APPENDIX D

Field Standard Operating Procedure

Surgical Tag Implantation Procedures Used in VAMP Studies

Purpose

To provide guidelines and standard protocols for surgical tagging of juvenile salmonids for VAMP studies.

Area of Applicability

All staff involved in surgical tagging of juvenile salmonids for VAMP studies.

References

Adams, N.S., Rondorf, D.W., Evans, S.D., Kelly, J.E. 1998. Effects of Surgically and Gastrically Implanted Radio Transmitters on Growth and Feeding Behavior of Juvenile Chinook Salmon. Transactions of the American Fisheries Society 127:128-136.

Kelsch, S. W., and B. Shields. 1996. Care and Handling of Sampled Organisms. Fisheries

Techniques, 2nd edition. American Fisheries Society 121-155.

Martinelli, T.L., H.C. Hansel, and R. S. Shively. 1998. Growth and physiological responses to surgical and gastric radio transmitter implantation techniques in subyearling Chinook salmon. Hydrobiologia 371/372: 79-87.

Summerfelt, R. C. and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213-272 in C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Materials Needed

- Thermometer
- YSI 55 dissolved oxygen (DO) meter
- Acoustic tags and acoustic tag equipment
- Chlorhexidine solution (30mL/L D-H2O)
- Saline solution (7g/L D-H2O)
- Tricaine methanesulfonate (MS-222; 100g/L),
- Sodium bicarbonate solution (buffer; 100g/L)
- Stress coat - stock concentration and 25% solution (250mL/L D-H2O)
- 70% ethanol or isopropyl alcohol solution
- 19 L bucket(s) marked at 10 L and clearly labeled 'Anesthesia'
- 19 L perforated recovery buckets (7 L holding capacity)
- 19 L bucket clearly labeled 'Reject' for fish that are not tagged
- Pair of gravity feed containers marked at 10 L, and connected by rubber tubing with in-line shut-off valves – one labeled 'anesthesia' and one labeled 'freshwater'
- Syringes for measuring anesthetic, buffer, and stress coat
- Oxygen delivery system or bubblers
- Dip nets
- Nitrile gloves
- Scale measuring to the nearest 0.1 g
- Large plastic weigh boats
- Measuring board with ruler to the nearest millimeter
- Surgery table (tray with foam pad and groove cut)
- Trays for holding solutions used to disinfect surgical tools
- Needle drivers

- Forceps
- Scalpel handle and blades
- Oxytetracycline (100 mg/mL concentration)
- Pipette (2-20 microliter (_L) volume) and tips
- Sutures (size: 5-0 and 4-0) with an RB-1 needle
- Spray bottles for alcohol
- Timer(s)
- Sharps container
- Datasheets and writing tools

Procedures

1) Collection and Pre-Tag Holding

A. The pre-tag holding period begins once the fish are placed in holding tanks. Prior to tag implantation, the pre-tag holding period should be at least 12-36 h. Fish should not have access to food during the pre-tagging holding period.

B. Each species collected is held in a separate holding tank to reduce stress. Record the species and collection date on each pre-tag holding container.

2) Fish Size Criteria

A. Size of fish tagged is dependent on the type of tag being used. A maximum tag weight to body weight ratio of 5% is used to calculate minimum fish size.

3) Pre-Tag Preparations

A. Environmental conditions

i. Dissolved oxygen (DO): will be measured as percent saturation in a pre- and post-tag holding tank or raceway during each tag session.

1. Measurements will be taken using a YSI model 55 DO meter

2. DO concentrations in pre- and post-tag holding tanks should be between 80% and 130% saturation.

ii. Temperature: will be measured in °C in a pre- and post-tag holding tank during each tag session.

1. Changes in water temperature exceeding 2°C require tempering (Kelsch and Shields 1996). "Tempering" means "to bring to a suitable state by mixing in or adding a usually liquid ingredient". Therefore, prior to exposing fish to a new water source the fish holding temperature and the temperature of the new water source need to be measured to ensure that the difference between the two water sources is ≤ 2°C. If the temperature difference is > 2°C then water in the container holding fish should be tempered at a rate of 0.5°C/15 min until the temperature difference between the two water sources is ≤ 2°C. New source water should be added in small amounts multiple times over 15 min to gradually change the temperature by 0.5°C. Once the temperature difference between the two water sources is ≤ 2°C fish can be transferred to the new water source.

B. Setup of equipment

i. Tags should be programmed and prepared for implantation.

ii. Disinfect all tags in chlorhexidine solution and thoroughly rinse in saline. Line tags up near the surgery table.

iii. Prepare surgical table and equipment for use.

iv. Setup measuring board and scale

1. Ensure the scale is functioning properly. Scales should be calibrated at the start of the season, checked each week for accuracy, and recalibrated as necessary.

2. Put approximately 1-2 mL of diluted stress coat on the weigh boat and the measuring board.

C. Recovery buckets must be filled with untreated river water and supplied with oxygen or a bubbler just prior to tagging. The concentration of DO in recovery buckets should be between 120 and 150% saturation.

D. Administration of anesthetic: The effectiveness of MS-222 as an anesthetic varies with factors such as temperature and fish density. Adjustments of the anesthesia concentration should be based on the amount of time it takes for a fish to lose equilibrium (induction time).

i. Fill the anesthesia bucket with 10 L of untreated river water. As a starting concentration, add 7 mL (1 mL= 1 cc) of MS-222 stock solution. This will yield an anesthetic concentration of 70 mg/L.

ii. Fill both gravity feed containers with 10 L of untreated river water. Add 2 mL of MS-222 stock solution to the container marked anesthesia. This will yield an anesthetic concentration of 20 mg/L.

iii. For each mL of MS-222 added to a container, add the same amount of bicarbonate solution (buffer).

iv. Water in all containers (anesthesia and gravity feed) should be changed periodically to minimize dilution of anesthesia water and temperature changes and to ensure you do not run out of water during a surgery.

v. Add a small amount of diluted stress coat for each liter of water in the anesthesia, gravity feed, and recovery containers to protect fish from loss/damage to the slime layer.

vi. Containers should be filled and prepared just prior to tagging to avoid temperature changes.

4) Implantation of Tags

A. Anesthetizing fish

i. Net one fish from the pre-tag holding source and place directly into an anesthesia bucket. Secure the lid as soon as the fish is in the bucket. Start a timer to keep track of how long a fish has been in the anesthesia bucket.

1. Time of sedation for a fish should normally be 2 - 4 minutes, with an average time of about 3 minutes. If loss of equilibrium takes less than 1 min or greater than 5 min, reject that fish. If after sedating a few fish, they are consistently losing equilibrium in more or less time than typical, adjust the concentration of the anesthetic (up or down) in 0.5 ml increments of stock MS-222 solution.

2. Remove the lid after one minute to observe the fish for loss of equilibrium. Once the fish loses equilibrium, visually screen the fish for tags, fin clips, fungus, disease, descaling, bloated belly, or any obvious abnormalities. Make sure to keep the fish submerged during this examination. Relay any information to the data recorder.

3. Keep the fish in the water for an additional 30 - 60 sec after it has lost equilibrium.

4. Rejects - If the fish is unacceptable for tagging, place the fish in the bucket labeled Rejects, and relay the information to the data recorder.

B. Recording fish length and weight

i. Transfer the fish to the scale and weigh the fish to the nearest 0.1 g.

ii. Transfer the fish to the measuring board and measure the fork length to the nearest millimeter (mm).

iii. Data must be vocally relayed to the data recorder to avoid data errors. The data recorder should then record this information and repeat numbers back to avoid any miscommunication.

iv. Any fish that is dropped on the floor during this process must be rejected. A fish dropped on the table during surgery may still be tagged. If a fish is dropped on the floor after it is tagged, remove the tag and reject the fish.

C. Surgery

i. Place the fish on the surgery table ventral side up. Anesthesia should be administered through the gravity feed tubing as soon as the fish is on the surgery table. The tubing must be placed just inside the mouth so the water flows across the gills. If the flow is too low, the fish will flare its opercula and become agitated. Adjust the flow so that the gilling rate of the fish is steady. Use the in-line valve to control the flow of anesthesia, fresh water, or a mixture of both. Start with a constant flow of anesthesia and monitor the condition of the fish.

ii. Using a scalpel, make an incision, approximately 5 mm in length (dependent on tag size), about 3 mm away from and parallel to the mid-ventral line. Start your incision a few millimeters in front of the pelvic girdle, approximately 20% of the distance from the base of the pelvic fins to the base of the pectoral fins, and draw the blade toward the head of the fish. (For example, in Figure 1, the distance between the base of the pelvic and pectoral fins is ~45 mm, so the incision should start ~9 mm in front of the base of the pelvic fins.) The incision should be just deep enough to penetrate the peritoneum (the thin membrane separating the gut cavity from the musculature), avoiding the internal organs. The spleen is generally near the incision point, so pay close attention to the depth of the incision. Refer to Figure 1 for location of internal organs and Figure 2 for placement of incision. Avoid getting anesthesia water in the incision.

Figure 1
Lateral view of a juvenile salmonid, showing the location of internal organs.

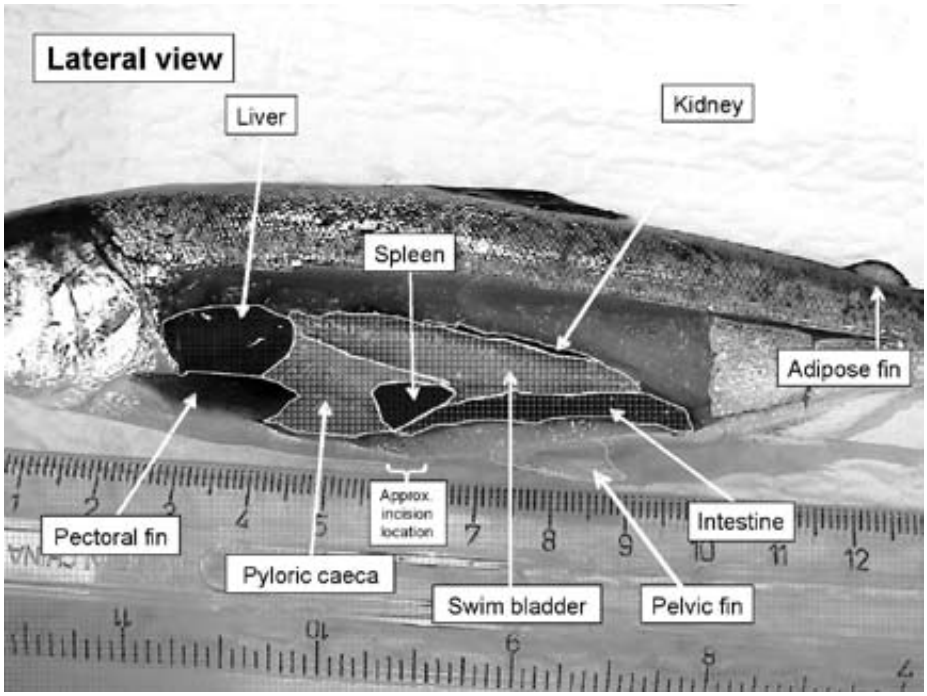
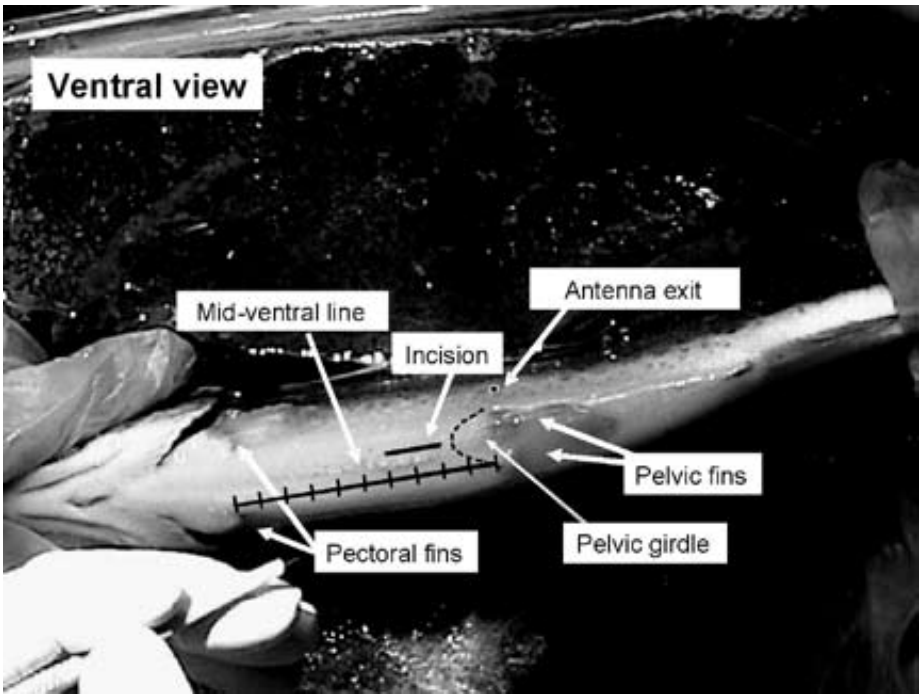


Figure 2
Ventral view of a juvenile salmonid, showing the location external organs and proper placement of incision and antenna exit (if applicable). This view corresponds to a left-handed surgeon's view and placement of the incision. For right-handed surgeons, the fish would be facing the right and the incision and antenna exit would be on the opposite side of the midline.



1. There is no exact specification for what size scalpel blade to use for each fish. We use a 5 mm blade for hatchery steelhead, which typically weigh more than 50.0 g. We use a 3 mm blade for smaller fish, such as yearling and subyearling Chinook salmon that typically weigh less than 50.0 g.

2. One scalpel blade can be used on about seven fish before it becomes dull. If the blade is pulling roughly or making jagged incisions, it needs to be changed prior to tagging the next fish.

3. Use forceps to open the incision to ensure you did not damage any internal organs or cause excessive bleeding. If you observe damage or think you damaged an organ, do not implant the tag, and reject that fish. Excessive bleeding should be noted on the datasheet.

iii. Gently push the tag into the body cavity, and position it so that it lies directly under the incision. This positioning will provide a barrier between the suture needle and internal organs. Through time the tag location will naturally move posterior in the fish.

iv. Use a pipette to administer oxytetracycline in the incision at a dosage of 50 mg/kg of body weight. Calculate the amount to administer for each fish using 1 _L of oxytetracycline for every 2 g of body weight (weight in g/2 = # of _L of oxytetracycline). For example, a 24.0 g fish would get 12 _L of oxytetracycline (Summerfelt and Smith 1990). Change the pipette tip after each fish.

v. Begin suturing the incision. Two or three interrupted stitches are used to close the incision, depending on the size of the tag and incision.

1. To make a stitch, lock the needle (at the end of the suture) in the needle drivers so the needle point faces you. Enter the outside edge of the incision on the side farthest from you and exit through the other edge of the incision, pulling the suture perpendicular through the two edges. The needle should enter and exit the skin as close to the edge of the incision as possible without tearing the skin (~ 2 mm from edge of incision). Pull the needle and suture through the skin to leave a tag end of about 2 - 3 cm of suture material protruding from the needle entrance location, then release the needle from the needle drivers. With your non-dominant hand, grasp the long end of the suture material (usually

with thumb and forefinger) at or below the needle, and make two forward wraps (i.e., away from your body) around the tip of the needle driver, which should be held in your dominant hand. With the two wraps still around the needle driver, grasp the short tag end of suture material with the needle driver and tighten the stitch by pulling the wraps off the needle driver and pulling both ends of suture material perpendicular to the incision. On the first knot, the dominant hand holding the needle driver should pull toward your body and the non-dominant hand should pull away from your body. Tighten the suture lightly, just so the edges of the incision meet, but do not overlap, pucker, or bulge the edges of the incision. The second knot is the same as the first, but in reverse order. On the second knot, grasp the long end of suture material with your non-dominant hand, make two reverse wraps (i.e., toward you body) around the end of the needle driver, grasp the short end of suture with the needle driver, and tighten the stitch. This time, the knot should be tightened by pulling your dominant hand (holding the needle drivers) away from you and your non-dominant hand toward you. The second knot can be slightly tighter than the first, again taking care not to overlap, pucker, or bulge the edges of the incision. The third knot is a repeat of the first and should be tightened snug to prevent the stitch from coming loose. This completes one stitch. Cut the suture with the needle drivers, leaving ends approximately 5 mm in length.

a. An alternative stitch consists of two knots, each with three wraps around the needle driver. The first knot consists of three forward wraps around the needle driver, and then is tightened by pulling the needle driver toward your body. The second is the same as the first, but in reverse order as described above.

b. When pulling a knot tight, be sure the knot lays flat and does not twist onto itself into a "balled-up" knot

2. There is no exact specification for what size suture to use. Generally, 4-0 suture is used for hatchery steelhead, which typically weigh greater than 50.0 g. For fish weighing less than 50.0 g, such as yearling and subyearling Chinook salmon, 5-0 suture is used.

3. Generally, a good time to switch the in-line valve on the gravity feed buckets to untreated river water is just prior to the last stitch. This initiates recovery from anesthesia as early as possible. However, if the fish appears to be inadequately gilling, provide a mixture or all fresh water as soon as possible. If the fish is too active to finish the surgery safely do not switch to fresh water, but maintain sedation.

4. If the incision is too long to close with two stitches, it is acceptable to add a 3rd stitch. Relay this information to the data recorder so they can note the extra stitch on the datasheet.

5. Because sutures are long, each individual suture (one packet) can be used on 2-4 fish. Rinse the suture material and the needle in the sanitizing solution used for instruments.

vi. Transfer the fish from the surgery table directly to a labeled recovery bucket. If a direct transfer is not possible, use a container filled with untreated river water to make the transfer.

vii. Between surgeries, the surgeon should prepare their tools for the next surgery. Disinfect the tools in chlorhexidine solution and rinse thoroughly with saline, load a new pipette tip, and ensure that the scalpel blade and suture are acceptable to use on the next fish.

viii. When all fish in a recovery bucket have spent 10 minutes in the bucket and gained equilibrium, transfer the bucket to the post-tag holding container (tank or raceway that has a constant flow of untreated river water).

5) Cleanup at the end of the tagging day

A. Wipe down all counter tops, scales and measuring boards with ethanol or isopropyl alcohol to disinfect.

B. Soak scalpels, catheters, forceps, and scissors in chlorhexidine solution for 15 minutes, rinse in saline solution, and thoroughly dry to prevent rusting.

C. Spray tagging platform (foam) with ethanol to disinfect.

D. Scrub needle drivers with a small brush and spray with ethanol or isopropyl alcohol.

E. Buckets should be rinsed thoroughly with untreated river water and placed upside down to dry. In addition, all buckets need to be cleaned weekly in accordance with Sterilization of 5 Gallon Buckets; FIE732.0.

APPROVED BY: _____ DATE _____
QUALITY ASSURANCE OFFICER

REVIEWED BY _____ DATE _____
LABORATORY SUPERVISOR