IACUC Guidance:	**TAMU-G-035**	Title:	**Guidelines for Performing Surgery in Fish**

Location	Effective Date	Review By
College Station/Dallas/Galveston/Kingsville	10/20/2022	06/30/2025
Houston	N/A	N/A

1. PURPOSE

1.1. To define surgical standards and best practices when planning and performing surgical procedures on fish species used for research, teaching, or other purposes at Texas A&M University.

2. SCOPE

2.1. Applies to fish species undergoing surgical procedures.
  2.1.1. Field conditions may require protocol-specific exemptions.
2.2. Does not contain a complete discussion of MS222, see TAMU-G-021
2.3. For guidelines specific to zebrafish, see TAMU-G-008
2.4. These guidelines are general recommendations and consequently do not factor in specific research associated concerns.

3. RESPONSIBILITY

3.1. Performing surgery in fish requires a knowledge and skill set that differ from that for mammalian surgery in a number of ways. Survival surgery in these species should not be attempted unless personnel have been properly trained and are competent to perform the procedures.
3.2. The **PI** is responsible for:
  3.2.1. Following these guidelines for approval of protocols which include surgery.
  3.2.2. Listing participants, with their qualifications to perform the activities or procedures selected, on the AUP when initially submitted.
  3.2.3. Ensuring that AUP personnel complete all training and BOHP enrollment activities as outlined in TAMU-G-029.
  3.2.4. Maintaining documentation of personnel training readily available to IACUC members or AWO staff.
  3.2.5. Awareness of the regulatory status of the species they intend to use in their studies. Regulations governing endangered species may be reviewed in Title 50 of the Code of Federal Regulations, part 17 for ESA regulations and part 23 for CITES (Convention on International Trade in Endangered Species of Flora and Fauna).
  3.2.6. Obtaining the necessary permits for wildlife studies.
3.3. The **IACUC** is responsible for:
  3.3.1. Reviewing the stated experience and qualification of protocol participants and identifying any needed additional training requirements.
  3.3.2. Inspecting and approving all surgical areas and procedure rooms (where surgery will occur) prior to use.
3.4. The **PI and the AV** share responsibility for ensuring that postsurgical care is appropriate.
3.5. **PI and Surgeon**: Responsible to ensure appropriate surgical preparation, technique and monitoring for each animal, as well as to oversee the animal's post-operative recovery period.

4. DEFINITIONS AND/OR ACRONYMS

4.1. **Analgesia:** Provision of pain relief without loss of consciousness
4.2. **Analgesic**: Drug used to relieve pain.
4.3. **Anesthesia**: Temporarily induces loss of sensation with or without loss of consciousness. Typically does NOT provide adequate post-procedural pain relief.
4.4. **Aseptic Surgical Techniques**: Well-established methods used to avoid the introduction of microbial contamination into tissues exposed and/or manipulated during surgery.

4.5. **AUP:** Animal Use Protocol. Document submitted by the PI indicating the housing and research procedures involving animals.
4.6. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
4.7. **BOHP**: Biosafety Occupational Health Program. Provides occupational health services to personnel at risk of exposure to animals or infectious biohazards in the course of their participation in IBC or IACUC permitted research, teaching or diagnostic activities.
4.8. **Fish**: A vertebrate animal that lives in water, typically breathes via gills, and usually have fins and scales. Includes all jawless (hagfishes and lampreys) and jawed vertebrates (cartilaginous and bony) fishes.
4.9. **Immersion:** A method of delivering drugs via direct contact with the skin or gills in a bath.
4.10. **Major Surgery:** Usually penetrates and exposes a body cavity, and includes the potential for significant impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. Examples: laparotomy, thoracotomy, joint replacement, and limb amputation.
4.11. **MS222**: Tricaine methanesulfonate is an FDA-approved drug ("Tricaine-S") for temporary immobilization (sedation, anesthesia) of finfish, amphibians, and other aquatic, cold-blooded animals.
4.12. **Non-survival surgery**: A surgery in which animals are euthanized under general anesthesia prior to anesthetic recovery.
4.13. **PI**: Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
4.14. **Preemptive Analgesia**: The administration of preoperative and/or intraoperative analgesia that is intended to take effect before the experience of pain.
4.15. **Sedation**: Central depression causing stupor where the animal is unaware of its surroundings but still responsive to painful procedures.
4.16. **Surgery**: Cutting into the body through the use of a tool such as a scalpel blade, surgical scissors, laser, or other suitable device. May also refer to an invasive measurement under anesthesia.
4.17. **Survival surgery**: A surgery in which animals are expected to recover from anesthesia following the procedure.

5. GUIDELINES OR PROCEDURE

5.1. **Handling and Restraint**

5.1.1. Wear clean, wet exam gloves when handling fish.
5.1.2. Moisten gloves with clean dechlorinated water, or water from the animal's home tank or environment.
5.1.3. Completely rinse powder from gloves before handling, if present.
5.1.4. In some field conditions, clean wet hands (without gloves) are acceptable.
5.1.5. Use nets to handle fish whenever possible as fish have a protective mucus layer on their skin and are slippery and difficult to hold.
5.1.6. Working surfaces for medical/surgical procedures must be non-abrasive but also prevent the fish from sliding, such as a soft moist cloth.
5.1.7. Anesthetize fish during any procedure that is stressful or likely to cause pain.
5.1.8. Cover fish eyes to prevent retinal damage from surgical lighting and surgical manipulations. Consider sterile gauze over a layer of ophthalmic ointment, especially for fish without eyelids.
5.1.9. Ensure fish remain wet during procedures out of the water. Water can be dripped, poured, or sprayed on exposed areas of the fish.
5.1.10. For fish from aquaria, use water from the home tank wherever possible; clean non-chlorinated water is also acceptable.
5.1.11. Fish should be maintained at the temperature for which they are acclimated. For cooling, ice cubes may be frozen in advance using water sources as indicated above. Ensure ice does not come in direct contact with the fish's skin and that cubes are removed and replaced as they begin to melt to maintain temperature, and salinity in the case of saltwater species.

5.2. **Anesthetics**

5.2.1. All animals should have a pre-surgical assessment to ensure they are not overtly ill.

5.2.2. Topical or local anesthetics are used infrequently.

5.2.3. Immersion or injection, either intraperitoneal (IP) or intramuscular (IM), is used to administer general anesthetics.

5.2.4. Hypothermia is **not** a humane or acceptable method of anesthesia.

5.2.5. **Injectable Anesthetic Agent**

5.2.5.1. If used, the gauge of the needle should be considered in relation to the size of the fish.

5.2.5.1.1. 25-28 gauge needle should be used for small fish,

5.2.5.1.2. 18-23 gauge needles are appropriate for larger fish.

5.2.5.2. Intra-peritoneal injections should be made in the ventral midline while aiming towards the head and spine (anterodorsal) to avoid the spleen.

5.2.5.3. Intramuscular injections may cause the loss of scales or cyst formation, for these reasons the pectoral muscle is the preferred IM injection site.

5.2.5.4. Food should be withheld for up to 24 hours before anesthesia to reduce the risk of vomiting, which impairs gill function. Contact AV, or designee for fasting recommendations of specific species.

5.2.6. **Immersion**

5.2.6.1. Immersion is the anesthetic method of choice for fish; see TAMU-G-021 for information regarding the use of MS222.

5.2.6.2. Clove oil and its extracts have become popular as anesthetic agents for freshwater and marine fish because of their wide availability, low cost, and shorter induction times when compared with MS222.

5.2.6.2.1. When compared with MS222 as an anesthetic agent, eugenol was found to have a more rapid induction, prolonged recovery, and narrow margin of safety, as it can cause rapid onset of ventilatory failure at high concentrations (> 400 mg/L).

5.2.6.2.2. For small tropical fishes, 0.05 mL of clove oil per 500 mL of water is the most efficient dose for studies where fish will be released back to their natural habitats.

5.2.6.3. For fish from aquaria, when possible, use water from the home tank as the source of water for the anesthetic tank.

5.2.6.4. Check the water quality parameters after adding the anesthetic, especially pH.

5.2.6.4.1. Buffer to a pH near the ambient environmental pH of the study species by the addition of sodium bicarbonate (NaHCO3), or seawater.

5.2.6.4.2. PH may be checked with either a calibrated pH meter or pH paper to ensure neutrality.

5.2.6.5. If fish must be anesthetized for longer than a few minutes, continuous delivery of an anesthetic agent in water to the gills will be required. Recirculating or continuous flow systems using aerated water containing anesthetic can be used after induction, to provide both continuous anesthesia and artificial ventilation.

5.2.6.5.1. 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.

5.2.6.5.2. 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 depend on the size of the animal being anesthetized.

5.2.6.5.3. Adjust the rate such that water flows into the mouth, gently over the gills and out the opercula for either flow anesthesia approach. Inappropriate flow rates can interfere with gas exchange or force water into the gastrointestinal tract.

5.3. **Monitoring Anesthesia**

5.3.1. 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 Table of Anesthesia Stages.

5.3.2. Activity decreases and the righting reflex is lost as fish become anesthetized.

5.3.3. Opercular movement (respiratory rate) progressively decreases with deepening anesthesia.

5.3.4. Hypoxemia can occur in fish despite good ventilatory efforts and can be recognized by pallor of the gills and the fin margins.

5.3.5. The heart rate can be directly monitored using a Doppler blood flow probe or ECG leads.

5.3.5.1. Monitor trends when using the Doppler or ECG; reference ranges are not known for many fish species.

5.3.6. Surgical planes of anesthesia can be confirmed by a lack of response to a firm squeeze at the base of the tail.

5.3.7. **Table of Anesthesia Stages**

Stage	Description	Fish Behavior
0	Normal	Reacts to stimuli; normal opercular rate and muscle tone.
1	Light Sedation	Slight decrease in reaction to stimuli; slight slowing of opercular rate; equilibrium unaffected.
2	Deep Sedation	Marked decrease in reactivity, only responds to strong stimuli, slight decrease in opercular rate, equilibrium unaffected.
3	Partial loss of equilibrium	Decrease in muscle tone, erratic swimming, increased opercular rate, reacts only to strong stimuli.
4	Total loss of equilibrium	Complete loss of muscle tone and equilibrium, loss of spinal reflexes; slow but steady opercular rate.
5	Loss of reflex reactivity	Loss of all reactivity, loss of all reflexes, slow and irregular opercular rate, bradycardia.
6	Medullary collapse; asphyxiation	Opercular movement stops, pending cardiac arrest and death.

5.4. **Emergency Care**

5.4.1. Immediately place the animal in untreated, aerated water and initiate forced ventilation if respiration stops altogether. Forced ventilation stimulates the buccal flow/heart rate reflex and provides support while speeding the elimination of the anesthetic.

5.4.2. Forced ventilation is accomplished by placing a tube from a small submersible pump into the fish's mouth and holding the fish upright until movement is resumed. Alternatively, forced ventilation may be performed 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.

5.4.3. For any fish with questionable opercular movement, gentle forced ventilation can help speed initial recovery. Forced ventilation enhances opercular movement and increase the passage of fresh, oxygenated water over the gills.
5.4.4. Respiratory arrest can precede cardiac arrest by several minutes; continue resuscitation efforts for several minutes despite a lack of immediate improvement. Contact the AV, or designee for emergency care.

5.5. **Tonic Immobility**
5.5.1. Some sharks and large bony fishes can be induced into a state of paralysis.
5.5.2. This tonic state and be exploited to conduct simple procedures (such as tagging) where anesthesia is impractical or unsafe for fish and/or personnel. The AV, or designee can consult on the use of this technique and recommend analgesics, where appropriate.
5.5.2.1. Include use of tonic immobility in the AUP.

5.6. **Analgesia**
5.6.1. The perception of pain in fish varies among species, which contributes to the controversy surrounding this issue.
5.6.2. All fish have the capacity for nociception. All fish exhibit behavioral and physiological effects from undergoing surgery or other types of stressors, such as lack of appetite, increased ventilation, opercular movement, and heart rate.
5.6.3. Administer analgesics where their use correlates with a reduction in behavioral or physiological stress reactions.
5.6.3.1. Include justification for withholding analgesia in the AUP.
5.6.4. Analgesics, which may be helpful in postoperative pain management, include butorphanol, morphine, and ketoprofen.
5.6.5. Analgesic properties of clove oil are currently unknown.
5.6.6. See **Appendix A** for a list of analgesic effects and side effects of analgesic drugs tested in fish
5.6.7. Consult with the AV, or designee to determine if the benefit of multiple doses of analgesics overrides the stress of handling.

5.7. **Asepsis**
5.7.1. The Guide and PHS Policy require survival surgery to be conducted using aseptic technique.
5.7.2. Instruments must be sterilized for survival surgery.
5.7.3. In cases where multiple surgeries will occur and instruments are reused, the instruments should be cleaned, soaked in a sterilizing solution (with adequate contact time) such as Cidex ™, and then rinsed in sterile saline/water to reduce tissue irritation.
5.7.4. Hot bead sterilizers may also be used to re-sterilize instruments. Ideally, a sufficient quantity of surgical instruments should be prepared so a sterile pack is available for each surgery.
5.7.5. Cap, mask, sterile gloves, and in some cases, sterile gown (preferable for major survival surgery) are recommended during surgery, but may not be possible in field settings. In all settings, the surgeon should avoid contamination of the surgical field.
5.7.6. The surgical area is disinfected by a modified aseptic technique utilized to protect the integrity of the mucus layer. The traditional surgical scrub should be replaced with either rinsing the skin with sterile saline or lightly wiping with a sterile saline moistened cotton swab. Another method of skin preparation is to gently swab the incision site with sterile saline and dilute iodine or chlorhexidine solution to reduce gross contamination.
5.7.7. Scales should only be removed if they are substantial enough to interfere with the incision. Scale removal will compromise skin integrity.

5.7.8. The use of a clear plastic surgical drape is highly recommended, because it helps create a moist environment for the fish, prevents strikethrough, and provides a sterile working surface. Incising the skin through the sterile plastic drape will enhance aseptic technique.

5.7.8.1. A variety of drape materials have been used (including rectal gloves). Drapes are usually held in position by moisture, Vaseline, or temporary suture. Large, clear, transparent plastic drapes with no adhesive or precut apertures, applied with a small bead of lubricating jell, are the most useful for isolating the surgical field.

5.8. **Surgical Technique**

5.8.1. Performing surgery in fish differs significantly from mammalian surgery. The fish's skin should be kept moist at all times; presoaking a foam V-tray and using clear plastic drapes help maintain a moist environment. Unlike mammals, fish have taut adherent skin, which makes closure of large defects challenging.

5.8.2. Fish do not have a linea alba. The surgeon should avoid making incisions in the lateral line, and the incision should follow the longitudinal axis of the fish. The coelomic cavity should be carefully entered, because the musculature of the body wall and the intestines look very similar in color. The body wall is fairly rigid and internal organs are not freely mobile, which cause the surgeon to have to work within the coelomic cavity rather than exteriorize organs. In some fish, such as the koi carp, visceral adhesions are normal and must be bluntly dissected to fully access the coelomic cavity.

5.8.3. **Suture Material**

5.8.3.1. Monofilament sutures such as polydioxanone or polyglyconate are preferred over multifilament sutures such as silk.

5.8.3.2. Suture size depends on the size of the fish; 2/0 to 4/0 sutures are commonly used.

5.8.3.3. Cutting tip needles cause less skin trauma and are favored over other needle types.

5.8.3.4. Both interrupted and continuous suture patterns can be used to close wounds.

5.8.3.5. Depending on the thickness of the body wall, a single or two-layer closure is appropriate. The skin is the strength layer of the closure. Fish are not prone to incision swelling, so sutures should be snug to ensure a water-tight seal. Both absorbable and non-absorbable material may be used. Absorbable sutures may not be readily absorbed in fish. Where practical, skin sutures should be removed once the wound has healed. Tissue glue and staples have been used in fish, but wounds closed by either method tend to have more complications.

5.9. **Post Procedural Care and Monitoring**

5.9.1. Fish should be monitored continuously until fully recovered from anesthesia, as evidenced by the return of the righting reflex and normal swimming behavior. Forced ventilation as described above under Emergency Care section may be utilized to aid in recovery.

5.9.2. When possible, fish from aquaria should be recovered in water from their home tank in a darkened and quiet area.

5.9.3. Once recovered from anesthesia, the fish should be monitored at least daily for general health and recovery from surgery. Fish should be carefully observed for the following complications: surgical wound breakdown, infection, osmotic imbalances, feed intake, weight loss, abnormal behavior, etc.

5.9.4. There may be some risk if the recuperating fish is placed in its home tank with other fish present because there is some risk of wound cannibalization and competition for food. Providing the recuperating fish with a shelter from other fish and an optimal environment will hasten wound healing and recovery.

5.9.5. Antibiotic therapy in fish should be given careful consideration. Imprudent use of antibiotics promotes the growth of antibiotic-resistant strains of bacteria and may require withdrawal times in food fish.

5.9.6. Due to regulatory requirements related to the use of antibiotics and other agents in wild fish, consultation with state fish and wildlife officials is highly recommended.

5.10. **Records** are required for every animal that has undergone surgery, must be maintained for the duration of AUP approval, and should contain:
- 5.10.1. Date of procedure
- 5.10.2. Protocol number
- 5.10.3. Identification of the type of surgery performed; e.g. “laparotomy”
- 5.10.4. Species and animal or cage identifier
- 5.10.5. The name of the surgeon and any assistants
- 5.10.6. Pre-surgical assessment
- 5.10.7. Pre-op preparation, as applicable
  - 5.10.7.1. Surgical scrub or site preparation
- 5.10.8. Event times or total time under anesthesia
- 5.10.9. Vital parameters monitored
- 5.10.10. Times of monitoring
- 5.10.11. A notation of any complication or abnormality identified
- 5.10.12. Drugs administered: dose, route and frequency of administration
  - 5.10.12.1. When DEA controlled substances are used, the date and drug usage volumes recorded in the controlled substance log and the dates and amounts recorded in the animal surgery records should match.
- 5.10.13. **Note:** Sample surgical records are available on the RCB/Animals web page and may be modified as needed

6. EXCEPTIONS

6.1. The PI may request an exception to the above standards by describing the departure in the AUP

6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

7.1. References/Resources:

- 7.1.1. CMP: https://vpr.tamu.edu/directory/comparative-medicine-program
- 7.1.2. CITI Working with Fish in Research Settings
  - 7.1.2.1. Web page: https://about.citiprogram.org/en/homepage/
  - 7.1.2.2. Instructions: https://rcb.tamu.edu/animals/training
- 7.1.3. www.ivis.org/advances/Anesthesia_Gleed/bowser/ivis.pdf
- 7.1.4. American Fisheries Society *Guidelines for the Use of Fishes in Research*
- 7.1.5. Chatigny F, Creighton CM, Stevens ED. Updated Review of Fish Analgesia. J Am Assoc Lab Anim Sci. 2018;57(1):5–12.
- 7.1.6. Fernandes, IM et al. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Braz J Biol. 2017 Jul-Sept;77(3):444-450.
- 7.1.7. Weber et al. Anesthesia, Diagnostic Imaging, and Surgery of Fish. Surgery Compendium. 2009. (Vol 31, No 2).
- 7.1.8. Wildgoose, William H. Fish surgery: An overview. Fish Veterinary Journal. Number 5. 2000.
- 7.1.9. "Fish anesthesia delivery system. An effective and economical recirculating fish anesthesia system uses a commercially available power head, plastic tubing and clamps from a hardware store, a 10-gallon aquarium, a custom-made acrylic support, and an open-cell foam V-tray cut to fit the patient. The primary flow of the anesthesia-laden water is delivered through the mouth to the gills, and trickles down through the foam to the aquarium reservoir for recirculation. A secondary flow can be diverted to keep the skin moist. Plastic rodent cages work well in place of the aquarium". Source: Harms, CA.

Surgery in Fish Research: Common Procedures and Postoperative Care. Lab Animal Vol. 34, No. 1, January 2005.

7.1.10. Additional information on antibiotic therapy in fish may be found in Stoskopf, M.K. (1993) Fish Medicine. 902pp. Philadelphia: W.B. Saunders.

7.1.11. Additional information on flow rates may be found in Stetter MD. Fish and Amphibian Anesthesia. Veterinary Clinics of North America: Exotic Animal Practice. 2001,4(1):69-82.

7.2. IACUC/AWO Referenced Documents (requires TAMU NetID authentication): Texas A&M IACUC Guidance – Division of Research (tamu.edu)

7.2.1. Sample surgical record forms – see **Sample Documents** section of web page

7.2.2. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards

7.2.3. TAMU-G-008 Guidelines for Working with Zebrafish

7.2.4. TAMU-G-010 Guidelines for the Use of Pharmaceutical and Non-Pharmaceutical Grade Drugs and Compounds

7.2.5. TAMU-G-021 Guidelines for Preparing MS222

7.2.6. TAMU-G-029 Guidelines for Animal Use Protocol Participation and Handling

7.3. Acknowledgements

7.3.1. This document was partially prepared using materials obtained from the University of Michigan.

8. HISTORY

Effective Date	Version #	Description
08/02/2021	000	College Station/Galveston: New Document
03/24/2022	001	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
07/01/2022	002	College Station/Galveston: Renewal; updated procedural section to add maintenance of temperature, additional method of forced ventilation, examples of stress reaction, surgical record retention, sample surgical record availability. Updated references and added Exceptions section. Reviewed and approved via email.
10/20/2022	003	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.

Appendix A: Analgesic effects and side effects of analgesic drugs tested in fish (adapted from Chatigny, et al.)

Agent (schedule)	Dose	Species (route)	Side Effects	Beneficial effects
Opioids				
Buprenorphine (III)	5µM , 0.005–0.2 µg/mL	Zebrafish (W)	Hyperactivity	Reversed thermal aversion Ameliorated behavioral changes
	0.01–0.1 mg/kg	Rainbow trout (IM, SC)	Depressed activity at 1 mg/kg	Ameliorated ventilation and heart rates
Butorphanol (IV)	0.4 and 10 mg/kg	Carp (IM)	Decreased ventilation rate and buoyancy problems	Mild behavior-sparing effect Improved food consumption
	0.25-5 mg/kg	Chain dogfish (IM)	—	—
	0.1-0.4 mg/kg	Goldfish (IM)	—	—
	0.2 and 0.5 mg/L	Zebrafish(W)	—	—
Morphine (II)	10–50 mg/kg and 0.12–48 mg/L	Goldfish (IM, W)	Hyperactivity	MAC reduction Ameliorated pain related behaviors
	40 and 300 mg/kg	Rainbow trout (IM, IP)	—	ED_{50} = 6.7 Ameliorated pain related behaviors and ventilation
	17 mg/kg IV and 40 mg/kg IP	Winter flounder (IP, IV)	Bradycardia followed by a prolonged increase in cardiac output and heart rate	Blocked cardiovascular response to a noxious stimulus
	3 and 6 mg/kg and 1, 2, and 48 mg/L	Zebrafish (IM, W)	Hyperactivity	Possible anxiolytic effect Ameliorated activity
	300 mg/kg	Atlantic salmon (IM, IP)	—	Possible anxiolytic effect
	5 and 20 mg/kg	Carp (IM, IP)	Hyperactivity	Ameliorated ventilation and food consumption Antiinflammatory effects
Tramadol (IV)	10–100 nmol/g	Carp (IM)	—	Increased nociceptive threshold
	10 µg/fish	Zebrafish (IM)	Hyperactivity and surface respiration	—
NSAID				
Aspirin (OTC)	1 and 2.5 mg/L	Zebrafish (W)	—	Ameliorated activity and ventilation

Agent (schedule)	Dose	Species (route)	Side Effects	Beneficial effects
Carprofen (Vet)	1–5 mg/kg	Rainbow trout (IM)	Depressed activity at 5 mg/kg	—
Flunixin (Vet)	0.5 mg/kg	Rainbow trout (IM)	—	—
	8 and 20 mg/L	Zebrafish (W)	—	—
Ibuprofen (OTC)	5–470 mg/L	Fathead minnows (W)	—	Antiinflammatory effects
	400 µM	Zebrafish (W)	—	—
Ketoprofen (Rx)	2 mg/kg	Carp (IM)	—	Reduced postsurgical muscle damage
	1–4 mg/kg	Chain dogfish (IM)	—	—
	0.5–2 mg/kg	Goldfish (IM)	—	MAC reduction
	2 mg/kg	Rainbow trout (IM)	—	—
Ketorolac (Rx)	0.5 mg/kg	Rainbow trout (IM)	—	—
Local anesthetics				
Lidocaine (Rx)	4.5–18 mg/kg	Rainbow trout (SC)	—	—
	1–5 mg/L	Zebrafish (W)	—	Ameliorated activity and ventilation
Others				
Medetomidine (Vet)	0.01–0.025 mg/kg	Goldfish (IM)	—	MAC reduction

—, no pertinent information reported; MAC, minimum anesthetic concentration; Nd, no data; OTC, over the counter; Rx, not restricted but prescription required; vet, for veterinary use only; W, in the ambient water

* When preemptive analgesia is used, consider reducing the dose of anesthetic 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.
* See TAMU-G-010 for a discussion of pharmaceutical and non pharmaceutical grade drugs and compounds.