INFLUENCE OF INCISION LOCATION ON TRANSMITTER LOSS, HEALING, INCISION LENGTH, SUTURE RETENTION, AND GROWTH OF JUVENILE CHINOOK SALMON

By

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To the Faculty of Washington State University:

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Abstract

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Many studies in fisheries research use acoustic and radio transmitters to track fish movements and estimate survival within populations. Currently, some researchers implant transmitters through an incision anterior to the pelvic girdle on the mid-ventral line (linea alba) of the fish to avoid bisecting muscle tissue. Others make an incision 2-10 mm lateral to and parallel to the linea alba to reduce disturbance of the incision site from the river bottom or tank. In the human medical literature, there is a growing trend toward “muscle-sparing” incisions that extend parallel to the underlying muscle fibers, thus preserving muscle strength and integrity. We measured differences in survival and growth, incision openness, transmitter loss, wound healing, and erythema among abdominal incisions on the linea alba, lateral and parallel to the linea alba (muscle-cutting), and extending parallel to the underlying muscle fibers (muscle-sparing). A total of 936 juvenile Chinook salmon (*Oncorhynchus tshawyscha*) were implanted with both Juvenile Salmonid Acoustic Telemetry System (JSATS) acoustic transmitters (0.43 g dry) and passive integrated transponder (PIT) tags. Fish were held at 12°C (n=468) or 20 °C (n=468) and examined once weekly over 98 days. Survival and growth did not differ among incision groups or between temperature groups. Incisions on the linea alba had less openness than muscle-cutting and muscle-sparing incisions during the first 14 days when fish were held at

12°C or 20°C. Transmitter loss was not affected by incision location by day 28 when fish were held at 12°C or 20°C. However, incisions on the linea alba had greater transmitter loss than muscle-cutting and muscle-sparing incisions by day 98 at 12°C. Relatively more incisions on the linea alba were closed during the first 28 days than muscle-cutting or muscle-sparing incisions at 12°C or 20°C. Through day 28, erythema was less prevalent on incisions on the linea alba when fish were held at 12°C but more prevalent when fish were held at 20°C, and this difference was likely related to how erythema was recorded. Results from our study will be used to improve tagging procedures for future studies using acoustic or radio transmitters.

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Dedication

To my Heavenly Father and His Son Jesus,

who created me with the capacity to complete this project.

CHAPTER ONE: INTRODUCTION

Surgical implantation of transmitters has become widely accepted for use in long-term studies of fish behavior using acoustic and radio telemetry (Winter 1996). The goal of surgically implanting transmitters remains the same across studies: ensure the transmitter is retained within the fish long enough to collect the desired data while minimally impacting survival and behavior. Survival studies involving transmitter implantation operate under the assumption that the method of implantation does not influence the behavior and survival of the fish (Peven et al. 2005). However, surgical implantation is invasive by nature, requiring an incision that bisects skin and muscle tissue, which may adversely affect the health of the fish.

The primary function of the integument of teleost fish is to provide a barrier to pathogens in the surrounding aquatic environment (Roberts 1989). The integument also aids in osmoregulation and, by producing mucus, helps decrease friction when fish move through the water by producing mucus (see review by Shephard 1994). Numerous studies have demonstrated that the integument plays a vital role in maintaining internal homeostatis and protection from pathogens, and damage to the integument may lead to death (Noga 2000). For example, in *Saprolegnia*-infected brown trout (*Salmo trutta*), loss of serum electrolytes and protein was found to be proportional to the percentage of integument affected (Richards and Pickering 1979). Atlantic salmon (*Salmo salar* L.) affected by invasive ulcers or "winter sores" extending into the underlying musculature died from osmoregulatory problems or bacterial septicemia (Lunder 1992). Furthermore, Atlantic salmon with either artificial wounds or a non-intact mucus layer had higher mortality than controls when all three groups were exposed to bath challenges of *Vibrio anguillarum* (causative agent of Vibriosis) and *Aeromonas salmonicida* (causative agent of Furunculosis; Svendsen and Bogwald 1997). Damage to the integument can sometimes result in 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. Research comparing healing rates in different kinds of tissues within the same fish species is sparse, with no known experimental studies. Although some studies mention anecdotal observations of incisions made in different locations (Pautzke and Meigs 1941), other studies only consider macroscopic appearance and transmitter loss among different incision locations (Schramm and Black 1984; Wagner and Stevens 2000; Dieterman and Hoxmeier 2009). In humans, healing takes longer in connective tissue with little or no blood supply, such as cartilage and tendons, than in highly-perfused tissue such as muscle (Barbul 2005). The blood supply drives the inflammatory response, and is essential for complete wound healing (Barbul 2005; Franz et al. 2008; Sen 2009). There is very little evidence that incisions in different types of fish tissue heal at different rates, and comparisons of healing among different fish tissues have not been well quantified with histology.

The effects of temperature on wound healing and transmitter expulsion should be considered 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). Because fish are poikilotherms, their physiology, immune function, and rates of healing are influenced by environmental temperature (Anderson and Roberts 1975; Le Morvan et al. 1998). Most species at a given temperature have similar rates of response for phagocytosis, inflammation, and wound healing, as well as responses to toxic and septicaemic microbial diseases (Roberts 1989). However, as temperature increases, the rate of responses accelerates (Roberts 1989). For example, Ream et al. (2003) showed that as temperature increased, so did the motility of epithelial wound-healing cells (keratocytes), which migrate across wounded areas to inhibit infection (Radice 1980 a, 1980 b; Euteneuer and Schliwa 1984). Anderson and Roberts (1975) found the white mountain cloud minnow (*Tanichthyes albonubes*) experimentally wounded (2-4mm long, 0.6-1mm deep) with a scalpel healed more quickly when held at elevated temperatures than the Atlantic salmon (*Salmo salar*) held at lower temperatures. In addition to delaying the healing process, lower temperatures have led to immunosuppression (Bly and Clem 1992; Le Morvan et al. 1998; and Nikoskelainen et al. 2004), which may influence the fish's ability to overcome any infection resulting from a surgical procedure. Studying differences in healing at both high and low temperatures may provide valuable insight into the healing process that might not be apparent at only one temperature.

Several locations for implantation of transmitters in fish have been mentioned in the literature. Incisions have been made in the connective tissue of the ventral midline (the linea alba; Marty and Summerfelt 1986; Lucas 1989; Knights and Lasee 1996; Wagner and Stevens 2000), across the white muscle tissue off of and parallel to the linea alba, anterior to the pelvic girdle (hereafter termed *muscle-cutting*; Adams et al. 1998; Wagner and Stevens 2000), or perpendicular to and several millimeters from the linea alba (vertical incision; Cobb 1933; Schramm and Black 1984). The concept of an incision that followed the lines of the underlying myomeres (hereafter termed *muscle-sparing*) was mentioned by Pautzke and Meigs (1941). There has been no research on the healing outcome of muscle-sparing incisions.

The objective of this 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 among three incision types. The first incision was on the linea alba, the second was off of and parallel to the linea alba (muscle-cutting), and the third extended from the linea alba towards the dorsum at a 45° angle, between the parallel lines of myomeres (muscle-sparing; Figure 1). All three incisions were made in the same vicinity, anterior to the pelvic girdle.

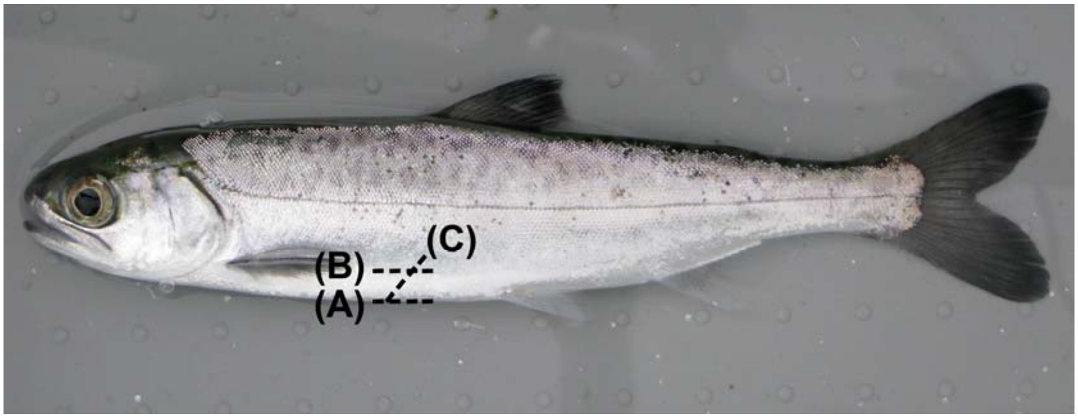

Figure 1. Incision locations used in the study were on the linea alba (A), muscle-cutting (B), or muscle-sparing (C).

I hypothesized that fish with incisions on the linea alba would have better incision closure with less erythema than those with muscle-cutting and muscle-sparing incisions. I also hypothesized that incisions on the linea alba would have a higher rate of transmitter loss because the transmitter rests partially or fully on the incision (Schramm and Black, 1984). The effect of environmental temperature on all variables was evaluated by executing the same study at a high and a low temperature (20ºC and 12ºC). Finally, I hypothesized that all incisions would heal more quickly at the high temperature (Anderson and Roberts 1975) and I expected incisions of fish held at the high temperature to have a higher rate of transmitter loss than incisions of fish held at the low temperature (Bunnell and Isely 1998).

CHAPTER TWO: METHODS

Study Site

Juvenile fall Chinook salmon (*Oncorhynchus tschawyscha*), originally obtained as eyed eggs from the McKenzie River Hatchery in Leaburg, Oregon, were raised at the Aquatic Research Laboratory at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. Fish used in the study (95-121 mm fork length [FL], mean 105 mm) were stocked into two indoor circular tanks (each tank was 1.2 m in diameter and 0.5 m deep, each holding 608 L of water), and the lights in the laboratory automatically simulated the natural photoperiod. Water temperature was maintained at 12 ± 1°C and 20 ± 1°C with flow-through well water, and fish were held at desired temperatures ±1ºC for 14 days prior to surgery. Fish were fed a daily maintenance ration of BioDiet Starter moist pellets (Bio-Oregon, Longview, Washington); food was withheld 24 hours prior to anesthetization for surgical implantation and weekly observations. The maintenance, handling, and testing procedures for the fish were approved by the PNNL Animal Care Committee, which meets the standards of the American Association for Accreditation of Laboratory Animal Care.

Surgical Procedure

The first trial was performed at 12°C in October, 2008 and a second trial was performed at 20°C in February, 2009. For each temperature trial, fish were selected randomly to create four groups of 156 fish each (three incision treatments and one control). An initial power analysis performed on a pilot study found that 104 fish per incision location were required to detect a 5% difference among incision locations (with 95% confidence and 80% power). An additional 52 fish were included to provide fish to collect for histology and to provide an even number of fish per surgeon. Because the final sample size was 156 fish per treatment, three surgeons were required to perform all of the surgeries in one day.

Fish were individually anesthetized (80 mg/L MS-222) until they reached stage 4 anesthesia (Summerfelt and Smith 1990), and then weighed (grams), and measured (millimeters FL) before being placed in a V-shaped neoprene trough. The trough was coated with a water conditioner (PolyAqua, Kordon LLC, Hayward, California) to protect the mucus layer, and a soft rubber tube was inserted in the fish's mouth to continuously perfuse the gills with a maintenance dose of anesthetic (40 mg/l MS-222). Control fish were anesthetized, weighed, and measured similar to treatment fish, but did not undergo surgery.

Prior to surgery, transmitters were disinfected by immersion in 70% ethanol for 10 minutes and surgical instruments were autoclaved. Instruments were disinfected by immersion in 70% ethanol for 8-10 minutes and rinsed in deionized water between each operation to minimize the spread of aquatic pathogens among fish. All surgeons wore sterile medical examination gloves. According to a predetermined random order, a 7-9 mm incision was made at one of three locations. The first was on the linea alba, the second was 3 mm from and parallel to the linea alba anterior to the pelvic girdle (muscle-cutting), and the third extended from the linea alba dorsally and posteriorly at approximately a 45° angle (between the myomeres; muscle-sparing). Both a Juvenile Salmonid Acoustic Telemetry System (JSATS) acoustic transmitter (12.0 × 5.3 × 3.7 mm, and 0.44 g in air, 0.30 g in water, 0.144 mL) and a passive integrated transponder (PIT) tag (12.5 × 2.1 mm, 0.10 g in air, 0.06 g in water, 0.036 mL) were implanted in the body cavity of the fish. The fish were double-tagged 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. Incisions were closed with two simple interrupted 5-0 Monocryl sutures (Ethicon, Rahway, New Jersey) approximately 2-3 mm apart.

Immediately following surgery, fish were placed ventral-side up on a different V-shaped neoprene trough coated with PolyAqua® (Novalek, Inc., Hayward, California) and an image of the incision was taken with a PixelLink™ PL-A66X firewire camera (0.5x magnification lens; Ottawa, Ontario, Canada) attached to a Zeiss microscope (0.65 magnification; Chester, Virginia). Fish were placed in a recovery bucket with bubbled air and upon full recovery 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, imaged, and graded once a week post-surgery for 98 days. Treatment and control fish were individually anesthetized (80 mg/l MS-222), weighed, measured, and placed on a V-shaped neoprene trough coated with PolyAqua®. During these weekly evaluations, a soft rubber tube was inserted in the fish's mouth to continuously perfuse the gills with a maintenance dose of anesthetic (40 mg/l MS-222) and the incision site was photographed with the same microscope camera previously described. One trained individual determined the presence or absence of the sutures, incision closure and apposition, and the presence of erythema on the incisions. Following examination, fish were placed in a recovery bucket with bubbled air and upon full recovery were returned to the holding tank.

Incision closure was defined as the point when the incision edges were touching and scale regeneration was nearly complete, similar to 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 "healed." Erythema in the incision was defined as any visible redness on the surface of the scales and integument. Other studies have referred to redness around the incision as inflammation (Hart and Summerfelt 1975; Lowartz et al. 1999; Walsh et al. 2000; Deters et al. in press), erythema (Hart and Summerfelt 1975), or exudate (Lowartz et al. 1999; Cunha da Silva et al. 2005). The criteria in Figure 2 was used to score apposition of the incision. Scores represented the proportion of the incision that was apposed, folding inward, overlapping, and/or gaping apart. Similar to Wynne et al. (2004) apposition scores (Figure 2) were ranked for analysis according to the following: no separation of layers (score of “1,” “2,” “1,2,” or “5”)

2. <50% superficial separation (score of “1,6,” “2,6,” “3,6,” or “4,6”)
3. >50% superficial separation (score of “1,7,” “2,7,” or “3,7”)
4. 100% separation of layers (score of “8”).

Incision lengths and openness were measured on digital images of the incisions using Image Pro® Plus Version 6.2 digital imaging software (Media Cybernetics Inc., Bethesda, Maryland). Incision lengths were measured only on day 0 by calibrating the measuring tool to a scale bar in the image and tracing the incision line from one end to the other. For incisions whose apposition scores indicated partial or full openness (Figure 2), the open area was measured by outlining the perimeter of the incision where scales ceased and muscle-tissue and viscera were visible in the incision. Any tissue around the incision that was damaged by the tearing of a suture was excluded from the open area that was measured.

Review of the digital images of fish held at 20°C revealed that 34 (65%) of surgeon A’s fish with muscle-sparing incisions were perpendicular to the linea alba rather than between the myomere lines. Therefore, these fish were excluded from analysis.

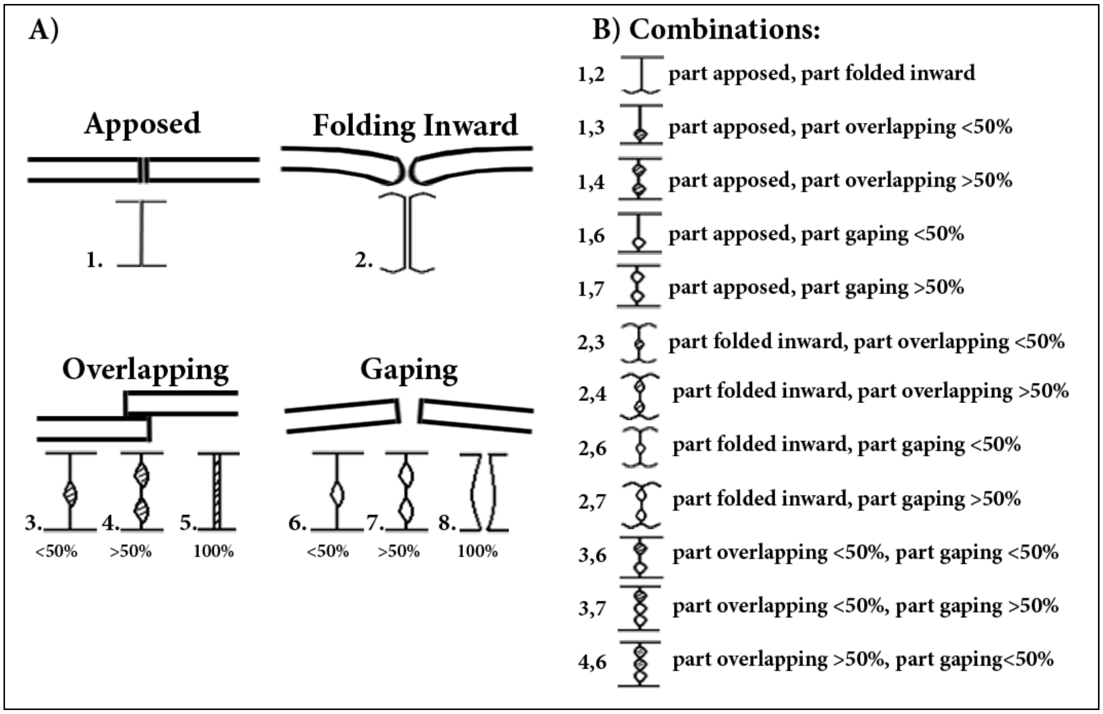

Figure 2. Criteria used to grade external appearance of tissue apposition of incision. A) Eight possible outcomes of apposition (apposed, folding inward, some portion overlapping, some portion gaping); B) combinations of the possible outcomes, i.e., “1,4” would mean more than 50% of the edges were overlapping and the rest was apposed.

Histology

Tissue samples were collected for histological examination from both temperature groups on days 0, 3, 7, 10, 14, 21, 42 and 98 following surgery. Three fish from each treatment group and one fish from each control group at each time point were examined. All fish were euthanized in 250 mg/L of MS-222 and fish tissue samples were prepared similar to the method in Elston et al. (1997). A rectangular-shaped area of tissue around the incision approximately 3 × 2 cm was excised. Tissue samples were immersion fixed with 10% buffered neutral formalin for 48 hours and stored in formalin. Tissues were processed by conventional paraffin embedding and stained with hematoxylin and eosin. Three transects were made across each incision. The first transect was made between the anterior end of the incision and the rostral suture, the second was directly across the middle of the incision, and the third was between the caudal suture and the posterior end of the incision. Due 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, none of these incisions were included in the analysis. Single-blind histological analysis was performed on the two remaining transects per incision using the criteria in Table 1, and ratings for the two sections were averaged for each fish.

Table 1. Histological criteria used to evaluate wound healing in juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through one of three incision locations (incision on the linea alba, muscle-cutting incision, or muscle-sparing incision).

Measure	Rating	Description
Tissue alignment	1	Normal-appearing, no misalignment
	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	Absent in wound area
	1	Normal presence 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 cells present
	2	Moderate amount of cells present
	3	Severe amount of cells present
Wound healing[c]	1	No evidence of wound present
	2	Considered nearly complete, with minimal degree of fibrosis and/or mildly less than normal muscle density
	3	Substantial fibrosis and/or inflammation; in case of dermis/epidermis, lack of alignment, fusion or abnormal skin regrowth indicating active but incomplete healing process
	4	Similar to a rating of 3, with lack of normal tissue replacement in wound area and/or abnormal healing pattern; includes incisions separated in two pieces where ingrowth of dermis/epidermis on one or both pieces shows failure of wound to close
Separation of tissue along wound axis	0	Tissue pieces were not separated
	1	Tissue pieces were separated
Ingrowth of dermis along wound axis	0	Dermis is not ingrown along wound axis
	1	Dermis is ingrown along wound axis
Myocytes less dense	0	Myocytes appear normal
	1	Myocytes less dense and smaller in diameter (typical of myocyte regrowth)
Thickened epidermis	0	Epidermis is normal thickness
	1	Epidermis is thickened (typical of regrowth over wound area)

[a] The serosal surface, musculature, and dermis/epidermis were graded separately for fibrosis, inflammation, and wound healing.

[b] Inflammatory cells included polymorphonuclear cells, mononuclear cells, lymphocytes, and neutrophils.

[c] Wound healing score was fish pathologist's expert opinion of healing by incorporating all criteria listed.

Statistical Analysis

Binary data (incision closure, incision erythema, and transmitter loss) were analyzed with a generalized linear model using a binomial error structure and a logit link function via analysis of deviance (ANODEV). Continuous variables (incision openness, mean suture presence, incision lengths, and ranks of incision apposition scores) were analyzed with ANOVA with repeated measures through day 28, because the majority of fish within the field study this laboratory study was funded to support have migrated to the ocean within 28 days (Smith et al. 2003). ANOVA with repeated measures was not used through day 98 (except with mean suture presence) because values approached or remained at zero from day 35 through 98, obscuring or eliminating the correlations between weeks that repeated measures determines. Therefore, ANOVA without repeated measures was used on days of biological significance and significance to project goals. Ranks of apposition scores were considered continuous variables because the ranks were based on an underlying continuous scale (Snedecor and Cochran 1989). Pairwise comparisons were made among incision locations and surgeons with a Bonferonni correction. An alpha of 0.05 was used for all tests.

The main effects of incision location and surgeon on all variables were evaluated. Results of the three surgeons remained pooled regardless of interactions, making the results applicable to most surgeons with 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 size differences between the two groups and the time lapse between research on the two groups (4 months apart). However, general comparisons between groups of fish held at 12ºC and 20ºC were still made.

The instantaneous growth rate (IGR) of treated fish were calculated with the formula from Isely and Grabowski (2007, p. 204, Equation 5.7)

$$G = \frac{\log_e L_2 - \log_e L_1}{t_2 - t_1}$$

where G is instantaneous growth rate, L_1 and L_2 are the lengths at t_1 (initial time) and t_2 (final time). Results were analyzed with ANOVA. Control fish were included in the study only to determine if mortality (if any) was caused by something other than the surgery (i.e. waterborne pathogens) and these fish were not individually marked. Therefore, growth rates were not calculated for control fish.

CHAPTER THREE: RESULTS

Transmitter Loss

Transmitter loss was influenced by water temperature and the time from implantation. Among fish held at 12°C, fish with incisions on the linea alba generally had the highest transmitter loss rate among incision types over the entire course of the study. However, much of the transmitter loss occurred later in the experiment. Transmitter loss was not significantly different ($P > 0.05$) among incision types through day 28, when transmitter loss began steadily increasing for incisions on the linea alba (Figure 3). By day 98, fish with incisions on the linea alba had significantly ($P = 0.003$) greater transmitter loss than fish with muscle-cutting and muscle-sparing incisions.

Among fish held at 20°C, transmitter loss occurred sooner and over a smaller time period (Figure 3). The majority of transmitter loss occurred between 18 and 24 days after implantation. During this period, 7 transmitters were lost on 7 consecutive days (1 each day) through incisions on the linea alba. However, there was not a significant difference ($P = 0.137$) in transmitter loss among incision types during the 98 day holding period.

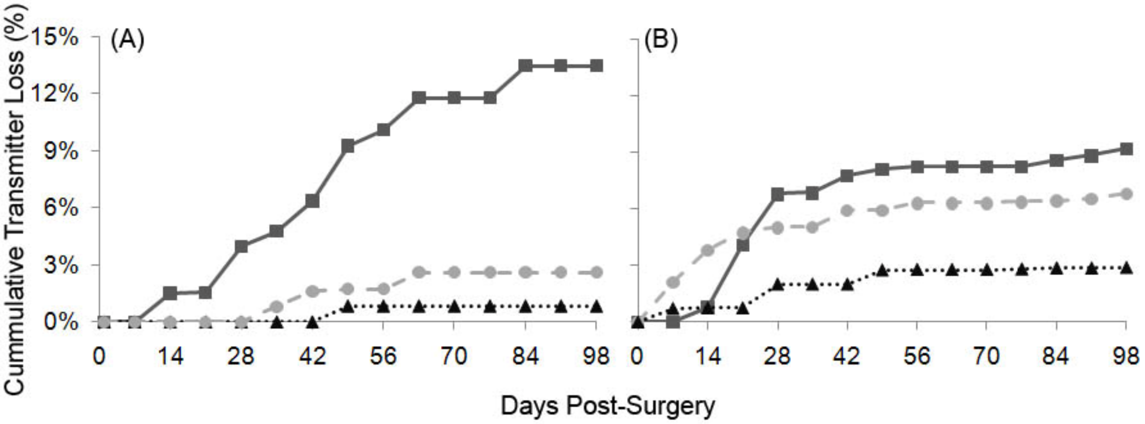

Figure 3. Cumulative transmitter loss among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through an incision on the linea alba (-■-), muscle-cutting (-●-), or muscle-sparing (·▲·) incision and held at 12°C (A) or 20°C (B).

Healing

The open area was significantly different among incision types ($P < 0.001$) during the first 21 days at both temperatures (Figure 4). When fish were held at 12°C, incisions on the linea alba were significantly ($P < 0.001$) less open than muscle-cutting and muscle-sparing incisions on day 7, 14, and 21 and significantly ($P = 0.031$) less open than muscle-cutting incisions on day 28. When fish were held at 20°C, incisions on the linea alba were significantly ($P < 0.002$) less open than muscle-cutting and muscle-sparing incisions on day 7 and 14, and less open than muscle-sparing incisions on day 21 ($P = 0.013$). The open area of muscle-cutting and muscle-sparing incisions peaked on day 14 at 12°C and on day 7 at 20°C. However, the open area of incisions on the linea alba peaked later on day 56 at 12°C and on day 21 at 20°C.

Incision apposition ranks differed significantly ($P < 0.001$) among incision types during the first 28 days at both temperatures (Figure 4). Apposition ranks for incisions on the linea alba were significantly ($P < 0.001$) lower (indicating better incision apposition) than muscle-cutting and muscle-sparing incisions on day 7 and 14 at both temperatures and on day 21 ($P < 0.001$) for fish held at 12°C. However, at 12°C, incisions on the linea alba had significantly ($P < 0.05$) higher apposition ranks than muscle-cutting and muscle-sparing incisions from day 42-98. When fish were held at 20°C, apposition ranks did not differ ($P = 1.000$) among incision types from day 21 through the end of the study.

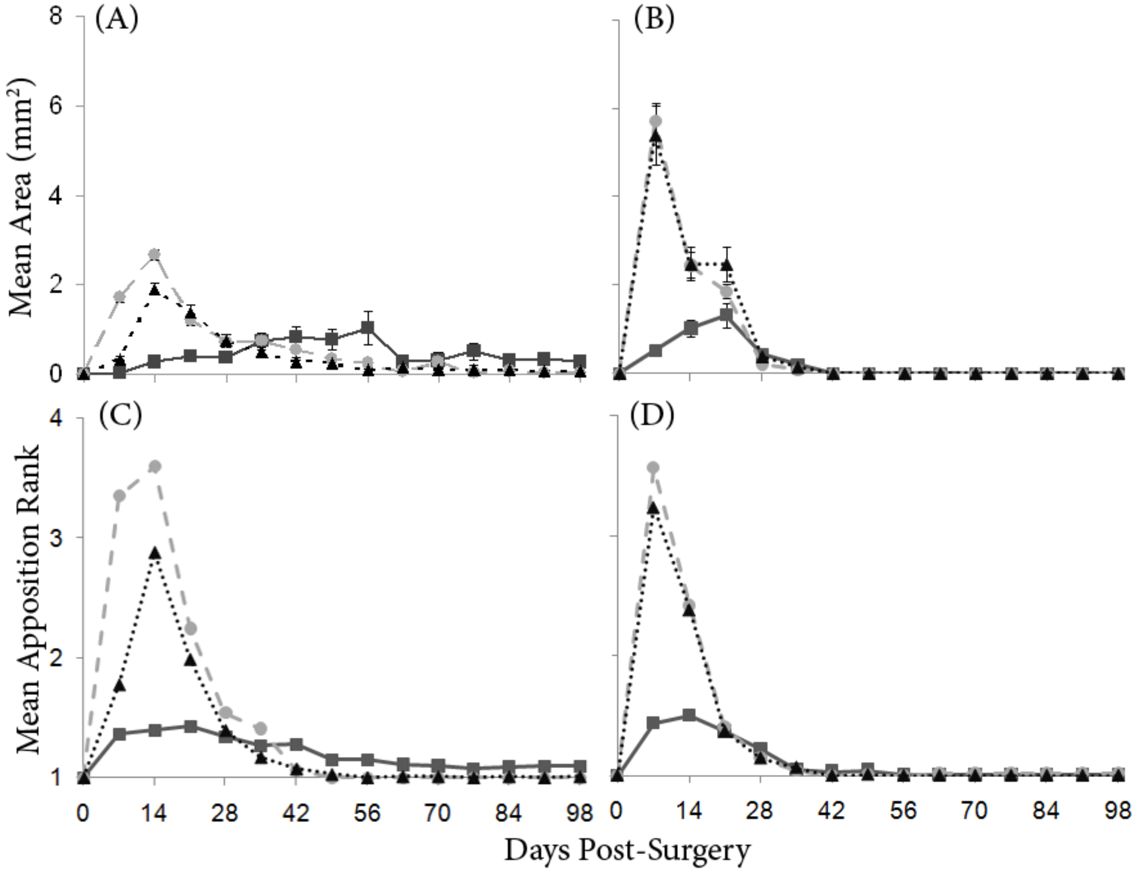

Figure 4. Open area of incisions in mm^2 (A,B) and mean ranks of apposition scores (C,D) among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through an incision on the linea alba (-■-), muscle-cutting (-●-), or muscle-sparing (·▲·) incision and held at 12°C (A,C) or 20°C (B,D). A lower apposition rank indicates better apposition. Error bars represent standard error.

Trends of erythema on incisions differed among incision types based on water temperature and time from implantation. At 12°C, significantly (*P* < 0.05) more muscle-cutting incisions than muscle-sparing incisions had erythema on day 14 and 28, and significantly (*P* < 0.001) more muscle-sparing incisions had erythema than incisions on the linea alba on day 7 and 14 (Figure 5). However, among fish held at 12°C, significantly (*P* < 0.05) more incisions on the linea alba had erythema than muscle-sparing incisions on day 42 and 49 and muscle-cutting incisions on day 49. Among fish held at 20°C, significantly (*P* < 0.05) more incisions on the linea alba had erythema than muscle-sparing incisions on day 14 and muscle-cutting incisions on day 14 and 21. Significantly (*P* = 0.003) more muscle-cutting incisions than muscle-sparing incisions had erythema on day 7 at 20°C.

Differences in incision closure (point at which incisions appeared healed) among incision types were influenced by water temperature and time from implantation (Figure 5). There was no significant difference (*P* = 1.000) in incision closure among incision types from day 7-21 at 12°C. By day 28, significantly (*P* < 0.01) more incisions on the linea alba were closed than muscle-cutting or muscle-sparing incisions on fish held at 12°C. Over time, this trend reversed and from day 70-91, significantly (*P* < 0.05) more muscle-sparing and muscle-cutting incisions were closed than incisions on the linea alba. By day 98, 12% of incisions on the linea alba were still not 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 significantly (*P* < 0.001) more incisions on the linea alba were closed than muscle-cutting and muscle-sparing incisions. Among fish held at 20°C, at least 75% of all three incision types were closed by day 28 and 99% were closed by day 63.

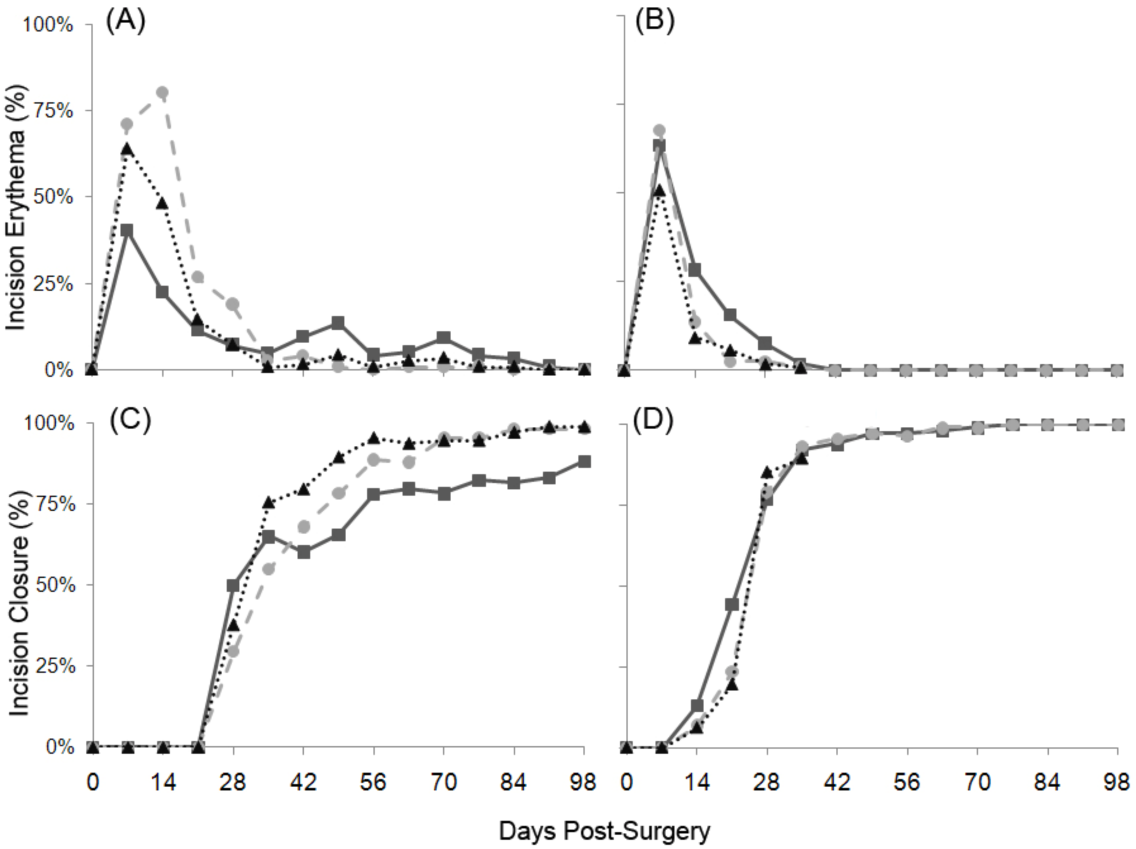

Figure 5. Percent of incisions with erythema (A,B) and incision closure (C,D) among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through an incision on the linea alba (-■-), muscle-cutting (-●-), or muscle-sparing (·▲·) incision and held at 12°C (A,C) or 20°C (B,D).

Incision Lengths

Incision lengths differed with water temperature and incision type. Mean lengths among incision types ranged from 7.0-7.9 ± 0.06 mm at 12°C and 7.5-8.0 ± 0.07 mm at 20°C (Figure 6). Mean lengths of incisions on the linea alba were significantly ($P < 0.001$) longer (7% on average) than muscle-cutting and muscle-sparing incisions at both temperatures. Muscle-sparing incisions were significantly ($P < 0.001$) shorter (by 7%) than muscle-cutting incisions at 12°C, but not significantly different ($P = 1.00$) at 20°C.

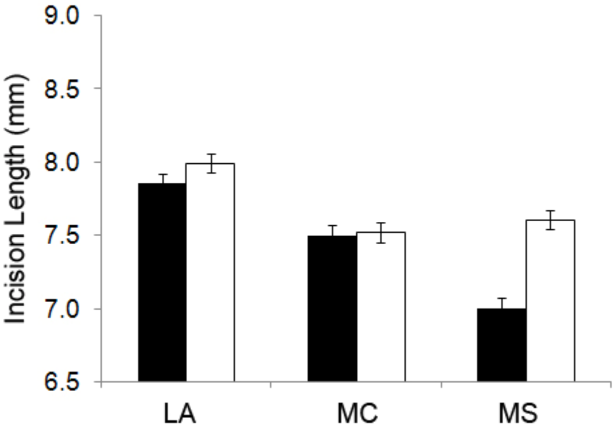

Figure 6. Mean incision lengths (±SE) among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through an incision on the linea alba (LA), muscle-cutting (MC), or muscle-sparing (MS) incision held at 12°C (black bars ■) or 20°C (white bars □). Error bars represent standard error.

Suture Presence

Fish with muscle-sparing incisions lost their sutures significantly ($P < 0.05$) more quickly than those with muscle-cutting incisions or incisions on the linea alba at both temperatures. There was no significant difference ($P > 0.05$) in mean suture retention between incisions on the linea alba and muscle-cutting incisions at 12°C and only on day 7 ($P < 0.001$) and 14 ($P = 0.014$) at 20°C, when incisions on the linea alba had higher suture retention than muscle-cutting incisions (Figure 7). At 12°C, incisions on the linea alba had significantly ($P < 0.05$) greater mean suture retention than muscle-sparing incisions on day 21, 28, and 49-98. Among fish held at 12°C, mean suture retention for muscle-cutting incisions was significantly ($P < 0.05$) greater than muscle-sparing incisions on day 28, 84, and 98. Among fish held at 20°C, suture retention differed between incisions on the linea alba and muscle-sparing incisions on day 14-84 with incisions on the linea alba having significantly ($P < 0.05$) greater mean suture retention. At

20°C, muscle-cutting incisions had significantly ($P < 0.05$) greater mean suture retention than muscle-sparing incisions on day 21-77.

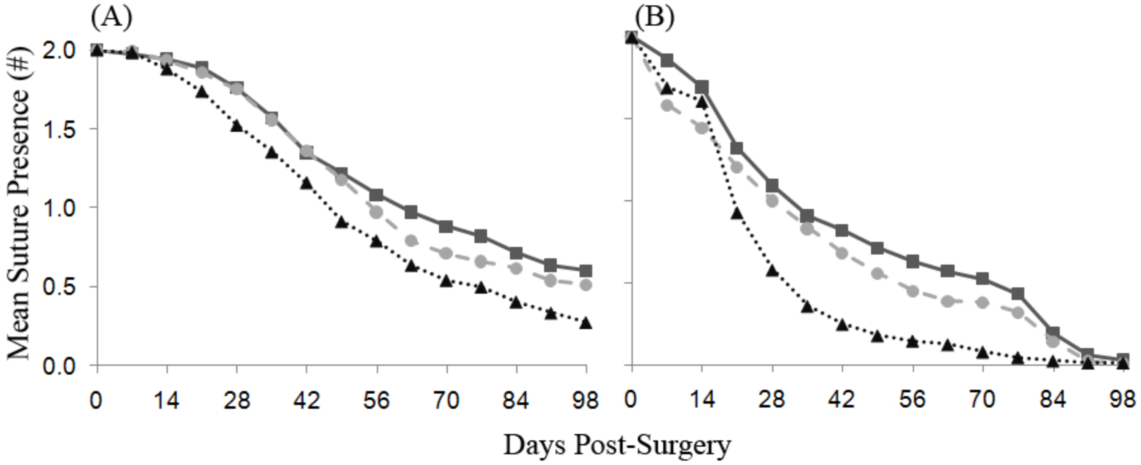

Figure 7. Mean suture presence among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through an incision on the linea alba (-■-), muscle-cutting (-●-), or muscle-sparing (·▲·) incision and held at 12°C (A) or 20°C (B).

Mortality and Growth

Mortality of surgically implanted fish was generally low (Table 2). There were no mortalities among fish held at 12°C. Although there was 11-20% mortality among fish held at 20°C, there was no significant difference ($P = 0.181$) among groups with different incision locations.

During most of the study period (all but day 91 for fish held at 12°C), there was no significant difference ($P > 0.05$) in growth among incision location groups held at both temperatures. On day 91, fish with muscle-cutting incisions at 12°C had significantly ($P = 0.02$) higher growth than fish with incisions on the linea alba. However on this day, growth among fish with muscle-cutting incisions and incisions on the linea alba were not significantly different ($P = 0.69$) from fish with muscle-sparing incisions.

Table 2. Instantaneous growth rate (IGR), cumulative mortality, and transmitter loss over 98 days among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag through an incision on the linea alba, muscle-cutting, or muscle-sparing incision.

Temp. (C)	Treatment	N	Growth (IGR)	Mortality (Cumulative %)	Transmitter loss (Cumulative %)
12	Linea alba	156	0.09	0 (0%)	16 (13%)
	Muscle-cutting	158	0.10	0 (0%)	1 (1%)
	Muscle-sparing	155	0.09	0 (0%)	3 (3%)
	Control	155	0.10	0 (0%)	--
20	Linea alba	156	0.20	20 (20%)	9 (9%)
	Muscle-cutting	156	0.19	17 (16%)	7 (7%)
	Muscle-sparing	121	0.20	11 (13%)	2 (3%)
	Control	157	0.21	12 (11%)	--

Surgeon Effects

The three surgeons participating in this study had previously performed hundreds of surgeries with similar survival results and received feedback on their surgery performance. However, subtle differences in suturing techniques were evidenced by interactions between surgeons and incision types on select days across all variables. There was no clear pattern to explain the interactions among surgeons and incision locations. Therefore, results for all three surgeons were combined and are considered applicable to surgeons with similar techniques. Surgeon effects may still be of interest and are included in Appendix A.

Histology

Healing of incisions followed a similar progression among fish held at both temperatures, with the various responses occurring more quickly at 20°C. The inflammatory response in the epidermis, dermis, and musculature generally peaked on day 21 among fish held at 12°C compared to day 14 among fish held at 20°C. The amount of fibrotic tissue within the incision peaked on day 21 among fish held at 12°C compared to day 10 among fish held at 20°C.

Because no difference in the progression of healing occurred among fish held at either temperature, the following results are presented only for fish held at 12°C.

Healing progressed in a similar manner among the three incision types during the first 14 days among fish held at 12°C (Figure 8). 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 lacked fusion of the dermis, epidermis, and musculature from day 3-14, or were so weakly fused that the layers broke apart when transects across the incisions were cut prior to embedding and staining (Figure 8). Neovascularization within the incision was present as early as day 3 in all incision types and increased steadily during the first 14 days. Moderate amounts of inflammatory cells were present in the serosal surface and musculature by day 3, and were noted in the dermis and epidermis on day 10. Muscle-cutting and muscle-sparing incisions had greater numbers of inflammatory cells in the serosal surface and musculature than incisions on the linea alba. The dermis and the epidermis of both edges of 92% of incisions on the linea alba were folded inward. In contrast, 54% of muscle-cutting and 42% of muscle-sparing incisions had at least one or both edges of the incision folded inward.

By day 21, the most notable difference among incision types of fish held at 12°C was the amount of fibrotic tissue present in the incision (Figure 9). Muscle-cutting and muscle-sparing incisions had much greater amounts of fibrotic tissue within the incision compared to incisions on the linea alba. The dermis remained ingrown on both edges of the majority (83%) of incisions on the linea alba, compared to ingrowth of one or both sides of muscle-cutting (50%) and muscle-sparing (17%) incisions. Inflammatory cells were observed at their highest levels in all three incision types on day 21, with the amounts being similar. Tissue layers on either side of the incision were misaligned in the majority (77% on average) of all incision types. Healing was still in progress by day 49 for all incision types, and the amount of fibrotic tissue and inflammatory cells decreased. However, muscle-cutting and muscle-sparing incisions still had greater amounts of fibrotic tissue present in the incision compared to incisions on the linea alba.

Most of the incisions of fish held at 12°C were fully healed by day 98 (Figure 10). Epidermal and dermal layers were fused and muscle tissue had regenerated where the incision had been made. Small pieces of fibrotic tissue (fibrotic tags) on the serosal surface were present in varying quantities on all three incision types.

Some anomalous situations existed where some incisions of fish held at 12°C were not healed by day 98. An incision on the linea alba (Figure 11) failed to close, with no regrowth of muscle and large amounts of fibrous granulation tissue between the incision edges. The epidermis was hyperplasic (excessively proliferating) and appeared chaotic. An image of the outside of the incision on day 98 revealed a large mat of *Saprolegnia sp.* covering the incision. One muscle-sparing incision (Figure 12) had severe misalignment of incision edges, with extensive fibrosis throughout the incision. The epidermis was hyperplasic (excessively proliferating) and the dermis was protruding into the incision area. An image of the outside of the incision on day 0 showed the edges of the incision were overlapping (Figure 12a). The edges of the incision were together by day 49 but indented (Figure 12b). Although the surface of the incision appeared to be cosmetically resolved by day 98, histology revealed the underlying muscle and tissue layers were not fully healed (Figure 12c).

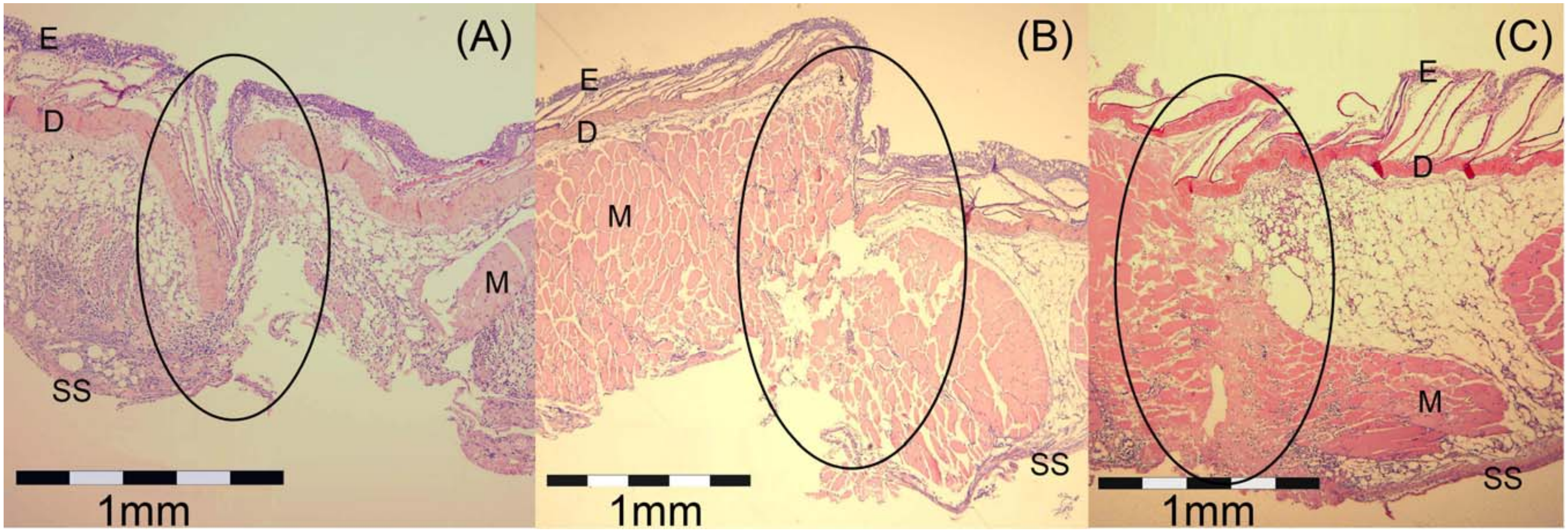

Figure 8. Transverse section of an incision on the linea alba (A), muscle-cutting incision (B), and muscle-sparing incision (C) on day 14. Incisions (within black ovals) are healing in a similar manner. The epidermis (E) and dermis (D) are not aligned and the dermis lacks fusion. Number of inflammatory cells was greater in the serosal surface (SS) and musculature (M) of muscle-cutting and muscle-sparing incisions than incisions on the linea alba.

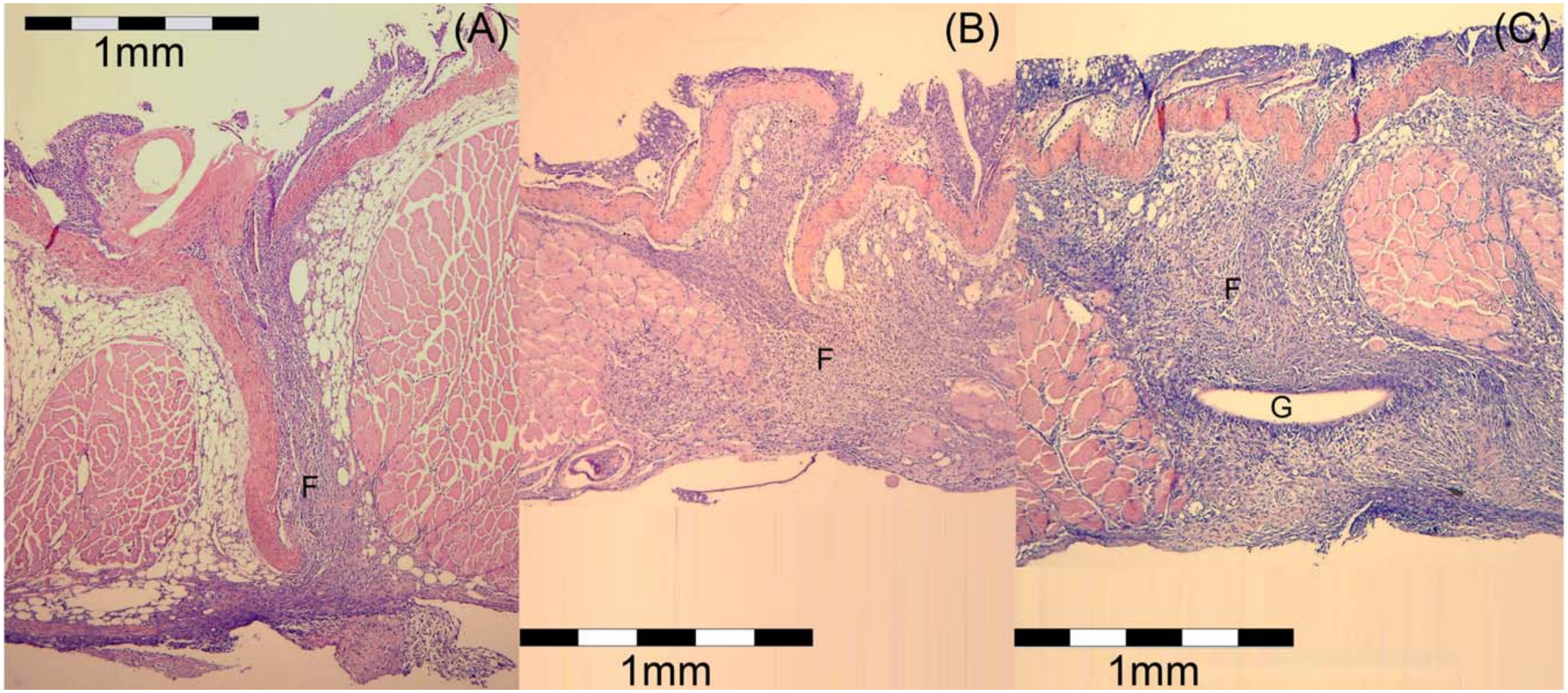

Figure 9. Transverse section of an incision on the linea alba (A), muscle-cutting incision (B), and muscle-sparing incision (C) on day 21. Fibrous granulation tissue (F) is present in greater quantities in the muscle-cutting and muscle-sparing incisions than in the incision on the linea alba. The gap (G) in the fibrous granulation tissue of the muscle-sparing incision is an artifact from processing.

Figure 10. Transverse section of the three incision locations on day 98. The incision in the adipose tissue (Ad) of the linea alba (A) is completely healed as are the incisions in the musculature (M) of the muscle-cutting (B) and muscle-sparing incision (C). The epidermis (E) and dermis (D) are continuous and fused and the fibrous granulation tissue has been replaced with normal tissue. Fibrotic tags (Ft) were present on the serosal surface of all three incision locations.

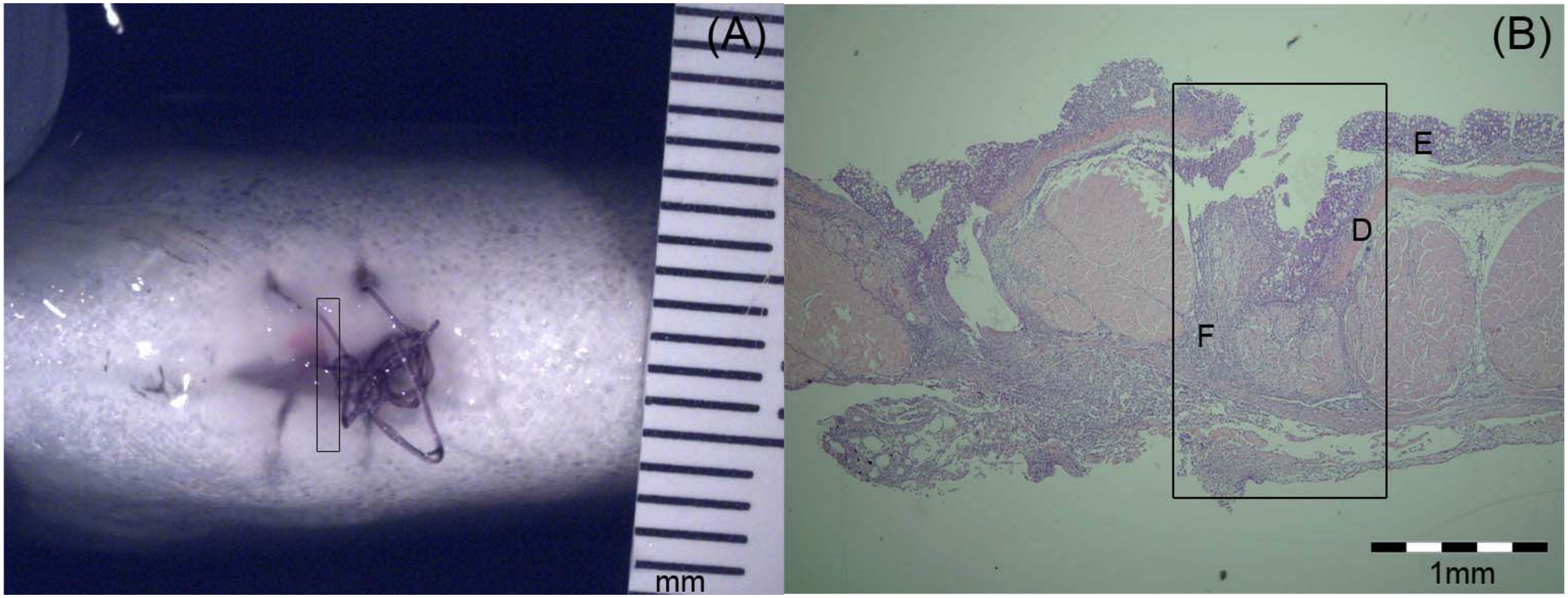

Figure 11. Anomalous healing of an incision on the linea alba on day 98. Externally (A) the incision is covered with *Saprolegnia sp.* The rectangle in image (A) shows the cross-section of the incision that was processed for histology. The transverse view of the cross-section (B) shows a thickened epidermis (E), discontinuous dermis (D), and large amounts of fibrotic tissue (F) between the edges of the incision.

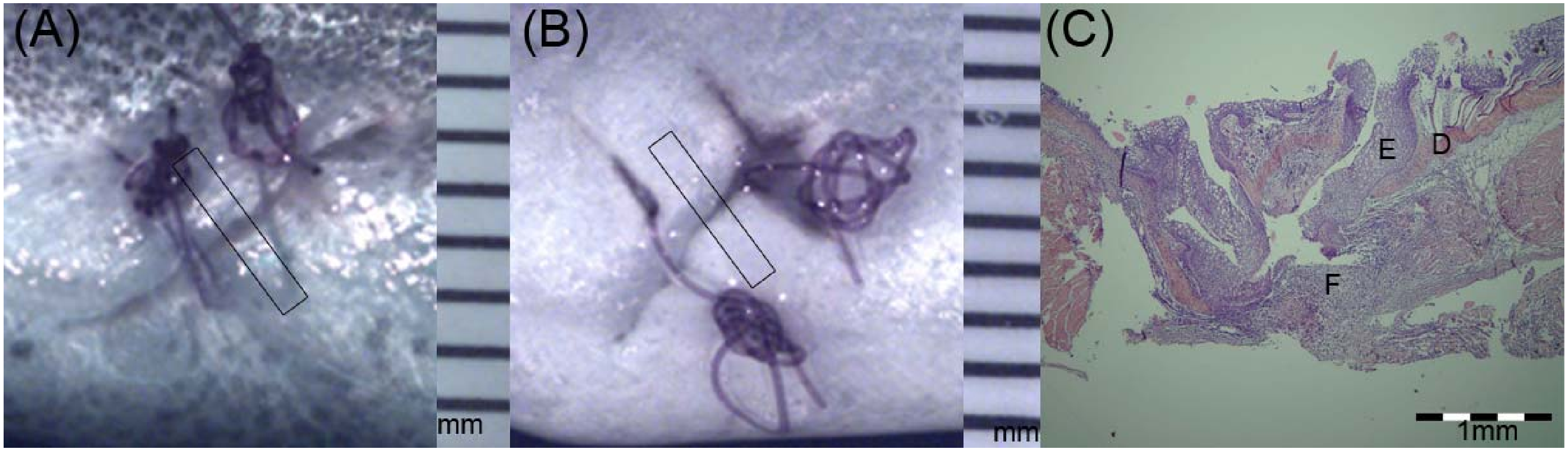

Figure 12. Anomalous healing of muscle-sparing incision. Externally on day 0 (A), the incision edges were overlapping. By day 49 (B), the incision edges were together, but remained indented. The rectangle in images (A) and (B) shows the cross-section of the incision that was processed for histology. The transverse view of the cross-section (C) on day 98 shows a thickened epidermis (E), discontinuous dermis (D), and large amounts of fibrotic tissue (F) between the edges of the incision.

CHAPTER FOUR: DISCUSSION

Transmitter Loss

Transmitter loss was not significantly greater for incisions on the linea alba during the first 28 days of both temperature treatments. A separate study on various suture knot patterns was conducted on similarly sized juvenile Chinook salmon implanted with the same JSATS transmitter and a PIT tag through an incision on the linea alba in 2009 (K.A. Deters, PNNL, personal communication, 2010). Throughout that 28-day study, only 1 of 225 acoustic transmitters was lost from fish held at 17ºC; in addition, only 2 PIT tags and no acoustic transmitters were lost from 262 fish held at 12ºC. In my study, the transmitter loss of incisions on the linea alba during the first 28 days was very small (4%) and was not significantly higher than loss from muscle-cutting and muscle-sparing incisions.

Transmitter loss was highest among fish with incisions on the linea alba by day 98 among fish held at 12ºC, and may have been related to how the incisions were healing. Histological results showed that as incisions on the linea alba were healing, 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 likely overcame the weak bond between the incision edges, causing the incision to dehisce and the transmitter to be lost through the incision (i.e. gravity; Marty and Summerfelt 1986; Sakaras et al. 2005). All incisions healed faster at 20ºC, and it is possible that the strength of the incision increased quickly enough to support the transmitter, thus preventing more transmitters from being lost. Therefore, the results of this study suggest that the odds of losing a transmitter through an incision on the linea alba may decrease as temperature increases and the rate of healing increases.

Healing

It is clear that incisions on the linea alba had better initial apposition and less openness than muscle-cutting and muscle-sparing incisions during the first 14 days at 12º or 20ºC. This difference was likely related to natural resting tension in the myomeres. When human muscle is cut, it retracts similar to a cut rubber band (Dederich 1963) because of its viscoelastic properties (Heidemann et al. 1999; Long et al. 2002). I observed that when the lateral muscles of an anaesthetized fish were cut with the muscle-cutting incision, the resting tension caused the edges of the incision to retract and gape open. Retraction made the tissue edges extremely difficult to appose and muscle-cutting incisions consequently had the greatest amount of open area among incision types. Additionally, when the fish recovered from anesthesia and began swimming, it is probable that the active contraction of the muscles continued to pull apart the edges of the muscle-cutting incision, further contributing to the greater openness observed in muscle-cutting incisions. The bending of the fish's body during swimming may have caused muscle-sparing incisions to exhibit greater openness relative to incisions on the linea alba. Openness and apposition in muscle-cutting and muscle-sparing incisions may improve with better surgical techniques, but the natural tension in muscle tissue and the fish's swimming movements likely will limit these improvements.

The way in which erythema was measured in this study may have made it a poor metric to assess healing. Early in the study (day 0 – 14), incisions on the linea alba had less erythema than the other two incision types at 12ºC, but the opposite was true at 20ºC. 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, many muscle-cutting and some muscle-sparing incisions at 20ºC expelled sutures that tore scales and integument away from the incision edges, making it difficult to observe erythema. Furthermore, the importance of erythema was uncertain because the mechanism producing the erythema was not identifiable by gross examination. Our histology results indicated that substantial inflammation of the tissues could occur without any visible erythema. Erythema could also be present without any inflammation in the underlying tissues. Without knowing the mechanism of how erythema was produced, and without tracking changes in erythema over time for individual fish, it did not appear to be a useful predictor in the final outcome of the healing of the incision. 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).

The results of this study showed that incisions on the linea alba had less evidence of healing by secondary intention, and much less open area, both of which could prove beneficial to the health of the fish. Less openness of incisions on the linea alba may benefit the fish by requiring less energy to heal and decreasing the chance for pathogens to enter. When incisions are not apposed to heal by primary intention (edges of the incision are first aligned and secured together with sutures), they take longer to heal by a process called secondary intention (Barbul 2005; McCallum 2007). During the process of secondary intention, a gap exists between the incision edges and 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. Both muscle-sparing and muscle-cutting incisions appeared to heal by 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 linea alba. Therefore, muscle-sparing and muscle-cutting incisions likely required more energy to heal (Barbul 2005). Additionally, the openness in muscle-cutting and muscle-sparing incisions will likely lead to a larger pathway for pathogens to enter (Svendsen and Bogwald 1997; Noga 2000) and may interfere with efficient osmoregulation (Lunder 1992).

Suture Presence

Sutures were lost faster from muscle-sparing incisions, most likely due to the angle of the sutures on the body. As the fish swam, the force of the water on the sutures of muscle-sparing incisions may have pushed the sutures towards the incision, enabling them to be expelled more easily. In contrast, on muscle-cutting incisions and incisions on the linea alba, the force of the water pushed the sutures parallel to the incision, allowing the suture to remain in the body wall longer.

Suture loss from all incisions was higher in the warmer (20°C) group than in the colder (12°C) group. Similarly, Walsh et al. (2000) found more than 50% of the absorbable monofilament sutures implanted in hybrid striped bass were expelled 60 days post-surgery at warm temperatures (mean 25.5°C), but at cold temperatures (mean 15°C) less than 25% were expelled, even at 120 days post-surgery. Deters et al. (2009) also found suture loss to be higher (36%) in juvenile Chinook salmon held at 17°C compared to suture loss (18%) in fish held at 12°C after 14 days.

If suture material persists in fish tissue, the risk of infection or irritation from the suture rubbing back and forth against the tissue may increase over time. Many studies have sought to determine the best suture material for fish surgeries and commonly report that monofilament sutures elicit less of a tissue reaction than other materials such as silk, catgut, or nylon (Kaseloo et al. 1992; Gilliland 1994; Wagner et al. 2000; Hurty et al. 2002; Mulcahy 2003; Harms 2005; Deters et al. 2009). However, the minimal tissue reaction may be why monofilament sutures persist for so long, and the constant rubbing of suture material against the fish's tissue may elicit an adverse reaction. Caputo et al. (2009) reported absorbable monofilament sutures were retained in largemouth bass from 362 to 733 days post-surgery and there were redness and signs of infection around the sutures. Roberts et al. (1973) reported that external tags, which were attached to the dorsal musculature of ocean-going Atlantic salmon with monofilament nylon suture remained attached for over two years. Histological analysis on the dorsal musculature showed much greater necrosis and bacterial presence around suture material connected to a trailing tag that could move than an embedded tag that could not move (Roberts et al. 1973). Irritation around monofilament suture decreases or disappears once the suture is lost (Walsh et al. 2000) or cannot move (Roberts et al. 1973). Therefore, although impractical in most field studies, it would seem ideal to use monofilament sutures to close the incision, removing the sutures once the incision heals and thus avoid any negative effects (i.e. infection or decreases in growth) that may come from the sutures rubbing against fish tissue.

Mortality and Growth

Mortality and growth were not affected by incision type in the controlled laboratory setting. However, mortality or adverse effects from incision openness may increase in a field setting where bacterial loads and environmental factors vary.

Conclusions and Recommendations

This study found the incision on the linea alba to be a better choice over muscle-cutting and muscle-sparing incisions. Transmitter retention was similar among the three incision locations during the first 28 days when fish were held at 12ºC or 20ºC. Incisions on the linea alba were much less open than the muscle-cutting and muscle-sparing incisions, suggesting a decreased risk of invading pathogens. Apposition of incisions during the first 14 days at both temperatures was better for incisions on the linea alba than muscle-cutting and muscle-sparing incisions, because the muscle retracted when cut and was more difficult to appose. The results from histology confirmed that incisions on the linea alba were healing with much less fibrotic tissue than were the muscle-cutting or muscle-sparing incisions, suggesting more incisions were healing by primary rather than secondary intention. Incision closure (external healing) was better for incisions on the linea alba than muscle-cutting and muscle-sparing incisions on 28 days at 12ºC and 21 days at 20ºC. Suture presence was higher for incisions on the linea alba than muscle-sparing incisions by day 28 at both temperatures. Therefore, I concluded that incisions on the linea alba are a better choice over muscle-cutting or muscle-sparing incisions for studies up to 28 days in length.

Several areas of study were identified for future research. One would be to examine possible links between suture presence and decreased growth over the long-term. A second study could quantify how various suture bites and varying amounts of tension different surgeons place on the suture knots influences healing rates and transmitter expulsion.

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APPENDIX A

RESULTS OF SURGEON EFFECTS

Transmitter Loss

Transmitter loss was not influenced by surgeon at either temperature. There was no significant difference ($P > 0.05$) in transmitter loss among fish implanted by surgeons A, B, and C and no significant interaction ($P > 0.05$) among surgeon and incision type at both temperatures (Figure 13).

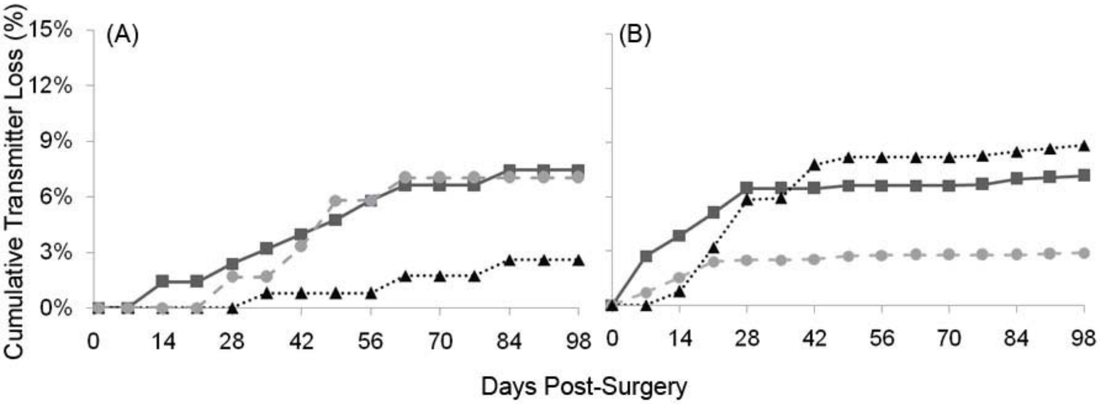

Figure 13. Cumulative transmitter loss among juvenile Chinook salmon held at 12°C (A) or 20°C (B) and implanted with an acoustic transmitter and PIT tag by surgeon A (-■-), B (-●-), or C (·▲·).

Healing

The open area of incisions varied over time at both temperatures among fish implanted by surgeons A, B, and C. There was a significant ($P < 0.05$) interaction among surgeon and incision type on day 14-28 at 12°C and on day 7-21 at 20°C. Incisions made by surgeon A had significantly ($P < 0.05$) greater open area than surgeons B and C, from day 14-35 at 12°C and on day 7 and 14 at 20°C (Figure 14). However, at 20°C, incisions made by surgeon C had significantly ($P < 0.05$) greater open area than surgeons A and B on day 28 and 35.

Incision apposition ranks differed among fish implanted by different surgeons over time and with temperature (Figure 14). There was a significant ($P < 0.05$) interaction among surgeon and incision type on day 7-28, 42, and 49 at 12°C and on day 7 and 14 at 20°C. When fish were held at 12°C, apposition ranks for fish implanted by surgeon A were significantly ($P < 0.001$) higher than fish implanted by surgeon B and C on day 21 and 28 and higher than fish implanted by surgeon C on day 35. At 20°C, apposition ranks of fish implanted by surgeon A were significantly ($P < 0.05$) higher than fish implanted by surgeon B and C on day 7. However, apposition ranks of fish implanted by surgeon C were significantly ($P < 0.05$) higher than fish implanted by surgeon A from day 21-35 and fish implanted by surgeon B on day 28 and 35.

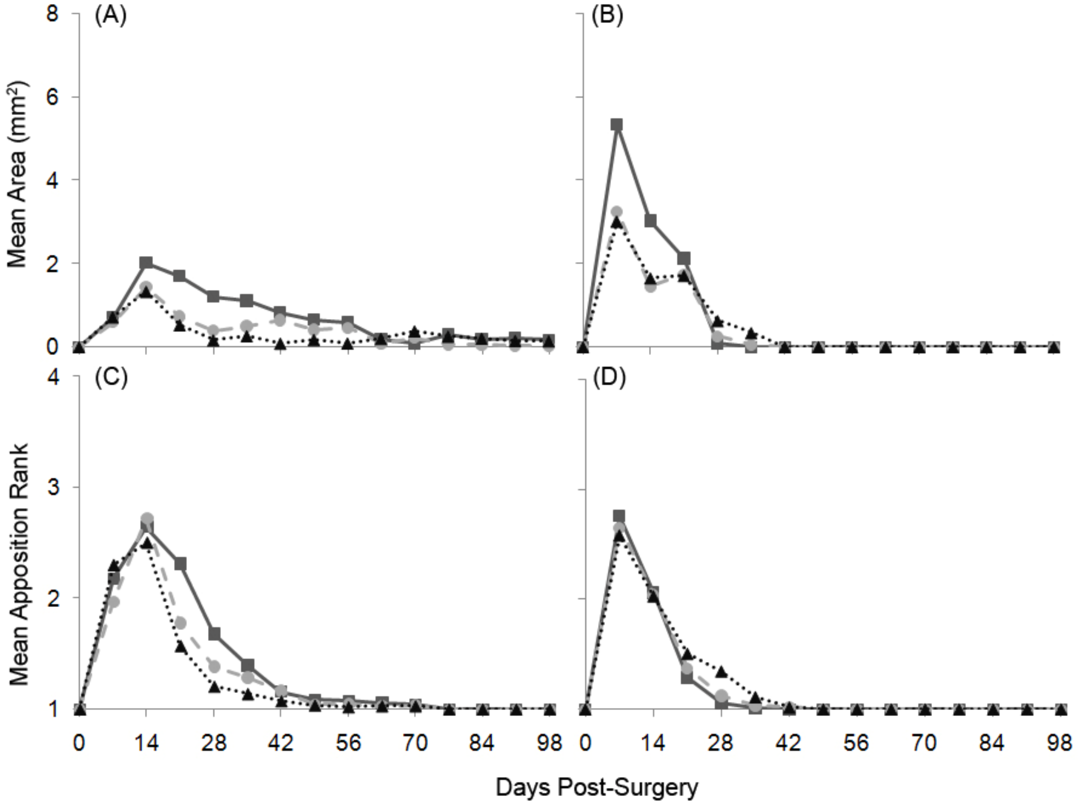

Figure 14. Mean open area (A,B) and mean apposition ranks (C,D) among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag implanted by surgeon A (-■-), B (-●-), or C (·▲·) held at 12°C (A,C) or 20°C (B,D).

Instances of erythema differed by surgeon and the time from implantation. Significant ($P > 0.05$) interactions occurred among surgeon and incision type on day 35-49 and day 63-84 at 12°C and on day 21 and 28 at 20°C. When fish were held at 12°C, significantly ($P < 0.004$) more incisions made by surgeon A had erythema than incisions made by surgeons B and C on day 14-28 (Figure 15). On day 49, significantly more incisions made by surgeon C had erythema than incisions made by A and B, among fish held at 12°C.

Incision closure differed by surgeon and time from implantation (Figure 15). There was a significant ($P > 0.05$) interaction among surgeon and incision type on day 49-98 at 12°C, and on day 7, 14, 49, and 63 at 20°C. For fish held in 12°C water, significantly ($P < 0.001$) more incisions made by surgeon B and C were closed than incisions made by surgeon A on day 28. On day 35, significantly ($P < 0.01$) more incisions made by surgeon C were closed than incisions made by surgeon A and B. Among fish held at 20°C, significantly ($P < 0.01$) more incisions made by surgeon B were closed than incisions made by surgeon A and C on day 14. By day 28, significantly ($P < 0.003$) more incisions made by surgeon A and B were closed than incisions made by surgeon C, and on day 35, significantly ($P < 0.05$) more incisions made by surgeon A were closed than incisions made by surgeon C. Incision closure was not significantly different ($P > 0.05$) among those made by surgeons A, B, or C from day 42-98.

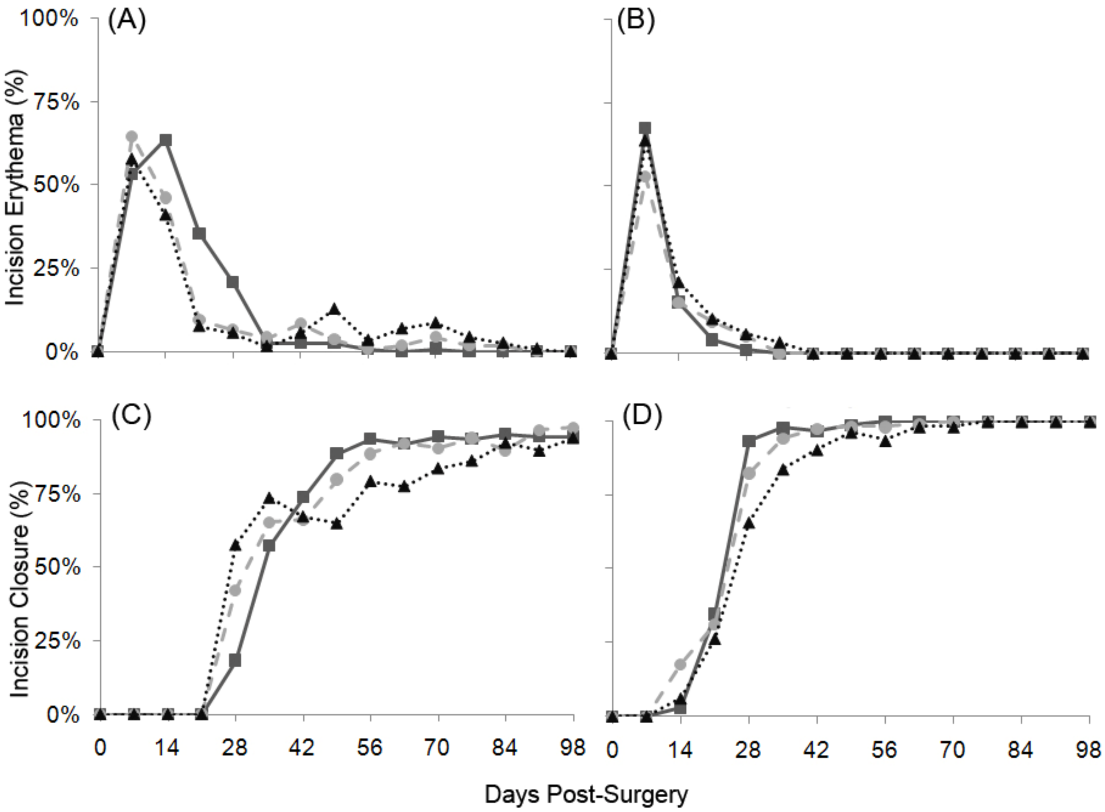

Figure 15. Percent of incisions with erythema (A,B) and closed incisions (C,D) among juvenile Chinook salmon implanted with an acoustic transmitter and PIT tag implanted by surgeon A (-■-), B (-●-), or C (·▲·) held at 12°C (A,C) or 20°C (B,D).

At the time of surgery, incision lengths varied by surgeon. There was a significant interaction (*P* < 0.05) among surgeon and incision type at both temperatures. Lengths of all incision types differed significantly (*P* > 0.05) among surgeons, except for muscle-sparing incisions made by surgeon A and C on fish held at 12°C, which were not significantly different (*P* > 0.05; Figure 16). Lengths of incisions on the linea alba and muscle-cutting incisions were not significantly different (*P* > 0.05) for fish implanted by surgeon A, with the same being true for surgeon C at 12°C.

Among fish held at 20°C, muscle-sparing and muscle-cutting incisions made by surgeon A were not significantly different (*P* > 0.05), but both were significantly (*P* < 0.05) shorter than incisions on the linea alba made by the same surgeon. All incision types made by surgeon B differed significantly ($P < 0.05$), with incisions on the linea alba being the longest and muscle-cutting incisions being the shortest. Incisions on the linea alba and muscle-sparing incisions made by surgeon C were not significantly different ($P > 0.05$), but both were significantly ($P < 0.05$) longer than muscle-cutting incisions.

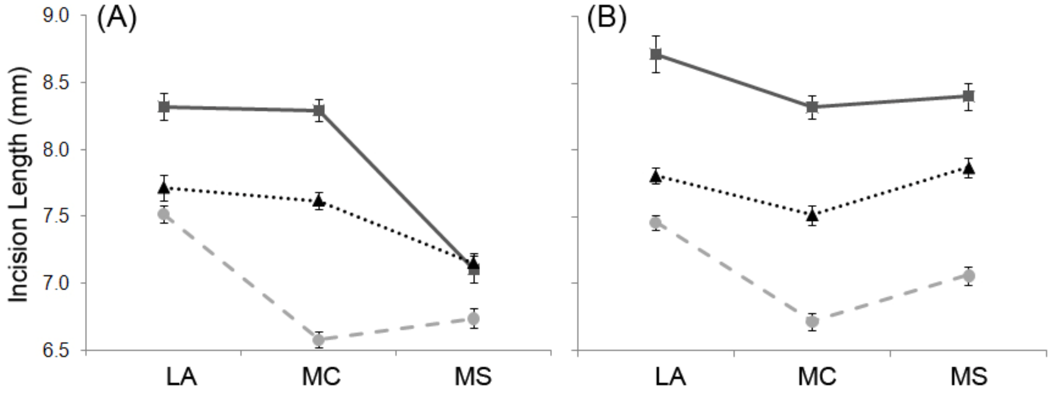

Figure 16. Incision lengths among juvenile Chinook salmon held at 12°C (A) or 20°C (B) and implanted with an acoustic transmitter and PIT tag implanted by surgeon A (-■-), B (-●-), or C (·▲·).

Suture Presence

Mean suture presence was significantly different ($P < 0.05$) among all three surgeons at both temperatures (Figure 17). Among fish held at both temperatures, the presence of sutures placed by surgeon A decreased significantly ($P < 0.05$) faster than those placed by surgeon B and C through the end of the study. Suture presence for fish implanted by surgeon B was between fish implanted by surgeon A and C, with surgeon A having the lowest and surgeon C the highest level of suture presence. A mean of 58% of sutures placed by surgeon C was still retained by day 98 compared to 0% by surgeon A and 13% by surgeon B. Among fish held at 20°C, 30% of sutures placed by surgeon C were still present by day 77 compared to 1% by surgeon A and 9% by surgeon B. However, less than 1% of sutures placed by surgeon A, B, and C were still present by 98 days among fish held at 20°C.

Review of the incision pictures revealed subtle differences in how far the suture entry and exit points were from the incision edges (suture bite). Surgeon C had the greatest suture bite, and surgeon A had the smallest suture bite.

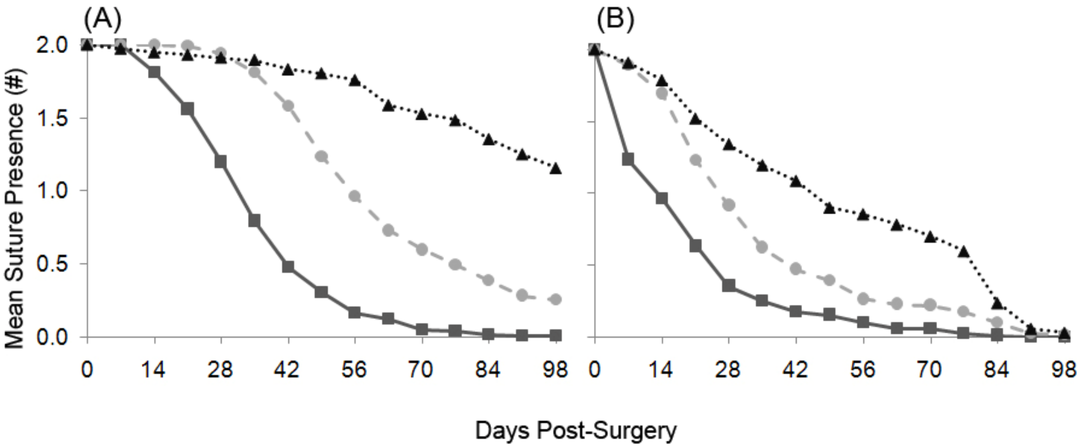

Figure 17. Mean suture presence among juvenile Chinook salmon held at 12°C (A) or 20°C (B) and implanted with an acoustic transmitter and PIT tag implanted by surgeon A (-■-), B (-●-), or C (·▲·).

Mortality and Growth

Mortality was not significantly different ($P > 0.05$) among surgeons at either temperature. At 12°C, growth differed among surgeons on day 77, 91, and 98 when fish implanted by surgeon C had a significantly ($P < 0.05$) lower growth rate (14% lower) than fish implanted by surgeon A. Growth was not significantly different ($P > 0.05$) for fish implanted by surgeon B compared to surgeons A and C. There was no significant correlation ($P > 0.05$) between growth rate and the number of sutures present on day 98.

DISCUSSION OF SURGEON EFFECTS

Healing

The variability in incision lengths among incision types and surgeons stresses the importance of continued practice for surgeons. Incision lengths differed over time (four months between temperature trials) even within a single surgeons' work, suggesting surgeons exhibit variability in their own techniques over time. There was little variability in the length of muscle-cutting incisions among surgeons and between temperatures, likely because all surgeons had the most experience using this location to implant transmitters. Incisions on the linea alba may have been longer than muscle-cutting incisions because the surgeons reported it was easier to cut a longer incision on the linea alba and they had less experience making incisions in this location. The length of muscle-sparing incisions had the most variability between the two temperature groups and presumably this was because it was the most dissimilar from the other two incision types. The surgeons received a small amount of practice with the muscle-sparing incision before performing it on the fish held at 12ºC. However, four months passed until the surgeons performed the muscle-sparing incision again, and surgeons likely needed continued practice with this incision to master it. The difference in incision lengths among surgeons further emphasizes the need for advanced training, feedback on results, and continued practice to reduce differences among surgeons.

Suture Presence

This research suggests that growth over the long term may be partially influenced by suture retention. Growth rates of fish held at 12°C were very low throughout the study, which may explain why there was no significant correlation ($P > 0.05$) between growth and suture retention on day 98. However, the higher number of sutures and the associated irritation of rubbing across the tissue of fish with incisions made by surgeon C (58%) compared to surgeon A (0%) remains the best suggestion for why growth differed between the two groups.

Surgeon Effects

The differences found among surgeons in suture retention and growth confirms the need for detailed, standardized protocols for performing fish surgeries at minimum (Cooke and Wagner 2004; Deters et al. 2009) with an additional need for feedback training. Cooke et al. (2003) found that surgeons with less experience had lower precision and lower retention of sutures than experienced surgeons. Some authors are responding to these findings by only using one experienced surgeon to perform all surgeries within a study, in order to reduce the variability from multiple surgeons (Brown et al. 2007; Hanson et al. 2008). However, some studies require large sample sizes and it is not possible for one surgeon to perform every surgery. All of the surgeons in this study had large amounts of experience, each having done hundreds of surgeries on juvenile salmonids. However, the observation that the greater distance between suture exit and entry points (suture bite) likely leads to higher suture retention suggests that even surgeons with extensive training and experience can have differences in techniques that can influence surgical outcomes. Deters et al. (2009) confirmed that surgeons with similar experience levels may differ in their techniques, which could affect surgical outcomes of suture and tag retention, incision openness, and healing. Future research may be needed to quantify the relative tightness of suture knots or size of a suture bite is and how these variables relate to transmitter retention and the healing rate.

APPENDIX B

Appendix B contains all of results for the main effects of incision location of fish held at both 12°C and 20°C in tabular format. Table 3 contains the results of an ANOVA of mean open area and table 4 contains the results of an ANOVA of mean ranks of apposition among incision locations. Table 5 contains the results of logistic regression of the presence or absence of erythema among incision locations. Table 6 contains the results of logistic regression the presence or absence of incision closure among incision locations. Table 7 contains the results of an ANOVA on the mean number of sutures present among incision locations.

Temp.	Location	7	14	21	28	35	42	49	56	63	70	77	84	91	98
		--Day--													
12°C	Linea alba	0.0 ± 0.0 _a_	0.3 ± 0.1 _a_*	0.4 ± 0.1 _a_*	0.4 ± 0.1 _a_*	0.7 ± 0.2 _a_	0.8 ± 0.2 _a_	0.8 ± 0.2 _a_	1.0 ± 0.4 _a_	0.3 ± 0.1 _a_	0.3 ± 0.2 _a_	0.5 ± 0.2 _a_	0.3 ± 0.1 _a_	0.3 ± 0.1 _a_	0.3 ± 0.1 _a_
	Muscle-cutting	1.7 ± 0.1 _b_	2.7 ± 0.1 _b_	1.2 ± 0.1 _b_	0.7 ± 0.1 _a,b_	0.8 ± 0.1 _a_	0.5 ± 0.2 _a_	0.3 ± 0.1 _a,b_	0.2 ± 0.1 _b_	0.1 ± 0.0 _a_	0.3 ± 0.1 _a_	0.0 ± 0.0 _b_	0.1 ± 0.0 _a_	0.0 ± 0.0 _b_	0.0 ± 0.0 _a_
	Muscle-sparing	0.3 ± 0.0 _c_	1.9 ± 0.1 _c_	1.4 ± 0.2 _b_	0.7 ± 0.1 _b_	0.5 ± 0.1 _a_	0.3 ± 0.1 _a_	0.2 ± 0.1 _b_	0.1 ± 0.0 _b_	0.2 ± 0.1 _a_	0.1 ± 0.1 _a_	0.1 ± 0.1 _a,b_	0.1 ± 0.1 _a_	0.1 ± 0.1 _b_	0.0 ± 0.0 _a_
20°C	Linea alba	0.5 ± 0.1 _a_*	1.0 ± 0.2 _a_*	1.3 ± 0.3 _a_*	0.4 ± 0.1 _a_	0.2 ± 0.1 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_
	Muscle-cutting	5.7 ± 0.4 _b_	2.5 ± 0.3 _b_	1.9 ± 0.2 _a_	0.2 ± 0.1 _a_	0.1 ± 0.1 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_
	Muscle-sparing	5.4 ± 0.7 _b_	2.5 ± 0.4 _b_	2.5 ± 0.4 _b_	0.4 ± 0.1 _a_	0.1 ± 0.1 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_	0.0 ± 0.0 _a_

Table 3. ANOVA on mean open area among incision locations by week at 12°C and 20°C. An asterisk (*) indicates interaction between incision location and surgeon.

Temp.	Location	7	14	21	28	35	42	49	56	63	70	77	84	91	98
		--Day--													
12°C	Linea alba	1.4± 0.05 _a_*	1.4± 0.06 _a_*	1.4± 0.06 _a_*	1.3± 0.05 _a_*	1.3± 0.05 _a_	1.3± 0.06 _a_*	1.2± 0.04 _a_*	1.2± 0.05 _a_	1.1± 0.03 _a_	1.1± 0.04 _a_	1.1± 0.02 _a_	1.1± 0.03 _a_	1.1± 0.03 _a_	1.1± 0.03 _a_
	Muscle-cutting	3.3± 0.07 _b_	3.6± 0.07 _b_	2.2± 0.10 _b,c_	1.5± 0.08 _a_	1.4± 0.06 _a,b_	1.1± 0.03 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_	1.0± 0.00 _b,c_
	Muscle-sparing	1.8± 0.06 _c_	2.9± 0.07 _c_	2.0± 0.09 _c_	1.4± 0.06 _a_	1.2± 0.04 _a,c_	1.1± 0.03 _c_	1.0± 0.02 _c_	1.0± 0.00 _c_	1.0± 0.01 _c_	1.0± 0.01 _c_	1.0± 0.00 _c_	1.0± 0.01 _c_	1.0± 0.00 _c_	1.0± 0.01 _c_
20°C	Linea alba	1.4± 0.07 _a_*	1.5± 0.07 _a_*	1.4± 0.06 _a_	1.2± 0.05 _a_	1.1± 0.02 _a_	1.0 ± 0.02 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_
	Muscle-cutting	3.6± 0.06 _b_	2.4± 0.09 _b_	1.4± 0.06 _a_	1.2± 0.04 _a_	1.0± 0.02 _a_	1.0 ± 0.00 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_
	Muscle-sparing	3.0± 0.08 _c_	2.0± 0.08 _b_	1.4± 0.06 _a_	1.2± 0.05 _a_	1.1± 0.03 _a_	1.0 ± 0.00 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_	1.0 ± 0.0 _a_

Table 4. ANOVA on mean ranks among incision locations by week at 12°C and 20°C. An asterisk (*) indicates interaction between incision location and surgeon.

				--Day--											
Temp.	**Location**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**98**
12°C	Linea alba	40% *a*	22% *a*	11% *a*	7% *a*	5% *a*	10% *a*	13% *a*	4% *a*	5% *a*	9% *a*	4% *a*	3% *a*	1% *a*	0% *a*
	Muscle-cutting	71% *b*	80% *b*	27% *b*	19% *b*	2% *a*	4% *a,b*	1% *b*	0% *a*	1% *a*	1% *a*	1% *a*	0% *a*	0% *a*	0% *a*
	Muscle-sparing	64% *b*	49% *c*	15% *a*	7% *a*	1% *a*	2% *b*	4% *b*	1% *a*	3% *a*	3% *a*	1% *a*	1% *a*	0% *a*	0% *a*
20°C	Linea alba	63% *a*	28% *a*	16% *a*	8% *a*	2% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*
	Muscle-cutting	68% *a*	14% *b*	2% *b*	3% *a,b*	1% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*
	Muscle-sparing	51% *b*	9% *b*	6% *b*	2% *b*	1% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*	0% *a*

Table 5. Logistic regression on proportion of fish with erythema on incisions at 12°C and 20°C. An asterisk (*) indicates interaction between incision location and surgeon.

				--Day--											
Temp.	**Location**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**98**
12°C	Linea alba	0.0% *a*	0.0% *a*	0.0% *a*	50% *a*	65% *a,b*	60% *a*	66% *a*	78% *a*	80% *a*	78% *a*	82% *a*	82% *a*	83% *a*	88% *a*
	Muscle-cutting	0.0% *a*	0.0% *a*	0.0% *a*	30% *b*	55% *a*	68% *a*	78% *a*	89% *a*	88% *a*	96% *b*	96% *b*	98% *b*	98% *b*	98% *a*
	Muscle-sparing	0.0% *a*	0.0% *a*	0.0% *a*	38% *b*	76% *b*	80% *b*	90% *b*	95% *b*	94% *a*	95% *b*	95% *b*	97% *b*	99% *b*	99% *a*
20°C	Linea alba	0.0% *a*	13% *a*	44% *a*	77% *a*	92% *a*	94% *a*	97% *a*	97% *a*	98% *a*	99% *a*	100% *a*	100% *a*	100% *a*	100% *a*
	Muscle-cutting	0.0% *a*	7% *a*	24% *b*	79% *a*	93% *a*	96% *a*	97% *a*	96% *a*	99% *a*	99% *a*	100% *a*	100% *a*	100% *a*	100% *a*
	Muscle-sparing	0.0% *a*	8% *a*	22% *b*	83% *a*	88% *a*	95% *a*	99% *a*	98% *a*	100% *a*	100% *a*	100% *a*	100% *a*	100% *a*	100% *a*

Table 6. Logistic regression on proportion of fish with incision closure at 12°C and 20°C. An asterisk (*) indicates interaction between incision location and surgeon.

		--Day--						
Temp.	**Location**	**7**	**14**	**21**	**28**	**35**	**42**	**49**
12°C	Linea alba	2.0 ± 0.01 *a*	1.9± 0.02 *a**	1.9± 0.03 *a**	1.8± 0.05 *a**	1.6± 0.06 *a**	1.3± 0.07 *a**	1.2± 0.08 *a**
	Muscle-cutting	2.0 ± 0.01 *a*	1.9± 0.02 *a*	1.9± 0.03 *a,b*	1.8± 0.05 *a*	1.6± 0.06 *a*	1.4± 0.07 *a*	1.2± 0.07 *a,b*
	Muscle-sparing	2.0 ± 0.01 *a*	1.9± 0.04 *a*	1.9± 0.05 *b*	1.5± 0.07 *b*	1.4± 0.07 *a*	1.2± 0.08 *a*	0.9± 0.08 *b*
20°C	Linea alba	1.9± 0.03 *a**	1.7± 0.05 *a**	1.3± 0.07 *a*	1.1± 0.08 *a*	0.9± 0.08 *a**	0.8 ± 0.07 *a**	0.7 ± 0.08 *a**
	Muscle-cutting	1.6± 0.06 *b*	1.4± 0.07 *b*	1.2± 0.07 *a*	1.0± 0.07 *a*	0.8± 0.07 *a*	0.7 ± 0.07 *a*	0.6 ± 0.07 *a*
	Muscle-sparing	1.7± 0.06 *a,b*	1.6± 0.06 *b*	0.9± 0.08 *b*	0.6± 0.07 *b*	0.4± 0.06 *b*	0.2 ± 0.05 *b*	0.2 ± 0.04 *b*

		56	**63**	**70**	**77**	**84**	**91**	**98**
12°C	Linea alba	1.1± 0.08 *a**	1.0± 0.08 *a**	0.9± 0.08 *a**	0.8± 0.08 *a**	0.7± 0.08 *a**	0.6± 0.08 *a*	0.6± 0.07 *a**
	Muscle-cutting	1.0± 0.08 *a,b*	0.8± 0.08 *a,b*	0.7± 0.08 *a,b*	0.7± 0.07 *a,b*	0.6± 0.07 *a*	0.5± 0.07 *a,b*	0.5± 0.07 *a*
	Muscle-sparing	0.8± 0.08 *b*	0.6± 0.08 *b*	0.5± 0.07 *a*	0.5± 0.07 *b*	0.4± 0.05 *b*	0.3± 0.06 *b*	0.3± 0.05 *b*
20°C	Linea alba	0.6 ± 0.08 *a**	0.6 ± 0.08 *a**	0.5 ± 0.07 *a**	0.4 ± 0.07 *a**	0.2 ± 0.04 *a**	0.1 ± 0.02 *a**	0.0 ± 0.02 *a*
	Muscle-cutting	0.5 ± 0.07 *a*	0.4 ± 0.06 *a*	0.4 ± 0.06 *a*	0.3 ± 0.06 *a*	0.1 ± 0.03 *a,b*	0.0 ± 0.01 *a*	0.0 ± 0.01 *a*
	Muscle-sparing	0.1 ± 0.04 *b*	0.1 ± 0.04 *b*	0.1 ± 0.03 *b*	0.0 ± 0.02 *b*	0.0 ± 0.02 *b*	0.0 ± 0.01 *a*	0.0 ± 0.01 *a*

Table 7. ANOVA on mean number of sutures retained by fish at 12°C and 20°C. An asterisk (*) indicates interaction between incision location and surgeon.