Surgical Protocols for Implanting JSATS Transmitters into Juvenile Salmonids for Studies Conducted for the U.S. Army Corps of Engineers

Version 1.0

Prepared by:
Surgical Protocols Steering Committee (listed alphabetically)

Gordon Axel, NOAA Fisheries
John Beeman, USGS
Rich Brown, PNNL
Brad Eppard, USACE, NWP
Scott Fielding, USACE, NWP
Eric Hockersmith, NOAA Fisheries
Theresa Liedtke, USGS
Christopher Peery, USFWS
Christa Woodley, PNNL

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U.S. Army Corps of Engineers, Portland District
Portland, Oregon

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1. Introduction

Biotelemetry has become a standard tool in the Columbia River to assess behavior and survival of downstream migrating anadromous salmonids. Use of passive integrated transponder (PIT) tags as well as radio telemetry (RT) has been common in the Columbia Basin (see Skalski et al. 1998; Bickford and Skalski 2000; Hockersmith et al. 2003). Technological advances have improved the capacity and reliability of these methods, but each has inherent limitations. For example, radio signals used for aquatic telemetry (e.g. 30 to 300 MHz range) are attenuated proportionally with water depth, making signals difficult to detect if fish are relatively deep (>15 to 20 ft). In salt water, radio signals are attenuated at an even greater rate because of the associated conductivity. Radio telemetry transmitters also typically incorporate an external antenna which may affect swimming behavior and long term survival of small fish. Alternatively, acoustic signals (30 to 500 Hz) travel well through fresh and saltwater, their transmitters do not require an external antenna, and this technology provides the potential for three-dimensional positioning. In an effort to create an effective and affordable salmon research tool, the U.S. Army Corps of Engineers (USACE) has developed an acoustic telemetry system for use with juvenile salmon evaluations within the Federal Columbia River Power System (FCRPS). The juvenile salmon acoustic telemetry system (JSATS) is being used in many parts of the FCRPS system for juvenile salmon evaluations and shows promise for use with other species and life stages. As use of JSATS expands, efforts to coordinate and standardize methods among research groups will be critical to maintain the quality and comparability of data that are collected. Subsequently, there is a need to develop surgical procedures for the intraperitoneal implantation of transmitters in fish while maintaining welfare status and ensuring that tagged fish are representative of untagged conspecifics.

To provide oversight of these protocols a Surgical Protocol Steering Committee has been formed consisting of research scientists from within the Columbia River Basin. The protocols described in this document are intended to provide strict guidelines as to how research fish utilized in Andromous Fish Evaluation Program (AFEP) funded passage and survival studies should be handled throughout the collection, tagging, and holding process prior to release. Surgical protocols documented here were established based on a thorough review of the scientific literature, current research, group discussions among experts in the field of biotelemetry and fish physiology and a consensus of the Steering Committee. As such, protocols that require the use of specified materials or techniques are not subject to change without discussion and consent of the Steering Committee prior to the start of the research field season. Anticipated or planned deviations from these protocols should be presented to the Surgical Protocol Steering Committee at least 90 days in advance of the research field season. Information pertaining to changes should be prepared and presented to the committee allowing for time to review and discuss proposed changes. Based on the discussion and decision of the Steering Committee, a waiver for deviation from a given protocol may be granted. This deviation shall be clearly documented in the Methods section of the annual research report. All waivers, granted and denied (and reasons for denial) will be documented by the Steering Committee for future reference.

The Surgical Protocol Steering Committee recognizes that unplanned issues and challenges are inherent to all scientific research therefore other protocols were established to allow for flexibility on the part of individual researchers. These protocols are intended to function more as guidelines however every effort should be made to follow protocols where practical. When possible a project lead should attempt to get a waiver from the Steering Committee by emailing its members prior to making any changes. In the event that this is not possible the USACE point of contact, Steering Committee members, and other affected researchers should be notified as soon as practical. All deviations from these protocols shall be documented and reported in the Methods section of the annual research report.

Similar documents that standardize other aspects of JSATS research (i.e. receiver deployment, data structure and processing methods, etc.) are in development. These are intended to be living documents. We anticipate that this document will be regularly updated as improvements to surgical techniques are developed, more data become available on current techniques, or logistical challenges associated with execution of these protocols are encountered. The scientific literature review and the most recent version of this document can be found under the AFEP Final Report/FCRPS section at

http://www.nwp.usace.army.mil/environment/home.asp

2. Pre-surgical Handling

Fish collection

In most cases juvenile salmonids *Oncorhynchus spp*. to be used for biotelemetry studies will be collected from juvenile fish facilities at USACE operated hydroelectric dams on the Columbia and Snake rivers. Dams with fish collection capabilities include Bonneville, John Day, McNary, Ice Harbor, Lower Monumental, Little Goose and Lower Granite dams. Fish may also be tagged at regional hatcheries when large numbers of study animals are needed in a short period of time or when potential impacts to wild fish need to be minimized.

While current ESA permitting requires that fish be held at dams for no more than 48 hrs by the COE before being released or transported, it is possible for fish to hold behind screens or in gatewells for extended amounts of time before arriving at juvenile facilities (Beeman and Maule 2001; Axel et al. 2002)[1,2]. Researchers should be aware of these potential delays when fish are being processed for telemetry programs.

Pre-surgical holding

The pre-surgery holding time starts once the group of fish to be tagged has been collected and the fish are considered in the possession of the researcher. Researchers should plan tagging operations so that the pre-surgery holding time is about 24 hr, plus or minus 6 hr (18 to 30 hr). Twenty-four hrs is believed sufficient to allow physiological recovery from the collection and sorting process and to allow for gut evacuation and for standard dynamic action to at least partially subside, but not too long so as to incur additional holding stress (Oldenburg et al. *In press*). Researchers should be aware that collection time prior to the start of pre-surgery holding can be up to 48 hrs, depending on fish abundance, and should plan accordingly to tag fish as quickly as possible. If fish are collected at a hatchery or other location where they are typically fed, food should be withheld for 24 h prior to tagging.

[1] Axel et al. (2002) reported median residence times in gatewells at McNary Dam for subyearling Chinook salmon smolts at McNary Dam were 2.6 to 8.6 hrs, median collection channel travel times were 4.5 to 10.5 minutes, and that time in gatewells represented 90 to 98% of time to transit the bypass system.

[2] Beeman and Maule (2001) reported median residence times in gatewells were 8.9 hr for Chinook salmon and 3.2 hr for steelhead, making up 83% and 96% of total transit times.

A tagging session can take from <1h to 12 hrs, from first to last fish, depending on the number of fish to be tagged and the structure and size of the tagging crew. Total surgery time begins (and pre-surgical holding ends) when the first fish is anesthetized for tagging. Post-surgical holding should generally be 24 hrs with an allowable range of 18 to 36 hrs. The post-surgical time begins (surgery time ends) when the last fish has been tagged and has been placed into the recovery container. ***Researchers should target total holding time, from researchers taking possession of fish to release (or loading onto a barge for transport studies) of around 72 hrs.***[3] Researchers should document total pre-surgical hold time, overall surgical time (from first fish in the knock-down anesthetic to the last fish placed into recovery container) and post-surgery holding times for each tagging group.

Note, surgical time for each fish is individually tracked (see below) and is a separate time measure than the total surgery period described here.

Exemptions from these protocols may be sought from the Steering Committee if a study design requires large release groups, or if fish abundances are low, such that more than one day of tagging is required to be pooled into one release group, requiring that some fish may have total holding time greater than 72 hrs. See above for description on how to obtain waivers from these protocols.

Holding conditions

Factors such as holding conditions, containers used, and distance of holding containers from tagging facility/trailer will vary by project. Typically, fish are transferred from the holding area to tanks at the collection location, then moved from these tanks to an anesthetic container. Researchers shall strive to reduce the number of times that fish are transferred and handled using equipment and methods that maximize water-to-water transfer. Environmental conditions should be as optimal as possible to reduce stress and maintain fish health prior to, during, and following surgery; these include:

- The water source for holding fish shall be river water where possible or well water if tagging/holding fish at a hatchery; treated water (i.e. tap water) shall never be used to hold fish.
- Dissolved oxygen shall be maintained at 80-110% saturation in continuous flow-through tanks (ideally) or by adding oxygen from an aeration system or via oxygen tanks through air stones in static tanks.
- Total dissolved gas levels shall be maintained below 105%. De-gassing columns can be used as needed to reduce gas levels.
- Water temperature deviations shall be less than 2˚C from ambient.
- Maximum water temperature limits for handling and tagging operations are generally set by USACE and NOAA Fisheries in the Fish Passage Plan[4] and collection/scientific permits.
- Fish shall be held in dark and covered tanks when possible (provide shading at minimum) and only dark containers (i.e. buckets) should be used.
- Holding densities should be less than 50g/L for all container types.

Researchers shall monitor and record water temperatures at regular intervals (i.e. Hobo temperature loggers recording at least hourly) and dissolved oxygen periodically, and appropriate steps

[3] Congleton and Wagner (2006) demonstrated that blood chemistry indicators of fish nutritional state (plasma proteins, lipids, ALP, etc.) significantly declined in Chinook salmon smolts after 7 to 14 days of fasting. After 3 days of fasting (hatchery Chinook salmon only) blood-chemistry variables were measurably lower (e.g. 3.65 vs. 3.35 g/dl plasma protein) for fasted fish, but no comparison was statistically significant.

[4] See: http://www.nwd-wc.usace.army.mil/tmt/documents/fpp/2008/index.html

shall be taken prior to reaching threshold levels. Precautions should be made by researchers to monitor water conditions appropriate to risks. For example, pumped water supplies may be more prone to interruption than gravity feed and should involve closer monitoring. Monitoring water quality overnight shall be considered if it is determined that the water supply is not reliable. Researchers shall document water quality conditions in their report if temperature or dissolved oxygen falls outside acceptable ranges as described above so this information can be taken into consideration when data are used later.

3. Surgical technique and materials

Fish collection and pre-sorting

Fish used for studies are usually collected at juvenile bypass facilities by non-research personnel and possibly research project designated assistants. It is important that study fish be representative of the untagged population. Selection of fish at this stage by non-researchers should be based solely on species and age/size group required for the study to avoid the chance that fish may be high-graded prior to coming into possession of research teams. Extra fish should be collected to insure that the researcher has the required number of fish needed in case some fish are rejected during the sorting/tagging process.

Fish sorting

Once researchers take possession of fish, the period of holding begins. Total time from this point until tagged fish are release should not exceed 72 hrs (see above). Fish shall be inspected prior to surgery and shall be excluded from the study group if one of the following conditions exists;

- Health or condition of the fish is poor to the point that short-term survival is questionable. These conditions include;
  - Greater than 20% descaling
  - Significant injury (open wound, significant deformity, bleeding)
  - Missing or mostly missing opercles.
  - Fungus
  - Moribund from existing disease (such as bacterial kidney disease or gas bubble trauma)
- Fish that are already tagged other than coded-wire-tag (CWT).
- Fish that are too small-- ***< 95 mm fork length***.

The total numbers and percent of fish rejected and reason for rejection for the study shall be documented in report appendices. The goal is 1% or lower rate of rejection over the course of a study. This goal does not include surplus fish collected above those needed for tagging. A rejection rate or holding mortality approaching or exceeding 1% for 2-5 d should be an indication of a chronic problem and researchers should discuss with appropriate USACE personnel on whether tagging should proceed.

Anesthetizing fish

MS-222 (tricaine methanesulfonate) is the anesthetic selected for tagging juvenile salmon. All precautions for use as noted in material safety data sheets (MSDS) shall be followed by researchers, in particular, when handling the drug in its crystalline powder form.

- Powdered MS-222 should be stored in a secure location (i.e. to limit potential exposure during day-to-day operations) and refrigeration is recommended.
- It is recommended that researchers make a stock solution[5] in amounts that can be used within one week (i.e. make fresh weekly).
- Stock solution should be stored in dark bottles since the chemical degrades in sunlight.

Recommended dosage for knock-down anesthesia is 60-80 mg/L. Researchers should adjust dosage as needed to ensure fish lose equilibrium (stage 4 anesthesia; Summerfelt and Smith 1990) within 2 to 3 minutes after being placed into the knock-down solution. Fish should be kept in anesthesia for an additional 30 to 60 seconds to assure they are sufficiently sedated for surgery.

MS-222 anesthesia solutions should be buffered with sodium bicarbonate. Typically researchers use a stock solution[5] with buffer added to anesthesia container just prior to use[6]. It is best if this buffer is stored in same location as stock anesthesia.

A mucus protecting water conditioner (e.g. 'Stress Coat', 'Poly Aqua') should be used in knock-down and, if necessary, recovery containers (and used to coat all surfaces fish may come in contact, see below) according to manufactures directions.

Water quality (temperature and D.O.) should be same as for holding requirements. Anesthesia water should be changed before the temperature exceeds ± 2˚C from ambient water temperature and dissolved oxygen levels should be at 80-110% of saturation at all times.

All fish transfers shall be water-to-water using sanctuary nets (or in small containers) so fish are always submerged.

Digital timers should be used to help track the time that individual/batches of fish are anesthetized.

Any fish inadvertently exposed to knock-down anesthesia for 10 minutes or more (includes total time in anesthesia, including time to weigh and measure fish prior to transfer to surgical cradle) should be censored from the study, allowed to recover and released.

Surgeries

[5] Example; 100 g MS222 powder to 1 L of water. 8 ml of stock solution in 10 L water = 80 mg/L concentration. Use same recipe for buffer (100 g in 1 L water) and add equal amount as MS222 stock solution used.
[6] Mixing MS222 and buffer stock solutions prior to use will cause precipitate to form.

Fish shall be held ventral side up in a rubber or foam cradle and kept moist with water and water conditioner. During surgery, a maintenance dose of anesthesia between 16-40 mg/L should be delivered to the fish through a soft tube placed in the fish's mouth. Each surgeon shallhave access to 2 sources of water: fresh water and water dosed with 40mg/L MS-222. The two water sources should be fitted with valves that feed into hoses. The two hoses should converge and attach to the tube placed in the fish's mouth. The surgeon can determine the mixture of anesthesia and freshwater throughout the duration of the surgery by adjusting the valves for the two water containers. The water supplied to the fish during surgery shall be oxygen saturated and the temperature should be within ±2˚C of ambient.

The posterior aspect of the incision shall be 3 to 5 mm anterior to the pelvic girdle. The incision should be made on the linea alba and shall be no longer than is necessary to easily insert the tag, ~6-8 mm. The incision location and size are critical. An incision placed too far posterior risks injury to the pelvic girdle. Conversely, placing the incision too far forward and/or making the incision too long risks injury to internal organs. Training on this procedure will need to be closely monitored.

Following insertion of the transmitter and PIT tag (if used), the incision is closed using two simple interrupted stitches using 5-0 monofilament (e.g. Ethicon Monocryl) suture material with attached needle (tapered or reverse cutting). Stitches shall be secured using a knot consisting of four single-wrap throws in alternating directions.

Fish shall be placed into a recovery container immediately following suturing and observed until they have recovered equilibrium. Fish that have not recovered equilibrium (as noted at the last point they can be observed prior to release) shall be rejected. It is recommended that the recovery vessel also serve as the release container to reduce extra handling. The density of tagged fish in any holding container shall not exceed 50 g/L.

Atypical events during surgery shall be documented and recorded in report appendices.

4. Procedures for sterilization and disinfection

All surgical equipment shall be sterilized in an autoclave at least daily and between tagging sessions as often as possible (some supplies will come sterilized from manufacturer).

All surgical equipment, including sutures and needles, shall be disinfected by soaking in Chlorhexadine or 70% ethanol (30% distilled water) for a minimum of 10 minutes, and then rinsed with distilled water between surgeries. Sufficient number of instruments should be available per surgeon to allow each set of tools to soak for at least 10 minutes. The disinfectant baths need to be changed regularly as organic debris (scales, mucus, blood) dramatically reduces the effectiveness of disinfectants. A toothbrush or other small brush should be used to remove organic debris from instruments prior to disinfection to maximize effectiveness.

Transmitters shall be soaked in Chlorhexadine or 70% ethanol (30% distilled water) and rinsed with distilled water prior to implantation. Typically, transmitters are disinfected the night or morning prior to tagging, and rinsed prior to being implanted. It is recommended that disinfected forceps or gloved hands be used to handle transmitters to minimize exposure prior to implantation.

Surgeons and all researchers handling fish shall wear surgical gloves while tagging.

The surgical table, cradles, buckets, and all other pieces of equipment and surfaces that come in contact with fish shall be disinfected regularly using Virkon Aquatic. All items or surfaces should be

exposed for 10 minutes and then rinsed with clean water (non-river where available). Where appropriate, Virkon should be neutralized prior to disposal. It is recommended that this procedure be used during the day when time allows (e.g. during lunch break).

Products mentioned here are those recommended for disinfection but local permitting/requirements may limit use of certain chemicals. Researchers should check with local POCs to determine what chemicals and disposal methods are acceptable prior to the study.

Antibiotics shall not be used.

5. Post-surgery holding

Post-surgery holding shall generally be 24 hrs with an allowable range of 18 to 36 hrs. This time frame allows fish to recover from the physiological stresses of surgery[7]. This time period will begin once the last fish has been tagged and will end when the fish have been released to the river or transportation barge. Researchers shall target total holding time, from collection to release, of around 72 hrs. Holding conditions shall be as described for the pre-surgery period, (see above) except fish are often held in individual buckets within holding tanks.

6. Transportation and release of tagged fish.

Transport and release methods will vary with the location and study goals. Once fish have recovered from anesthesia associated with surgery, researchers shall attempt to avoid any additional handling. The dissolved oxygen levels in transport containers shall be maintained at 80-110% saturation and shall be less than 2°C differential from river water at point of release. If the container temperature varies by more than 2˚C from that of receiving waters, the container water shall be tempered to a level within 2˚C by gradually exchanging water between holding container and receiving water so that temperature does not change faster than 1˚C in 15 minutes. During poor weather conditions, releasing surgically implanted fish shall be conducted in a manner safe to the researchers and the tagged fish.

7. Surgery training

A number of laboratory studies conducted in recent years have shown that taggers with feedback training perform better than taggers that have not had the same level of training (e.g. Deters et al 2010). For studies utilizing surgical implantation of transmitters this can result in negatively biased results and/or failure to determine actual differences between treatments. To minimize these effects the Surgical Tagging Protocol Steering Committee developed the following protocols for surgeon training.

Training Protocols

[7] For example, MS-222 typically is no longer detectable in the system at 24 hrs post-exposure Committee for Veterinary Medicinal Products. 1999. Tricaine Misilate Summary Report. The European Agency for the Evaluation of Medicinal Products.

The following components of surgeon training are mandatory for USACE funded projects where surgical implantation of acoustic transmitters is utilized. These are minimum requirements.

1. All trainees must tag a minimum of 75 live fish.
    a. All fish must be implanted in a single day. (NOTE: if a study design calls for tagging fewer than 50 fish per tag day trainees may tag the 75 fish over two days)
    b. All 75 tagged fish shall be held for a minimum of 14 days post tagging to evaluate survival and tag retention.

2. The last 20 fish tagged by each tagger shall be held separately from the other fish and used for evaluation of suture retention and incision healing. (NOTE: if tagging is conducted over 2 days, the last 10 fish from each day shall make up this group)
    a. Images of each fish incision shall be taken for the 20-fish group on day 0, 7, and 14.
    b. Images shall be made available to surgeons to provide feedback on surgery performance.
    c. Images of fish implanted by the trainer will be included for every group of trainees.

3. Fish used for training shall be of the species being studied. The size distribution of fish used for training shall also be representative of the fish planned for study. It is recommended that fish used for training are in the lower end of the expected size distribution for study fish. In the event that multiple species are being targeted for study surgeons should be trained on the smaller of the two species. For example, if planning to tag Chinook and steelhead, training should be done on Chinook.

4. All surgeons must be re-evaluated every year and retrained if there is a modification to surgical protocols.
    a. For previously trained surgeons this will include only implanting and evaluating at least 20 live fish
    b. If the previously trained surgeon fails this evaluation, they would be subjected to full surgery retraining.

5. A record of results including images from days 0, 7, and 14 shall be maintained for each research project and made available to the USACE upon request.

Evaluation Criteria

Since fish health can be an issue during training, the performance of all trainees will be examined in relation to the trainer. Surgeon grading will be based upon images taken directly after surgery, and 7 and 14 d after surgery. If any of the following conditions are present at a rate empirically higher than the trainer, the trainee will need to have further training and be retested:

1. Mortality
2. Tag expulsion
3. Lack of suture functionality prior to day 14
    a. The suture has pulled through one side of the incision
    b. The suture is untied and is not holding the incision closed
    c. The suture is not present
4. Major tissue trauma is present (an example is shown in figure 11)

Suggested Training Outline

The following is a suggested outline for a training module (Brown et al. 2010; T. Liedtke and J. Beeman USGS, *pers. com.*). It is suggested that the following topics be included in a training program, however, format or use of the outline is placed at the discretion of the agency and project leaders.

Learning Objectives
At the end of the training period, it is suggested that the trainee should:

- Recognize and understand the importance of conducting surgery in a manner that put the fish on a trajectory to survive with negligible sublethal impairments.
- Develop an understanding of anesthesia and recovery of fish.
- Understand basic information about fish biology and surgical techniques (including principles of sterilization) needed to properly handle and care for fish during surgery.
- Exhibit proficiency in fish surgical procedures, including the handling and use of tools and completion of all phases of the surgical procedure.
- Understand body positioning and posture needed to reduce surgical fatigue and reduce chances of surgeon injury or exhaustion.
- Recognize the types and level of practice needed to maintain skills and be willing to subject themselves to testing (surgical evaluation).

1. Introduction
    a. Importance of training program – In general, trainers need to emphasize the importance of following protocols, taking care of the study animals and conducting good science so information collected provides the greatest benefit for all interested groups. Positive attitude by researchers may significantly affect (improve) quality of data that is produced. Topics that may be included are:
        i. Care of animals
        ii. Conducting good science
        iii. Producing comparable and reliable information
    b. Your research project
        i. Background
        ii. Goal
        iii. Objectives
        iv. Study plan, etc.
    c. Project personnel introductions--experience, background, roles
    d. Go over training manual/materials
        i. Include a CD/DVD with photographs, video of procedure when possible.
        ii. Describe training outcomes and evaluation criteria (see below).

2. More background information
    a. Basics
        i. Fish biology/physiology facts (e.g. how fish breath)
        ii. Key points of fish handling (e.g. stress kills)
        iii. Fish identification
    b. Surgical tagging procedure (PowerPoint slides and/or video)

c. Importance of using protocols for sterile and clean work environments

3. Equipment Familiarization
    a. Blades, catheters, suture, gravity feeds, etc.
4. Suture demo/training.
    a. Practice suturing on a non-fish model such as a piece of foam or a banana until proficient in technique and able to suture quickly
        i. Discuss body position (the surgeon must be comfortable)
        ii. Discuss technique
    b. Suture dead fish until proficient with the technique able to suture quickly.
        i. Discuss incision location, length, depth and tag placement
    c. Conduct dissections to inspect tag and suture placement
    d. Repeat on dead fish until surgery times are 4-5 (maybe this should be 2-4 min which is more representative of actual tagging times) minutes per fish
    e. The trainer should provide detailed personal instruction to trainees to aid in time saving measures and to improve suturing technique.

5. MS-222 Training
    a. Preparing solutions, buffering, dosing buckets, maintenance buckets
    b. Water temperature and dissolved oxygen monitoring
    c. Fish behavior and fish handling guidelines
    d. Netting
    e. Fish recovery
    f. Timing of procedures

6. Data recording
    a. Datasheets
    b. Computer programs
    c. Storing and archiving

The surgeon should practice verbalizing observations/data to the data recorder while performing surgeries.

Appendix A: Examples of good and poor surgery technique

Examples of good suturing technique

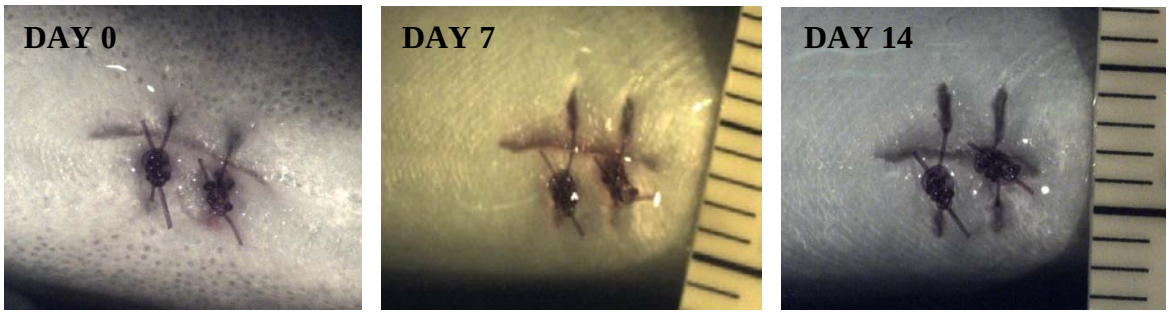

Figure 1. Good closure with two 2x2x2x2 sutures. Nice small incision but with slight overlapping (this can be very hard to avoid on subyearling fish or fish with very little muscle tissue). Sutures are in a compact ball even on days 7 and 14, indicating they were tied correctly. The tissue bites were adequate to hold the wound closed without tearing too soon. By day 14, the sutures are starting to migrate/tear toward the incision, but they are still keeping the tissue edges apposed.

Figure 2. Good closure with two 2x2x2x2 sutures. The knots are in a compact ball. The tissue edges are apposed correctly on day 0 which will lead to faster healing. Since the tissue bites were substantial and the knots were tied correctly (notice the compact "ball" of suture), the sutures continue to provide good apposition. By day 14, the sutures are starting to tear or be expelled out of the skin. This is good timing because the incision is starting to close up at this time as well.

Figure 3. Another example of good closure with a single 1x1x1x1 suture.

Examples of poor suturing technique

1. **Poor knot construction** (Figures 4, 5, and 6). Incorrectly tied sutures can result in knots coming untied which may lead to gaping incisions and possible tag loss. Occasionally, the sutures remain intact, but the suture "ball" is so large that it can cause excessive irritation to the fish's skin which could lead to necrosis and tag loss. It can be difficult to identify incorrectly tied sutures immediately after surgery. That is why it is important to examine fish several days/weeks later after the fish has had a chance to engage in active swimming. Correctly tied sutures should look like a tight ball of suture with very few gaps. Incorrectly tied sutures appear as a high stack (Figure 11) or have large gaps within the "ball" (left suture Figure 6).

Figure 4. Poor knot construction can cause increased irritation (redness, ulceration of the skin) due to the larger surface area of the suture. Large gaps in the suture knots indicate incorrectly tied sutures.

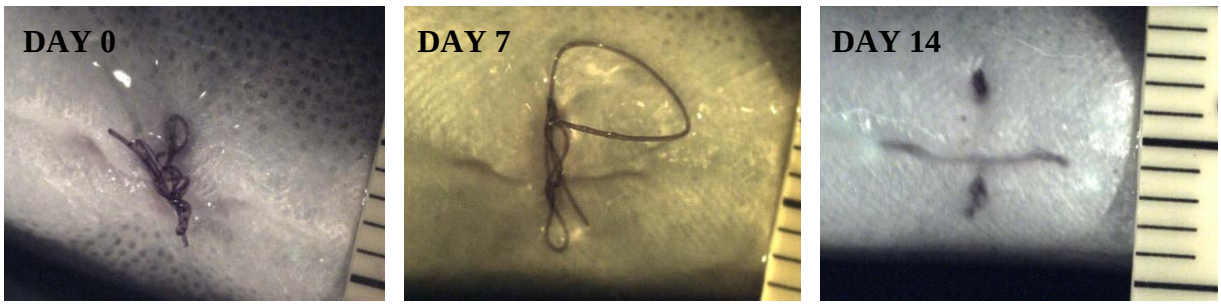

Figure 5. This is a poorly-constructed single 2x2x2x2 suture. Since the suture is still functional (just barely) on day 7, this would not count as a lost suture.

Figure 6. Inadequate suture bite can lead to loss of sutures. Note: this would not count as a lost suture since the suture is still functional on day 7. These are also examples of poorly-constructed knots. The consecutive throws should "lock" down on one another, not stack up high; these appear to be "granny" (slip) knots.

2. **Inadequate bite** (Figure 6). When surgeons insert the suture needle through the body wall too close to the incision, the suture does not bite an adequate amount of skin and the suture is likely to tear through the skin. This leads to a loss of closure on the incision and can lead to a gaping, open incision. Inadequate bites can be hard to gauge in pictures due to overlapping of the tissue edges, 2-D pictures, etc. Therefore, grading will likely be based on tearing, torn through to the incision, or lost sutures.

3. **Too much pressure on sutures** (puckering / tearing; Figures 7, and 8). The application of too much pressure when tying sutures leads to a puckering effect. The edges of the incision are typically not apposed appropriately when puckering occurs. Incision edges are typically apposed at the site of the suture but not apposed along the rest of the incision. This leads to loss of sutures.

Figure 7. Poor apposition coupled with too much tension/puckering can result in wounds opening.

Figure 8. Too much pressure on sutures/puckering (most noticeable on day 7) and poor knot construction lead to ripping sutures and irritation to the tissue.

4. **Poor apposition** (Figures 7 and 8). Misaligned suture entry and exit points and unbalanced or excessive pressure when tying sutures can cause skin overlapping or puckering which results in poor apposition of the two tissue edges. Poor apposition requires increased healing time since similar tissue layers have a greater distance to cover in order to reconnect. Poor apposition alone does not lead to surgeon failure (unless it is consistent), but when coupled with one or more other bad techniques will not be accepted.

5. **Tissue trauma** (Figures 9, 10, 11 and 12). Incorrect usage/force of tools during surgery can lead (whether moderate [Figure 9] or severe [Figure 11]) to poor healing and likely increases the chance of tag expulsion, fungal growth, and abnormal behavior. Some trainees squeeze tools (forceps) too tightly while conducting surgery (Figure 12). This can lead to death of the tissue (necrosis), poor healing, and fungus. Tissue trauma can take extended periods for healing as can be seen by wounds remaining on the fish exhibited in Figure 10, 28 days after surgery.

Figure 9. Moderate tissue trauma (visible over the incision) associated with incision closure on a juvenile salmonid. Tissue puckering is also evident in these pictures.

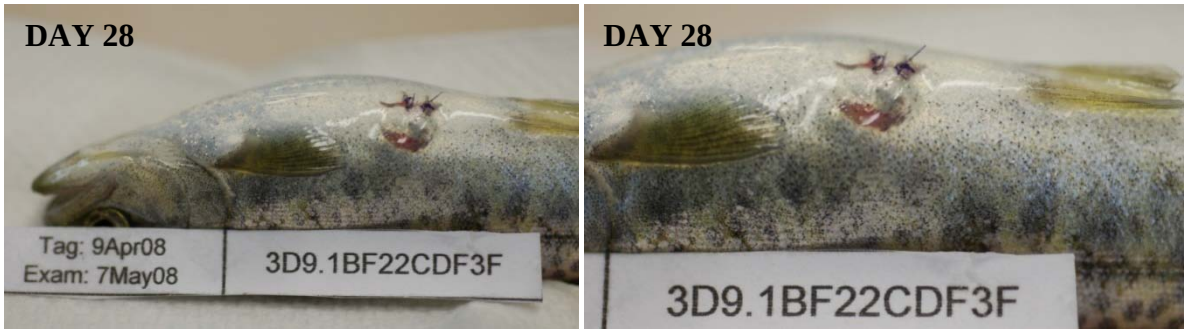

Figure 10. Tissue trauma (visible below the incision) associated with incision closure on a juvenile salmonid. Images were taken 28 d after surgery (Images provide by NOAA Fisheries).

Figure 11. Severe tissue trauma (visible above incision) associated with incision closure on a juvenile salmonid.

Figure 12. Tissue necrosis (visible above the incision) due to too much pressure applied to surgical tools.

6. **Loss of suture** (Figures 13, 14 and 15)**.** Sutures are critical to the retention of transmitters, especially when exposed to the low pressure environments of turbines. Effort is being made to identify techniques that will allow the use of just a single suture to close the incision. This makes the loss of even a single suture unacceptable. However, at some point after the implantation of a transmitter, sutures are naturally expelled. Thus any suture that is non-functional within the first seven days after surgery will be considered unacceptable. Non-functional sutures include missing sutures, untied sutures and sutures that have torn through to the incision (on either side).

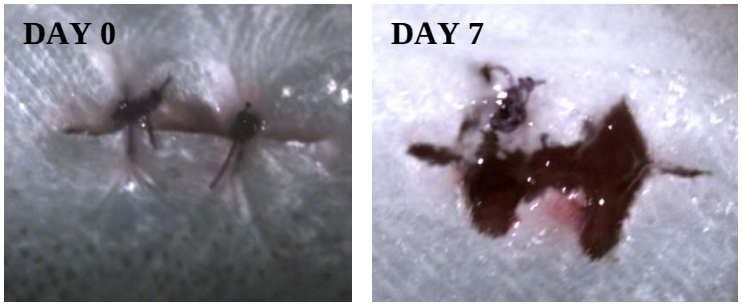

Figure 13. Loss of sutures due to too much pressure being applied when tying suture (i.e. puckering).

Figure 14. Suture loss on day 7 is due to poor knot construction during surgery. Loss of the suture can lead to an open incision and poor healing. In the day 0 image, gaps in the suture ball indicate that this knot was tied incorrectly (look at examples of good suture technique in Figures 14 -17 below; no gaps are visible on day 0). As a result, the knot has come untied and the tag is visible on day 7. We assume loss of the tag would be very likely in a river environment. For this reason, suture loss on day 7 is unacceptable.

Figure 15. Gaping wound as a result of sutures tearing out.

7. **Gaping wounds** (Figures 15 and 16). Non-functional sutures and poor apposition can cause wounds to gape open which could lead to tag loss. There are many other factors that may cause wounds to open such as tissue necrosis and inadequate wound closure during surgery. Therefore, any technique that results in wounds opening greater than 3 mm will be considered failure.

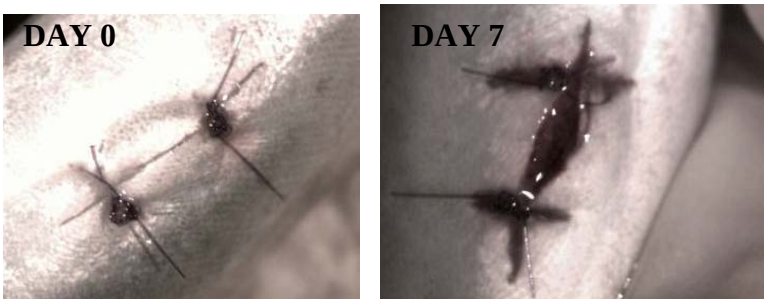

Figure 16. Incisions may begin to open up due to inappropriately-sized incisions and incorrectly spaced sutures. Sutures should be evenly spaced between the ends of the incision with erring on the side of too close to the center rather than too close to the ends.

USACE will work with the research agencies to provide source of fish and dummy transmitters for training sessions.

Instructors should be experts and should be present throughout the training process.

8. Documentation

Due to the nature of fisheries research, unexpected changes to study designs or implementation plans do occur. Therefore flexibility in how work is carried out is sometimes required. Researchers should endeavor to adhere to these protocols and where important deviation occurs the project sponsor should be notified as soon as possible. Further, deviation from these protocols should be documented in the annual research report with an explanation as to what protocol(s) was altered and why. This document should be considered a living document; changes based on sound science will be considered and discussed by the steering committee prior their adoption. This document will be updated as changes to these protocols are made.

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