

**UNIVERSITY OF CALIFORNIA, SAN DIEGO  
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

**Guidelines for the Care and Use of Ectothermic Vertebrates**

INTRODUCTION

Inherent respect for all systems and forms of life should be a characteristic of any individual participating in field or laboratory research on animals. Consistent with the University's longstanding interests in education and research, this document is intended as a standard for the humane care and use of ectothermic vertebrates (fishes, amphibians, and reptiles) by faculty, support staff, and students at the University of California San Diego. Because of the extensive number of ectothermic vertebrates (more than 25,000 species of fish, 4,000 species of amphibians, and 6,000 species of reptiles) and their staggering range of ecological diversity, no concise compendium of approved laboratory and field methods for all these groups is practical. Instead, we present general guidelines based on the most current information, to advise the investigator regarding techniques that are known to be appropriate and effective. Ultimate responsibility for the ethical conduct of the investigation and the scientific validity of the results must rest with the investigator.

A number of considerations underlie all aspects of the care and use of live ectothermic vertebrates:

1. The number and ecological diversity of ectothermic vertebrates is precisely what makes these animals attractive for educational exhibit and research ranging from biomedical to populational. This diversity dictates that it is generally impossible to generate specific guidelines for groups larger than a few closely related species. In fact, inappropriate application of specific guidelines could actually preclude humane care, as well as hinder research.
2. Ectothermic vertebrates, with their great diversity, present far more potential problems for adequate care than do domesticated birds and mammals. This diversity requires that a researcher be willing to devote substantial attention and effort to determining the unique needs of species for which insufficient information is available. Unlike the situation with endotherms, particularly mammals, with ectotherms, the researcher is likely to become (or already be) the individual with the greatest expertise regarding the care of a particular species; housing of the animals in close proximity to the researcher's offices and laboratories may be crucial.
3. Although ectothermic vertebrates do not usually generate sufficient excess metabolic heat to raise their body temperatures above ambient levels, they are not "cold blooded" under normal conditions, they do regulate their body temperatures by behavioral means, often at high levels and within narrow limits. Thus, their resting metabolic rates, aerobic capacities, and overall energy needs are far lower than those of endotherms (birds and mammals) of comparable size.
4. Most ectothermic vertebrates are not available from commercial breeding colonies; they are frequently collected in the wild by an investigator, purchased from an amateur collector, or received as a gift from another researcher or governmental institution. Availability is often constrained by seasonal activity of the animals and by the political climate in their country of origin. The precise taxonomy may be problematic at the time of acquisition, and the acquisition of an unexpected species often provokes a new direction in the research. Therefore, it will often be necessary for investigators to submit blanket protocols covering a large number of related species.
5. Because it is impossible to obtain most ectothermic vertebrates through licensed suppliers, University personnel should assure themselves that they are legally obtaining an animal from whatever commercial or individual source. The Institutional Animal Care and Use Committee should be notified immediately of any indication of illegal trade or improper care of animals by a supplier. Similarly, in collecting animals in the field, University personnel are responsible for knowing the status of the species in question and are bound by governmental restrictions or prohibitions.
6. Ectothermic vertebrates are frequently used for both exhibit and research purposes; investigators or

units such as the Birch Aquarium may routinely maintain individuals of various species for the duration of the animals' lives.

## GENERAL GUIDELINES

The following guidelines are generally applicable to the care of most fishes, amphibians, and reptiles.

1. The animals should be housed in such a way as to inhibit the spread of disease. Procedures to assure same will vary with the species. Separate quarantine facilities are ordinarily not required for ectotherms; however, newly acquired animals should be isolated for varying periods before being housed with resident animals.
2. The animals should be housed in such a way that their needs regarding temperature, humidity, and light are met, ideally under conditions that mimic their natural environment.
3. The animals should be housed so that the population density of the enclosure is such that detrimental behavior or injury does not result from overcrowding, inappropriate isolation, or predation.
4. Enclosures should be labeled to identify the number and species enclosed (both common and scientific names). Every room containing live animals should have prominently displayed the name and department of the investigator, as well as office and emergency phone numbers. If animals belonging to more than one investigator are housed in the same room, the enclosures should be labeled so that the responsible individual can be immediately determined. Animal Use Protocols and appropriate permits should be on file. If marking of individuals is necessary, this should be accomplished in a humane and appropriate manner.
5. The enclosures should contain the necessary accoutrements (for example, rocks, twigs, appropriate substrate) to mimic, as much as is feasible, the animals' natural habitat. When appropriate, the animals should be provided with a place to hide.
6. The enclosures should be such that escape is not feasible. Animals should not be subjected to inappropriate stressful stimuli in their immediate environment.
7. Animals should be maintained on a diet appropriate in quality and quantity to their needs.
8. Animals should be checked regularly for signs of injury or disease, the frequency of such checks to be determined by the species, general condition of the individuals, and length of residence in the laboratory.
9. Procedures for handling, restraining, anesthetizing, and euthanizing animals should be painless, humane, and carried out by properly trained personnel. Approved anesthetics currently include sodium pentobarbital, ketamine, ethyl m-aminobenzoate methanesulfonate, benzocaine, halothane, isoflurane, 2-phenoxyethanol. Approved methods of euthanasia include lethal application of the above anesthetics. The use of physical means, such as decapitation, is discouraged unless justified, and such means should be followed by brain pithing unless intact brain tissue is a requirement of the experiment.
10. Ectotherms that normally hibernate for some period may be appropriately maintained in cold torpor for a period up to six months or until the individual has experienced a 20% loss of body weight or shows other signs of poor health.

## FISHES

The physical requirements to be considered, according to the species, number of specimens, and duration of holding each animal, should include the following:

1. An adequate electrical supply for filter pumps, air supply, and heating/cooling systems.
2. A mechanism for appropriate temperature regulation of the entire room and/or individual tanks, when

appropriate for the species.

3. An emergency power source to assure the continuance of fresh air flow to tanks, when inhabitants of the tanks depend on artificial air.
4. A sink with hot and cold running water is desirable and in some circumstances essential.
5. A floor drain is desirable and in some circumstances essential.
6. A light source on a 24-hour timer is important for some situations.
7. A central water supply tank, incorporating filtration, oxygenation, temperature control, and ultraviolet sterilization, may also be useful. Separate supply tanks are needed for fresh water and sea water; ideally, these should contain three to five times the volume needed for the individual tanks.

The local fresh water available on the San Diego campus is generally not suitable, directly from the tap, for most fishes. Precipitation of potentially lethal additives is generally less efficient than mixing the desired fresh water from a combination of distilled water and appropriate salts. Piped sea water is available to some UCSD facilities; otherwise, it can also be mixed from distilled water and appropriate combinations of marine salts. Investigators should determine the specific needs of their species, as desirable pH and ionic concentrations can vary considerably.

The dietary needs of fishes also vary tremendously, and the investigator must determine the most appropriate food items and amounts according to the species and age of individuals. Commercial fish chows are appropriate for some species; the dietary needs of some others can best be met by commercially prepared fish or seafood items; live food needs can frequently be fulfilled by brine shrimp or black worms. All food sources should be stored appropriately to avoid contamination or spoilage.

As with water and food requirements, the investigator should be acquainted with the temperature requirements of a particular species. Appropriate water temperature may be even more crucial than diet, on a short-term basis, as many fish diseases erupt in conditions of desirable water temperature. Many species are particularly susceptible to problems caused by sudden changes in water temperature. It is therefore generally advisable to "float" a plastic bag containing new fish, in the water used for their transport, until the ambient water temperature is gradually equalized, before introducing new animals to a tank.

### References

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American Fisheries Society, 2004. Guidelines for the Use of Fishes in Research.

## AMPHIBIANS

Amphibians generally require cool, moist environments, although tropical species may prefer room temperature. Containers should not be airtight, but should be covered if evaporation is a problem.

Terrestrial caecilians are burrowers and require clean, moist soil. Aquatic caecilians do well under the same conditions as fresh-water tropical fish, although most also need some form of shelter. An overturned clay pot with an entrance hole is appropriate.

Many species of terrestrial salamanders do well on a substrate of moist paper towels, replaced at intervals sufficient that accumulated feces do not grow mold. Most species also do well on an earth/twig/leaf substrate. In either case, a small pool at one end of the enclosure and pieces of clay pots for shelter are appropriate additions.

Axolotls should be maintained in containers of at least one liter of water per adult animal, at a temperature of 18-20°C. An aeration system is usually required for long term care of axolotls and other aquatic salamanders, especially for stream-dwelling species.

Frogs and toads should be housed in containers of sufficient depth that they do not injure themselves by leaping against the top. Rough wire screen or other abrasives should be avoided in places that the animals will contact. Generally, a few inches of water in the bottom of the container, with rocks protruding above the surface, represent optimal conditions. Totally aquatic anurans, such as African clawed frogs, should be kept in approximately one liter of water per adult animal, at a temperature of 16-25°C.

Dietary needs of amphibians vary with the species; for long term maintenance, it is important to ensure that the diet is nutritionally as well as calorically sufficient. A chronic diet of mealworms, for example, is inappropriate, as this food item may eventually clog the digestive tract and result in calcium/potassium imbalance.

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## REPTILES

Temperature, proper lighting, sufficient space, and appropriate food are the key aspects of reptile care. Cages must be escape proof and, for most species, should not have rough wire screen in places that animals can reach with their noses.

Temperature. As most reptiles actively thermoregulate during the photophase and exhibit voluntary hypothermia during the scotophase, heat in enclosures should form a gradient, with high temperatures not exceeding 40°C. Thigmothermal heat sources are preferable. The use of infrared heat lamps should be avoided for non-desert species because of their intense drying effect. If lamps are used as a heat source, their intensity should be appropriate to the enclosure and species.

Lighting. A photocycle is preferable to continual light or continual dark, unless the photoperiod is an integral part of the experiment. Quality of the light is also important. A variety of fluorescent lamps mimic the color spectrum, while others produce the ultraviolet spectrum in the range that converts vitamin D2 to D3. Without exposure to these wavelengths, animals in long-term maintenance will eventually develop severe disorders in calcium metabolism.

Food. Reptiles can generally be divided into insectivores, carnivores, and herbivores. For long-term maintenance, insectivores, the small lizards, and some snakes, should be fed a diet of mixed insects, rather than one consisting largely or exclusively of mealworm larvae or crickets. Both of these food sources will eventually cause severe calcium deficiency. Insects should be dusted with a calcium carbonate/vitamin powder at least once each week. Carnivorous reptiles that feed on live prey should, if possible, be trained to eat killed prey items, in order to prevent accidental injury. Herbivores should be fed a complete diet consisting of a variety of vegetable matter, never lettuce or other greens alone. Supplemental protein sources, such as commercial dog/cat food, are preferable to mixed vegetable diets for some species.

Habitat. Aquatic turtles and crocodilians require clean water and a dry place to emerge. Lizards, amphibians, and snakes generally require mostly dry conditions with adequate water. Species from moist areas often require less ventilation and frequent spraying.

Venomous Reptiles. The California Department of Fish and Game currently requires the following prior to issuance of a permit to maintain live venomous reptiles in the state:

1. All venomous reptiles and any progeny shall be confined in escape-proof enclosures. Entrances providing immediate access to the reptiles shall be kept locked to prevent entry of unauthorized persons. Access to the reptiles shall be restricted to the permittee and his/her authorized representatives or associates. All individual cages or enclosures shall be of such design and construction as to preclude the escape of any reptile from any human or natural cause, including earthquake.
2. All windows or other openings, including vents, in any room where venomous reptiles or their progeny are maintained, shall be screened and barred to prevent the escape of animals into the wild or the entry of unauthorized persons into the room.
3. Any room where venomous reptiles are confined shall be equipped with adequate temperature control to ensure that the animals are humanely maintained.
4. An up-to-date list of all subject anti-venom sources in California shall be posted in the immediate vicinity of the room where venomous reptiles are maintained.

Inspecting officers will not be limited by these requirements; they may impose additional restrictions which they feel are necessary in any particular case.

In addition to these requirements, it is required that all personnel dealing with these animals on campus be approved by the appropriate University personnel. Any cage containing a venomous reptile must be labeled with the common name, family, genus, and species of the animal in such a way that the label can be easily removed and taken with a victim to the hospital. A first aid kit and typed instructions for treatment of snake bite (both first aid and initial hospital care) must be present and prominently displayed in each room

containing live venomous reptiles. Venomous animals must be transported on campus only by approved personnel, and the animals must be in secure, properly marked containers. Whenever possible, venomous reptiles should be manipulated by indirect means (e.g., hooks or shift cages), rather than handled directly.

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