Florida Atlantic University
Institutional Animal Care and Use Committee
Standard Operating Procedure
Fish Anesthesia and Surgical/Biological Sampling Procedures – Field Studies

Performance Standard:
To minimize the risks associated with anesthesia and surgical procedures and reduce post-operative pain. This is accomplished by ensuring animals are appropriately assessed and monitored during pre-, intra-, and post-procedural periods. To assure proper documentation is provided for confirmation of quality care.

Background/Purposes:
This SOP in conjunction with FAU Policy 10.4.24 is intended to outline the pre-, intra-, and post-procedural requirements for fish that undergo general or localized anesthesia for experimental (i.e. imaging, ultrasound), surgical, and/or biological sampling procedures in a field setting, either onshore or on/alongside a boat.

Responsibilities:
1. Researcher/Investigator:
   a. Describe all anesthetic/surgical procedures and the methods used to monitor animals during pre-, intra-, and post-procedural periods in the animal protocol that will be given to the animal post-surgery.
   b. Ensure compliance with all relevant IACUC SOPs and policies regarding animal anesthesia, surgery, and euthanasia.
   c. Ensure appropriate training of personnel and documentation of procedures in accordance with this and other relevant IACUC SOPs and policies.
   d. Ensure that all anesthesia and surgical procedures are followed and documented per approved protocols.
   e. Assure animals receive adequate post-surgical/procedural care.
   f. Assuring that any anesthetic drug administered to animals is within the manufacturer provided expiration date.
   g. Contact the IACUC whenever you see unexpected complications associated with the procedure that have not been identified in the protocol.
   h. Contact a veterinarian if there are post procedural/ post-surgical health related concerns.

2. IACUC:
   a. Review and approve protocols/amendments to protocols.
   b. Assure adequate training of personnel.
   c. Inspect animal facilities/laboratories at least semiannually to ensure records are maintained and approved procedures are followed.
   d. Review/ report animal welfare issues.
   e. Check that anesthetic drugs are administered to animals as described in the IACUC protocol and are within the manufacturer provided expiration date.

f. Post approval review of anesthetic and surgical procedures

3. Research Integrity:
   a. Serve as liaison between the IACUC and PIs to ensure surgical procedures are consistent with regulatory requirements.
   b. Keep relevant training records
   c. Coordinate communication between research personnel, CM and Training Coordinator to assure training requirements are met in accordance to IACUC Policy.

4. Attending veterinarian and/or designee:
   a. Provide guidance/oversight on surgery programs and post-surgical care.
   b. Provide consultation services to investigators on the appropriate choice of anesthetic agents.
   c. Assist with training personnel and participate in procedures as required to ensure animal health and well-being.
   d. Verify proficiency of personnel in approved experimental procedures as determined by the IACUC.
   e. Provide support regarding proper maintenance of equipment.

SOP Outline:

A. SPECIFIC REQUIREMENTS FOR SURVIVAL PROCEDURES

1. Pre-procedural planning

a. Pre-procedural planning is critical to the success of surgical and anesthetic procedures in animals and must include input from all members of the surgical team.
b. The plan has to identify personnel, their roles, and experience in those roles so training requirements can be identified and addressed; equipment and supplies required for the procedures planned. The first goal of fish surgery should be to minimize handling of fish.
c. Field studies with fish are typically performed either on a boat or onshore near water.
d. The overall goal of fish surgery should be to minimize handling of the fish. Thus, the importance of basic planning of the procedure cannot be overstated.

2. Preparation of the surgical arena

a. When surgical procedure has to be performed in a field setting or on a boat, the surgical arena should be located in an area with the least amount of traffic.
b. There should be an area specifically designated for surgery where no other procedures are performed during the surgical procedure. In certain situations (i.e., large fishes), it is acceptable to implant transmitters alongside the boat with the animal in the water.
c. The surgical "table" must be stable and must be constructed of a non-porous material that can be disinfected using appropriate agents (see Appendix A: Table 1). On a boat, a designated flat surface of the deck or part of the bow/stern can be used. A surface disinfectant must be available at all times in the designated procedural area(s).
d. Disinfect the surgical area and equipment prior to each surgery. On a boat, when multiple surgeries are to be performed, the surgical area should be sanitized with water

and/or a bleach dilution (followed by a water rinse) between surgeries, and then thoroughly sanitized between capture sites or (minimally) at the end of the day.
    i. Ensure that surface disinfectants will not be toxic to fish that are subsequently placed upon those surfaces. See **Appendix A: Table 1** for suggested hard surface disinfectants.
e. The area immediately surrounding the surgical area should also be wiped down prior to surgery to decrease dust borne contaminates if applicable.

3. Sterilization of surgical instruments

NOTE: Alcohol is NOT a sterilant and will not be approved as such by the IACUC

a. All surgical instruments must be sterile.
b. Instruments sterilization can be achieved in a number of ways. Heat sterilization via steam (autoclave) is the preferred method. (See Appendix A: Table 2)
c. Chemical sterilants:
    i. Virkon Aquatic (Western Chemical, Inc.) is an oxidizing agent with potassium peroxymonosulfate as the active ingredient. It is approved for use in aquaculture with labeled efficacy against some fish pathogens.
    ii. Aldehydes must be thoroughly rinsed off of instruments with sterile saline or sterile water before use.
    iii. Peracetic Acid is a very powerful oxidant. It is compatible with most materials. Needs to be made fresh.
    iv. Chlorine products will corrode stainless steel instruments and have extreme toxic effects on fish, and therefore should not be used.
d. Sterilized instruments stored in sterile unopened packages must be labeled with the date of sterilization and have 6-month shelf life if stored properly.
e. In field research settings where there are multiple surgeries performed and instruments reused, instruments should be thoroughly cleaned and held in a disinfecting solution for a sufficient time (at least 10 minutes), and then rinsed well with sterile saline.
    i. It is preferable to have a sufficient quantity of surgical instruments prepackaged and autoclaved prior to the surgeries.
    ii. Within a given cohort of fish, multiple sets of instruments can be rotated through the surgery and disinfection processes. This would involve a series of instrument baths so that the flow of the procedures is not interrupted. Instruments should be manually cleaned prior to disinfection, and care taken to meet the required contact times of chemical disinfectants.
f. Surgical instruments, suture materials, and surgical implants such as transmitters should be considered contaminated at the first contact with nonsterile objects or surfaces (e.g., the outside of a fish, a surgery platform, or unsterile water).
g. All materials to be implanted into animals must be **sterile and biocompatible**. When available, industry-made implants that are available in sterile packages should be used.
    i. Ideally, transmitters should be sterilized (**Appendix A: Table 1**) several days before implantation procedures, with sterilization performed in the controlled conditions

of a laboratory. Sterile transmitters can then be transported to the surgery site in sterile sample bags or other sterile packaging.

4. **Pre-surgical/procedural evaluation of the animal**
   a. Evaluate the fish to ensure its health. The animal should be alert and responsive, with clear eyes. Attention should be given to the fish's level of exhaustion, particularly if its capture involved a prolonged struggle.
   b. Researchers should establish minimum standards for fish health before beginning a project. The maximum proportion of descaling that will be tolerated, whether fish with visible lesions will be tagged, and the acceptable parasite load for participating fish are examples of criteria necessary to optimize study outcomes.
   c. Whenever possible, weigh animals pre-operatively and record weight on surgical log. A hanging/spring scale attached to a cloth bag or soft net can be useful for weighing larger fish. Animal weights are especially useful if drug dosages need to be calculated.

5. **Infection prevention**
   a. A single application of povidone iodine ointment to the closed incision before returning the fish to the water may be successful in reducing the incidence of oomycete infection, although retention time is limited.

6. **Anesthesia**
   a. Anesthetic drug use in research fish released to the wild following surgery raises regulatory dilemmas if the fish has the potential for entering the human food supply.
      i. Consultation with state fish and wildlife agencies is advisable.
   b. Limited options exist for anesthesia in field research settings.
   c. The first step in a successful surgical procedure is to establish a safe and effective anesthetic protocol.
   d. For short procedures (< 5 min), use of local anesthesia or submersion in anesthetic "to effect" may be adequate.
   e. Whenever possible, general anesthesia should be used when fish undergo surgical implantation procedures.
      i. Carbon dioxide – legally allowable in fish that will be immediately returned to the wild
         1. CO_2 anesthesia should be administered in conjunction with pure O_2 introduced with an ultrafine diffuser (e.g., bubbles <1 mm) to ensure supersaturated O_2 (>100% saturation) conditions.
         2. Implementation of CO_2 anesthesia requires training and experience. The IACUC will work directly with researchers to develop safe and effective CO_2 anesthetic protocols.
      ii. Tonic immobility – induces immobility with rapid recovery for manipulation of elasmobranchs during field procedures. To date, however, experimental data are lacking for the induction of a true unconscious state or full lack of

pain perception in fishes subjected to tonic immobility. The FAU IACUC will review protocols using tonic immobility for surgeries/painful procedures on a case-by-case basis.

f. Stages of sedation with general anesthesia – see **Appendix A: Table 5**
g. Local anesthesia
    i. Local anesthetics interrupt nerve conduction in a specific region of the body, thereby preventing the noxious stimulus from being conducted to the central nervous system. They do not provide analgesia, only regional anesthesia.
    ii. Lidocaine or lidocaine/bupivacaine blocks should be performed by one or more subcutaneous injection(s) of around the incision site, allowing 5 minutes for absorption prior to cutting.
        1. Each injection should be $\leq$ 1 ml
    iii. Dosages should be species-specific and based on the relevant literature, and protocols should state a maximal total dose.
    iv. Lidocaine is acidic and can cause pain and inflammation upon injection; therefore, it should be diluted 3:1 with sodium bicarbonate to yield a neutral pH.

7. Surgical preparation of the animal

a. Move the animal to the surgical area and make sure to position the patient correctly. Stabilize the animal in the correct position.
    i. In certain field situations involving very large fishes, some surgical procedures may be performed along the side of the boat with the animal in dorsal recumbency.
b. Surgical preparation should minimize disruption of the skin and natural mucus, because these are natural barriers to infection.
c. A simple swipe along the intended incision site with a cotton swab soaked in sterile saline, dilute povidone iodine (e.g., Betadine), or dilute chlorhexidine solution to reduce gross contamination suffices in place of a traditional surgical scrub.
d. Removing scales along the incision line facilitates a smooth entry; however, this practice should be limited to fish with scales robust enough to interfere with the incision and to the minimum amount necessary, as scale removal further disrupts the skin.
e. Draping the surgical field with a clear plastic sterile drape presents many advantages and should be used whenever practicable, especially for longer or more invasive surgical procedures.

8. Preparation of the Surgeon

a. The surgeon should thoroughly clean his/her hands and don sterile surgical gloves if appropriate to the circumstances. (See Appendix A: Table 3). Take care to put gloves on aseptically. Gloves must be changed between individual animals.

9. Intra-operative care and monitoring

a. Keep the skin moist throughout the surgical procedure, while taking care to avoid irrigating the incision site with water.
b. Incision placement will depend on the body type and size or life stage of fish.

i. Researchers should strive to make the smallest incision possible for the procedure, to minimize the time needed for surgery and the number of sutures required.

c. Minimize handling of the skin along the incision line to reduce post-surgical inflammation.
d. The muscle color of the body wall in fish is often very similar to that of the underlying intestines, necessitating a carefully controlled entry into the coelomic cavity to prevent intestinal damage.
e. Organs are not freely mobile, so surgeons must perform manipulations within the coelomic cavity rather than exteriorizing the organs. Many cyprinids normally have visceral adhesions that must be bluntly separated to navigate the coelomic cavity if necessary.
f. Intra-operative monitoring should include monitoring of ventilation rate, movement, and the use of a Doppler ultrasound (if available) to monitor heart rate.
g. Evaluation of anesthetic depth is important. **Caution:** the zone between enough anesthesia and too much is very narrow in fish.

10. Closure of the coelomic cavity

a. An abundance of freely mobile skin is rarely encountered in fish, so skin defects due to incision may be difficult to close.
b. Many suture materials have been used successfully in fish. Monofilament sutures such as polydioxanone (PDS; See Appendix A: Table 4) or polyglyconate are preferable to multifilament sutures such as polyglactin 910 (Vicryl, Ethicon), due to the ability of multifilament sutures to wick contaminants into the tight seal.
    i. Fish may not readily absorb nominally absorbable sutures.
    ii. In wild fish for which suture removal is not feasible, closure materials should be considered as permanent or semi-permanent regardless of their classification.
c. Tissue glue (i.e., cyanoacrylate tissue adhesive) and staples can be problematic for incision closure in fish, causing dermatitis and leading to incision dehiscence, and therefore are not recommended.
d. Holding tissue layers such as skin should be sutured using simple continuous, simple interrupted, horizontal mattress, or continuous Ford interlocking suture pattern.
    i. Continuous patterns reduce drag, minimize knot surface area, and reduce surgery time, but may be more prone to loosening if adequate tension is not maintained through the entire line and if knots at either end are not secure.
        1. Interrupted patterns may be more appropriate for inexperienced surgeons.
    ii. Suture pattern choice will depend on the size, location, and depth of the incision.
    iii. Sutures should be tied snugly (but without excessive tension) to ensure a water-tight seal.
    iv. Needles with a cutting tip facilitate skin penetration.
e. Single or two-layer closure is usable depending on the thickness of the body wall; skin is the strength layer of the closure.

i. The subcutaneous layer is minimal in fish, with dermis tightly adhered to the underlying muscle, so there is rarely any dead space to eliminate.

11. Providing appropriate post-surgical/procedural care

a. Wild-caught fish should not be released into natural waters until they have fully recovered from the effects of anesthesia to avoid predation or impingement in natural substrates.
b. Observe all fish until completely recovered from anesthesia. Complete recovery is defined as the ability of the animal to maintain an upright position and make purposeful, coordinated movements.

12. Documentation requirements

a. Maintain records until the end of the study with all other study related documents.
b. Documentation must include: PI name, IACUC protocol number, species, experimental procedure performed, date of the procedure, administration of anesthetic agents, including dose, volume injected, route, and time of administration and any complications encountered during or after the procedure.
c. All records must be available on demand from the IACUC, outside agencies (e.g., AAALAC, NIH OLAW), and the veterinary staff.

Appendix A:

Table 1: Hard Surface and Instrument Disinfectants

NAME	EXAMPLES	COMMENTS
Aldehydes	Glutaraldehyde (Cidex®, Cide Wipes®)	Rapidly disinfects surfaces, only 12 min contact time required for disinfection of surgaces and/or instruments between animals. Compatible with most materials. Toxic. OSHA has set exposure limits. Many hours required for sterilization. Consult safety representative on proper use.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.
Bleach (sodium hypochlorite)	10–20% bleach solution	Rapidly disinfects surfaces and instruments. Environmentally friendly (rapidly breaks down in salt and water). Be sure to use non-chlorine (“color-safe”) bleach solutions.
Potassium peroxymonosulfate	Virkon® Aquatic	Approved for use in aquaculture. Effective against a broad range of fish pathogens.

*The use of brand names as examples does not indicate a product endorsement.
Always follow manufacturer's instructions.

Table 2: Methods of Initial Instrument Sterilization		
AGENTS	**EXAMPLES** *	**COMMENTS**
Physical: Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs. 131°C for 3 min).
Physical: Dry Heat[1]		Make sure instruments are clean and free of debris before using this method for effective sterilization
Aldehydes[2]	Glutaraldehyde, Dialdehyde, Ortho-phthalaldehyde (Cidex OPA®)	Many hours required for sterilization. Consult safety representative on proper use. Only 12 min contact time required for moderate disinfection, 20 min for high disinfection. 10 hours required for true sterilization. Compatible with most materials.

[1]This method should only be used between animals with instruments previously sterilized with another method.
[2]Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before use.

Table 3: Skin/Tissue Disinfectants		
AGENTS	**EXAMPLES**	**COMMENTS**
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbe killing action. Works best in pH 6–7.
Chlorhexidine	Nolvasan®, Hibiclens®	Rapidly bactericidal and persistent. Effective against many viruses. Presence of blood does not interfere with activity. Do not use near eyes.

The use of common brand names as examples does not indicate a product endorsement.
NOTE: Alternating disinfectants is more effective than using a single agent.

Table 4. Suture Selection	
SUTURE	**CHARACTERISTICS AND FREQUENT USES**
Vicryl®, Dexon®	Absorbable; 60–90 days. Braided. Inert. Nonantigenic. Non-collagenous. Excellent knot security. *Braided sutures have a wicking effect and are not recommended for closure of skin incisions.* Ligate or suture tissues where an absorbable suture is desirable.
Dexon™ S	Absorbable. Monofilament. Inert. Nonantigenic. Non-collagenous. Excellent knot security. Ligate or suture tissues where an absorbable suture is desirable.
PDS® or Maxon®	Absorbable; 6 months. Monofilament. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Prolene®	Nonabsorbable. Inert. Monofilament.
Nylon	Nonabsorbable. Inert. Monofilament. General skin closure.

The use of common brand names as examples does not indicate product endorsement.

Table 5. Stages of Anesthesia in Fishes			
Stage	**Plane**	**Category**	**Behavioral Response of Fish**
0		Normal	Swimming actively Reactive to external stimuli Equilibrium normal Muscle tone normal
I	1	Light sedation	Voluntary swimming continues Slight loss of reactivity to visual and tactile stimuli Respiratory rate normal Equilibrium normal Muscle tone normal
I	2	Deep sedation	Voluntary swimming stopped Total loss of reactivity to visual and tactile stimuli Slight decrease in respiratory rate Equilibrium normal Muscle tone slightly decreased Still responds to positional changes
II	1	Light narcosis	Excitement phase may precede increase in respiratory rate Loss of equilibrium Efforts to right itself Muscle tone decreased Still responds to positional changes weakly
II	2	Deep narcosis	Ceases to respond to positional changes Decrease in respiratory rate to approximately normal Total loss of equilibrium No effort to right itself Muscle tone decreased Some reactivity to strong tactile and vibrational stimuli Suitable for external sampling, gill biopsies, fin biopsies
III	1	Light anesthesia	Total loss of muscle tone Responds to deep pressure Further decrease in respiratory rate Suitable for minor surgical procedures
III	2	Deep anesthesia	Total loss of reactivity Respiratory rate very low Heart rate very slow
IV		Medullary collapse	Total loss of gill movements followed in several minutes by cardiac arrest
Adapted from Stoskopf 1985			

Appendix B: Literature Cited

1. Brown LA. Anaesthesia for fish. 2011. Vetfish 8(2): 68–70.
2. Chatigny F, Kamunde C, Creighton CM, Stevens ED. 2017. Uses and doses of local anesthetics in fish, amphibians, and reptiles. Journal of the American Association for Laboratory Animal Science. 56(3): 244–253.
3. Harms CA. 2005. Surgery in fish research: common procedures and postoperative care. Lab Animal 34(1): 28–34.
4. Kessel ST, Hussey NE. 2015. Tonic immobility as an anaesthetic for elasmobranchs during surgical implantation procedures. Canadian Journal of Fisheries and Aquatic Sciences 72: 1–5.
5. Mulcahy DM. 2003. Surgical implantation of transmitters in fish. ILAR Journal 44(4): 295–306.
6. Stoskopf MK. 1985. Manual for the Aquatic Workshop, American Association for Laboratory Animal Science, National Capital Area Branch, Washington, D.C.
7. Stoskopf M. 2015. Biology and management of laboratory fishes. In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT (eds). Laboratory Animal Medicine, 3rd Ed. Elservier, Inc.
8. Wargo Rub AM, Jepsen N, Liedtke TL, Moser ML, Weber III S. 2014. Surgical insertion of transmitters and telemetry methods in fisheries research. American Journal of Veterinary Research 75(4): 402–416.
9. Kugio K, Tamaru S, Hisatomi Y, Sakaguchi T. 2016. Long-duration carbon dioxide anesthesia of fish using ultra fine (nano-scale) bubbles. PLoS ONE 11(4): e0153542.