Preclinical Research Center
Ben-Gurion University of the Negev

STANDARD OPERATING PROCEDURE 202
FISH AND AQUATIC AMPHIBIAN ANESTHESIA

1. PURPOSE

This Standard Operating Procedure (SOP) describes methods for anesthetizing fish and aquatic amphibians.

2. RESPONSIBILITY

The Principal investigator (PI) and their research staff.

3. MATERIALS

3.1. Anesthetic agent (MS-222) or Clover oil (Eugenol)
3.2. Absorbent pad
3.3. Personal protective equipment: gloves, protective clothing (lab coat), eye protection
3.4. Holding and recovery tank

4. FISH ANESTHESIA

4.1. If using a new anesthetic protocol or species, anesthetize a few fish and follow them through full recovery to ensure safe drug dosages and techniques and provide sufficient anesthetic depth for the intended procedures.
4.2. Maintain adequate oxygenation:
  4.3.1. Supply via an air pump or similar device.
  4.3.2. Oxygenate all water chambers during anesthesia and recovery.
  4.3.2 Flush gills with an oxygenated anesthetic solution.
4.4. Use water from the original fish-holding tank for transport, anesthetic, and recovery chambers. If using another water source, closely duplicate the original holding tank's water quality parameters (i.e., chlorine, temperature, pH, and ammonia).
4.5. Maintain water temperature at the species' normal optimum during anesthesia and recovery.
4.6. Anesthetic agents for fish:

ANESTHETIC AGENT	DOSE	COMMENTS
MS-222* (tricaine methanesulfonate)	75–125 mg/L (induction) and 50–75 mg/L (maintenance)	Sodium bicarbonate should be added to the stock solution to maintain neutral pH. Only FDA-approved anesthetic for fish (21-day withdrawal).
Clover oil - Eugenol	250 microliter/L	Stock solution dissolved in ethanol
Lidocaine 2%	gel	Local application

* Light-sensitive chemical: should be kept in a dark container or a cabinet/drawer

4.8. Stages of anesthesia in fish:

STAGE 1 Deep sedation	STAGE 2 Deep narcosis	STAGE 3 Surgical anesthesia
Cessation of voluntary swimming Loss of response to stimuli.	Decreased muscle tone Equilibrium loss The appropriate level for fin and gill biopsies.	Slow respiration and heart rate Total loss of activity to stimuli.

4.9. Assess the surgical plane of anesthesia by monitoring:

4.9.1. Total loss of equilibrium and muscle tone

4.9.2. Decreased respiratory rate

4.9.3. No response to stimuli firmly squeezes at the base of the tail to determine response to stimuli.

4.10. Evaluate respiratory rate and gill color:

4.10.1. Observe the movement of the operculum (the rigid flap that covers the gills) as it opens and closes to assess the rate.

4.10.2. Observe gill color: should be dark pink to light red.

4.10.3. If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume.

5. AQUATIC AMPHIBIAN ANESTHESIA

5.1. Anesthesia methods are achieved by immersion in an anesthetic solution.

5.2. Keep amphibians moist all the time out of water.

5.3. Induce anesthesia in a closed container to prevent the animal from jumping or falling out to avoid injury.

5.4. Anesthetic induction may produce an excitement phase.

5.5. Anesthetic Agents:

ANESTHETIC AGENT	DOSE	COMMENTS
MS-222 (tricaine methanesulfonate)	1-2 g/L	After immersion for 20 minutes, it provides surgical anesthesia for 30 min. (1g/L) to 60 min. (2g/L).

5.5.1. MS-222: Buffer solution with sodium bicarbonate to maintain neutral pH.

5.5.2. Wide safety margin.

5.6. Pulmonary respiration will cease during anesthesia; therefore, respiratory rate cannot be used to monitor anesthetic depth; however, cutaneous respiration is sufficient to prevent clinical hypoxia.

5.7. Monitor heart rate during anesthesia by direct observation (ventral midline, caudal to the shoulders)

5.8. Stages of Anesthesia in Amphibians:

INDUCTION	LIGHT ANESTHESIA	SURGICAL ANESTHESIA
Decreased gular movement and diminished withdrawal reflex.	Loss of righting reflex and absence of abdominal respirations.	No withdrawal reflex (toe pinch) and cessation of gular movements.

5.9. Allow animals to reach an appropriate level of anesthesia for planned procedures.

5.10. Remove the animal from the anesthetic bath, rinse it with fresh water, or remove the topical preparation by rinsing.

5.11. The animal will remain anesthetized for 10 to 20 minutes.

5.12. Determine full recovery from anesthesia by monitoring when the righting reflex returns and the animal can move normally. It typically takes 30 to 90 minutes after the animal is rinsed with fresh water.

5.13. Do not raise the amphibian's body temperature above average room temperature to speed recovery.

5.14. Increased body temperature will increase metabolism and oxygen requirements.

5.15. Cutaneous respiration may not be sufficient to maintain adequate oxygenation in this situation.

5.16. Do not apply alcohol or other preparations that contain alcohol directly to the skin of an amphibian, as absorption of these products through the skin may dissolve normal secretions that protect the animal from dehydration and infections.

6. SAFETY PRACTICES

6.1. MS-222:

6.1.1. Wear protective clothing, gloves, and eye protection when handling the MS-222 powder.

6.1.2. Wear gloves to handle animals exposed to MS-222