

Standard Operating Procedures for Fishes

- A. Classification of fishes
 - 1. Fishes are classified by use:
 - a. Research
 - b. Teaching
- B. Identification

All procedures to ensure appropriate identification of fishes will be responsibility of the Faculty member in charge of the fishes, or by those designated by the Faculty member and certified by FIU's Animal Care Program.

- 1. Each aquarium/tank or set of aquaria that house the same species of fish will display an appropriate identification card.
 - 2. Soiled aquarium identification cards should be replaced as required.
- C. Housing

All procedures to ensure appropriate housing of fishes will be the responsibility of the Faculty member in charge of the fishes, or by those designated by the Faculty member and certified by FIU's Animal Care Program. Because of the diversity of the fishes and their natural habitats, no general guidelines can be provided for housing fishes.

- D. Feeding

Because of the diversity of fishes and their natural habitats, no general guidelines can be provided for the feeding and watering of fishes. Specific schedules for feeding should be kept and made available to veterinary or IACUC inspectors for each species to be housed.

- E. Environmental

Because of the diversity of fishes and their natural habitats, no general guidelines can be provided for fish environments. For each type of fish to be housed, temperature, lighting and water conditions should be specified.

Water Conditions

- A. Each aquarium/tank should include adequate aeration and filtration for the fishes being housed.
- B. Water quality should be measured (nitrates, nitrites, pH, salinity). This can vary but for high-density tanks two times per week is recommended. If only a few small fish are housed in a big tank or microcosm, at least once every six months. Researches must use their best judgments to determine where their tanks fall within these extremes.

Transporting Fish

The most important single factor in transporting fish is the provision of adequate concentrations of dissolved oxygen. The importance of supplying adequate levels of

dissolved oxygen cannot be overemphasized. Failure to do so results in severe stress and possibly hypoxia or buildups of blood lactic acid that may contribute to fish kills two to three days after stocking. The amount of oxygen that can be dissolved in water is based on water temperature. When the upper level is reached the water is referred to as being "saturated with oxygen". Dissolved oxygen saturation is higher for cool water than for warm water.

Sometimes a chemical anesthetic may be beneficial by producing a light sedation. The only Food and Drug Administration (FDA) approved anesthetic for food fish is Finquel (tricaine methanesulfonate). Finquel may be used at a rate of 0.1-0.5g/gal of water.

Probably avoiding stress can prevent 80-90% of disease in captive fish. Stress weakens fishes' immune systems, leading to increased susceptibility to disease. Diseases and pathogens are almost always present in tanks, but a healthy fish's immune system will prevent them from being a problem.

Common Stressors for Captive Fish

- Poor water quality: measurable ammonia or nitrates, or very high nitrates.
- The water temperature is fluctuating more than 2 degrees F per day excepting shallow water species that are tolerant of radical temperature swings or where research interests are involved as approved by IACUC.
- Incompatible species in the tank.
- Too many fish in the tank.
- The tank is too small for the fish.
- The water is too warm or too cold for the species
- Wrong pH for species.
- PH fluctuations greater than 0.2 units per day.
- Insufficient cover or hiding places present.
- Wrong water hardness for the species.
- Insufficient oxygen in the water.

Anesthesia

Finquel (tricaine methanesulfonate) AKA: MS-222

Recommended Dosage: 0.01% or 125mg/L in bath. Note: the needed concentration for a given level of anesthesia can vary by an order of magnitude between species. Experiment for your species, starting at lower doses.

There is wide latitude in the permissible dosage and recommended amounts. Fish are placed in a water/MS-222 solution until the desired level of narcosis/anesthesia is attained. The sustaining dose for prolonged anesthesia should be much more dilute. Watch out for cessation of respiration (such fish may be respired through the mouth with a siphon or peristaltic pump)

Solutions of MS-222 gradually lose their activity but a 10% solution will remain fully active if stored in a brown bottle for up to 3 days. Wear gloves when using MS-222 as it is reportedly carcinogenic.

Euthanasia

Concentrated solutions of MS-222.

Several methods of euthanasia have been historically used with fishes, including exposure to cold ice water for an extended period of time (~5 minutes). However, the most common means of euthanasia involves use of a commercial anesthetic called MS-222 (3-Aminobenzoic Acid Ethyl Ester Methanesulfonate salt, also called ethyl m-aminobenzoate; $C_9H_{11}NO_2-CH_4SO_3$; Sigma Cat # A-5040 or equivalent). A 0.4% stock solution is typically made in tris-buffered water as follows:

MS-222 stock solution (0.4%, 100 ml)

400 mg tricaine

97.9 ml autoclaved/distilled water

2.1 ml 1 M Tris-Cl (pH 9)

Adjust pH to 7.0. Store this solution in a dark container. Some individuals freeze this stock solution, while others keep it at room temperature. Regardless of storage parameters, tricaine can get old and lose potency.

MS-222 working solution (0.02%, 100 ml)

To use tricaine as an anesthetic combine the following in a Lee specimen container:

5 ml MS-222 stock solution

95 ml clean tank water (preferably from the same tank that the fish came from)

Euthansia of fish

Fish can be euthanized by over-anesthetizing a fish in the working solution (100 ml of 0.02% anesthetic (MS-222; Note: if numerous fish need to be euthanized, one can utilize a 0.06% solution). A fish is typically placed into a dedicated anesthetic container. The swimming movements of the fish will slow down gradually (approximately 30 seconds, depending on the size of the animal). It will lose its ability to stay upright in the water and keel over on its side or totally lie on the bottom upside down. The mouth and gill arches will still be moving, albeit very slowly. It will take ~10 minutes for the fish to become fully over-anesthetized, at which point it should be removed and disposed of properly. Proper notes regarding age, phenotypic information etc. must be recorded at this time.