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Category: Animal Husbandry Author(s): T. Schoenfeld

1.0 Purpose

This SOP describes the procedures for routine care and husbandry of Rana frogs (*R. pipiens*, or northern leopard frogs; *R. catesbeiana*, or American bullfrogs) housed in the vivarium.

2.0 Policy

- 2.1 The care and husbandry of frogs housed in the FSU vivarium must be pursued according to the standards set forth in this SOP, which are to be considered routine.
- 2.2 Variations in this routine must be approved by the IACUC through protocol review.
- 2.3 Only approved personnel, whether faculty, students, or staff, may have access to the vivarium and participate in the care and husbandry of housed animals.
 - 2.3.1 Such personnel must be formally identified on an approved IACUC protocol, whether proactively or retroactively listed.
 - 2.3.2 Such personnel must also be formally identified on animal room cards and frog room log sheets.
 - 2.3.3 Such personnel must be fully trained in approved methods of animal care and husbandry, by:
 - 2.3.3.1 Completing assigned CITI training modules;
 - 2.3.3.2 Reviewing all pertinent FSU SOP's; and
 - 2.3.3.3 Receiving face-to-face instruction from an experienced PI.
 - 2.3.4 It is the responsibility of the PI to ensure that all personnel handling animals covered by his/her protocol are approved in all of these ways before they begin such work.

3.0 Materials

- 3.1 Animal room card (see SOP 140)
- 3.2 Frog room log sheets (see SOP 151)
- 3.3 Tanks and tank rack
 - 3.3.1 All frogs not isolated in quarantine or actively involved in an experimental procedure are housed in holding room 101G or 101H.
 - 3.3.2 10-gal and 20-gal glass aquarium tanks are used to house 1-2 or 2-4 frogs, respectively (depending on size), placed on shelving in 101G or 101H and covered with secured wire mesh covers.
 - 3.3.3 Rocks are provided to enable frogs to sit partially or entirely out of the water (added to a depth of 2-3 cm).
 - 3.3.4 Before added to a frog tank, tap water is dechlorinated with dechlorinating chemicals (liquid, tablets, etc.) that are designed for aquatic animals, following the manufacturer's instructions.
- 3.4 Animal housing ID cards (tank cards)

- 3.4.1 All tanks must be outfitted with an animal housing ID card (see SOP 120 for details), held in a card holder or otherwise affixed to the tank.
- 3.4.2 Additional colored cards may be employed to identify special circumstances, such as the presence of dead animals (see SOPs 120 and 151, and below).
- 3.4.3 Animals found housed in a tank without a tank card may be euthanized, by order of PI.

3.5 Food

- 3.5.1 Live small animals such as crickets are fed to frogs in a separate dry tank (to prevent drowning of crickets).
- 3.5.2 Crickets will be gut-loaded by feeding them fresh fruit/vegetables and/or mouse chow and/or commercial cricket food to raise their nutritional value as food for frogs housed for more than 2 weeks.
 - 3.5.2.1 Calcium fortification will be emphasized, by either dusting fresh foods with powdered calcium supplement, feeding foods high in calcium (kale and other greens, squash, cabbage, half green grapes, orange slices), or employing calcium fortified commercial diets, such as mouse chow or cricket food (e.g., Nature Zone Total Bites). Acceptability by crickets will determine which is used.
 - 3.5.2.2 Crickets will be housed and fed daily in their own "faunarium" for 1 week preceding feeding to frogs.

3.6 Room Environment

- 3.6.1 A 12h:12h light:dark (12:12 L:D) cycle, with lights on at 0700, will be the default light cycle for rooms 101G and 101H, unless a variation is called for in an approved protocol.
 - 3.6.1.1 Only a PI with designated use of animals being housed in the room shall be authorized to change the set light cycle.
 - 3.6.1.2 Caretakers and researchers may be authorized to enter the room during the dark phase, employing the red ceiling light that is controlled by the hallway switch outside the room.
 - 3.6.1.3 Adjustments for transitions between daylight savings and standard time will be authorized and implemented only by the PI using the room.
 - 3.6.1.3.1 PI's may opt not to change the settings over these transitions, meaning that lights on/off will shift between 0700/1900 (EST) and 0600/1800 (EDT).
 - 3.6.1.3.2 PI's are advised to consider these seasonal time changes when scheduling experiments during the fall or spring that require entry into the holding room close to lights on or lights off.
- 3.6.2 Routine ambient temperature will be maintained at 18-21°C (65-70°F).
 - 3.6.2.1 Variations in ambient temperature for these rooms can be requested through Capital Planning.

4.0 Procedure

- 4.1 Changing tank water and feeding
 - 4.1.1 Wear a lab coat and nitrile gloves when preparing to handle frogs within a tank.
 - 4.1.2 Bring a dry tank into the holding room, on a cart with sufficient room to hold the housing tank as well.
 - 4.1.3 Carefully move the occupied tank onto the cart, then transfer the frog(s) (along with their tank card) to the dry tank, grasping firmly around the abdomen or hind legs and making sure that no frogs are able to escape from opened tanks.
 - 4.1.4 Add crickets to the dry tank (2-4 live full-grown crickets per frog), then move the dry tank back to the shelving until the feeding session is completed.
 - 4.1.4.1 Caretaker may need to separate frogs further if some frogs are being denied their fair share of crickets.
 - 4.1.5 In the meantime, change the tank water by pouring used water down the drain (floor drain or sink drain) and replacing with an equivalent volume of dechlorinated tap water.
 - 4.1.6 At the end of the feeding session, transfer fed frogs (along with their tank cards) back to the original tank with fresh water and return to shelving.

4.2 Daily care

- 4.2.1 Check on all tanks with animals, noting:
 - 4.2.1.1 the health of each animal,
 - 4.2.1.2 whether there are dead animals
- 4.2.2 Complete the appropriate row of the frog room log sheet (see SOP 151) by logging:
 - 4.2.2.1 health status
 - 4.2.2.2 the census (# adult animals)
 - 4.2.2.3 temperature (current/min/max)
 - 4.2.2.4 humidity (current/min/max)
- 4.2.3 Remove dead animals and transfer to freezer (see section ??).
 - 4.2.3.1 Be sure to add dead animal card (red) for follow-up by PI if living animals remain in tank.
- 4.2.4 Room maintenance:
 - 4.2.4.1 Sweep floor and empty trash container as needed.
 - 4.2.4.2 Check vermin traps.

4.3 Care 3X per week

- 4.3.1 Change tank water
- 4.3.2 Feed (typically, crickets in a separate dry tank)
- 4.3.3 Spray carts with disinfectant and air dry once returned to cage washing room

4.4 Weekly care

4.4.1 Sweep and mop the floor at least weekly, more often as needed.

- 4.4.2 Flush floor drains with the soapy water from mop bucket and follow with a bucket-full of clean water.
- 4.4.3 Wipe door knobs with disinfectant.
- 4.5 Care every 2 weeks
 - 4.5.1 For frogs housed for longer than 2 weeks, and at 2 week intervals thereafter:
 - 4.5.1.1 transfer animals into a cleaned tank at water changing.
 4.5.1.1.1 Soiled tanks and rocks are cleaned by hand in the cage washing sink with hot tap water, followed by final rinse with RO water.
 - 4.5.1.2 gut-load crickets to improve nutritional quality for frogs.
 4.5.1.2.1 House with fresh fruit, vegetables and/or rodent
- 4.6 Biannual care (when holding room is not being used to house animals)
 - 4.6.1 Wipe down all walls and surfaces with disinfectant.
 - 4.6.2 Wipe down tank rack with disinfectant.
- 4.7 Special care procedures
 - 4.7.1 Dead animals
 - 4.7.1.1 Dead animals found in a tank during routine observations are placed in a plastic bag containing a red card attached.
 - 4.7.1.1.1 Information from tank card should be transferred to the red card.
 - 4.7.1.1.2 An additional red card should be added to the tank card holder to identify removal of a dead animal where other living tankmates remain.
 - 4.7.1.1.3 The dead animal should be noted in both the log sheet and the disposition log (see SOP 160).
 - 4.7.1.1.4 Plastic bag should be placed in the freezer designated for dead animals (either in 101B, off cage washing room, or in SCI 103 research lab, accessed through vivarium procedure room 101D).
 - 4.7.2 Injured or distressed animals (see SOP 300)
 - 4.7.2.1 Place a red card in the card holder of the tank, noting the date and time and specific observation warranting the card.
 - 4.7.2.2 Contact the PI for further action, which could include:
 - 4.7.2.2.1 Consultation with the Attending Veterinarian
 - 4.7.2.2.2 Euthanasia
 - 4.7.2.3 Enter comments in the room log and disposition log., noting the specific tank.
 - 4.7.3 Escaped animals (discovered outside of feeding sessions)
 - 4.7.3.1 Capture the animal and place into a tank of its own, identified as "escaped animal" with a red card.
 - 4.7.3.2 Contact the PI responsible for the room for further action.
 - 4.7.3.3 Enter comments in the room and disposition logs.

5.0 References

- 5.1 Grass frogs (Sargent-Welch/Ward's)
 https://www.sargentwelch.ca/www.sargentwelch.ca/images/Grass_Frogs-Tadpoles.pdf
- 5.2 Care of Rana or Bull Frogs (Western Michigan University WMUAF-32-2014)

 https://wmich.edu/sites/default/files/attachments/u245/2015/iacuc sops 10">https://www.mich.edu/sites/default/files/attachments/u245/2015/iacuc sops 10">https://www.mich.edu/sites/attachments/u245/2015/iacuc s

SOP REVISION HISTORY

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