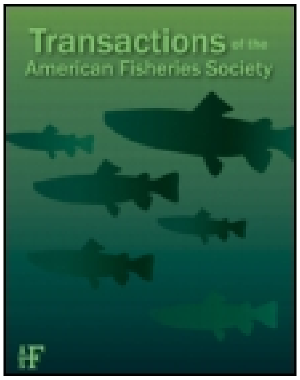


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# Optimal Suturing Technique and Number of Sutures for Surgical Implantation of Acoustic Transmitters in Juvenile Salmonids

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**ARTICLE**

# Optimal Suturing Technique and Number of Sutures for Surgical Implantation of Acoustic Transmitters in Juvenile Salmonids

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***Abstract***

**The size reduction of acoustic transmitters has led to a reduction in the length of the incision needed to implant a transmitter. Smaller suture knot profiles and fewer sutures may be adequate for closing an incision used to surgically implant an acoustic transmitter. As a result, faster surgery times and reduced tissue trauma could lead to increased survival and decreased infection for implanted fish. The objective of this study was to assess the effects of five suturing techniques on mortality, tag and suture retention, incision openness, ulceration, and redness in juvenile Chinook salmon *Oncorhynchus tshawytscha* implanted with acoustic transmitters. Suturing was performed by three surgeons, and study fish were held at two water temperatures (12°C and 17°C). Mortality was low and tag retention was high for all treatments on all examination days (7, 14, 21, and 28 d postsurgery). Because there was variation by surgeon in suture retention among treatments, further analyses included only the one surgeon who received feedback training in all suturing techniques. Incision openness and tissue redness did not differ among treatments. The only difference observed among treatments was in tissue ulceration. Incisions closed with a horizontal mattress pattern had more ulcerations than did other treatments among fish held for 28 d at 17°C. Results from this study suggest that one simple interrupted 1 × 1 × 1 × 1 suture is adequate for closing incisions on fish under most circumstances. However, in dynamic environments, two simple interrupted 1 × 1 × 1 × 1 sutures should provide adequate incision closure. Reducing bias in survival and behavior tagging studies is important when making comparisons with the migrating salmon population. Therefore, by minimizing the effects of tagging on juvenile salmon (reduced tissue trauma and reduced surgery time) researchers can more accurately estimate survival and behavior.**

Biotelemetry studies are often used to assess survival and behavior of migratory and resident fishes (Pegg et al. 1997; Jepsen et al. 1998; Skalski et al. 1998, 2001; Hockersmith et al. 2003; McMichael et al. 2010). Although injected passive integrated transponder (PIT) tags (Muir et al. 2001) and archival tags (Svedäng et al. 2007) are used in studies of survival and behavior, surgically implanted radio (Jacobsen et al. 2002; Ovidio et al. 2002; Hockersmith et al. 2003; Moser et al. 2007) and acoustic (Humston et al. 2005; Jepsen et al. 2008; Rechisky et al. 2009; McMichael et al. 2010) transmitters are more

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widely used (Cooke et al. 2011b). The implantation of radio and acoustic transmitters typically requires the surgical incision to be closed with sutures to promote healing and subsequent transmitter retention. Complications from the surgical procedure (e.g., incomplete or delayed incision healing, infection) can lead to mortality of fish or premature suture loss, which can result in transmitter loss (Deters et al. 2010). Assumptions of behavioral and survival studies assume that tags are retained and the behavior of the fish is not abnormal. Therefore, the minimization of postsurgical effects and transmitter loss in fish implanted with biotelemetry devices is important to ensure accurate interpretation of study results.

Various suture materials have been used to close surgical incisions in fish implanted with biotelemetry devices. Mono- and multifilaments with varying absorptive properties constitute the majority of commonly used sutures, and monofilament is more widely used in fish surgeries than other suture materials (Wagner and Cooke 2005). With the use of monofilaments, tissue inflammation is reduced and incision healing is accelerated in some fish species, and so they are more widely used compared with multifilaments (Kaseloo et al. 1992; Thoreau and Baras 1997; Wagner et al. 2000; Hurty et al. 2002; Deters et al. 2010; Wagner et al. 2011). The structure of multifilaments can contribute to the wicking of bacteria and subsequent inflammation of tissue surrounding suture entry and exit points (Hurty et al. 2002). Thus, monofilament suture materials are likely to minimize some postsurgical effects in implanted fish.

An adequate number of sutures are needed to ensure proper tissue apposition and postsurgery healing in fish. General guidelines for mammalian surgery suggest suture spacing (i.e., distance between consecutive sutures) to be the same distance as suture width (Swaim 1980); however, no fish-specific guidelines for suture spacing were found in the literature. Suture spacing (inferred from reported incision length and suture numbers) varied from approximately 2.5 to 6 mm in several studies where fish were implanted with biotelemetry devices (Hart and Summerfelt 1975; Harvey et al. 1984; Wagner and Stevens 2000; Anglea et al. 2004; Brown et al. 2006; Caputo et al. 2009; Deters et al. 2010). If adequate apposition and incision closure can be obtained with fewer sutures, then potential benefits to fish include a reduction in tissue trauma (fewer entry and exit points and less suture-to-skin contact), shorter surgery and anesthesia durations, and overall reduced stress on fish.

Other factors that may influence postsurgery healing characteristics in fish, and may be considered to be knowledge gaps in surgical implantation of electronic tags as identified by Cooke et al. (2011b), are the suture pattern used to close incisions and the knot type (number of wraps per throw or number of throws per suture) used to secure sutures. For clarification of the terminology used in this study, a wrap refers to the encircling of the suture material around the needle holder, a throw is the tying down of the suture material, and knot refers to the knotting technique as a whole; for example, the 2 × 1 × 1 × 1 knot type (the term "knot" can also refer to two consecutive throws, that is, all knotting techniques in this study consist of two complete knots for each suture). For example, a 2 × 1 × 1 × 1 knot consists of two wraps on the first throw followed by three single-wrap throws (each in alternating directions). Numerous suture patterns exist for incision closure, and varying the number of wraps and throws used to secure sutures creates a trade-off between knot security and increased surface area of the knot. Additional wraps (e.g., a surgeon's knot in which two wraps are used on the first throw; Figure 1) and throws can increase knot security, particularly when monofilaments are used (Dunn 2007). However, this also increases the surface area of the knot. Increased surface area can provide more attachment points for fungal or bacterial growth and may increase drag. Incorrectly tied knots, or knots without sufficient wraps or throws to secure the knot, can lead to untying and premature suture loss. If suture loss occurs before healing is adequate, tag loss can occur. Therefore, it is important to understand the balance between knot security and surface area and how these relate to the potential benefits to fish implanted with transmitters.

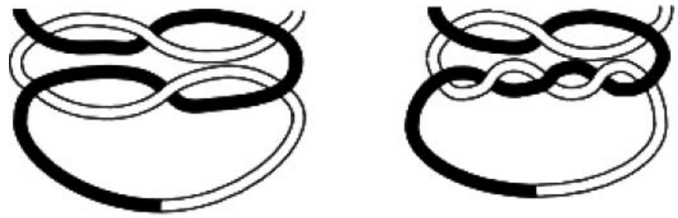


FIGURE 1. Two types of knots used for suturing incisions: a square knot and a surgeon's knot, each consisting of two throws.

Recently, the miniaturization of acoustic transmitters has led to a reduction in the length of incision needed to surgically implant the transmitter. A shorter incision may increase the likelihood that one suture could adequately close the incision. However, the effects, or potential benefits, of incision closures with a single suture on postsurgery healing characteristics and tag retention in juvenile salmon are unknown.

Thus, the objective of this study was to assess the effects of suture number, suture pattern, and knot type on mortality, tag and suture retention, incision openness, ulceration, and redness in juvenile Chinook salmon *Oncorhynchus tshawytscha* implanted with acoustic transmitters. Results from this study will provide insight into the feasibility of single-suture incision closures and the effect of knot size on postsurgery healing characteristics in juvenile salmonids. Moreover, this study will aid in the refinement of fish surgery protocols for biotelemetry research on various species and sizes of fish.

## METHODS

*Experimental fish.*—Hatchery spring Chinook salmon ($N =$ 539) obtained as eyed eggs in October 2008 from the Leavenworth National Fish Hatchery (Leavenworth, Washington) and reared at the Pacific Northwest National Laboratory (PNNL)

TABLE 1. Sample size, means, and ranges of fork length (FL), weight, and tag burden in Chinook salmon for each of the five treatments.

| Variable | Treatment | | | | |
|---|---|---|---|---|---|
| | 1 × 1 × 1 × 1 | 2 × 1 × 1 × 1 | 2 × 2 × 2 × 2 | 2 × 2 × 2 × 2 double | Horizontal mattress |
| Sample size (*n*) | 108 | 108 | 107 | 108 | 108 |
| FL (mm) | 109 | 110 | 110 | 110 | 110 |
| Range in FL | 99–119 | 97–116 | 97–118 | 101–120 | 96–121 |
| Weight (g) | 16.1 | 16.4 | 16.2 | 16.3 | 16.6 |
| Range in weight | 11.0–22.1 | 11.2–21.3 | 11.5–21.5 | 10.6–21.7 | 9.8–20.8 |
| Tag burden (%)[a] | 3.4 | 3.3 | 3.3 | 3.3 | 3.3 |
| Range in tag burden | 2.4–4.8 | 2.5–4.7 | 2.5–4.6 | 2.4–5.0 | 2.5–5.4 |

[a]Combined burden of acoustic transmitter and PIT tag.



Aquatics Research Laboratory (ARL) in Richland, Washington, were used in this study. Mean (±SE) fork length (FL) and weight of fish were 110 ± 0.2 mm (range, 96–121 mm) and 16.3 ± 0.1 g (range, 9.8–22.1 g), respectively (Table 1). Fish were gradually (rate of <2°C/d) acclimated to their study temperature (12°C or 17°C well water) and held at that temperature (±2°C) for at least 2 weeks before experimentation. After the first round of surgeries for fish held at 17°C, the immediate mortality and erratic swimming behavior of a large proportion of the fish made it clear that these fish were not healthy enough to be used in this study. The fish were culled, and the surgeries were repeated with fish from another tank but from the same batch. These fish were not acclimated to their study temperature (17°C) but had been held in 15°C well water before surgery. Water temperatures of 12°C and 17°C were used to simulate the mean water temperatures during out-migration of yearling and subyearling Chinook salmon, respectively, in the Columbia River basin. Throughout the study, fish were held in eight 1.2-m-diameter circular fiberglass tanks at the PNNL ARL. The tanks ranged in volume from 490 to 735 L. Four tanks were maintained at 12°C; the remaining four were controlled to 17°C. To minimize tank effects, tank assignment within each temperature regime was rotated weekly following postsurgery examinations. For example, fish were rotated from one 12°C tank to another 12°C tank each week following examination. All fish were fed once daily with Biodiet pellets (Bio-Oregon, Longview, Washington) at 1.1% of their body weight and were exposed to a photoperiod of 12 h light : 12 h dark for the duration of the study. Fish were not fed for 24 h before surgery.

*Surgical equipment and procedures.*—Surgeries were performed on subyearling Chinook salmon on October 12–13, 2009. Three surgeons performed all surgeries. All surgeons had previously practiced on all suturing techniques used in this study; however, only one surgeon received feedback training on his performance of all techniques. The other two surgeons had previously received feedback training on one of the five techniques (2 × 2 × 2 × 2 double). Feedback training consisted of conducting surgeries and holding fish for 2 weeks, which is the length of time that has been deemed important for increased transmitter and suture retention (Deters et al. 2010; Cooke et al. 2011a). During the 2-week holding period, images of incisions were taken on days 0, 7, and 14. After the 14-d holding period, the surgeon was able to link the progression of incision healing or any suture loss to an incision closure technique used during surgery.

Suturing technique and surgeon orders were assigned randomly without replacement. Each surgeon used the same suturing technique to perform surgery on one fish before switching to the next technique in the treatment order. Each surgeon performed an equal number of surgeries with each suturing technique. Fish from a different water temperature (12°C and 17°C) were assigned to the surgeons after four full rotations of the treatment order. New random assignments were made for the second day of surgeries.

Before surgery, all fish were brought to stage 4 anesthesia (as described by Summerfelt and Smith 1990) with tricaine methanesulfonate (MS-222) at a concentration of 80 mg/L in water. After FL (millimeters) and weight (grams) were obtained, fish were placed ventral side up on a foam-rubber pad and supplied with a maintenance anesthesia dose of 40 mg/L MS-222. Surgical incisions were made with a BD Beaver Micro-Unitome knife with a 3-mm blade (Becton, Dickinson and Company, Franklin Lakes, New Jersey). An incision approximately 8 mm long was made on the linea alba, anterior to the pelvic girdle (similar to Panther et al., in press). All fish were implanted with a Juvenile Salmon Acoustic Telemetry System acoustic transmitter (model SS-208, Advanced Telemetry Systems, Isanti, Minnesota) and a passive integrated transponder (PIT) tag (Destron Technologies, St. Paul, Minnesota). All fish were double-tagged (acoustic and PIT) to simulate field studies on the Snake and Columbia rivers where the presence of a PIT tag prevents fish from being sorted into transport barges or trucks at juvenile bypass facilities within dams. Acoustic transmitters were 12.0 mm long, 5.2 mm wide, 3.8 mm high, and weighed 0.43 g in air (0.30 g in water; 0.14 mL volume). The PIT tags used were 12.5 mm long, 2.1 mm wide, and weighed 0.10 g in air (0.06 g in water; 0.04 mL volume). Tag burden (mean ± SE) for all fish was 3.3 ± <0.1% (range, 2.4–5.4%; Table 1). After the incision was made, the PIT tag and the acoustic transmitter were inserted into the peritoneal cavity of the fish. The incision was closed with

TABLE 2. Suture pattern type and number and type of knots used for each treatment on Chinook salmon in this study.

| Pattern type | Number of knots | Knot type |
|---|---|---|
| Simple interrupted | 1 | 1 × 1 × 1 × 1 |
| Simple interrupted | 1 | 2 × 1 × 1 × 1 |
| Simple interrupted | 1 | 2 × 2 × 2 × 2 |
| Simple interrupted | 2 | 2 × 2 × 2 × 2 |
| Interrupted horizontal mattress | 1 | 1 × 1 × 1 × 1 |



violet 5–0 absorbable monofilament suture (Monocryl, Ethicon, Somerville, New Jersey) in one of the five techniques described below. After recovery from surgery, fish were released to their respective holding tanks. Feeding resumed 1 d after surgery.

The knots for all five techniques consisted of four throws in alternating directions. The number of wraps (around the needle holder) per throw (Figure 2; Table 2) and the number of knots used varied by technique as follows:

(1) 1 × 1 × 1 × 1 = one wrap on each throw
(2) 2 × 1 × 1 × 1 = two wraps on the first throw followed by one wrap on consecutive throws
(3) 2 × 2 × 2 × 2 = two wraps on each throw.

In addition, the type of suture pattern varied by technique. A simple interrupted (over and over) suture pattern passes through each side of the incision once, and the ends are tied in a knot (Figures 3 and 4). An interrupted horizontal mattress pattern passes through each side of the incision twice before the ends are secured with a knot (Figures 3 and 4).

*Response examinations.*—All fish were examined at 7, 14, 21, and 28 d postsurgery, and mortality and tag retention were assessed and recorded daily. With the use of a microscope, a single evaluator graded all fish on all examination days. Examinations consisted of anesthetizing fish with 80 mg/L MS-222 and obtaining FL (millimeters) and weight (grams). Fish were then placed ventral side up on a foam-rubber pad and supplied with a maintenance anesthesia dose of 40 mg/L MS-222. Evaluations were made on both the anterior and posterior sutures separately and on the incision. Similar to Deters et al. (2010), a retained suture was defined as any suture that was present in the fish, remained knotted, and did not tear through the body wall of the fish. The percentage of suture retention was assessed for all fish and graded as 0, 50, or 100% retention based on the initial number of knots at the time of surgery.

The extent of ulceration, redness, and incision openness were quantified on each fish. A stereomicroscope (0.65 × magnification; Stemi 2000-CS, Zeiss AG, Jena, Germany) connected to a computer and monitor was used for viewing and taking images of the fish incision area. At the beginning of the day, a ruler at a fixed height under the microscope was calibrated with image analysis software (Image-Pro Plus and Image-Pro Analyzer, version 7.0.1, Media Cybernetics, Bethesda, Maryland). The fish was then elevated so that the incision was in the same plane as the ruler. Area of incision openness, wound ulceration, and redness were then outlined on examination photographs with the imaging software; area was calculated in square millimeters. Fish having large areas of fungus covering the incision at the time of examination could not be graded for openness, ulceration, or redness for that day.

*Data analysis.*—Categorical covariates included surgeon at one or three levels, surgery day at two levels, and suture treatment at five levels. All three surgeons were included for the response variables mortality, tag retention, and suture retention, whereas analysis for the response variables redness and ulceration (and openness) included the one surgeon who had received feedback training. Data from this surgeon (surgeon 1) were only used since a suture × treatment interaction was found for suture retention. This interaction was probably due to the lack of feedback training for surgeons 2 and 3, and thus lower suture retention was observed for these two surgeons. Redness and ulceration analyses were limited to fish that had 100% suture retention because the extent of redness and ulceration are dependent upon the presence of the suture and the amount of time since the suture was lost. Response variables were analyzed separately for fish held at 12°C and 17°C, on days 7, 14, 21, and 28 (i.e., temperature was not included as a covariate).

The response variables were modeled with a linear model. The mean proportion of the response variables were treated as continuous variables between 0 and 1. The response


FIGURE 2. The three knot types used in this study to close incisions.

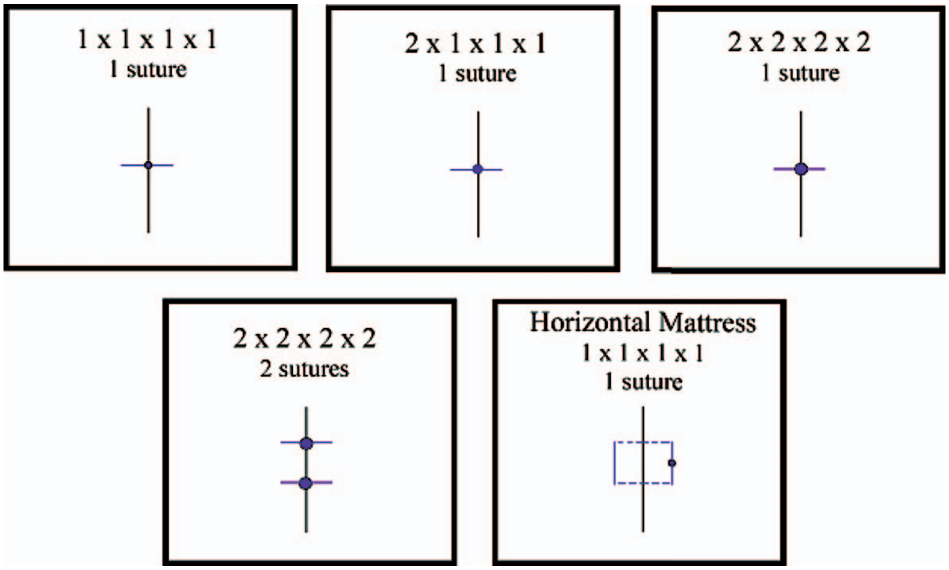


FIGURE 3. The five suturing techniques (treatments) used in this study. The long line in the center of the diagrams represents the incision. Shorter lines and circles represent the suture material and knots. Dashed lines represent suture material within the peritoneal cavity. [Figure available online in color.]

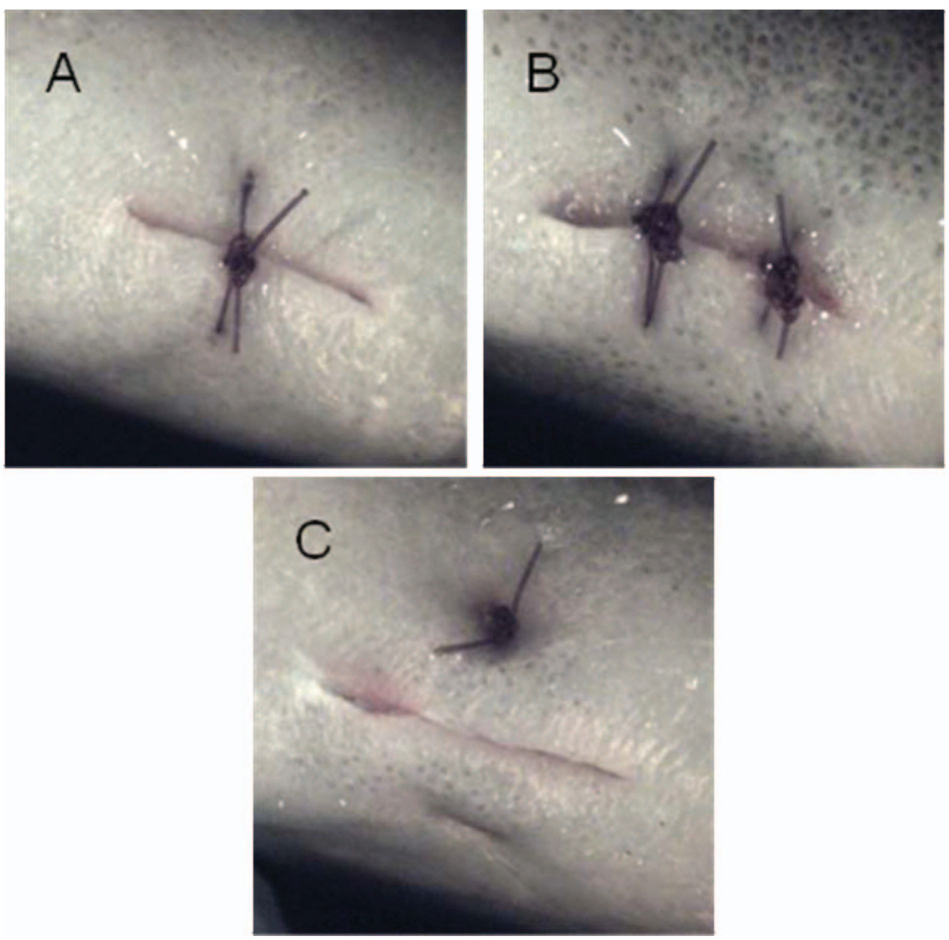


FIGURE 4. Day-0 images of three treatment patterns: **(A)** a single simple interrupted suture with $1 \times 1 \times 1 \times 1$ knot, **(B)** two simple interrupted sutures with $2 \times 2 \times 2 \times 2$ knots, and **(C)** an interrupted horizontal mattress suture with $1 \times 1 \times 1 \times 1$ knot. [Figure available online in color.]

variables were calculated as the average proportion across all fish at a given level of surgery day, surgeon, and suture treatment. Analysis of variance (ANOVA) was performed by means of a randomized complete block design; surgery day served as the blocking factor to assess the significance of surgeon and suture treatment.

For ulceration data, in cases where there were significant differences between suture treatments, sequential pairwise differences between treatments were tested. Differences between treatments were ordered from largest to smallest. Pairwise *t*-tests were performed sequentially, starting with the treatment pairs that produced the largest difference, followed by the treatments that produced the second largest difference, and so on. Once a pairwise *t*-test was not significant, no further tests were done. To control for the increased probability of a type I error, a Šidák correction was used to adjust the rejection region, depending on the number of pairwise tests:

$\alpha_{\text{family}} = 1 - (1 - \alpha_{\text{comparison}})^{1/t}$, where $t$ is the number of paired *t*-tests

$\alpha_{\text{family}}$ = the new familywise error rate

$\alpha_{\text{comparison}} = 0.05$

## RESULTS

### Mortality

Overall, mortality was low among fish held at both 12°C (2.6%) and 17°C (1.7%) and did not differ significantly ($P >$ 0.05) among treatments or surgeons at either water temperature. In addition, mortality did not differ significantly by surgery day for fish held at either 12°C ($P = 0.09$) or 17°C ($P = 0.12$).

### Tag Retention

Expulsion of acoustic transmitters or PIT tags was less than 1% in this study; one acoustic transmitter and two PIT tags were expelled. All expelled acoustic transmitters and PIT tags originated from fish implanted by surgeon 2; however, tag retention did not differ significantly ($P = 0.22$ and 0.30) among surgeons, nor did it differ significantly ($P = 0.65$ and 0.66) among treatments, or between surgery days ($P = 0.09$ and 0.17) at either 12°C or 17°C, respectively.

### Suture Retention

Among fish held at 12°C, suture retention varied among surgeons and treatments, but not between surgery days. Suture retention differed significantly ($P < 0.01$) among surgeons on all examination days. In addition, suture retention differed significantly among the five treatments on day 7 ($P < 0.01$) and day 21 ($P < 0.01$). However, suture retention did not differ significantly among treatments on day 14 ($P = 0.12$) or day 28 ($P = 0.07$). There was a significant surgeon × treatment interaction on days 7 ($P < 0.01$), 21 ($P < 0.01$), and 28 ($P = 0.02$). This indicates that the differences among treatments are probably related to low retention by one or more surgeons. Sutures by surgeon 2 had much lower retention in several treatments than those of the other surgeons (Figure 5) on all examination days. Surgeon 3 also had lower suture retention than surgeon 1 on days 21 and 28. Surgeon 1 was the only surgeon that had received feedback training on all suturing techniques.

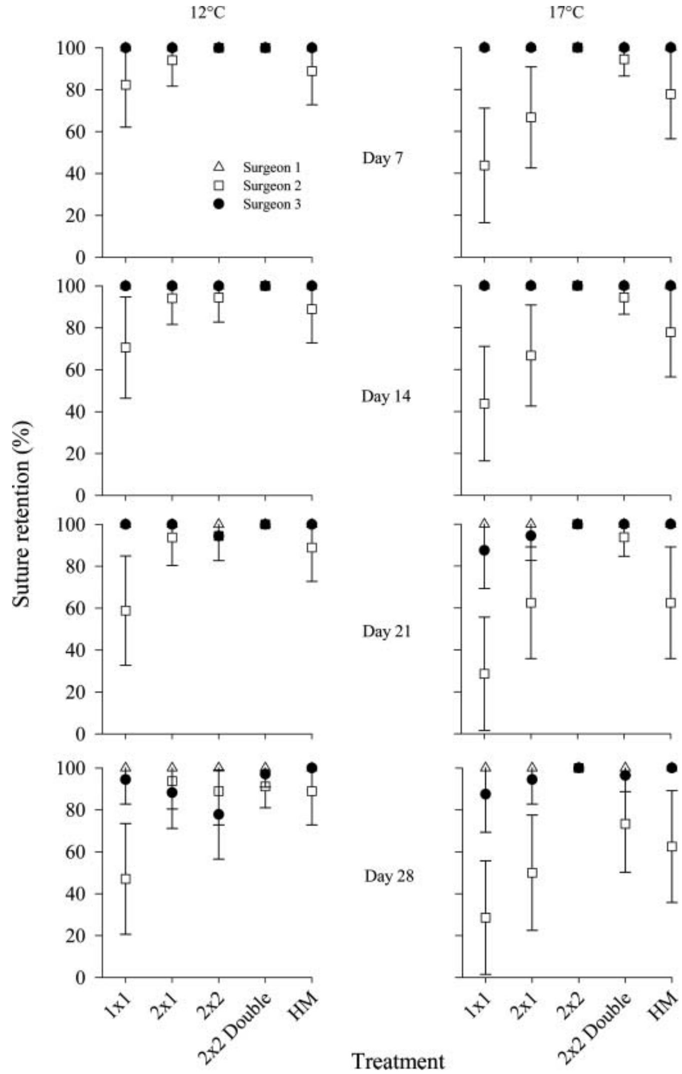


FIGURE 5. Percentage of suture retention by treatment, temperature, and examination day for each of the three surgeons. HM: horizontal mattress technique.

Among fish held at 17°C, suture retention varied among surgeons and treatments and between surgery days. Suture retention differed significantly ($P < 0.01$) among surgeons on all examination days. In addition, suture retention differed significantly among the five treatments on day 21 ($P < 0.01$) and day 28 ($P < 0.01$). However, suture retention did not differ significantly among treatments on day 7 ($P = 0.10$) or day 14 ($P = 0.11$). There was a significant surgeon × treatment interaction on both day 21 ($P = 0.03$) and day 28 ($P < 0.01$). This indicates that the differences among treatments are probably related to low retention by one or more surgeons. Surgeon 2 had much lower suture retention in several treatments than the other surgeons (Figure 5).

Owing to the surgeon × treatment interactions, we attempted to examine differences in suture retention among the treatments for the one surgeon who had received feedback training with all suture techniques (surgeon 1). However, suture retention was 100% for surgeon 1 among all treatments on all examination days. This finding precluded any statistical analysis.

## Openness

We attempted to examine differences in incision openness among the treatments for surgeon 1 by measuring any areas in which wound edges were gaping or not approximated. However, all incision edges remained 100% approximated during the 28-d study. A lack of incision openness across all treatments, surgery days, temperatures, and examination days precluded any statistical analysis.

## Ulceration and Redness

Neither ulceration nor redness differed significantly ($P > 0.05$) among treatments at 12°C on any examination day for sutures of surgeon 1 (Figure 6). At 17°C, there was also no significant ($P > 0.05$) difference among treatments in redness on any examination day. However, ulceration at 17°C differed significantly ($P < 0.01$) among treatments on day 28. On this day, ulceration was significantly ($P < 0.01$) higher for fish with horizontal mattress sutures than for all other treatments. Aside from ulceration on day 28 at 12°C, there were no other significant differences ($P > 0.05$) between surgery days for ulceration and redness at either 12°C or 17°C.

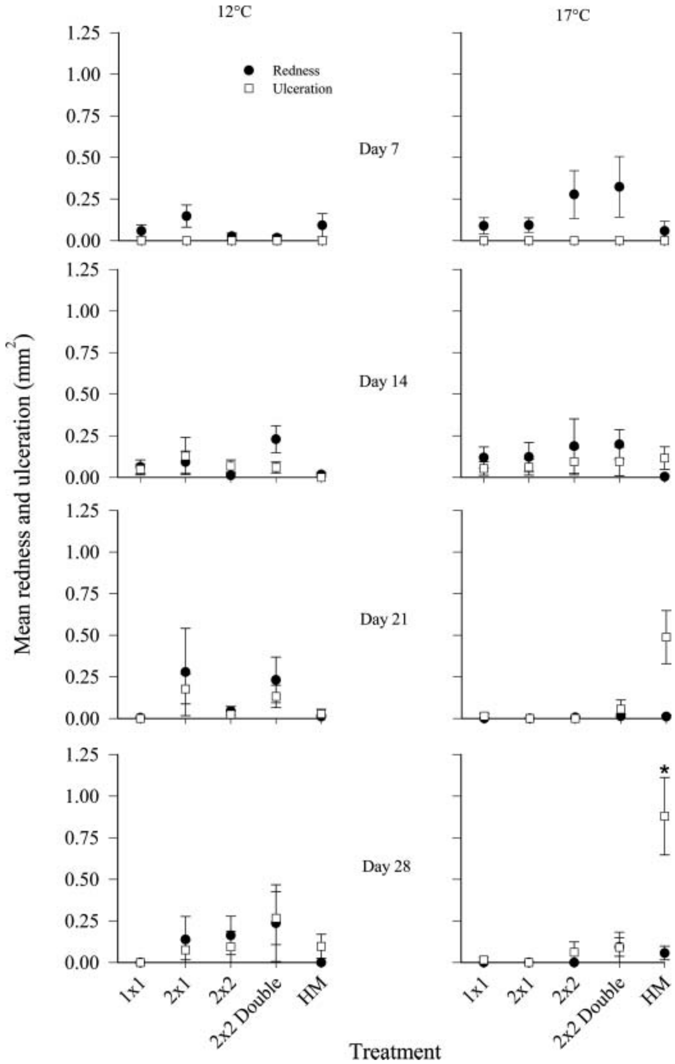


FIGURE 6. Mean redness and mean ulceration by treatment, temperature, and examination day for surgeon 1. The asterisk on day 28 denotes significant differences among the treatments for the horizontal mattress (HM) technique.

## DISCUSSION

The lack of a difference in transmitter loss among treatments in this study suggests one suture is adequate for closing surgical incisions (approximate length 8 mm) on the linea alba and retaining acoustic transmitters and PIT tags (combined weight of 0.53 g in air) implanted in juvenile Chinook salmon up to 28 d. Panther et al. (in press) observed 4% expulsion within 28 d of implantation of a 0.54-g transmitter through the linea alba in similar-sized Chinook salmon when incisions were closed with two 2 × 2 × 2 × 2 monofilament sutures. Chittenden et al. (2009) noted 0% and 22% expulsion of 0.9- and 1.5-g tags, respectively, in similar-sized fish (95–120 mm) implanted through the linea alba and held 50 or 35 d, respectively, when incisions were closed with two monofilament sutures (with an unspecified knot type).

Although suture retention was variable among treatments when data from all surgeons were examined, results from surgeon 1 (who had feedback training for all knot types) alone showed no variation among the five treatments. No sutures were lost by this surgeon during the 28-d study, and all transmitters were retained. Several texts in surgical techniques suggest that when closing a wound, one should use the simplest knot that can provide adequate wound closure (Turner and McIlwraith 1989; Slatter 2003; Dunn 2007). It is also believed that once an individual becomes proficient at suturing, the two wraps of a surgeon's knot are unnecessary, and the single wrap of a square knot is adequate (Nealon 1979). In addition, Fossum et al. (2007) suggested against using a surgeon's knot with monofilament sutures unless wound tissue tension is very high. It is important to mention that the 2 × 2 × 2 × 2 knotting technique is not likely to be explained or suggested by any surgical texts because it was a technique created by Deters et al. (2010) to incorporate and standardize the various knotting techniques used by several experienced fish surgeons within the Pacific Northwest region of the United States. The results for surgeon 1 indicate that surgical proficiency was attained (100% suture retention) and that the simplest knot (1 × 1 × 1 × 1) successfully performed the task (high tag retention, high suture retention, and low levels of redness, ulceration, and mortality).

As described by Deters et al. (2010), surgical technique (i.e., suture retention) may be improved with the implementation of feedback training. This finding is supported by the high tag and suture retention and low degree of tissue trauma exhibited across all five treatments in fish implanted by surgeon 1 in this study. In addition, the higher suture loss observed in fish implanted by surgeon 2 may have been avoided. Feedback training pictures on day 14 would have shown that the throws were not being set down properly as square knots (Nealon 1979) and this could have been corrected before the start of the study. Therefore, it is highly advised that surgeons receive feedback training in the suturing technique to be used for a study. For surgeons who have demonstrated proficiency in a specific technique via feedback training, smaller knots with fewer wraps (i.e., the 1 × 1 × 1 × 1 knot) are probably adequate and will further reduce tissue trauma and time spent under anesthesia.

Ideally, sutures should be retained in fish only as long as needed to ensure proper apposition and healing of the surgical incision. Thus, suture retention should be regarded as beneficial only up to the point in time when they are no longer needed. Sutures that remain in fish when no longer needed may contribute to inflammation and infection (Caputo et al. 2009). These complications could ultimately lead to alterations in fish behavior or death. Location of the surgical incision may influence the length of time sutures need to be intact. For example, incisions on the linea alba may heal faster than those placed in muscle tissue (Panther et al., in press); therefore, longer suture retention times may be unnecessary. Tissue bite (the amount of tissue sutured on either side of the incision) also may be an important factor in suture retention time. It is likely that sutures are not dissolving, but rather being actively (physiologically) or passively (via drag on the suture) expelled by fish (K. A. Deters, personal observation). In humans, dyed Monocryl loses all tensile strength by day 28, but absorption is not complete until 91–119 d postsurgery (Dunn 2007). One might expect the fish's aquatic environment to hasten hydrolysis, the process by which synthetic absorbable sutures (like Monocryl) are dissolved (Dunn 2007). However, the fish's foreign body reaction (resulting in suture expulsion) may be faster than suture absorption at these water temperatures. Throughout this study, sutures were observed to move closer to the incision location while the suture and knot remained intact. A reduction in tissue bite may hasten suture expulsion to coincide more closely with incision healing. The implementation of a smaller tissue bite by surgeon 1 could have resulted in lower suture retention by day 28 than we saw in this study. By this time, incision healing was probably sufficient for tag retention. There is a need for further research that would investigate healing rates of incisions (including tensile strength of incisions) in salmonids at various water temperatures to better understand when sutures are no longer needed.

Overall, openness, redness, and ulceration were very low in fish implanted by the surgeon who had feedback training in all suturing techniques (surgeon 1). Fish with incisions closed with one suture pass (1 × 1 × 1 × 1, 2 × 1 × 1 × 1, and 2 × 2 × 2 × 2) experienced one-half the number of suture entry and exit points (thus approximately one-half the surface area of suture-to-skin contact) compared with fish that had incisions closed with two suture passes (2 × 2 × 2 × 2 double and horizontal mattress). According to other researchers (Wagner et al. 2000), less tissue trauma (i.e., fewer suturing points and less skin contact) evokes a reduced inflammatory response. With the exception of ulcerations occurring by day 28 at 17°C (ulceration was higher for the horizontal mattress pattern than for all other treatments), the number of suture passes across the incision did not influence the response variables in this study. Owing to the higher tissue ulceration associated with the horizontal mattress sutures, this technique is not recommended for implanting acoustic transmitters in juvenile Chinook salmon.

The successful wound closure and tag retention with single sutures in this study led to the development of another study that examined tag and viscera expulsion, mortal injury, and suture and incision tearing in fish that experienced simulated turbine passage (rapid decompression: Boyd et al., in press). During that study, researchers compared incisions on juvenile Chinook salmon closed with a single 2 × 2 × 2 × 2 suture to those closed with two 2 × 2 × 2 × 2 sutures. Although incision length was only 6 mm (~2 mm shorter than those used in this study), there was a greater occurrence of viscera expulsion through incisions closed with a single suture than those closed with two sutures. In addition, occurrence of viscera expulsion was higher for smaller fish (which have a higher tag burden) and those experiencing a greater ratio of pressure change (acclimation pressure divided by exposure pressure). This present study suggests that a single suture is adequate for incision closure (a lack of incision openness for surgeon 1) in fish not experiencing rapid pressure changes. However, based on the findings of Boyd et al. (in press), it is not recommended that incisions be closed with a single suture if fish may experience rapid decompression such as that associated with turbine, spillway, or other routes of passage at hydroelectric dams.

The simplest suturing technique (simple interrupted 1 × 1 × 1 × 1 knot) that was examined performed as well as or better than all other treatments when tied by a surgeon who had feedback training. This knot was the fastest to tie and led to reduced surgery time. When tied by a surgeon who did not receive feedback training in this technique, suture retention rates were lower. We recommend the use of a single simple interrupted 1 × 1 × 1 × 1 suture for closing incisions (~8 mm or less) associated with implantation of acoustic transmitters in juvenile salmonids when experienced surgeons have received satisfactory feedback training. All surgical candidates should be fully trained in the technique and should receive feedback on mortality, tag and suture retention, and tissue trauma at several intervals over a 2- to 3-week period. Only candidates who can produce satisfactory results (e.g., high tag and suture retention, low mortality and tissue trauma) should be used as surgeons in telemetry studies. However, until further research is completed we caution against the use of a single 1 × 1 × 1 × 1 suture when fish will be monitored through a dynamic riverine environment, such as rapid pressure changes associated with passage at hydroelectric dams. For this type of study, two simple interrupted 1 × 1 × 1 × 1 sutures will probably provide adequate wound closure.

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