

## Standard Operating Procedure for the Husbandry of Immature *Xenopus sp.*

### Purpose

*Xenopus laevis* and *Xenopus tropicalis* are susceptible to stress and water condition related diseases such as red leg and gas bubble disease. These husbandry procedures will be followed to ensure their health and well-being. Additional information is available in “*The Laboratory Xenopus sp.*” handbook.

### Holding Conditions

1. Adult frogs are housed in modular units from Aquaneering. These units provide a temperature, conductivity and pH controlled environment of recirculating water that is constantly undergoing mechanical, biological and UV filtration.

2. The water conditions can be varied to account for different life stages

*X. laevis* Tadpole/Froglet Parameters

Water Temperature: 75F (range 73 – 77F), pH 7.4 (range 7.2 - 7.6), Conductivity: 1000  $\mu$ S (range 900 - 1100)

*X. tropicalis* Tadpole/Froglet Parameters

Water Temperature: 78F (range 75 – 79F), pH 7.0 (range 6.8 - 7.2), Conductivity: 1000  $\mu$ S (range 900 - 1100)

Water Quality

NH<sub>3</sub>/NH<sub>4</sub>: 0 (0 – 0.5), NO<sub>2</sub>: under 1, NO<sub>3</sub>: under 40, Alkalinity: over 40

### Feeding

1. Immature *Xenopus laevis* or *Xenopus tropicalis* are fed depending on size.
2. Juveniles are fed *twice* daily.
3. Turn the water flow off individually to each tank of tadpoles or turn off the air stone in static tanks before feeding and leave off for at least 2 hours after feeding.
4. Feed larger tadpoles a combination of Sera-micron with the 1:1 mixture of ground brine shrimp flakes and Nasco crushed post-metamorphic brittle resuspended in water from the frog tanks.
5. Place measured food into 50ml Falcon Tube for each tank. Fill the tube up with system water, shake, and pour into tank.
6. Smaller froglets are fed a 1:1 mixture of Nasco post-metamorphic crushed frog brittle (medium brittle)/Bio-Oregon Bio Vita Fry 1.2 mm.
7. Larger froglets are fed a 1:1 mixture of Nasco post-metamorphic frog brittle (medium brittle)/Bio-Oregon Bio Vita Fry 2.0 mm.
8. After an initial feeding, add additional pellets as needed to any tanks that are still actively feeding.
9. Check cleanliness of the tanks 1-3 hours after feeding; remove any uneaten food as necessary using a different net for each tank.
10. To introduce trout pellets to a diet begin with 3 parts Nasco frog brittle to 1 part trout pellets. Gradually reduce the frog brittle to a ratio of 2 parts brittle to 1 part trout pellets and eventually a 1:1 mixture of frog brittle and trout pellets.
11. Food is sourced from the following companies:
  - Bio-Oregon Products ([www.bio-oregon.com](http://www.bio-oregon.com))
    - Bio Vita-Fry: Feed sizes 1.2mm and 2.0mm
    - BioTrout: Feed size 4.0 mm
  - Nasco Products ([www.enasco.com/xenopus/](http://www.enasco.com/xenopus/))
    - Post-Metamorphic Brittle: SB29027(LM)MX
    - Crushed Post-Metamorphic Brittle: SA05964(LM)MX
    - Adult Brittle: SA02764(LM)MX
  - Sera Products ([www.sera.de/en/home.html](http://www.sera.de/en/home.html))
    - Sera Micron 50 ml: 00720

### **Tadpole Husbandry and Feeding**

1. Week 1 - Tadpoles are kept in petri dishes and media is exchanged daily (0.1x MMR with gentamycin). Tadpole density is kept to no more than 50 tadpoles in a 100mm petri dish in an incubator set at 64°F (*laevis*) or 80°F (*tropicalis*).
2. Week 2 - Tadpoles are put into static tanks or 200ml+ Beakers. Media is exchanged daily (0.1xMMR no gentamycin) with a small amount of Sera Micron added.
3. Week 3 - Tadpoles are placed into static tanks and air hoses are added.
4. Week 4-5 – Tadpoles are placed on the Aquaneering system or other flow-through tanks.
5. Tadpoles are fed *twice* daily.
6. Feeding for tadpoles:
  - Day 1: 1L 0.1 x MMR + 1L of clean system water with 0.1g sera micron
  - Days 2-4: 0.1g sera micron
  - Days 5 & 6: 0.25g sera micron
  - Days 7-11: 0.4g sera micron
  - Days 12-21: 0.6g sera micron
  - Days 21-28: 0.4g sera micron + 0.2g finely ground brine shrimp flakes mixture
  - Days 28-35: 0.4g sera micron + 0.4g finely ground brine shrimp flakes mixture
  - Days over 35: 0.5g sera micron + 0.5g finely ground brine shrimp flakes mixture
7. Place measured food into 50ml Falcon Tube for each tank and add system water to the 50 ml mark, shake, and pour into tank.
8. Tadpoles that have completely metamorphosed into froglets will be transferred to the Aquaneering system.

### **Tank Density**

1. Tadpoles are kept at 2-3 tadpoles per liter (40-50 tadpoles in 16L Nursery Tank).
2. Post metamorphic froglets can be kept at 1-2 froglets per liter (16-30 froglets in 16L Tank).
3. Juvenile *X. laevis* (3-5 month olds) or *X. tropicalis* (3-8 month olds) should be kept at no more than 1 frog per 2 liters (8 frogs in 16L tank).