

Standard Operating Procedures

#AU 000027 Goldfish Holding

Note: While this SOP specifically discusses goldfish, the principles of holding can be applied to virtually any other species of fish.

Note: This SOP deals specifically with holding fish in static tanks with filter units.

Tank setup

- 1) Tanks for fish holding need to be up and running 1-2 weeks prior to bringing in fish. This allows the biological filter sufficient time to set up
- 2) The minimum volume per fish is 1 L; however, if the fish are of considerable size (<20 g), a greater volume of water per fish is required
 - a. Often it is not clear how much water per fish is required, the only way to be certain is to closely monitor water quality once animals are being housed, if it is clear that the filter is unable to deal with the amount of fish present, an additional tank will need to be set up and animals transferred to the new tank
- 3) Once a tank has been selected based on size, fill it with water
 - a. If a dechlorinated water source is available, use this to fill the tank
 - b. If a dechlorinated water source is not available, either use aged water or fill the tank with tap water and age the water for a minimum of 48 hours
 - i. A dechlorinating chemical may also be added to speed up aging

NOTE: dechlorinated water made using carbon filtration is superior to aged or treated water as carbon filtration will remove metals and other potential contaminants in addition to chlorine, making dechlorinated water preferable to other water sources

- 4) After filling the tank with water, add substrate to the bottom of the tank. This can take the form of clean marbles, gravel, and/or spawning tiles
 - a. Marbles and gravel are useful to provide substrate for the biological filter, spawning tiles are a form of enrichment for goldfish
- 5) Affix a filter unit to the tank appropriate to the volume of water being filtered. The filter unit should consist of a mechanical filter to remove particles, a chemical filter (i.e., carbon) to remove harmful chemicals, and a biological filter
 - a. Prior to starting the filter add an appropriate amount of Cycle or other such treatment designed to form a biological filter directly into the filter over top of the biological filter substrate
 - b. Run the filter for 1-2 days prior to adding fish, this will allow the biological filter time to set up
 - c. If possible, holding a few fish of the same species in the tank prior to adding new fish will aid in setting up the biological filter and conditioning of the tank
- 6) **Aeration:** Aerators should be used to ensure sufficient oxygenation of the water. Be sure to rinse air stones and airline tubing prior to placement into the tank
- 7) **Temperature:** A heater or a chiller may be added to keep the tank at an appropriate temperature if needed. The optimal range for temperature for goldfish is 20°C ± 2°C. Higher temperatures increase the probability of growth of pathogens, lower temperatures push goldfish out of their preferred thermal zone

- 8) **Light cycle:** Fish may be held on a 16 hours light:8 hours dark, a 12 hours light:12 hours dark, or a natural photoperiod. If it is not possible to hold fish in a room with photoperiod controls, the fish should be kept in a room with a window and kept close to the window to allow for exposure to a natural light cycle
- 1) Affix a waterproof tag to the tank containing the following information:
 - a. Tank number
 - b. AUP number
 - c. Initial number of fish in tank
 - d. Arrival date
 - e. Emergency contact information for PI and other contact people

Acclimation and Evaluation of Condition

- 1) Animals **MUST** be procured from reputable sources. Purchasing fish from a commercial pet store is not permissible as quite often the holding conditions are subpar
 - a. Prior to placing animals into the holding tank, inspect the animals in the transport bag for signs of injury and disease. If parasites or potential diseases are noted, a salt bath is recommended to remove any parasites and to aid in heal of any external infections
 - i. A salt bath consists of placing fish in 20 g/L of NaCl in tank water for 5 minutes
NOTE: Do **NOT** use iodized salt, use NaCl from a chemical supplier or sea salt.
 - b. If you perform a salt bath, fish will need to be immediately placed into the tank, meaning the acclimation steps in 2) a) and b) are omitted
- 2) Goldfish are usually transported in a bag containing water that has had pure oxygen added to assist with transport
 - a. Upon receipt place the bag into the tank and allow the temperature to equilibrate for 1-2 hours
 - b. Add a volume of water (not greater than 20% of the volume of the bag) from the tank to the bag and allow the fish to acclimate for 1-2 hours
 - c. Slowly allow the fish into the tank, minimizing the amount of waste being added to the tank from the transport bag
- 3) All fish need to be acclimated to holding conditions for a minimum of 1 day before being fed and 2 weeks prior to being used for any experiments. During this time, fish should be carefully inspected for a variety of conditions and behaviours, including:
 - a. Fin damage, this may be a sign of infection
 - b. Discolouration or necrotic tissue, which may be a sign of infection
 - c. External parasites such as ich, which can appear as small white dots
 - d. Malformation, emaciation, or bloating, which may indicate nutritional deficiencies
 - e. An inability to maintain balance in the water column
 - f. Flashing or rubbing themselves against course objects (both are signs of parasitic and other infections)

Any fish displaying any of the conditions above should be removed from the population and either treated (if possible) or euthanized via overdose of MS-222.

- 4) During the acclimation period water chemistry (nitrates, nitrites, ammonia, and oxygen) should be monitored daily, with water changes (up to 40%) occurring as required. This information shall be recorded on the animal husbandry forms detailed below

General Housing

- 1) A binder containing animal husbandry forms shall be used to maintain records of all animals being held. The forms should contain the following information:
 - a. Tank number, AUP number, initial number of fish, arrival date, and emergency contact information for the PI and other responsible staff

- b. The following columns should be included in the sheets as well:
 - i. Date
 - ii. Nitrate
 - iii. Nitrite
 - iv. Ammonia
 - v. Mortalities
 - vi. Notes
 - vii. Signature

- 2) All fish should be fed and inspected twice a day, excluding weekends where once a day is sufficient. The PI should *IMMEDIATELY* be informed if there is a spike in mortalities as this may require increasing holding or euthanizing sick or injured fish.

- 3) Fish are fed twice a day, once with commercial flake food, once with frozen brine shrimp (artemia)
 - a. Feeding is 5-minute *ad libitum*, meaning as much food as the fish will consume in a five-minute period. Overfeeding will result in fish overeating and/or pathogens thriving in the tank
 - b. After feeding any additional food is removed from the tank via a net, and waste is removed via a suction hose

- 4) It is important to maintain proper water chemistry in the tank. After the 2-week acclimation period where water is tested daily for nitrate, nitrite, and ammonia, water chemistry may be measured twice a week provided the concentrations of the various compounds is stable. If a spike in one of the parameters is noted, a 20-40% water change should be performed in order to stabilize the chemistry. Additional cycle should also be added to reinoculate the tank with a biological filter.