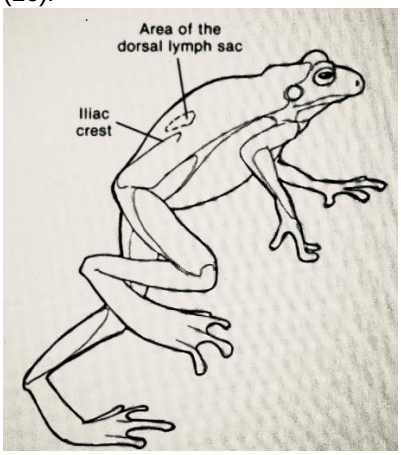
Species Specific Anesthetic & Analgesia Recommendations

AMPHIBIANS

Aquatic frogs such as *Xenopus laevis* or *X. tropicalis* should be handled with soft nets during unanesthetized exams and/or procedures. When indicated, physical restraint should be firm but gentle and care must be taken to preserve the integrity of the protective mucous layer. Use of rinsed and moistened non-powdered gloves are recommended when handling amphibians in order to protect their delicate skin and prevent handler contact with glandular secretions, which may be toxic. Thoroughly rinsed vinyl gloves appear to be the safest option, especially when it comes to the handling of tadpoles (4).

Chemical restraint is required for prolonged or invasive procedures. Some frog species including *Xenopus* have paired lymphatic structures called dorsal lymph sacs (16) (see figure 1) located subcutaneously under the skin of the back. These structures communicate with the venous circulation and are an excellent site for injection. Other routes including intracoelomic, intramuscular and intravenous are also frequently used.

Figure 1. In some anurans, a pair of dorsal lymph sacs lies immediately lateral to the base of the sacrum (16).



A light plane of anesthesia is characterized by a loss of righting reflexes, but withdrawal reflexes and gular (throat) respiratory efforts remain. As the anesthetic level deepens, abdominal respiration is lost, followed by slowing of gular (throat) movements, which stop as a surgical level is reached. The cardiac impulse (visible heartbeat) should be retained; slowing or loss indicates an anesthetic overdose.
Skin should be kept moist during recovery. Ambient recovery temperatures of 60 - 70^0 F are appropriate for most species; avoid unnecessary warming because cutaneous respiration cannot meet the metabolic demands of increased body temperature (7).

MS-222, benzocaine, and eugenol (clove oil) are highly irritating to skin and mucous membranes. Be sure to wear gloves and lab coat/disposable coat when handling. Work under a fume hood to help minimize risk of exposure. Alternatively, use goggles and a respirator (note: individual needs to be medically evaluated, and dependent on the type of respirator, be fitted). Rinse skin and mucous membranes thoroughly in cases of exposure.

Immersion Anesthesia*

Agent(s)	Immersion Bath Dosages	Comments/Reference(s)
Tricaine methanesulfonate (MS-222, tricaine, Finquel®, Syncaine®)	Larvae: 200 - 500 mg/liter *more sensitive than adults Adult frogs (*Xenopus*) & salamanders: 500 mg/liter – 2 grams/ liter Toads: 1 - 3 g/liter	Anesthetic of choice for *Xenopus*. Safest for long and/or repeated procedures - **1g/liter** provides at least 30 min of surgical anesthesia in this spp. All MS-222 **working** solutions must be buffered: $NaHCO_3$ 420 - 1,050 mg/liter (10-25 mEq/liter). Unbuffered solutions result in a prolonged induction time and are irritating to skin (7). Do **not** buffer **stock** solutions. Store MS-222 powder in the original sealed container in a dry location at room temperature until the expiration date noted by manufacturer on packaging. Ideally MS-222 stock solutions are utilized the same day as preparation per vendor recommendation. When necessary, stock solutions of MS-222 may be kept up to 30 days. They must be refrigerated and stored in tinted (amber) or opaque bottles. Stock solutions of MS-222 that are older than 30 days, or that have not been properly stored must not be used. All MS-222 powder and stock solution containers must be appropriately stored, labeled (concentration and preparation or expiration date), and used prior to expiration date. Contact Health, Safety, and Environment (HS&E) for appropriate disposal methods as MS-222 solutions cannot be poured down the drain or introduced into the general water supply.
Benzocaine	Larvae: 50 mg/liter to effect Adult frogs (*Xenopus*) & salamanders: 200 - 300 mg/liter to effect	Immersion anesthetic related to MS-222 but with greater potency, more rapid induction & narrower margin of safety. Less water soluble than MS-222, must be dissolved in acetone or ethanol (7).
Eugenol (clove oil)	360 mg/liter for 15-20 minutes. 310 - 473 mg/liter * large variation by species	Does not need to be buffered. (8). Not recommended. Highly variable anesthetic duration, narrow safety margin, prolonged recovery. Reversible gastric prolapse reported in 50% of leopard frogs (3, 7).

*Important: Pharmaceutical-grade MS-222 like Finquel® and Syncaine® is the recommended agent and should be used instead of the non-pharmaceutical grade MS-222 or eugenol. If non-pharmaceutical grade compounds are used, this must be justified in the ACUC protocol.

Injectable Anesthesia

Agents(s)	Dose	Comments/Reference(s)
Ketamine	50 - 150 mg/kg SC, IM, IV, or dorsal lymph sacs (considered same as IV)	For minor surgeries or restraint; induction time, depth of anesthesia and recovery time vary greatly with species (7). DEA required.
Alfaxalone	18 mg/kg IM, IV, intracelomic	African clawed frogs/ deep sedation for 1 – 3 hour (IM, IV), 10 – 60 min intracoelomic (9). DEA required.

Inhalant Anesthesia

Agent(s)	Dose	Comments/Reference(s)
Isoflurane	Induction: 3 – 5% Maintenance: 1 – 2.5%	Induction time can exceed 20 min. Ensure chamber is moistened. Larger amphibians with lungs can be intubated (7).

Analgesia

Agent(s)	Dose	Comments/Reference(s)
Buprenorphine	14 mg/kg in dorsal lymph sac	DEA required. (7, 15).
Butorphanol	25 mg/kg intracoelomic	DEA required, 12 hour duration. (7, 15).
Xylazine	10 mg/kg intracoelomic	12 - 24 hour duration.
Flunixin meglumine (Banamine®)	25 mg/kg intracoelomic	4 hour duration.
Meloxicam	0.1 mg/kg 0.4 – 1.0 mg/kg PO, SQ, intracoelomic q 24 hours	American bullfrogs/ decreased circulating prostaglandin E2 (PGE2) levels measured 24 hours post muscle biopsy (14). Analgesia (3).
Lidocaine	2 mg/kg local infiltration, dilute 3:1 with sodium bicarbonate solution, duration 30–60 min.	Do not exceed 5 mg/kg **total** dose either topically or intra-incisional (5).

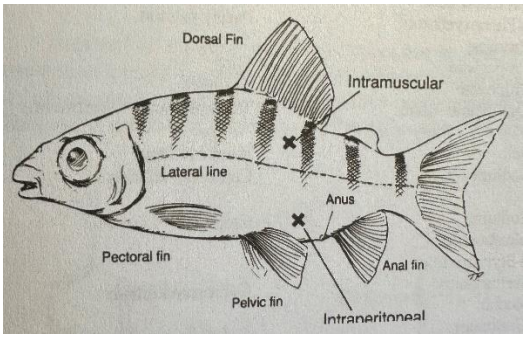
FISH

Immersion is the preferred method of anesthesia. This technique is analogous to gas induction chamber use for mammals. Agents are absorbed across the gills and exert their impact centrally. It is recommended that two separate tanks of water be used: one for induction and the other for recovery. In addition, animals should be fasted for 24 hours prior to anesthesia and maintained in a calm state until induced. Fish should be handled with wet gloved hands.

Loss of the equilibrium represents the first stages of anesthesia. Surgical anesthesia is attained when there is no response to stimuli and respiration rate is very slow. Gill movements should be maintained through anesthesia. If spontaneous gill movement ceases during anesthesia, the fish should be placed in a recovery bath to increase oxygenation through the gills.

In addition to emersions, traditional routes of drug administration used for mammalian anesthesia and analgesia may also be used with fish, including oral, intramuscular (IM, given above the lateral line under the dorsal fin), intravenous (IV), and intraperitoneal (IP) injection (2) (see figure 2).

Figure 2. Routine sites for intraperitoneal (intracelomic) and intramuscular injections in fish (2).



Although hypothermia to immobilize fish has been well established, there is little evidence to date demonstrating the process provides sufficient anesthesia or analgesia (13).

MS-222 and eugenol (clove oil) are highly irritating to skin and mucous membranes. Be sure to wear gloves and lab coat/disposable coat when handling. Work under a fume hood to help minimize risk of exposure. Alternatively, use goggles and a respirator (note: individual needs to be medically evaluated, and dependent on the type of respirator, be fitted). Rinse skin and mucous membranes thoroughly in cases of exposure.

Immersion Anesthesia*

Agent(s)	Immersion Bath Dosages	Comments/Reference(s)
Tricaine methanesulfonate (MS-222, tricaine, Finquel®, Syncaine®)	Species specific variation: Induction: 100 - 200 mg/liter Maintenance: 50 - 100 mg/liter	Anesthetic of choice for fish. MS-222 **working** solution (10g/liter) can be buffered with NaHCO3 at 10g/liter or to complete saturation to reach pH of 7.0 - 7.5. Aerate water to prevent hypoxemia; narrower margin of safety in younger and/or warm water fish (3, 7). MS-222 **stock** solutions should **not** be buffered. See dose calculator at: Tricaine-S Concentration Calculator (syndel.com) Store MS-222 powder in the original sealed container in a dry location at room temperature until the expiration date noted by manufacturer on packaging. Ideally MS-222 stock solutions are utilized the same day as preparation per vendor recommendation. When necessary, stock solutions of MS-222 may be kept up to 30 days. They must be refrigerated and stored in tinted (amber) or opaque bottles. Stock solutions of MS-222 that are older than 30 days, or that have not been properly stored must not be used. All MS-222 powder and stock solution containers must be appropriately stored, labeled (concentration and preparation or expiration date), and used prior to expiration date.

		Contact Health, Safety, and Environment (HS&E) for appropriate disposal methods as MS-222 solutions cannot be poured down the drain or introduced into the general water supply.
Eugenol (clove oil)	10 - 100 mg/liter bath for sedation to handleable 17 - 25 mg/liter bath	Aqui-SE contains 50% eugenol and Aqui-S20E, 10% eugenol, a compound mixture of eugenol and polysorbate 80 (for solubility); lower doses (6 mg/liter) will produce sedation without anesthesia (3).

*Important: Pharmaceutical-grade MS-222 like Finquel® and Syncaine® is the recommended agent and should be used instead of the non-pharmaceutical grade MS-222 or eugenol. If non-pharmaceutical grade compounds are used, this must be justified in the ACUC protocol.

Injectable Anesthesia

Agents(s)	Dose	Comments/Reference(s)
Ketamine	10 - 80 mg/kg IM	Immobilization for short procedures; complete recovery can take > 1 hour (3). DEA required.
Ketamine + Medetomidine	(K) 1 - 2 mg/kg + (M) 0.05 - 0.1 mg/kg IM	Immobilization; reverse (M) with **atipamezole** 0.2 mg/kg IM (10). DEA required.

Analgesia Note: Considerable evidence supports the presence of mu and kappa opioid receptors in teleost fish and thus endogenous opioid system that might be manipulated to provide analgesia. In general, however, specific drug and dosing regimens are still lacking for most fish including zebrafish (*Danio* spp.), the species those most commonly used in biomedical research (13).

Agent(s)	Dose	Comments/Reference(s)
Butorphanol	0.05 - 0.1 mg/kg IM	General fish dose range for post-operative analgesia. (3) DEA required.
Morphine	5 mg/kg IM	Koi/analgesia (1). DEA required.
Ketoprofen	2 mg/kg IM	As a post-operative analgesic in koi (10).
Lidocaine	1 – 2 mg/kg 5 mg/L **immersion** for 30 minutes	Local anesthetic; use with caution in small fish; do not exceed 2 mg/kg **total** dose (11). Higher activity levels and faster return to baseline opercular beat rate after fin clipping as compared to fish receiving no analgesia (6).
Aspirin	2.5 mg/L **immersion** for 30 minutes	Higher activity levels and decreased opercular beat rate after fin clipping as compared to fish receiving no analgesia (6).

VI. References

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