

Algoma University Animal Care Committee	
AU #0035 Fish anesthesia with clove oil (adapted from Carleton University's SOP FAR-05)	
Issue date: March 18, 2025	Last revised: March/2025

1. Purpose

Anesthetics are valuable tools used to immobilize fish and reduce physical damage and stress during fisheries research and aquaculture practices (e.g., transport, sorting, spawning, vaccination), stock assessment (e.g., enumeration of fish, collection of aging structures), and experimental procedures (e.g., telemetry tag implantation).

Clove oil (eugenol), which is also the active ingredient in commercially available Aqui-S, is a widely used fish anesthetic and can be used in both laboratory and field settings. Fish anesthetized with clove oil in field settings can be immediately released following manipulation, unlike fish anesthetized with MS-222 which require a withdrawal period (5-21 days, depending on jurisdiction). This protocol outlines ways to minimize stress on fish when using clove oil as an anesthetic.

2. Policy

CCAC guidelines and regulations

3. Responsibility

It is the responsibility of the Principal Investigator to ensure that this SOP is followed and that those handling fish are appropriately trained to do so.

4. Training Required

Animal Research Ethics
 Handling/training in the relevant species
 Bio-methodology training in the relevant technique and species

5. Materials Required

Nitrile surgical gloves
 Eye protection
 Clove Oil
 95 % Ethanol
 Graduated cylinder

Anesthesia container
Research container
Recovery container
2 Aerators
Water circulation pump (variable speed preferred)
Hosing for water circulation pump
Thermometer
Dip net (sized appropriately for fish)
Clean water
MSDS Sheet

6. Procedures

Preparing the anesthetic, research, and recovery baths

1. Prepare the anesthetic mixture in advance of working with fish. Clove oil and ethanol need to be mixed in a 9:1 ratio and stored in a dark container (clove oil is light sensitive).
2. Although a 60 ppm solution is usually safe to anesthetize fish (induction), make sure you conduct a literature search for clove oil anesthetic dosages for your study species. If fish go down too quickly (<2 min) try 40 or 50 ppm. If they go down too slowly (>5 min) then try 70 ppm. Keep in mind that water temperature, size, species, etc. will influence the rate of anesthesia in an unpredictable manner.
3. Put on nitrile gloves and safety glasses.
4. Begin preparing the anesthesia container by filling it with water. Make sure there is enough water so that fish will be able to swim up and down in the water column. Prepare your desired dosage (ppm) of anesthesia and then add the appropriate amount of 9:1 clove oil to ethanol mixture to the anesthesia container (e.g., 60 ppm in Table 1).
5. Begin preparing a research container by filling it with water. The water should be deep enough so that the gills of the fish are submerged, but the surface of the fish requiring access (if applicable) is not.
6. If experimental procedures will take longer than 2-3 minutes to complete after anesthetic induction, prepare a research container that will be filled with water and a weak anesthetic (approximately half the dosage used in the anesthesia container) to keep fish in during manipulation (e.g., surgery). This container needs to have a circulation device that pumps water through the gills of the fish, such as a small water pump with plastic tubing.
7. Fill a third container with water to be used as a recovery bath. This container should be sufficiently large enough so that fish will be able to swim up and down in the water column.

8. Use aerators to constantly supply oxygen to all three containers.

9. Use a thermometer to make sure the water temperature in all three containers and the original water source (where the fish came from) remains even. Temperature in the containers should not deviate more than ± 1 °C from the original water source. If the water becomes too warm or cold, empty the containers and refill them.

Table 1. Mixture rates for the preparation of clove oil as an anesthetic to create a 60 ppm bath.

<u>60 ppm bath</u>		
clove oil (ml)	ethanol (ml)	water (L)
0.12	1.08	2
0.24	2.16	4
0.36	3.24	6
0.48	4.32	8
0.6	5.4	10
0.72	6.48	12
0.84	7.56	14
0.96	8.64	16
1.08	9.72	18
1.2	10.8	20
1.32	11.88	22
1.44	12.96	24
1.56	14.04	26

Anesthetizing fish

1. Put on nitrile gloves.

2. Use a dip net to transfer a single fish into the anesthesia container.

3. Fish should be fully anesthetized after 5 minutes of exposure to the anesthetic. When fully anesthetized, fish will lose equilibrium completely, decrease their respiratory rate and will not respond to stimuli (such as a firm tail squeeze with fingers). If the respiration rate of a fish becomes extremely slow or stops, move it to the recovery container immediately. If, on the other hand, the fish is not fully anesthetized after 5 minutes you will need to increase the dosage of the anesthetic.

4. Do not perform any procedures until the fish is fully anesthetized.

5. When the fish is fully anesthetized, move it into the research container ventral side up, making sure the gills are fully submerged. Perform any short-term procedures that can be completed in 2-3 minutes (e.g., blood sampling, weight and length measurements, fin or gill clipping).

6. If performing a surgery requiring ongoing anesthetic delivery, gently insert a water circulation hose into the mouth of the fish and turn on the pump. Regulate the water flow so that it provides fresh water and oxygen, but not so forceful that it might harm the fish. As above, the maintenance bath for a surgery should be roughly half that of the induction dose (e.g., if fish is anesthetized with 60 ppm then use a 30 ppm maintenance bath).

7. After manipulation, place the fish gently into the recovery container. The fish should show signs of improvement within 2 to 3 minutes of entering the recovery tank (e.g., regain equilibrium and increased respiratory rate). Recovery may take longer at low water temperatures typical of winter seasons.

8. Once fully recovered, use the dip net to transfer the fish back into their original water source (e.g., lake, stream, housing tank).

Disposal

Clove oil must be disposed of according to acceptable federal, provincial / territorial, and municipal regulations for the disposal of chemical materials. Larger volumes can be spread out on gravel roads during the day to evaporate provided it is away from entering a waterway (via sewer or runoff or other) while smaller volumes (e.g., <20 liters) of waste could be transported back to Algoma University via a sealable watertight container and disposed of via the department's chemical disposal system.

REVISION HISTORY		
Revision #	Revision Date	Summary of Changes
1	March 18, 2025	ACC accepted with no revisions