HHS Public Access

Author manuscript

Cold Spring Harb Protoc. Author manuscript; available in PMC 2020 October 08.

Published in final edited form as:

Cold Spring Harb Protoc. ; 2020(10): pdb.prot106138. doi:10.1101/pdb.prot106138.

Animal maintenance systems: *Xenopus laevis*

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Abstract

Modular recirculating animal aquaculture systems incorporate biological, mechanical, activated carbon, and UV sterilization and create nearly self-contained stable housing systems. Nonetheless, minimal water exchange is necessary to mitigate accumulation of metabolic waste and regular weekly, monthly, and yearly maintenance to ensure accurate and efficient operation. This protocol describes the methods for establishing a new recirculating system and the necessary maintenance, as well as water quality parameters required for keeping *Xenopus laevis*.

MATERIALS

Reagents

Ammonia Fresh Water and Salt Water Test Kit (API, Chalfont, PA, USA).

Bleach (6.0% NaClO).

Dilute with Type II water (ASTM International 2018) *to make a 10% bleach solution (final concentration of NaClO is 0.6%)*

Nitrite NO_2 Fresh Water and Salt Water Test Kit (API, Chalfont, PA, USA).

Nitrate NO_3 Fresh Water and Salt Water Test Kit (API, Chalfont, PA, USA).

Dechlorinator ($Na_2S_2O_3$). ProLine brand (Pentair AES, Apopka, FL, USA).

Contains both the anhydrous (CAS# 7772-98-7) and pentahydrate salt (CAS# 10102-17-7) molecules. 1.6–2.6 ppm of $Na_2S_2O_3$ per 1 ppm of chlorine is typically sufficient to dechlorinate water.

Ethanol (190 proof).

Dilute to 70% with Type II water.

Nitrifying bacteria (ProLine brand, Pentair AES, Apopka, FL, USA).

Reef Salt from Seachem Laboratories (Madison, GA, USA).

Sodium Bicarbonate ($NaHCO_3$) (ProLine brand, Pentair AES, Apopka, FL, USA).

Virkon Aquatic (DuPont, Wilmington, DE, USA).

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Method

Establishing a new recirculating system

1. Rinse biomedia with Type II water and place in the biofilter.
2. Disable the water effluent exchange and UV sterilization, also keep the carbon filter empty.

It is necessary to initially limit filtration and sterilization functions and allow nitrogenous waste products to build up sufficiently to support healthy growth of the biomedia.

1. Allow flow from a single tank on the end of each rack and add 5–10 frogs to the system.
2. After 24 hours, disable the water flow but keep the biomedia aerated.

Biomedia can be aerated using an external air pump.

1. Add nitrifying bacteria to the biofilter.

Larger systems require more nitrifying bacteria. Follow the instructions outlined by ProLine.

1. Wait 1–2 hours before restarting the water flow.

During this brief period, the frogs present in the system should be kept in their tanks. The absence of water flow should not be detrimental to their health.

1. Perform daily NH_3/NH_4^+, NO_2^-, and NO_3^- level measurements.

Wait for NH_3/NH_4^+ levels to measure at least 1–2 ppm.

1. Initiate UV sterilization, water exchange, and add carbon to the system.

Flow to 1–2 additional tanks can be started.

1. The levels of NO_2^- and NO_3^- should begin to increase.
2. Allow for NH_3/NH_4^+ and NO_2^- levels to reduce to 0.5 ppm before adding more frogs to the system.

The entire process can take 2 weeks to 2 months. Nitrifying bacteria can thrive when water parameters are consistent. If dosing reservoirs are not present, alkaline buffers can be used to stabilize pH. Once NH_3/NH_4^+ and NO_2^- levels reach the desired level, gradually add frogs to the system while monitoring water quality paramenters.

Daily Water Assessment and Maintenance Required for Proper Operation

1. Record system readings.

This allows the user to deduce trends and identify potential issues that may be occurring.

1. Inspect the UV bulb.

2. Regularly scrape internal sides of the tanks to remove any algae buildup.

Algal buildup will vary depending on several factors including lighting and dissolved nutrients. Excessive buildup should be removed as needed.

1. In flood and flush systems shake the stand pipes to clear them. Tanks with only an overflow bulkhead will require daily active removal of detritus to keep them clean.

2. Exchange 10% of the system water.

3. Measure pH and conductivity dosing reservoirs daily.

Use sodium bicarbonate to buffer and regulate pH and sea salt to control conductivity.

1. Observe carbon and mechanical filters for buildup of waste and replace as needed once passage of water through them is significantly impeded.

2. Observe individual tanks for accumulation of waste and film on the bottom and sides and replace ones that are particularly dirty.

To clean tanks: scrub and rinse with Type II water, spray with 10% bleach and let sit for 1 h, rinsing again with Type II water, spray with 70% ethanol and let sit for 1 h, rinse once more with Type II water, and allow to air dry.

Weekly Water Assessment and Maintenance Required for Proper Operation

1. Assess NH_3/NH_4^+, NO_2^-, and NO_3^- levels with API test.

Acceptable measurements: NH_3/NH_4^+ = 0 ppm (0–0.5 ppm), NO_2^- < 1 ppm, NO_3^- < 40 ppm, alkalinity >40 ppm. If levels are out of range perform a 25% water exchange

1. Test pH and conductivity with a reliable external probe as a reference for internal system probes.

If the external and internal probes are not in agreement, clean the system probe and check that it is correctly reading calibration solutions. Recalibrate the probe if necessary.

1. Measure temperature with an external thermometer

Heaters and chillers are used to keep the temperature stable.

Annual Water Assessment and Maintenance Required for Proper Operation

1. Replace UV bulbs and check to ensure that the quartz sleeve is intact

2. Replace pH electrodes.

3. Remove and clean return pipes

4. Replace all rubber tubing.

Water quality parameters for Xenopus laevis—The water quality parameters and animal stacking density necessary for optimal growth will vary dependent on the life history stage of *X. laevis* (Green 2009; Hilken et al. 1995). These parameters are listed in the table below.

Age	Water Temperature (°C)	pH	Conductivity (μS)	Tank Density
Tadpole/Froglet	24 (23–25)	7.4 (7.2–7.6)	1000 (900–1100)	2–4 per 1 L
Juvenile	22 (20–24)	7.8 (7.0–8.5)	1600 (1200–1800)	1 per 1–2 L
Adult	20 (18–20)	7.8 (7.0–8.5)	1600 (1200–1800)	1 per 3 L

System sterilization

1. Disable all system components including the water pump, biomedia agitators, and probes.
2. Discard biomedia or sterilize in a 10% bleach solution.
3. Scrub the inside of the sump and associated parts and remove all detritus.
4. Remove and dispose of all filter pads, cartridges, and carbon.
5. Disassemble and scrub all the tanks and accessories.
6. Rinse the tanks with water, coat with 10% bleach and let stand for 1 hour.
7. Rinse the tanks with water, coat with 70% ethanol and let stand for 1 hour.
8. Rinse the tanks with Type II water and let air dry.
9. Soak accessories in 10% bleach for 1 hour.
10. Soak accessories in 10 g/L solution of $Na_2S_2O_3$ in Type II water for 1 hour.
11. Soak accessories in 70% ethanol for 10 minutes, rinse, and air dry.
12. Drain 75% of the sump volume.
13. Reconstruct the system but keep carbon out.
14. Start the system.
15. Add bleach to the system.

Ensure the final concentration of NaClO in the system is 0.06%.

1. Reduce the effluent rate to its lowest setting.

Do not reduce the daily water exchange rate to less than 1%.

1. Wait 2–3 days, then increase the daily water exchange rate to approximately 50%.

This will begin removing the bleach from the system.

1. Begin testing the pH of the system after a week.

2. Add Virkon to the system once the system water pH matches the influent water pH.

The concentration of Virkon should be 3.2 g per 1 m^2 of estimated internal surface area of the entire system.

1. Reduce the effluent rate back to its lowest setting.

Virkon can cause foam accumulation and in some cases, may over flow. To help mitigate this, limit agitation of the water and spray 70% ethanol directly on the foam to help disperse bubbles.

1. Wait 7 days then increase the daily water exchange rate to approximately 50%.

This will wash the Virkon out of the system.

1. Allow the system to run for 2–3 days, replace all rubber tubing, and follow the protocol for establishing a new recirculating system described above to stabilize the system and allow for housing animals.

Discussion

Optimal eggs and embryos are produced by healthy laboratory population of *Xenopus laevis*. Regular monitoring of water quality parameters, as well as daily monitoring and visual inspection of the animals is crucial for prevention and early identification of developing conditions that may be detrimental to overall colony health. Use of tap water is discouraged and starting with a good water source providing Type II water will aid in maintenance of optimal water parameters as well as limit the chances of introducing pathogens and toxic contaminants. Municipal tap water may also be basic and thus may add the additional complication of needing to use acid buffers to aid in stabilizing the pH. The stability of the parameters will also be affected by the animal maintenance system configuration, with recirculating systems being more cost effective and efficient than flow-through systems and also requiring much less maintenance than static systems. Furthermore, animal health is best when the accumulation of nitrogenous waste is low, thus the initial effort made to establish a stable biofilter in a recirculating system is absolutely necessary. Nitrifying bacteria used in the biofilter aid in establishing an ammonia/nitrogen cycle in which toxic ammonia is oxidized by *Nitrosomonas* bacteria producing toxic nitrite that is further oxidized by *Nitrobacter* bacteria into nontoxic nitrate (Hem et al. 1994). Once this cycle is established, animals can be safely kept in the system and should remain healthy with regular maintenance to keep the system clean and water parameters within the tolerance range of the animals (McNamara et al. 2018). If the systems are operating well and the water parameters are within range yet the animals are not thriving, a potential presence of pathogens should be considered and tested. Presence of highly virulent pathogens can greatly affect animal quality of life and may require euthanasia off all the frogs housed in the system. In such a case, complete system sterilization is necessary before it can be safely used to house animals again.

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