

SOP #: 317.01Title: SOP - African Clawed Frog (Xenopus laevis) Care and Husbandry

Approvals:

Attending Veterinarian

Date:

Principal Investigator

Date:

1. Purpose

1.1 This standard operating procedure is to provide instruction in the appropriate handling and care of Xenopus frogs.

2. Responsibility

2.1 It is the responsibility of all Mukhopadhyay Laboratory personnel to understand and practice the proper husbandry and care of Xenopus adults housed in the laboratory.

3. Definitions

3.1 Tap water: Water out of the tap from public water supplies. It contains up to 4 ppm (4 mg/L) of chlorine, (EPA), and usually less than 1 ppm ammonia/ammonium.

3.2 AmQuel Plus: commercial water conditioner. Used at a dilution of 5 ml per 10 gallons of water to detoxify chlorine, chloramines, nitrates and nitrites and kept for at least 30-60 min before it is ready for the animals.

4. Guidelines**4.1 Housing**

4.1.1 Frogs are housed in the Academic Health Center 1 (AHC1), laboratory #425

4.1.2 As soon as the frogs from Xenopus Express arrives in the laboratory facility, are transferred into a Plexiglas tank (90X40X40 cm) with a capacity of 38 Gallons, where the water is pretreated with Amquel plus.

4.1.3 A maximum of three frogs will be housed in the tank at any given time.

- 4.1.4 Pieces of 7" by 2-1/2" PVC pipe and/or synthetic lily pads are used for enrichment and hiding places

- 4.1.5 **Water quality**

- 4.1.5.1 The pool has a 38 gallon capacity; add approximately 15 gallons of water treated with Amquel Plus to tank.
- 4.1.5.2 Use tap water from room #425.
- 4.1.5.3 Fill the tank with 15 gal water and let water stand for at least 72 hours before using to de-gas and dechlorinate the water and to bring to room temperature. Thiosulfate could be used to remove chlorine.
- 4.1.5.4 Treat the water with Amquel Plus 5 ml/ 10 gal of water
- 4.1.5.5 Bring water to room temperature (70° F) to avoid temperature shock to the frogs (a 1 or 2 degree change in temperature can be detrimental to the frogs)
- 4.1.5.6 A water thermometer with a probe in the tank automatically measures the water temperature and gives a readout
- 4.1.5.7 Dissolved gases (oxygenation) are not considered a critical parameter for Xenopus frogs—adult Xenopus are air breathers that must come to the water's surface to breathe. Some gases may be exchanged through the skin, but it is possible for Xenopus frogs to drown if kept underwater for several hours.
- 4.1.5.8 **Nitrogenous wastes** (values in parts per million—ppm)
 - 4.1.5.8.1 Nitrification of water will be controlled by
 - 4.1.5.8.1.1 biological filter (bacteria that break down ammonia and nitrites)
 - 4.1.5.8.1.2 water replacement
 - 4.1.5.8.2 Frog wastes contain urates that are converted to ammonia.
 - 4.1.5.8.3 Nitrosomonas or Nitrosococcus spp. convert ammonia to nitrite
 - 4.1.5.8.4 Nitrobacter spp. convert nitrite to nitrate.
 - 4.1.5.8.5 Nitrate (the least toxic of the nitrogenous components of frog waste breakdown) will be removed by water replacement.
 - 4.1.5.8.6 Levels of nitrogenous compounds in frog system water will be monitored. The following water quality standards will be applied:
 - 4.1.5.8.6.1 Ammonia (NH₃) acceptable range concentration: 0 to 0.8 ppm

4.1.5.8.6.2 Nitrite (NO₂) acceptable range concentration: 0 to 0.75 ppm

4.1.5.8.6.3 Nitrate (NO₃) acceptable range concentration: 0 to 20 ppm

4.1.5.9 The tank is cleaned every other day. The water filter and circulator should be cleaned regularly. It should be switched on with the addition of Amquel to the water.

4.1.5.10 While cleaning the tank the animals are kept in a smaller tank with pre-treated water.

4.1.5.11 Animal record is maintained using African Clawed Frog (*Xenopus laevis*) Care and Husbandry form 917 - current revision.

4.1.5.12 Water will be tested weekly for Nitrates, Nitrites and Ammonia using the kit protocol from Aquarium Pharmaceuticals.

4.1.5.13 Test records are maintained using African Clawed Frog (*Xenopus laevis*) Water Testing form 918 - current revision

4.1.6 General water considerations

4.1.6.1 Frogs are not to be placed in distilled or deionized (e.g., ultra-pure RO) water.

4.1.6.2 Do not use tap water from sink for frogs. The sink in the frog room is for hand washing only. The chlorine and chloramine present in city water are caustic to frogs' skin.

4.1.6.3 Once removed, frog system water must not be returned to the system. Frog system water that has been removed for frog transport or other uses (e.g., anesthetic agent diluent, egg harvest solution, etc.) must be discarded after use.

4.1.6.4 Do not add any chemical agents to frog water without specific instructions from LAR vet staff or the Principal Investigator. The frog water composition is controlled, and any alterations in osmotic pressure or pH of the water could prove harmful to frogs.

4.1.7 Temperature Range

4.1.7.1 The correct water temperature for adult *Xenopus* is 19-22 °C (66-71°F)

4.1.7.2 Room temperature should be 68°F (20.0°C) ± 3°F (± 1.7°C)

4.1.8 Light cycle and intensity (controlled automatically by automated system)

4.1.8.1 Circadian periodicity = 14 hours on, 10 hours off (14/10)

4.1.8.2 Lights on at 6:00 am, lights off at 20:00 (8:00 pm) Eastern Time. Light timer clock will be changed to coincide with local time (i.e., adjusted for Daylight Savings in spring and fall).

4.1.8.3 Incursions into the room during the dark cycle are discouraged, as the abrupt provision of light will startle the frogs.

4.1.8.4 Light bulbs provide full-spectrum lighting

4.1.9 Feeding

4.1.9.1 Frogs should be fed every alternate day with an adult *Xenopus* diet (commercially available from *Xenopus Express*).

4.1.9.2 Frogs may eat segments that are shed from their skin—this is normal.

4.1.9.3 The quantity of food supplied will target what frogs will eat within 1 hour following feeding.

4.1.9.4 Frog food intake will be monitored in conjunction with feeding. Frogs that appear underweight may be housed 2 per cage to reduce competition for food. Single housing is to be avoided, as it has been noted that the visual stimulation of another frog feeding may be important in activating a frog's desire to eat.

4.1.9.5 Pre-anesthetic food restrictions: frogs should be fasted 12 hours prior to anesthesia induction to reduce food regurgitation.

4.1.10 Procedures for Room Sanitation

4.1.10.1 The floor is cleaned once a week and waxed once a year.

4.1.10.2 The use of soaps, detergents, disinfectants, and other chemical agents will be avoided as much as possible. Cleansing chemicals will be used only when necessary and only under the direction of authorized personnel.

4.1.10.3 The whole room is UV treated once a year. No animals are kept in the lab during UV treatment.

4.1.11 Emergency Procedures

4.1.11.1 The animals will survive short power outages, even for a couple of days. However, during hurricanes, we will sacrifice all animals and dispose of the carcasses on the last day before the University shuts down.

4.1.11.2 The water tank will be emptied, dried and stored in a secure place.

4.1.12 Personal Protective Equipment: nitrile gloves and labcoat.