Florida Atlantic University
Institutional Animal Care and Use Committee
Standard Operating Procedure
Fish Anesthesia, Analgesia, and Biological Sampling Procedures – Captive Fishes

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 and that appropriate analgesic regimens are provided. To assure proper documentation is provided for confirmation of quality care.

Background/Purposes:
Florida Atlantic University (FAU) is committed to the improvement of human and animal health through research and the advancement of science. It is FAU policy to meet or exceed all laws and regulations governing animal care and use in research, teaching and testing. Humane treatment and well-being of animals used in research are considered highest priority. 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) and/or surgical procedures at FAU.

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 including analgesic provisions that will be given to the animal post-surgery.
    b. Ensure compliance with all relevant IACUC SOPs and policies regarding animal anesthesia, surgery, analgesia 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, analgesia and surgical procedures are followed and documented per approved protocols.
    e. Assure animals receive adequate post-surgical/procedural care.
    f. Assuring that any anesthetic, sedative or analgesic 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, or if pain is not alleviated by IACUC approved analgesic regimens.

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, sedative, and analgesic drugs are administered to animals as described in the IACUC approved 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 the 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 and analgesic 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. This plan should be detailed in an approved IACUC protocol and must include input from all members of the surgical team, including the surgeon/anesthetist, veterinarian, research technician, and investigator.
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 location where the procedure will be performed; preoperative animal health assessment; and post-operative monitoring and care if applicable.

2. Preparation of the surgical arena

a. 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.
b. Pre-designated surgical procedure rooms adjacent to the fish housing facility should be used whenever feasible. If the procedure has to be performed outside of an

animal facility, justification must be provided in the protocol and approved by the IACUC. If the procedure is to be performed within a research laboratory, the area used for surgery must be located in an area with the least amount of traffic.

c. There should be an area specifically designated for surgery where no other procedures are performed during the surgical procedure.
d. 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). Lab benches or tables work well for this purpose. A surface disinfectant must be available at all times in the designated procedural area(s).
e. Disinfect the surgical area and equipment prior to each surgery.
    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.
f. The area immediately surrounding the surgical area should also be wiped down prior to surgery to decrease dust borne contaminates in the area.

3. Sterilization of surgical instruments

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

a. Suitable instruments for fish surgery often depend on the fish's size.
    i. For smaller fish, an ocular or microsurgical pack is warranted. Head loupe magnification with center-mounted illumination is helpful for visualizing structures that are small or deep within the coelomic cavity.
    ii. Gelpi or Weitlander retractors for larger fish and self-retaining ocular retractors for smaller fish considerably improve access to the internal organs.
b. All surgical instruments must be sterile.
c. Instruments sterilization can be achieved in a number of ways. Heat sterilization via steam (autoclave) is the preferred method. (See Appendix A: Table 2)
d. 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.
e. Sterilized instruments stored in sterile unopened packages must be labeled with the date of sterilization and have 6-month shelf life if stored properly.
f. In smaller fish (< 8 inches length), a sterile surgical pack can be used consecutively in a group of animals provided a hot bead sterilizer is used to sterilize the cutting end/tips of the instruments prior to use between animals. Care must be taken to remove blood and

debris before re-sterilizing using this method. Caution should be taken when using the hot bead sterilizer. Instruments should be left in the sterilizer for a minimum of 10 seconds. Ensure adequate cooling time to prevent tissue damage. After 10 seconds, instruments can be placed back onto the sterile field.

g. 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).
h. 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 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.
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. Withholding food is not necessary in fish unless specifically mandated by the protocol or surgical procedure.
d. 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. While antimicrobial use should not be considered as a substitute for proper aseptic technique, prophylactic preoperative antibiotic therapeutics can be used in fish.
b. 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. The first step in a successful surgical procedure is to establish a safe and effective out-of-water anesthesia protocol.
b. For short procedures (< 5 min), use of local anesthesia or submersion in anesthetic "to effect" may be adequate.
c. Whenever possible, general anesthesia should be used when fish undergo surgical implantation procedures.

d. For procedures of longer duration, induction by immersion in a known concentration of anesthetic, followed by continuous delivery to the gills of known concentrations of anesthetic in water is required.
    i. Low-cost and portable recirculating systems can be readily acquired or devised according to a researcher's needs. One such system (Appendix A: Figure 1) uses a standard head pump, flexible plastic tubing and clamps, open-cell foam surgery platform cut to fit the patient, and an acrylic support fitted to an aquarium of suitable size.
    ii. After induction by immersion in a known concentration of anesthetic, the fish can be removed to the surgery platform. Water containing anesthetic can be pumped from the tank over the gills; the water then percolates through the foam platform and off the acrylic support back into the tank for recirculation.
    iii. Such systems can allow fish to be maintained on anesthesia for procedures lasting over 3 hours.
e. The most commonly used fish anesthetic is tricaine methanesulfonate (MS-222). Refer to the **FAU Guidelines for the Preparation and Use of MS222 (TMS, tricane methanesulfonate) for Animal Procedures** for more information. Note that MS-222-based products require a 21-day withdrawal time before the fish can be released into the wild.
f. Other general anesthetics include:
    i. Benzocaine – dissolved in 95% ethanol – dosage: 15–500 ppm. 3-day withdrawal period required before fish can be released into the wild.
    ii. Injectables – rarely used for bony fishes, and none are FDA-approved for use in fish.
    iii. Metomidate (Aquacalm™) – not a true anesthetic, has a tranquilizing effect; associated with longer recovery times – dosage for tranquilization: 2.5–5.0 mg/l (freshwater and tropical marine fish); dosage for transportation: 0.06–0.20 mg/l (tropical marine fish). No withdrawal times established – not approved for use in food fishes.
    iv. 2-Phenoxyethanol – rapid induction, rapid recovery; induces immunosuppression, is an irritant to human eyes and skin – dosage: 200–300 μl/l (salmonids); 100–500 μl/l (general [non-elasmobranchs], cyprinids); 1.5 mg/l – 600 mg/l (depending on species). Not approved for use in food fish in the USA.
    v. AQUI-S 20E (Eugenol) – can be applied for zero withdrawal with Investigational New Animal Drug (INAD) approval from the Aquatic Animal Drug Approval Partnership (AADAP) program with the U.S. Fish & Wildlife Service.
g. Stages of sedation with general anesthesia – see Appendix A: Table 5
h. 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.
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 for the incision, since scale removal further disrupts the skin.
e. A clear plastic sterile drape presents many advantages for fish surgery, helping to retain moisture around the fish while disallowing moisture to leak through and compromise the surgical field, and provides a sterile working surface. Draping the surgical field should be used whenever practicable, especially for longer or more invasive surgical procedures.

8. Preparation of the Surgeon

a. The surgeon should thoroughly scrub his/her hands. (See Appendix A: Table 3).
b. It is appropriate to don standard surgical attire (cap, mask, sterile gloves) just prior to starting the surgery. Standard laboratory exam gloves are **not** acceptable alternatives. Take care to put sterile gloves on aseptically.
c. Gloves, drapes, and instruments should be replaced with new sterile components if the sterile field is compromised.

9. Intra-operative care and monitoring

a. Keep the skin moist throughout the surgical procedure, taking care to avoid irrigating the incision site with anesthesia water. Pre-soaking the open-cell foam V-tray and using a clear plastic surgical drape help prevent desiccation of skin and fins.
b. Incision placement will depend on the body type and size or life stage of fish. For surgical implants, incisions should be placed exterior to a suitable body cavity to accommodate the selected transmitter.

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. The rigid body wall is poorly pliable, unless the coelomic cavity is distended. Adequate retraction with self-retaining retractors helps the surgeon maintain coelomic visualization for more invasive procedures.
f. 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.
g. Intra-operative monitoring should include monitoring of ventilation rate, movement, and the use of a Doppler ultrasound (if available) to monitor heartrate.
h. Evaluation of anesthetic depth is important. Techniques for monitoring this state vary slightly with the agent used. Absence of a response to physical manipulation or negative stimuli (e.g., hemostat pinch) is one indicator of the appropriate level of anesthesia. **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. For fish in captive care, these must be managed for second-intention healing.
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. Ideally, skin sutures should be removed when the incision is healed, usually in 2–3 weeks for uncomplicated cases. Suture removal typically eliminated the source of inflammation and can speed the final stages of incision healing.
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. Incision swelling is generally not a problem in fish, so 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. Recover animals in a clean, quiet, dark, protected tank with a lid/cover to minimize stress, prevent contamination of the incision site, and prevent escape.
   i. For recovery tanks, ensure that there is adequate tank space for holding fish prior to and after surgery as well as clean containers to facilitate the transfer of fish through each stage of the procedure, as needed.
   ii. Recovery tanks should contain large amounts of high-quality, well-oxygenated water of a temperature suitable for the species of fish and resembling the environment to which they will be returned. Oxygen concentrations should be monitored and supersaturation of oxygen should be avoided. Temperature in recovery tanks should be maintained within 2°C of source water temperature. pH in recovery tanks should be consistent with that of the original source water.
   iii. Multiple water changes may be required to replenish clean water in recovery tanks as fish metabolites accumulate over time.
   iv. For freshwater fish, salt added to the recovery tank water at 1–3 g/l (ppt) helps reduce the fish-environment osmotic gradient.
   v. As a general rule, it is better to release the fish as soon as it is fully recovered than it is to hold them for more than a few hours.
   vi. Some studies require additional confinement (e.g., for extended postsurgical monitoring or suture removal) or transport to another location for tagging or release. These studies will likely require additional accommodations for holding or transport of fish, which should be pre-arranged.
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. Do not leave animals unattended until they are completely recovered and returned to general housing. Monitor animals regularly until completely recovered.
c. Monitor the animal for signs of postoperative complications as indicated in the IACUC approved protocol. Examples include the following: lethargy, labored/abnormal breathing, increased or decreased movement, self-mutilation, abnormal posturing, redness or swelling around the surgical site or partial/total opening of the wound. Monitoring methods, treatments, and humane endpoints should be clearly stated in the approved animal protocol.
d. Contact a veterinarian if you see post-operative complications.

e. Captive fish need to be provided with supportive care for at least 2–5 days post-operatively, which might include but is not limited to appropriate feed and feed supplements, and a clean and quiet environment.
f. Assess fish for pain at least once daily even when analgesics are administered during the immediate post-operative period. Fishes undergoing minor surgical procedures should be monitored for three (3) days and those undergoing major surgical procedures should be monitored for five (5) days.

12. Provision of Analgesics

a. Pre- or intra-operative/procedural analgesia and most often post-operative analgesia should be used whenever pain is expected. All drugs administered must be within manufacturer provided expiration dates.
b. No analgesics are currently approved by the FDA for use in fish destined for human consumption.
c. Various opioids, NSAIDs, and local anesthetics may provide analgesia in some fish.
d. Any exception to the use of analgesics must be scientifically justified in the protocol and approved by the IACUC.
e. Additional/supplementary analgesics have to be provided if an animal is still showing pain either during or beyond the regular analgesic regimen as described in the IACUC protocol on an as needed basis. The AV or designee should be consulted.
f. Although single-dose analgesic delivery is not optimal from a pain management perspective, it is important to consider the detrimental effects of capture and handling of fish for additional dosing.

13. Documentation requirements

a. Documentation must be maintained by the research staff and kept in the animal housing room until the end of the post-operative period (i.e. when sutures are removed and/or the wound is healed).
b. Maintain records until the end of the study with all other study related documents in the research lab.
c. Documentation must include: PI name, IACUC protocol number, species, experimental procedure performed, date of the procedure, administration of anesthetic and analgesic agents, including dose, volume injected, route, and time of administration and any complications encountered during or after the procedure. Any fish undergoing major survival surgery must be monitored individually. Records can be adjusted to the needs of an individual study in collaboration with the veterinarian.
d. 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 Disinfectants		
NAME	**EXAMPLES** *	**COMMENTS**
Aldehydes	Glutaraldehyde (Cidex®, Cide Wipes®)	Rapidly disinfects surfaces. Toxic. OSHA has set exposure limits.
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. 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
Ionizing radiation	Gamma Radiation	Requires special equipment.
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. Compatible with most materials.
Peracetic Acid	Perasafe®	Compatible with most material. May adversely affect anodized aluminum. Needs only 10 min contact time. Must be made fresh.

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

[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.

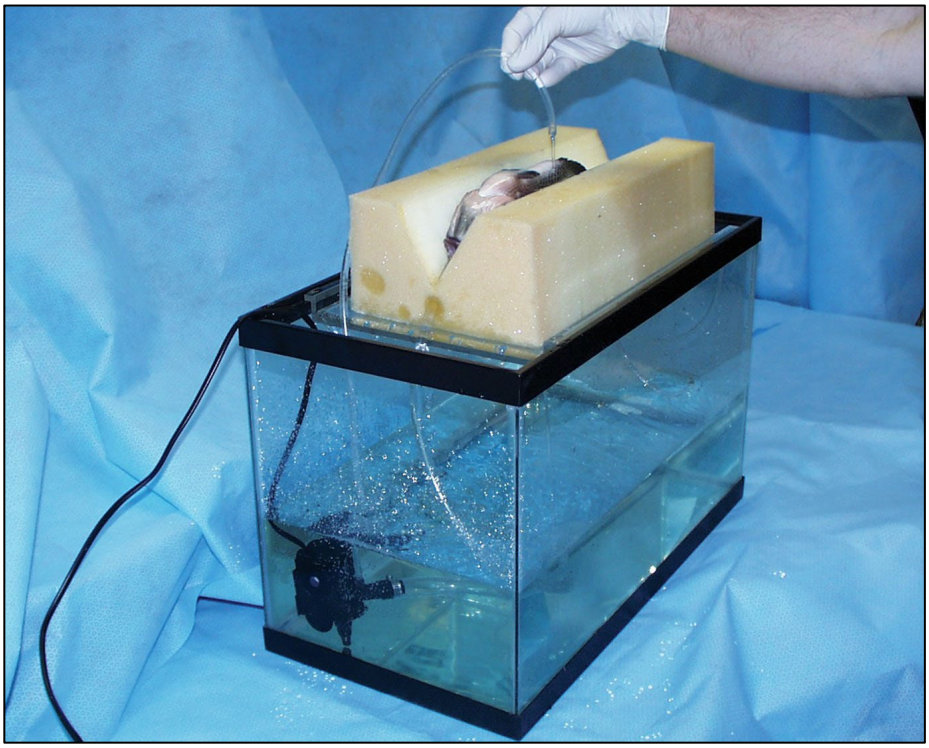

Figure 1. Fish anesthesia delivery system. An effective and economical recirculating fish anesthesia system uses a commercially available power head, plastic tubing and clamps from a hardware store, a 10-gallon aquarium, a custom-made acrylic support, and an open-cell foam V-tray cut to fit the patient. 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. Image credit: Harms 2005.

Table 4. Suture Selection	
SUTURE *	**CHARACTERISTICS AND FREQUENT USES**
Vicryl®, Dexon®	Absorbable; 60–90 days. Braided. Inert. Nonantigenic. Non-collagenous. Excellent knot security. 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.
NOTE: Braided sutures have a wicking effect and are not recommended for closure of skin incisions.

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

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