

	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL	POLICY # 24.01 Originally Issued: 6/20/2018 Revised:
	Aquatic Vertebrates	

I. Background and Purpose

The Guide for the Care and Use of Laboratory Animals, 8th edition (National Academies Press, 2010) includes general guidelines and recommendations for the care and use of aquatic species in research; UCSD is obligated to follow these guidelines and recommendations as they apply to all animals used in research and teaching.

While some aquatic species (e.g., frogs, zebrafish) are available from commercial vendors or breeding colonies, other species may be collected in the wild or obtained from other institutions; therefore, the procedures used for acquiring mammals or other species may not apply. ACP follows all Federal, State and local regulations and guidelines for acquiring certain species, including many ectothermic vertebrates. For further guidance, please refer to the ACP website for Animal Acquisitions.

II. Who Should Read This Policy

All personnel with assigned responsibilities for handling aquatics on an approved protocol, ACP veterinary staff and ACP animal care staff.

III. Definitions

Term	Definition
Aquatic Vertebrate	Any hatched, live animal that has a backbone or spinal column and lives most of its life in the water. Aquatic species may breathe oxygen through the air or water. Most commonly used species include fish, amphibians (e.g. frogs) and reptiles (e.g. turtles). Zebrafish are considered hatched at 3 days post fertilization (dpf).

IV. Policy

1. All aquatic animals, including those to be obtained from non-commercial sources, must be ordered through the ACP Animal Acquisitions process. ACP will verify IACUC approval, allocation and availability of housing space, if necessary. Acquisition of animals in the field must be described in the protocol and the number of animals must be reported to the IACUC.
2. Zebrafish larvae may be housed in the lab for up to 7 dpf without requiring a satellite facility. A description of the housing conditions and location must be described in the IACUC approved protocol. Zebrafish over 7 dpf and all other species held outside of an approved vivarium for over 12 hours must be housed in an IACUC approved satellite facility as per IACUC Policy 28.

3. Animals must be housed in conditions that meet their needs regarding temperature, light, humidity, water flow, and space. Population density must be controlled in order to prevent overcrowding, or inappropriate isolation. Environmental enrichment, such as appropriate substrates, climbing platforms or hiding places must be provided as appropriate for the species. Primary enclosures must be secure to prevent accidental escape. Animal housing practices must inhibit the spread of disease. Specific procedures will vary with the species.
4. Enclosures must be labeled in accordance with IACUC Policy 36. If marking/identification of individual animals is performed, the method must be described in the Animal Use Protocol and be accomplished in a humane and appropriate manner (*i.e. tags, fin snips, etc.*).
5. Animals and their environments must be monitored daily. Records documenting checks of daily health and feed and micro/macro environments must be completed daily and maintained in all housing rooms. Records of room and equipment sanitation and maintenance must be completed and maintained in all housing rooms and retained in accordance with ACP procedures. The respective responsibilities of laboratory staff and ACP for these daily checks must be agreed upon in writing.
6. Daily reporting to ACP of health issues, disease or unexpected deaths of animals is required. Researchers can contact the ACP veterinary staff for assistance in developing Standard Operating Procedures for care and maintenance of aquatics.
7. Training of lab personnel in appropriate methods is the responsibility of the PI. Documentation of such training is required and must be available for inspection. Training is also available through ACP.
8. Where animals are bred, breeding colony reports must be submitted monthly to the IACUC.
9. California Fish and Wildlife regulations apply to *Xenopus*, Axolotls, and transgenic zebrafish. These regulations prohibit these animals or their progeny from being released into the environment. Refer to the SOP for each species and also see below for Transgenic Aquatic Species.
10. Euthanasia methods must be described in the Animal Use Protocol and be consistent with USDA policy and AVMA guidelines.
11. All aquatic animal carcasses and tissues, both transgenic and non-transgenic, must be rendered non-viable by appropriate use of bleach or other methods, prior to disposal. Zebrafish are considered pathological waste and must be disposed of per EHS requirements.

V. Related Documents

UCSD Documents	Policy 13 Euthanasia Policy 28 Satellite Facility Policy 35 Surgery in Rodents, Birds, Reptiles, Amphibians and Fish Policy 36 Animal Identification Animal Carcass Disposal Pathway Biohazard Waste Disposal Guidelines
Other Documents and References	AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

VI. Additional information

General Guidelines for the Care and Maintenance of Aquatic Species

Standard Operating Procedures

ACP will ensure, in conjunction with the investigator, the needs for specific species, Standard Operating Procedures (SOPs) for the daily husbandry and care of animals, and the cleaning and maintenance of all housing areas and equipment are prepared in accordance with standard ACP procedures. The SOPs must describe in detail all aspects of the care provided to the animals, including:

1. Feeding (feed type and vendor, frequency of feeding, method of feeding)
2. Regular monitoring of both the animals and their environment, including:
 - Daily checks for animal health, including weekends and holidays
 - Daily recording of critical environmental parameters, depending on the species
 - For aquatic species: examples of critical parameters include water temperature and pH
 - Regular recording of additional environmental parameters, depending on the species, such as concentration of nitrates, nitrites, ammonia, salinity, hardness and dissolved oxygen in the water of aquatic housing systems.
 - The frequency of testing will vary depending on the species, life stage, system and specific parameter.
 - Newly-established aquatic systems generally require more frequent testing.
3. Standards for acceptable ranges of environmental parameters for the species concerned e.g. room temperature and humidity, water conditions, and response plans in the event that conditions are outside the acceptable ranges.
4. Description of the housing system (e.g. static, flow-through or recirculating aquatic system, biological filtration system, size of primary enclosures).
5. Description of the source and treatment of water for aquatic or semi-aquatic species (e.g. municipal water, ocean water, RO water, distilled water, filtration type, any water conditioners or additives such as salts or dechlorinating agents).
6. Description of handling procedures required for routine husbandry (e.g. capture of animals to transfer them to different primary enclosures).
7. Cleaning methods and frequency of primary enclosures and secondary enclosures rooms. In general, each room should have its own stock of supplies, including nets and other animal handling devices and cleaning supplies. For aquatic species, this should include a description of the frequency and amount of water replaced.
8. Maintenance, cleaning, and replacement guidelines for all equipment used to house animals, including primary enclosures, filters, pumps, heaters, UV lights, nets, and water-quality probes.
9. Any regularly used disease prevention and/or treatment protocols.
10. Procedures used to acquire and introduce new animals. This generally includes a quarantine period to monitor and address potential disease problems, as well as allowing new animals to acclimate.

11. Procedures used to monitor the health status of the existing population, including any procedures for monitoring animals and/or their environments for the presence of disease-causing agents or conditions.
12. In conjunction with ACP veterinary staff, an effective response plan for an animal disease outbreak, including procedures for large-scale culling of sick animals and for cleaning and disinfection of tanks and water systems.
13. Procedures for anesthesia, transportation (out of the vivarium), euthanasia (consistent w/ AVMA guidelines), confirmation of death, and disposition of animals.
14. An appropriate response plan in the event of equipment malfunction or failure, both on a small scale and large scale. This must include contact information for appropriate personnel, and plans for possible relocation or euthanasia of animals.

NOTE: SOPs must be submitted to the IACUC for review and approval as part of the animal use protocol. Once approved, a copy of the SOPs should be kept in all areas where animals are housed.

In addition, aquatics housing facilities should have:

1. An adequate electrical supply for filter pumps, water pumps, air supply, and/or heating/cooling systems, as well as an emergency power source to assure the continuance of fresh water and aeration to tanks.
2. Ground Fault Interrupt (GFI) electrical outlets.
3. A mechanism for appropriate temperature regulation of the entire room and/or individual tanks, when appropriate for the species.
4. An appropriate light source and light-dark cycle in all rooms or enclosures. Gradual changes in room light intensity are recommended, as sudden changes may elicit a startle response in some species (e.g. Fish). Some aquatic and semi-aquatic species may need full-spectrum lighting and/or heat lamps to provide supplemental heating to facilitate adequate physiological function.
5. Sinks with hot and cold running water and functional floor drains are desirable in fish-housing areas.
6. Large, heavy equipment (e.g. racks, tanks, large filter canisters) must be secured to walls or constructed to prevent them from falling in the event of an earthquake.
7. Standards for acceptable water conditions appropriate to the types of animals housed.
8. Personnel managing aquatic systems must be trained on relevant aspects of water chemistry, how to monitor water quality, and how water quality can impact animal health.

Transgenic Aquatic Species

Creation of new transgenic animals (including cross breeding of transgenic strains) requires approval in an animal use protocol, approved by the UCSD IACUC.

Transgenic aquatic species require special procedures to comply with California Fish and Wildlife Regulations and the Federal NIH Guidelines for Research Involving Recombinant DNA Molecules, as follows:

1. All transgenic aquatic animals shall be held, raised, and transported in a closed-water system or in a system which treats effluent discharge from the facility with a disinfection system adequate to ensure against the inadvertent release of live animals into the building drain.
2. Release of transgenic aquatic animals or their progeny into waters of the state is prohibited.
3. Access to facilities containing transgenic aquatic animals must be restricted through means determined to be adequate by ACP to assure against unauthorized removal of animals.
4. Movement of live transgenic aquatic animals from facilities is prohibited unless specifically permitted by ACP.
5. If transgenic aquatic animals are held with non-transgenic animals of the same species, all such animals that commingle with transgenic animals shall be treated as transgenic for the purposes of regulation and may not be introduced into waters of the state. Non-transgenic individuals that can be individually identified as non-transgenic may be exempt from this provision with prior ACP approval.

Amphibians

Specific requirements for amphibians vary by species. As with other ectothermic vertebrate species, expert advice should be sought when designing appropriate housing and husbandry SOPs.

1. Amphibians generally require cool, moist environments, although tropical species prefer warmer temperatures. Primary housing enclosures should not be airtight, but should be covered if evaporation is a problem.
2. As with other ectothermic species, consideration should be given to species-appropriate temperature and lighting and maintenance of a power source. Light wavelength requirements for amphibians are largely unknown. Many amphibians can be maintained for long periods of time with standard fluorescent room lights, and no supplemental lighting at the tank level.
3. 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.
4. Axolotls should be maintained in containers of at least one liter of water per adult animal, at a temperature of 16-24°C. An aeration system is usually required for long term care of axolotls and other aquatic salamanders, especially for stream-dwelling species.
5. Anurans (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 for semi-aquatic anurans. Totally aquatic anurans, such as African clawed frogs (e.g. *Xenopus laevis*), may be housed in approximately two liters of water per adult animal, but a wide variety of housing systems are currently utilized in research settings. Researchers are encouraged to monitor on-going research and evaluate space needs as necessary, documenting their findings in the individual SOP and/or protocol as appropriate.