	Washington State University Institutional Animal Care and Use Committee	Standard Operating Procedure
	Minimum Standards for *Xenopus* Husbandry Approved: 8/26/2020 (Replacing version 12/19/2018)	
Author: Office of the Campus Veterinarian-CVS	Document Number: IACUC SOP-14	

I. Purpose:

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

The selection of appropriate housing systems requires professional knowledge and judgment and depends on the nature of species and age of *Xenopus* used, and the design of the experiments.

Sanitation frequency, system maintenance and water quality monitoring will vary based on the species, aquatic system and research needs. Unique species or research requirements may be further defined in specific IACUC approved protocols or Standard Operating Procedures.

III. Procedure:

In addition to the procedures below, all facilities housing *Xenopus* must follow the conditions specified in WSU's **Aquatic Invasive Species Permit** for African Clawed Frogs from the State of Washington Department of Fish and Wildlife. For example, all waste water must be treated prior to release, all specimens must be confined to a secure facility that will prevent escape and no specimens can be transferred without director approval. A copy of this permit must be posted near or on the facility door.

Daily Procedures: (365 days a year without exception)

Observe each tank and check for health issues.

- Observe all animals for signs of illness or distress. Signs to look for include red (or other) discoloration of the skin, abnormal behavior, loss of body condition, open cuts or abrasions, bloating, and lethargy.
- Record feeding: the feeding interval should be based on species, life stage, the nutritional quality and quantity of the food fed. Feeding should range from daily to 2 times per week. Frogs should be left undisturbed for 3-5 hours after feeding.
- Check that each tank is identified, clean, free of damage and that the water level is sufficient.
- Document all transfers, deaths and euthanasia of frogs
- Contact the Office of the Campus Veterinarian (OCV) to report sick frogs and

mortalities or log entries in the OCV health database. Some mortality is anticipated in developing tadpoles. If within expected ranges, mortality data can be provided to OCV in a monthly summary. Unanticipated and higher than normal rates of mortality must be promptly reported to OCV.

- Check and record water temperature, which should be maintained at 17-24° C (*X. laevis*) or 24-28° C (*X. tropicalis*).
- Document parameters listed above in addition to room activities on room log sheet (feeding, health check, temperature of water or room depending on aquatic system).

<u>Sanitation</u> (twice weekly, monthly as appropriate):

Standing/Static water tanks:
Siphon solid wastes from tanks as needed, typically after feeding. Clean tanks to remove mild algae accumulation on an "as needed" schedule so that algal growth does not interfere with daily observation of animals. Replace a percentage of system water volume as appropriate with conditioned water as determined by nitrate levels, total ammonia nitrogen, and/or pH. Document procedures on room log sheet.

Recirculating systems with central filtration and flow-through water tanks:
Same as above but also back-flush, clean and/or replace filters associated with mechanical filtration systems as needed and monitor biological filtration system media levels.

Sanitation monitoring: Both *Xenopus* and the supportive biological filter necessary for denitrification are extremely sensitive to chemical exposure, so disinfection of aquatic system components should be appropriate for the research and level of risk. At minimum, projects involving multiple animal sources or exposure to infectious disease need to participate in the sanitation monitoring program. Please see the *Guide,* pg. 86 and the WSU sanitation monitoring SOP for more detail.

Waste water:
All aquarium/holding tank water is to be treated for 30 minutes with ¼ cup of regular household bleach per 10 gallons water prior to disposal into a municipal wastewater treatment system. An alternate method of equal to or more stringent decontamination effectiveness could be used on approval (requires approval from Washington Department of Fish and Wildlife).

Housing Rooms:
All animal rooms should be regularly cleaned and disinfected.

Water Quality monitoring:

In addition to daily temperature checks, check and record water quality parameters based on the species, aquatic system, density, and with a new or modified system. If water quality values are out of the normal range, action is required to correct the issue.

- The below parameters should be tested at least weekly in static systems & at least monthly in established recirculating or flow-through systems. (More frequent testing is required with new or newly modified systems):
  - *pH - 6.5-8.5 (in recirculating systems a minimum pH of 7.0 is advisable)*
  - *Conductivity (500-2000 µS/cm (X. laevis), 500-1000 µS/cm (X. tropicalis)*
  - *Alkalinity ($CaCO_3$) 50-200 mg/L*
  - *Ammonia (NH_3) < 0.02 mg/L*
  - *Nitrite (NO_2-N) < 0.5 mg/L*
  - *Nitrate (NO_3-N) < 50 mg/L*
- *Other testing recommended at least monthly*
  - *Dissolved Oxygen > 7 mg/L (X. laevis) and >5 mg/L (X. tropicalis) monthly*
  - *Hardness ($CaCO_3$) 175-300 mg/L (X. laevis) and 100-300 mg/L (X. tropicalis)*

Feeding:

Frogs should be fed palatable, non-contaminated, and nutritionally adequate food daily or according to their requirements, unless the protocol under which they are being used requires otherwise.

Pelleted feed should be stored in properly labeled vermin-controlled containers. It should be discarded either 6 months after being received or opened or at the manufacturer's expiration or best-by date. If bulk feed is stored frozen to extend the shelf-life, the manufacturer's verification of the extended shelf life should be kept on file and provided on request.

Raw liver can be frozen until used If not fed out fresh after receipt. Refrigeration of fresh or thawed liver should not exceed 5 days depending on the degree of freshness on receipt.

Feeding interval should be based on species, life stage, and specific feeding behavior.
All feedings should be recorded in room logs.

Identification and animal counting:

Each tank/tub should be individually identified. The number of animals acquired through breeding, purchase or other means must be entered and tracked on the IACUC database. Please refer to the WSU IACUC Guideline for Counting Animals. In addition,
Each research laboratory using Xenopus will need to submit the annual usage and disposition to the Animal Welfare Program Office by January 15 each year so that the

campus permit can be maintained. Fish and Wildlife requests that the annual report contain the number of animals, size, disposition, and the general nature of the research.

Environmental Enrichment:

Enrichment should elicit species appropriate behaviors and should be evaluated for safety and utility. Recommendations include social housing, a shelter, hide, artificial lily pad, or aquarium plants. Ensure that structural enrichment does not have any sharp edges which may cause abrasions on the frog's skin. Xenopus should be grouped housed however aggression, illness or project-related reasons may occur warranting individual housing. Refer to the Environmental Enrichment Policy for additional information.

Physical plant:

For interior facilities, floors should be moisture-resistant, nonabsorbent, impact-resistant, and relatively smooth. Walls should be moisture resistant and have GFCI electrical outlets that are properly positioned to eliminate possible safety hazard. Any non-GFCI circuits necessary for essential equipment must be elevated out of the "splash" zone so either high on the wall or on the ceiling. All electrical equipment located in aquatics areas that are powered under a non-GFCI protected outlets must be in good working order, have waterproof connections, and should be inspected regularly for damage. Outlets should be water resistant or fitted with waterproof covers. Pipes used for transporting water into and around the system must not be galvanized or copper, due to heavy metal leaching that can occur.

Temperature, Humidity and Illumination:

Heating and air in frog rooms should be controlled in a manner that supports species-specific needs. Depending on the system, room or water temperature should be recorded on a room log sheet. Humidity does not directly impact aquatic animals but high levels of humidity in frog rooms can be detrimental to electronic equipment and can promote microbial growth. Illumination levels, photoperiod and wavelength should be appropriate to the species and sufficient to allow visualization of the animals for health and well-being. Installation of emergency lights for when the light cycle is off is recommended. Xenopus typically avoid bright light, so tanks should be set up so that animals can retreat from direct illumination.

Tanks and Density:

Frogs should be housed in non-porous and non-abrasive primary enclosures that meet their general needs (i.e. proper size tank for species-specific requirements and for maintaining appropriate densities for group housed frogs). The needs of each situation must be evaluated by the IACUC in consultation with the Principle Investigator or possible outside experts. Space recommendations and housing density varies with species, age, life support and research but must designed to restrict escape (Xenopus do jump out) or accidental entrapment and allow for normal movement and postural adjustments. Published density recommendations are 1 adult frog per 2 liters of water or 6-8 larvae per liter. Depth must

be adequate to allow adult frogs to fully submerge in normal posture (> 6 inches).

References:

1. American Association for Laboratory Animal Science. Animal Care and Use Courses. Aquatic Animal Husbandry and Management. https://www.aalas.org/
2. NASCO (2003) 'NASCO On-line catalogs' http://www.enasco.com/prod/Static?page=xenopus
3. Laboratory Animal Science Association (LASA) (2001) 'Good Practice Guidelines – Xenopus Husbandry' www.aalas.org
4. *Guidance for the housing and care of the African clawed frog,* Xenopus laevis. Reed BT (2005), RSPCA, Horsham, UK. Report can be downloaded at: www.rspca.org.uk/xenopus
5. The Laboratory Xenopus sp., Sherril L. Green, 2010, A Volume in The Laboratory Animal Pocket Reference Series.