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UC Davis
Institutional Animal Care and Use Committee (IACUC)

Title: Surgery Guidelines for Aquatic Amphibians and Fish

I. Purpose:

This document explains the current requirements for performing survival surgery in aquatic amphibians and fish at UC Davis.

II. Policy:

General requirements for survival surgery include:

1. Surgical area approved in an IACUC protocol.
2. Use of sterile instruments.
3. Anesthesia and analgesics as outlined in approved IACUC protocol(s).
4. Aseptic technique whenever possible.
5. Monitoring and care of animal's well being.
6. Post-operative care.
7. Records maintained.
8. Training of personnel documented prior to initiating surgery.

Investigators may conduct survival surgery on amphibians and fish in IACUC-approved investigator laboratories under special circumstances; however, use of an animal facility procedure room is preferred. While there is no requirement for a dedicated surgical facility for aquatics, there are expectations for how surgery must be conducted.

III. Procedure:

A. **Acclimation Period:** Per the Guide for the Care and Use of Laboratory Animals (*The Guide*), newly received animals should be given a period for physiologic, psychological, and nutritional stabilization before their use. Animals should have a minimum 3-day acclimation period prior to anesthesia, surgery, or similarly invasive procedures. It is the responsibility of the Principal Investigator (PI) to ensure the acclimation period has been met. Additional information can be found in the IACUC Policy "Animal Acquisition, Acclimation and the Animal Tracking System".

B. **Use of Sterile Instruments:** Surgical instruments must be sterilized for use in survival surgery. Several techniques (e.g., steam, dry heat, gas sterilization or chemical agents) can be used to sterilize instruments and other materials that will come in contact with animal tissues. Sterilization with chemical agents should ensure that no chemical residues are left on instruments, as these residues may cause severe tissue reactions. Steam or dry heat are the preferred methods to sterilize surgical instruments. Another acceptable method of instrument use includes "tips only" practice. Using clean exam gloves and a "tips-only" technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field.

C. **Anesthesia**

1. MS-222 is a common anesthetic agent for fish and aquatic amphibians. Any proposed anesthetics must be included in the approved IACUC protocol.
    i. If using MS-222 please refer to SC-40-406 MS-222 Preparation and Use for more information on safe preparation and use.
2. Monitoring anesthetic depth in amphibians:

    The following signs are listed in the order in which they occur during onset of anesthesia:

    i. Erythema (reddening) of skin of caudal abdomen
    ii. Loss of righting reflex (inability to remain upright)
    iii. Cessation of abdominal respiratory movements
    iv. Cessation of spontaneous movement
    v. Loss of corneal reflex (if applicable)
    vi. Loss of throat movements
    vii. Loss of superficial pain reflex in response to skin pinch

3. Monitoring anesthetic depth in fish:

    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.

    i. Activity decreases, the righting reflex is lost, and muscle tone decreases as fish become anesthetized.
    ii. Opercular movement (respiratory rate) progressively decreases with deepening anesthesia.

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

4. Maintain the animal in a surgical plane of anesthesia throughout the duration of the procedure.

D. **Surgical preparation:**
1. A recovery tank should be prepared before the surgery is started. The water temperature in the recovery tank should match water temperatures used during the surgical procedure.
2. Wash hands with a disinfecting soap and rinse thoroughly.
3. Required attire for the surgeon:
    i. Moistened non-powdered gloves (latex-free depending on species)
    ii. Mask
    iii. Clean scrub top, lab coat, or disposable gown
4. Ensure animals remain wet during procedures when out of water with care taken to prevent irrigating the incision site with contaminated anesthetic or tank water.
5. Surgical preparation of the incision site should minimize disruption of skin and mucus layer.
6. The skin at the incision site should be irrigated with copious amounts of sterile saline. If greater antimicrobial activity is desired, the skin can be irrigated with a dilute solution of povidone iodine (1:20). Application of harsher chemical disinfectants and alcohol may irritate the skin and increase the risk of tissue damage and postoperative morbidity and mortality.
7. A sterile clear plastic drape should be positioned over the animal to help isolate the incision site, create a sterile field, and help retain heat and moisture.

E. **During Surgery:** The surgical field must be kept aseptic throughout the procedure.
Sterile instruments must be prevented from contacting non-sterile surfaces.
Instruments must be placed on a sterile surface when not in use.
Monofilament suture or absorbable (e.g. nylon. PDS, PGA) is recommended to reduce inflammatory reactions in Xenopus and fish.

F. **Monitoring and Care of Animal's Well-being:** Monitor the animal carefully during the surgical procedure. Maintain the animal in a surgical plane of anesthesia throughout the duration of the procedure. Anesthetized animals must not be left unattended during the procedure.

G. **Analgesia:** Consult with veterinary staff for advice on analgesics specific for the species and when writing the animal care and use protocol.

H. **Postoperative Care:**
   1. Fish:
      i. Recover fish in aerated, conditioned, fresh or original housing water.
      ii. 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.
      iii. Most fish are fully recovered from immersion anesthetics within 15 minutes of placement in fresh water. Prolonged recoveries (> 30 minutes) indicate excessive anesthetic depth or a compromised animal. Recovery from parenteral anesthetics can be highly variable.
      iv. 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 demonstrate vigorous movement and may need to be carefully restrained to prevent self-injury.
   2. Aquatic Amphibians:
      i. Rinse the animal with sterile saline or clean water to remove any remaining anesthetic after incisional closure if using immersion or topical anesthetics.
      ii. Partially submerge the animal in fresh water with the head and nares held above water or place in a closed but not airtight container with a moist paper towel on the bottom during recovery. Other methods are also acceptable if the skin is kept moist and relative humidity in the immediate environment maintained at > 70%.
      iii. DO NOT submerge aquatic amphibians in water until fully ambulatory and recovered from anesthesia as drowning can occur.
      iv. Single housing or small group housing for several days after surgery should be considered as part of the post-surgical care.

I. Post-Operative Documentation

1. Surgical/post-operative records must be maintained until the wound is healed or sutures are removed. Daily observations and treatments must be recorded on the post-operative record, for example, the UC Davis Campus Veterinary Services Anesthesia Record.
2. Amphibians must be monitored for appetite as well as any complications such as dehiscence or infection. Such adverse effects would be reasons for immediate euthanasia.
3. Record any analgesia provided as approved in your animal care and use protocol.
4. Record any suture or wound clip removal.

J. Xenopus Oocyte Harvesting via Laparotomy

1. Multiple major surgical procedures on a single animal are acceptable only if they are included in and an essential component of a single research protocol, and it has been scientifically justified (i.e., multiple surgeries on a single animal are justified considering consistency of oocyte quality and the reduction in the total number of animals used). The total number of laparotomies must be described in the approved animal care and use protocol. The total number of laparotomies approved per frog cannot exceed five (5) recovery surgeries (a 6th must be terminal) and will depend on the health and lifespan of the animal, as well as the duration of oocyte production.
2. A minimum of one month of recovery time is required between survival surgeries.
3. Investigators should consider rotating frogs so that the interval between surgeries is maximized for a particular animal, or alternating oocyte collection between the left and right ovaries.
4. The protocol must include a written description of the method used to identify animals to ensure adequate time has lapsed between surgeries (e.g., skin marking or tattooing, tank rotation).
5. Close any laparotomy site in two layers (muscle and skin) to prevent incisional dehiscence.
6. Skin sutures and wound clips, if non-absorbable, must be removed 2-3 weeks after surgery.

K. Training

1. PIs are required to ensure all staff conducting or assisting with amphibian and fish survival surgeries are appropriately trained and that training has been documented. Researchers conducting surgical procedures must have appropriate training to ensure that good surgical technique is practiced – that is, aseptic technique, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns. Personnel must obtain training on aseptic techniques from other knowledgeable personnel or by attending the Aseptic Techniques instructor led training. PIs and staff may also receive hands-on training for specific surgical techniques from others proficient in the procedure.

2. Please contact Campus Veterinary Services (530-752-0514, or lahc@ucdavis.edu) for questions regarding animal health, anesthetic support, surgical wound care, postoperative analgesia, or other questions regarding these guidelines.

IV. Resources:

1. ILAR, Guide for the Care and Use of Laboratory Animals
   http://nap.edu/12910
2. Standard of Care 50-103 "Sanitation and Sterilization Quality Assurance and Monitoring"
   https://research.ucdavis.edu/wp-content/uploads/SC-50-103.pdf
3. UC Davis Campus Vet Services Anesthesia Record
   https://research.ucdavis.edu/wp-content/uploads/Anesthesia-Record.pdf
4. Standard of Care-40-404 "Animal Care Program Medical and Health Records"
   https://research.ucdavis.edu/wp-content/uploads/SC-40-404.pdf
5. Aseptic Technique Courses
   https://research.ucdavis.edu/policiescompliance/animal-care-use/training-classes/
6. UC Irvine "Surgery Policy & Guidelines"
   https://research.uci.edu/animal-care-and-use/policies-and-guidance/surgery-policy-and-guidelines/
7. University of Michigan "Guidelines on Fish Anesthesia Analgesia and Surgery"
   https://az.research.umich.edu/animalcare/guidelines/guidelines-fish-anesthesia-analgesia-and-surgery
8. University of Michigan "Guidelines on Amphibian Anesthesia Analgesia and Surgery"
   https://az.research.umich.edu/animalcare/guidelines/guidelines-amphibian-anesthesia-analgesia-and-surgery