

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

**Title: Animal Care Plan {*Lithobates catesbeianus* (Bullfrog), Goldberg, Airport Gardens}**

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**I. Procedure:**

Daily (365 days a year without exception):

- Observe each animal and check for health concerns (*Guide* pg. 112).
- Clean and organize room, anterooms, and surrounding premises (*Guide* pg. 72).
- Record daily completion of tasks, environmental monitoring, initial, and date daily animal care sheet (*submit copy of sheet*).
- Report on the OCV Health Database M-F.
  - Any abnormal animals must be reported to the Office of the Campus Veterinarian (Health database), or if an emergency call 5-6246 or 509-335-1871.
- *Food type and method of water provision must be described along with where the food is stored and how storage conditions are monitored (humidity/temperature).*
  - *Animals will be fed in diet and frequency based upon life stage*
  - *Adult bullfrogs will be fed two times per week (every 3-4 days)*
    - *Diet will consist of crickets, earthworms, and wax worms*
    - *All food items will be obtained from a pet food supplier and maintained in the lab*
    - *Vitamin and mineral supplements will be administered once per week by dusting crickets prior to feeding*
    - *Monitoring of consumption of food items will be recorded on the daily care sheet.*
  - *Tadpoles will be fed every 2 days*
    - *Diet will consist of boiled lettuce or spinach, rabbit pellet powder, or fish flakes*
    - *Food items will be obtained from pet food suppliers and maintained in the lab*
- *Pest monitoring and/or control devices (define type) and documented on daily care sheet.*
  - *Sticky traps (non rodent) will be used to control insects.*
- *For aquatic species, water quality assessments must be outline (table preferred).*
  - *Water quality will be tested at time of Dechlorination treatment for: Ammonia, Nitrate, Nitrite and daily for the tadpoles. Room temperature and humidity is recorded daily. Routine ambient temperatures should be between 65-75 F. Humidity should ideally be 30-60%.*

Other Interval or As needed:

- *Describe what activities if any might be done on an alternative interval or on an as needed basis (sanitizing feed barrels, changing filters, aquatic enclosure cleaning, water testing device calibration, stall stripping, etc)*
  - *Containers will be cleaned every 10 days unless water quality requires more*

## Washington State University Standard of Veterinary Care for Satellite Animal Housing Location

*frequent.*

- All primary enclosures will be cleaned by soaking a 10% bleach solution for at least 2 minutes, rinsed with fresh water, and dried completely
- More frequent water changes will be implemented if water quality conditions approach:
  - Ammonia levels 0.2mg/L (keep below these amounts)
  - Nitrite levels 0.5 mg/L
- **Animal rooms and support area will be swept and cleaned. Areas will be disinfected between experiments.**

### Facilities

- *Describe where the animals will be housed (room, building, outdoor facilities).*
  - *Airport Gardens Building*
- *Provide date and findings of the ventilation assessment (air changes/hour and flow pattern) for interior facilities(Call the Animal Welfare Program for this information 57951)*
  - *No HVAC data available*
- *Describe illumination and mechanism of controlling light cycle. When was the last measurement taken? (AWP)*
  - *This is a natural lighting facility with windows*
- Interior room surfaces shall be moisture-resistant, non-absorbent, impact resistant, and sanitizable.

### Housing

- *Describe animal housing (cage, pen, tank, etc.) size and material*
  - *Adult bullfrogs will be housed individually in 10-gallon aquaria or 5 gallon buckets with secure lids that allow ample air flow*
    - *The bottom of the containers will be covered with 3-4 inches of treated water and a PVC tube or other structure sticking out above the water will be provided as a basking/perching spot*
  - *Tadpoles will be housed individually in approximately 1-gallon containers with secure lids that allow ample air flow*
  - *Bullfrog eggs will be housed in plastic shoebox containers with up to 50 eggs collected from the same location housed together*
    - *Eggs will be housed keeping kin groups together (eggs that are attached to the same vegetation)*
  -
- *Describe environment enrichment that will be provided*
  - *Adults are provided basking perch areas*
- *Describe if the animals will be housed socially or individually*
  - *Adults and tadpoles will be housed individually per ASAF*

## II. Sanitation Monitoring

- *Describe Sanitation Monitoring Program per SOP #5, if applicable.(Contact OCV at 509-335-6246 or [or.ocv.alert@wsu.edu](mailto:or.ocv.alert@wsu.edu) to enroll in sanitation monitoring)*
  - *See facility SOP*
  - *Housing containers or tanks will be swabbed once a year while in use after cleaning.*

## III. Waste Disposal

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

- *Describe where carcasses will be disposed*
  - Carcasses will be retained frozen in Heald 503 as needed for tissue specimens or otherwise incinerated.
- *Describe where soiled bedding or for aquatics where water will be disposed and how?*
  - Soiled water will be disposed of down the drain or bleached and disposed outside the facility in the gravel area.
- *Describe how hazardous waste is disposed if applicable*
  - N/A

**IV. Animal Numbers and Tracking**

- *List the person's name that will be tracking animals on My Research*
  - 38 will be responsible for reporting numbers to the IACUC office

**V. Signage, Emergency Information (List of posted signs and locations)**

- *Describe location of the following mandated signage and verify posting*
  - Guidelines for Reporting Animal Concerns
  - Emergency contact information for Satellite Housing Location Personnel
  - Any biological, chemical, radiation or other hazard signage as required

**VI. Security**

- *Describe how the facility is secured (electronic key, standard key and number)*
  - 41

**VII. Disaster Plan**

- See separate plan template below. Templates are available for all WSU locations

References

[https://www.fitchburgstate.edu/sites/default/files/documents/2021-03/210\\_01\\_Frog\\_Husbandry\\_122017.pdf](https://www.fitchburgstate.edu/sites/default/files/documents/2021-03/210_01_Frog_Husbandry_122017.pdf)

*Italics listed above are intended to be filled in with specific information about your species*  
Non italic wording should be left or modified to meet your specific needs

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

**Washington State University-Pullman  
Disaster Plan  
Animal Care  
Goldberg Lab- Airport Gardens**

The primary goal for this document is to give general procedures and information for research animal care and support that are to be followed in the case of an emergency. This is secondary to the employee disaster plan and will only be implemented when conditions provide a safe working environment for employees.

**Emergency Plan**

WSU telephone service has temporary emergency back-up power and would work in situations of power failure. Employees would be notified of problems and work plan by [REDACTED] 38 [REDACTED] 38 [REDACTED] would also be responsible for notifying Facility Operations, EH & S, and OCV of emergency situations in a timely period. Employees would report to work, as they are physically able to. In cases of extended power failure and/or loss of HVAC, animals would be triaged and cared for in priority. Water supply will continue functioning unless physically damaged. There are back up supplies of food and bedding for the animals. [REDACTED] 38 [REDACTED] will supervise care for the animals during a disasters situation with advisory aid from OCV. In the absence of [REDACTED] 38 [REDACTED], Caren Goldberg would take charge of such care.

**Phone List**

Name	Title	Contact number
Dr. Caren Goldberg	PI	[REDACTED] 14 [REDACTED]
[REDACTED] 38 [REDACTED]	[REDACTED] 38 [REDACTED]	[REDACTED] 38 [REDACTED]
Facility Operations		509-335-9000
EH & S		509-335-3041
Office of the Campus Veterinarian		509-330-1871 emergency cell 509-335-6246 office
Campus Security		911 (emergency) 509-335-8548(non- emergency)
Campus Fire Department		911 (emergency)

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

**Evacuation Plan:**

**In case of an emergency, such as a fire, everyone is required to leave the building immediately. Calmly exit the building or leave the impacted outdoor area and meet in the open grassy area between the building and the mesocosm area.**

## **SOP Bullfrogs- Airport Gardens**

Goldberg Lab

ASAF 6668

### **Animal Observation**

1. All animals will be observed daily, including weekends and holidays.
  - a. The PI and graduate students/lab technicians on the project will provide animal care after appropriate training has been completed and documented
  - b. During daily observation environmental conditions will be recorded

### **Animal Housing**

2. Housing of animals will depend on life stage
  - a. Adult bullfrogs will be housed individually in 10-gallon aquaria or 5 gallon buckets with secure lids that allow ample air flow
    - i. The bottom of the containers will be covered with 3-4 inches of treated water and a PVC tube or other structure sticking out above the water will be provided as a basking/perching spot
  - b. Tadpoles will be housed individually in approximately 1-gallon containers with secure lids that allow ample air flow
  - c. Bullfrog eggs will be housed in plastic shoebox containers with up to 50 eggs collected from the same location housed together
    - i. Eggs will be housed keeping kin groups together (eggs that are attached to the same vegetation)
3. Water used to house amphibians will be tap water treated with commercial dechlorinator
4. Environmental enrichment will be provided to adult bullfrogs in the form of PVC pipe pieces for basking/perching

### **Cleaning Procedures**

5. Containers will be cleaned after the 10-day experimental/holding period unless water quality requires more frequent changes
  - a. All primary enclosures will be cleaned by soaking a 10% bleach solution for at least 2 minutes, rinsed with fresh water, and dried completely
  - b. More frequent water changes will be implemented if water quality conditions approach:
    - i. Ammonia levels 0.2mg/L (keep below these amounts)
    - ii. Nitrite levels 0.5 mg/L

### **Animal Feeding**

6. Animals will be fed in diet and frequency based upon life stage
  - a. Adult bullfrogs will be fed two times per week (every 3-4 days)
    - i. Diet will consist of crickets, earthworms, and wax worms
    - ii. All food items will be obtained from a pet food supplier and maintained in the lab

## **SOP Bullfrogs- Airport Gardens**

Goldberg Lab

ASAF 6668

- iii. Vitamin and mineral supplements will be administered once per week by dusting crickets prior to feeding
- iv. Monitoring of consumption of food items will be recorded on the daily care sheet.
- b. Tadpoles will be fed every 2 days
  - i. Diet will consist of boiled lettuce or spinach, rabbit pellet powder, or fish flakes
  - ii. Food items will be obtained from pet food suppliers and maintained in the lab

### **Animal Handling**

- 7. Animals will be handled using disposable gloves (changed with each individual) that have been rinsed with water or a clean net that has been disinfected and rinsed at least five times with treated water

### **Housing Conditions**

- 8. Temperature
  - a. Room controls will be used to keep housing temperatures between 20C and 30C
  - b. Temperature will be monitored daily
- 9. Humidity
  - a. Will be monitored daily with room hygrometer
  - b. All life stages will have access to adequate water, if water levels are reduced by evaporation between water changes, additional water will be added to containers
- 10. Animals will be housed on a natural light/dark cycle via the windows in the barn

### **Pest Control**

- 11. The most probable pest/malady while rearing amphibians are bacterial infections. If any infections are detected on animals, we will disinfect all containers using a dilute bleach solution and place all animals in thoroughly rinsed disinfected containers with fresh water. If infection persists, we will discuss treatment with the campus veterinarian.
  - a. Prior to reuse, disinfected containers will be rinsed at least five times with water to remove traces of the chemical disinfectants
  - b. We do not anticipate pest problems in food storage of aquatic or terrestrial food sources (crickets, worms, daphnia, brine shrimp, etc.). If such problems arise, we will take appropriate pest removal measures dependent upon the situation.

### **Acclimation**

## **SOP Bullfrogs- Airport Gardens**

Goldberg Lab

ASAF 6668

12. Animals transferred from the field to the lab will undergo a 3-day acclimation period prior to any experimental sampling

### **Animal Room Cleaning**

13. Animal rooms and support spaces will be cleaned monthly and disinfected between experiments.

### **Documentation**

14. Documentation will be maintained of daily observations, cage changing, room cleaning and measurements of temperature and humidity.
15. Animal health will be reported on My Research OCV Animal Health Database: *Goldberg Eastlick B74A* Monday to Friday. Either there were no issues “No Change” or enter abnormal animal issues. If there is an emergency, or a serious animal issue call 5-6246 or 509-330-1871.

Sources for guidance:

Poole VA, Grow S. 2012. Association of Zoos and Aquariums Amphibian Husbandry Resource Guide. Edition 2.0.

<https://assets.speakcdn.com/assets/2332/amphibianhusbandryresourceguide.pdf>

Schaffer DO, Dleinow DM, Krulish L. 1992. The Care and Use of Amphibians, Reptiles, and Fish in Research. Scientists Center for Animal Welfare.

Odum RA, Zippel K. 2011. Water Quality for Amphibians. Amphibian Ark.

<http://www.amphibianark.org/wp-content/uploads/2018/08/Water-Quality-Odum-Zippel-2011.pdf>



# SUMMARY OF STANDARD OPERATING PROCEDURES WASHINGTON STATE UNIVERSITY

**TITLE:** XENOPUS HUSBANDRY

**PURPOSE:** TO OUTLINE THE PROPER PROCEDURES FOR THE CARE AND FEEDING OF XENOPUS SPECIES IN THE LABORATORY

The following SOP was developed based on the husbandry of Xenopus in consultation with Gay Lynn Clyde, DVM, and many published resources.

## **1. SUPPLY AND TRANSPORT**

### **A. Source**

- <https://www.mbl.edu/xenopus/Xenopus1>, Dexter, MI  
<http://www.xenopus1.com/Xenopus1/HOME.html>
- National Xenopus Resource Center, Woods Hole, MA <https://www.mbl.edu/xenopus/>

### **B. Transportation**

- Frogs or tadpoles may be shipped in thermos jug or plastic bag in an insulated container (Styrofoam). Size and density should be followed the IATA guide for live animal shipping.
- Well soaked sphagnum moss or wet sponges should be placed inside the secondary container to prevent drying out of the frog.
- Density in each container for transport depending on the species. Some amphibians may need to separate by sex. There should be only one type of species in each shipping container.
- Xenopus do not require food for short term shipment.
- The temperature for shipping xenopus is best between 10°-33° C. Heat packs or cold packs can be used if temperature out of the range. The animals should not have direct contact with the heat or cold packs.
- Shipment should be by overnight courier or air post. The shorter the time of the shipment will reduce the chance of complications.
- Upon arrival, the frogs or tadpoles should be transferred immediately into a previously prepared and clean container. This is especially important to save the water in the shipping container for aquatic amphibians. Rapid change in their water environment may result in shock or death.
- Gradually replace by slow dilution from shipment water to the facility water over a few hours to prevent shock of the new frogs.

### **C. Quarantine**

- All amphibians should be quarantine upon arrival. This can be accomplished by **housing them** in an isolated tank.
- There should be a separate quarantine room for the frogs and reptiles that come from wild or suspicion infectious disease carriers.

- A dedicated quarantine tank for frogs come from credible purpose breeder should be placed in the room with other research frogs. The water system of the quarantine tank should be completely separated from the other research frogs in the room.
- Wild origin frogs quarantine period is 2 to 3 months or more. For those that are captive reared and bred frogs, the quarantine period is 2 weeks.
- The new arrival frogs need to be assessed daily to look for the activity level, any discoloration, ulceration, petechia, abdominal swelling, and any other changes. If the new arrivals show any abnormal behavior or illness, they should be immediately removed from the colony and notify OCV.

## **2. HOUSING AND CARE.**

### **A. Lighting**

- Photoperiod
  - A cycle of 12 hours of light, 12 hours of dark is recommended
  - The relationship between photoperiod and *Xenopus* oogenesis is not known. Bellerby and Hogben (1933) suggest that photoperiod does not play a significant role in *Xenopus* oogenesis, but it may still need more investigation.
  - Some amphibian tadpoles (e.g. *Rana temporaria* & *Rana pipiens*) may require darkness to induce their growth rate and survival rate.
- Spectrum
  - Full spectrum wavelength bulb should be used
- Intensity
  - Avoid using bright light
  - Shelter or shaded area should be provided for the animals to retreat from light if they desire

### **B. Noise/Vibrations**

- Loud noises should be avoided. Low voices should be used in the room, along with reduction of vibration noises such as carts, cleaning equipment, etc.
- No music is allowed in the amphibian rooms

### **C. Water Provisions**

- Volume/Population Density
  - Adult *Xenopus*: 1 adult/large juvenile frog per 2 liters. At least 6 inches depth of water is needed for them to submerge completely and adequately covered.
  - Larvae: up to 6-8 larvae and recent metamorphs per liter
- Temperature and Humidity
  - Both *X. laevis* and *X. tropicalis* prefer warm water (see table below).
  - Temperature lower than normal range can make them lethargic, anorexic, and their metabolism and immune system will be depressed which will make them more susceptible to disease.
  - Higher than 30° C is lethal.
  - Changing of temperature should be done gradually. A sudden change of greater than 2° – 5° C will result in mortality

- Humidity should be maintained at about 80% in the room. Airflow should be adjusted or lowered to prevent desiccation and drying out of the habitats and the animals.
- Temperature in the room should be held similar to the temperature range in the tank since water in the tank may equilibrate with the room air temperature.

iii. Water Quality

- Dechlorinated water must be used.
- Pipes used for transporting water into and around the system should not be galvanized or copper, due to heavy metal leaching that can occur.
- Water quality conditions for *Xenopus* species are as follows:

Parameter	<i>Xenopus laevis</i>	<i>Xenopus tropicalis</i>	Testing Frequency
pH	6.5-8.5	6.5-8.5	Daily
Temperature °C	18-25	24-26	Daily
Conductivity uS	500-3000	500-1000	Daily
Chlorine/chloramine	0	0	Weekly
Alkalinity mg/L as CaCO <sub>3</sub>	50-200	50-200	Weekly
Hardness mg/L as CaCO <sub>3</sub>	175-300	100-300	Weekly
Ammonia mg/L	<0.02	<0.02	Weekly
Nitrite mg/L	<0.5	<0.5	Weekly
Nitrate mg/L	<50	<50	Weekly

iv. Cleaning and Disinfection

- Standing Water Tank (used for *Xenopus laevis*, Eastlick Vivarium Room F)
  - Standing water tanks will need manual water changes. Sive et al 2003,
  - Minimum water changes: 2 times per week.
- Flow-through system (used for *Xenopus tropicalis*, Heald Room 230)
  - Biofilter recirculated water system. Tanks are disinfected and changed every 2 weeks including all components of the tank. All lids are changed as needed due to excess food accumulation.
  - Trap of biofilter needs to be cleaned once a week.
- Use of Cleaning Agent
  - All tanks and equipment will be cleaned by manual scrubbing with warm deionized water to remove biofilm and debris.
  - All tanks and equipment are disinfected with a 1.98% solution of bleach with a 30-minute soak time. All tanks and equipment are then rinsed thoroughly in a commercial bottle washer the reaches 180 degrees F with deionized water. All tanks and equipment are then allowed to fully dry before reuse.
  - Fine mesh nets are rinsed with deionized water after each use, then soaked in a commercial net-disinfection or bleach solution, for example: (active ingredients, benzalkonium chloride and methylene blue, Net Soak, Jungle Labs, Cincinnati, OH)

or (bleach solution) for 30 minutes. Nets are then rinsed free of disinfectant with deionized water, and air dried before next use.

- Extreme caution will be used to ensure that all chemicals are thoroughly rinsed from all equipment before returning to use.

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v. **Recording**

- Action Log will be kept that tracks daily, weekly, monthly, morbidity and mortality and will be submitted to veterinary services monthly.

**D. Tank Housing**

i. **Labeling**

The minimal required information must be documented either on the tank or room (if single PI) Color coding with tape can be used to indicate PI, etc.:

- 1) Principle Investigator and contact phone number
- 2) Species/Genetic background
- 3) Date of receipt or hatch date
- 4) Sex
- 5) ASAF number

- ii. Lids and drain covers will be provided on juvenile and adult in tanks to prevent from jumping out or escaping.

**E. Identification and marking techniques**

- Pictures of *Xenopus* skin patterns can be useful for identification.

**F. Group Housing**

- Frogs group in the same tank should be the same species and similar sizes
- It is recommended to have at least 5-6 frogs per tank.
- Frogs will develop hierarchies within territories. Keep the same group of frogs together can prevent stress and injuries.

**G. Catching and Handling**

i. **Adult frog**

- Gloves should be used when handling amphibians. Do not use powdered or latex gloves and abrasive paper towels for these animals.
- Gloves should be well rinse and moistened with dechlorinated water when handling to prevent desiccation of the mucus layer of the frog.
- Soft fine mesh net can be used. The net should be appropriate size to comfortably hold the animal.
- Aquatic species can be transferred or held in plastic or glass well covered containers with tank water to prevent desiccation and protect the sensitive gills in aquatics.
- Physical restraint of adult frog for injection can be done with one-handed technique. See pictures below.

ii. **Larvae/aquatics**

- Larvae or aquatics can be transferred from tank to tank with net or glass or plastic pipet.



## H. Feeding regime and Food Types and Frequency

### i. Processed feeds

#### 1. Larval diets:

- Start feeding larvae once they start swimming at a 45-degree angle with the head pointing at the bottom of the tank.
- Feedings should be finely grounded. Since larvae are filter feeder, large particles may damage their filter feeding mechanism which may cause death.
- Larvae are fed powdered nettle (freshwater fish/amphibian food) or any other feed that is finely grounded.
- Fresh food slurry should be made fresh daily and fed twice per day.
- It may take 4-5 hours for the larvae to clear up the feed. Adjustment of quantity can be made if it is too soon or too long time to clear the feed. It is recommended to turn off the water system for 2 hours after feeding.
- Larvae stops feeding once the forelimbs erupt.

#### 2. Froglet (from half of tail absorbed to metamorphosis complete): Processed feeds can be utilized as exclusive food source for froglets beyond the larval stage if they are nutritionally balanced.

- When half of the tail is absorbed, froglets are fed diced meat (e.g., calf liver) or pellet chow provided by Xenopus1, similar to other commercial feed: Nasco post-metamorphic crushed brittle and Bio-Oregon Bio Vita Fry 1.2 m (MBL National Xenopus Resource)

#### 3. Juvenile or Adult:

- Adults will be fed pellets provided by Xenopus1, similar to other commercial feed: 1:1 of Post-metamorphic frog brittle (medium brittle)/Bio-Oregon Bio Vita Fry 2m (MBL National Xenopus Resource)
  - Larger adult: 6 pellets/frog
  - Juvenile/smaller adult: 10-12 pellets/frog
- It is recommended to feed adult frogs 2-3 times a week
- Amount may be adjusted whether the frogs clear up too fast or too slow. The frogs will finish most of the feed in 30 minutes normally. If the frogs finish the food in 15 minutes, that indicates inadequate of feed. In contrast, food leftover hours after feeding indicates feeding in excess and the amount need to be reduced.

- Remove leftover feed after several hours of feeding which helps to maintain the water quality and prevent water mold development

## **I. Environmental Enrichment**

### **- Group housing**

- Refuge cover should be provided for aquatic species. It can be floating neoprene “lily pads,” clay pots, earthenware pipes, cups hollow aquarium logs, rocks and acrylonitrile-butadiene-styrene (ABS) pipe. All materials need to be routinely disinfected.
- Torreilles and Green (2007) have found that providing a refuge cover with ABS pipe can reduce bit wound incident in high density stock.
- Live feeds such as tubiflex, mealworms, maggots can be a type of environmental enrichment

## **J. Assessment of Health and Disease Prevention**

### **i. Diagnosis of ill health**

- Observe the frogs without disturbing the primary enclosure. Look for signs:
  - Change in attitude or behavior
  - Excessive distension of the coelomic cavity (bloating)
  - Any open wound, lesion, ulceration, petechia, or discoloration on skin
  - Erosion or tremors on limbs
  - Excessive mucous production or shedding of skin
  - Weight loss
  - Buoyancy/righting problem
- A gentle tap on the side of the tank will trigger the normal frog to dive or search for refuge, but the sick frog may be slowed to respond or not respond at all.

### **ii. Common Disease (See attached Section)**

## **K. Biosecurity**

### **i. Prevent Introduction of Pathogens**

- All imported amphibians should have a source history
- A strict quarantine SOP should be followed (see Quarantine SOP above).
- Control personnel access and procedures. Limit entrance to facility to vivarium personnel, lab, and veterinary services. Use disinfection floor mats, booties, PPE for any other personnel coming into the facility.
- A morbidity and mortality report (online) should be submitted to the Office of the Campus Veterinarian daily

### **3. SCIENTIFIC PROCEDURE**

#### **A. Recognizing Pain**

- i. Behavior suggestive of pain or distress
  - Sudden movement
  - Avoid stimuli or escape behavior
  - Wiping or rubbing irritated area
  - Decreased or increased activity level
  - Loss of appetite

#### **B. Sedation, Anesthesia and Analgesia**

- i. Caution for sedation/anesthesia/analgesia:
  - Keep the frog skin moisten throughout the procedure by using a moisten container or spray the frog with dechlorinated fresh oxygenated water to prevent desiccation
  - Beware of temperature, an excessive sudden increase or decrease in temperature will kill the frog
  - During anesthesia, the frog may cease ventilation by the lungs. Gas exchange through the moisten skin can be sufficient for maintaining resting metabolism
  - Assess the depth of anesthesia by withdrawal reflex (toe pinch), corneal reflex, and righting reflex.
  - If immersion method is used, the induction chamber should have lid to prevent escape. Also, the anesthetic solution should not cover the head and nares of the frog during induction to prevent drowning. It is recommended to keep the level of anesthetic solution below the humero-scapular joint.
  - Frogs must be fully recovered from general anesthesia and no sedation signs from analgesic before returning to regular housing after surgery because they may drown. They can be placed into a shallow warm water bath for recovery or rinse them with well oxygenated fresh water until they recover.
- ii. Anesthesia: (induction of unconsciousness, e.g. examination, surgery, placement of chips or tags for ID, manual gamete removal.)
  - 1. Tricaine Methanesulfonate (MS-222) is available as pharmaceutical grade reagent (Western Chemical)
    - Stock solution (5-10 g/L)
      - Light sensitive, store in dark colored or foil wrapped bottles
      - Stock dated when made and stored at -20 C
      - Stable for 3 months
      - Discard stock solution when the color changes or an oil film develops
    - Working solution from stock solution
      - 1. Dilute the stock solution to working solution to
        - Adult: 1-5g/L
        - Larval/aquatic: 200-500 mg/L or 0.2-0.5 g/L
      - 2. Buffered to neutral pH 7.4 with sodium bicarbonate
      - 3. Stock dated when made and stored at -20° C

4. Stable for 1 month
  5. Discard solution when any color change or an oil film develops
  6. There are human safety concerns, handle in chemical fume hood, wear PPE, make MSDS available.
- Administration of MS-222
    - Immersed in buffered MS-222 at 1-5 g/L for adult and 200-500 mg/L for larval or aquatic
      - Immerse the frog in the buffered working solution 1-5g/L MS-222
      - Sedation may take 10-20 minutes after immersion
    - Injection into coelomic (peritoneum) cavity
      - 50-200 mg/kg
2. Benzocaine hydrochloride
    - Gel 7.5% or 20% (e.g., Oragel or Ambesol)
      - 0.5-1 cm length for 20% gel apply to ventrum
    - Stock solution (10% w/v in 100% ethanol)
      - Light sensitive, store in dark colored or foil wrapped bottles
      - Stock dated when made and stored at RT
      - Stable for 3 months
      - Discard stock solution when the color changes or an oil film develops
    - Working solution from stock solution. Dilute the stock solution to working solution to (make fresh prior to use and discard after use):
      - Adult: 1% suspension in aquarium water
      - Larval/recent metamorph: 0.005-0.01% suspension in aquarium water (pH ~7.5) for anesthesia depending on size of larvae, 0.02-0.03% for recently metamorph/larger juveniles.
        - Immerse the frog in benzocaine solution
        - Sedation may take 1-3 minutes after immersion
      - Injection into coelomic cavity
        - 50-200 mg/kg
- iii. Analgesia
    1. Buprenorphine
      - 38 mg/kg SC (Dorsal lymph sac with 22-28 gauge ½-¾ inch needle)

## C. Euthanasia

In accordance with the AVMA Euthanasia Guidelines 2013, an acceptable physical method of euthanasia of amphibians involves injection, immersion or topical application\* Any method of euthanasia must be described in a WSU IACUC ASAF protocol.

- Euthanasia of amphibians with injection, immersion, or topical application must be followed by an approved secondary method immediately to ensure death.
  - i. Primary method
    - a) Injection into coelomic cavity (intraperitoneum) or dorsal lymph sacs (see picture below)



- Overdose of pentobarbital: 60-100 mg/kg
- b) Immersion
  - Buffered MS-222
    - If using >5 g/L bath for less than 1 hour, a secondary method must be used right after the frog is removed from the solution.
    - 5-10 g/L bath for 1 hour
  - Buffered benzocaine hydrochloride
    - >250 mg/L bath
- c) Topical application
  - 20% benzocaine gel (2 cm X 1 mm) ventral abdomen application
  - This may take 7 minutes for the animal to loss right reflex and withdrawal reflex. Death occurred in 5 hours.
- ii. Secondary Methods to Insure Death
  - Frog must have loss of consciousness or anesthetized before using any of the secondary method
    - a) Decapitation
      - Decapitation must follow by pithing method for adults
    - b) Double pithing
      - Insertion of a sharp needle (22-25 gauge 5/8 inch) or metal probe at atlanto-occipital joint and enter the foramen magnum with the needle back and forth to transect the proximal spinal cord. After this, the needle/probe will be inserted in the foramen of the spinal cord to sever those neurons. See pictures below.
- Disposal: medical or hazardous waste for incineration

A



B



C



# Intracoelomic Injection

#### 4. NATURAL BREEDING (both *X. laevis* and *X. tropicalis*)

##### A. Female

- breeding age from 8 months of hatching to 5 years
- To ensure the quality of oocytes, it is recommended breeding every 3-6 months
- Sexually mature sign – cloacal valves are larger and visible

##### B. Male

- Breeding age can be as early as 4-6 months of hatching
- Sexually mature sign – darkened nuptial pads on the inner forelegs and fingers

##### C. Induction of ovulation with hCG (human chorionic gonadotropin)

**The first, or "priming", injection is optional: 10 U of hormone for males and females in a volume of 0.1ml of sterile phosphate buffered saline (i.e. dilute stock 10:1) for *X. tropicalis* and 50 U for males and 100 U for females (which are larger) in *X. laevis* (Sive et al., 2000). This is followed by a second, "boosting", injection of 100-200 units in 0.1ml (undiluted stock) for *X. tropicalis*, and 500-800U for females (depending on size) and 50U in males in *X. laevis*. Frogs will usually mate/lay without the priming injection, just a little more slowly. The highest doses for *X. laevis* are largely attributed to a larger body size.**

Injections are made into the dorsal lymph sac or the coelom (body cavity) by inserting the needle just beneath the skin between the dorsal lateral line stripes (see the Cold Spring Harbor Xenopus manual for proper injection technique, Sive et al. 2000). Avoid intramuscular injection.

After the first injection return the frogs to their original containers and leave them in a quiet place at 22-25°C overnight. The priming injection should be done 12 to 72 hours before the boosting injection. After the boosting injection, the animals should be divided into male/female pairs and placed in clean biofiltered water (or 0.05 X Marc's Modified Ringer, pH 7.6-8.0) in a fresh container. The mating tanks should be put in a quiet dark place. Amplexus should begin 1-3 hours after boosting and can continue for up to 6 hours. For some females, however, it could take 12-18 hr (overnight) or not occur at all. This is thought to be related to stress or reproductive condition.

##### *Timetable for natural mating:*

- 1 to 2 days prior to mating: Prime females and males
- 3 to 4 hours prior to mating: Boost each animal with HCG
- Place male/female pairs in mating containers, at room temperature in a quiet area
- Mating: Observe animals periodically w/o disturbing
- Collect eggs at desired intervals
- After adults breed, males and females are placed in clean water either singly or in pairs by sex in separate containers, and monitored for 48 hr for normal feeding and activity to ensure that breeding did not induce an infection (see section 2.J.)

#### 5. GUIDELINES FOR *IN VITRO* FERTILIZATION

This procedure is used primarily for *Xenopus laevis* when embryos at a particular stage are required at the same time (e.g, during Biol 321 laboratory when students are observing all stages of embryonic development, including the cortical rotation and initial cleavage, CRISPR injections). In this procedures females are primed as if a natural breeding will occur, but instead, eggs are manually extracted from females, testes are dissected from euthanized males, and sperm from these testes fertilize oocytes in petri dishes. Like natural breeding, it is recommended breeding every 3-6 months to ensure the quality of oocytes.

- Prime and boost females as described in "Natural" mating protocol above. **After the boost injection, females are ready for egg extraction (i.e., squeezing) at approximately 3-4 hrs. To verify the specific time, females will be observed after the injection and the procedure will continue when eggs become visible in the cloaca.**
- Sacrifice male by immersion in RT in 0.2% MS222 or 0.2% benzocaine (followed by cervical dislocation/pithing), dissect testis into RT L15 media + 10% calf serum (i.e., fetal bovine serum). Testes are manually macerated to disrupt sperm-containing tubules (unused testes can be stored in L15+CS @14°C for several days to a week).
- Squeeze eggs from a laying female into a dry petri dish (or with a few drops of 1xMMR to prevent premature activation).
- Place 1/4 of a testis (or more, depending on the volume of eggs to be fertilized) into the dish with the eggs and dissociate with forceps, mixing the testis directly with the dry eggs.
- Wait 3-5 min, then flood with 1/20x MMR. Eggs can also be squeezed into 1XMMR, where they can be stored before use for 30 min or so, or into 0.4XMMR, in which some activation may occur, but where sperm attachment may be optimal.
- Place females into a 5-liter aquarium with fresh water and 5 mg/L gentamycin for recovery (24 hr) with or without 1 x MMR. She should be observed for signs of lethargy, anorexia, lesions on skin, sloughing of skin; consult OCV if any of these symptoms persist after 24 hr. If female appears fit, she can return to stock tanks.

*Timetable for natural mating:*

- 1 to 2 days prior to egg collection: Prime females and males
- 3 to 4 hours prior to egg collection: Boost each animal with HCG
- Sacrifice male to obtain testes
- Squeeze females to collect eggs in Petri dish(es)
- Spread masticated testes over eggs in Petri dish for fertilization

**Rearing Eggs and Larvae**

- a. Rearing eggs at 18°-22° C. Hatching occurs on second or third day.
- b. Transfer the larvae with glass or plastic tip pipet to larval tank 3 days after they start feeding
  - i. Count and record the number of larvae in each tank
  - ii. It is recommended to clean the tank and change water on a 3 days interval if the density keeps between 6-8 larvae per liter.

- c. Metamorphosis starts at about 5-6 weeks after fertilization and takes 15-20 days to complete. Soon after the forelimbs erupt, the larvae stop feeding. This can be seen when the water does not clear up after feeding.
- d. Approximately a month after the completion of metamorphosis, these juveniles can be treated as adults.

## 6. REFERENCES

1. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
2. Anderson, L. C., Fox, J. G., Otto, G., Pritchett-corning, K. R., & Whary, M. T. (Eds.). (2015). *Laboratory animal medicine* (3rd ed.) Elsevier.
3. Green, S. L. (2010). *The laboratory xenopus sp.* Taylor and Francis Group, LLC.
4. Guidance for the housing and care of the African clawed frog, *Xenopus laevis*. Reed BT (2005), RSP CA, Horsham, UK.
5. ILAR Journal - Use of amphibians in the research, laboratory, or classroom setting. (2007). 48(3)
6. Koustubhan, Punita, David L. Kaplan, and Michael Levin. "Humane Anesthesia and Pain Management in Amphibian Limb Surgery of *Rana Pipiens*." *Cold Spring Harbor protocols* 2013.2 (2013): 149–155. *PMC*. Web. 29 June 2017.
7. McNamara, S., Wlizla, M., Horb, M.E. 2018. Husbandry, general care, and transportation of *Xenopus laevis* and *Xenopus tropicalis*. *Methods Mol Biol.* 2018 ; 1865: 1–17. doi:10.1007/978-1-4939-8784-9\_1.
8. National Research Council (US) Subcommittee on Amphibian Standards. Amphibians: Guidelines for the breeding, care, and management of laboratory animals. Washington (DC): National Academies Press (US); 1974. VI, Amphibian Management and Laboratory Care. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK217594/>
9. Poole, T. (Ed.). (1987). *The UFAW handbook on the care & management of laboratory animals* (6th ed.) Churchill Livingstone Inc. New York.
10. Torreilles S, McClure DE, Green SL. (2009) Evaluation and Refinement of Euthanasia Methods for *Xenopus laevis*. *Journal of the American Association for Laboratory Animal Science* 48:512–516.
11. Sive HL, Grainger, RM, Harland, RM. (2000) Early development of *Xenopus laevis*. *A laboratory Manual* Cold Spring Harbor Laboratory Press . 2000 p. 8-10.
12. Wright, KM. 2001. Restraint techniques and euthanasia, p. 111-122. In: K. M. Wright and B. R. Whitaker (eds.). *Amphibian medicine and captive husbandry*. Krieger Publishing, Melbourne, Florida.

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

**Title: Animal Care Plan {*Xenopus laevis and tropicalis*, Crespi, Eastlick B74, Eastlick B56 }**

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**I. Procedure:**

Daily (365 days a year without exception):

- Observe each animal and check for health concerns (*Guide* pg. 112).
- Clean and organize room, anterooms, and surrounding premises (*Guide* pg. 72).
- Record daily completion of tasks, environmental monitoring, initial, and date daily animal care sheet (*submit copy of sheet*).
- Report on the OCV Health Database M-F.
  - Any abnormal animals must be reported to the Office of the Campus Veterinarian (Health database), or if an emergency call 5-6246 or 509-335-1871.
- *Food type and method of water provision must be described along with where the food is stored and how storage conditions are monitored (humidity/temperature). (SEE SOP Xenopus 2018 for further details)*
  - Larval diets:
    - Start feeding larvae once they start swimming at a 45-degree angle with the head pointing at the bottom of the tank.
    - Feedings should be finely ground. Since larvae are filter feeder, large particles may damage their filter feeding mechanism which may cause death.
    - Larvae are fed powdered nettle or any other feed that is finely ground.
    - Fresh food slurry should be made fresh daily and feed twice per day.
    - It may take 4-5 hours for the larvae to clear up the feed. Adjustment of quantity can be made if it is too soon or too long time to clear the feed. It is recommended to turn off the water system for 2 hours after feeding.
    - Larvae stops feeding once the forelimbs erupt.
  - Froglet (from half of tail absorbed to metamorphosis complete): Processed feeds can be utilized as exclusive food source for froglets beyond the larval stage if they are nutritionally balanced.
    - When half of the tail is absorbed, froglets are fed diced meat (e.g., calf liver) or pellet chow provided by Xenopus1, similar to other commercial feed: Nasco post-metamorphic crushed brittle and Bio-Oregon Bio Vita Fry 1.2 m (MBL National Xenopus Resource)
  - Juvenile or Adult: (SEE Juvenile Xenopus Feeding Protocol)
    - Adults will be fed pellets provided by Xenopus1, similar to other commercial feed: 1:1 of Post-metamorphic frog brittle (medium brittle)/Bio-Oregon Bio Vita Fry 2m (MBL National Xenopus Resource)
      - Larger adult: 6 pellets/frog
      - Juvenile/smaller adult: 10-12 pellets/frog
    - It is recommended to feed adult frogs 2-3 times a week
    - Amount may be adjusted whether the frogs clear up too fast or too slow. The frogs will finish most of the feed in 30 minutes normally. If the frogs finish the food in 15 minutes, that indicates inadequate of feed. In contrast, food leftover hours after feeding indicates feeding in excess and the amount need to be reduced.

## Washington State University Standard of Veterinary Care for Satellite Animal Housing Location

- Remove leftover feed after several hours of feeding which helps to maintain the water quality and prevent water mold development
- *Calf Liver is stored in the freezer in Heald 331 and is brought down before feeding for the adults*
- *The pellets are stored at 27-29 C in an enclosed plastic container. Env. Monitoring of temperature is recorded on the daily care sheet.*
- *Pest monitoring and/or control devices (define type) and documented on daily care sheet.*
  - *Two sticky traps for “insect only” are placed at opposite ends of the room, the traps are checked daily and replaced at least every 2 weeks (See Pest Management Protocol)*
- *For aquatic species, water quality assessments must be outline (table preferred).*

Parameter	<i>Xenopus laevis</i>	<i>Xenopus tropicalis</i>	Testing Frequency
pH	6.5-8.5	6.5-8.5	Daily
Temperature °C	18-25 (rm temp)	24-26 (water temp)	Daily
Conductivity uS	500-3000	500-1000	Daily
Chlorine/chloramine	0	0	Weekly
Alkalinity mg/L as CaCO <sub>3</sub>	50-200	50-200	Weekly
Hardness mg/L as CaCO <sub>3</sub>	175-300	100-300	Weekly
Ammonia mg/L	<0.02	<0.02	Weekly
Nitrite mg/L	<0.5	<0.5	Weekly
Nitrate mg/L	<50	<50	Weekly

### Weekly or Twice Weekly:

- *Describe what activities if any might be done once a week (cage changing, floor sweeping & mopping, water bottle changes, emergency eye wash flushing, etc.)*
  - *Standing water tanks will need manual water changes: minimum of:*
  - *1x/week for laevis*
  - *2x/week for tropicalis and tadpoles*
  - *Floors are swept at least weekly*

### Biweekly (not to exceed every 14 days):

- *Describe what activities if any might be done every 2 weeks (feeder or enrichment device sanitation, ventilated cage changing, dusting, etc.)*
  - *Refill stock tank (charcoal filtered water from Eastlick) with water and test water quality (water is conditioned at this time) for Heald 102 B (X. laevis).*
  - *Flow through systems tanks are scrubbed and siphoned every 2 weeks, disinfected as needed.*
  - *See SOP 6/2/21 for details*

### Facilities



**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

- *Describe where the animals will be housed (room, building, outdoor facilities).*
  - *Frogs are housed in Heald 102B, Eastlick B74F, Heald, 323,*
- *Provide date and findings of the ventilation assessment (air changes/hour and flow pattern) for interior facilities (Call the Animal Welfare Program for this information 57951)*
  - *HVAC measurement: Not applicable for aquatics*
- *Describe illumination and mechanism of controlling light cycle. When was the last measurement taken? (AWP)*
  - *Heald 102B is on a light timer with a 12:12 light cycle (SEE SOP Xenopus 2018 for further details)*
  - *Heald 230 is on a light timer with a 12:12 light cycle*
  - *Date of last measurement: 5/21*
- *Interior room surfaces shall be moisture-resistant, non-absorbent, impact resistant, and sanitizable.*

**Housing**

- *Describe animal housing (cage, pen, tank, etc.) size and material*
  - *Flow through tanks ~50 gallons (plastic circular)*
  - *Static tanks ~10-15 gallon (plastic rectangular)*
  - *Static tank ~2 gallon (plastic rectangular)*
  - *Static container (individual) ~0.2 gallon (plastic)*
- *Describe environment enrichment that will be provided (See SOP Xenopus 2018)*
  - *Refuge cover should be provided for aquatic species. It can be floating neoprene “lily pads,” clay pots, earthenware pipes, cups hollow aquarium logs, rocks and acrylonitrile-butadiene-styrene (ABS) pipe. All materials need to be routinely disinfected.*
  - *Torreilles and Green (2007) have found that providing a refuge cover with ABS pipe can reduce bit wound incident in high density stock.*
- *Describe if the animals will be housed socially or individually*
  - *Social housing is the default for all frogs not on experiments with social housing exemption.*

**II. Sanitation Monitoring**

- *Describe Sanitation Monitoring Program per SOP #5, if applicable. (Contact OCV at 509-335-6246 or [pr.ocv.alert@wsu.edu](mailto:pr.ocv.alert@wsu.edu) to enroll in sanitation monitoring)*
  - *See Cleaning and Disinfection section of Crespi SOP Xenopus Husbandry 2021*
  - *Sanitation monitoring will be performed annually on microenvironment enclosures and feed containers by OCV.*

**III. Waste Disposal**

- *Describe where carcasses will be disposed*
  - *Carcasses are placed in freezer Heald 331 until enough for EH&S to pick up for final disposal in STI*
- *Describe where soiled bedding or for aquatics where water will be disposed and how?*
  - *X. tropicalis water is filtered with biological filter and then water goes in to the municipal water system*
  - *X. laevis water is treated with 1.98% bleach for 20-30 minutes before the water goes to the municipal water system*

**IV. Animal Numbers and Tracking**

- *List the person's name that will be tracking animals on My Research*
  - *Robyn Reeves or Erica Crespi report every 6 months to the AWP*

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

**V. Signage, Emergency Information (List of posted signs and locations)**

- *Describe location of the following mandated signage and verify posting*
- *All posting below will be maintained on the entrance to the animal housing locations:*
  - Guidelines for Reporting Animal Concerns
  - Emergency contact information for Satellite Housing Location Personnel
  - Emergency contact and Notification for Veterinary assistance (OCV or other veterinarian)
  - Any biological, chemical, radiation or other hazard signage as required

**VI. Security**

- [REDACTED] 41 [REDACTED]
- [REDACTED] 41 [REDACTED]
- [REDACTED] 41 [REDACTED]
- [REDACTED] 41 [REDACTED]
- [REDACTED] 41 [REDACTED]
- [REDACTED] 41 [REDACTED]

**VII. Disaster Plan**

- *The PI will develop a phone tree for immediate notification of animal emergencies.*
- *If there is a [REDACTED] 41 [REDACTED] and the PI is notified by FacOps.*

**References**

*Italics listed above are intended to be filled in with specific information about your species*  
Non italic wording should be left or modified to meet your specific needs

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

**Washington State University-Pullman  
Disaster Plan  
Animal Care  
{PI} Lab- {location}**

The primary goal for this document is to give general procedures and information for research animal care and support that are to be followed in the case of an emergency. This is secondary to the employee disaster plan and will only be implemented when conditions provide a safe working environment for employees.

**Emergency Plan**

WSU telephone service has temporary emergency back-up power and would work in situations of power failure. Employees would be notified of problems and work plan by {INSERT NAME}. {INSERT NAME} would also be responsible for notifying Facility Operations, EH & S, and OCV of emergency situations in a timely period. Employees would report to work, as they are physically able to. In cases of extended power failure and/or loss of HVAC, animals would be triaged and cared for in priority. Water supply will continue functioning unless physically damaged. There are back up supplies of food and bedding for the animals. {INSERT NAME} will supervise care for the animals during a disasters situation with advisory aid from OCV. In the absence of {INSERT NAME, INSERT NAME} would take charge of such care.

**Phone List**

Name	Title	Contact number
Erica Crespi	PI	14
Robyn Reeves	Animal Care Lead	14
Paul Wheeler		14
Facility Operations		509-335-9000
EH & S		509-335-3041
Office of the Campus Veterinarian		509-330-1871 emergency cell 509-335-6246 office
Campus Security		911 (emergency) 509-335-8548(non- emergency)
Campus Fire Department		911 (emergency)

○

**Evacuation Plan:**

**In case of an emergency, such as a fire, everyone is required to leave the building immediately.  
Calmly exit the building and meet at {INSERT LOCATION }.**

**Washington State University Standard of Veterinary Care for  
Satellite Animal Housing Location**

[illegible]

Obtained by Rise for Animals.  
Uploaded to Animal Research Laboratory Overview (ARLO) on 10/30/2023