Title: Husbandry Care for Xenopus

I. Purpose:

The purpose of this policy is to outline the minimum standards of care for *Xenopus laevis* Frogs.

II. Policy:

All departments providing care for Xenopus must meet or exceed these minimum requirements which are based on the Public Health Service Policy, and the ILAR *Guide for the Care and Use of Laboratory Animals*.

III. Procedure:

All facilities housing Xenopus must follow the conditions specified in the UCD’s California Department of Fish and Game Permit to import, transport, or possess Research Detrimental Species (Permit # 537). For example water being drained from Xenopus tanks must be screened or treated to prevent escape of Xenopus and the release of reproductive material. A copy of this permit must be posted near or on the Vivarium door.

**Daily Procedures: (365 days a year without exception)**

Observe each animal and check for health issues. Signs to look for include red (or other) discoloration of the skin, failure to feed properly (or weight loss), open cuts or abrasions, bloating, and lethargy. Contact Campus Veterinary Services to report sick frogs. Check and record water temperature which should be maintained at 17-24°C (*X. laevis*) 24-28°C (*X. tropicalis*). Check that each tank is individually identified and has a total frog count, and adjust posted frog count as needed. Disinfect nets after each use. Record deaths and euthanasia in the room log. Feeding should range from daily to 2 times per week. (This depends on the nutritional quality and quantity of the food fed. Feeding interval should be based on feed manufacturer’s recommendations. Frogs should be left undisturbed for 3-5 hours after feeding.) Document parameters listed above in addition to room activities in room log sheet (feeding, animal number, room temperature).

**Weekly Procedures:**

Clean tanks at least once per week. Check and record water quality, pH (6.5-8.5, in recirculating systems a minimum pH of 7.0 is advisable) and conductivity (500-2000 μS (*X. laevis*), 500-1000 (*X. tropicalis*) μS). Feed containers must be cleaned on a weekly basis.
Water Quality and Temperature:
Water should be dechlorinated and chloramine free water with 1 adult frog per 2 liters of water and 5-10 cm in depth. Check and record water quality. If water quality values are out of the normal range contact your supervisor or PI to correct the issue. Document tank checks on room log sheet.

Suggested water quality should be kept in the following ranges for both X. laevis and X. tropicalis unless specified to maintain optimal growth and maintenance:
- 175-300 mg/L (X. laevis) and 100-300 mg/L (X. tropicalis) Hardness (CaCO₃)
- 50-200 mg/L Alkalinity (CaCO₃)
- > 7 mg/L (X. laevis) and >5 mg/L (X. tropicalis) Dissolved Oxygen
- < 0.02 mg/L Ammonia (NH₃)
- < 0.5 mg/L Nitrite (NO₂⁻)
- < 50 mg/L Nitrate (NO₃⁻)

Standing water tanks:
Transfer frogs to a clean tank containing water as described in water quality section. Use dedicated nets and accessories to that specific tank. Drain the water from the dirty tank. If the tank is hand cleaned, use a clean sponge or brush to aid in removing deposited debris. For disinfection, food grade hypochlorite, (e.g. ‘Klorite’) at 200ml/70L (0.28% solution), 3-10% bleach or Betadine scrub should be used. It is critical that the tanks are thoroughly rinsed clean of any residual chemical before placing frogs into the tank.

Drip Through water tanks:
Clean tanks in place with a brush to remove mild algae accumulation on an “as needed” schedule. For heavily soiled cages, net the frog and transfer it to a clean tank. Stop water flow to that specific tank and remove the tank from the system.

Biweekly Procedures:
Sweep/squeegee floors to remove dust, dirt, and excess water. Wipe down shelves used for housing when containers are removed for washing. Follow the UCD Housekeeping Policy.

Monthly Procedures:
Disinfect and sanitize room and scrub brushes, sponges, enrichment devices, holding containers and floors.

Feeding:
Frogs should be fed palatable, non-contaminated, and nutritionally adequate food at least twice a week or according to their particular requirements, unless the protocol under which they are being used requires otherwise. Feed should be kept in properly labeled vermin controlled containers and properly stored. It should be discarded either 6 months after being received or opened, or when properly stored at the manufacturer’s expiration date. (e.g. Nasco feed recommends if feed is kept dry and at room temperature, the food is stable for one year. This shelf life is extended indefinitely if feed is kept frozen. BUT, it also states a more frequent rotation of feed may be used when using X. laevis for research). Feed container should be
cleaned as needed or when replenished.

**Identification:**
Each tank should have an individual identification and total frog count.

**Environmental Enrichment:**
All frogs should have a refuge to serve as environmental enrichment, such as PVC pipe cut lengthwise. Ensure that there are not any sharp edges which may cause abrasions on the frog's skin. Other forms of enrichment are acceptable as long as they are non-porous, do not harm the frogs, and can be cleaned and disinfected.

**Euthanasia:** 2013 AVMA Guidelines on Euthanasia

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<tr>
<th>Acceptable Methods (S.7.3.4)</th>
<th>Acceptable with Condition (S3.7.5)</th>
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<tbody>
<tr>
<td>Injectables (barbiturates, dissociative agents and anesthetics as specified), External or topical Agents (pharmaceutical-grade buffered tricaine methanesulfonate (MS 222), benzocaine hydrochloride)</td>
<td>Inhaled anesthetics as specified, CO2, manually applied blunt force trauma to the head (requires secondary pithing), rapid freezing</td>
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*require IACUC approval*

**Facilities & Monitoring:**
Floors should be moisture-resistant, nonabsorbent, impact-resistant, and relatively smooth. Tanks may not be directly housed on the floor. Walls should be moisture resistant and have GFI electrical outlets that are properly positioned to eliminate possible safety hazards.

**Temperature, Humidity and Illumination:**
Heating and air in frog rooms should be controlled in a manner that supports species-specific needs (optimal room temperatures should be between 19-25°C/66-77°F). Room temperature should be recorded on a room log sheet and close to the temperature of the tank water to prevent having to adjust tank water temperatures as a result of fluctuations in room temperatures. Humidity at the room level does not directly impact Xenopus however high levels of humidity can be detrimental to electronic equipment. Regular monitoring of the HVAC system is important and is best performed at the room level. A lighting regime of between 12 and 14 hours of light, and 12 and 10 hours dark is recommended. All new frogs should be quarantined 14 days in a separate tank before entering the established colony. Care should be provided for quarantine tanks after all other tanks are attended to.

**Caging:**
Tanks should be constructed of non-porous material that can be cleaned and disinfected regularly and allow for daily observation of the animals. Tanks should provide a safe environment and not be constructed of material that may cause injury to the frogs. Frogs should be housed such that their primary enclosure meets their general needs based on species needs, behavior, and goals of the study. Generally Xenopus should be grouped housed however aggression, illness or project-related reasons may occur warranting individual housing.

**References:**
1. **American Association for Laboratory Animal Science.** Animal Care and Use Courses. Aquatic Animal Husbandry and Management. [https://www.aalas.org/](https://www.aalas.org/)


   - Xenopus Husbandry [www.aalas.org](http://www.aalas.org)


6. Effect of Water Hardness on Oocyte quality and Embryo Development in the African Clawed Frog, (Xenopus laevis) Godfrey, E.W., Sanders G.E, Comparative Medicine 2004, April; (2) 170-175.
