NRAC Bulletin No. 112-1993

New York Sea Grant

Northeastern Regional Aquaculture Center

University of Massachusetts
Dartmouth
North Dartmouth
Massachusetts 02747

A Manual for Nonlethal Surgical Procedures to Obtain Tissue Samples for Use in Fish Health Inspections

G. A. WOOSTER, H.-M. HSU And P. R. BOWSER[1]
Department of Avian and Aquatic Animal Medicine
College of Veterinary Medicine
Cornell University, Ithaca, New York 14853

[1] To whom correspondence should be addressed.

INTRODUCTION

Fish health inspections procedures currently require sacrificing of fish to detect specific fish pathogens. For salmonids, relevant pathogens include the viruses for infectious pancreatic necrosis (IPN), viral hemorrhagic septicemia (VHS) and infectious hemopoietic necrosis (IHN); the bacteria Aeromonas salmonicida, Yersinia ruckeri and, Renibacterium salmoninarum; and the parasites Myxobolus cerebralis and Ceratomyxa shasta. Specific tests performed to detect these pathogens follow procedures suggested by the Fish Health Section of the American Fisheries Society (Amos 1985).

Inspection procedures are necessary to prevent the spread of virulent pathogens to states, hatcheries and watersheds where the pathogens are not likely to exist. Sample procedures lethal to fish may be acceptable when the sampled fish come from a large population. Typically, 60 fish per lot are sampled to provide 95% confidence that an infected fish will be detected if infection exists at a 5% prevalence (Amos 1985). However, broodfish of rare or economically important strains are not usually held in large numbers. Additionally, current fish health inspection techniques restrict the development of interstate commerce of live aquaculture products because fish hatchery manager would risk losing a substantial investment should a fish health inspection be performed that would include the sacrifice of these fish. Thus, there is great need for the development of non-lethal sampling protocols for use in fish health inspections.

Many anesthetic compounds have been investigated and used on fish for shipping stress reduction, surgical anesthesia and euthanasia. They permit survivable surgery and humane handling of fish. These compounds include: benzocaine, 2-methylquinoline (quinaldine), tricaine methanesulfonate (MS-222), metomidate and etomidate along with many other compounds (Klontz 1965, Amend, Goven, and Elliot 1982, Brown 1987, 1988 and 1993, Gingerich and Drottar 1989). MS-222 is the most commonly known and used and with carbon dioxide and sodium bicarbonate constitute those anesthetics currently approved by the FDA for use with food fish (Marking and Meyer 1985, Schnick, Myer and Gray 1989, See Appendix A, page 21).

Surgical procedures on fish are uncommon but not new. Gonadal castration is reported as early as 1745 (Akhtar 1984) and 1755 (Tull 1755). Many investigations have employed surgery on fish for a variety of purposes including: installation of chronic fistulas in the gastrointestinal tract of fish (Krayukhin 1962), urophysectomy (Ireland 1969), extirpation of caudal neurosecretory system (Fridburg, Nishioka, Bern, and Fleming 1966), implantation of ultrasonic transmitters (Hart and Summerfelt 1975), pinealectomy (Goetz, Hoffman, and Pancoe 1977), partial hepatectomy for carcinogenicity studies (Kyono-Hamaguchi 1984, Ostrander, Blair, Stark, and Hurst 1993), stanniosomatiectomy (Kenyon, Chester-Jones, and Dixon 1980), pancreatectomy (Lewis, Parke, and Epple 1977), tissue biopsy sampling for genetic and electrophoretic analysis (Morizot, Schmidt, Carmichael, Stock, and Williamson 1990, Mair 1989, Harvey, Noble, Neill and Marks 1984), Physiological studies (Pavlovskii 1962, Smith and Bell 1967, Klontz and Smith 1968, Tytler and Hawkins 1981), kidney biopsy for disease diagnostics (Noga, Levine, Townsend, Bullis, Carlson, and Corbett 1988) and gonadectomy (Robertson 1958, Akhtar 1984, Bart 1988, Bart and Dunham 1990).

Purpose

The purpose of this manual is to instruct fish health personnel the procedures for liver and kidney biopsies in live salmonid broodfish. The tissues collected will be suitable for fish disease diagnostic or fish health inspection purposes. Kidney tissues alone may be all that are necessary for health inspection purposes; however, both procedures are outlined in detail. The intent of the biopsy is to detect vertically transmissible diseases or pathogens that can be passed into eggs or fingerlings that might be shipped from one point to another.

The manual is divided into ten sections:
(1) Materials; (2) Preparation; (3) Anesthesia; (4) Liver Biopsy Surgery; (5) Kidney Biopsy Surgery; (6) Suturing; (7) Postoperative care; (8) Biopsy sample processing; (9) Appendices; (10) References.

1. MATERIALS

The suggested materials listed here were selected in an effort to keep procedural costs to a minimum. See Appendix A for sources of materials and supplies listed by numbers. Note that identification of a source or product does not constitute an endorsement. Any suggestions or modifications that reduce cost or that improve or simplify the procedures are encouraged. Please send any suggestion to the principal author (address in on title page).

Personnel

Two skilled, technicians familiar with salmonid internal anatomy are necessary to conduct the procedures. At least one individual must possess some veterinary surgical experience. Teamwork is essential for expedient and efficient time management.

Consumables

1. Cheesecloth / gauze pad (6 ply, 68.6 meters [75 yards] non-sterile cut 23 x 41 cm, 70 pieces/box) Sterilize gauze in autoclave with autoclavable pan with lid or aluminum foil covering.
2. Gauze sponges (4 x 4 x 8 ply or 12 ply, 200/bag)
3. Surgical face mask
4. Surgical head cover or hair net
5. Surgical gown or lab apron
6. Surgical gloves or N-Dex nitrile gloves (non-sterile). Use new set of gloves disinfected with 70% ethanol, for each fish.
7. Sterile polyester tip applicator
8. Sterile #10 scalpel blades
9. Sterile polyethylene bulb transfer pipettes (for aspiration)
10. 15 ml polypropylene round bottom tubes, sterile
11. Polyglactin 910 sutures 2-0 (3.0 metric, 70 cm) with swaged-on FS-1 cutting needle
12. Stainless steel ring jaw tags or other tag/marking scheme

Chemical supplies

13. Ethanol (ethyl alcohol, 95% for flame sterilizing, 70% for hand rinse between fish)
14. MS-222 (3-aminobenzoic acid ethyl ester, Tricaine methanesulfonate, Tricaine or ethyl m-aminobenzoate)
15. Potassium permanganate
16. Phosphate-buffered saline (PBS, 0.1 M) (see Appendix B for formulation.)
17. Betadine povidone iodine surgical scrub
18. VetBond Tissue Adhesive #1469 (n-butyl cyanoacrylate, for hemostasis)

Surgical tools

19. Senn blunt end retractor or Foerster sponge clamp
20. Lewis lens loop 14 cm (5 1/2" smooth loop, 5.5 x 8.0 mm)
21. Tissue dressing forceps, 13 cm (5") - serrated curved tips
22. 14 cm long Wietlaner retractor
23. Two Adson tissue forceps
24. Sharp point iris scissors - 10 cm (4") straight or 10 cm (4") angled on side
25. Two aneurysm needles (16.5 cm [6 1/2"] , blunt tip with eye) or two dissecting tenaculum (16.5 cm [6 1/2"] , sharp point, will need to blunt the tip), or two Hupp trachea hooks (16.5 cm [6 1/2"] sharp point, will need to blunt the tip)
26. Two Crile-Wood needle holders (serrated points, 12 cm and 15 cm [5" and 6"])
27. A 9 cm Backhaus towel clamp with stainless steel rounded edge washer (2.5 cm diam.,3 mm thick, 3 mm center hole) or a stainless steel teaspoon with nonpatterned handle (no burrs or rough edge)

V-trough and gill irrigation system:

28. Two submersible pumps (Model 1A, Little Giant Pump Co., Oklahoma City, OK)
29. Digital timer (100 hr. timer/stopwatch)
30. Polyproplyene wash bottle (for 70% ethanol)
31. Small gooseneck or other suitable lamp
32. Candle (for flame sterilizing)

33. Binder clips (25 mm or 12 mm [1" or 1/2"])
34. 114 to190 l (30 to 50 gal) rubber or plastic garbage can
35. Two insulated coolers, 382 mm dia. x 382 mm width x 762 mm length dimension, 60 l capacity (15" d X 15" w X 30" l, 16 gal)
36. Six radiator hose clamps
37. Plexiglas acrylic sheet 6 mm x 915 mm x 610 mm (1/4" x 36" x 24")
38. 12.7 mm (1/2") cpvc pipe, one 2.4 m (8') length to be cut
39. Five 12.7 mm (1/2") cpvc elbows 90°
40. Three 12.7 mm (1/2") cpvc "T"
41. Four 12.7 mm (1/2") cpvc or sch40 pvc gate valves
42. Two 9.5 mm male thread x 6.3 mm female thread (3/8" x 1/4") coupling sch80 pvc
43. 16 mm (5/8") ID flexible tygon tubing (approx. 9 meters [30 '] total length) to be cut
44. 12.7 mm (1/2") ID tygon tubing 76 mm (3") long
45. 9.5 mm (3/8") ID tygon tubing 38 mm (1.5") long
46. 9.5 mm (3/8") ID rigid plastic (polystyrene) or glass tubing 76 mm (3"), long bent into 90° elbow
47. Aquarium air pump (heavyduty)
48. Aquarium air line tubing and air stone diffusers.

2. PREPARATION

It is important to schedule adequate time for the procedures. Surgical procedures described in this manual are labor-and time-intensive when a statistically appropriate number of fish are chosen (usually 60 fish) for a fish health inspection. If rare broodfish or a small number of broodfish are available then the number of fish should be modified according to Amos (1985) and any appropriate governmental regulations. It is advisable to practice on a few expendable fish before working on important broodfish.

Be sure that fish chosen for surgery are taken off feed for 48 hours before surgery is performed. This will allow the gastrointestinal tract (GIT) to become void of most fecal and undigested material that could expand the GIT and interfere with surgery. Additionally, it should be noted that a voided flaccid GIT is less likely to ruture or be cut during surgery. Klontz and Smith (1968) reported that for optimum response to anesthesia, fish should not be fed 24 to 48 hours prior to anesthetization. Being off feed also reduces complications from regurgitation (Brown 1988).

Select a opperating site where fish chosen for surgery can be held in a raceway or tank. Set up the V-trough surgical support table near or over the raceway or tank, if convenient. Be sure that electrical services for pumps, lighting, etc. are safely held away from wet areas. Lay out all materials and surgical instruments before starting (Figure 1) and check items against materials list. Be sure tissue sample tubes are properly labeled ahead of time. Immediately before surgery, all personnel should wash their hands with a Betadine surgical scrub and dress in surgical attire. Surgical instruments should be soaking in 95% ethanol so that they can be flame-sterilized as needed.

Figure 1. Overall view of surgical facilities.

3) ANESTHESIA:

Before surgery, anesthetize each fish individually in an insulated container (41 cm d x 44.5 cm w x 84 cm l) with 60 l of water (temperature equal to the raceway or tank water in which the fish are held) using nonbuffered MS-222 at 100 mg/l. MS-222 lowers pH of water. In soft, low alkalinity (< 50 mg/l $CaCO_3$), or poorly buffered waters you may want to check pH before and after adding MS-222 to the anesthetic reseservoir. If pH is too low you may want to buffer the anesthetic solution with physiological buffers (Brown's 1988 formula for Phosphate buffered saline is given in Appendix B). Approximately 2 minutes are required for deep surgical anesthesia (depending on the size of the fish and the water temperature) and the fish's response should be closely monitored. Onset of deep surgical anesthesia is marked by cessation of opercular movements, decreased muscular tone, and no response to external stimuli (McFarland 1960, Stoskopf 1985). Cutting of skin and muscle does not cause reflex muscular contraction. The 100 mg/l dose of MS-222 allows adequate anesthesia but prevents the fish from going beyond deep anesthesia into a terminal state. Higher doses or prolonged exposure to MS-222 will lead to mortalities.

Before positioning the fish, place a length of 13 mm (1/2") cpcv pipe in the notch of the V-trough (Figure 2) so anesthetic-containing water will flow freely beneath the fish and cheesecloth draping without the fish or gauze damming the trough and flooding the surgery site. Drape two pieces of 23 cm x 41 cm cheesecloth into the trough over the length of cpcv pipe. Allow water to wick into the cheesecloth. This will help keep the fish skin moist and cool. After approximately 2 minutes, take the fish out of the anesthesia bath and place it in latero-dorsal recumbency on the V-trough surgical support table so that the left side of the fish faces up, with the head to the left. Immediately, place the gill irrigation tube into the fish's mouth (Figure 3) so that water from the upturned (right angle) of the tube irrigates the left or upside gills and flows down over the lower (downside) gills. Adjust flows with the valves (Figure 4) so that water does not exit the left operculum and flow near the incision site (Figure 3).

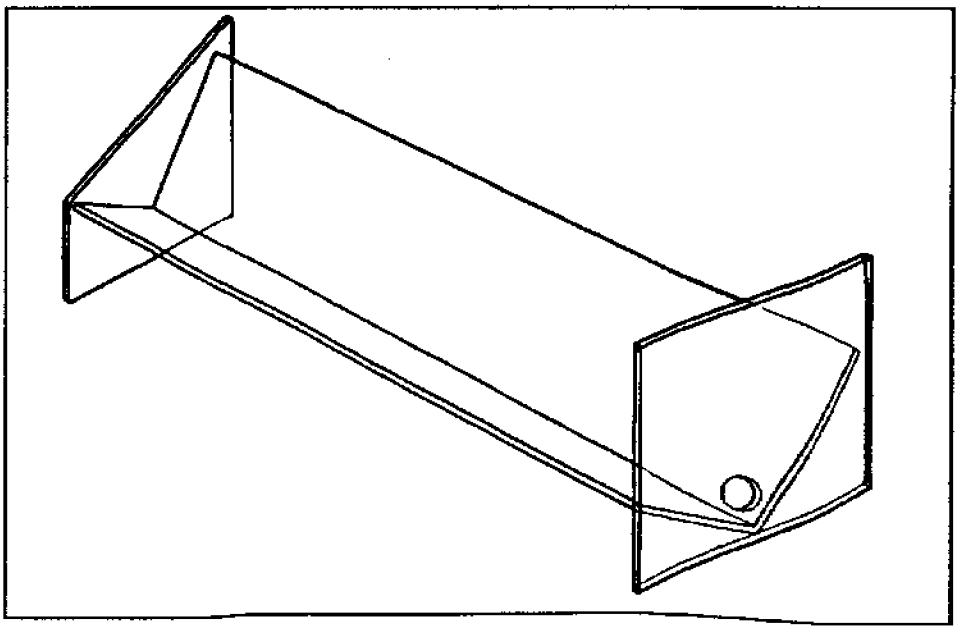


Figure 2. V-trough surgical support table.

Figure 3. View of gill irrigation tube in mouth of fish.

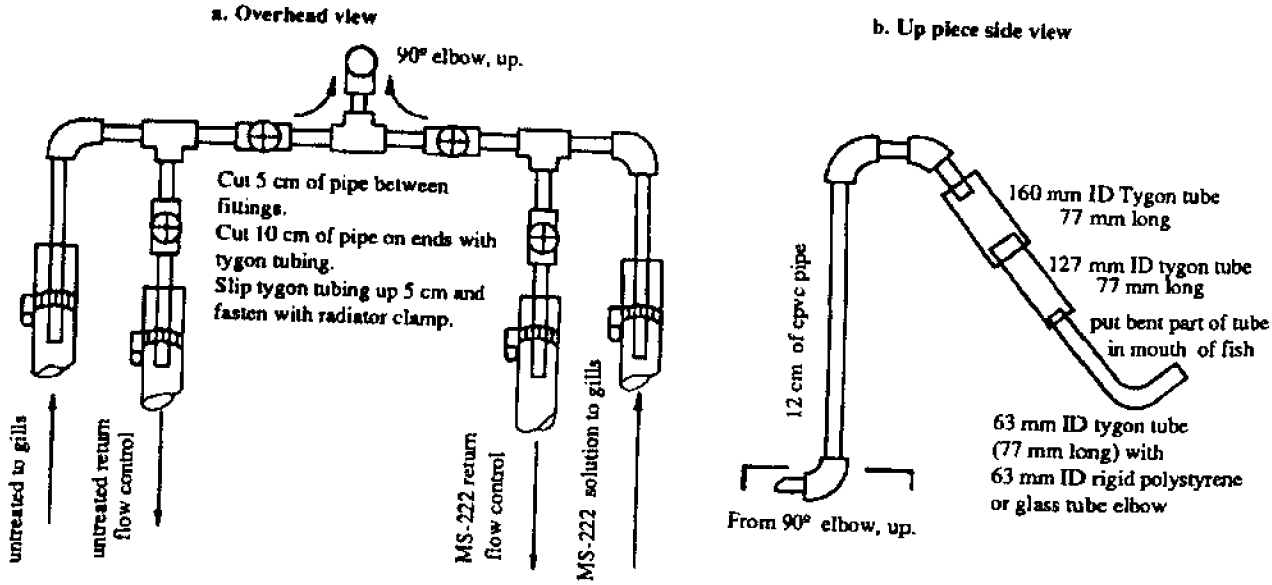


Figure 4. Valve schematic for gill irrigation system.

When all water flows are properly adjusted, the surgical procedure may begin (next section). Maintain flow of anesthetic solution for 2 to 5 minutes then, using the valves, switch to a fresh flow of untreated chilled water. When opercular movement is first noted or muscle contractions are observed switch back to the MS-222 anesthetic solution. The change from anesthetic gill irrigation to nonanesthetic gill irrigation during surgery is designed to help prevent the fish from going into terminal anesthesia (medullary collapse). Also, during longer surgery procedures gill irrigation helps to keep the fish's body wet and cool. Cooling aids anesthesia by reducing activity and blood flow.

Fish larger than 1 kg can suffer serious oxygen deficiency resulting in neurological damage when under deep anesthesia for long periods (Smith and Bell 1967). The irrigation technique is desirable for long-term procedures (> 5 minutes) because depth of anesthesia can be maintained easily by switching from anesthetic solution to untreated water; vigorous aeration with air stones in the anesthetic and untreated solution helps prevent oxygen deficiency. It is highly desirable to maintain untreated water and anesthetic solution at the same temperature as the raceway or tank water in which fish are held. A reservoir of anesthetic solution for gill irrigation can be maintained this way by setting a clean or new 114 to 190 l (30 to 50 gal) plastic or rubber garbage can in the raceway/tank and sinking it with enough anesthetic solution that it is stable and not floating. Untreated water for gill irrigation can come directly from the raceway or tank and return to the tank (Figure 5) or be discharged down a drain. Anesthetic solution is returned to the anesthetic reservoir and recirculated (Figure 5). Renew the anesthetic solution after 8 to 10 surgical procedures.

4. LIVER BIOPSY SURGERY

Incision dimensions are described are appropriate for salmonid broodfish 30 cm to 53 cm (12" to 21") in size. If using smaller or larger fish adjust dimensions accordingly. Total time for procedure is approximately 15 to 20 minutes per fish.

When the fish is in deep anesthesia and positioned on the V-trough surgical support table, drape a piece of sterile 23 cm x 41 cm 6 ply cheesecloth over the caudal region of the fish to provide a "sterile field" leaving only the head and the liver biopsy incision site exposed (Figure 6). The "sterile field" is set up to keep secreted mucus off the sutures and instruments. Do not cover the head or left operculum, as a clear view of the left operculum is necessary to monitor depth of anesthesia. A moistened sterile 4" x 4" gauze sponge may be placed over the left eye to minimize photosensitivity stress. Care must be taken to keep the gauze moist during the surgery; if allowed to dry, the sponge may adhere to and damage the eye.

The incision site should not be aseptically swabbed or prepared. The fish's skin is easily damaged by alcohol and surgical scrub solutions. Additionally, swabbing removes mucus, which has some antimicrobial activity. A gentle swabbing of excess mucus from the incision site with a gauze sponge is all that is needed.

Draw the left pectoral fin anterior from the incision site with a Senn blunt-end retractor or Foerster sponge forceps secured to the surgical trough with a binder clip. Start an incision (Figure 7) with a small skin-deep cut in the flank using a sterile #10 scalpel. Using a curved tissue-dressing forceps, gently grasp the skin at the incision site to lift the abdominal wall so the scalpel can penetrate without damaging underlying organs. Produce a small opening into the abdominal cavity, then use the scalpel, blade up, to finish opening along the length of the incision (Figure 8). Making the incision with the scalpel blade up prevents accidental penetration of the liver and other organs that lie immediately below and against the abdominal wall. Usually the pyloric cecae are located immediately below the start point for the incision. With proper retraction of the skin, adequate space is present between the point where the blade enters the abdominal cavity and the underlying organs. On rare occasions a fish will have a reversed internal anatomy and the liver will be lying against the right abdominal wall. Should this happen, carefully move aside obstructing organs.

Spread the incision open with a sterile Weitlaner retractor to provide an incision field approximately 7 cm wide (Figure 9). Lift up an expose a lobe of the liver with an Adson-type tissue forceps and excise a piece of tissue measuring

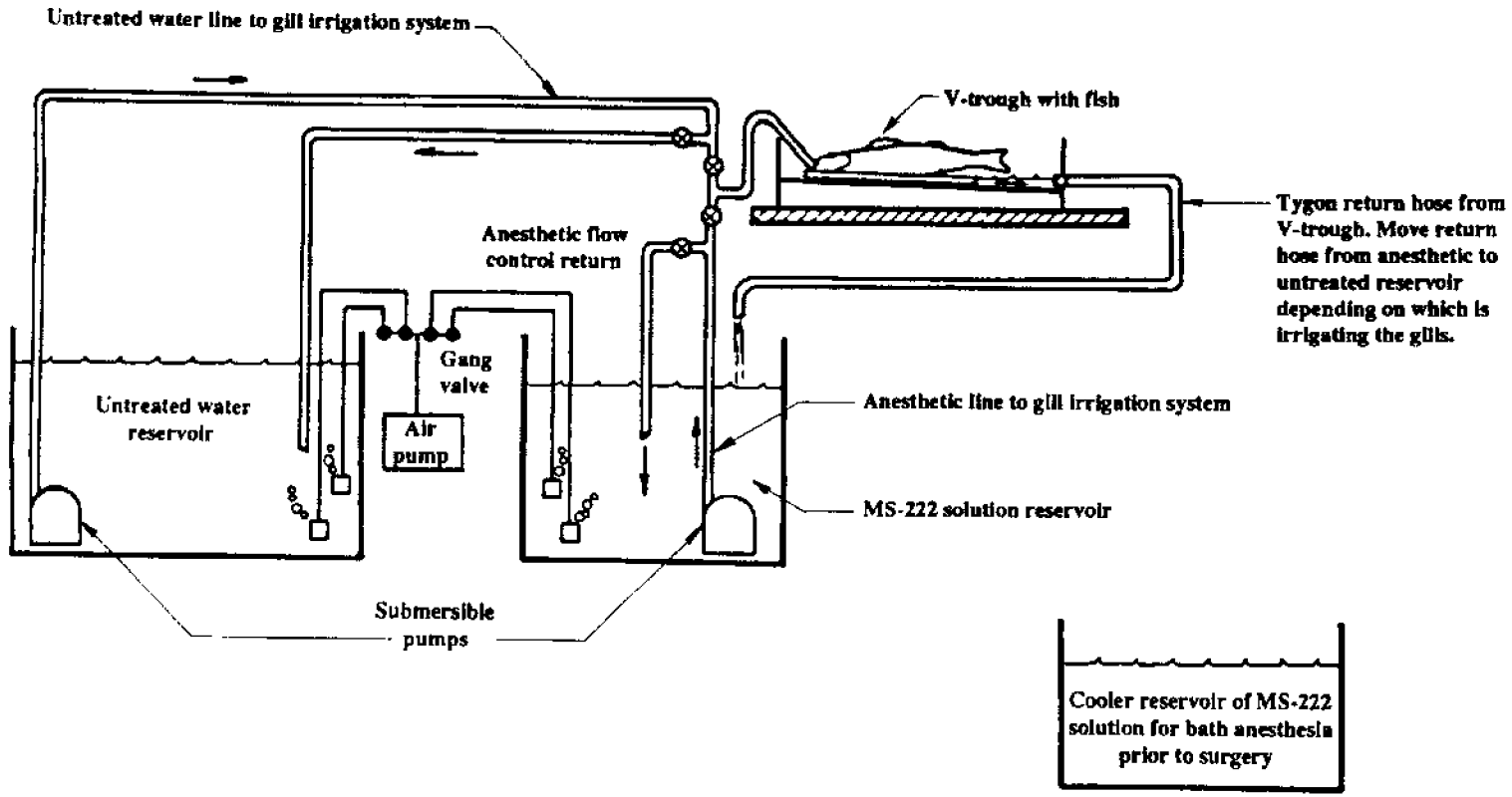


Figure 5. Flow diagram for delivery of water with MS-222 and fresh water in support of anesthesia during surgery on salmonids.

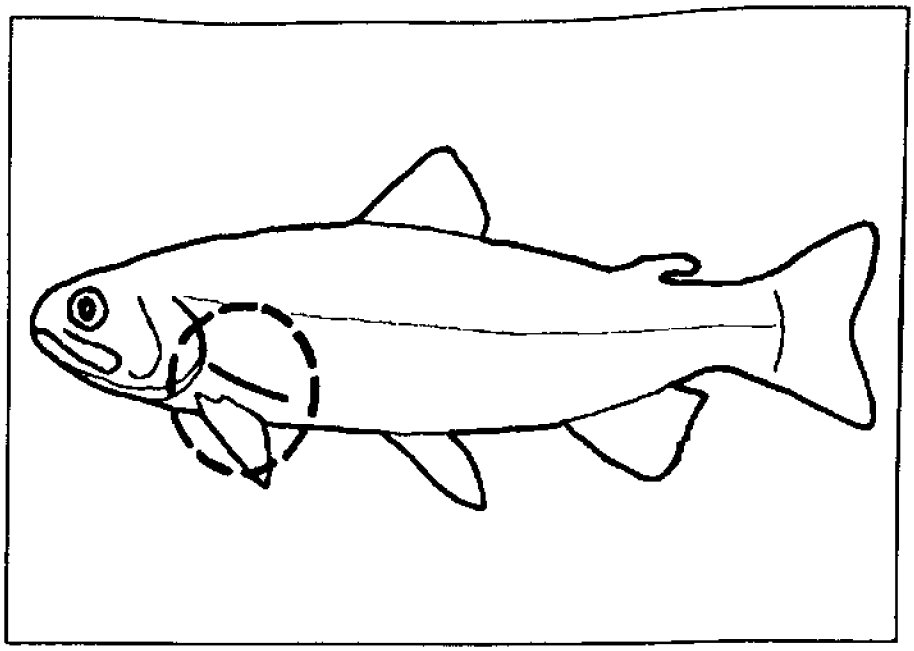


Figure 6. Site for abdominal incision in liver biopsy procedure.

Figure 7. Location of initial abdominal incision for liver biopsy procedure. Lift skin and underlying tissues to prevent damage to internal organs.

Figure 8. Completed abdominal incision for liver biopsy procedure.

Figure 9. Retraction of abdominal incision for liver biopsy. Note: Liver "L" and Pyloric cecae with adipose tissue "P".

Figure 10. Excision of liver tissue using iris scissors and Adson tissue forceps.

approximately 1 cm^3, using sterile sharp-point iris scissors (Figure 10). Take special care to avoid damaging or cutting large blood vessels. No cauterization or ligation is needed. However, after the liver biopsy is taken, heavy bleeding may ensue. Aspirate blood with a sterile polypropylene transfer pipette and gently blot the clots with a sterile 4"x 4" gauze sponge. Place the sample in a sterile15 ml polypropylene tube or equivalent container and place it in a cooler with crushed ice.

Proceed to suturing (Section 6).

5. KIDNEY BIOPSY SURGERY

Incision dimensions described are appropriate for salmonid broodfish ranging 30 cm to 53 cm (12" to 21") in size. If using smaller or larger fish adjust dimensions accordingly. Total time for the procedure is approximately 25 to 35 minutes per fish.

When the fish is in deep anesthesia and positioned on the V-trough surgical support table drape a piece of sterile 23 cm x 41 cm 6-ply cheesecloth over the midsection of the fish anterior to the kidney incision site (Figure 11). An additional drape covering the caudal region posterior to the incision site may also be used. Drape the left eye as previously described in the liver biopsy section, being sure to keep the head and left opercular uncovered to facilitate observations for the state of anesthesia.

Using the incision strategy described in the liver biopsy section, start the incision with a #10 scalpel at a point midway between the lateral line and the left pelvic fin (Figure 12). Do not cut across the lateral line. Cut the incision from anterior to posterior to a point dorsolateral and 4 cm to 5 cm anterior to the anus (Figure 13). Take care not to cut through the abdominal wall into the intestine or urinary tract or through any bone or cartilage. Use a 14 cm Weitlaner retractor to open the incision approximately 8 cm wide (Figure 14) to allow access to the left lateral side of the posterior kidney. To avoid damaging the underlying organs with the prongs of the retractor, lift up the abdominal muscle wall up with aneurysm needles while positioning the retractor. Carefully move aside internal organs. Obstructing adipose and gonadal tissue may be excised from the fish (Figure 15) to expose the gas bladder. Cut the connective mesentery as needed, or carefully tease and tear it either with forceps or the blunt tip of an aneurysm needle taking care to avoid

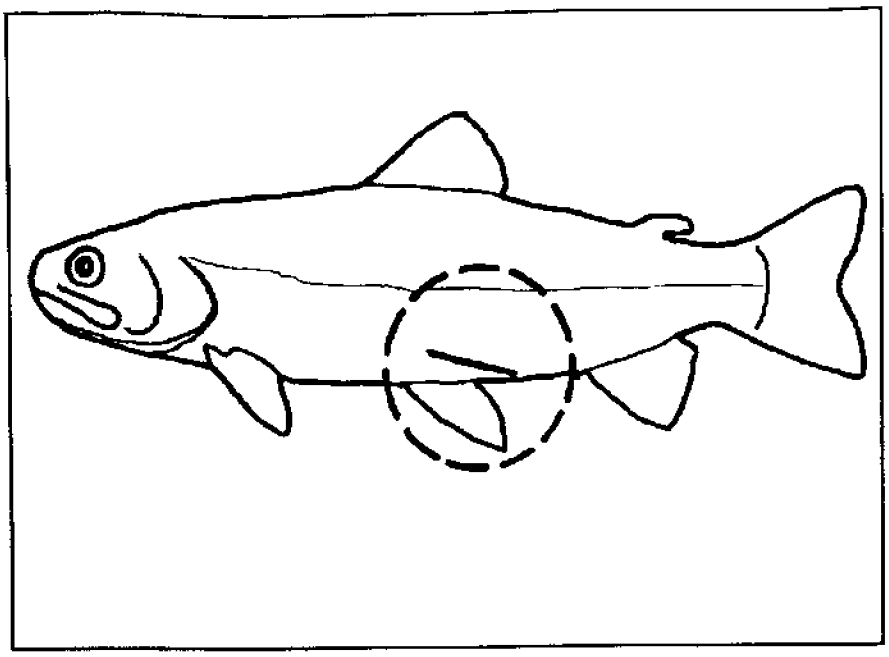


Figure 11. Site for abdominal incision in kidney biopsy procedure.

Figure 12. Location of initial abdominal incision for kidney biopsy procedure.

Figure 13. Completed abdominal incision for kidney biopsy procedure.

Figure 14. Retraction of abdominal incision for kidney biopsy.

major blood vessels. Tease the gas bladder away from the kidney biopsy site approximately 4 cm posterior from the Corpuscles of Stannius using two blunt aneuryism needles or blunted trachea hooks to tear the connective tissue (Figure 16). Exercise care to not damage or puncture the gas bladder. Using the sterile 9 cm Backhaus towel clamp to hold a stainless rounded edge washer, or by using a sterile stainless steel teaspoon gently depress the gas bladder away from the kidney biopsy site (Figure 17). Do not tease the whole gas bladder away from the total length of the kidney. Expose only the biopsy site.

After the biopsy site is exposed, use a sterile # 10 scalpel to cut a rectangular piece of kidney (approximately 0.8 cm x 2.5 cm x 1 cm deep) from the left lateral side of the kidney through the left opisthonephric duct, avoiding damage to the right opisthonephric duct draining the right side of the kidney (Figure 18). Also avoid cutting too close to the urinary bladder.

Extensive bleeding will occur at this point. Aspirate the blood from the biopsy site to allow a clear view. Push the 5.5 mm x 8 mm smooth oblong of the Lewis lens loop into the short side of the rectangular cut and draw it through along its length (the length of the rectangular cut) to cut the bottom of the kidney tissue (Figure 19).

Aspirate the blood as needed and use serrated tissue forceps to remove the kidney tissue. Place the biopsy in a labeled polypropylene tube and store as described in the liver biopsy section. Bleeding will be heavy, from approximately 10 ml to 15 ml per fish. No ligation or cauterization is necessary; however, a drop of tissue adhesive cyanoacrylate (VetBond) may be placed on the scalpel blade just prior to making the kidney incision. This will clot blood and may reduce bleeding. It has been our experience (Wooster, Hsu and Bowser 1993) that no differences in the success rate of the surgery could be found between hemostasis and cyanoacrylate hemostasis.

Be sure to use an animal-grade cyanoacrylate formulated for tissue or surgical use. Commercial cyanoacrylates (ie., "super glues") have different formulations and can cause inflammation, be toxic to tissues, and stimulate adhesions (Olsen and Bruce 1987).

6. SUTURING

Following the biopsy, aspirate the abdominal cavity with sterile polypropylene transfer pipettes and blot clots with sterile 4"x 4" gauze sponges. Close the abdominal incision using an absorbable synthetic suture (Polyglactin 910, braided 3.0 metric, Vicryl) with swaged-on cutting edge needle (FS-1). Use a simple interrupted suture with reinforced surgeons square knot (Figure 20a, 20b) (Knecht, Allen, Williams, and Johnson 1987). Use the curved serrated tissue forceps and a serrated Crile-Wood needle holder to aid suturing. Try to keep the suture line coiled upon the "sterile field" of cheesecloth to avoid contaminating it in the flowing trough water. When closing the incision, push the needle through the skin approximately 2 to 3 mm lateral to the incision line. Insert the suture through the muscle on one side (but not through the peritoneum or into the abdominal cavity), pass it through an equal amount of muscle and skin on the opposite side, and tie (Figure 21). The knot should be offset, so as not to rest on the incision and the loose ends should be cut. The next simple interrupted suture is placed approximately 0.5 to 1.0 cm from the first (Figures 20a and 22). Care should be taken not to apply excessive tension on the tissue when tying the sutures, as eversion of the incision, tearing, and tissue damage can result. Also, check that the suture isn't tied too loosely by using the forceps to test the knot and suture tension. The incision is correctly closed when the forceps cannot easily retract an opening into the incision.

The simple interrupted suture pattern is used so that a loosened knot will not open the whole incision. In the aquatic environment of the fish this is particularly important. Knecht, et al. (1987) reviews suture patterns and surgical techniques and the reference is highly recommended as an aid to understanding correct suturing.

After each fish biopsy including postoperative care, is completed, wash surgical instruments in clear raceway or tap water, using a scouring pad if needed. Soak instruments in 95% ethanol and flame them to sterilize instrument tips that come in contact with tissue. After flaming, lay out the instruments on a field of sterile cheesecloth in a shallow pan. Before proceeding to the next fish, change surgical gloves and rinse gloved hands with 70% ethanol.

Figure 15. Excision of obstructing gonadal tissue to expose the gas bladder.

Figure 16. Retraction and teasing of mesenteries from gas bladder and kidney biopsy site.

Figure 17. Depression of gas bladder with stainless steel washer to expose the kidney biopsy site.

Figure 18. Kidney biopsy site (body wall musculature and all obstructing organs removed) with biopsy tissue removed and incision made through left opisthonephric duct, "L".

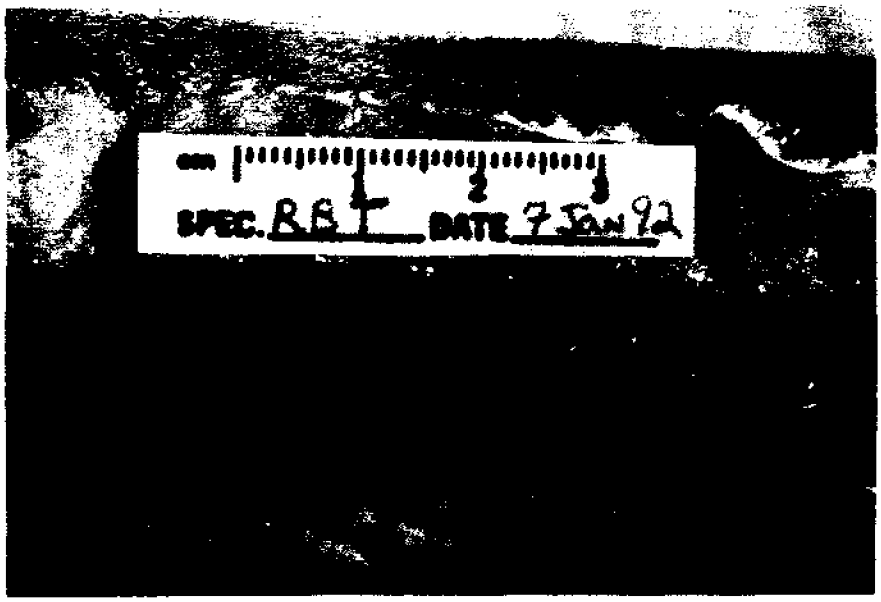


Figure 19. Use of a Lewis lens loop to obtain the kidney biopsy (body wall musculature and obstructing organs removed).

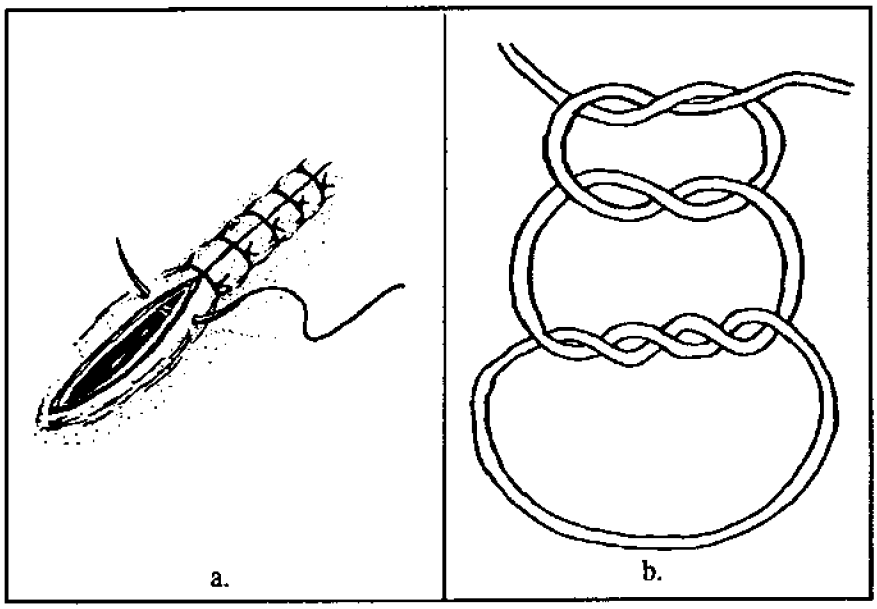


Figure 20. Interupted suture pattern (a), Reinforced surgeon's square knot.(b).

Figure 21. Suture procedure showing proper passage of needle through the abdominal muscle.

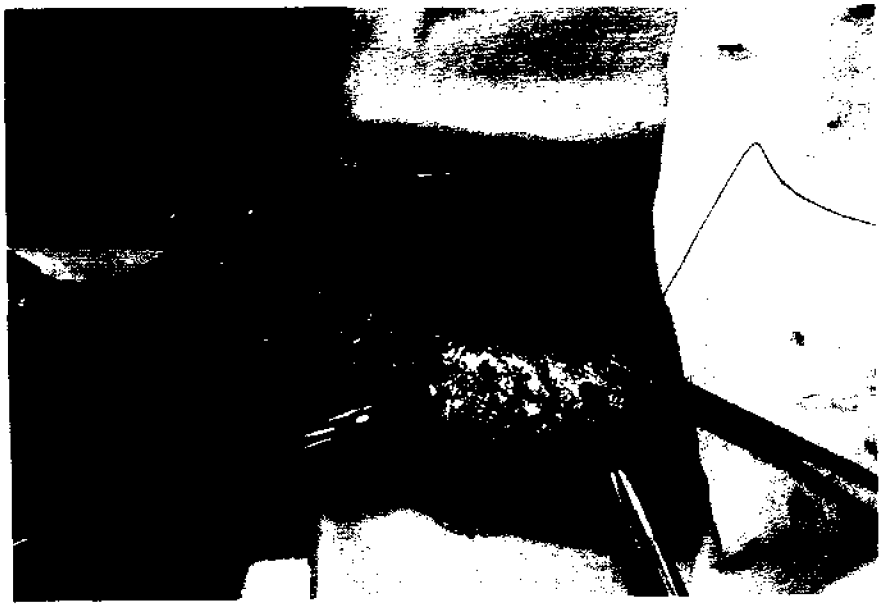


Figure 22. Completed suture pattern on liver-biopsied fish.

7. POSTOPERATIVE CARE

After suturing, tag each fish in the jaw with a stainless steel ring tag or by another suitable method to facilitate individual identification. After tagging, apply potassium permanganate to the abdominal incision at a 1:1 (weight:volume) ratio with distilled water on a sterile polyester tip applicator to help prevent fungal and bacterial infections. Immediately place the fish carefully into an indoor raceway or tank with flowing water for observation. Permanganate is a strong oxidant and it is important to have it washed from the incision so tissue damage doesn't occur.

Recovery from anesthesia may take up to15 minutes. If needed, place an air stone diffuser or source of water flow near the fish's mouth. When it is coming out of anesthesia, the fish may dart about erratically. Observe it closely to be sure no injury occurs and that the suture remains intact. If a suture opens or bleeding into the water is observed, immediately removed and resuture the fish.

After 48 hours the fish may be transferred to an appropriate holding raceway or tank. Use MS-222 at 25 ppm to sedate the fish during transport. Feed may be presented 72 hours postoperatively, according to standard hatchery methods. Antibiotics are not necessary and should be considered only if a bacterial infection of known drug sensitivity is diagnosed.

Should a bacterial or fungal problem occur any time after the operation use standard treatment protocols (Bowser and Buttner 1991). However, special attention should be given to the fish and if stress from treatment is observed, immediately withdraw treatment. The postoperative fish may be more sensitive to therapeutic treatments than normal fish.

8. BIOPSY SAMPLE PROCESSING

The size of the sample taken (Figure 23) should be sufficient for most standard diagnostic screening procedures. Impression smears or squash preparations may be made before the biopsy is stored on ice. These initial samples may be used for bacterial staining or fluorescent antibody tests. In addition bacterial plates may be streaked by taking a bacterial loop of material from the kidney before the animal is sutured. Samples should be stored and tests performed according to the procedures outlined by Amos (1985) or other appropriate guidelines.

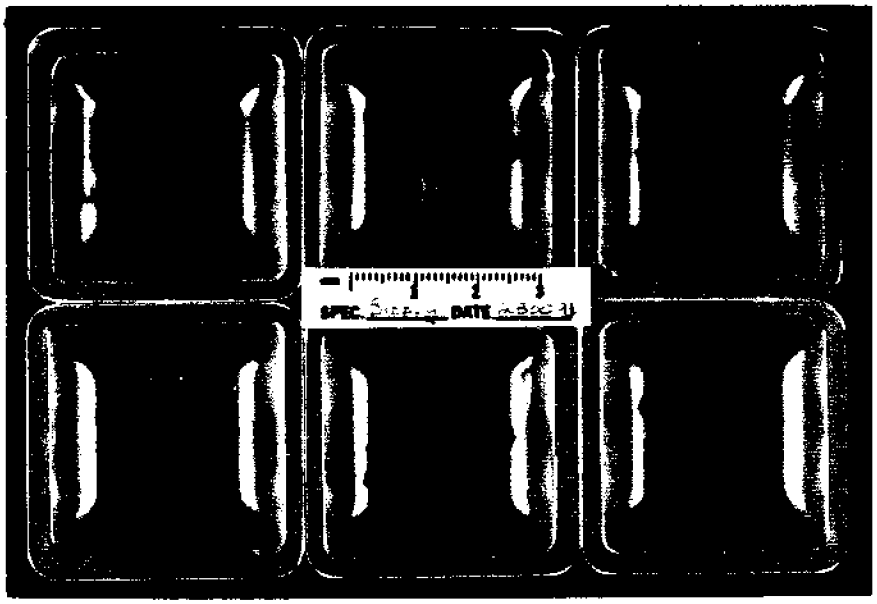


Figure 23. Representative example of liver (top row) and kidney (bottom row) tissues obtained by the nonlethal surgical technique.

9. APPENDICES

Appendix A: Listing of sources for materials and supplies. Other sources may be available locally or found in appropriate trade publications. Note: Listing of a source or manufacturer does not imply an endorsement.

Item(s):	Source:
1	John R. Lyman Co. P. O. Box 15 Chicopee MA 01014 800-628-9260
2-6	J. A. Webster Inc. 86 Leominster Rd. Sterling MA 01564-2114 800-225-7911
6	N-Dex nitrile gloves are available from: Krackler Scientific Inc. P.O. Box 1849 Albany NY 12201 800-334-7725
7-10	Fisher Scientific Inc. 711 Forbes Ave. Pittsburgh PA 15219 412-562-8300
11	Pittman-Moore 421 E. Hawley Rd. Mundelien IL 60060 800-525-9480
12	Salt Lake Stamp Co. 380 W. 2nd South St. P. O. Box 2399 Salt Lake City UT 84110 801-364-3200
13	Fisher Scientific Inc. See above.
14-16	Sigma Chemical Co. P.O. Box 14508 St. Louis MO 63178 800-325-3010 Argent Chem. Lab. 8702 152nd Ave. NE Redmond WA 98052 800-426-6258

Item(s):	Source:
14	MS- 222 also available from: Crescent Research Chemicals Inc. 5301 N. 37th Place Paradise Valley AZ 85253 602-893-9234
17	Purdue Fredrick 100 Connecticut Ave. Norwalk CT. 06850 800-877-5666
18	Butler Co. 156 Mushroom Blvd. Rochester NY 14623 800-288-5378
19-27	Miltex Inst. Company Inc. 6 Ohio Drive Lake Success NY 10042 516-775-7100 (NY, overseas) 800-645-8000 (others)
28	Purchase from local electric motor, pump, or plumbing store.
29, 30	Fisher Scientific Inc. (see above).
31-42	Purchase from local store.
43-46	Fisher Scientific Inc. (see above).
47, 48	Aquatic Ecosystems Inc. Or purchase from local pet store. 2056 Apopka Blvd. Apopka FL 32703 407-886-3939

Note: The most recent information on the current regulatory status of FDA-approved drugs and chemicals used in food fishes can be accessed by calling FARAD access center located at the University of Florida (904-392-4085).

Appendix B: Formula for a Phosphate Buffered Saline.[1]

Phosphate-buffered saline (PBS) 0.1 Molar

For 4 l:	Sodium dihydrogen phosphate (NaH_2PO_4 [monobasic])	1.8 gm
	Disodium hydrogen phosphate (Na_2HPO_4 [dibasic])	7.2 gm
	Sodium chloride (NaCl)	29.6 gm

1. Add distilled water to 4 l.
2. Adjust the pH to 7.5 with 1 Molar Sodium hydroxide (NaOH).

[1] (Brown 1988).

Appendix C: Additional Figures

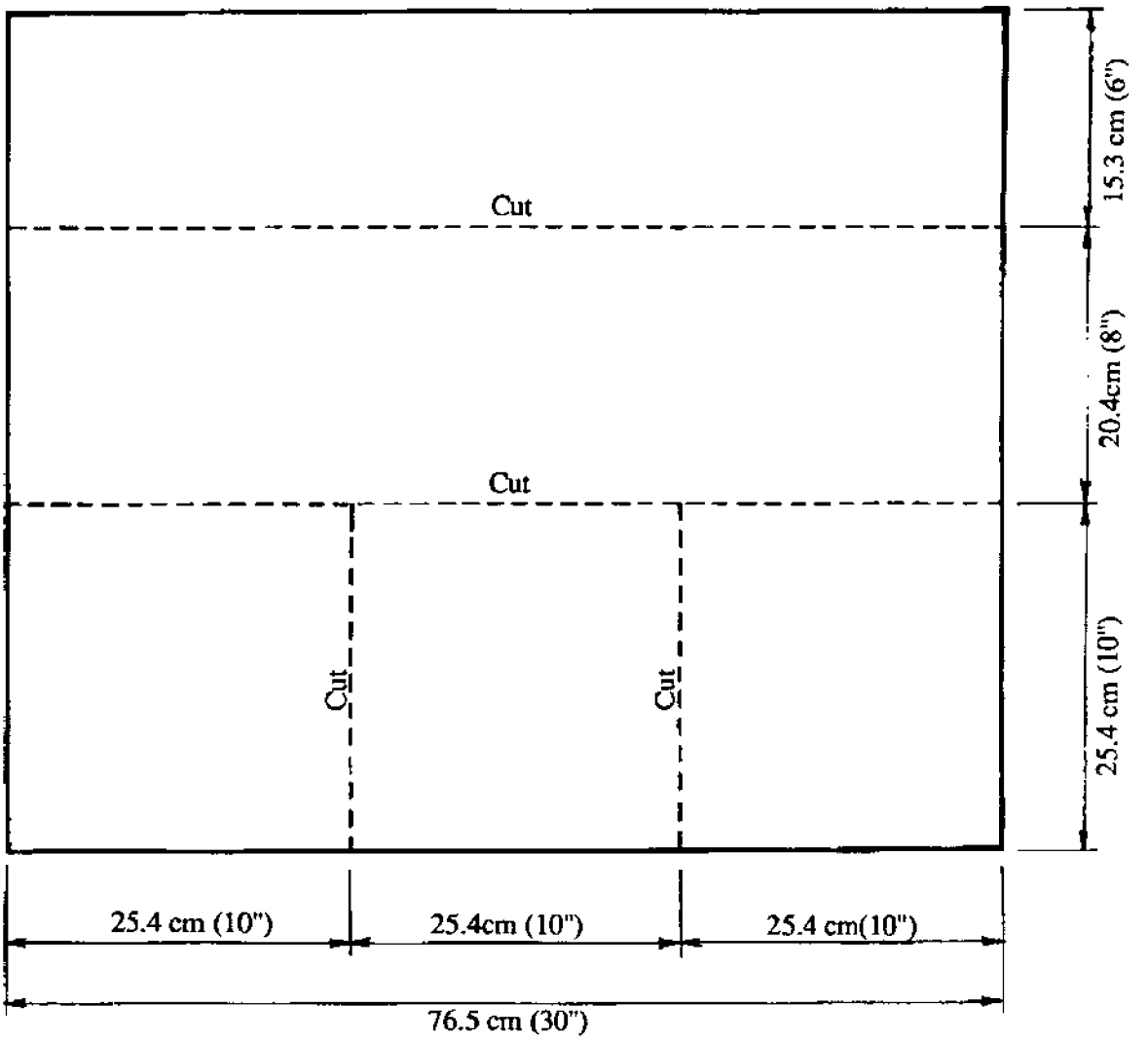
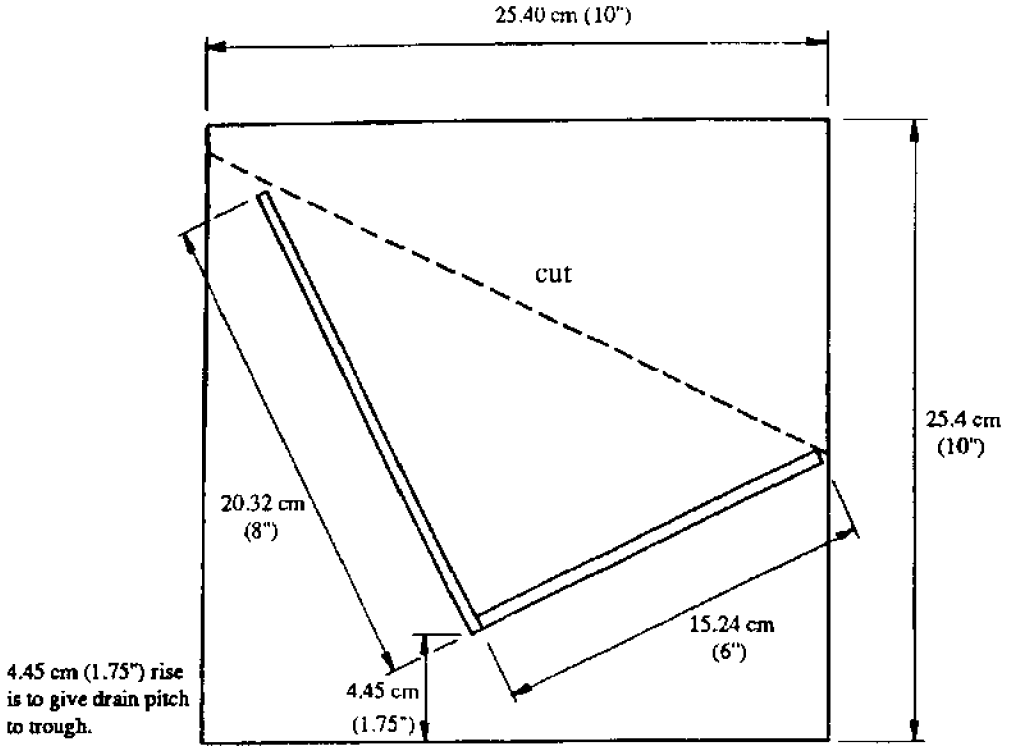
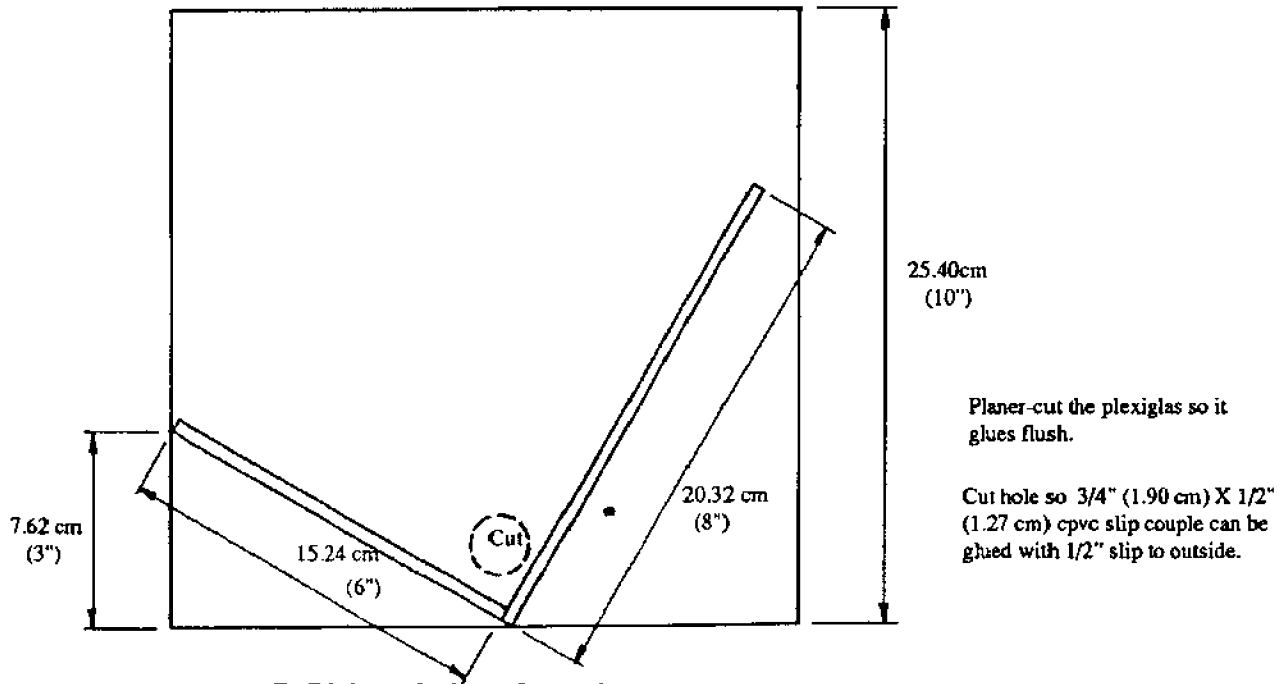


Figure C-1. Plans for constructing the V-trough surgical support table. Template for cutting Plexiglas sheet.



A. Left end view of trough.



B. Right end view of trough.

Appendix Figure C-2. End views of constructed V-trough surgical support table.

10. REFERENCES

Akhtar, N. 1984. Anesthesia, Abdominal Surgery and Efficacy of Ovariectomy and Subsequent Androgen Treatments in Inducement of Sex Reversal in Tilapia nilotica. Doctoral Dissertation. Deptartment of Fisheries and Allied Aquacultures, Auburn University, AL.

Amend, D. F., B. A. Goven, and D. G. Elliot. 1982. Etomidate: Effective Dosages for a New Fish Anesthetic. Transactions of the American Fisheries Society 111:337 - 341.

Amos, K. H., editor. 1985. Procedures for the Detection and Identification of Certain Fish Pathogens. 3d ed. Fish Health Section, American Fisheries Society. Corvallis, OR.

Bart, A. N. 1988. Effects of Anesthesia, Surgical Procedure, Temperature, Antibiotics, Reproductive Cycle and Size on Survival of Channel Catfish and Blue Catfish After Surgical Removal of Testes. Master's Thesis. Department of Fisheries and Allied Aquacultures, Auburn University, AL.

Bart, A. N., and R. A. Dunham. 1990. Factors Affecting Survival of Channel Catfish After Surgical Removal of Testes. The Progressive Fish Culturist 52:241 - 246.

Bowser, P. R., and J. K. Buttner. 1991. General Fish Health Management. Bulletin No. 111-1991. Northeastern Regional Aquaculture Center, USDA. North Dartmouth, MA. 12 pp.

Brown, L. A. 1987. Recirculation Anesthesia for Laboratory Fish. Laboratory Animals 21:210 - 215.

Brown, L. A. 1988. Anesthesia in Fish. Pages 317 - 331 in: M. K. Stoskopf, editor. Veterinary Clinics of North America: Small Animal Practices 18(2)

Brown, L. A. 1993. Anesthesia and Restraint. Pages 79-90 in: M. K. Stoskopf, editor. Fish Medicine. W. B. Saunders Co. Philadelphia, PA.

Fridburg, G., R. S. Nishioka, H. A. Bern, and W. R. Fleming. 1966. Regeneration of the Caudal Neurosecretory System in the Cichlid Teleost Tilapia mossambica. Journal of Experimental Zoology 162:311 - 336.

Gingerich, W. H., and K. R. Drottar. 1989. Plasma Catecholamine Concentrations in Rainbow Trout (Salmo gairdneri) at Rest and After Anesthesia and Surgery. General and Comparative Endocrinology 73:390 - 397.

Goetz, F. W., Jr., R. A. Hoffman, and W. L. Pancoe. 1977. A Surgical Operating Apparatus for Fish and Its use in the Pinealectomy of Salmonids. Journal of Fish Biology 10:287 - 290.

Hart, L. G., and R. C. Summerfelt. 1975. Surgical Procedures for Implanting Ultrasonic Transmitters into Flathead Catfish (Pylodictis olivaris). Transactions of the American Fisheries Society 104:56 - 59.

Harvey, W. D., R. L. Noble, W. H. Neill, and J.E. Marks. 1984. A Liver Biopsy Technique for Electrophoretic Evaluation of Largemouth Bass. Progressive Fish Culturist 46:87-91.

Ireland, M. P. 1969. Effect of Urophysectomy in Gasterosteus aculeatus on Survival in Fresh Water and Sea Water. Journal of Endocrinology 43:133 - 134.

Kenyon, C. J., I. Chester-Jones, and R. N. B. Dixon. 1980. Acute Responses of the Freshwater Eel (Anguilla anguilla) to Extracts of the Corpuscles of Stannius Opposing the Effects of Stanniosomatectomy. General Comparative Endocrinology 41:531 - 538.

Klontz., G. W. 1965. Anesthesia of Fishes. Pages 350-370 in: D. C. Sawyer, editor. Experimental Animal Anesthesiology. USAF School of Aerospace Medicine; Aerospace Medical Division (AFSC), Brooks Air Force Base, TX.

Klontz, G. W., and L. S. Smith. 1968. Methods of Using Fish as Biological Research Subjects. Pages 323 - 385 in: W. Gay, editor. Methods of Animal Experimentation, Vol. 3. Acedamic Press, London.

Knecht, C. D., A. R. Allen, D. J. Williams, and J. H. Johnson. 1987. Fundamental Techniques in Veterinary Surgery 3d. edition. W. B. Saunders Co. Philadelphia, PA.

Krayukhin, B. U. 1962. Methods of Installing Chronic Fistulas in the Digestive Tract of Fish. Pages 125-137 in: E. N. Pavlovskii, editor. Techniques for the Investigation of Fish Physiology. (Translated from Russian by the Israel Program for Scientific Translations. Jerusalem, 1964.) U.S. Dept. of Commerce. Washington, D.C. IPST#1130.

Kyono-Hamaguchi, Y. 1984. Effects of temperature and Partial Hepatectomy on the Induction of Liver Tumors in Oryzias laptipes. Pages 337 - 344 in: K. L. Hoover, editor. Use of Small Fish Species in Carcinogenicity Testing. Symposia Proceedings. May 1984. National Cancer Institute Monograph #65. NCI, Bethesda, MD.

Lewis, T. L., W. W. Parke, and A. Epple. 1977. Pancreatectomy in a Teleost Fish, Anguilla rostrata (American Eel). Laboratory Animal Science 27(1):102 -109.

Mair, G. C. 1989. A Technique for Sampling Muscle Tissue From Live Fish. Journal of Fish Biology 35:159 - 160.

Marking, L. L. and F. P. Meyer. 1985. Are Better Anesthetics Needed in Fisheries? Fisheries 10(6):2 - 5.

McFarland, W. N. 1960. The Use of Anesthetics for Handling and the Transport of Fish. California Fish Game 46:407 - 431.

Morizot, D. C., M. E. Schmidt, G. J.Carmichael, D. W. Stock, and J. H. Williamson. 1990. Minimally Invasive Tissue Sampling. Pages 143-156 in: D. H. Whitmore, editor. Electrophoretic and Isoelectric Focusing Techniques in Fisheries management. CRC Press. Boca Raton, FL.

Noga, E. J., J. F. Levine, K Townsend, R. A. Bullis, C. P. Carlson, and W. T. Corbett. 1988. Kidney Biopsy: A Non-lethal Method for Diagnosing Yersinia ruckeri infection (Enteric Redmouth Disease) in Rainbow Trout (Salmo gairdneri). American Journal of Veterinary Research 49(3):363 - 365.

Ostrander, G. K., J. B. Blair, B. A. Stark, and J. G. Hurst. 1993. Response of Rainbow Trout Liver to Partial Hepatectomy. Aquatic Toxicology. 25:31 - 41.

Olsen, M. E. and J. Bruce. 1987. Cyanoacrylate Tissue Adhesives. Lab Animal 16 (2):27-30.

Pavlovskii, E. N., editor. 1962. Techniques for the Investigation of Fish Physiology (Translated from Russian by the Israel Program for Scientific Translation. Jerusalem, 1964.) U.S. Department of Commerce, Washington D.C.

Robertson, O. H. 1958. Accelerated Development of Testes After Unilateral Gonadectomy, with Observations on Normal Testes of Rainbow Trout. U.S. Fish and Wildlife Service. Fisheries Bulletin 127, Vol. 58. U.S. Government Printing Office, Washington D.C.

Schnick, R.A., F. P. Myer, and D. L. Gray. 1989. A Guide to Approved Chemicals in Fish Production and Fishery Resource Management. University of Arkansas Cooperative Extension Service. Little Rock, AR.

Smith, L. S. and G. R. Bell. 1967. Anesthetic and Surgical Techniques for Pacific Salmon. Journal of Fisheries Research Board Canada 24(7):1579 - 1588.

Stoskopf, M. K. 1985. Manual for the Aquatic Animal Workshop. American Association for Laboratory Animal Science, National Capital Area Branch. Washington D.C.

Tytler, P. and A. D. Hawkins. 1981. Vivisection, Anaesthetics and Minor Surgery. Pages 247 - 278 in: A. D. Hawkins, editor. Aquarium Systems. Academic Press, London.

Tull, S. 1755. Method of Castrating Fish. The Gentleman's Magazine. London, England. 25:416 - 417.

Wooster, G. A., H.-M. Hsu, and P. R. Bowser. 1993. Non-Lethal Surgical Procedures for Obtaining Tissue Samples for Use in Fish Health Inspections. Journal of Aquatic Animal Health 5(3):157-164.