University of California, Berkeley

OFFICE OF LABORATORY ANIMAL CARE

Working Instructions

WIN Number:	**203**	**Frog Life Support System Maintenance**	**Revision #:**	0
Date Effective:	8/3/23		**Supersedes:**	0

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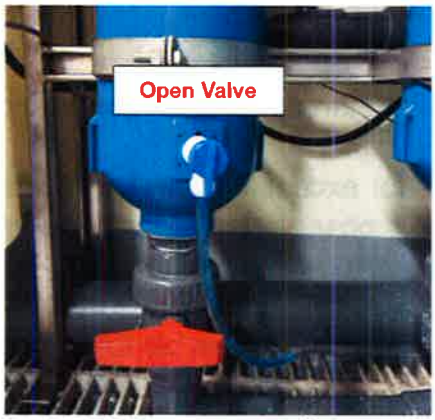
PROCEDURE

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

1. **Filter pads and socks:**
    a. Instructions
        - Slide the tray at the top of the sump tank to expose socks and filter pads located at the top of the sump tank.
        - Inspect the socks and filter pads for excessive debris or obstructions. If obstructions or excessive debris is observed, replace the socks and filter pads. (new filters/socks located in 30F)
        - Place the filter pads and socks back into the original location.
        - Use a flashlight to look into the sump for excess bio load buildup or if pump intake is getting blocked.
        - Discard used filter pads, and socks in a red biohazard bag and dispose in an approved carcass freezer or cooler.
    b. Reproductive material of *Xenopus* species
        - Inspect filter pads and socks for reproductive material. If reproductive material is present, discard in a red biohazard bag and dispose in an approved carcass freezer or cooler.
    c. FSI/Aquaneering
        - Check the pressure valves and if the pressure is above 20 psi complete the following steps

- Slowly turn both orange ball valves from vertical position to the horizontal off position.

- Turn the blue pressure release valve from horizontal closed position to vertical open position at the bottom of the canister to drain the residual water out of the canister filter.



- Turn the filter lid clockwise to unscrew and remove the filter lid
- Remove the filter bag, clean the filter sleeve and canister
- Place a new bag filter in the filter sleeve and place the filter sleeve back into the canister. (new bag filter located on rack outside of the room)
- Screw the canister lid back on turning counterclockwise until the lid is snug and the pressure gauges are positioned outward.
- Slowly turn both orange ball valves from the horizontal off position to the vertical on position.

- Turn the blue pressure release valve from the vertical open position to the horizontal closed position
- Wait five (5) minutes to be sure there are no leaks from the canister. If a leak is visible, adjust the lid slowly until there is no longer a leak.
- If the pressure remains above 20 psi after completing the above steps report to facility supervisor or assistant supervisor via OLAC approved reporting software

  Note: room 1E

2. **Check Dosing Carboys**
   - If the liquid in Carboy is below the ⅓ mark, add the respective solution (Sodium Bicarbonate, Cichlid Lake Salt, Acid buffer) at 25g per liter of DI water. (Solutions are in room 30F)

     Safety note: Don appropriate PPE; extended cuff chemical resistant gloves, apron, eye protection

3. **Probes:**
   a. Assess probe placement.
      - Remove the plexi-glass slide from the top of the system sump.
      - Use a light source to illuminate the left corner inside the sump.
      - Check the probes to ensure that ⅓ of the electrode body is submerged in water and the gray float valve is fully submerged.

   b. Calibrate probes **(conductivity)**
      - Prepare 1413uS solution by placing it in a closed container in an empty system tank's water overnight.
      - Unfasten the conductivity probe from its holder located in the system sump tank and feed the probe through the hole in the sump tank cover
      - Check for any damage and/or cracks. **Note:** Use caution when handling and hang the probe in the air to avoid breakage.
      - The conductivity on the control panel should read "OuS"
      - Press ***RETURN*** button on the system to enter settings
      - Scroll to "***SENSOR SETTINGS"*** and press the ***RETURN*** button
      - Scroll to "**Conductivity"** and press the ***RETURN*** button
      - Scroll to **"Calibration"** and press the ***RETURN*** button, select calibration value:1 and press the **RETURN** button, select Temp Sensor and press the **RETURN** button. Input the temperature of the system water (taken before the calibration procedure)
      - System will instruct you to hold the probe in the air, press ***RETURN*** button to start
      - Submerge the probe in the 1413uS solution, then press ***RETURN*** button to start the calibration
      - Timer will sound once calibration is complete

        Note: system will default to "Save now": select yes".
      - Watch the control panel home screen for the conductivity to display 1413uS (while the probe is still inserted into solution). If the conductivity is +/- 50uS from

1413uS, report to Supervisor or assistant supervisor via OLAC approved reporting software

- Dry and replace probe back into fastener in the system sump tank and ensure probes are properly in place and the float valve is submerged.

c. Calibrate probes **(pH)**

- Prepare yellow 10.0 and blue 7.0 buffer solution by placing 1 oz of each in a closed container in an empty system tank's water overnight
- Unfasten the conductivity probe from its holder located in the system sump tank and feed the probe through the hole in the sump tank cover
- Check the probe for damage and/or cracks.
- The conductivity on the control panel should read "OuS"
- Press ***RETURN*** button on the system to enter settings
- Scroll to "**pH-value"** and press the ***RETURN*** button
- Scroll to **"Calibration"** and press the ***RETURN*** button, select calibration value:1 and press the **RETURN** button
- System will instruct you to place the probe in the yellow 7.0 buffer, press ***RETURN*** button to start
- Timer will sound once the process is complete
- System will now instruct you to place the probe in the blue 10.0 buffer, press ***RETURN*** button to start
- Timer will sound once calibration is complete
  Note: system will default to "Save now": select yes".
- Watch the control panel home screen for the pH to display 10pH (while the probe is still inserted into solution). If the pH is +/- 0.2 units from 10pH, report to the Supervisor or assistant supervisor via OLAC approved reporting software
- Dry and replace probe back into fastener in the system sump tank and ensure probes are properly in place and the float valve is submerged.

d. Change pH & Conductivity probes

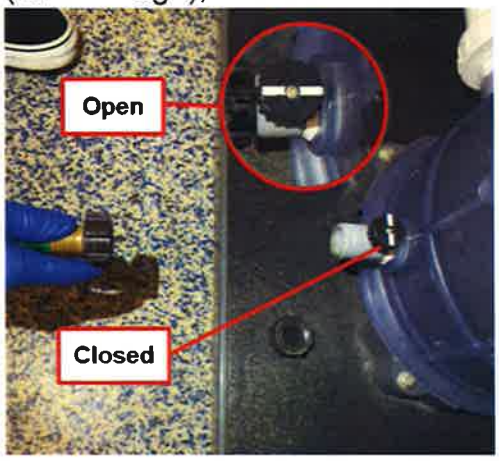
- Open the System 3 controller box by pressing down on the top and bottom pressure lock located on the left side of the box
- Unscrew the brace at the bottom of the controller box to loosen the cable going to the motherboard
- Remove the old probe from out of the system sump
  - Remove outer magnet
  - Unscrew cable box
- In the left bottom section of the motherboard there is a plugged in cable labeled "Cond" and "pH", this is the outlet for the conductivity and pH probes.
- Unplug the cables from the motherboard and take out the cables from the bottom of the controller box
- Plug in the new probe cables into the controller board
  Note: You will hear a "click" when it is in place.
- Close and lock the controller box by shutting and pressing on the left side until you hear a snapping sound.

- Calibrate probes following steps 7b. (conductivity probe) and/or 7c (pH probe) above
- Place the new, calibrated probe in the system sump
- Tighten the line brace at the bottom of the controller box

4. **Flush/scrub trough weekly:** Scrub visible algae and flush with water as needed.

5. **Clean canister cartridge:**
   a. Instructions:
      - Select **Feed Pause #1** on the control panel and turn **OFF** main water valve (large orange valve at the bottom)

      - Open the drain valve on the canister. Remove drain cap and attach hose (for drainage), aim hose toward drain



      - Loosen top pressurized cap (on canister) and open valve to allow drainage.
      - Detach lid and canister gasket/clamp (circular metal ring) on filter
      - Scrub the inside of canister with an approved cleaning device as the water drains
      - Reassemble the canister cartridge and secured the lid, then place pressure cap back on lid (at the top)
      - Close the drain valve on the canister, remove the attached hose and place the drainage cap on tight.
      - Turn on orange water valve (located to the right of the canister)
      - Reselect **Feed Pause # 1** to turn the system back on.

- Fill canister to the top, and close knob before all air escapes (there should be about 1-3 inches of air space)
- Carefully turn the top pressurized knob to allow air to escape the canister and fill with water
- Check to make sure water is not leaking from top lid or sides of canister
- Wipe down equipment and rack with a towel or other approved device after servicing the system.

6. **Siphon and clean the Sump tank:**
   a. Instructions for a 2-rack system
      - Clean Sump Tank & Perform a Water Change: Keep system running (do NOT select **Feed pause**)
      - Scrub sump tank(s) to loosen debris
      - Start a siphon on rack #1 (the rack closest to the control panel) and aim the siphon into the floor drain.
      - Place a water hose in the sump tank of rack #2, turn on the water with a steady stream (moderate flow) using **cold water only.**
      - Check the water quality metrics on the control panel and manually dose as needed.
   b. Instructions for a 1-rack system
      - Clean Sump Tank & Perform a Water Change: Keep system running (do NOT select **Feed pause**)
      - Scrub sump tank(s) to loosen debris
      - Start a Siphon in the rack sump and siphon out all loose debris and biofilm
      - Check the water quality metrics on the control panel and manually dose as needed.

7. **UV bulbs:**
   a. Instructions:
      - Select **Feed Pause # 1** on the control panel (lights will go off)

- Unscrew the plug cap from the UV bulb reactor chamber.

- Pull the bulb out beyond the quart sleeve and carefully remove the plug from the bulb, then carefully remove the expired UV bulb out of the quart sleeve.
- Slide the new UV bulb carefully into the quart sleeve.
  Note: The bulb should not be visible when properly placed.
- Plug the UV bulb into the power socket and screw the plug cap back on the reactor chamber
- Label the cord with date of bulb change
- Select **Feed pause #1** to restart the system and ensure the UV bulbs are illuminated.
- Place expired UV bulbs in original packaging and labeled used.
- Give used UV bulbs to the facility supervisor/assistant supervisor for disposal

8. **Dosing head:**
   a. If there has not been any level change in the carboys and a drop in water levels and/or the dosing head appears discolored, oxidizes, and/or darkened.
   b. Specific instructions:
      - Set the system in **Feed pause** mode
      - Open the control panel box by pressing the latches on the left of the box

      - Unscrew and loosen the tube fastener under the control panel box using a screwdriver

- Disconnect the dosing head in need of repair by simultaneously pressing the side latches and carefully pulling the head off of the motor rod, then disconnect the lines leading to the dosing head.

- Carefully place the new dosing head on the motor rod and press down firm until all four head latches lock in place with a click.
- Run the new head tubes through the tube fastener
- Connect the top tube from the new dosing head to the tube from the respective carboy and the bottom tube from the new dosing head to the tube going to the system sump
- Tighten the tube fastener at the bottom of the control panel box and close the panel
- Press and hold the pump button to bleed the lines, then watch for air to leave the tubes and visualize liquid moving in the lines

9. **Check and refill Dosing Carboys:**
    a. Check Dosing Carboy Levels (Acid, Base or Salt) these sit below the Control Panel.
        - If the fluid levels are below the ⅓ mark 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 or assistant supervisor via OLAC approved reporting software.

REFERENCE DOCUMENTS

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