

## WVU IACUC Guidelines: Aquatic Vertebrate Guidelines

### Purpose

The purpose of this document is to provide guidance for care and management of aquatic facilities used for research and teaching at West Virginia University (WVU). The *Guide for the Care and Use of Laboratory Animals* (NRC, 2011) provides guidance regarding the care and use of aquatic animals in research. The appropriate management of aquatic vertebrates will vary based on species.

### Definitions

**Aquatic Vertebrate:** Any hatched, live animal that has a backbone or spinal column and lives most of its life in the water. Aquatic species may breathe oxygen through the air or water. Regulations apply to larval forms of fish and amphibians and offspring of egg-laying vertebrates only after hatching. Zebrafish are considered hatched at 3 days post-fertilization (dpf). Commonly used species include fish, amphibians, and reptiles.

**Life Support System:** Physical structure used to contain the water and the animals as well as the ancillary equipment used to move and/or treat the water.

**Satellite Facility:** A satellite facility is any containment outside of a core facility or centrally designated or managed vivaria in which aquatic animals are housed for more than 24 hours. (see WVU IACUC Satellite Housing Policy #21-001)

### Guidelines

1. Most aquatic vertebrates are housed outside of the centrally managed vivaria.
2. Laboratories that manage aquatic colonies **must** have SOPs in place for review by the IACUC and the Office of Laboratory Animal Resources (OLAR) veterinary staff which describe care and husbandry for the species.
3. Animal Acquisition and Quarantine
  - a. Aquatic satellite facilities order animals and manage census outside of the centralized animal ordering system.
  - b. Appropriate records **must** be maintained to ensure animal numbers in protocols are not exceeded. The laboratory is responsible for communicating with the IACUC regarding animal number use.
  - c. Animals should be counted on the animal use protocol based on the definition above of an aquatic vertebrate animal.
  - d. When receiving animals of varying health status, there should be appropriate quarantine procedures in place to maintain health status of the existing colony.

#### 4. Physical Plant

- a. Holding areas for aquatic species should be provided with drains of a suitable size and number to accommodate water released during system operation and maintenance, or as a result of life support system or tank failure.
- b. Materials used for floors, walls, and ceilings should be impervious to water while floors should be slip resistant and able to withstand the loads inherent with large quantities of water.
- c. Electrical receptacles or circuits should be ground fault interrupted (GFI) to prevent electrocution of personnel and animals.
- d. Doors and frames, supply diffusers, exhaust registers, lighting fixtures, HVAC ducts and components (exposed to high levels of moisture or corrosives), and other metallic elements should be made of moisture- and corrosion-resistant materials.
- e. Sufficient ventilation should be provided to prevent moisture buildup on room surfaces and maintain suitable temperatures for the species.

#### 5. Water Quality

- a. Water quality is essential to the health and well-being of aquatic animals.
- b. Routine measurements and documentation of water quality **must** be performed.
- c. Water quality parameters will vary by species and life support system.
- d. Satellite facility SOPs should describe parameters which are monitored, expected range, frequency of monitoring, and process for management of animals if water parameters deviate from set points.
- e. Critical life support parameters should be identified and monitored remotely to ensure animal health.
- f. Chlorine and chloramines used to disinfect water for human consumption (e.g., city water) are toxic to fish and amphibians and **must** be removed or neutralized before use in aquatic systems.

#### 6. Life Support System

- a. Biofilters **must** be of sufficient size to process the bioload entering the system.
- b. Source water selection should be based on the provision of a consistent or constant supply, incoming biosecurity level requirements, water volumes needed, species selection, and research considerations.

#### 7. Environmental Parameters

- a. Water temperature may be controlled at its source, within the life support system, or by controlling the macroenvironment.
- b. The macroenvironment may influence water temperature even if the life support system provides control.
- c. Room temperature setpoints should be established based on each species and appropriate remote alarm systems established at the room level.
  - Deviations in room temperature should be reported to facilities via phone and the Attending Veterinarian (email).
- d. Acceptable room humidity levels are defined by safety issues, equipment needs and staff comfort.
- e. Acceptable room air exchange rates are determined by thermal and moisture loads.

- f. Appropriate photoperiod and automatic light cycle should be established based on species, life stage, and experimental use.
- g. Mechanical failure of equipment which results in animal loss or injury **must** be reported to the Attending Veterinarian.

#### 8. Husbandry and Care

- a. Appropriate husbandry standards should be established based on species.
- b. Tanks housing fish are changed and cleaned/sanitized as often as necessary to allow for visualization of all fish housed in the tank, maintenance of colony health, and appropriate water quality parameters.
  - Algae growth is acceptable providing it does not prevent visualization of the fish for health assessment.
- c. System components such as lids on fish tanks, which may accumulate feed, may require sanitation as often as weekly depending on the frequency and type of feed and the system's design.
- d. Ideally, nets should be designated to each system based on health status. Appropriate cleaning and disinfection protocols should be in place after use.
- e. All husbandry methods **must** be validated on a routine basis using ATP testing methodology or other method approved by the IACUC committee. ATP testing can be scheduled by contacting [OLARhusbandrysupervisors@hsc.wvu.edu](mailto:OLARhusbandrysupervisors@hsc.wvu.edu) to set up a time to test after cleaning.
  - Commonly used procedures include mechanical scrubbing followed by soaking in a dilute bleach solution and rinsing.
- f. If mechanical washers are used, water temperature **must** be validated each day a load is ran. Temperature can be validated using temperature test strips. Water temperature should reach at least 160 degrees (~15 second contact time) or greater.
- g. Cleaning, sanitizing, or changing of other equipment and system components (e.g. filters, UV bulbs) is performed according to specific laboratory SOPs.
- h. Routine cleaning of the room (sweeping/mopping) should be done on an established basis as determined by the needs of the area in order to maintain cleanliness. Cleaning agents should be chosen based on compatibility with aquatic species.
- i. Feeding
  - Animals **must** be fed at least once daily an appropriate diet based on species.
  - Food **must** be maintained in a sealed container, stored according to manufacturer recommendations, and expiration dates written on containers (NON-live food).
  - Live food sources should be maintained and managed to ensure a steady supply and the health and suitability of the organism as a food.
- j. Animals **must** be checked and fed daily by an approved individual. This includes weekends/holidays/emergencies. OLAR can assist with health checks if an emergency arises. Please contact [Olarvetstaff@hsc.wvu.edu](mailto:Olarvetstaff@hsc.wvu.edu) if needed.

#### 9. Enclosure Space and Density

- a. Animals **must** have enough space to make normal postural adjustments (e.g., the animal should be able to completely rotate without touching the sides of the enclosure).

- b. For semi-aquatic reptiles, terrestrial areas should be provided to allow for normal behaviors as appropriate to the species (e.g., basking, feeding, digesting, and oviposition).
- c. Adult zebrafish (*Danio rerio*) in typical biomedical research settings are generally housed 5-12 adult fish per liter of water.
- d. If fish are single housed, they **must** be appropriately identified based on the WVU IACUC Social Housing Policy #18-001.

#### 10. Environmental Enrichment

- a. Enrichment should be provided based on the WVU IACUC Guidelines Environmental Enrichment for Animals #11-011.

#### 11. Personal Protective Equipment

- a. Personnel working with fish or any aquatic species should wear disposable gloves if in direct contact with fish, fish water, tanks and other equipment in that come into contact with fish.
- b. Disposable palpation sleeves can be used for cleaning tanks or reaching into deeper aquaria. These are available from OLAR.
- c. **Door signage should identify potential risks to personnel, where relevant.**

#### 12. Veterinary Care

- a. Any sick animal identified by the laboratory **must** be reported to the OLAR veterinary staff.
- b. An increase in colony morbidity or mortality above baseline levels **must** be reported to OLAR veterinary staff immediately. Labs should provide a monthly colony mortality to the clinical veterinarian.
  - Mortality logs are submitted to veterinary staff on a monthly basis.
- c. Laboratory personnel **must** work with OLAR vet staff to establish an animal health surveillance program for the colony that includes routine health testing. Contact [OLARvetstaff@hsc.wvu.edu](mailto:OLARvetstaff@hsc.wvu.edu) to setup a routine colony health surveillance program and animal submission schedule.
- d. OLAR veterinary staff will provide routine health check visits for aquatic species (see WVU IACUC Satellite Housing Policy #21-001).
- e. OLAR veterinary contact information will be provided and **must** be posted within housing space.

#### 13. Records

- a. The following records **must** be maintained and available for review by the IACUC or Veterinary staff
  - Daily room log (animal health status, mortality, feeding, temperature of life support system, water quality parameters, water changes, tank change/cleaning, equipment maintenance, room cleaning, room environmental parameters--temp./humidity).
  - Animal daily census
  - Training records for staff
  - Laboratory husbandry SOPs
  - Validation records for cleaning methods
  - Mortality log (submitted monthly to OLAR Vet Staff at [OLARvetstaff@hsc.wvu.edu](mailto:OLARvetstaff@hsc.wvu.edu))

## **References**

1. [Guide for the Care and Use of Laboratory Animals](#), National Research Council, 2011.
2. “A review of the role of temperature time in an effective cage sanitization program” October 1994 [Contemporary topics in laboratory animal science / American Association for Laboratory Animal Science](#) 33(5):66-8.
3. “Appropriate rearing density in domesticated zebrafish to avoid masculinization: links with the stress response” March 2017 [The Journal of experimental biology](#) 220(Pt 6): 1056-1064.