PURPOSE:
To provide the highest quality of care for fish housed in Texas Tech animal facilities in order to prevent the development of disease or disorders that could compromise research studies.

I. ROOM MAINTENANCE

A. Daily

1. The Principle Investigators, Graduate Students, Research Assistant and/or Animal Care Staff will observe all tanks/aquaria.
2. Check the room for operational deficiencies. Report any problems to the PI, Unit Manager or Building Work Order Coordinator.
3. Observed all fish for any evidence of illness or a change in behavior and the “DAILY OBSERVATION FORM” is completed. Report sick fish and treatment on the “TREATMENT/OBSERVATION FORM”.
4. Remove any dead fish and document in project notebook.
5. Check temperature in tanks/aquaria.
6. Feed accordingly.
7. Mop up any water on the floors.
8. When the Principle Investigators, Graduate Students, Research Assistant, Animal Care Personnel and/or Animal Care Staff require veterinary assistance they should follow the directions under Health Care.

B. Weekly

1. Check water parameters.
2. Nitrites: should always remain at 0 ppm, reduce if elevated.
3. Ammonia: should always remain at 0 ppm, reduce if elevated.
4. pH: should be in the range of 6 - 9 pH units and adjusted according to optimal conditions for the species being held.
5. Dissolved Oxygen should be >4.5 mg/L.

C. New tanks

1. Check water parameters every other day until stabilized (4-6 weeks) then weekly.
2. Use “seasoned” biological filters.
3. Life support systems must be set up and acclimated prior to receiving new fish.
   Life support systems typically fall into three categories: closed recirculating, flow-
through or static. The water may be fresh, brackish or salt and is maintained at specific temperatures depending on the species’ needs.

Closed Recirculating
a. The biological filtration system for a closed recirculating system should be “primed” by the addition of either a approved hardy disease-free fish or ammonium chloride (5 mg/L/day). Since the detoxifying capacity of a biological filter adapts to its nutrient supply, the capacity of a seeded or “seasoned” biofilter should be considered prior to the introduction of the entire fish population.
b. Water parameters (e.g. ammonia and nitrite levels) of the system should initially be monitored daily to every other day until they stabilize to safe levels.
c. Considering the naturally high pH levels of water available at Texas Tech University and the tendency of ammonia to exist in its more toxic form in alkaline waters, measurement of the nitrogenous waste products is critical in newly established aquatic systems.
d. The testing interval can be lengthened to weekly checks for pH, ammonia, nitrites and salinity (for marine systems) once these parameters measure within the safe range.

Flow-through
a. Water is constantly replaced and may use large volumes of water.

Static
a. Water is stationary and periodically replaced and may use mechanical devices to move and aerate water.

D. Water supply

1. If a municipal water supply is used the water must be dechlorinated. All water should be analyzed prior to use.
2. Use a Daily Observation Form and the Required Water Analysis chart to record data. Check water quality prior to use and adjust accordingly.

E. New Aquariums

1. Initial record of various water quality parameters should be made regarding the natural quality of the water source prior to its use in an artificial aquatic system to document both the high alkalinity and pH values. The following water quality parameters should be made on new aquariums:
   a. pH
   b. Alkalinity
c. Hardness  
d. Chloramines  
e. Dissolved oxygen

**F. Personnel Safety**

1. Rubber boots or rubber-soled shoes should be worn by caretakers to allow better traction and reduce the risk of electric shock.  
2. Use exam gloves when handling fish and wash hands with anti-microbial hand soap (unless chemicals within antimicrobial soaps may interfere with ongoing or future research in the laboratory).  
3. Periodically check all electrical cords, fixtures, and outlets.

**G. Aquatic Health Monitoring**

1. The Principle Investigators, Graduate Students and/or Animal Laboratory Technician daily observe aquaria and tanks.  
2. All aquaria and tanks containing fish are observed for any evidence illness or a change in behavior and the “DAILY OBSERVATION FORM” is completed.  
3. All animal health comments must be recorded on an individual “TREATMENT/OBSERVATION FORM”.

**H. Health Care**

It is everyone’s responsibility to inform the University Veterinarian when an animal becomes ill or a change in behavior is noted. Seriously ill animals should be reported IMMEDIATELY to the veterinarian. When an investigator, technician or animal care personnel requires veterinary assistance they should:

1. Complete the “TREATMENT/OBSERVATION FORM” in the Notebook. Indicate date, room number, animal number/cage ID, problem observed and name or initials of the person making the report.  
2. Contact the University Veterinarian or ACS Manager:

   Dr. Tiffanie Brooks, University Veterinarian, Animal Care Services.  
   806-834-8588 Office  
   806-239-2120 Cell Phone

   Carla Grisham, Manager, Animal Care Services.  
   806-834-3437 Office  
   254-913-5156 Cell Phone
3. Provide all the above information to the individual contacted above, who will give advice and authorization for the action(s) that should be taken.

II. AQUATIC HEALTH RECOMMENDATIONS

A. Water Quality Maintenance

DO levels need to be checked both early in the a.m. and late p.m. for outdoor systems, especially during periods of elevated water temperatures.

B. Disease Transmission Prevention

1. All fish populations intended for research purposes are initially quarantined and the initial health status determined prior to starting the research project.
2. Health checks would need to be conducted upon arrival and 1 week later to alert a researcher to any potential health problems.
3. Monitoring and recording water quality parameters and fish feeding/behavioral patterns at regular intervals would then need to be conducted for the duration of a research project.
4. Suspect fish can be submitted to the Texas Veterinary Medical Diagnostic Lab in College Station, TX or Amarillo, TX for diagnostic evaluation or evaluated on-site using diagnostic techniques.
5. Dip nets should be assigned to a specific aquarium or disinfected prior to use between different aquaria. A “dip net” bucket can be used for this purpose (e.g. 10 ml household bleach to 1 L water as a 1 hour dip). Bleach must be mixed fresh daily or as needed. Nets then need to be rinsed thoroughly after this treatment to remove chlorine residues prior to netting fish. Since chlorine is highly toxic to fish, sodium thiosulfate (or other dechlorinator) should be on-hand for emergency dechlorination purposes.
6. Transport stress can result in the occurrence of secondary infections in fish by aquatic opportunistic pathogens. Quarantine and acclimation of new fish populations for 2-4 weeks prior to initiation of a study is recommended to prevent the interference such an event would cause during a research project. A physical examination (e.g. gill biopsy, etc.) of a sample set from a new population may also be conducted to give an indication of the initial health status of a fish population upon its arrival to the facility.
7. The presence of multiple aquaria in large open recirculating systems increases the likelihood of disease transmission by potential pathogens. Disinfection of water exiting the biofiltration system by ozonation or UV-radiation should be considered as a deterrent against disease transfer between aquaria in these systems.
C. Handling and Restraint

1. The use of electro anesthesia in field studies is considered a viable method of capture and restraint for short minor procedures by some authorities that allow both rapid induction and recovery times, while avoiding residue problems. However, it does present safety hazards to both the operator/handler, as well as the fish. Spinal injuries and muscular hemorrhages have been reported in fish. The type of current and the orientation of fish in respect to the electrodes appear to be critical to the immobilizing effect of this technique (Ross & Ross, 1984; Anesthetics and sedative techniques for fish). Use of low frequency (> 30 Hz) direct currents has been reported to help reduce the incidence of these injuries. Water conductivity should measure 100-500 uS/cm 3-6 amps to effectively transmit electric current to shock fish (Reynolds, J. 1983. Electro Fishing. Pages 147-163 in L.A. Nielsen and D.L. Johnson, eds. Fisheries Techniques. American Fisheries Society, Bethesda, MD) considering its long history of use as a fish collection technique, electrofishing can be conducted with minimal risk to crewmembers providing proper safety procedures are followed.

2. Gill netting is another collection technique used in the field to collect fish that involves the use of a static netting system that can kill or injure fish that are entrapped in the netting for long periods of time. Periodic checking of positioned nets is therefore recommended.

3. The uses of salt at (500-1000 ppm) or anesthetics at low sedative doses (e.g. MS-222: 15-66 ppm) are additional considerations reportedly useful for stress reduction in transported fish (Jensen, G.: Louisiana Cooperative Extension Service; SRAC Publication 392). Adequate aeration and controlled cooled water temperatures are additional important factors that can be easily addressed with the use of aerators and “Kool-paks”.

4. Variation in euthanasia dosages for Tricaine Methanesulphonate (MS-222) was cited in different proposals and may reflect species variation noted in different references. The standard dosage reported by DeTolla in Guidelines for the Care and Use of Fish in Research is 500 mg/L. Severing the spinal cord just posterior to the level of the opercula to ensure non-recovery can follow chemically induced euthanasia.

5. The anesthetic dosage of this compound is reported to be between 100-200 mg/L. The anesthetic effect of MS-222 is affected by both the dosage and duration of exposure. Since it acts as a respiratory depressant prior to causing cardiac arrest, the opercular movements of fish can be used to determine the plane of anesthesia. Fish should be removed from the anesthetic-treated water as opercular movements slow and response to stimuli ceases. If opercular movements cease, the fish should be placed in untreated water and forcibly moved open-mouthed through the waters until respiration recovers.
6. All methods of immobilization should probably be tested on the study species (if possible) prior to their actual utilization in a study due to species variation that is encountered in response to various techniques.

D. Personnel Safety Measures

1. Use of rubber boots or rubber-soled shoes by caretakers in the aquatic labs would allow better traction on wet floors, reduce the potential for electrical shock and prevent transmission of pathogens with the ancillary use of disinfectant foot baths.

2. Use of exam gloves in handling fish and antimicrobial soaps for hand cleaning would decrease the likelihood for transmission of pathogens with zoonotic potential.

3. Electrofishing techniques utilized for electro-anesthesia of fish in the field. Adequate safety procedures and precautions should be explained to students by the trainer/instructor. Specialized training of the research biologist in handling electrofishing gear and CPR is recommended, as well as, development of an emergency plan to the nearest medical facilities prior to any electrofishing expedition due to the chance of electrical shock.

4. Only commercially available electrofishing gear should be used that has recently been inspected.

5. Dip nets need to be made of non-conducting material and all crewmembers should wear rubber gloves and boots (that have been inspected for leaks). Please see fish sampling considerations presented by the Southern Division of the American Fisheries Society (in an abstract from its meeting in Lexington, KY regarding evaluation of fisheries techniques; www.sdafs.org/) for more information on commonly used fish collection techniques.