WVU IACUC SOP:
Fish and Aquatic Frog - Satellite Care

Instructions:

Use this document as a guide in constructing an SOP for husbandry and care of your specific species.

Below is a template that should be used to create this SOP. Be sure to fill-in all sections.

As this document details the care of animals under your direct supervision, it may apply to more than 1 IACUC protocol, and copies should be submitted with each. This document outlines details for successful approval of your unique species care SOP by the IACUC, and takes into consideration natural history considerations not adequately covered by generic sops for animal care. The following guidelines are reasonable, but they may not cover all species or situations-it is up to the PI to recognize this and add these other sections or details where relevant to your operation, (e. g larval and adult care, special experimental conditions that influence animal care and well-being, etc.). If your situation seems exceptional and a deviation from standard animal care practices (i. e. observe all animals each day, assure water quality is acceptable daily to minimize disease or mortality, disease and mortality records, quarantine procedures, etc.), please provide both a justification and data or documentation to justify any procedure or guideline you use that is outside the norm. Use of "we have always done it this way" is not likely to be acceptable by itself as the sole rationale. Overall, be careful not to include overlap in details with those provided in your IACUC protocol. You may wish to refer to this SOP for husbandry details in your IACUC protocol rather than repeating them twice. If this is your first submission, or a 3 year renewal, please submit a copy of your revised SOP with your IACUC submission for review. Be aware that the WVU IACUC needs to inspect your satellite facility and conditions of intended operation before satellite care can start, even if approval has been granted.

Remember deviations from optimal husbandry and procedures (typically outlined here), such as suboptimal water quality parameters (etc.) for experimental reasons, are only allowed if scientifically justified in your IACUC protocol. This would necessitate labeling this use of animals as Category E and the requirement to justify on scientific grounds the need to create more than minor distress, disease, or pain that goes unmitigated. Under the law, convenience or cost cannot be used as a part of this category E justification. Legally, and in accordance with WVU IACUC policies, satellite housing (keeping animals 24 or more hours outside a vivarium) requires prior IACUC approval. Please remember that daily animal observations is required for satellite housing, this includes keeping daily records (mortalities, room temperature, room humidity, etc.). If you are considering a new site for satellite housing, ask OLAR to inspect; they can provide helpful guidelines to facilitate this process with the IACUC.

I. **<u>Documentation Requirements (see below):</u>**

a. **Daily observations** of animals, mortalities, and medical treatments per tank, as well as room temperature and humidity must be available during inspections for site and tank in the PI's possession.

b. Water Quality Logs: An up-to-date daily log containing water quality measurements (see section G) is required. The only exception to this is where direct IACUC approval for variations on this requirement has been given. (An example daily water quality/observation log is available as an appendix to this SOP).

c. **Daily records including water quality logs should include mortality counts by tank id, results of exams or diagnostics, and therapies.** (See: sick fish SOP for signs and treatment regimes, if warranted.)

II. **<u>Occupational Health Concerns:</u>** Certain zoonotic diseases are found in captive and wild-caught fish, including a variety of atypical mycobacteria (aquarist's rash … *M. avium, M. xenopi, M. marinum,* etc.), *Erysipelothrix rhusiopathiae, Nocardia* spp., *Salmonella* spp., *Klebsiella* spp., *Leptospira icterohaemorrhagica*, *Vibrio vulnificus* and *Edwardsiella tarda* and related species. Some wild fish also harbor inapparent zoonotic diseases. Although they may appear unlikely to shed the organism frequently, the risk is still there, and especially for people with uncertain immune status. Although human infections for some of these diseases are rare, the mortality rate can be high in humans. For this reason, personnel working with fish or any aquatic species should wear disposable gloves if coming into contact with fish, used in fish water or tanks and other equipment in contact with fish. Disposable palpation sleeves can be used for cleaning tanks or reaching into deeper aquaria. These are available from OLAR. **Door signage should identify potential risks to personnel where relevant.**

III. **<u>Quarantine:</u>** Procedures will vary with the intended use of fish/frogs in captivity. Records of procedures and monitoring are expected from satellite quarantine facilities. For establishment of long-term colonies, especially if animal quality is unknown, or when adding stock to these animals, longer quarantines of up to 60-90 d may be required. If animals are all-in all-out, housed for short-term (e. g. a few weeks) the quarantine may be relatively short such as 2 weeks, especially if animals are treated prophylactically, or come from an approved disease-free source, <u>AND</u> if animals can be separated by equipment, isolation practices, and source.

Separation from field personnel activities, fish species, their equipment and boots, and colony housed fish should be preserved at all times during quarantine and for established colonies. Separation between lots of fish and between species, are also prudent steps to take as captive stress can bring out diseases and increase the shedding of pathogens. Likewise, caring for sick, quarantined but healthy and healthy/low risk animals in that order is expected.

a. <u>Short-term or low risk housing.</u> For short-term studies, up to three weeks in length, and/or employing certified clean fish, minimal quarantine procedures (treat parasites, if sick or prophylactic treatment due to stress) may be employed if the animals remain healthy, and no other fish are housed in the same water body. Beware of leaky overhead return piping in recirculating systems leading to cross-contamination.

b. <u>Long-term or high risk housing.</u> A formal quarantine may be warranted if wild animals or those from uncertified sources are collected. This may be 30-60 d depending on the risk. Treatments are rendered during this period, and isolation procedures are followed (access these animals last, limit access, separate nets, isolation housing, separate water supplies, etc.). Beware of leaky overhead return piping in recirculating systems leading to cross-contamination.

IV. **<u>Water Quality Testing:</u>** All new tank system set-ups should be tested at least daily for Ammonia and Nitrite until values stabilize in the normal range at expected stocking densities; once in maintenance mode, ammonia and nitrite testing can be done weekly on the highest density or metabolically active tanks for each animal class. **<u>Temperature and pH should be checked daily on all captive man-made enclosures.</u>** Testing values must be documented as well as the source of the measurement (i.e., which tank(s) the sample was taken from) and should be made available to inspectors upon request along with other required observations detailed above. **<u>When out of range values are found, logging more frequent tests to document corrective actions until back in the acceptable range is expected.</u>**

<u>New Tank parameters</u>: Unless IACUC approved, thresholds for intervention for UIA (NH_3) and Nitrite (NO_2-) are as follows: $NH_3 < 0.05$ mg/L (i. e. Calculated UIA from total ammonia (TAN) using a pH/temperature conversion table); for Nitrite < 1.0 mg/L. **<u>TAN values without calculating UIA may be misleading-PI's are responsible for making this conversion. While new tank set-up parameters may have excursions outside these values, records should show a corrective path back into the acceptable range.</u>**

Template for Aquatic Husbandry and Care SOP:

A. **PI name and current protocol number:**

B. **Housing**
   1. Building:
   2. Room:
   3. Tank IDs:

C. **Species name (common and scientific):**

D. **List all sex and age classes that will be housed*:**
   *****Examples – eggs, larvae, fry, adult males, adult females, etc. Define groups by differing practices such as housing, food or care (e.g., sex and age may influence husbandry/care practices such as larval frogs requiring different diets and density allowances or sexually mature fish fighting or eating young)

E. **Density matrix:** Density rules of thumb that are used are described below (select and complete what is relevant)
   a. ☐ **Aquatic Frogs density**
      - ☐ Adult (size?)
      - ☐ Sub-adult (size and adult weight equivalent)
      - ☐ Fry (size and density allowed)
      - ☐ Larvae (size and density allowed)
      - ☐ Eggs (density allowed)

   b. ☐ **Fish density**
      - ☐ No greater density than at least two body lengths apart in all directions.
      - ☐ Other:

   c. ☐ **We use ammonia/nitrate monitoring levels to address density and either increase biofiltration or reduce numbers accordingly by humane euthanasia.**

F. **Water system used:** Select those that apply
   - ☐ Flow-through – List turnover rate:
   - ☐ % water change/dump and fill – List turnover rate:
   - ☐ Recycled filtered**
   - ☐ Other: - List turnover rate:

   **Check the types of filters that are in use:
   - ☐ Biological
   - ☐ Chemical
   - ☐ Mechanical

   Indicate how the filtration and UV systems were sized to give the desired water condition:
   OR
   Provide water quality data over the last month, if you have it, demonstrating conditioning is sufficient.

   Check those that are relevant:
   - ☐ A modular system is used that has self-contained heating/cooling, 3 types of filtration, and continuous monitors (temperature, pH and conductivity).
   - ☐ A continuous tank ammonia indicator is used in addition to intermittent indicator strip testing for ammonia.

☐ Outside tanks or natural wetlands are used.

G. Water Quality Testing

Maintenance Value Table

Parameter	Units	Frequency	Acceptable Range	Typical Normals
Conductivity*	uS			250-1000 (fresh H_2O)
TAN	mg/L			< 0.1
pH				
Temperature	°C			
DO (25°C)	%			> 60%
NH_3 (UIA)*	mg/L			< 0.0
Nitrite*	mg/L			< 0.2
Turbidity/Algae**				Animals must be visible at all times
Chlorine(s)	mg/L			0.0
Hardness (GH)	mg/L			
Alkalinity (KH)	mg/L			

* Salinity or ion build-up: Evaporative water losses lead to ion build-up over time. Conductivity, specific gravity, refractive index, chlorine test kit testing are typical measures of NaCl and/or ion build-up. You will need to test intermittently if you routinely add salt, or if you recycle, and you add water to compensate for evaporation.
Nitrate levels can be measured (especially marine systems), but generally, regular tank cleaning keeps organic load down; also nitrate is less of an issue in fresh water systems. However, excessive organic detritus accumulation can lead to anaerobic conditions (H2S toxicity) and mass mortality without regular tank cleaning. Regular spot removal of detritus can prevent this problem from developing to begin with.
**Excessive algae build-up can lead to acute mortality if lighting is restricted suddenly (e.g move from a sunlight room).

NOTE: Response to out of range values should be documented on daily care sheets. Treatment corrections should be documented with follow-up testing to show that corrective measures were effective.

H. Cleaning Procedures

a. ☐ Check here to request an exception to monthly room sanitation procedures of fish satellite housing rooms (rooms are still to remain clean and unclutters with GFC receptacles, etc.), due to the risk it poses to the fish from aerosolized disinfectant getting into the tanks.

b. Detail the room sanitization procedure frequency and products used:

c. Detail the tank sanitization procedure frequency and products used:

d. Detail the frequency of chemical (charcoal) filter changes:

e. Detail the frequency of mechanical filter changes:

f. What percent over-sized in the UV bulb array output (or total wattage):

g. How many bulbs are in the UV array?

h. If applicable, what is the UV quartz sleeve cleaning maintenance schedule:

i. Check those which apply to the frequency of UV bulb changes:
   - ☐ The UV effluent culture results are checked monthly and if the culture is positive, the bulb is changed.
   - ☐ The UV C output is checked with a UV C meter and if the output is < 80%, the bulb is changed.

I. **Feeding:** A species-specific complete diet is preferred, unless native food and diversity are available and are pathogen and parasite free. Diet diversity and/or intermittent vitamin/mineral supplementation are considered optimal practices if a complete formulated diet is not available.

a. What is the amount of food fed at one time?

b. How long is food present?

c. What are the ID diet(s) and/or supplements that are fed?

d. If live food is being fed, describe how disease is controlled:

J. **Sick Animal Disposition**

a. What are the signs indicating that treatment or euthanasia are needed?

b. Euthanasia is selected based on the following criteria:

c. What are the procedures for new animal introduction after transport (if relevant)?

d. Are animals being purchased from an approved source that is free of disease?

e. In the wild/field, are precautions being taken to ensure that diseases are not introduced or spread via repatriation to new areas or comingling animals from different sources that will be returned to a hatchery or commercial stocks or the wild?

f. What treatments are being done in quarantine or at entry?

K. **Quarantine**
Describe the quarantine procedures by risk group:

L. **Disaster Planning**
Select one or more options in each section or describe how these disasters would be addressed:

a. Loss of cooling for an extended period (power could also be lost)
   - ☐ Use individual tank coolers
   - ☐ Place ice in the tanks in place of water additions, or with conditioning salts
   - ☐ Other:

b. Loss of heating for an extended period
   - ☐ Add warm water pre-heated elsewhere

☐ Add individual tank heaters
☐ Other:

c. Loss of water recirculation/uv and ammonia toxicity
☐ Stop feeding
☐ Decrease density, add zeolite, do 25-50% water changes
☐ Lower pH gradually (fresh water only)
☐ Continue to monitor for a nitrite peak if biofiltration still occurs
☐ Add more active biofilter
☐ Other:

d. NH_3/Nitrite Toxicity
☐ 25-50% water changes
☐ Add chloride (NaCl), decrease biomass, reduce temperature, reduce feeding
☐ Add more active biofilter
☐ Increase DO (aeration) as much as possible
☐ Other:

e. Extended total system failure (loss of power and water access), or labor support failure
☐ Euthanasia by MS-222 or benxocaine (250 mg/L for 10 minutes beyond the termination of gilling for either)
☐ Euthanasia using a conditionally approved method such as decapitation or electrostunning plus a secondary method as recommended in the AVMA 2013 Guide on Euthanasia https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
☐ Other:

References

1. Noga, E, Fish Diseases. Wiley-Blackwell, 2nd Ed. 2010.
2. Ostrander GK, ed. The Laboratory Fish. Academic Press 2000.
3. Brown L, Aquaculture for Veterinarians. Pergamon Press 1993.
4. Stoskopf M, Fish Medicine. Saunders 1993.

Appendix 1 – Some Useful Definitions

Alkalinity (Carbonate Hardness) - Alkalinity is a measure of the buffering or acid neutralizing capacity of water and is typically measured in German degrees of carbonate hardness (dKH) by most commercially available test kits. You will also find alkalinity expressed as mg/1 CaCCh or meq/liter. Alkalinity is important to keep track of, especially in "closed" recirculating systems, to prevent wide swings in pH. Carbonate and bicarbonate are the alkaline ions in water that bind hydrogen ions and acids that would otherwise raise pH. There are a number of products on the market (bicarbonate based) that increase alkalinity and thereby buffer water. These can be added using an automatic dosing system controlled by a pH meter or in response to readings obtained with an alkalinity test kit.

Aquatic Biological Cycle (simplified) - Usually referred to as the nitrogen cycle, the control of which is absolutely critical to the health of fish and amphibia. In brief, waste products arising from respiration and digestion produce NH3 (ammonia), which can rapidly accumulate to toxic levels (0.7-0.8 mg/1). In systems with sufficient growth of Nitrosomonas species of bacteria, ammonia is converted to nitrite (N02), which in turn is converted to the less toxic nitrate (NO3) by Nitrobacter species. Denitrification (conversion and ultimate removal of nitrate) is essential to the health of aquatic species but can only be accomplished by regular water changes or more exotic methods such as "algal turf scrubbers" or chemoautotrophic methods (involving growth of anaerobic bacteria). In practice, frequent water changes will be the only reasonable method available. Note, however, that 100% water changes should be avoided if possible (in favor of 10-50% water changes) in order to maintain the benefits of "conditioned" water.

Biomass - Usually expressed as pounds or inches (snout to tail) of a fish or amphibian per gallon of water even though "pounds" and "inches" are not units of mass. Bio-Mass is largely an empirically determined value and can differ depending on the animal and the type of life support system used to house them. Density refers to animal number/ unit volume and is somewhat subjective when referring to large numbers of individuals such as larval tanks.

Bio-Media for Bio Filter - Any material suitable for bacterial colonization. In aquaria this can include gravel beds, porous (e.g., ceramic, volcanic) substrates or a variety of commercially available porous plastic "balls" with large surface areas. The important feature of such materials is that they be kept wet but well oxygenated. Typically found in "wet-dry" applications or submerged and subject to high flow conditions to ensure oxygenated supply of water. NOTE: NEVER DISINFECT A BIOLOGICAL FILTER UNLESS COMPLETE BREAK DOWN AND CLEANING OF SYSTEM IS WARRANTED IN ORDER TO STEM A DISEASE OUTBREAK. ALWAYS REMOVE THE BIOLOGICAL FILTER WHEN ANTIBIOTIC AND OTHER TANK TREATMENTS ARE APPLIED.

Filter - Any device that removes non-dissolved particulate matter from the water. In some situations, mechanical filters also serve as an important substrate for bacterial growth.

Fluidized Bed - Typically a cylinder partially filled with fine sand and gravel through which water is pumped at a high flow rate sufficient to keep the sand "bed" in suspension. Properly engineered fluidized beds can provide an enormous surface area for bacterial colonization and therefore make ideal biofilters that can also be very compact. If water flow is disrupted for extended periods, however, death of beneficial aerobic bacteria can occur rapidly.

Hardness - This can be a very confusing area because there are several different measures of hardness and they are expressed in different units in the US and Europe. The following should help depending on the test kit being used:

GH = total hardness (often expressed as German degrees of hardness dGH) KH = carbonate hardness (also expressed as degrees of hardness dKH) PH = permanent hardness

GH = PH + KH

Carbonate hardness (KH) is based on bicarbonate and is not "permanent" because the C02 can be removed by boiling water. The calcium and magnesium that remains after boiling is, therefore, referred to as permanent hardness. Kits are available to measure both KH and GH. For amphibian or fish husbandry, GH is the more useful measure. One degree of total hardness (dGH) is equivalent to 10 mg/L of CaO and/or MgO.

Using this measure, a dGH of 1-4 is considered very soft, 4-8 dGH soft, 8-12 dGH medium hard, 12-18 dGH fairly hard, 18-30 dGH hard, and over 30 dGH is considered very hard. Many investigators feel that X. laevis does best at medium to fairly hard levels of hardness. For fish species optimal GH and KH will depend on where the fish originate.

Ozone Reactor - Device used to mix water with ozone for the purposes of sterilization. Not recommended for amphibian husbandry, or small fish syatems (see UV sterilization).

Parts Per Million (PPM) - For most purposes, one can assume 1 ppm is equivalent to 1 mg/L.

Sand/Gravel Filters - Several designs used but, in general, involves sand/gravel bed sitting on perforated plate separated from bottom of tank by a small space. Water is passed through sand bed by gravity or through the use of pumps. Provides good secondary mechanical and/or primary biological filtration.

Turnover Rate - The time it takes to turnover completely the contents of a tank of water, typically in relation to water passing through a device (e.g., filter, sterilizer) and being returned to a tank (or clean water being trickled in at one end of tank at rate equal to water outflow at other end). This is more complicated than one might think at first due to the fact that filtered or fresh water is constantly being mixed with non-filtered water when returned. The following turnover formula can be used to establish duration of turnover for a given tank and filter (after Escobal, 1996).

$T = a\,(G/F)$

T = Number of hours for all water in tank to pass through a filter F = Flow rate of water through filter (e.g., gallons/hour) a = Purity coefficient = 9.2 G = Total net volume in tank

So, for example, how long will it take for all the water in a 100 gallon tank to turnover (e.g., pass through a filter) assuming a flow rate of 50 gallons per hour through a filter?

$T = 9.2\,(100/50) = 18.4$ hours Thus, it takes nearly a day for one "complete" turnover.

This equation assumes that increasing resistance to water flow does not develop over time (i.e., in the case of a filter that slowly clogs reducing flow rate gradually - there are ways of calculating this, but for most purposes this is unnecessary, assuming a regular schedule of general filter maintenance).

Ultraviolet Sterilization - Typically a cylinder or cylinders containing one or more quartz glass sleeves, each of which enclose a Hg lamp producing UV light. Water is pumped into one end of the chamber and, depending on factors such as turbidity, flow, and "dwell-time", sterile water exits the chamber before being returned to the tank(s). These systems can be very effective if manufacturer's guidelines are followed regarding flow rates and capacities. Better quality units are designed to allow frequent cleaning of the quartz sleeve without needing to shut down or disassemble the unit. It is critical that UV bulbs be replaced at least twice per year to maintain necessary levels of radiation, othat UVC output is measured. The use of UV sterilization is HIGHLY RECOMMENDED on recirculating systems, AND ESPECIALLY ON THOSE that include return of filtered water to multiple tanks via a common manifold system. A problem exists that not all bulbs operate at 100% of their certification. If the UV system has insufficient redundancy in bulbs, dirty sleeves, turbid water, larger parasites or failing bulbs before their tiem can lead to significant disease or mortality.Wet-Dry System

Typically involves various Bio-media (e.g., plastic balls) enclosed in a plastic "biotower" such that water drips by gravity through the media, which remains emersed. This results in a highly oxygenated "wet-dry" environment ideal for the growth of aerobic bacteria needed to break down ammonia to nitrite and nitrate (see biological cycle).

Additional Information:

1. Adey, W.H. and Loveland, K. 1991. Dynamic Aquaria: Building Living Ecosystems. Academic Press, San Diego, CA.

2. Escobal, P.R. 1996. Aquatic Systems Engineering: Devices and How They Function. Dimension Engineering Press, Oxnard, CA.