Requirements for Enclosures Housing Aquatic and Semi-Aquatic Species
The following section is intended to provide a general description of institutional requirements for enclosures of aquatic and semi-aquatic species, as well as, the proper care, use, and the humane treatment of these species produced or used in research and teaching.

General Description of Requirements
Prior to bringing aquatic animals into an animal space for research or teaching activities, investigators are required to obtain an animal use protocol (AUP), establish a written set of standard operating procedures (SOPs) which describe routine care and monitoring, and have their completely set up animal space approved by the institutional animal care and use committee (IACUC). The AUPs, SOPs, and records of daily and routine monitoring must be maintained and readily available within the aquatic space at all times (either posted or maintained in a notebook). New or significantly revised SOPs should be prepared in consultation with and/or approved by qualified personnel and submitted to the university veterinarian for final approval.

An arrangement must be established to insure that all animals and their housing environment are assessed on a daily basis. Routine and emergency contact information must be clearly posted at the facility entrance, as well as, at the entrance of each animal space, if applicable. Each facility must maintain a disaster plan that is posted and available to all lab users.

Overall System Design
The aquatic animal facility should be structurally designed to resist damage from a humid environment and from accidental enclosure leakage, spills, and complete system failures which may result in a significant quantity of water on floor surfaces. In addition, there should be a mechanical or structural devise to contain water within the facility. Surfaces within the facility and animal spaces should be composed of materials and finishes that can be properly sanitized, when necessary, while maintaining animal safety and welfare.

Copper piping and lead-based solders should be avoided. Floor drains should be present in all aquatic facilities with adequate facility wide drainage which may require drains in several locations. Floors should have gentle slopes to promote drainage to designated areas and steeply angled slopes should be avoided.

A dry area should be provided for report writing, record keeping and any other activity which requires a dry surface. SOPs and AUPs should be displayed or stored in this area along with emergency procedures, contact information, feeding schedules, water quality reports and any other necessary data logs.

Life support systems for aquatic organisms refers to the physical structure used to contain the water, organism and necessary equipment used to move, filter or treat the water. The simplest system is a static system where water is stationary and replaced or replenished depending on species, organism
density or project objective. Flow through systems have a continual supply of source water and may use large volumes of water as it is not reused. Recirculation systems are common in indoor high-density housing systems and are designed to exchange a specific volume of water per unit of time. Recirculating systems are the most complex often containing components to aerate, degas, heat or chill water as well as carbon, biologic, ultraviolet and particulate filters. Recirculating systems may have multiple enclosures or just one enclosure. Not all components are present in every system and some components may perform multiple functions.

Biological filtration is critical for limiting ammonia and nitrite accumulation in recirculating systems. The biofilter must be of sufficient size to process the system bioload. Care must be taken to maintain an operating microbial ecology. Beneficial microbial communities can be adversely affected by rapid changes in water chemistry and temperature which could result in negative effects to the efficiency of biological filters with adverse effects to sensitive species.

All areas of the animal facility need to be kept clean. Rooms need to have an area, or in a larger facility, a centralized location where enclosures can be cleaned, sanitized, and dried. Care should be taken not to contaminate water from one tank to another and to have designated nets and cleaning brushes or pads for each culture. Rooms should also have a net sterilization system to reduce contamination between and amongst cultures.

**Housing**

Aquatic environments should be designed to meet the established physical and behavioral requirements of each species such as shelters, social grouping, overhead cover, substrates, basking or ‘dry’ areas, and lighting. The shape, color, depth, and volume of tanks should be appropriate for the species and life stage being held.

The ability to visually assess all aquatic animals in tanks and incoming water flows is essential as a first step in assessing animal health. Tanks should be designed to facilitate adequate means of regular cleaning. Good water quality should be maintained as retention of wastes in animal enclosures promotes the proliferation of pathogenic bacteria, protoza and fungi, and leads to oxygen depletion and foul, cloudy water. The accumulation of ammonia is extremely toxic to aquatic animals and should be monitored to determine the necessary cleaning frequency.

**Housing Densities**

It is almost impossible to provide stocking rates, even with regard to a particular proposal, because of the differences in sizes of aquatic animals, especially fish, size of aquaria, and holding system. Each species should be housed at a density that ensures the well-being of the animal while meeting experimental parameters. In some cases, the ideal environment for maintaining a given species will have to be developed using performance-based criteria such as growth or reproduction. Each tank must be identified with an identification number, species, population/strain, and number of or approximate mass of organisms contained within.

**Water Source**

Water source must be carefully considered at each facility. Water quantity, availability, organism of interest, and purpose of the experiment should be considered when selecting source of water. Deep wells are the best source of fresh water because they generally contain fewer infectious agents, toxic chemicals or water treatment additives. Municipal tap water usually contains chlorine/chloramines and must be treated to remove these before use with most aquatic organisms. Artificial or
reconstituted waters using reverse osmosis or distilled water amended with micro and macro salts can be utilized and is a good alternative to protected source waters. Surface waters from rivers, stream, lakes or ponds can also be used where deemed appropriate to accomplish specific project objectives. Artificial salt water may be created by adding appropriate salt to fresh water sources.

If fresh or sea water is drawn from an open body of water or a municipal source, it should be tested for, and treated to remove, contaminants and pathogens. A comprehensive analysis of water quality parameters (ions, pH, metals, pathogens, etc.) should be conducted before a fish holding/testing facility is planned to ensure that the water supply is suitable.

**Feed**
Feed should be purchased from sources that manufacture feed according to standards employed in the feed industry for fish and other domestic animals, and according to published nutrient requirements for said species, if available. Feed should be appropriate for species providing dietary needs, free from contaminants and kept in sealed containers. Feed should be stored away from cleaning or sterilizing agents and protected from contamination from miscellaneous pest organisms. Feed container should be labeled with received date, open date and expiration date if applicable. Bulk feed should be stored in a refrigerator or freezer. Daily or weekly feed containers may be kept in the animal room if labeled with received, opened and expired dates. The container must also be lidded and rodent proof.

Fish must be fed at appropriate interval and with a nutritionally adequate, properly sized feed. Optimal feeding techniques are essential for good health and well being, and to prevent the fouling of water with uneaten feed.

**Redundancy in Aquatic Life Support Systems**
All aquatic facilities should have an emergency contingency capacity, capable of maintaining aerated and filtered water, and heating and cooling of water as appropriate.

**System Maintenance**
Aquatic facilities should have written maintenance schedules developed specifically for the facility. Routine maintenance and equipment overhaul and replacement should occur while the equipment is still operating normally. All electrical equipment, life support equipment and air and filtration systems should be checked and serviced on a regular interval.

The facility, tanks and enclosures should be kept in a clean and orderly manner. Enclosures and equipment should be disinfected before and after every experiment in accordance with facility sanitation SOPs.

The inherent nature of aquatic facilities requires that there be constant maintenance, repair and upgrading. Trained personnel need to be on call 24 hours a day, 7 days a week. The facility must have a method for assuring a rapid emergency response by staff outside normal operating times.

**Water Quality**
Water quality parameters should be monitored at an appropriate frequency for each system and should allow for predictive management of water quality rather than only reactive management of crisis in water quality.
In general, recirculating systems should be monitored for a larger number of parameters including dissolved oxygen, temperature, salinity, pH, ammonia, nitrite, nitrate and total dissolved solids.

Water quality records should be maintained and available for retrospective analysis in the event of problems. Water quality monitoring frequency should account for species sensitivity, density and type of holding system. Water quality data sheets should have an acceptable range for each parameter tested. If the value falls outside an acceptable range, immediate action should be taken and action should be documented on the data sheet.

Health and Disease Monitoring
Healthy animals are pre-requisites for reliable data used for research and teaching. A veterinarian or other experienced personnel with aquatic medicine experience and training should be available to assist in the identification of disease and the development of SOPs designed to reduce the potential for disease. Disease management protocols should include a system for the detection and reporting of clinical signs, and criteria to distinguish between acceptable and unusual levels of mortality. Isolation and treatment of diseased animals, and the rapid removal of dead animals will help reduce the spread of disease.

Euthanasia
Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the AVMA Guidelines on Euthanasia. Euthanasia may be planned and necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives or other treatments. The act of euthanasia should be carried out in a manner that avoids distress to the animal. The selection of a euthanizing agent should take into consideration the number of fish to be euthanized, species, size, age and objectives of the protocol. It is essential that euthanasia be performed by personnel skilled in the method for the species in question and in a professional and compassionate manner. Death must be confirmed by trained personnel able to recognize cessation of vital signs in the species being euthanized. Often euthanasia is a two-step process with the second step designed to assure death.

Unexpected Mortality and Adverse Events
An adverse event or unexpected outcome is defined as any unfavorable or unexpected outcome that results in or causes the welfare of an animal to become compromised. Adverse events must be reported in writing to the IACUC and the attending veterinarian within 72 hours. Unexpected mortality or morbidity in excess of expected mortality or morbidity must also be reported to the IACUC and the attending veterinarian within 72 hours. This written statement should describe in detail the event, any actions taken to eliminate, modify or mitigate a reoccurrence of the event.

Dead Organism Storage and Disposal
Dead organisms should be placed in a plastic bag and stored in a dedicated freezer in a biohazard bag until picked up for disposal by Clemson University Environmental Health and Safety (EHS). It is the responsibility of the PI to call EHS for dead organism pick up.

Quality Assurance
Every effort is made to ensure that aquatic animal facilities, as well as, the welfare of aquatic animals are maintained at the highest standards. A standardized SOP and AUP template is used by all investigators to ensure that the correct information is present and to make it easy to access needed
information. In addition to regular, unannounced visits by Clemson University veterinarians, animal spaces are also routinely checked by laboratory management personnel to ensure that the micro and macro environments are clean and healthy and that the SOP of care and maintenance is being adhered to by all animal care personnel.