



Internal Regulation Title	Aquatic Guidelines				
Date Last Revised	09/17/2014	Date Created	08/22/2017	Revision #	
Regulation Number	18	Required by:	<input type="checkbox"/> OHRP	<input type="checkbox"/> Funding Agency	<input type="checkbox"/> OLAW
Applicability	<input type="checkbox"/> RGC Internal	<input checked="" type="checkbox"/> Researcher	<input type="checkbox"/> Institutional		
Subgroup	<input type="checkbox"/> NKU Compliance	<input type="checkbox"/> IRB	<input checked="" type="checkbox"/> IACUC	<input type="checkbox"/> IBC	

1.0 PURPOSE

This document outlines standard operating procedures for the care and use of aquatic animals in research.

2.0 GENERAL INFORMATION AND SCOPE

Principal Investigators who will maintain aquatic species at Northern Kentucky University for use in research, teaching or testing are required to establish written standard operating procedures (SOPs) which describe routine care and monitoring. SOPs along with records of routine monitoring must be readily available within the aquatic facility at all times (either posted or maintained in a notebook). The IACUC will assess the effectiveness of procedures during semiannual inspections.

An arrangement must be established to insure that all animals and their housing environment are assessed on a regular basis.

Routine and emergency contact information must be clearly posted at the facility entrance.

3.0 DETAILS

A. STUDY SPECIFIC STANDARD OPERATING PROCEDURES

The scope and complexity of the SOP may vary greatly depending on the species and type of housing. The list below provides suggestions for information to include. In some cases additional information may be necessary; in others only a few of the points below may need to be addressed.

- 1) Procedures for frequent observations
- 2) Procedures for cleaning aquariums (or other housing unit)
- 3) Procedures for water conditioning
- 4) Diet and general feeding plan
- 5) Record keeping procedures
- 6) Frequency of:
 - a. Cleaning
 - b. Feeding
 - c. Water quality monitoring
 - d. Water change
 - e. Room sanitation

- 7) Lighting schedule
- 8) Water temperature
- 9) Procedures for monitoring water quality
- 10) Parameters to monitor (temperature, pH, dissolved oxygen, ammonia, nitrites, nitrates, etc.)
- 11) Monitoring frequency
- 12) Procedures for room sanitation
- 13) Filter maintenance procedures
- 14) UV light change

B. AQUATIC ANIMAL HEALTH

FREQUENT OBSERVATIONS

Tanks are to be checked on a frequency approved in protocol review and consistent with the *Guide* and any dead or ill fish removed and recorded. Methods of prophylactics, diagnoses, control, and treatment of the disease and injuries follow currently accepted practices for aquatic vertebrates. Illnesses or deaths that occur at a frequency beyond anticipated levels should be reported immediately to the IACUC administrator.

ANESTHESIA, ANALGESIA, EUTHANASIA

Appropriate use of anesthetics and analgesics should be as described in the IACUC protocol. Methods for euthanasia must be consistent with the AVMA Guidelines on Euthanasia.

SICK/DISEASED FISH

In general, unless the fish are held at or near their optimum lethal temperature, the water temperature should be elevated 5°C and treated with iodized salt at a concentration of 3 g/l. Salt treatment is effective against fungi, protozoans, and other eukaryotes, as well as bacterial infections. Most freshwater fishes can tolerate concentrations as high as 10% and cichlids can tolerate concentrations as high as 60%, which is roughly twice the saline concentration as seawater. Salt treatment also has the advantage in that the salt is completely purged from the fish's body, once they are returned to freshwater. Fishes will then be treated with the recommended antibiotic. If the disease prevails, then the fish are destroyed, the tank cleaned with dishwashing liquid, and soaked in a Clorox solution for 24 hours. Any nets used to capture diseased fishes are rinsed in bleach immediately after being used.

NET DISINFECTION

Rooms also need to have a net disinfection system. Such a system should provide for one-time use of nets. A supply of sanitized or autoclaved nets are to be made available in a "clean bucket". Following each procedure, one-time-use nets are placed in a "dirty bucket" for cleaning. Nets are never to be shared among tanks or experiments.

SANITATION

Rooms need to have an area (centralized in larger facilities) where tanks can be cleaned, sanitized, and dried. Holding areas and tanks need to be kept clean. Care should be taken not to contaminate water from one tank with that from another. It is especially important to use clean nets for each application.

C. WATER SOURCE

The water source is carefully considered for all facilities. Deep wells are the best source of fresh water because they contain fewer infectious agents and/or toxic chemicals such as sewage or agricultural chemicals. Municipal tap water, in most areas, must be treated for the removal of chlorine/chloramine before use. Artificial fresh water and salt water can also be generated using DI or RO water. Water quality levels are monitored and maintained at levels appropriate for specific organisms. Each individual principal investigator will develop standard operating procedures for the organisms being held in her/his facility.

D. FEED

Feed is to be wholesome, palatable, free of contaminants, and kept in sealed containers. Cleaning agents are to be stored away from the food. Detailed information about the diet, including storage is to be provided in the SOP.

E. HOLDING DENSITIES

It is almost impossible to provide stocking rates, even with regard to a particular proposal, because of the differences in sizes of fish and sizes of aquaria. A density of more than 1.5 cm of fish per liter of water should not be exceeded, unless justification for higher densities is part of the experimental design and documented in a specific proposal. Each tank must be identified with an identification number assigned by the IACUC.

F. GENERAL FEATURES OF AQUATIC ANIMAL FACILITIES

COMPOSITION

Wall coverings, floor treatments, door thresholds, and to a lesser extent ceilings (depending upon height) are of materials impervious to water or made resistant as necessary. Doors to the holding rooms must have thresholds that prevent water escape.

PLUMBING FEATURES

Copper piping and lead-based solders should be avoided. Rooms should be provided with adequate facility-wide drainage, which requires drains in several locations. Steeply angled slopes to central floor drains must be avoided because it creates unstable footing for heavy aquarium racks.

Floor drains should be present in all facilities. Specialized drains are an important consideration. Water with infectious agents and or life stages of exotic (non-indigenous) species should not be discharged into surface waters. If not plumbed to sanitary lines (with proper chlorine disinfection) specialized systems to contain and treat contaminated waste will be constructed.

Polishing filters (particulate and/or charcoal) as well as water softeners can be provided to ensure the availability of conditioned water as necessary.

DRY AREA

A dry area is to be provided for report writing, record keeping, and other water-protected activities. This area should display procedures, emergency procedures, contact information, feeding schedules, water quality reports and any other important data.

G. TRANSPORTATION OF AQUATIC SPECIES

Fishes captured within driving distance of Northern Kentucky University should be placed in 100L coolers equipped with aeration devices. The air pumps for these systems can be powered from 120-volt outlets or from a vehicle cigarette lighter. Water temperatures need to be monitored and adjusted by adding ice or heat.

4.0 REFERENCES

Regulations Governing the Acquisition of Aquatic Species:

Lacey Act, Endangered Species Act (endangered and threatened wildlife and plants), and Convention of International Trade in Endangered Species of Wild Flora and Fauna (CITES).

Additionally, proper permits must be obtained and made available to USFWS personnel to collect these organisms. In certain cases (i.e. rare and or endangered species), permits are needed to hold these organisms, even if not collected by the Principal Investigator or her staff. With regard to specimens obtained outside the U.S., all import and export shipments must be declared. Obtaining clearance from an USFWS agent and filing a Form 3-177 is required at the time of entry or exit into or from the U. S. Within the U. S., the transportation of fishes across state lines without the proper permission and permits from the respective states and/or USFWS is in direct violation of the Lacey Act.

5.0 FORMS OR ATTACHMENTS

6.0 DEFINITIONS

Approvals

Title	Approved	Date Approved	Not Applicable
Manager of Research Compliance	<input checked="" type="checkbox"/>	06/13/2018	<input type="checkbox"/>
IACUC Chair	<input checked="" type="checkbox"/>	06/13/2018	<input type="checkbox"/>
Institutional Official	<input type="checkbox"/>		<input checked="" type="checkbox"/>

Revisions

Title	Approved	Date Approved	N/A	Summary
Manager of Research Compliance	<input type="checkbox"/>		<input type="checkbox"/>	
IACUC Chair	<input type="checkbox"/>		<input type="checkbox"/>	
Institutional Official	<input type="checkbox"/>		<input type="checkbox"/>	