

Policies and Procedures

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Purpose

The purpose of this policy is to describe clear guidelines care of bullfrogs (*Rana catesbeiana*) and leopard frogs (*Rana pipiens*) held in the Department of Biology animal rooms.

Application

Policy describes the standard of veterinary care expected to be provided by Principal Investigators who perform the care of bullfrogs (*Rana catesbeiana*) and leopard frogs (*Rana pipiens*) held in the Department of Biology animal rooms.

Procurement

Frogs are ordered from a commercial vendor (e.g. Carolina Scientific or Nebraska Scientific) and shipped in a cardboard box with air holes and damp sphagnum moss and/or paper to keep the frogs moist.

Frogs are placed in tanks on the day of arrival at Creighton. Newly-arrived frogs must be housed in tanks that do not contain any currently-housed frogs and remain separated for a period of three days or more to allow for assessment of health. Any frogs arriving dead should be recorded and reported to the PI of the relevant IACUC protocol. Frogs being transferred from shipping boxes to tanks will be rinsed with conditioned water (see Water below) before being placed in the tanks.

Tanks are labeled with:

- PI's name
- IACUC protocol number
- Species
- Number of animals,
- Vendor, and
- Date of arrival

Frogs to be used in survival experiments are given at least 72 hours to acclimate to their housing before use in experiments.

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Handling

Handling of frogs is minimized to avoid distress. Clean, non-powdered gloves, wetted with tank or conditioned water, are worn. If frogs are being picked up, they are held around the waist to reduce the chance they can escape by pushing off using their back legs.

Housing

Animal room

Frogs are housed in Rigge Science 527, the smaller of the two animal rooms in the Biology Department. Since bullfrogs and leopard frogs are wild species that normally experience seasonal daylight patterns, natural light rather than timed fixtures is used in this housing space. The temperature of this room is normally near 70°F, but as with light these species are used to considerable environmental variation and can experience temperatures ranging from 60-80°F without distress.

Tanks

The Department of Biology currently has three sizes of tanks that can be used to house frogs: 38L (50×25×30cm high) and 76L (60×33×50×43cm high) glass tanks and a 420L (210×50×40cm high) plexiglass tank.

Frogs are housed individually or in same-species groups in tanks at a density no greater than one individual per 500 cm² of floor space in the tank, with at least 2 liters of water per animal. In the large plexiglass tank, a bubbler is used to aerate the water.

Lids are used to prevent animals from escaping, with the lids containing holes or being constructed partially or fully of screening or netting to allow air exchange. Lids are secured or have sufficient weight to prevent frogs from knocking them off.

Tanks are kept on shelving or countertops, or in the case of the large plexiglass tank on stacked cinderblocks.

Enrichment

Enclosures of a cave-like nature are provided for retreat. These enclosures are made of bricks, plastic, stainless steel, or similar non-reactive material and are large enough for a frog to completely withdraw itself under cover.

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Containers, water levels, and enclosures are arranged in such a way that frogs can sit in or out of the water. The combination of these elements serves as environmental enrichment for the frogs.

Water

Preparation

Water quality is important to the health of the frogs, so a standard set of procedures is followed to prepare water for use in the tanks.

Standard Omaha tap water is used, since the pH and solute composition (except for chloramines; see below) are suitable for these frog species without adjustment of those factors.

Because tap water does contain chloramines, and to improve animal health and help maintain water quality over time, a combination of Novaqua and Amquel (or similar water conditioners from other manufacturers) is used to remove chloramines, support frogs' mucus coating and reduce buildup of ammonia, nitrates and nitrites in the water. All conditioners are used according to label directions and well-mixed with the water in a large container before use. The water is tested for pH before introduction into frog tanks.

Monitoring

Water condition is monitored at least every other day for nitrites, nitrates and pH using commercially available aquarium test strips (e.g., API 5-in-1 Test Strips or similar). Nitrites should be at or near zero, nitrates below 40 ppm and pH between 5.5 and 8.5.

Water changes

For animals kept in tanks with 4L or more water per animal, water is changed once per week, and for animals in tanks with 2–4L of water per animal water is changed twice per week. In the event the water in a tank becomes cloudy or malodorous, or is outside the acceptable range for nitrites, nitrates or pH, it will be changed that day regardless of the normal changing schedule.

Water changes involve removing 75% or more of the water volume by bailing, siphoning, or draining in a manner that minimizes disturbance of the frogs. In addition, any feces and dead crickets and worms are removed. Conditioned water is then added to the tank to the original volume, again in a manner that minimizes disturbance of the frogs.

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Waste water is disposed of in the city sewer system.

Feeding

Frogs are fed live crickets, nightcrawlers (earthworms) or waxworms every two to three days (generally Mondays, Wednesdays and Fridays) beginning the day after their arrival. Food species are obtained from commercial vendors or local pet stores.

For frogs being kept two or more weeks, the food species will be rotated to provide a mixed diet. In addition, crickets will be dusted with a calcium and vitamin powder such as ReptiVite to prevent dietary insufficiency of these substances.

Live food items are placed on dry areas in the tanks so they do not immediately drown. Frogs generally eat live prey items fairly quickly. Dead prey items will be removed during daily health inspections.

Tank cleaning

Tanks are normally given a complete cleaning and disinfecting only when all frogs in the tank have been permanently removed. The exception to this would be if a tank is in use for more than a month or in certain cases of disease, when frogs are transferred to fresh tanks and the previous tank(s) are disinfected for later reuse. Disinfection involves all surfaces in contact with the frogs or their water and includes tanks, enclosures, and any bubbler tubing.

Tanks are cleaned using a 0.2% solution of standard household bleach. Gloves are worn to protect the hands of the cleaning personnel. The tank is wiped and scrubbed as needed to remove debris. In addition, the bleach solution is normally left in the tank overnight. The bleach solution is then removed, the tank is given a thorough rinsing, and then fresh water is placed in the tank overnight. After emptying, the tank is checked for bleach aroma and re-rinsed if needed until no bleach is detectable.

Enclosures that are autoclavable (bricks, stainless steel, etc.) are scrubbed and rinsed with plain water and then autoclaved. Other enclosures and tubing (plastic) are scrubbed and rinsed with the 0.2% bleach solution and then rinsed thoroughly with fresh water.

Health assessment and record keeping

Animal health is checked daily. Signs of illness or distress in frogs include weight loss, lethargy, failure to eat, and change in appearance of skin, eyes or mucus membranes. If an animal becomes sick or moribund, the veterinarian will be consulted or the animal

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will be euthanized following the most straightforward procedure in the IACUC protocol covering the frog.

In addition to health, room temperature and general water condition (clear, clean-smelling) is checked and recorded daily, water chemistry (pH, nitrites, nitrates) is checked and recorded every other day, and feeding is conducted and recorded every two to three days.

When any frogs removed from a tank for terminal use, the number of animals and date of removal are recorded on the tank's tag.

Carcass disposal

Frog carcasses are bagged and placed in the freezer in the large animal room (Rigge Science 426) for pickup by SteriCycle.

Room maintenance

While in use for frog housing, Rigge Science 427 is swept and mopped and counters are cleaned at least once per week, or more often if needed.