

SOP Tiger Salamanders- Eastlick B74A

Goldberg Lab

Animal Observation

1. All animals will be observed daily, including weekends and holidays.
 - a. The PI and graduate students/lab technicians on the project will provide animal care after appropriate training has been completed and documented
 - b. During daily observation environmental conditions will be recorded

Animal Housing

2. Housing of animals will depend on life stage
 - a. Adult salamanders will be housed individually in plastic shoebox containers with a tight-fitting lid that allows for gas exchange with the surrounding environment
 - i. The bottom of containers will be covered with 3 layers of paper towels and moistened with treated water (no standing water)
 - b. Larval salamanders will be housed in approximately 5 gallons of water, with 3 larvae per container
 - i. This density was shown to be low enough to avoid cannibalistic morphologies (Collins and Cheek 1983)
 1. Additionally, we will keep kin together to reduce the onset of cannibalism (Pfennig and Collins 1993)
 2. If signs of cannibalism are observed, larvae will be separated by nets/screens to avoid cannibalism/injury or moved to being housed separately
 - c. Salamander eggs will be housed in one-gallon buckets with a single egg mass or group of eggs collected from the same location housed together
 - d. During development, individuals will be moved to the appropriate housing conditions
 - i. When observed, hatched larvae will be removed from containers housing egg masses and into larval housing conditions
 - ii. Metamorphosing salamanders with visible absorption of external gills will be transferred to a plastic shoebox container that is tilted so standing water is present on one end (enough to cover the individual) and moistened paper towels are present on the other end of the shoebox
 - iii. After metamorphosis is observed (animal is observed spending time outside the standing water in the container), it will be transferred to adult housing
 - e. Water used to house amphibians will be purified by reverse osmosis and treated using a commercially available water conditioner (e.g. RO right) to

- the appropriate electrolyte balance (according to water conditioner manufacturer's instructions for freshwater species)
- f. Environmental enrichment will be provided to adult salamanders in the form of PVC pipe pieces for shelter

Cleaning Procedures

3. Containers will be cleaned on a frequency depending on life stage
 - a. Adult salamander containers will be cleaned bi-weekly
 - i. Paper towels will be replaced and moistened with water
 - ii. Additional paper towel changes may be necessary if all food items are not consumed within 24 hours of feeding to prevent ammonia buildup
 - iii. PVC pieces will be rinsed with treated water
 - b. Larval salamander containers will undergo a water change weekly
 - i. At least 50% of water and all solid waste present will be removed and replaced with fresh treated water
 - ii. Frequency and amount of water changed may be adjusted based on water quality during development as larger larvae will eat more and produce more waste
 1. More frequent water changes will be implemented if water quality conditions approach:
 - a. Ammonia levels 0.2mg/L (keep below these amounts)
 - b. Nitrite levels 0.5 mg/L
 - iii. In the case where 100% water changes are necessary, larvae will be removed from their current container using nets, containers will be rinsed and refilled with treated water, and larvae will be returned to their respective containers
 - c. Salamander egg containers will undergo a weekly water change
 - i. Because excess handling of eggs and egg masses can damage them, eggs and egg masses will remain in their respective containers and at least 50% of water will be removed and replaced with clean treated water

Animal Feeding

4. Animals will be fed in diet and frequency based upon life stage
 - a. Adult salamanders will be fed two times per week (every 3-4 days)
 - i. Diet will consist of crickets, earthworms, and wax worms
 - ii. All food items will be obtained from a pet food supplier and maintained in the lab
 - iii. Vitamin and mineral supplements will be administered once per week by dusting crickets prior to feeding
 - iv. Monitoring of consumption of food items will be recorded on the daily care sheet.

- b. Larvae will be fed every 2 days
 - i. Diet will consist of daphnia and brine shrimp for smaller larvae and mosquito larvae, tubifex worms, or small earthworms for larger larvae
 - ii. Food items will be obtained from pet food suppliers and maintained in the lab

Animal Handling

- 5. Animals will be handled using disposable gloves (changed with each individual) that have been rinsed with water or a clean net that has been disinfected and rinsed at least five times with treated water

Housing Conditions

- 6. Temperature
 - a. Room controls will be used to keep housing temperatures between 10C and 20C
 - b. Temperature will be monitored daily
- 7. Humidity
 - a. Will be monitored daily with room hygrometer
 - b. All life stages will have access to adequate water, if water levels are reduced by evaporation between water changes, additional water will be added to containers
- 8. Animals will be housed on a 12:12 light/dark cycle

Pest Control

- 9. The most probable pest/malady while rearing amphibians are bacterial infections. If any infections are detected on animals, we will disinfect all containers using a dilute bleach solution and place all animals in thoroughly rinsed disinfected containers with fresh water. If infection persists, we will discuss treatment with the campus veterinarian.
 - a. Prior to reuse, disinfected containers will be rinsed at least five times with water to remove traces of the chemical disinfectants
 - b. We do not anticipate pest problems in food storage of aquatic or terrestrial food sources (crickets, worms, daphnia, brine shrimp, etc.). If such problems arise, we will take appropriate pest removal measures dependent upon the situation.

Acclimation

- 10. Animals transferred from the field to the lab will undergo a 3-day acclimation period
- 11. During the 3-day acclimation period for eggs and larvae pond water will slowly be transitioned to treated lab water 1/3 volume at a time.

Animal Room Cleaning

12. Animal rooms and support spaces will be cleaned monthly and disinfected between experiments.

Documentation

13. Documentation will be maintained of daily observations, cage changing, room cleaning and measurements of temperature and humidity.

Sources for guidance:

Poole VA, Grow S. 2012. Association of Zoos and Aquariums Amphibian Husbandry Resource Guide. Edition 2.0.

<https://assets.speakcdn.com/assets/2332/amphibianhusbandryresourceguide.pdf>

Schaffer DO, Dleynow DM, Krulish L. 1992. The Care and Use of Amphibians, Reptiles, and Fish in Research. Scientists Center for Animal Welfare.

Collins JP, Cheek JE. 1983. Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. American Zoologist 23:77-84.

Pfennig DW, Collins JP. 1993. Kinship affects morphogenesis in cannibalistic salamanders. Nature 362:836-838.

Odum RA, Zippel K. 2011. Water Quality for Amphibians. Amphibian Ark.
<http://www.amphibianark.org/wp-content/uploads/2018/08/Water-Quality-Odum-Zippel-2011.pdf>