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| Algoma University Animal Care Committee |  |
| <b>#AU 0032 Fish Husbandry</b>          |   |
| Issue date: October 2024                | Last revised: May 2025  |

### 1) Purpose

This Standard Operating Procedure (SOP) describes acceptable procedures for the keeping and husbandry of fish including the setup of tanks and monitoring of water quality.

**NOTE:** This SOP deals specifically with holding fish in static tanks with filter units

### 2) Policy

CCAC guidelines and regulations

### 3) Responsibility

Principal investigator, their research staff, and their student investigators

### 4) Training Required

Animal Research Ethics

Handling/training in the relevant species

Bio-methodology training in the relevant technique and species

### 5) Materials Required

Tank of appropriate size for fish being kept

Dechlorinated water of choice

Filters (Mechanical, Chemical, and Biological)

Cycle or other biological filter creating treatment

Substrate of choice (Gravel, Marbles, or Sand)

Enrichment (Spawning tiles, fake vegetation, fake coral, etc.)

Aerator

Heater/Chiller (Optional)

Timer to control photoperiod

Tank Label that includes tank number, AUP number, initial number of fish, arrival date, and emergency contact

Water Testing Kit

Animal Husbandry Form

Food that is appropriate to the fish being held

### 6) Procedures

Procedure: Tank Setup

1. Tanks for fish holding need to be up and running 1-2 weeks prior to bringing in fish to allow the biological filter sufficient time to properly form

2. **Fish density:** The normal minimum volume per fish is 1 Liter; however, if the fish are of considerable size (>20 grams), a greater volume of water per fish is warranted

a. Often it is not clear how much water per fish is required, the only way to be certain is to closely monitor the water quality once animals are being housed, if 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. **Tank Selection:** 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. **Substrate and enrichment:** Each tank requires substrate (e.g. gravel, marbles, or sand) and enrichment. The purpose of enrichment is to provide areas of refuge at the bottom and vertically within the tank. Enrichment can take the form of spawning tiles, fake vegetation, fake coral, or ornaments

5. **Filtration:** 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 weeks prior to adding fish, this will allow the biological filter time to form

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 is species specific; however, many commonly held species are amenable to room temperature ( $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). Prior to holding a new species of fish, determine the normal holding temperature and range for that animal. Higher temperatures increase the probability of growth of pathogens, lower temperatures push fish 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.

9. **Tank Label:** Affix a waterproof tag containing the following information:

- a. Tank number
- b. AUP (Animal Use Protocol) number
- c. Initial number of fish in tank
- d. Arrival date
- e. Emergency contact information for Principal Investigator (PI) and other contact people

**Procedure: Acclimation and Evaluation of Condition**

1. Animals **MUST** be procured from reputable sources. Purchasing fish from a commercial pet store or local bait shop 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 the healing of any external infections

- i. A salt bath consists of placing fish in 20g/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, skip right to step 2) c).

2. Fish 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
- c. Slowly allow the fish into the tank, minimizing the amount of water 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 or behaviors, 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)

**NOTE:** Any fish displaying any of the conditions above should be removed from the population and either treated if possible or euthanized following the protocols listed in SOP #AU0007 (Anaesthesia and Euthanasia of Fish MS-222)

4. During the acclimation period, water chemistry (nitrates, nitrites, ammonia, and pH) and temperature should be monitored daily, with water changes (up to 40%) occurring as required.

- a. This information should be recorded on the animal husbandry forms.
- b. The standard kits for testing will be test strip kits, which involve dipping a strip into the tank and

comparing the colour change to a template. See the instructions on the test strip containers for details on how the test is performed as it differs between kits.

**NOTE:** Not all test strips have nitrate, nitrite, and ammonia; meaning multiple strips from different test kits may be required. Appropriate kits include Tetra and API branded test kits

### **Procedure: 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. This information must also be on a waterproof tag on the tank, mentioned in step 9) of Tank Setup procedure

b. The following columns should be included on 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 an increase in mortalities as this may require increasing holding or euthanizing sick or injured fish

3. Fish are fed twice a day, with the diet depending on the species and age of fish being held. For holding goldfish, use commercial flake food, for holding minnows, feed once a day with commercial flake food and once a day with frozen brine shrimp (artemia). When staffing is low, feeders can be employed. This should not exceed more than three days

a. Feeding is 5-minutes *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, remove any additional food 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 (see below). After the two-week acclimation period where water is tested daily for nitrate, nitrite, pH, 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 to stabilize the chemistry. Additional cycles should also be added to reinoculate the tank with a biological filter

5. Fish must also be observed daily for a variety of endpoints that may show injury, disease, or distress. These include but are not limited to:

a. **Physical Appearance.** Including:

- i. Do fish appear normal/abnormal
  - ii. Eye condition
  - iii. Fin and skin condition
  - iiii. Damaged fins indicate injury or infection
  - iv. Mucous production
  - v. Colour change
    - Darkening of skin can indicate an infection
    - Some infections can have distinct colours, for example saprolegnia can cause white spots and then “furry” spots on the skin
- b. Clinical Signs.** Including:
- i. Issues with food consumption
    - This may be difficult to note, but if animals are not actively feeding after food has been provided, it indicates there may be a health issue with the fish
  - ii. Respiratory rate
    - Increased or decreased respiration may indicate an issue with oxygenation or water chemistry
    - Animals that appear to be gasping at the surface may also indicate a low dissolved oxygen concentration in the tank
  - iii. Posture in water
    - Animals should be upright in the water and actively swimming. Animals unable to retain their balance should be removed from the population and euthanized according to SOP #AU 0007 (Anesthetization and Euthanasia of Fish MS-222)
- c. Behavior.** Includes:
- i. Aggression between animals
    - If unusual aggression is noted, you may need to split up fish into addition tanks and/or increase enrichment
  - ii. Position in water column (are animals crowded or are they spread throughout the tank)
  - iii. Hyperactivity/Hypoactivity
    - Animals may try and jump and escape or swim at a higher than usual rate, also animals may not swim at all, indicating an issue
  - iv. Rubbing/Scraping
    - Indicates there may be a skin infection causing discomfort
  - v. Feeding
    - If animals are not feeding as expected, this indicates there may be an issue in the tank

### **Procedure: Water Quality Testing**

**NOTE:** Both physical and chemical parameters must be monitored in each tank.

1. **Physical Parameters:** The most important physical parameter to measure is temperature and must be done daily. Temperature can be measured by:

- a. **In-Tank Digital Thermometer:** This is the best option as the thermometer is always held within the tank, and in addition to giving an accurate reading; there is no chance of cross-contamination

b. **External Digital Thermometer:** These thermometers have a cable that runs from the thermometer readout and into the tank. These are a viable option for monitoring temperature; **HOWEVER**, there should be one thermometer per tank to prevent cross-contamination. If a thermometer needs to be transferred between tanks, it must be thoroughly disinfected first

c. **Temperature Logger:** A temperature logger can be used to monitor the tank for short intervals for a long period of time. For example, once every 10 minutes for multiple days. This type of logger can be used if there is concern there are daily fluctuations that are not captured by daily monitoring; however, they are not ideal for recording daily temperatures as they need to be removed from tanks to be read, which can be labour-intensive

**NOTE:** Do **NOT** use glass thermometers or analog floating thermometers as they are often inaccurate, and readings are often specific to individuals. In addition, glass thermometers are prone to breaking, which may introduce chemicals and glass to the tanks.

**NOTE:** Infrared thermometers can be used to measure surface temperature only, and because of this they may not be giving the true water temperature.

2. **Chemical Parameters:** The basic water chemistry parameters are pH, nitrate, nitrite, and ammonia and can be measured several ways. The primary method for measuring water chemistry will be test strips. Test strips often include multiple parameters per strip and require placing the strip into the tank (or in a sample of water from the tank) and reading the colours of each test to determine the value. Test strip kits are easy to source, have no chemicals to store, and can be found at local pet stores or online.

a. Other testing methods include water chemistry kits (both colourimetric and non-colourimetric) and in-tank sensors (both card and electronic sensors). These options can be much more accurate than test strips; however, they are often much more labour intensive or cost prohibitive

**NOTE:** API and Tetra are two common brands for test strips. Please note that not all test strips will contain the same parameters, and sometimes two or more strips are required to ensure measurement of pH, nitrate, nitrite, and ammonia.

3. **Frequency:** The frequency of measurement is dependent upon the importance of the parameter, the patterns of water quality, as well as the logistics of measurement.

a. **Water temperature** must be measured at least once a day. This will allow for long-term trends to be seen in the data. If needed, temperature loggers (as mentioned in 1) c) above) can be used to ensure there are no significant fluctuations during the day

b. **Water chemistry:** The frequency of water chemistry measurements is dependent upon several factors. If the water chemistry parameters are within an acceptable range, water chemistry must be measured once a week. During the two-week acclimation period, the water chemistry must be measured at least once every 2 days to ensure the filtration is sufficient for the tank. If there is a spike in values for the various parameters that warrant water changes (see below), then water chemistry should be measured every 1-2 days until the tank has stabilized.

4. **Remedies:** The remedy for water quality issues is dependent on the parameter.

a. **Temperature:** Consistently low temperatures can be dealt with by the addition of an appropriately sized heater to the tank. Consistently high temperatures can be dealt with by using a water chiller. Alternatively, the temperature of the room where the animals are held can be adjusted to ensure the water temperature in the tank is correct. If there is an issue with fluctuating temperatures, this is likely due to issues with temperature control within the room/building, and work must be done to ensure these fluctuations stop. Fluctuations may also be due to a tank being too close to a window, moving the tank may solve the issue.

b. **Water Chemistry:** Water chemistry issues can be dealt with by two primary methods, first, perform a water change (up to 40%), this will dilute the chemicals and allow for balance to be restored to a tank. Alternatively, if the water quality parameters are constantly going above what is allowed, it indicates the filtration is insufficient for the fish. Fish either need to be removed for the tank or additional filtration must be added to the tank.

| REVISION HISTORY |               |   |
|------------------|---------------|---|
| Revision #       | Revision Date | Summary of Changes                            |
| 1                | March 2025    | Addressed questions raised in comments of SOP |
| 2                | May 2025      | Reformatted to new SOP template               |
| 3                |               |   |
| 4                |               |   |