for 6 months at room temperature and for 24 months when kept frozen. Food should not be stockpiled as it has a short shelf life and nutrient values decline quickly. Refrigerate or freeze foods in sealed containers until use.
- 4.8. Water Quality:
  - 4.8.1. Parameters can be monitored by hand or with instruments.

- 4.8.2. Useful water parameters to monitor are concentrations of ammonia, nitrates, temperature, pH, conductivity (dissolved solids), and dissolved oxygen.
- 4.8.3. As a general rule, in well-established systems, temperature should be checked daily; pH, ammonia, and nitrates are checked once weekly. When fish are added to new tanks, monitor ammonia, nitrate daily until the biofiltration stabilizes.
- 4.8.4. The frequency of monitoring and the need to check additional parameters (e.g., O<sub>2</sub> concentration) will be increased in recirculating systems, in extensive housing, or new installations.
- 4.8.5. For zebra fish, pH range is 6.8 to 7.2; ammonia and nitrite levels should be below 0.3 ppm, and nitrate levels should be below 200 ppm. If these levels reach upper limits, a 10% water volume change at a time can be done.
- 4.8.6. Recirculating water systems rely more heavily than flow through systems on maintaining good filtration processes.
- 4.8.7. Any water used in the fish system should be dechlorinated. Water can be conditioned by adding Prime and a pinch of Biozyme and letting it stand for at least 24 hours, or by transferring water back and forth between two containers to precipitate evaporation of chlorine, and letting it stand for 24 hours.
- 4.8.8. A system with ultraviolet light filters to kill bacteria in the water will not provide one hundred percent protection against all organisms.
- 4.8.9. Quarantine fish racks should have a separate water system, monitoring probes, and supplies to avoid contamination.
- 4.9. Sanitation
  - 4.9.1. Good sanitation programs prevent cross contamination and disease.
  - 4.9.2. The most common disinfectant used on fish equipment is a bleach solution followed by thorough rinsing preferably in a cage washer (without soap) with hot water (86°C or 180°F). Soap should be avoided as inadequate rinsing will kill fish and disrupt bio filters. Other disinfectants, such as iodophores, can also be used. Allow adequate contact time according to manufacturer's recommendation or a minimum of 1 hour for a bleach solution.
  - 4.9.3. Equipment can be rinsed with chlorinated water as long as it is completely dry before use. This allows the chlorine to dissipate.
  - 4.9.4. All equipment for use on quarantine fish should be cleaned separately.
  - 4.9.5. Fish tanks do not need to be cleaned if water quality parameters are good, and are absent of diseases.
  - 4.9.6. Fish tanks are often cleaned when algae begins to obscure the view of the fish. Debris and slime buildup depends upon the numbers of fish and how often the fish are fed.
  - 4.9.7. Tanks should be cleaned when they are emptied.
  - 4.9.8. Debris accumulating in the tank should be removed daily to weekly, or when water quality is bad.
  - 4.9.9. In closed systems, about 30% of the water should be changed weekly. If water quality remains acceptable, simply top off using deionized water.
  - 4.9.10. Chemical and mechanical filters should be cleaned or replaced regularly according to manufacturer's recommendation and fish density.
  - 4.9.11. Fishroom floors should be mopped weekly. Shelves and counters should be wiped clean as needed.
- 4.10. Pest Control:
  - 4.10.1. Most commonly encountered pests are roaches. Eliminating hideouts, spilled food on floor and tank lids, and access with tight-door sweeps will control insects.
- 4.11. Emergency Plans:
  - 4.11.1. An emergency or disaster plan should be in place as well as back up (duplicates) of critical equipment and an electric generator. The system must be able to maintain the fish for 24 hours minimum.
  - 4.11.2. Weekend or holiday care should include once a day feeding for adults to maintain optimum egg production. Larval fish require normal care daily so it may be advisable to have fewer fish or none in the nursery over a long holiday break.

- 4.12. Identification and Records:
- 4.12.1. Identification labels placed on fish tanks should contain relevant information such as, for example, genetic background, stock number, and birth (fertilization) date. The PI name and protocol number can be included if there are more than one for a room or facility.
  - 4.12.2. Records can be kept in a computer database file.
  - 4.12.3. Records should be kept of temperature, water quality, feeding, and sanitation.
  - 4.12.4. Procedures conducted on the fishes must be documented in a medical record. A group record (instead of individual records) is acceptable.
- 4.13. Preventative Medicine:
- 4.13.1. All newly acquired fish should be quarantined.
  - 4.13.2. No fish from this isolated, self contained, set up should ever be mixed with the non-quarantine fish.
  - 4.13.3. Only surface sanitized (with mild bleach solution) embryos are introduced to the non-quarantine facility. Once the fish strain has been established from the embryos the adults in quarantine can be euthanized.
- 4.14. Observation and Control of Disease:
- 4.14.1. Fish should be observed daily for signs of illness or injury. Veterinary staff must be contacted if illness or injury is observed.
  - 4.14.2. Signs of disease can include color changes: (paleness, redness, hemorrhage), shape changes: (weight loss, bloating, skeletal deformities, masses/swellings, eye protrusion) external lesions: (ulcerations, fin erosion, fin clamping, protruding scales) behavioral changes: (rapid breathing, loss of equilibrium, lethargy, erratic movements, gathering at water surface) and increased mortality.
  - 4.14.3. If moribund or dead fish are noted water quality and husbandry should be reviewed.
  - 4.14.4. Diagnostic work-ups can include skin scrapings, fin or gill biopsy, necropsy, bacteriology, virology and histopathology. Using sentinel fish to monitor water systems can help disease investigation.
  - 4.14.5. Treatments and drugs should always be tested on a few fish before group treatments. Potential toxicity to biological filtration should be considered. Hospital tanks should be used to isolate and treat sick fishes.

## 5. REFERENCES

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- 5.1. Westerfield, M. The Zebrafish Book: A Guide for the Laboratory use of Zebrafish *Danio rerio* (University of Oregon Press, Eugene 2000).
- 5.2. Monte Matthews, Bill Trevanow, PhD, and Jennifer Matthews, DVM, PhD. A virtual Tour of the Guide for Zebrafish Users. *Lab Animal* Volume 31, No. 3. March 2002.
- 5.3. Courtland, Sam. Recirculating Systems for Zebrafish. *Lab Animal*, Volume 31, No. 3. March 2002.

### Species-Specific Information

Attach Completed Questionnaire to your Animal Use Protocol and submit to the FACC.

#### 1. INVESTIGATOR INFORMATION

- 1.1. Principal Investigator:
- 1.2. Protocol Number:
- 1.3. Location of animal housing: Building:                      Room:
- 1.4. Species: (Common name, scientific name optional):

#### 2. PERSONNEL AND EMERGENCY PROCEDURES

- 2.1. Who will be responsible for maintenance of aquaria and observation of animals?
  
- 2.2. Describe procedures for providing weekend and holiday care. Indicate who (e.g. regular animal care staff, students, part-time staff, etc.) provides and oversees care and what procedures are performed.

#### 3. DESCRIPTION OF TANK OR AQUARIUM

- 3.1. Number of tanks/aquaria:
- 3.2. Dimensions: (h x w x d):
- 3.3. Capacity (gallons):
- 3.4. Material:     Glass/silicone       Acrylic       Fiberglass       Other:
- 3.5. Type:         Fresh water       Marine (salt water)       Other:
- 3.6. How long are tanks maintained?

#### 4. WATER QUALITY

- 4.1. Water Source for aquaria:     Tap water     Well water     Other:
- 4.2. Treatment of water to be added to aquaria (check all applicable):
  - Filtration, describe:
  - Temperature control
  - Chlorine/chloramine treatment, describe:
  - UV sterilization
- 4.3. Testing of water to be added to aquaria (check all applicable):
  - Ph, give method:
  - Salinity, give method:
  - Hardness, give method:
  - Chlorine, give method:
  - Other:

4.4. Water quality maintenance (check all applicable):

Flow through system

Flow rate:

System capacity:

Static (non-flow through) system  Temperature control:

Water temperature:            °F            °C

Controlled by:             Room temperature             Water heater

Aeration

Filtration system: (Give manufacturer and model number of filtration system, if applicable.)

System description:

Filter flow rate:

Filter media:

Filter media change schedule:

Under gravel filter

Water changes

Frequency:

Percent of tank volume per change:

What provisions are made for preventing "new tank syndrome" (i.e. the build-up of ammonia and other nitrogen compounds that occur in a new tank before the bacterial biofilter is established)?

Algae control:

Gravel stirred and/or siphoned?

Frequency:

4.5. Light:     Incandescent             Fluorescent             Specialized lights, describe:

Controlled Photoperiod:

Time ON: \_\_\_\_\_ am pm    Time OFF: \_\_\_\_\_ am pm

4.5.1. Are aquarium lights on a timer?  Yes     No

4.5.2. Are room lights on a timer?     Yes     No

4.5.3. Does the room have windows?  Yes     No

4.6. Water quality monitoring:

4.6.1. Tests (check all applicable):

pH

Method:

Frequency:

Salinity

Method:

Frequency:

Hardness

Method:

Frequency:



6.1.3. List the cleaning/sanitizing agents (specify generic or broad category) used.

6.2. Cleaning and disinfection of room:

6.2.1. Describe animal room cleaning frequency, procedures (floors, walls, ceilings, ducts, exposed pipes, and fixtures), methods (hose, high pressure washer, vacuum cleaner, hand brushing, mopping, etc.), and cleaning/sanitizing agent(s) (generic name) used.

6.2.2. Describe the procedures for sanitizing cleaning implements such as mops and mop buckets.

6.3. Describe how the effectiveness of sanitization procedures is monitored (water temperature monitoring, microbiologic monitoring, visual inspections, etc.).

6.4. Describe the program for controlling pests (insects, rodents, etc.) noting the control agent(s) used, where applied, and who oversees the program and applies the agent(s).

6.5. Describe procedures for disposal of dead animals.

## 7. ANIMALS

7.1. Source:

7.2. Health status:

7.3. Stocking density:

7.4. Number of fish:

7.5. Size of fish:

7.6. Are animals compatible with respect to species, sex, etc.?  Yes  No

## 8. RECORDKEEPING

8.1. Daily and periodic animal care procedures should be recorded in a way that lab personnel, Laboratory Animal Services, the Facility Animal Care Committee, and regulatory site visitors can determine that such procedures are performed, and by whom. Please attach a sample, blank, form for each form you will use to record your animal care procedures.

## 9. ADDITIONAL INFORMATION

9.1. Please feel free to provide additional information that you feel will help document your aquatic animal care procedures. This could include existing written Standard Operating Procedures for your lab, references (citations or all or part of pertinent references), procedures from other labs, etc.