Guidelines on Fish Anesthesia Analgesia and Surgery

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1. Purpose
   1. This document is intended to serve as a guideline for anesthesia, analgesia, and surgery of fish. This is not intended to be an inclusive tutorial on all possible techniques that can be used in these species. The following guidelines are general recommendations they do not factor in species-specific or research-associated concerns.
   2. All surgical procedures, anesthetics, analgesics, antibiotics or other medications used on animals must be approved by the IACUC and described in the animal use protocol. Personnel handling animals and/or performing surgical procedures must be listed on the animal use protocol and appropriately trained prior to performing any surgeries or other procedures.
   3. Any divergence from the recommendations in this document must be described in the animal use protocol prior to implementing the deviation.
   4. If you have questions or comments about this document, please contact the ULAM veterinary staff at ulam-vets@umich.edu or 734-936-1696. The ULAM training core (ULAM-Trainingcore@umich.edu or 734-763-8039) can be contacted to provide training in these techniques at no charge. Sources of additional information are listed at the end of the document.
   5. For any concerns regarding animal health after work hours or on holidays/weekends, please contact DPS (3-1131) who will contact the on call veterinarian.

2. Responsibility
   1. Principal Investigator: Responsible to ensure appropriate anesthesia, monitoring, and analgesia is provided for all animals undergoing surgical or sedation procedures.

3. Definitions
   1. Anesthesia: Temporarily induces loss of sensation with or without loss of consciousness.
   3. A/A: Anesthesia and analgesia.
   4. Sedation: A mild degree of central depression in which the patient is awake but calm
   5. Immersion: A method of delivering drugs via direct contact with the skin in a bath.

4. Procedures
   1. Handling and Restraint
      1. Wear clean, wet exam gloves when handling fish.
      2. Moisten gloves with clean dechlorinated water, or water from the animal’s home tank.
      3. Completely rinse powder from gloves before handling, if present.
      4. In some field conditions, clean wet hands (without gloves) are acceptable.
      2. Fish have a protective mucus layer on their skin and are slippery and difficult to handle; use nets to handle fish whenever possible. Always use a net to restrain non-sedated fish.
      3. Work surfaces for medical/surgical procedures must be non-abrasive but also prevent the fish from sliding, such as a soft moist cloth.
      4. Fish must be anesthetized during any procedure that is stressful or likely to cause pain.
      5. Fish must remain wet during procedures out of the water. Water can be dripped, poured, or sprayed on exposed areas of the fish. Use water from the home tank wherever possible; clean non-chlorinated water is also acceptable.
   2. Anesthesia
      1. General Considerations
         1. There is tremendous inter-species variation, and anesthetic protocols that work for one type of fish may not be effective in another. Prior to use of any anesthetic regimen it is recommended to consult the literature and investigators with experience in the species of interest. Neiffer (2009) is a good starting resource for individual species options. Refer to Appendix A for general recommendations.
      2. Delivery Methods
         1. Immersion
1. This is analogous to inhalant anesthesia in air-breathing species and occurs when a diluted anesthetic is absorbed by the gills and/or the skin to produce anesthesia.
   1. Advantages of immersion anesthesia include consistent response and a relatively high safety margin, rapid recovery following removal to fresh water, and the lack of physical restraint for induction.
   2. Disadvantages include difficulty changing the anesthetic concentration in the water during the procedure, lack of suitability for longer procedures (>10 minutes), and the irritant nature of some anesthetics. Despite these potential disadvantages, anesthesia by immersion is generally the anesthetic method of choice.

2. Induce anesthesia in an anesthetic-treated tank. Monitor the animal to determine when it has reached an appropriate depth of anesthesia (see monitoring section below).

3. Several immersion anesthetics can significantly alter the pH of water; use buffers to establish and maintain a physiologically appropriate pH.

4. Fish are very sensitive to changes in water pH, temperature, and mineral content, and consistent water quality between the fish’s normal holding tank, and the anesthetic and recovery tanks is extremely important. Use water from the animal’s home tank in the anesthesia and recovery tanks whenever possible.

2. Flow Anesthesia
   1. For procedures out of water lasting more than a few minutes, recirculating or continuous flow systems using aerated water containing anesthetic can be used to provide both continuous anesthesia and artificial ventilation.
   2. When using flow anesthesia, induce anesthesia in using the immersion method above. When an appropriate level of anesthesia is reached, the animal can be transitioned to either of the methods listed below.
      1. The recirculating delivery system utilizes a submersible pump that is placed within a tank containing the anesthetic solution. The anesthetic water is pumped from this tank, into the animal’s mouth, over the gills and out through the opercula. The animal is positioned on a fenestrated board above the anesthetic water tank. This method is commonly used in larger fish.
      2. The non-recirculating method uses a new IV bag and drip set as the anesthetic water reservoir and delivery method. The size of the bag and the drip rate will depend on the size of the animal being anesthetized (see Stetter 2001 for specific size recommendations).
   3. For either flow anesthesia approach, adjust the rate such that water flows into the mouth, gently over the gills and out the opercula. Inappropriate flow rates can interfere with gas exchange or force water into the gastrointestinal tract.

3. Parenteral
   1. IM injections are administered into the epaxial muscles. There may be leakage of anesthetic as the muscles contract post-injection. Insert the needle between scales.
   2. IV injection produces rapid induction of anesthesia but requires manual restraint or prior sedative administration. The caudal vein lies ventral to the tail vertebrae and can be accessed by a ventral midline or lateral approach.
   3. ICo (intra-coeleomic) administration is analogous to IP injections in mammals. It is effective but induction times are inconsistent and there is risk of visceral damage.
   4. Additional considerations when working with injectable anesthetics include inconsistent sedation or anesthesia that may require supplementation with immersion techniques, and the potential for prolonged recovery requiring artificial ventilation.

3. Monitoring Anesthesia
   1. The depth of anesthesia in fish can be monitored by observing the behavior of the fish in water. While appropriate monitoring parameters can vary based upon anesthetics and species, several general guidelines can be used for monitoring anesthetic depth. See Appendix A for the stages of sedation and analgesia in fish.
      1. As fish become anesthetized their activity decreases and they lose their righting reflex.
      2. Opercular movement (respiratory rate) progressively decreases with deepening anesthesia.
         1. Hypoxemia can occur in fish despite good ventilatory efforts and can be recognized by pallor of the gills and the fin margins.
      3. The heart rate can be directly monitored using a Doppler blood flow probe or ECG leads.
         1. Monitor trends when using the Doppler or ECG; reference ranges are not known for many fish species.
         2. Surgical planes of anesthesia can be confirmed by a lack of response to a firm squeeze at the base of the tail.
   2. Fish Levels of Anesthesia
      1. Level 1: Light sedation: Reaction to some external stimuli (visual and tactile) is slightly reduced.
      2. Level 2: Deep sedation: Opercular movement rates are slightly reduced, and fish do not react to most external stimuli except pressure.
      3. Level 3: Narcosis: Muscle tone is decreased, swimming is erratic, and the opercular rate is increased. Fish continue to react to strong tactile and vibrational stimuli. This is appropriate for external sampling and fin/gill biopsies.
      4. Level 4: Light anesthesia: Equilibrium and muscle tone are lost, opercular rate decreases, fish respond only to strong pressure. This is appropriate for minor surgical procedures.
      5. Level 5: Surgical plane of anesthesia: Opercular movements are shallow and the heart rate is decreased. There is no reaction to external stimuli and no reflex reactivity.
      6. Level 6: Medullary collapse: Opercular movement ceases and the heartbeat is shallow to absent.

4. Anesthetic Agents
   1. Immersion/Flow Anesthetics
      1. MS-222 (Finquel, tricaine methanesulfonate): Recommended
      1. Pharmaceutical-grade MS-222 is required.
1. Two pharmaceutical-grade MS-222 preparations are currently available:
   1. FINQUEL MS-222 - 99.5% Pure Tricaine Methanesulfonate
   2. Western Chemical's TRICAINE-S (MS-222, TMS, tricaine methanesulfonate) is an FDA approved fish anesthetic (FDA ANADA 200-226)

2. MS-222 is a chemical powder that must be dissolved prior to usage. MS-222 is a acidic in solution and must be buffered to a physiologic pH (7.0-7.4) prior to usage.

3. MS-222 powder is stable for up to 5 years if stored in the original sealed container in a dry location at temperatures < 25°C. 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 will 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. (Alpharma, 2001 and Pharmaq, 2010)

4. MS-222 is a human health hazard and must always be prepared wearing gloves and in a biosafety cabinet or flow hood. Extended direct contact to skin can cause a reversible retinopathy. Please contact the University of Michigan's Occupational Safety and Environmental Health division (734 647-1143) for appropriate disposal methods.

5. The only substance approved by the FDA for field sedation of fishes is MS-222. However, MS-222 use in the field is limited because of an FDA requirement that food fish, including feral fishes that may be caught and eaten by humans, must go through a 21-day withdrawal period prior to release or slaughter for human consumption (American Fisheries Society, 2014; 21CFR592.2503). Therefore, any wild fish anesthetized with MS-222 that are of a species consumed by humans and of legal size (see table 4 in Appendix A) must either be held for the 21-day withdrawal period prior to release or euthanized. Alternatively, other methods of anesthesia or sedation requiring special permitting can be used. Please contact ULAM veterinarians if you require anesthesia or sedation of wild fish that will be released back into the wild.

6. If the animal begins to recover prior to completion of the procedure, there are several options:
   1. Apply a paper towel or gauze soaked in the original MS-222 solution directly to skin with care to avoid the surgical site.
   2. Drip the MS-222 solution directly onto the skin with care to avoid the surgical site.
   3. If there is no open incision, place the animal back into 50% of the original concentration of MS-222.

2. Benzocaine
   1. Parent compound of MS-222, which is less water soluble and less acidic.
   2. Low toxicity in humans and improved safety in species sensitive to MS-222.
   3. Fat-solubility may result in prolonged recovery, especially in old or gravid fish.

2. Parenteral Anesthetics - dosing depends upon species and route of administration
      1. Provides safe and effective anesthesia in some species; can cause respiratory depression, bradycardia, and poor immobilization in others
   2. Consistently effective as an aid to restraint or capture and for short, minor procedures.
   2. a2-agonists: dexmedetomidine:
      1. Medetomidine, in conjunction with ketamine, for has been used in fish but currently has limited availability. Dextemetomidine has not been evaluated in fish, but can be used at half the recommended medetomidine dose in other species.
      1. Usually used in combination with ketamine to improve muscle relaxation and anesthetic duration and depth
      2. Effects can be reversed with the administration of atipamezole.
      3. Xylazine can produce apnea and convulsant activity and is not recommended.

3. For anesthetic agents for use in fish, see Appendix A

4. Recovery/Post-Anesthetic Care
   1. Recover fish in aerated, untreated water.
   2. During recovery, a reversal of the stages of anesthesia should occur with a gradual increase in opercular movement, return of equilibrium, and eventual resumption of normal swimming.
   3. Most fish are fully recovered from immersion anesthetics within 5 minutes of placement in fresh water. Prolonged recoveries (> 10 minutes) indicate excessive anesthetic depth or a compromised animal.
   4. Recovery from parenteral anesthetics can be highly variable.
   5. Fish may pass through an excitement phase during recovery and may attempt to escape from the recovery tank. Stimulation can exacerbate the excitement phase and once fish are showing progressive signs of recovery (increased opercular and fin movement, increased muscle tone, and a return of equilibrium) it may help to cover the tank with a lid. Occasionally, fish will demonstrate vigorous movement and may need to be restrained to prevent self-injury.

5. Emergency Care
   1. If respiration stops altogether, immediately move the animal to untreated, aerated water and initiate forced ventilation. Forced ventilation stimulates the buccal flow/heart rate reflex and provides support while speeding the elimination of the anesthetic.
      1. Forced ventilation is accomplished by moving the fish gently and slowly FORWARD in a circular path through the water. Dragging the fish backward through the water may result in damage to the gills. For any fish with questionable opercular movement, gentle forced ventilation can help speed initial recovery. This will enhance opercular movement and increase the passage of fresh, oxygenated water over the gills.
   2. Respiratory arrest can precede cardiac arrest by several minutes; continue resuscitation efforts for several minutes despite a lack of immediate improvement.
3. Analgesia

1. General Considerations
   1. Pain and the use of analgesia remains a controversial issue in fish medicine. Marked differences in neuroanatomy and behavior exist between fish and higher, homeothermic vertebrates, and analgesic use in fish has not been systematically evaluated. In the absence of contrary evidence, conditions or procedures that are expected to cause pain or distress in a human are to be considered painful or distressing to fish and alleviated accordingly.

2. Analgesic Agents
   1. Opioids - Fish have mu and kappa receptors suggesting a role for opioids. Suggested opioids and doses are in Appendix B.
   2. NSAIDs - The analgesic effects of NSAIDs in fish are questionable, but the anti-inflammatory effects may provide benefit. See Appendix B.
   3. Topical analgesia - amino amides (lidocaine)

3. Signs of Stress, Distress, and Pain
   1. Fish react to noxious stimuli, distress, and discomfort. Signs of distress and discomfort in fish may include, but are not limited to, the following:
      1. Listlessness
      2. Decreased Appetite
      3. Loss of body condition
      4. Abnormal behavior
      5. Social isolation
      6. Abnormal orientation
      7. Darker coloring
      8. Rapid opercular movement
      9. Rapid gill or mouth movement
      10. Agitated swimming

4. Surgery

1. Preparation of the Surgical Area
   1. According to the Guide for the Care and Use of Laboratory Animals: Eighth Edition, "For most survival surgery performed on rodents and other small species...the space should be dedicated to surgery and related activities when used for this purpose, and managed to minimize contamination from other activities conducted in the room at other times." (pg. 144)
   2. The surgical area should be a room or a portion of a room that is easily sanitized and not used for any other purpose during the time of surgery.
   3. Clean and disinfect the surface upon which the surgery will be performed with an approved environmental disinfectant before beginning the surgical procedure.

2. Preparation of Surgical Supplies
   1. Surgical Instruments
      1. Use prepackaged aseptic surgical supplies whenever possible.
      2. Initial steam sterilization (autoclaving), plasma vapor sterilization, or ethylene oxide sterilization (for heat or pressure sensitive items) is required for all surgical instruments and items to be implanted.
   3. Preparation of the Animal
      1. Fasting
         1. Depending on animal size, species, and procedure, consider fasting animals for up to 24 hours prior to anesthesia. Consult a ULAM veterinarian to determine if fasting is appropriate/recommended.
      2. Anesthesia
         1. Use an approved agent appropriate for the species AND the procedure.
         2. The animal must be maintained in a surgical plane of anesthesia throughout the duration of the procedure
      3. Skin disinfection
         1. Due to the protective mucus layer present on fish skin, aggressive cleansing is not recommended. The skin should be prepared with a single wipe using sterile saline or a dilute povidone iodine or chlorhexiderm solution immediately before surgery. Removal of scales is generally not recommended and should be avoided or minimized when possible.
   4. Surgeon Preparation
      1. Wash hands thoroughly with a disinfecting soap such as chlorhexidine or iodine based surgical scrubs or 3M Avaguard® hand antiseptic.
      2. The surgeon must wear a mask, sterile or clean gloves, and a clean scrub top, clean disposable PPE gown, or clean lab jacket during the surgical procedure.
      1. Clean gloves include unused standard latex or nitrile lab gloves stored in a sealable bag or container to minimize dust and debris contamination.
   5. Performing Multiple Surgeries in Series
      1. Begin with at least 2 sets of sterile instruments.
      2. Between animals, clean the instruments and sterilize with a hot bead sterilizer.
      1. It is imperative that tools are completely cooled after sterilization to avoid thermal damage to the animal.
      2. Cold sterilization products require prolonged contact times, present health hazards the animals and/or surgeons, and are not recommended.
      3. No more than 5 animals should be operated on per pack of sterile instruments.
      4. Use new clean or sterile gloves for each animal.
      5. Clean the surgical area with an appropriate disinfectant between animals.
6. Suture Materials and Wound Closure
   1. Use monofilament suture material (e.g. PDS, Maxon, or Ethilon) to minimize tissue reactions and infection that could slow healing time. Braided materials such as vicryl or silk are generally contraindicated for skin closure in fish despite their historical use. Tie sutures snugly for a watertight seal. Post-operative swelling in fish is minimal, unlike mammals.
   2. Absorbable suture materials have been recommended as a means of avoiding additional handling for removal. Remove all skin sutures (absorbable or nonabsorbable) by 14 days after surgery if they are still present (provided the skin incision is adequately healed), unless described otherwise in a IACUC approved protocol or as recommended by a ULAM veterinarian to necessitate complete wound healing.
   3. Tissue adhesives (e.g. cyanoacrylate) are not recommended for use in fish (associated with tissue reactions and wound dehiscence). Similarly, surgical staples are regarded as inferior to appropriately placed sutures for wound closure.
   4. Routine prophylactic antimicrobial use is generally not necessary if appropriate aseptic technique is utilized. Contact the ULAM veterinary staff for cases that may warrant antibiotic treatment (contaminated wound repair, inadvertent bowel perforation, other break in aseptic technique).

7. Post-Operative Monitoring and Care
   1. Animals must be visibly observed and monitored every 15 minutes during recovery from anesthesia until they resume normal swimming (refer to the Recovery and Post Anesthetic Care section above).
   2. Post-operative medications including analgesics, antibiotics and/or anesthetic reversals should be administered during the early recovery period and according to the approved protocol or the advice of a ULAM veterinarian. Tanks/enclosures containing fish that have undergone surgery must be labeled.
      1. The label should include the date of surgery.
      2. Records should document animal condition, pre and post-operative drugs, concerns noted during surgery and post-operative recovery notes. Post-operative monitoring can be in the form of a post-surgical record or as poorly recovering post-operative fish clinically appear similar to a spontaneously ill fish, the post-operative monitoring can be incorporated into the daily health check process with associated daily documentation.
      1. Records must be kept near the animal, either in the animal housing room or in an adjacent procedure area.
   5. Monitoring should continue until skin sutures are removed, or if sutures are not placed, fish should be monitored specifically for post-operative complications in the first 24 hours following surgery and after that time their post-operative monitoring can be incorporated into daily health checks.

5. Appendices

1. Appendix A: Anesthetic Agents for Use in Fish

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose/Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immersion</strong></td>
<td></td>
</tr>
<tr>
<td>Tricaine</td>
<td>50-175 mg/L (zebrafish)</td>
</tr>
<tr>
<td>(Recommended)</td>
<td>25-700 mg/L (various species, consult veterinarian)</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>25-100 mg/L (zebrafish)</td>
</tr>
<tr>
<td></td>
<td>5-600 mg/L (various species, consult veterinarian)</td>
</tr>
<tr>
<td><strong>Parenteral</strong></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>2-5 mg/kg IM (when used with dexmedetomidine)</td>
</tr>
<tr>
<td>Dexmedetomidine</td>
<td>0.025-0.05 mg/kg IM (when used with ketamine 2-5 mg/kg)</td>
</tr>
<tr>
<td></td>
<td>Reverse with atipamezole, equal volume dexmedetomidine (2-10x the dose)</td>
</tr>
</tbody>
</table>

2. Appendix B: Analgesic Agents for Use in Fish

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Interval (hours)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Opioids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butorphanol</td>
<td>0.1-0.4</td>
<td>IM</td>
<td>24</td>
<td>Efficacious dose varies with species</td>
</tr>
<tr>
<td>(Recommended)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>0.3</td>
<td>IM</td>
<td></td>
<td>Used in research, dose varies with species.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Immersion delivery of morphine (10mg/L) has</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>demonstrated analgesia in animals challenged</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>with electric shock.</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Not reported</td>
<td></td>
<td></td>
<td>Wide range of safety and efficacy in other</td>
</tr>
<tr>
<td></td>
<td>in fish,</td>
<td></td>
<td></td>
<td>veterinary species</td>
</tr>
<tr>
<td></td>
<td>0.01-0.02 in dogs and cats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Strength</td>
<td>Route</td>
<td>Duration</td>
<td></td>
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<tr>
<td>---------</td>
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<td></td>
</tr>
<tr>
<td>Carprofen</td>
<td>2.0-4.0</td>
<td>IM</td>
<td>72-96</td>
<td></td>
</tr>
<tr>
<td>Flunixin</td>
<td>0.25-0.5</td>
<td>IM</td>
<td>72-96</td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2.0</td>
<td>IM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.1-0.2</td>
<td>IM</td>
<td>24-48</td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1.0-2.0 (max)</td>
<td>Topical, local injection</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

*When pre-emptive analgesia is used, consider reducing the dose of anesthetic (whether inhalant or injectable) to the low end of the recommended range. Anesthetic depth must be carefully monitored and drug doses may need to be titrated to maintain appropriate levels. With new projects, sexes, strains or anesthetic analgesic combinations, assess a subset of animals before expanding to use in a larger cohort.

6. **References**

18. Western Chemical. TRICAINE-S Brand of Tricaine Methanesulfonate For Anesthesia and Tranquilization of Fishes and Other Cold-Blooded Animals. 4/2014.