



- mesh covers or glass covers if the species is likely to jump out of the tank.
- 3.3.2.2 together with individuals from the same population, unless the species is a solitary species or is in a solitary phase of their life (e.g. many species are solitary during the breeding season but not solitary during the rest of the year). Individuals of the same species from different populations should not be housed together.
  - 3.3.3 Substrate such as gravel, yarn refuge, and/or artificial plants should be provided for those species that typically utilize such substrates in their natural habitat.
  - 3.3.4 Each tank should contain a filter of appropriate size for the size of the tank. Filter should be a three stage filter (mechanical, chemical, and biological) wherever possible.
    - 3.3.4.1 Use of a pre-filter on the filter intake is permitted and encouraged when used in tanks housing small/young fish that might otherwise be killed by the filter. Use of a pre-filter should be noted in the IACUC protocol.
  - 3.3.5 Tap water is
    - 3.3.5.1 dechlorinated prior to treatment with dechlorinating chemicals (liquid, tablets, etc.) that are designed for aquarium fish. Water should be dechlorinated following manufacturer instructions.
    - 3.3.5.2 made into saltwater through the use of a commercially bought salt designed for aquarium fish, if saltwater fish are being housed. Manufacturer instructions should be followed to achieve the desired salt concentration.
- 3.4 Animal housing identification (tank identification)
- 3.4.1 All tanks must be labeled with the experimental condition being tested (e.g. for an experiment testing different salinities, each tank must identify the salinity it is at). If no experimental condition is being tested, the label should indicate that it is a control or is a stock tank. If more than one species and/or population of a species is in the same room, the tank label must ALSO identify the species or population.
  - 3.4.2 Animals found housed in a tank without a label may be euthanized, by order of PI.
- 3.5 Food
- 3.5.1 Commercial fish flakes is appropriate for commercially-bought fish. For fish obtained from the wild, flakes should be supplemented with live or dead Brine shrimp, *Daphnia*, and/or Bloodworms until fish fully take to the commercial fish flakes.
- 3.6 Room Environment
- 3.6.1 A 12h:12h light:dark (12:12 L:D) cycle, with lights on at 0700, will be the default light cycle for rooms 101G and 101H, unless a variation is called for in an approved protocol.

- 3.6.1.1 Only a PI with designated use of animals being housed in the room shall be authorized to change the set light cycle.
- 3.6.1.2 Caretakers and researchers may be authorized to enter the room during the dark phase. As little light as possible should be used.
- 3.6.1.3 Adjustments for transitions between daylight savings and standard time will be authorized and implemented only by the PI using the room.
  - 3.6.1.3.1 PI's may opt not to change the settings over these transitions, meaning that lights on/off will shift between 0700/1900 (EST) and 0600/1800 (EDT).
  - 3.6.1.3.2 PI's are advised to consider these seasonal time changes when scheduling experiments during the fall or spring that require entry into the holding room close to lights on or lights off.
- 3.6.2 Routine ambient temperature will be maintained at 18-21°C (65-70°F).
  - 3.6.2.1 Variations in ambient temperature for these rooms can be requested through Capital Planning.

## 4.0 Procedure

### 4.1 Acclimating new fish

- 4.1.1 Tanks that will eventually permanently house the fish should be set up in advance of putting fish in. Tanks should ideally be set up and circulating new (dechlorinated) water a minimum of 1-2 days in advance of placing fish into the tanks.
- 4.1.2 Regardless of whether fish are obtained from a commercial vendor or directly from the wild, fish should be acclimated to the room slowly so as to prevent excess stress and distress
  - 4.1.2.1 For commercially bought fish, it is advisable to consult with the vendor about the best way to acclimate fish. Typically these fish acclimate sooner (and easier) than wild caught fish, but this is not always the case. General guidelines for acclimating commercially bought fish are:
    - 4.1.2.1.1 Allow fish to float inside bags/containers of their own water inside the tanks that will permanently house them for at least 30 minutes. It may be necessary to provide aeration to the bag/container, depending on the size/amount of the fish and the amount of water in the bag/container.
    - 4.1.2.1.2 For the first 5-10 minutes of this 30+ minute period, do not add any new water.
    - 4.1.2.1.3 After the first 5-10 minutes, slowly add new water to the bag/container. It is advisable to start by adding very small amounts of new water and

- gradually increase the amount of new water added over the remaining 20-25+ minute period. Periodically inspect fish for any signs of extreme stress or distress and adjust the rate of adding water if necessary.
- 4.1.2.1.4 After the 30+ minutes, if fish are not looking overly stressed or distressed, gently release them into the tank that will permanently house them. If fish seem overly stressed or distressed, allow them to float for another 30+ minutes and then re-evaluate (and then releasing them or repeat floating for longer).
  - 4.1.2.1.5 It is advisable to not feed fish immediately after transfer, but a small amount of food should be given the same day they are released into the new tank.
- 4.1.2.2 For wild caught fish
- 4.1.2.2.1 Fish should be kept in containers of the water they were collected in and allowed to adjust to room temperature at least 12-24 hours before any new water is added. Air should be delivered to the fish during this adjustment period with air pumps and air stones or other similar equipment. Lids should be kept on the containers as much as possible to reduce stress and prevent fish from escaping. Fish should also not be fed.
  - 4.1.2.2.2 After this initial 12-24 hour adjustment period, new (dechlorinated) water should slowly be introduced into the containers over the course of a minimum of 1-2 days. It is recommended that new water be added in small amounts at first and then gradually increased in amount. It is appropriate to remove small amounts of the water the fish were collected in and place it into the tanks that will eventually house the fish. Once the container contains approximately 60% or more of new water, fish can transferred to the tanks that will permanently house them.
- 4.1.2.3 Food can be given to fish after the initial 12-24 hour adjustment period, although it is recommended to give small amounts at first to prevent a build up of uneaten food or waste products that can degrade water quality. It is also recommended that no food be given to fish immediately after transferring them to tanks that will permanently house them.
- 4.1.3 Throughout the entire acclimation process, fish should be occasionally checked to ensure that no fish are dead or that signs of extreme stress requiring euthanasia are not required. Dead fish should be removed

immediately. Fish requiring euthanasia should follow euthanasia procedures.

#### 4.2 Feeding

- 4.2.1 Flake food and any live/dead food (see 3.4.1) should be mixed together in a separate container containing either distilled water or treated (dechlorinated) tap water. If frozen food is used, it should be fully thawed out in the container before being given to the fish.
- 4.2.2 Using a transfer pipette or other instrument, an appropriate amount of the food should be administered to each tank. What is appropriate should be determined by the PI based on the number of fish in the tank, size of fish in the tank, etc.
- 4.2.3 Fish should be fed once per day at least 5 days per week. Additional feedings per day or per week are permitted.
- 4.2.4 Feeding should be noted in the fish room log sheet (SOP 152).

#### 4.3 Daily care

- 4.3.1 Check on all tanks with animals, noting:
  - 4.3.1.1 the health of each animal,
  - 4.3.1.2 whether there are dead animals
- 4.3.2 Complete the appropriate row of the fish room log sheet (see SOP 152) by logging:
  - 4.3.2.1 health status
  - 4.3.2.2 room temperature (current)
- 4.3.3 Remove dead animals and transfer to freezer (see section ??).
  - 4.3.3.1 Be sure to note this in the Comments section of the fish room log sheet.
- 4.3.4 Room maintenance:
  - 4.3.4.1 Sweep floor as needed.
  - 4.3.4.2 Check vermin traps.

#### 4.4 Weekly care

- 4.4.1 Water quality check (and adjustment, if necessary). The use of commercially bought test kits and adjustment supplies is acceptable.
  - 4.4.1.1 pH and salinity (if applicable) matches home environment (if bought commercially, matches what was used by vendor)
  - 4.4.1.2 alkalinity of 7-11 dKH
  - 4.4.1.3 ammonia of <0.2 ppm
  - 4.4.1.4 nitrite/nitrate of <0.2 ppm

#### 4.5 Care as needed

- 4.5.1 Perform ~1/3 water change by removing water from tank and adding fresh, treated (dechlorinated) water
- 4.5.2 Clean filter and filter inserts. Replace if not performing adequately (due to age, becoming clogged, etc.).

#### 4.6 Biannual care (when holding room is not being used to house animals)

- 4.6.1 Wipe down all walls and surfaces with warm water.
- 4.6.2 Wipe down tank rack with warm water.

4.6.3 Disinfectants and cleaning chemicals should be avoided because of potential for contamination of tanks unless using chemicals are specifically designed for use in aquarium tanks. If uncertain, do not use chemicals.

#### 4.7 Special care procedures

##### 4.7.1 Dead animals

4.7.1.1 Dead animals found in a tank during routine observations are placed in a plastic bag and also noted on the fish room log sheet.

4.7.1.1.1 Plastic bag should be placed in the freezer designated for dead animals (either in 101B, off cage washing room, or in other approved research lab).

##### 4.7.2 Injured or distressed animals (see SOP 300)

4.7.2.1 Contact the PI for further action, which could include:

4.7.2.1.1 Consultation with the Attending Veterinarian

4.7.2.1.2 Euthanasia

4.7.2.2 Enter comments in the room log sheet, noting the specific tank.

##### 4.7.3 Escaped animals

4.7.3.1 Capture the animal and place into a tank of its own, identified as “escaped animal” on tank label.

4.7.3.2 Contact the PI responsible for the room for further action.

4.7.3.3 Enter comments in the room log sheet. Identify the specific tank it escaped from, if known.

## 5.0 References

- 5.1 Louis J. DeTolla, S. Srinivas, B. R. Whitaker, C. Andrews, B. Hecker, A. S. Kane, & R. Reimschuessel. 1995. Guidelines for the Care and Use of Fish in Research. *ILAR Journal* 37(4): 159-173.

## SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
220.01	12/20/17	Authored by D. Welsh