University of California, Berkeley

OFFICE OF LABORATORY ANIMAL CARE

Working Instructions

WIN Number:	**202**	**Frog Health, Room Check and Feeding**	**Revision #:**	0
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PROCEDURE

All work instructions must be demonstrated by the trainer and performed by the trainee.

1. Perform all daily health checks and tank check first thing in the morning (including weekends and holidays) unless instructed otherwise by the Facility Supervisor or Veterinarians.
    a. Check "Special Service Requests" posted on the back of the door before beginning husbandry duties. "Special Service Requests" are posted on the back of the door.
    b. PI maintained rooms check monthly care log if applicable.
    c. Record room temperature, humidity, and UV light on a daily checklist if applicable and report to the Facility supervisor or assistant supervisor if values are out of range and document communication in the "Notification of Facility Issues". (Reset Hygrometer)
    d. Specific instructions for Profilux 3, Aquatic Enterprise and Aquaneering recirculating systems:
        - Check water temp, pH level and conductivity via digital display on the control panel.
        - If out of range, check carboys and ensure the correct lines are in correct carboys
        - and have sufficient levels of liquids.
        - Check UV lights for illumination (bright bluish/green color). If not illuminated contact
        - Facility supervisor or assistant supervisor.
        - Check Dosing Carboy Levels (Acid, Base or Salt) these sit below the Control Panel.
        - If the fluid levels are lower than 50% refill.

- If any alarms or the display screen on the control panel is not on check the following:
  - Verify the carboys have adequate water levels
  - Ensure the lines in the carboys are in the correct carboy
  - Check the sump levels
  - If the alarm does not stop or the control panel does not come on after checking the above contact the Facility supervisor and/or assistant supervisor via OLAC approved reporting software. .

e. Check the tank stem pipe to ensure it is clear and not clogged. If clogged remove the stem pipe, clean and rinse with tap water.
f. Check Hose/Inflow - Check that water is running into each tank that has animals. The rate of water flow should be moderate.
g. Check the filtration system and ensure pre-filters and sock filters are flowing and not clogged. If the filters are not flowing properly or clogged, replace the filter.
h. Perform water quality test daily and record results: Nitrates/Nitrites
i. Mop/Squeegee floors weekly or as needed.

2. Assess every tank for health abnormalities, use a flashlight when necessary. Mid and high-level tanks/enclosures use a step stool and lower tanks/enclosures can be visualized by kneeling onto the knees.
   a. All health concerns should be marked with a pink health check Post-it® and enter into OLAC approved reporting software. If it is an urgent health concern also report via "Health Aquatic" slack group.
   b. Isolate any frog that has a health concern or found outside the tank.
      - Place isolated animals in a static tank in the original housing room.
      - Frogs with health concerns from the same tank may be isolated together.
      - Place animals found on floor in a clean tank and label "Found on Floor" with the date and your initials.
   c. **Exception**: Any frogs in PI maintained rooms will NOT receive a pink health check Post-it® and will **NOT** be isolated. These health cases will still be entered into OLAC approved reporting software. If it is an urgent health concern also report via "Health Aquatic" slack group.

Non-Emergency Health Concerns	Emergency Health Concerns
• Ulcerations around the mouth or on the skin • Masses or swelling: Anywhere on body with no open wounds • Lack of appetite • Thin appearance • Superficial wounds • Mucus layer sloughing	• Open mouth breathing • Prolapse (cloacal) • Weakness • Masses or swellings: anywhere on body with open wounds • Severe wounds: large or deep open wounds • Gas bubbles present between digits or near eyes • Bloating • Lethargy • Blood from mouth or nose

3. If you find a dead animal remove from the tank and place it in a bag labeled with appropriate sticker, put a red dot with the date and number found dead (FDIT) on the tank, enter the information in OLAC approved reporting software. **Note:** if multiple found dead in a tank report in OLAC approved reporting software and "Health Aquatic" slack group.
    a. Properly label the carcass bag, by indicating PI/Lab, tank ID number, rack number, location and date.
    b. Place the bagged carcass in the Weill Hall necropsy designated freezer (room 36).
    c. If a dead animal was found within a static tank, drain, and replace water.
    d. **Exception**: Any dead frogs in Dr. Hayes' rooms report in OLAC approved reporting software. **DO NOT** remove it from the tank.
    e. **Exception**: Any frogs found dead in PI maintained rooms check the monthly log and notify the Facility supervisor or assistant supervisor.

4. Feeding:
    a. Feed animals as indicated on the Frog Feeding Chart.
    b. In the Aquatic Enterprises System, press the "feed pause" button on the Profilux 3 control panel.
    c. If leftover feed or debris is present, siphon or drain and replace water no earlier than 3 hours after animals have completed eating to prevent regurgitation of food.
        - Static tanks: change tank water 3-4 hours after feeding.
        - Recirculating tank: siphon out any leftover food the next day after feeding.
        - If decreased appetite is noted label with a pink health check Post-it® and enter into OLAC approved reporting software.

Frog Feeding Chart:

Species	Feed Type	Portion	Frequency
African clawed frog (*Xenopus Laevis*)	Adult Brittle (Nasco) & Bio-Oregon Bio Trout 4mm" half and half mix	1 Tbsp. per 6 frogs	**Mon and Thurs**
Tropical clawed frog *(Xenopus Tropicalis)*	Fry feed 2.0mm (BioVita Fry 2.0mm from BioOregon)	1 tsp. per 3 frogs	**Mon, Wed, Fri**
	BioVita Fry 2.0mm from Bloodworms (Hikari Bio-Pure Frozen Bloodworms) (Backup diet if Fry feed is unavailable)	1 tsp. per 6 frogs	**Mon, Wed, Fri Choose either of these days**
Bullfrog (*Lithobates (Rana) catesbeiana*)	Cricket- 5wk old	4-6 crickets per 1 frog	**Mon and Wed**
Dwarf frogs (*Hymenochirus*)	BioVita Fry 2.0mm from BioOregon	1/8 tsp. per 1 frog	**Mon, Wed, Fri**
Lake Oku clawed frog *Xenopus longipes*	Fry Feed (BioVita Fry 2.0mm from BioOregon)	½ tsp per 6 frogs	**Mon, Wed, Fri**
	Bloodworms (Hikari Bio-Pure Frozen Bloodworms)	10mL thawed bloodworms per 3 frogs	**Mon, Wed, Fri**

5. Tank cleaning:
    a. Scrub or clean tank if algae and/or debris build up is 50% or more and/or interferes with the ability to visualize all frogs in the tank.
    b. Siphon or drain leftover feed/debris a minimum of three (3) hours after feeding.

Static tank:

- Remove the lid and siphon leftover feed/debris and water to a manageable weight
- Place screen over tank, take tank to the sink and dump the remaining water
- Bring the tank back, place it on the shelf and fill with system water then replace the lid.

Recirculating system:

- Turn off inline water and remove the lid
- Siphon leftover feed/debris and water to a manageable weight
- Transfer the animals and remaining water into a new tank in the room, then place the new tank back on the shelf, replace the lid, the inflow hose and turn on the inline water (flow rate of water should be moderate)
- Place the dirty tank on a cart or by the sink (flat surface), remove the stem pipe centerpiece and clean/scrub with brush, unscrew and remove the stem pipe to drain the remaining water, clean/scrub tank
- Dump the bucket of water/debris down the drain in the room.
- Place the clean tanks in room 48

Exception: Any tanks in PI maintained rooms that have algae and/or debris build up of 50% or more check the monthly log and notify the Facility supervisor or assistant supervisor.

6. **Water quality testing:**
    a. Test and record exact measurements of the water quality for all systems on the aquatic system checklist daily and/or weekly, excluding weekends and holidays.
    b. Recirculating system: the readings are displayed on the control panel.
    Note: Wait 10-15 minutes after conducting a water change or a manual dose for the most accurate reading.

Water Quality Testing:

If a parameter is out of range, report to facility supervisor and/or assistant supervisor via OLAC approved reporting software.

Parameter	Acceptable Range	Test Frequency	Instructions
Tank Temperature	**X. Laevis**-18-22°C (Setpoint: 17°C) **X. tropicalis**-21-25°C (Setpoint: 25°C) **X. longipes-** 17-21°C (Setpoint: 19°C) **Dwarf frogs-** 20-25°C	Daily	1. Read tank temperature from control panel (not set point temp)
Nitrate & Nitrite	Nitrate- <80mg/L Nitrite- <1.0mg/L **X. Laevis-** **X. tropicalis-** **X. longipes-** **Dwarf frogs-**	Daily (excluding weekends/ holidays)	1. Obtain 2 water samples per room. 2. Add 10 drops from bottle 1 (nitrate) & 10 drops from bottle 2 (nitrate) to vial 1 3. Add 5 drops Nitrite (nitrite) to vial 2 4. Cap vial & invert a few times to mix 5. Wait 5 minutes then read/record results
pH	6.5 - 7.5 **X. Laevis,** **X. tropicalis,** **X. longipes,** **Dwarf frogs-** (Setpoint: 7.0)	Daily (excluding weekends/ holidays)	1. Obtain 1 water sample per room. 2. Add 3 drops bottle 1 (pH) to vial **OR** place pH meter in tank & record results
Conductivity	**X. laevis** 500-900 µS (Setpoint: 700µS) **X. tropicalis** 750-900 µS (Setpoint: 850µS) **X. longipes** - 0-50 µS (Setpoint: 0µS) **Dwarf frogs-** 0-50 µS	Daily (excluding weekends/ holidays)	1. Read and record from the control panel. 2. If parameters are out of range check the carboy solution level, clear dosing lines and then verify that the float valve in the sump tank is level. 3. Following step (2) if conductivity is still out of range the following morning Report to facility supervisor and/or assistant supervisor
Ammonia	<0.8mg/L **X. Laevis-** **X. tropicalis-** **X. longipes-** **Dwarf frogs-**	Weekly	1. Obtain 1 water sample per room. 2. Add 8 drops from bottle 1 (ammonia) & 8 drops from bottle 2 (ammonia) to vial 1 3. Cap vial & invert a few times to mix 4. Wait 5 minutes then read/record results

Manual Dosing:

Issue	Recipes	Instructions/Notes
Low Conductivity	1.5 tsp cichlid lake salt + 10 gallons water= 8.8 units raised 10ml cichlid lake salt: 50 gal water = 200 µS	1. Calculate the amount needed for a specific aquatic system. 2. Don appropriate PPE; extended cuff chemical resistant gloves, apron, eye protection 3. Dose with cichlid salt in the sump tank or empty designated tank on recirculating system 4. Add ½ the calculated cichlid salt and wait 20 minutes 5. Record and monitor conductivity levels 6. If Conductivity is still low after 20 minutes, add small amounts of cichlid salt and monitor conductivity levels 7. If conductivity is too high, siphon water out of the sump and fill with cold water from the hose. 8. Repeat steps as needed until conductivity is in range.
High Conductivity	N/A	1. If conductivity is too high, siphon water out of the sump tank and fill with cold water from the hose.

7. Enrichment:
    a. Clean and replace enrichment during tank cleaning.

8. Monthly:
    a. Clean and sanitize all vertical and horizontal surfaces.

REFERENCE DOCUMENTS

REVISION HISTORY			
REVISION NUMBER	**AUTHOR(S)**	**EFFECTIVE DATE**	**REVISION(S)**