I. **Purpose:**

The purpose of this policy is to outline the WSU minimum standards of care for fish.

II. **Policy:**

All departments providing animal care for fish must meet or exceed these minimum requirements which are based on the Public Health Service Policy and the ILAR *Guide for the Care and Use of Laboratory Animals*.

The selection of appropriate fish housing systems requires professional knowledge and judgment and depends on the nature of species and age of fish used, and the design of the experiments.

Sanitation frequency, system maintenance and water quality monitoring will vary based on the species, aquatic system and research needs. Unique species or research requirements may be further defined in specific IACUC approved protocols or Standard Operating Procedures.

III. **Procedure:**

**Daily activities: (365 days a year without exception)**

Observe each tank and check fish for health issues.

- Observe all animals for signs of illness or distress. Signs to look for include abnormal swimming and/or feeding behavior, discoloration of the water or fish.
- Record feeding: the feeding interval should be based on fish species, life stage, and specific feeding behavior. Many species require daily or multiple daily feedings.
- Check that each tank is identified, clean, free of damage and that the water level is sufficient.
- Document all transfers, deaths and euthanasia of fish.
- Contact the Office of the Campus Veterinarian (OCV) to report sick fish and mortalities or log entries in the OCV health database. Some mortality is anticipated in large colonies and with developing fry. If within expected ranges, mortality data can be provided to OCV in a monthly summary. Unanticipated and higher than normal rates of mortality must be promptly reported to OCV.
- Check and record water temperature which should meet the species-specific requirements (cold water fishes 5-15°C, temperate water fishes 12-26°C, and warm water fish 18-35°C).
- Document parameters listed above in addition to room activities on room log sheet (feeding, health check, temperature of water or room depending on aquatic system).
Sanitation (weekly, monthly, quarterly, as appropriate):

Standing/Static water tanks:
Siphon solid wastes from tanks as needed. Clean tanks to remove mild algae accumulation on an “as needed” schedule so that algal growth does not interfere with daily observation of animals. Replace a percentage of system water volume as appropriate with conditioned water as determined by nitrate levels, total ammonia nitrogen, and/or pH. Document procedures on room log sheet.

Recirculating systems with central filtration and flow-through water tanks:
Same as above but also back-flush, clean and/or replace filters associated with mechanical filtration systems as needed and monitor biological filtration system media levels.

Sanitation monitoring: fish and the supportive biological filter necessary for denitrification are extremely sensitive so disinfection of aquatic system components should be appropriate for the research and level of risk. At minimum, projects involving multiple animal sources and infectious disease need to participate in the sanitation monitoring program. Please see the Guide, pg. 86 and the WSU sanitation monitoring SOP at https://iacuc.wsu.edu/documents/2016/06/sop_5.pdf/ for more detail.

Waste Water:
All aquarium/holding tank water from transgenic aquatic animals must be treated prior to disposal into a municipal wastewater treatment system, per recommendation by the NIH Guidelines and as approved by the WSU Institutional Biosafety Committee.

Housing Rooms:
All animal rooms should be regularly cleaned and disinfected.

Water Quality monitoring:
In addition to daily temperature checks, check and record water quality parameters based on the species, aquatic system, fish density, and with a new or modified system. If water quality values are out of the normal range, action is required to correct the issue.

- The below parameters should be tested at least weekly in static systems & at least monthly in established recirculating or flow-through systems. (More frequent testing is required with new or newly modified systems or):
  - pH
  - Alkalinity
  - Ammonia (NH₃)
  - Nitrite (NO₂⁻N)
  - Nitrate (NO₃⁻N)
Feeding:
Fish should be fed palatable, non-contaminated, and nutritionally adequate food daily or according to their requirements, unless the protocol under which they are being used requires otherwise. Feed should be stored in properly labeled vermin-controlled containers. It should be discarded either 6 months after being received or opened or at the manufacturer’s expiration or best-by date. If bulk feed is stored frozen to extend the shelf-life, the manufacturer’s verification of the extended shelf life should be kept on file and provided on request. The feeding frequency can range from continuous (i.e. automatic feeders which should be checked regularly to ensure proper functioning) to 2-3 times per week. This depends on the nutritional quality and quantity of the food fed. Feeding interval should be based on fish species, life stage, and specific feeding behavior. All feedings should be recorded in room logs.

Identification and animal counting:
Each tank should be individually identified. The number of animals acquired through breeding, capture, purchase or other means have to be entered and tracked on the IACUC database. Please refer to the WSU IACUC Guideline for Counting Animals at https://iacuc.wsu.edu/documents/2016/06/guidelines_counting_animals.pdf/

Environmental Enrichment:
Enrichment should elicit species appropriate behaviors (schooling, substrates for reproduction, etc.) and should be evaluated for safety and utility. Refer to the Environmental Enrichment policy at https://iacuc.wsu.edu/documents/2016/06/policy_30.pdf/.

Physical plant:
For interior facilities, floors should be moisture-resistant, nonabsorbent, impact-resistant, and relatively smooth. Walls should be moisture resistant and have GFCI electrical outlets that are properly positioned to eliminate possible safety hazard. Any non-GFCI circuits necessary for essential equipment must be elevated out of the “splash” zone so either high on the wall or on the ceiling. All electrical equipment located in aquatics areas that are powered under a non-GFCI protected outlets are to be in good working order, have waterproof connections, and should be inspected regularly for damage. Outlets should be water resistant or fitted with waterproof covers. Pipes used for transporting water into and around the system must not be galvanized or copper, due to heavy metal leaching that can occur. Naturalistic environments such as “ponds” and outside housing areas should protect against predation.

Other testing recommended at least monthly
- Total Suspended Solids (turbidity)
- Dissolved Oxygen
- Conductivity
- Hardness (CaCO₃)
Temperature, Humidity and Illumination:
Heating and air in fish rooms should be controlled in a manner that supports species-specific needs. Depending on the system, room or water temperature should be recorded on a room log sheet. Humidity does not directly impact aquatic animals but high levels of humidity in fish rooms can be detrimental to electronic equipment and can promote microbial growth. Illumination levels, photoperiod and wavelength should be appropriate to the species and sufficient to allow visualization of the animals for health and well-being. Gradual changes in light intensity are recommended. Installation of emergency lights for when the light cycle is off is recommended. For light-sensitive species, use a red light.

Tanks and Density:
Fish should be housed in primary enclosures that meet their general needs (i.e. proper size tank for species-specific requirements and for maintaining appropriate densities for group housed fish). The needs of each situation must be evaluated by the IACUC in consultation with the Principle Investigator or possible outside experts. Primary enclosure requirements will be based on species needs, behavior, and goals of the research, but must be designed to restrict escape or accidental entrapment. Space recommendations and housing density varies with species, age, life support and research but must allow for normal movement and postural adjustments. There are published density recommendations for multiple common species.

References:

2. Canadian Council on Animal Care Guidelines on: The Care and Use of Fish in Research, Teaching and Testing; Canadian Council on Animal Care, 2005
3. Reed, B., & Jennings M., Guidance on the housing and care of Zebrafish. 2011