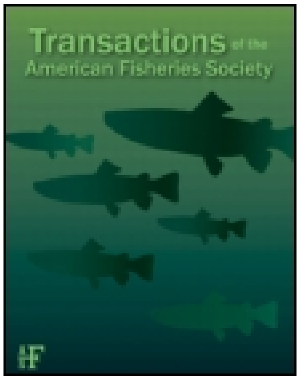

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Influence of Incision Location on Transmitter Loss, Healing, Survival, Growth, and Suture Retention of Juvenile Chinook Salmon

Jennifer L. Panther [a c], Richard S. Brown [a], Greg L. Gaulke [a], Katherine A. Deters [a], Christa M. Woodley [a] & M. Brad Eppard [b]

[a] Pacific Northwest National Laboratory, Post Office Box 999, Richland, Washington, 99354, USA

[b] U.S. Army Corps of Engineers, Portland District, 333 Southwest First Avenue, Portland, Oregon, 97204, USA

[c] Confederated Tribes of the Colville Reservation, Fish and Wildlife Department, 25B Mission Road, Omak, Washington, 98841, USA

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ARTICLE

Influence of Incision Location on Transmitter Loss, Healing, Survival, Growth, and Suture Retention of Juvenile Chinook Salmon

Jennifer L. Panther,*[1] Richard S. Brown, Greg L. Gaulke, Katherine A. Deters, and Christa M. Woodley
Pacific Northwest National Laboratory, Post Office Box 999, Richland, Washington 99354, USA

M. Brad Eppard
U.S. Army Corps of Engineers, Portland District, 333 Southwest First Avenue, Portland, Oregon 97204, USA

Abstract
Fisheries research involving surgical implantation of transmitters necessitates the use of methods that minimize transmitter loss and fish mortality and optimize healing of the incision. We evaluated the effects of three incision locations on transmitter loss, healing, survival, growth, and suture retention in juvenile Chinook salmon *Oncorhynchus tshawytscha*. The three incision locations were (1) on the linea alba (LA incision), (2) adjacent and parallel to the LA (muscle-cutting [MC] incision), and (3) extending from the LA towards the dorsum at a 45° angle, between the parallel lines of myomeres (muscle-sparing [MS] incision). A Juvenile Salmon Acoustic Telemetry System acoustic transmitter (0.44 g in air) and a passive integrated transponder tag (0.10 g in air) were implanted into each fish (total $N = 936$ fish). The fish were held at 12°C or 20°C and were examined weekly for 98 d. The progression of healing among incision locations and the variability in transmitter loss made it difficult to identify one incision location as the best choice. The LA incisions had a much smaller wound extent (area of visible subepidermal tissue) than MC and MS incisions during the first 28 d of the study. In both temperature treatments, apposition of incisions through day 14 was better for LA incisions than for MC and MS incisions. However, MC and MS incisions were less likely than LA incisions to reopen over time and thus were less likely to allow transmitter loss through the incision.

Surgical implantation of transmitters has become widely used in acoustic and radiotelemetry studies of fish behavior (Winter 1996). The goal of surgically implanting transmitters remains the same across studies: to ensure that the period of transmitter retention within the fish is long enough to allow the collection of the desired data while minimally affecting behavior and survival. Survival studies involving surgical implantation operate under the assumption that the method of implantation does not influence fish behavior or survival (Peven et al. 2005). However, surgical implantation requires an invasive incision that bisects skin and muscle tissue and therefore may adversely affect fish health by damaging the integument.

The primary function of the integument of teleost fish is to provide a barrier to pathogens found in the surrounding aquatic environment (Roberts 1989). Damage to the integument may cause mortality, usually by increasing a fish's susceptibility to disease (Logan and Odense 1974) or decreasing efficient osmoregulation (Noga 2000). In addition to disrupting the integument, surgical incisions on fish can bisect different types of tissue.

*Corresponding author: jennifer.panther@colvilletribes.com
[1]Present address: Confederated Tribes of the Colville Reservation, Fish and Wildlife Department, 25B Mission Road, Omak, Washington 98841, USA.
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Several anatomical locations for transmitter implantation in fish have been mentioned in the literature. Incisions have been made in the connective tissue of the linea alba (LA; Marty and Summerfelt 1986; Lucas 1989; Knights and Lasee 1996; Wagner and Stevens 2000), which lies between two cylindrical connective tissue sheaths that surround a pair of muscles called the infracarinalis anterior (Greene and Greene 1915). Incisions have also been made parallel to the LA in the hypaxial musculature, which consists predominantly of white muscle fibers (hereafter, muscle-cutting [MC] incision; Videler 1993; Wagner and Stevens 2000). Another type of incision is one made perpendicular to and several millimeters from the LA (vertical incision; Cobb 1933; Schramm and Black 1984). The concept of an incision that follows the lines of the underlying myomeres (hereafter, muscle-sparing [MS] incision) was mentioned by Pautzke and Meigs (1941). Research comparing healing rates in different kinds of tissues within the same fish species is sparse, and to our knowledge there have been no published experimental studies of this subject. Although some studies have mentioned anecdotal observations of incisions made in different locations (Pautzke and Meigs 1941), other studies have considered only the macroscopic appearance and transmitter loss among different incision locations (Schramm and Black 1984; Wagner and Stevens 2000).

The objective of our study was to determine differences in survival, transmitter loss, healing (as measured by incision closure, presence of abnormal redness of the skin around the incision [erythema], and histological indicators), growth, and suture retention in juvenile fall Chinook salmon *Oncorhynchus tshawytscha* that received three incision types. The incision locations were (1) on the LA (hereafter, LA incision), (2) 3 mm from and parallel to the LA (MC incision), and (3) extending from the LA towards the dorsum at a 45° angle, between the parallel lines of myomeres (MS incision; Figure 1). All three incisions were made 4–5 mm anterior to the pelvic girdle.

Because an LA incision receives a similar amount of tension from both sides of the body and is located in less-perfused connective tissue, we hypothesized that fish with LA incisions would have better incision closure and less erythema than fish with MC and MS incisions. We also hypothesized that LA incisions would have a higher rate of transmitter loss than the other incision types because the transmitter rests partially or fully on the LA incision (Schramm and Black 1984). Because healing rates are temperature mediated (Anderson and Roberts 1975) and temperature may influence transmitter expulsion (Knights and Lasee 1996; Bunnell et al. 1998; Bunnell and Isely 1999), the effect of environmental temperature on all variables was evaluated by executing the study at two temperatures (20°C and 12°C) that are encountered by migrating juvenile salmon in the Snake and Columbia rivers (Columbia Basin Research, School of Aquatic and Fishery Sciences, University of Washington 1993). Finally, we hypothesized that all incisions would heal more quickly at 20°C than at 12°C (Anderson and Roberts 1975), and we expected the incisions of fish held at 20°C to have a higher rate of transmitter loss than the incisions of fish held at 12°C (Bunnell et al. 1998; Bunnell and Isely 1999).

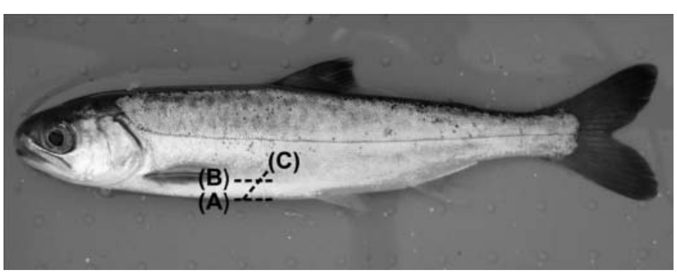

FIGURE 1. Photograph depicting the incision locations used in juvenile Chinook salmon (A = on the linea alba; B = muscle-cutting incision; C = muscle-sparing incision).

METHODS

Study site.—Fall Chinook salmon juveniles were raised at the Aquatic Research Laboratory at the Pacific Northwest National Laboratory (PNNL) in Richland, Washington. Fish (fork length [FL] range = 95–121 mm; mean FL = 105 mm) were stocked into two circular tanks (each tank was 1.2 m in diameter and 0.5 m deep and held 608 L of water), and the lights in the laboratory automatically simulated the natural photoperiod. Water temperature was maintained at either 12 ± 1°C or 20 ± 1°C with flow-through well water, and fish were held at the desired temperatures (±1°C) for 14 d before surgery. Fish were fed a daily maintenance ration of BioDiet Starter moist pellets (Bio-Oregon, Longview, Washington); food was withheld for 24 h prior to anesthetization for surgical implantation and weekly observations.

Surgical procedure.—The first trial was performed at 12°C in October 2008, and the second trial was performed at 20°C in February 2009. For each temperature trial, fish were selected randomly to create four groups (three incision treatments and one control) of 156 fish each. An initial power analysis performed on a pilot study found that 104 fish/incision location were required to detect (with 95% confidence and 80% power) a 5% difference in suture retention, erythema, and incision closure among incision locations. An additional 52 fish were included in each group to provide enough fish for histological examination. Because the final sample size was 156 fish/treatment, three surgeons were required to perform all of the surgeries in 1 d.

Fish were anesthetized (tricaine methanesulfonate [MS-222] at 80 mg/L of water) until they reached stage 4 anesthesia (Summerfelt and Smith 1990). Fish were then weighed (g), measured for FL (mm), and placed in a V-shaped neoprene trough. The trough was coated with a water conditioner (PolyAqua; Kordon, Hayward, California), and a soft silicone tube was inserted into the fish's mouth to continuously perfuse the gills with a maintenance dose of anesthetic (MS-222 at 40 mg/L). Control fish were anesthetized, weighed, and measured in a similar manner as treatment fish but did not undergo surgery.

Before surgery, transmitters were disinfected by immersion in a 70% solution of ethanol for 10 min and surgical instruments were autoclaved. Instruments were disinfected by immersion in 70% ethanol for 8–10 min and rinsed in deionized water between each operation to minimize the spread of aquatic pathogens among fish. All surgeons wore medical examination gloves. According to a predetermined random order, a 7–9-mm incision was made at one of the three anatomical locations: (1) on the LA (LA incision), (2) 3 mm from and parallel to the LA and anterior to the pelvic girdle (MC incision), or (3) extending from the LA dorsally and posteriorly at an approximately 45° angle (MS incision). Myomere lines were externally visible on fish, and surgeons attempted to make the cut parallel to the lines as closely as possible. Both a Juvenile Salmon Acoustic Telemetry System (JSATS) acoustic transmitter (12.0 × 5.3 × 3.7 mm, 0.44 g in air, 0.30 g in water, 0.144-mL volume) and a passive integrated transponder tag (12.5 × 2.1 mm, 0.10 g in air, 0.06 g in water, 0.036-mL volume) were implanted into the coelomic cavity of each fish. Although JSATS transmitters of this size can have a variable life span depending on the rate at which they emit a signal, they are typically programmed for 15 or 30 d of use (McMichael et al. 2010). The range of transmitter burdens was 3–7% of body weight for fish held at 12°C and 3–6% of body weight for fish held at 20°C. The fish were double-tagged to simulate field studies on the Snake and Columbia rivers, where the presence of a passive integrated transponder tag prevents fish from being sorted into transport barges or trucks at juvenile bypass facilities. Incisions were closed with two simple interrupted sutures (5–0 Monocryl; Ethicon, Rahway, New Jersey) located approximately 2–3 mm apart.

Immediately after surgery, fish were placed ventral side up on a V-shaped neoprene trough coated with PolyAqua, and an image of the incision was taken with a firewire camera (0.5× magnification lens; Model PL-A66X; PixeLINK, Ottawa, Ontario) that was attached to a microscope (0.65× magnification; Zeiss, Chester, Virginia). Fish were placed into a recovery bucket supplied with bubbled air, and upon recovery they were returned to a circular tank. All treatment and control fish were held in the same circular tank.

Macroscopic evaluation.—Incisions on fish were examined under light magnification (0.65×), imaged, and graded once per week for 98 d postsurgery. During weekly evaluations, treatment and control fish were individually anesthetized (MS-222 at 80 mg/L), weighed, measured, and placed on a V-shaped neoprene trough coated with PolyAqua. A soft silicone tube was inserted in the fish's mouth to continuously perfuse the gills with anesthetic (40 mg/L) as described above, and the incision was photographed with the microscope camera. One trained individual determined the presence or absence of the sutures, incision closure and apposition, and the presence of erythema on the incisions. After examination, fish were placed into a recovery bucket and were later returned to the holding tank.

Incision closure was defined as the point when the incision edges were touching and scale regeneration was nearly complete (Walsh et al. 2000). The point at which incision closure occurred was a qualitative assessment made by the observer to define when the incision appeared to be "healed." Erythema in the incision was defined as any visible redness on the surface of the scales and integument, without necessarily confirming the cause (i.e., inflammation, active bleeding, or infection). The criteria presented in Figure 2 were used to grade apposition of the incision. Grades represented the proportion of the incision that was apposed, folding inward, overlapping, gaping apart, or a combination of these (Figure 2). Similar to Wynne et al. (2004) apposition grades were scored for analysis according to the following criteria:

(1) No separation of layers (grade of 1; 2; 1,2; or 5);
(2) Less than 50% superficial separation (grade of 1,6; 2,6; 3,6; or 4,6);
(3) Greater than 50% superficial separation (grade of 1,7; 2,7; or 3,7); and
(4) 100% separation of layers (grade of 8).

The area (mm^2) of visible subepidermal tissue (termed "wound extent"; see Lazarus et al. 1994) was measured on digital images of the incisions with Image-Pro Plus software (Media Cybernetics, Bethesda, Maryland). For incisions with apposition scores that indicated visible subepidermal tissue (Figure 2), the wound extent was calculated in the software by outlining the perimeter of the wound where scales ceased and where muscle tissue and viscera were visible. Any tissue around the incision that was damaged by the tearing of a suture was excluded from the wound extent estimate.

A review of images of fish held at 20°C revealed that for one surgeon, 34 (65%) of the fish in the MS incision group had incisions that were made perpendicular to the LA rather than between the myomere lines. These fish were excluded from analysis.

Histology.—Tissue samples for histological examination were collected from fish in both temperature treatments on days 0, 3, 7, 10, 14, 21, 42, and 98. Three fish from each treatment group and one fish from each control group were examined at each time point. All fish were euthanized in MS-222 at 250 mg/L, and fish tissue samples were prepared similar to the methods of Elston et al. (1997). A rectangular-shaped area (∼3 × 2 cm) of tissue around the incision was excised. Samples were fixed by immersion in a 10% solution of neutral buffered formalin for 48 h and were stored in formalin. Tissues were processed according to conventional paraffin embedding techniques and were stained with hematoxylin and eosin. Three transects were made across each sampled incision. The first transect was between the anterior end of the incision and the rostral suture, the second was across the middle of the incision, and the third was between the caudal suture and the posterior end of the incision. Owing to a processing error, many of the transects between the anterior end of the incision and the rostral suture were sectioned incorrectly and did not include the incision; therefore, these transects were excluded from analysis. Single-blind

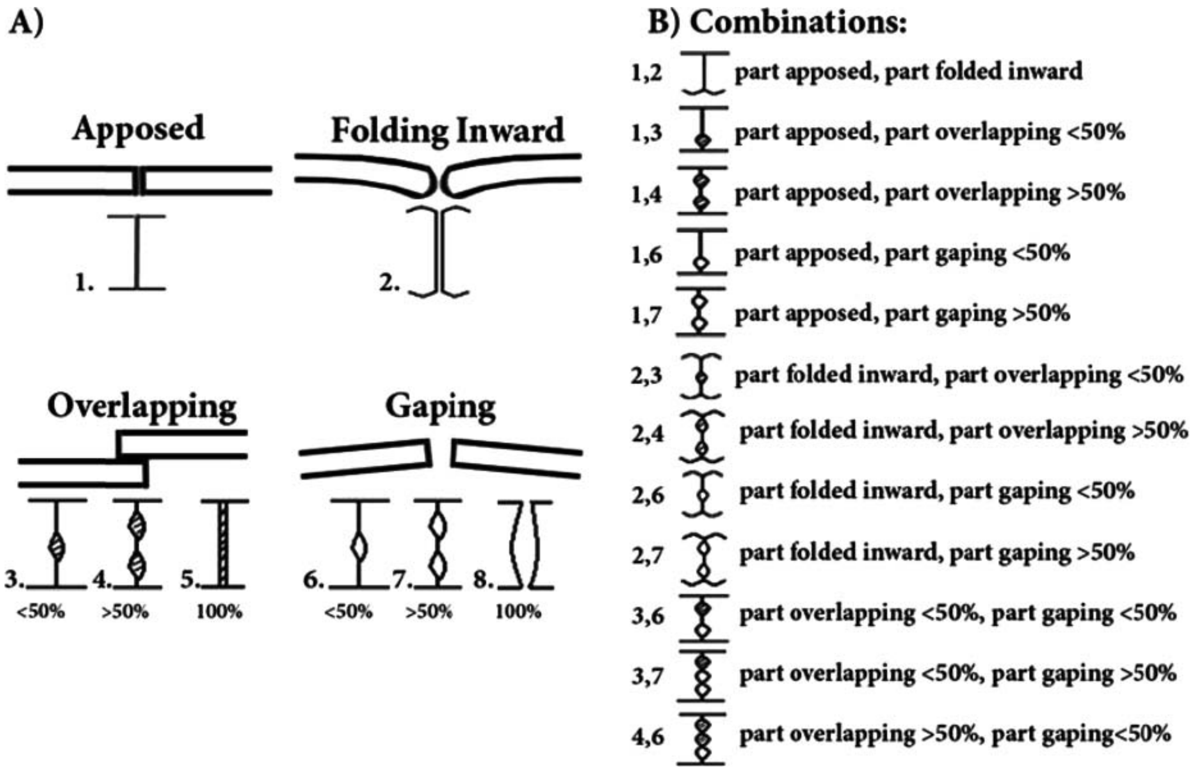

FIGURE 2. Criteria used to grade the external appearance of tissue apposition of incisions made in juvenile Chinook salmon: **(A)** eight possible outcomes of apposition (apposed, folding inward, some portion overlapping, some portion gaping); and **(B)** combinations of the possible outcomes (e.g., a grade of 1,4 means that the edges overlapped by more than 50% and the rest of the incision was apposed).

histological analysis was performed on the two remaining transects per incision by use of the criteria listed in Table 1, and ratings for the sections were averaged for each fish.

Statistical analysis.—Binary data (incision closure, incision erythema, and transmitter loss) were analyzed by use of a generalized linear model with a binomial error structure and a logit link function via analysis of deviance. Continuous variables (wound extent, mean suture presence, and apposition score) were analyzed for each observation day by use of two-way analysis of variance with incision location and surgeon as factors. A repeated-measures analysis of variance was not used because many values approached or remained at zero on days 35–98, thus obscuring or eliminating the correlations between weeks that repeated measures would determine. Apposition score and mean suture presence were treated as continuous variables because both metrics were based on an underlying continuous scale (Snedecor and Cochran 1989). Pairwise comparisons (with a Bonferroni correction) were made among incision locations and surgeons. An α of 0.05 was used for all statistical tests.

The main effects of incision location and surgeon on all variables were evaluated. Results of the three surgeons remained pooled regardless of interactions; our results are therefore applicable to most surgeons who use similar techniques or to many research projects in which multiple surgeons are required because of large sample sizes. Temperature was not included as a factor because of the elapsed time between the two trials (i.e., 4 months apart) and the size differences between the two groups (12°C: FL range = 95.0–116.0 mm, mean ± SD = 101.3 ± 4.5 mm; 20°C: FL range = 95.0–121.0 mm, mean ± SD = 108.4 ± 4.4 mm). However, general comparisons were made between the groups of fish held at 12°C and 20°C. The Results and Discussion emphasize day 28 because juvenile Chinook salmon similar to those used in this study typically migrate to the ocean within 28 d (Smith et al. 2003) and transmitter life was 30 d.

The instantaneous growth rates in lengths of treated fish were calculated with the formula presented by Isely and Grabowski (2007). Results were analyzed with analysis of variance. Control fish were included in the study only to determine whether mortality (if any) was caused by some factor other than the surgery (e.g., waterborne pathogens), and these fish were not individually marked. Therefore, growth rates were not calculated for control fish.

RESULTS

Transmitter Loss

Transmitter loss among incisions differed with water temperature and the time since implantation. Among fish that were held at 12°C, fish with LA incisions generally had the highest

TABLE 1. Histological criteria used to evaluate wound healing in juvenile Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag implanted through one of three incision locations: incision on the linea alba, muscle-cutting incision, or muscle-sparing incision (see Methods).

Measure	Rating	Description
Tissue alignment	1	Normal in appearance; no misalignment of tissue.
	2	Some apparent misalignment; may be masked by inflammatory reaction or plane of section.
	3	Misaligned, with missing muscle or moderate to severe misalignment of myomeres.
Scales over wound area	0	Scales are absent in wound area.
	1	Normal presence of scales in wound area.
	2	Increased scale density.
Fibrosis[a]	1	Mild degree of wound-healing fibrosis present.
	2	Moderate degree of fibrosis present.
	3	Advanced inflammatory infiltrate or fibrosis, depending on indicated stage of inflammation.
Inflammation[b]	1	Minimal amount of inflammatory cells present.
	2	Moderate amount of inflammatory cells present.
	3	Severe amount of inflammatory cells present.
Wound healing[c]	1	No evidence of wound present.
	2	Healing considered nearly complete; minimal degree of fibrosis and/or mildly less-than-normal muscle density.
	3	Substantial fibrosis, inflammation, or both; in case of dermis and epidermis, lack of alignment, fusion, or abnormal skin regrowth indicates an active but incomplete healing process.
	4	Similar to a rating of 3; normal tissue replacement is lacking in wound area, an abnormal healing pattern is apparent, or both; includes incisions that are separated into two pieces where the ingrowth of dermis and/or epidermis on one or both edges shows a failure of the wound to close.
Separation of tissue along wound axis	0	Tissue pieces are not separated.
	1	Tissue pieces are separated.
Ingrowth of dermis along wound axis	0	Dermis is not ingrown along wound axis.
	1	Dermis is ingrown along wound axis.
Myocyte density	0	Myocyte density appears normal.
	1	Myocytes are less dense and are smaller in diameter (typical of myocyte regrowth).
Thickness of the epidermis	0	Epidermis is of normal thickness.
	1	Epidermis is thickened (typical of regrowth over wound area).

[a]The serosal surface, musculature, and dermis–epidermis were graded separately for the fibrosis, inflammation, and wound healing measures.
[b]Inflammatory cells include polymorphonuclear cells, mononuclear cells, lymphocytes, and neutrophils.
[c]Wound healing score was based on fish pathologist Ralph Elston's (AquaTechnics, Sequim, Washington) expert opinion of healing by incorporating all criteria listed.

transmitter loss over the entire course of the study. However, much of the transmitter loss occurred later in the experiment. Transmitter loss was not significantly different among incision types through day 28 ($P > 0.05$), the point when transmitter loss began to steadily increase for fish with LA incisions (Figure 3). For days 49–98, fish with LA incisions had significantly greater transmitter loss (16 transmitters by day 98; $P = 0.003$) than fish with MC incisions (3 transmitters) or MS incisions (1 transmitter).

Among the fish that were held at 20°C, transmitter loss occurred sooner and over a smaller time period (Figure 3) and most of the losses were observed between days 18 and 24. During this period, seven transmitters were lost through LA incisions on seven consecutive days (1 transmitter each day). However, transmitter loss by day 98 was not significantly different among the incision types ($P = 0.137$; 9 transmitters from LA incisions; 7 transmitters from MC incisions; 2 transmitters from MS incisions).

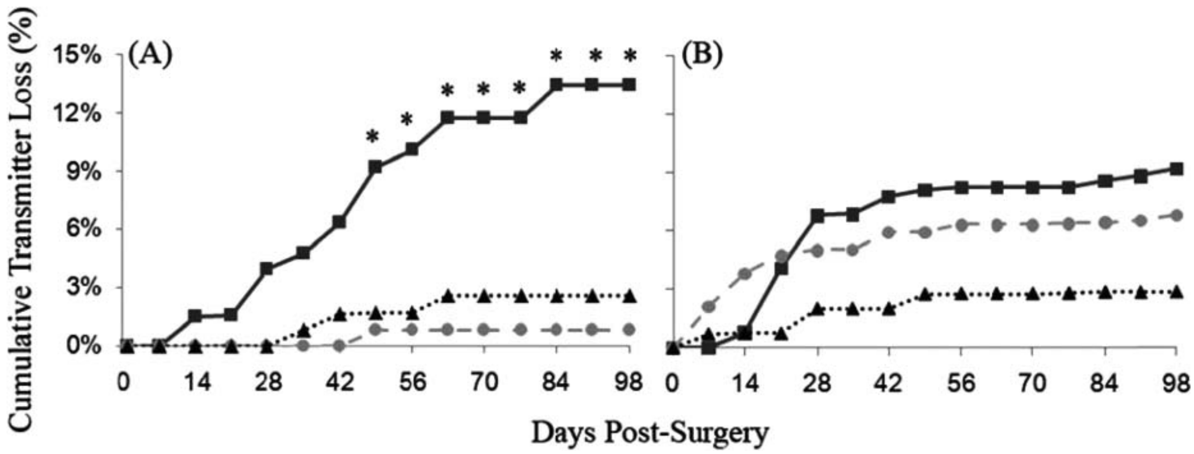

FIGURE 3. Cumulative transmitter loss among juvenile Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag implanted through one of three incision types (dark-gray squares = incision on the linea alba; light-gray circles = muscle-cutting incision; black triangles = muscle-sparing incision) and that were held at either **(A)** 12°C or **(B)** 20°C. An asterisk indicates a significant difference among treatments ($P < 0.05$).

Healing

Wound extent differed among incision types through day 21 at both temperatures (Figure 4). For fish that were held at 12°C, those with LA incisions had significantly smaller wound extents on days 7, 14, and 21 than fish with MC and MS incisions ($P < 0.001$); fish with LA incisions had significantly smaller wound extents on day 28 than fish with MC incisions ($P = 0.031$). For fish held at 20°C, LA incisions had significantly smaller wound extents than MC and MS incisions on days 7 and 14 ($P < 0.002$), and LA incisions had significantly smaller wound extents than MS incisions on day 21 ($P = 0.013$). Wound extents for MC and MS incisions were greatest on day 14 at 12°C and on day 7 at 20°C. However, wound extents of LA incisions were greatest on day 56 at 12°C and on day 21 at 20°C, since some incisions reopened after initially appearing to be closed.

Incision apposition scores differed among incision types through day 14 at both temperatures (Figure 4). Apposition scores for LA incisions were significantly lower (indicating better incision apposition) than those for MC and MS incisions on days 7 and 14 of both temperature trials ($P < 0.001$) and on day 21 of the 12°C trial ($P < 0.001$). However, at 12°C the LA incisions had significantly higher apposition scores than MC and MS incisions for days 42–98 ($P < 0.05$). At 20°C, apposition scores did not differ among incision types for days 21–98 ($P = 1.000$).

Trends of erythema on incisions differed among incision types based on water temperature and time since implantation. Among fish held at 12°C, significantly more MC incisions than MS incisions had erythema on days 14, 21, and 28 ($P < 0.05$), and significantly more MS incisions than LA incisions had erythema on days 7 and 14 ($P < 0.001$; Figure 5). However, at 12°C, significantly more LA incisions than MS incisions had erythema on days 42 and 49 ($P < 0.05$), and significantly more LA incisions than MC incisions had erythema on day 49 ($P < 0.05$). Among fish held at 20°C, significantly more LA incisions than MS incisions had erythema on days 7–28 ($P < 0.05$), and significantly more LA incisions than MC incisions had erythema on days 14 and 21 ($P < 0.05$). Significantly more MC incisions than MS incisions had erythema on day 7 at 20°C ($P = 0.003$).

The percentage of incisions exhibiting closure (i.e., point at which incisions appeared to be healed) varied among incision types in relation to water temperature and time since implantation (Figure 5). There was no significant difference in incision closure among incision types on days 7–21 at 12°C ($P = 1.000$). By day 28, the percentage of incisions that were closed was significantly greater among LA incisions than among MC or MS incisions on fish held at 12°C ($P < 0.01$). Over time, this trend reversed; for days 70–91, incision closure was significantly greater for MS and MC incisions than for LA incisions ($P < 0.05$). By day 98, 12% of LA incisions were still not completely closed. Among fish held at 20°C, there was no significant difference ($P = 1.000$) in incision closure among incision types except on day 21, when the percentage of closed incisions was significantly ($P < 0.001$) greater for LA incisions than for MC and MS incisions. During the 20°C trial, at least 75% of the incisions (all types) were closed by day 28 and 99% of incisions were closed by day 63.

Suture Presence

At both temperatures, fish with MS incisions lost sutures sooner than fish with MC or LA incisions. There was no significant difference in suture retention between LA and MC incisions at 12°C ($P > 0.05$); at 20°C, suture retention differed only on days 7 ($P < 0.001$) and 14 ($P = 0.014$), when LA incisions had higher suture retention than MC incisions (Figure 6). At 12°C, LA incisions had significantly greater mean suture retention than MS incisions on days 21, 28, and 49–98 ($P < 0.05$); MC incisions had significantly greater suture retention than MS

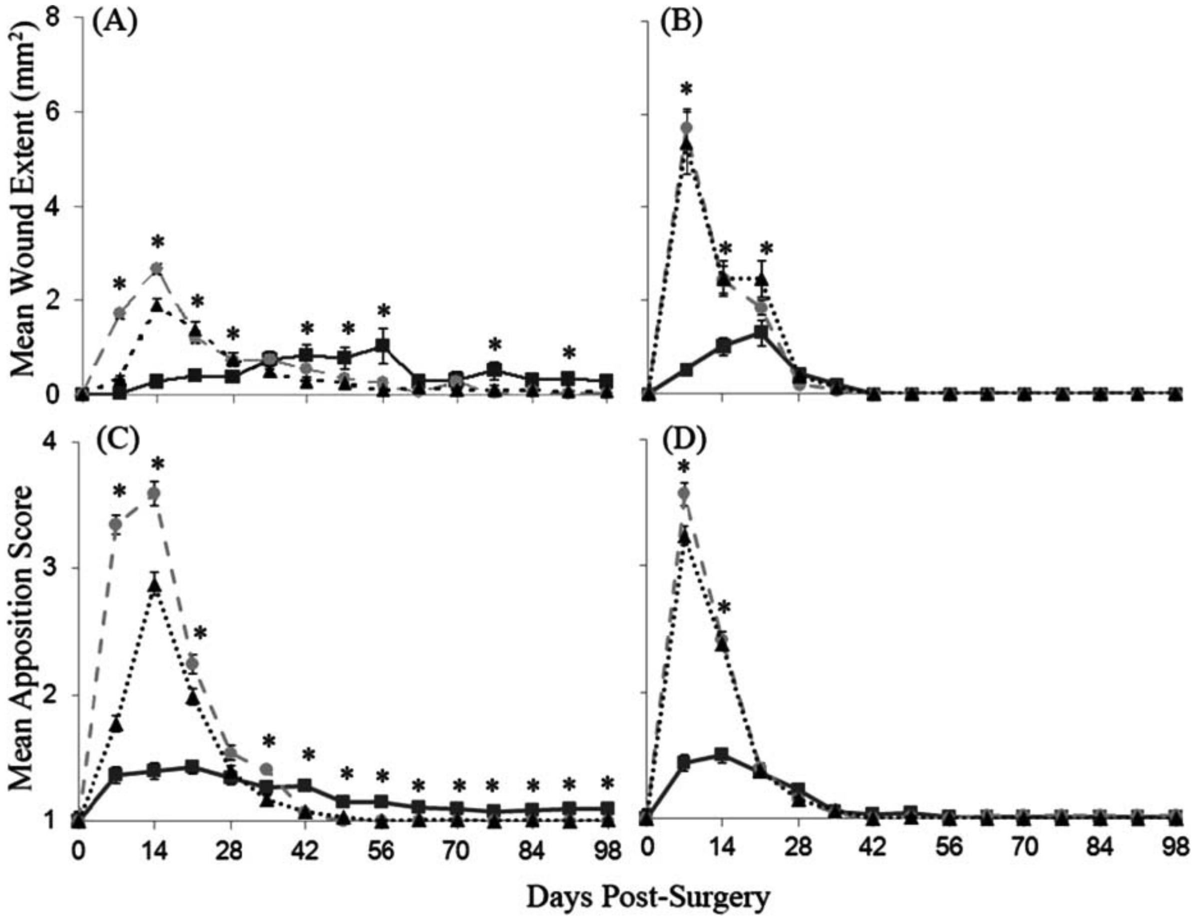

FIGURE 4. Means (±SE) of wound healing criteria for juvenile Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag implanted through one of three incision types (dark-gray squares = incision on the linea alba; light-gray circles = muscle-cutting incision; black triangles = muscle-sparing incision): **(A)** area (mm^2) of wound extent for fish held at 12°C; **(B)** area of wound extent for fish held at 20°C; **(C)** apposition score for fish held at 12°C; and **(D)** apposition score for fish held at 20°C. A lower apposition score indicates better apposition. An asterisk indicates a significant difference among treatments ($P < 0.05$).

incisions on days 28, 84, and 98 ($P < 0.05$). For fish held at 20°C, suture retention differed between LA and MS incisions on days 14–84, when LA incisions had significantly greater retention ($P < 0.05$). At 20°C, MC incisions had significantly greater suture retention than MS incisions on days 21–77 ($P < 0.05$).

Mortality and Growth

Mortality of fish that received surgically implanted transmitters was generally low (Table 2). There were no mortalities among fish that were held at 12°C. Although there was 11–20% mortality by day 98 among the fish that were held at 20°C, there was no significant difference in mortality owing to incision location ($P = 0.181$). Through day 84, the mortality among fish with LA incisions was similar (≤5% difference) to the mortality of the control fish or the fish with other incision types. However, the range in mortality increased to 7% on day 91 (9–16%; 9% for the control, 12% for the MC incision group, 15% for the MS incision group, and 16% for the LA incision group) and to 9% on day 98 (11–20%; 11% for the control group, 16% for the MS incision group, 17% for the MC incision group, and 20% for the LA incision group).

During most of the study period (all but day 91 for fish held at 12°C), there was no significant difference in growth among incision groups held at both temperatures ($P > 0.05$). On day 91 of the 12°C trial, fish with MC incisions had significantly higher growth than fish with LA incisions ($P = 0.02$).

Surgeon Effects

The three surgeons participating in the study had previously performed hundreds of surgeries with similar survival results and had received feedback on their surgery performance. However, subtle differences in suturing techniques were evidenced by surgeon × incision type interactions on select days across all variables. There was no clear pattern to explain these interactions. Therefore, results for all three surgeons were combined and are considered applicable to surgeons who use similar techniques.

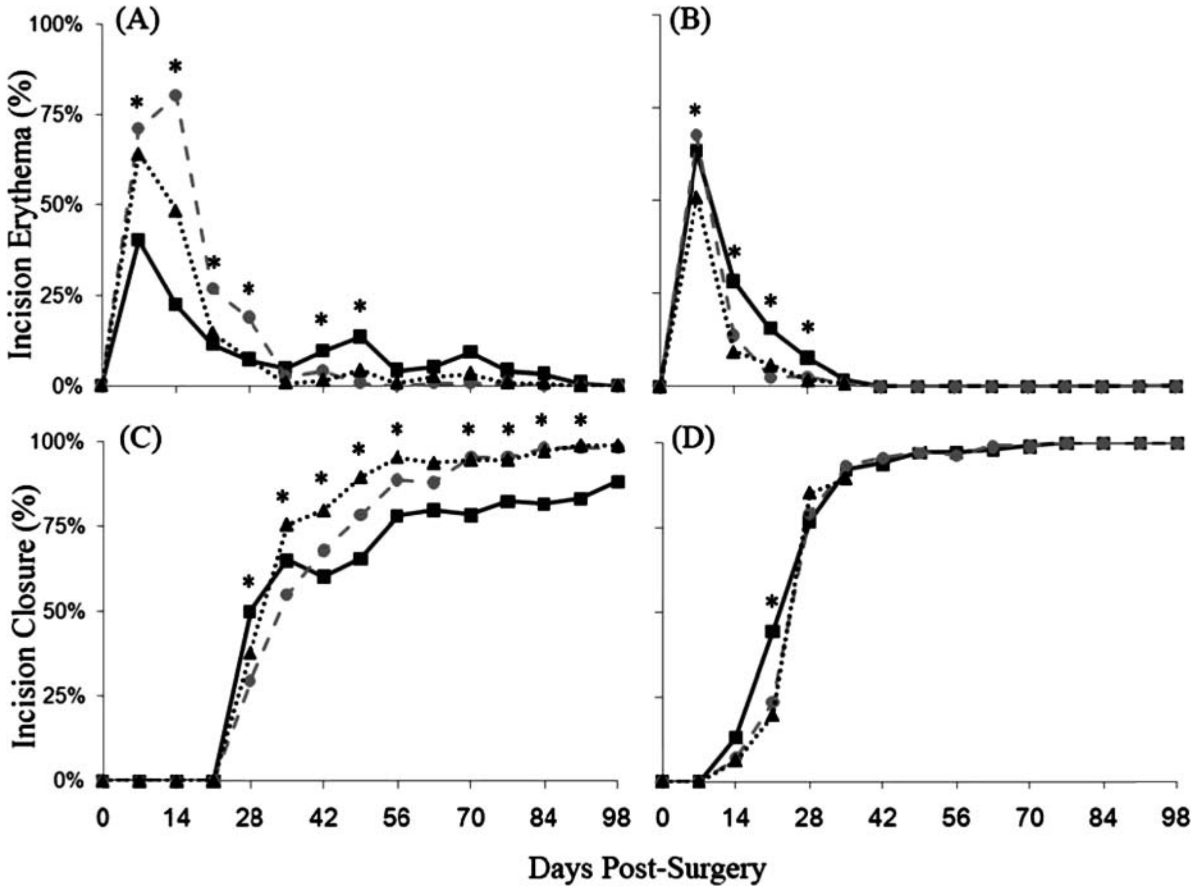

FIGURE 5. Means of wound healing criteria for juvenile Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag implanted through one of three incision types (dark-gray squares = incision on the linea alba; light-gray circles = muscle-cutting incision; black triangles = muscle-sparing incision): **(A)** percentage of incisions with erythema among fish held at 12°C; **(B)** percentage of incisions with erythema among fish held at 20°C; **(C)** percentage of incisions that were closed among fish held at 12°C; and **(D)** percentage of incisions that were closed among fish held at 20°C. An asterisk indicates a significant difference among treatments ($P < 0.05$).

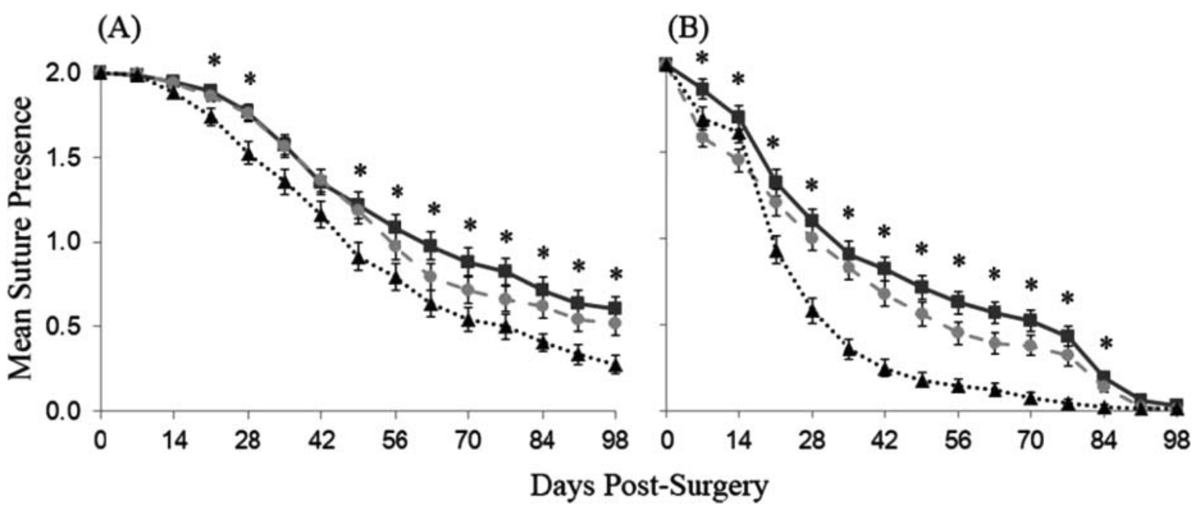

FIGURE 6. Mean (±SE) suture presence among juvenile Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag through one of three incision types (dark-gray squares = incision on the linea alba; light-gray circles = muscle-cutting incision; black triangles = muscle-sparing incision) and that were held at **(A)** 12°C or **(B)** 20°C. An asterisk indicates a significant difference among treatments ($P < 0.05$).

TABLE 2. Instantaneous growth rate (IGR) in length, number of fish that died (cumulative mortality percentage is given in parentheses), and number of transmitters that were lost (cumulative loss percentage is given in parentheses) through day 98 among juvenile Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag implanted through an incision on the linea alba (LA), a muscle-cutting incision (MC), or a muscle-sparing incision (MS).

Temperature (°C)	Treatment	*N*	IGR	Mortality (cumulative %)	Transmitter loss (cumulative %)
12	LA	156	0.09	0 (0)	16 (13)
	MC	158	0.10	0 (0)	1 (1)
	MS	155	0.09	0 (0)	3 (3)
	Control	155	0.10	0 (0)	
20	LA	156	0.20	20 (20)	9 (9)
	MC	156	0.19	17 (17)	7 (7)
	MS	121	0.20	11 (16)	2 (3)
	Control	157	0.21	12 (11)	

Histology

Healing of incisions followed a similar progression among fish held at both temperatures, although the various responses occurred more quickly at 20°C than at 12°C. The inflammatory responses in the epidermis, dermis, and musculature generally peaked on day 21 in fish held at 12°C and on day 14 in fish held at 20°C. The amount of fibrotic tissue within the incision peaked on day 21 in fish held at 12°C and on day 10 in fish held at 20°C. Because the progression of healing did not differ between fish that were held at the two temperatures, results are presented only for the fish in the 12°C trial.

In fish that were held at 12°C, healing progressed in a similar manner for the three incision types during the first 14 d (Figure 7). Thickening of the epidermis occurred as epithelial cells proliferated and migrated around the cut edges of the incision, covering any exposed muscle or viscera. On average, 75% of all incision types either (1) lacked fusion of the dermis, epidermis, and musculature during days 3–14 or (2) were so weakly fused that the layers broke apart when transects were cut across the incisions before embedding and staining (Figure 7). Neovascularization within the incision was present as early as day 3 for all incision types and increased steadily through day 14. Moderate numbers of inflammatory cells were present in the serosal surface and musculature by day 3 and were observed in the dermis and epidermis on day 10. The MC and MS incisions had greater numbers of inflammatory cells in the serosal surface and musculature than LA incisions. For 92% of the LA incisions, the dermis and epidermis of both edges of the incision were folded inward. In contrast, 54% of MC incisions and 42% of MS incisions had one or both edges of the incision folded inward.

By day 21, the most notable difference among incision types for fish held at 12°C was the amount of fibrotic tissue present in the incision (Figure 8). The amount of fibrotic tissue within the incision was much greater for the MC and MS incisions than for the LA incisions. For the majority (83%) of LA incisions, the dermis remained ingrown on both edges, whereas ingrowth (on one or both edges) was less frequent among the MC (50%) and MS (17%) incisions. Inflammatory cells were observed at their highest levels in all three incision types on day 21, and abundances were similar among the incision types. Tissue layers on either side of the incision were misaligned in the majority (77% on average) of all incision types. For all incision types, healing was still in progress by day 49, but the amounts of fibrotic tissue and inflammatory cells had decreased. However, the amount of fibrotic tissue present in the incision was still greater for MC and MS incisions than for LA incisions.

Of the incisions that were examined histologically (i.e., from fish in the 12°C trial), most were fully healed by day 98 (Figure 9). Epidermal and dermal layers were fused and muscle tissue had regenerated at the incision site. Small pieces of fibrotic tissue (fibrotic tags) on the serosal surface were present in varying quantities for all three incision types.

DISCUSSION

The progression of healing at incision locations and the variable transmitter loss made it difficult to identify one incision location as the best choice. The LA incisions had much smaller wound extents than the MC and MS incisions during the first 28 d postsurgery, suggesting a decreased risk of invading pathogens or osmoregulatory problems. Apposition of incisions through day 14 in fish from both temperature treatments was better for LA incisions than for MC and MS incisions. The results from histology confirmed that LA incisions were healing with much less fibrotic tissue than were the MC or MS incisions. However, MC and MS incisions were less likely to reopen over time, and thus the likelihood of transmitter loss was lower for these incision types. Although our results for the first month after implantation indicate that there are advantages to the use of LA incisions, none of the incision locations are clearly superior when the entire 98-d study period is considered; however, the expected battery life for a transmitter of this limited size is unlikely to exceed 30 d.

During the 12°C trial, transmitter loss by day 98 was highest among fish with LA incisions, which may have been related

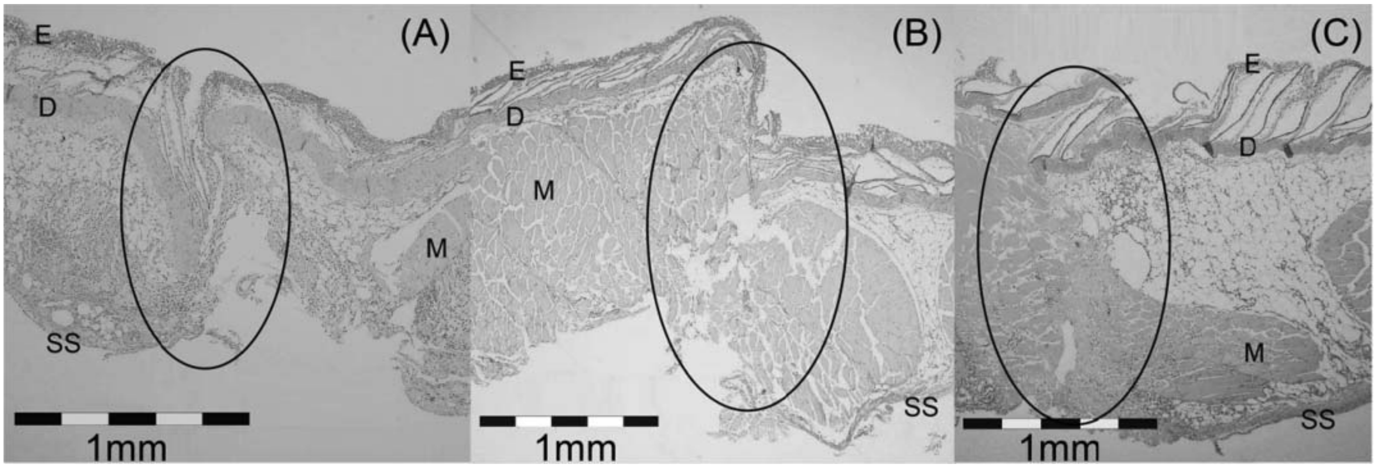

FIGURE 7. Transverse sections of incisions in juvenile Chinook salmon at day 14 postsurgery: **(A)** an incision on the linea alba, **(B)** a muscle-cutting incision, and **(C)** a muscle-sparing incision. Incisions (denoted by black ovals) are healing in a similar manner. The epidermis (E) and dermis (D) are not aligned, and the dermis lacks fusion. The number of inflammatory cells was greater in the serosal surface (SS) and musculature (M) for muscle-cutting and muscle-sparing incisions than for incisions on the linea alba.

to how the incisions were healing. Histological results showed that as LA incisions healed, the edges curled inward and were connected only weakly with a thin layer of epithelial cells, as described by Phromsuthirak (1977). The weight of the transmitter on the incision may have caused the sutures to fail (Schramm and Black 1984) and probably overcame the weak bond between the incision edges, causing the incision to dehisce and the transmitter to be lost through the incision (i.e., by gravity; Marty and Summerfelt 1986). All incisions healed faster at 20°C, and it is possible that the strength of the incision increased quickly enough to support the transmitter, thus preventing a greater loss of transmitters. Differences in healing between temperature treatments may be attributable to size differences between fish in the two trials, although the difference in mean size was relatively small (7 mm). Healing might also have differed if observed at temperatures within the range between the two study temperatures.

Although more transmitters were lost through LA incisions than through the other incision types by day 98, transmitters were lost through all three incision locations. Indeed, a large

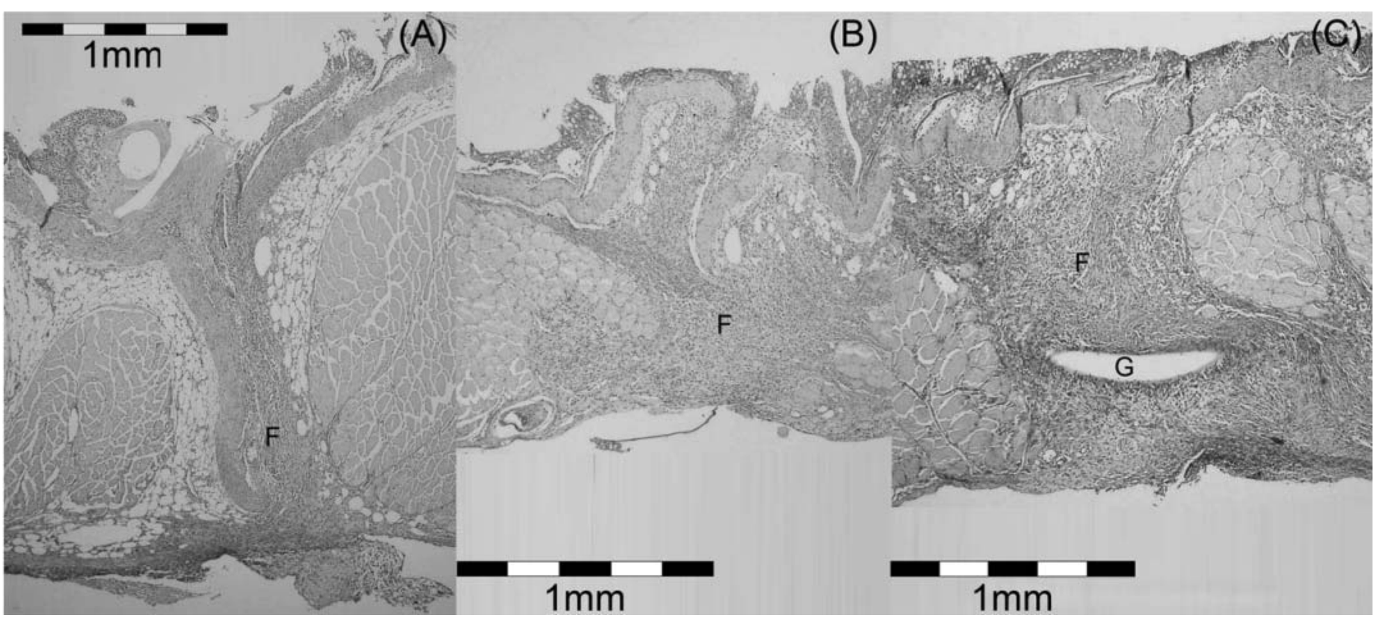

FIGURE 8. Transverse sections of incisions in juvenile Chinook salmon at day 21 postsurgery: **(A)** an incision on the linea alba, **(B)** a muscle-cutting incision, and **(C)** a muscle-sparing incision. Fibrous granulation tissue (F) is present in greater quantities for the muscle-cutting and muscle-sparing incisions than for the incision on the linea alba. The gap (G) in fibrous granulation tissue of the muscle-sparing incision is an artifact from processing.

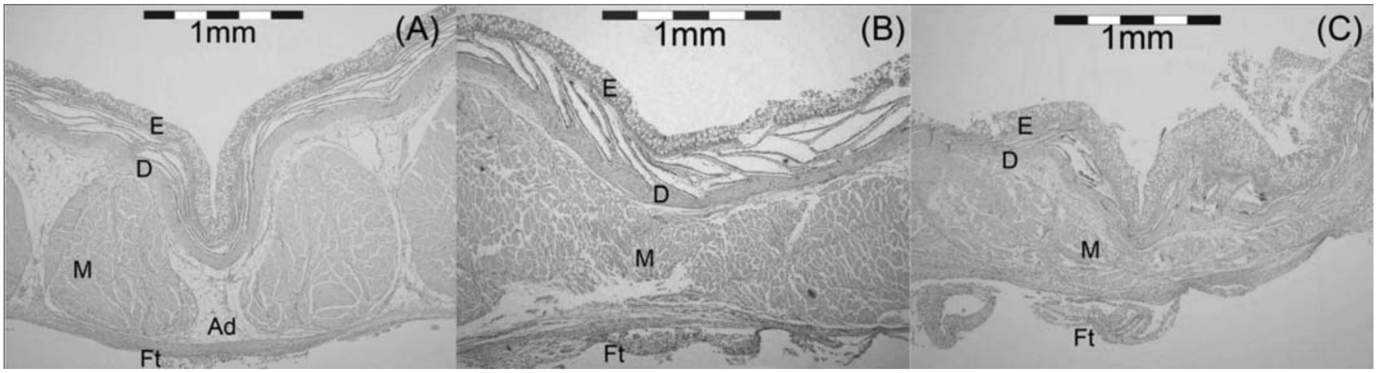

FIGURE 9. Transverse sections of incisions in juvenile Chinook salmon at day 98 postsurgery: **(A)** an incision on the linea alba, **(B)** a muscle-cutting incision, and **(C)** a muscle-sparing incision. The adipose tissue (Ad) at the linea alba incision is completely healed, as is the musculature (M) at the muscle-cutting and muscle-sparing incisions. The epidermis (E) and dermis (D) are continuous and fused, and the fibrous granulation tissue (Figure 8) has been replaced with normal tissue. Fibrotic tags (Ft) are present on the serosal surface of all three incision locations.

number of studies (Schramm and Black 1984; Marty and Summerfelt 1986; Lucas 1989; Knights and Lasee 1996; Bunnell et al. 1998; Bunnell and Isely 1999; Walsh et al. 2000; Deters et al. 2010b) have shown that transmitter loss for a given incision location can occur over a range of fish sizes, a variety of species, and a range of temperatures. However, a recent study by Deters et al. (2010a) found no transmitter expulsion or mortality among juvenile Chinook salmon (FL range = 96–121 mm; transmitter burden = 2–6% of body weight) that received 0.43-g JSATS transmitters implanted through an LA incision. The fish in that study were held for 28 d at both 12°C and 17°C, and transmitters were implanted by a surgeon with feedback training (see Deters et al. 2010b for details). Despite this, our results and the results of previous studies suggest that regardless of the incision location employed, some way of estimating transmitter loss should be included in the study design.

The way in which erythema was measured in this study may have made it a poor metric for the assessment of healing. Early in the study (days 0–14), LA incisions had less erythema than the other two incision types at 12°C, but the opposite was true at 20°C. These results were somewhat misleading and were related to how erythema was recorded. Erythema was noted when it was visible against the scales and integument. However, at 20°C, sutures were expelled from many MC incisions and some MS incisions, thus tearing scales and integument away from the incision edges and making it difficult to observe erythema.

The faster loss of sutures from MS incisions relative to the other incision types was probably due to the angle of the sutures on the body. As the fish swam, the force of the water on the sutures of MS incisions may have pushed the sutures towards the incision, enabling them to be expelled more easily. In contrast, for MC and LA incisions, the force of the water may have pushed the sutures parallel to the incision, thus allowing the sutures to remain in the body wall longer.

Suture loss from all incisions was higher during the 20°C trial than during the 12°C trial. Similarly, Walsh et al. (2000) found that more than 50% of the absorbable monofilament sutures used on hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) were expelled by 60 d postsurgery at warm temperatures (mean = 25.5°C), whereas at cold temperatures (mean = 15°C) less than 25% of sutures were expelled even by 120 d postsurgery. Deters et al. (2010b) also found that suture loss after 14 d was higher in juvenile Chinook salmon held at 17°C (36%) than in fish held at 12°C (18%).

The results showed that LA incisions had less evidence of healing by secondary intention (defined below) and much smaller wound extents during the early part of the study (first 28 d); both of these factors could prove beneficial to the health of the fish. Smaller wound extents of LA incisions may benefit the fish by requiring less energy to heal and by permitting efficient osmoregulation to be reestablished more quickly. When incisions are not apposed to heal by primary intention (i.e., the edges of the incision are first aligned and then secured together with sutures), they take longer to heal by a process called secondary intention (Barbul 2005). During secondary intention, a gap that exists between the incision edges must first be filled with fibrous granulation tissue, healing from the bottom up (Barbul 2005). The fibrous granulation tissue eventually contracts, pulling the edges of the incision into apposition. Although the MS and MC incisions were initially closed with sutures, they exhibited evidence of secondary intention because histological examination revealed that both contained greater amounts of fibrous tissue in the musculature than incisions within the adipose and connective tissue on the LA.

More histological research on healing is needed to determine whether LA incisions actually heal by primary intention rather than secondary intention or whether the less-perfused tissue of the LA naturally heals with less fibrotic tissue. Future studies should consider using other means (such as histology) to accurately assess wound healing in addition to tracking changes in erythema, changes in wound area or perimeter, color, signs of infection, and wound closure (Lazarus et al. 1994; Franz et al.

2008). Additionally, healing and transmitter loss in fish that are swimming and foraging in the natural environment (rather than in the laboratory) should be evaluated with field research.

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