

## **Guide for the ethical treatment of fish, amphibians and reptiles for research purposes**

We generally follow the guidelines developed by consensus among conscientious members of the Animal Behavior Society (published *Animal Behavior*, 2012), the Fisheries Society of the British Isles (published in the *Journal of Fish Biology* 2006) and the American Society of Ichthyologists and Herpetologists ([www.asih.org](http://www.asih.org), 2004).

1. Choice of species and non-animal alternatives
  - a. Investigators should avoid species that are rare, endangered or listed as species of concern unless the study purports to contribute to species conservation.
  - b. Preference should be given to species that are well suited for captivity to minimize stress induced by captivity.
  - c. The species should be well-suited for the study question at hand.
2. The number of individuals.
  - a. The number of animals used should be the minimum that are sufficient to accomplish the stated research goals.
  - b. Animals collected from the wild should not deplete or detrimentally impact natural populations.
3. Procedures
  - a. Ethical treatment of fish, amphibians and reptiles assumes that these animals are sentient and sufficiently complex to be cognizant of pain and suffering. Therefore, researchers should take the same general measures followed to minimize pain and suffering in warm-blooded vertebrates.
  - b. Field studies:
    - i. Investigators must always consider and minimize the effect of their study on the natural resident population and the habitat on which it depends. Researchers bear the responsibility of acquiring knowledge of the local habitat and population in order to minimize their effect on the natural population.
    - ii. Trapping: live traps should be checked frequently (“frequently” depends on the biology of the species)
    - iii. Marking and telemetry: ensure that removed tissue does not affect animal’s survival and general killing:
  - c. Aggression, predation and intraspecific killing:
    - i. Reasonable measures should be taken to minimize harm to study animals, including the use of models in lieu of staged encounters with predators, and pre-determined end points.
  - d. Aversive stimuli, deprivation and motivation:
    - i. Care should be exercised to ensure that levels of manipulation are no greater than necessary to produce the desired effect.

- e. Social isolation or crowding:
    - i. An understanding of the natural behavior of an animal is necessary to minimize these sources of stress. Some species are stressed by crowding while others are stressed by social isolation.
  - f. Deleterious conditions:
    - i. Inducement of disease, increase in parasite load, exposure of animals to pesticides and homeostatic stressors should address possible treatment or alleviation of the condition induced.
4. Housing needs vary for each species. The investigator bears the responsibility to ensure adequate space, shelter, food and water, photoperiod, temperature and hygienic living conditions for their study animals. There is not usually any reason to house each species in separate rooms.
  5. Safety: all facilities housing aquatic animals should be equipped with ground fault interrupter circuits to protect against electrical shock.
  6. Water quality assurance: the single most important parameter to the health and welfare of fish and amphibians is proper care of water quality.
    - a. Dechlorination: city tap water contains chlorine and chloramines. These are both highly toxic to fish and must be removed using either a dechlorinating agent or a filter (e.g. charcoal tower.)
    - b. Filtration: water filtration can be achieved through a variety of means. There are two main types of filtration: mechanical and biological. Coarse media such as sponges or polyester cartridges mechanically remove suspended particles from the water. Filtration media become clogged with debris and must be inspected regularly and cleaned and/or replaced as necessary. Undergravel filters, sponge filters and trickle filters create large surface area for *Nitrosomonas* and *Nitrobacter* bacteria to colonize. These bacteria biologically oxidize ammonia (highly toxic) to nitrite (highly toxic) to nitrate (low toxicity). Regular water changes remove nitrate and prevent it from accumulating.
    - c. Water replacement: The general rule of thumb for water replacement is 10% replacement per week. A gravel vacuum should be used to remove feces and uneaten food from the tank floor when siphoning out water during a water change. Turtles and *Xenopus* may require more frequent water changes depending on stocking density and tank volume.
    - d. Temperature: Depending on the temperature range of the species, heaters or chillers may be required.
  7. Feeding: regular feeding
    - a. Adequate amount: poikilotherms eat small amounts of food. Excess food fouls the water and clogs the filtration system.
    - b. Adequate frequency: for many species, one feeding per day is adequate. More than three feedings per day runs the risk of over-feeding and should usually be limited to larvae.
    - c. Appropriate food type: most fish eat generic flake food, but wild fishes in particular (e.g. stickleback, darters, mudminnows, etc) refuse flakes and must be fed specialized food (e.g. brine shrimp)

## 8. Holding conditions

- a. Appropriate density: crowding increases stress, suppresses the immune system and increases the rate of transmission of parasites and pathogens.
- b. Appropriate availability of refuge: if aggressive individuals are housed together then refuge is required to protect the subordinate individuals from injury or death.
- c. Lighting: fish should not be exposed to constant light or constant dark. A 24-hour L:D cycle of 12:12 to 6:18 should be maintained by use of automatic timers.
- d. Algae: alga is a natural and beneficial part of the natural environment. Algae should be scraped clean of the front viewing pane so that people can easily view the fish and monitor their health and welfare.

## 9. Disease Control

- a. Hygiene: regular water changes (10 % per week for fish) keeps animals stress low and keeps pathogens at low density
- b. Use of quarantine tank for newly arrived organisms
- c. An antiseptic net dip to prevent transfer of disease organisms among tank
- d. When any of these signs develop: inactivity, loss of appetite, drooping fins, labored swimming, labored ventilation, bloat, red-lined rays in the fins, exophthalmia, fungal hyphae on mouth or body surface:
- e. Remove and euthanize the affected individual by cervical dislocation or by overdose of anesthetic
- f. Increase the frequency and proportion of water changes to 50% of tank volume or more per day until health of the tank's inhabitants stabilizes